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CONTENTS—PART 1

Program Committee iv
Chronological List of Sessions v
Thematic List of Sessions xii
Abstracts in Session Order*
Monday, October 30–Thursday, November 2..... 1

*8,138 volunteer abstracts, 17 symposia.

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CHRONOLOGICAL LIST OF SESSIONS

(see page xii for Thematic List of Sessions)

Session Number and Title	Page
SUNDAY	
Public Lecture—8:00 p.m.	
1. Neural Networks for Vision and Memory in Humans. A.R. Damasio	No abstract
MONDAY	
Symposia—8:30 a.m.	
2. The Neurobiology of Neuropeptide Y (NPY). *Chaired by:* W.F. Colmers	1
3. Functional Organization of the Thalamus. *Chaired by:* S.M. Sherman	1
Slide Sessions—8:30 a.m.	
4. Visual system: development and plasticity I	1
5. Human behavioral neurobiology: memory	3
6. Serotonin I	5
7. Neural-immune interactions I	7
8. Transplantation I	9
9. Cell lineage and determination I	11
10. Neuroglia	13
11. Calcium channels I	15
12. Trophic agents I	17
13. Neural plasticity in adult animals I	19
14. The aging process	21
15. Biological rhythms and sleep: neuroregulators	23
16. Transmitters in invertebrates I	25
17. Brain metabolism and blood flow	27
Poster Sessions—8:30 a.m.	
18. Mental illness: affective disease	29
19. Comparative neuroanatomy I	31
20. Motivation and emotion I	33
21. Alcohol, barbiturates, benzodiazepines I	36
22. Degenerative disease: Parkinson's I	39
23. Ischemia: pharmacological protection	42
24. Epilepsy: genetic models	46
25. Control of posture and movement I	48
26. Control of posture and movement II	52
27. Motivation and emotion II	55
28. Alcohol, barbiturates, benzodiazepines II	58
29. Acetylcholine I	62
30. Motor systems I	65
31. Motor systems II	66
32. Motor systems III	68
33. Association cortex and thalamocortical relations I	70
34. Association cortex and thalamocortical relations II	72
35. Potassium channels I	74
36. Ion channels: chloride and miscellaneous	78
37. Learning and memory: physiology I	80
38. Long-term potentiation I	84
39. Endocrine control and development I	87
40. Sprouting and sprouting mechanisms I	89
41. Sprouting and sprouting mechanisms II	91
42. Endocrine control and development II	93
43. Genetic models of nervous disorders I	95
44. Genetic models of nervous disorders II	97
45. Respiratory regulation I	98
46. Differentiation, morphogenesis and development: position and form	101
47. Somatic and visceral afferents I	102
48. Uptake, storage, secretion and metabolism I	106
49. Auditory system: cortex and integration	110
50. Auditory system: avian connections and processing	114
51. Retina I	115
52. Visual cortex I	117
53. Visual psychophysics and behavior I	121
54. Degenerative disease: Parkinson's II	123
55. Specificity of synaptic connections	125
56. Trauma I	128
57. Trauma II	131
58. Hypothalamic-pituitary-adrenal regulation: CRH	134
59. Neurotoxicity: dopamine	137
60. Neurotoxicity I	140
61. Opiates, endorphins and enkephalins: tolerance and dependence	143
62. Opiates, endorphins and enkephalins: physiological effects I	146
63. Pain modulation: CNS pathways	149
64. Pain modulation: opioid mechanisms	153
65. Cytoskeleton, transport and membrane targeting	156
Special Lecture—11:45 a.m.	
66. Proto-Oncogenes and Transcription Factors in the Brain: A Role in Adaptation? T. Curran	No abstract
Symposia—1:00 p.m.	
67. New Opportunities for Study of Mechanisms of Central Nervous System Ischemia. *Chaired by:* J.A. Zivin	161
68. From Early Embryogenesis to Circuits: Cellular and Molecular Strategies Applied to the Analysis of Vertebrate Brain Development. *Chaired by:* D. Goldowitz and P. Levitt	161
Slide Sessions—1:00 p.m.	
69. Visual cortex II	161
70. Synaptogenesis I	163
71. Long-term potentiation II	165
72. Second messengers I	168
73. Catecholamine receptors	170
74. Control of posture and movement III	172
75. Subcortical visual pathways I	174
76. Ion channel modulation and regulation I	176
77. Cerebellum I	179
78. Pain modulation	181

Session Number and Title	Page
79. Postsynaptic mechanisms I	183
80. Peptides: biosynthesis, metabolism and biochemical characterization I	185
81. Peptides: anatomical localization I	187
82. Neuroendocrine regulation I	188
Poster Sessions—1:00 p.m.	
83. Presynaptic mechanisms I	190
84. Pharmacology of synaptic transmission	193
85. Sodium channels I	196
86. Excitatory amino acids: receptors I	198
87. Excitatory amino acids: receptors II	201
88. Retina II	204
89. Auditory system: hair cells	207
90. Auditory system: cochlea	209
91. Peptides: physiological effects I	212
92. Peptides: physiological effects II	216
93. Serotonin receptors I	219
94. Serotonin II	223
95. Interactions between neurotransmitters I	226
96. Regional localization of receptors and neurotransmitters I	229
97. Other biogenic amines and purines: adenosine and histamine	232
98. Epilepsy: human studies	236
99. Oculomotor system I	238
100. Biological rhythms and sleep: sleep	241
101. Human behavioral neurobiology: memory and language	244
102. Behavioral pharmacology: analgesics and NMDA	247
103. Drugs of abuse: biogenic amines	249
104. Drugs of abuse: cocaine I	252
105. Synaptic structure and function I	256
106. Aging I	259
107. Regulation of autonomic functions I	263
108. Transplantation: cortex and brainstem	265
109. Psychotherapeutic drugs: dopamine and neuropeptics	268
110. Stress, hormones and the autonomic nervous system	272
111. Differentiation, morphogenesis and development: forebrain	276
112. Trophic agents II	277
113. Cortex I	280
114. Cortex II	282
115. Basal ganglia and thalamus I	284
116. Basal ganglia and thalamus II	287
117. Auditory, olfactory and other sensory systems	290
118. Biochemical and pharmacological correlates of development I	292
119. Neural-immune interactions: stress	295
120. Neural plasticity in adult animals: motor systems	298
121. Learning and memory: anatomy I	302
122. Staining and tracing techniques	306
123. Somatosensory cortex and thalamocortical relationships I	311
124. Somatosensory cortex and thalamocortical relationships II	313
125. Regeneration: general	315
126. Regeneration: GAP43	318
127. Regeneration: CNS I	319
Special Lecture—4:15 p.m.	
128. Genes that Control Aspects of the Development of a Nematode. H.R. Horvitz	No abstract
Presidential Symposium—8:00 p.m.	
129. Visual Pigments, Color Vision, and Color Blindness. J. Nathans. Mechanisms of Phototransduction in Retinal Rods and Cones. K.-W. Yau. Signal Flow in Visual Transduction. D.A. Baylor	No abstract

TUESDAY

Symposia—8:30 a.m.	
130. Hormones, Neural Circuits and Communication. *Chaired by:* A.P. Arnold	322
131. The Initial Events in Taste: Chemosensory Transduction in the Vertebrate Taste Bud. *Chaired by:* S.D. Roper	322
Slide Sessions—8:30 a.m.	
132. Visual cortex III	323
133. Excitatory amino acids: receptors III	325
134. Differentiation, morphogenesis and development: cellular and molecular studies I	327
135. Alzheimer's disease I	329
136. Process outgrowth, growth cones and guidance mechanisms I	331
137. Regeneration I	333
138. Cardiovascular regulation I	335
139. Potassium channels II	337
140. Epilepsy: basic mechanisms I	339
141. Learning and memory: anatomy II	341
142. mRNA regulation I	343
143. Peptides: receptors I	345
144. Neuroethology I	347
Poster Sessions—8:30 a.m.	
145. Neuroglia: myelin and myelin-forming cells	349
146. Neuroglia: active membrane responses	351
147. Calcium channels II	354
148. Ischemia: excitability and neurotransmission	357
149. Ischemia: mediators of neuronal death	360
150. Transmitters in invertebrates II	364
151. Peptides: biosynthesis, metabolism and biochemical characterization II	368
152. Pain modulation: spinal opioid mechanisms	370
153. Differentiation, morphogenesis and development: neuromuscular development	372
154. Comparative neuroanatomy II	374
155. Hormonal control of behavior I	377
156. Neural-immune interactions II	380
157. Subcortical somatosensory pathways I	383
158. Subcortical somatosensory pathways II	385

Session Number and Title	Page
159. Spinal cord and brainstem I	389
160. Control of posture and movement IV	392
161. Control of posture and movement V	395
162. Long-term potentiation III	398
163. Hippocampus and amygdala I	400
164. Cerebellum II	404
165. Acetylcholine II	407
166. Monoamines and behavior I	410
167. Alcohol, barbiturates, benzodiazepines III	414
168. Serotonin III	417
169. Serotonin receptors II	420
170. Catecholamine receptors: dopaminergic (D2)	423
171. Catecholamine receptors: dopaminergic (D1)	428
172. Catecholamine receptors: dopaminergic	431
173. Catecholamine receptors: adrenergic	432
174. Second messengers II	434
175. Neuronal death: models and mechanisms	436
176. Somatic and visceral afferents II	439
177. Neuronal death: lesion induced	442
178. Differentiation, morphogenesis and development: fiber guidance and synaptogenesis	445
179. Brain metabolism and blood flow I	446
180. Brainstem systems	450
181. Epilepsy: kindling I	453
182. Visual system: development and plasticity II	455
183. Subcortical visual pathways II	458
184. Biological rhythms and sleep: invertebrates	461
185. Learning and memory—pharmacology: NMDA	463
186. Learning and memory—pharmacology: monoamines	465
187. Pain pathways: spinal cord	468
188. Pain pathways: primary afferents	470
189. Presynaptic mechanisms: calcium	472
190. Presynaptic mechanisms: facilitation and depression	475
191. Human behavioral neurobiology: event related potentials	476
History of Neuroscience Lecture—11:45 a.m.	
192. Revolutions and Breakthroughs in Nerve and Muscle. Sir A. Huxley	No abstract
Symposia—1:00 p.m.	
193. Optical Imaging of CNS Development, Organization and Function. *Chaired by:* A. Grinvald	479
194. Recent Advances in the Biology of Affective Disorders. *Chaired by:* C.B. Nemeroff	479
Slide Sessions—1:00 p.m.	
195. Excitatory amino acids: excitotoxicity I	479
196. Human behavioral neurobiology: other I	481
197. Presynaptic mechanisms II	483
198. Serotonin receptors III	485
199. GABA and benzodiazepines I	487
200. Transplantation II	489
201. Biological rhythms and sleep: other I	492
202. Visual system: development and plasticity III	494
203. Cholinergic receptors	496
204. Differentiation, morphogenesis and development: cellular and molecular studies II	498
205. mRNA regulation II	500
206. Vestibular system	502
207. Spinal cord and brainstem II	504
208. Learning and memory: physiology II	505
Poster Sessions—1:00 p.m.	
209. Neuroglia in disease	507
210. Neuroglia: structure and biology	510
211. Vestibular system: VOR and integration	513
212. Vestibular system: receptor organs and vestibular nuclei	517
213. Muscle I	519
214. Muscle II	522
215. Postsynaptic mechanisms II	524
216. Pharmacology of synaptic transmission: amino acids and calcium channels	528
217. Excitatory amino acids: receptors IV	531
218. Excitatory amino acids: receptors V	533
219. Sodium channels II	536
220. Potassium channels III	538
221. Opiates, endorphins and enkephalins: physiological effects II	542
222. Pain modulation: biogenic amines I	545
223. Pain modulation: biogenic amines II	547
224. Alcohol, barbiturates, benzodiazepines IV	550
225. Serotonin IV	553
226. Acetylcholine III	554
227. Monoamines and behavior II	557
228. Peptides: anatomical localization II	561
229. Peptides: receptors II	563
230. Membrane composition and cell surface macromolecules I	567
231. Process outgrowth, growth cones and guidance mechanisms II	569
232. Process outgrowth, growth cones and guidance mechanisms III	573
233. Endocrine control and development III	576
234. Endocrine control and development IV	578
235. Interactions between neurotransmitters II	581
236. Regional localization of receptors and neurotransmitters II	584
237. Differentiation, morphogenesis and development: neurogenesis and control of neuron number	587
238. Differentiation, morphogenesis and development: cell surface components	590
239. Cardiovascular regulation II	592
240. Cardiovascular regulation: hypertension	595
241. Cell lineage and determination II	598
242. Uptake, storage, secretion and metabolism II	600
243. Control of posture and movement VI	604
244. Learning and memory: anatomy III	607
245. Cerebellum III	611
246. Ingestive behaviors I	614
247. Neuroethology II	617
248. Epilepsy: anti-epileptic drugs	621
249. Visual psychophysics and behavior II	623
250. Hypothalamic-pituitary-gonadal regulation I	626
251. Regulation of autonomic functions II	630
252. Behavioral pharmacology: benzodiazepines and stimulants	632

Session Number and Title	Page
253. Drugs of abuse	636
Presidential Special Lecture—4:15 p.m.	
254. The Relation of Speech to Other Specializations. A. Liberman	No abstract
Grass Foundation Lecture—8:00 p.m.	
255. Ionic Channels: Modulation by G Protein Coupled Receptors and Second Messengers. B. Hille	No abstract

WEDNESDAY

Symposia—8:30 a.m.	
256. Progress in Research on D1 Dopamine Receptors. *Chaired by:* R.E. Chipkin	639
257. Computing Motion in Flies, Monkeys and Man: Linking Physiology with Psychophysics and Computational Theory. *Chaired by:* C. Koch	639
Slide Sessions—8:30 a.m.	
258. Learning and memory: anatomy IV	639
259. GABA and benzodiazepine receptors I	641
260. Excitatory amino acids: anatomy and physiology I	643
261. Gene structure and function I	645
262. Alzheimer's disease: amyloid I	647
263. Process outgrowth, growth cones and guidance mechanisms IV	649
264. Calcium channels III	651
265. Genetic models of nervous disorders III	653
266. Ingestive behaviors II	655
267. Second messengers III	657
268. Sensorimotor integration	659
269. Regulation of autonomic functions III	661
270. Opiates, endorphins and enkephalins: physiological effects III	663
271. Transmitters in invertebrates III	665
Poster Sessions—8:30 a.m.	
272. GABA and benzodiazepines II	667
273. Peptides: receptors III	670
274. Serotonin receptors IV	673
275. Nicotinic receptors	675
276. Synaptic structure and function II	680
277. Adrenal medullary regulation	683
278. Developmental disorders: genetic and chemical models	684
279. Neurotoxicity II	686
280. Differentiation, morphogenesis and development: glia	689
281. Control of posture and movement VII	691
282. Spinal cord and brainstem: cord anatomy	694
283. Mental illness: schizophrenia	697
284. Epilepsy: basic mechanisms II	700
285. Opiates, endorphins and enkephalins: anatomy and chemistry I	702
286. Trophic agents III	704
287. Trophic agents IV	708
288. Neuropeptides and behavior	711
289. Neural-immune interactions III	713
290. Hypothalamic-pituitary-adrenal regulation I	715
291. Neural-immune interactions IV	718
292. Neuroendocrine regulation: prolactin	721
293. Biological rhythms and sleep: other II	724
294. Human behavioral neurobiology: other II	728
295. Learning and memory—pharmacology: acetylcholine I	731
296. Learning and memory—pharmacology: acetylcholine II	733
297. Transmitters in invertebrates IV	736
298. Peptides: anatomical localization III	739
299. Auditory system: cochlear nucleus	742
300. Auditory system: brainstem and higher nuclei	745
301. Chemical senses: peripheral olfaction	748
302. Chemical senses: taste and carotid receptors	751
303. Spinal cord	754
304. Hormonal control of behavior II	758
305. Excitatory amino acids: excitotoxicity II	761
306. Excitatory amino acids: excitotoxicity III	764
307. Excitatory amino acids: excitotoxicity IV	768
308. Regional localization of receptors and neurotransmitters III	771
309. Learning and memory: physiology III	773
310. Epilepsy: kindling II	777
311. Neural plasticity in adult animals: monoamines and ACH	780
312. Oculomotor system II	783
313. Cortex III	786
314. Cortex IV	788
315. Subcortical visual pathways III	790
316. Visual system: development and plasticity IV	793
Presidential Special Lecture—11:45 a.m.	
317. Visual Perceptions in People and Machines. V.S. Ramachandran	No abstract
Symposia—1:00 p.m.	
318. Mesopontine Cholinergic Neurons: The Neuronal Substrate of the Ascending Reticular Activating System? *Chaired by:* P.B. Reiner	797
319. Hebbian Synapse: Learning Rules and Mechanisms. *Chaired by:* J. Lisman	797
Slide Sessions—1:00 p.m.	
320. Visual cortex IV	797
321. Hypothalamic-pituitary-adrenal regulation II	799
322. Drugs of abuse: cocaine II	801
323. Ischemia	803
324. Oculomotor system III	806
325. Morphogenesis and differentiation	808
326. Interactions between neurotransmitters III	810
327. Cardiovascular regulation III	812
328. Uptake, storage, secretion and metabolism III	814
329. Somatic and visceral afferents III	816
330. Catecholamines I	818
331. Blood-brain barrier I	820

Session Number and Title	Page
Poster Sessions—1:00 p.m.	
332. Calcium channels IV	822
333. Ligand-gated ion channels: cholinergic	826
334. Ligand-gated ion channels: glutamate	828
335. GABA and benzodiazepine receptors II	830
336. Second messengers: protein phosphorylation	832
337. Peptides: anatomical localization IV	835
338. Peptides: biosynthesis, metabolism and biochemical characterization III	838
339. mRNA regulation III	841
340. Opiates, endorphins and enkephalins: behavioral effects	844
341. Pain modulation: afferent mechanisms	847
342. Psychotherapeutic drugs: antidepressants	850
343. Brain metabolism and blood flow II	854
344. Alzheimer's disease: transmitters and behavior	858
345. Trophic agents V	864
346. Trophic agents VI	867
347. Regeneration: CNS II	870
348. Process outgrowth, growth cones and guidance mechanisms V	873
349. Process outgrowth, growth cones and guidance mechanisms VI	875
350. Regeneration: cell biology	878
351. Regeneration: PNS	881
352. Cell lineage and determination III	884
353. Nutrition and prenatal factors	886
354. Learning and memory: anatomy V	888
355. Membrane composition and cell surface macromolecules II	891
356. Ingestive behaviors III	894
357. Regulation of autonomic functions IV	897
358. Basal ganglia and thalamus III	900
359. Basal ganglia and thalamus IV	904
360. Basal ganglia and thalamus V	907
361. Basal ganglia and thalamus VI	910
362. Basal ganglia and thalamus VII	912
363. Motor systems: reflex function I	914
364. Motor systems: reflex function II	917
365. Spinal cord and brainstem: cord physiology	919
366. Retina III	922
367. Chemical senses: olfactory pathways and processing	926
368. Chemical senses: gustatory pathways	929
369. Degenerative disease: other I	931
370. Degenerative disease: other II	934
371. Degenerative disease: Parkinson's III	937
372. Excitatory amino acids: anatomy and physiology II	940
373. Excitatory amino acids: anatomy and physiology III	943
374. Excitatory amino acids: anatomy and physiology IV	946
375. Neuroendocrine regulation: photoperiod/pineal	949
Presidential Special Lecture—4:15 p.m.	
376. Cognitive Illusions in Judgement and Choice. A. Tversky	No abstract

Session Number and Title	Page
THURSDAY	
Symposia—8:30 a.m.	
377. The Basal Ganglia: Structure and Function. *Chaired by:* M.R. DeLong	952
378. Instructive Effects of Activity in the Developing Visual Pathway. *Chaired by:* M. Constantine-Paton	952
Slide Sessions—8:30 a.m.	
379. Trophic agents VII	953
380. Excitatory amino acids: receptors VI	955
381. Gene structure and function II	957
382. Process outgrowth, growth cones and guidance mechanisms VII	959
383. Neural plasticity in adult animals II	961
384. Ingestive behaviors IV	963
385. Cardiovascular regulation IV	965
386. Retina IV	967
387. Ligand-gated ion channels I	969
388. Peptides: physiological effects III	971
389. Receptor modulation: up and down regulation I	974
Poster Sessions—8:30 a.m.	
390. Neural plasticity in adult animals: limbic system	976
391. Limbic system I	979
392. Opiates, endorphins and enkephalins: anatomy and chemistry II	980
393. mRNA regulation IV	984
394. Peptides: receptors IV	987
395. Potassium channels IV	990
396. Potassium channels: modulation and regulation	992
397. GABA and benzodiazepine receptors III	994
398. GABA and benzodiazepines III	997
399. Catecholamines II	999
400. Second messengers: calcium	1003
401. Second messengers: phosphoinositide turnover	1005
402. Catecholamines: neurotoxicity	1009
403. Catecholamines III	1011
404. Differentiation, morphogenesis and development: transmitters and enzymes	1015
405. Biochemical and pharmacological correlates of development II	1017
406. Differentiation, morphogenesis and development: channels and currents	1020
407. Neurotoxicity: metals and organics	1021
408. Nutrition and prenatal factors: substances of abuse	1023
409. Blood-brain barrier II	1025
410. Process outgrowth, growth cones and guidance mechanisms VIII	1027
411. Epilepsy: basic mechanisms III	1030
412. Neuromuscular disease	1033
413. Process outgrowth, growth cones and guidance mechanisms IX	1036
414. Alzheimer's disease: neuropathology	1038
415. Circuitry and pattern generation: vertebrates	1044
416. Circuitry and pattern generation: invertebrates and models	1046
417. Somatosensory system I	1050

Session Number and Title	Page
418. Somatosensory cortex and thalamocortical relationships III	1051
419. Visual cortex V	1054
420. Biological rhythms and sleep: other III	1058
421. Interhemispheric relations	1060
422. Behavioral pharmacology: dopamine	1063
423. Neuropeptides and behavior: CCK	1067
424. Neuropeptides and behavior: CRF	1068
425. Neuropeptides and behavior: oxytocin and vasopressin	1069
426. Regulation of autonomic functions V	1071
427. Epilepsy: benzodiazepines and inhibitor amino acids	1073
428. Neuroendocrine regulation: neurohypophysial peptides	1075
429. Hypothalamic-pituitary-adrenal regulation III	1079
430. Hypothalamic-pituitary-gonadal regulation II	1082
431. Hypothalamus	1085
432. Drugs of abuse: dopamine mechanisms	1090
433. Transplantation: hippocampus and basal forebrain	1093
434. Drugs of abuse: CNS pathways	1095
435. Hormonal control of behavior III	1099
436. Long-term potentiation IV	1101
437. Learning and memory: anatomy VI	1104
Warner-Lambert Lecture—11:45 a.m.	
438. Mechanisms Underlying the Self-Organization of Visual Cortex Functions. W. Singer	No abstract
Symposia—1:00 p.m.	
439. Neuropeptide Regulation of Reproduction. *Chaired by:* P.E. Micevych	1106
440. Inhibitory Influences on Growth Cones and Cells. *Chaired by:* M.E. Schwab	1106
Slide Sessions—1:00 p.m.	
441. Visual cortex VI	1106
442. Biochemical and pharmacological correlates of development III	1108
443. Alzheimer's disease II	1110
444. Trauma III	1112
445. Auditory system	1114
446. Invertebrate learning and behavior I	1116
447. Circuitry and pattern generation	1118
448. Differentiation, morphogenesis and development: cellular and molecular studies III	1120
449. Mental illness	1122
Poster Sessions—1:00 p.m.	
450. Gene structure and function III	1124
451. mRNA regulation V	1127
452. Ingestive behaviors V	1130
453. Monoamines and behavior III	1133
454. Neuroethology III	1135
455. Invertebrate learning and behavior II	1139
456. Ion channels: cell function	1142
457. Calcium channels: modulation and regulation	1147
458. Ligand-gated ion channels II	1150
459. GABA and benzodiazepine receptors IV	1152
460. Monoamines and behavior IV	1155
461. Excitatory amino acids: receptors VII	1159
462. Excitatory amino acids: receptors VIII	1162
463. Excitatory amino acids: receptors IX	1165
464. Learning and memory—pharmacology: other I	1169
465. Learning and memory—pharmacology: other II	1172
466. Behavioral pharmacology: other	1174
467. Cardiovascular regulation V	1177
468. Cardiovascular regulation VI	1180
469. Drugs of abuse: stimulants	1185
470. Pain pathways: CNS	1188
471. Respiratory regulation II	1191
472. Acetylcholine IV	1194
473. Control of posture and movement VIII	1198
474. Control of posture and movement IX	1199
475. Oculomotor system IV	1202
476. Retina V	1206
477. Visual system: development and plasticity V	1210
478. Epilepsy: excitatory amino acids	1213
479. Receptor modulation: up and down regulation II	1216
480. Blood-brain barrier III	1219
481. Neural plasticity in adult animals: sensory systems	1221
482. Catecholamines IV	1225
483. Catecholamines: anatomy	1229
484. Neurotoxicity: MPTP and excitotoxic	1231
485. Psychotherapeutic drugs	1233
486. Clinical CNS neurophysiology	1237
487. Transplantation: spinal cord	1241
488. Limbic system II	1243
489. Hippocampus and amygdala II	1246
490. Hippocampus and amygdala III	1249
491. Interactions between neurotransmitters IV	1252
Special Lecture—4:15 p.m.	
492. Microtubules and Cell Morphogenesis. M. Kirschner	No abstract

FRIDAY

Symposium—8:30 a.m.	
493. Melatonin: New Light on CNS Mechanisms of Action. *Chaired by:* D.N. Krause & M.L. Dubocovich	1255
Special Lecture—8:30 a.m.	
494. Ion Channel Proteins: Structure from Function. C. Miller	No abstract
Special Lecture—10:00 a.m.	
495. Neuronal Organization in the Cerebral Cortex. A. Peters	No abstract
Special Lecture—11:30 a.m.	
496. Signal Processing and Neural Networks in the Oculomotor System. D.A. Robinson	No abstract
Slide Sessions—8:30 a.m.	
497. Visual psychophysics and behavior III	1255
498. Regeneration II	1257
499. Trophic interactions I	1259

Session Number and Title	Page
500. Process outgrowth, growth cones and guidance mechanisms X	1261
501. Invertebrate learning and behavior III	1263
502. Pain pathways	1265
Poster Sessions—8:30 a.m.	
503. Gene structure and function IV	1267
504. mRNA regulation VI	1271
505. Uptake, storage, secretion and metabolism IV	1274
506. Peptides: biosynthesis, metabolism and biochemical characterization IV	1275
507. Ingestive behaviors VI	1277
508. Monoamines and behavior V	1281
509. Invertebrate learning and behavior IV	1283
510. Invertebrate sensory systems I	1286
511. Invertebrate sensory systems II	1290
512. Neuroethology IV	1292
513. Invertebrate motor function	1296
514. Ion channel modulation and regulation II	1299
515. Muscarinic receptors	1302
516. Postsynaptic mechanisms III	1307
517. Catecholamines V	1310
518. Catecholamines VI	1314
519. Receptor modulation: up and down regulation III	1316
520. Second messengers: adenylate cyclase	1320
521. Differentiation, morphogenesis and development: cytoskeleton	1323
522. Biological rhythms and sleep: other IV	1324
523. Differentiation, morphogenesis and development: molecular correlates	1327
524. Differentiation, morphogenesis and development: tissue culture models	1330
525. Somatosensory system II	1331
526. Developmental disorders: human diseases	1333
527. Visual system: development and plasticity VI	1335
528. Hypothalamic-pituitary-gonadal regulation III	1339
529. Ischemia: energy metabolism and ischemic models	1342
530. Neurotoxicity III	1346
531. Neurotoxicity IV	1349
532. Synaptogenesis: neuromuscular junction	1351
533. Transplantation: striatum I	1353
534. Transplantation: striatum II	1356
535. Trophic interactions II	1359
536. Trophic interactions III	1361
537. Trophic interactions IV	1364
538. Transplantation: retina	1367
539. Transplantation III	1368
540. Neuroendocrine regulation II	1371
541. Alzheimer's disease: amyloid II	1375
542. Aging II	1379
543. Infectious disease	1385
544. Synaptogenesis II	1387
545. Subcortical visual pathways IV	1391
546. Retina VI	1395
547. Visual cortex VII	1397

THEMATIC LIST OF SESSIONS

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

Session Number	Session Title	Type	Day and Time
Theme A: Development and Plasticity			
106.	Aging I	Poster	Mon PM
542.	Aging II	Poster	Fri AM
117.	Auditory, olfactory and other sensory systems	Poster	Mon PM
118.	Biochemical and pharmacological correlates of development I	Poster	Mon PM
405.	Biochemical and pharmacological correlates of development II	Poster	Thu AM
442.	Biochemical and pharmacological correlates of development III	Slide	Thu PM
9.	Cell lineage and determination I	Slide	Mon AM
241.	Cell lineage and determination II	Poster	Tue PM
352.	Cell lineage and determination III	Poster	Wed PM
238.	Differentiation, morphogenesis and development: cell surface components	Poster	Tue PM
134.	Differentiation, morphogenesis and development: cellular and molecular studies I	Slide	Tue AM
204.	Differentiation, morphogenesis and development: cellular and molecular studies II	Slide	Tue PM
448.	Differentiation, morphogenesis and development: cellular and molecular studies III	Slide	Thu PM
406.	Differentiation, morphogenesis and development: channels and currents	Poster	Thu AM
521.	Differentiation, morphogenesis and development: cytoskeleton	Poster	Fri AM
178.	Differentiation, morphogenesis and development: fiber guidance and synaptogenesis	Poster	Tue AM
111.	Differentiation, morphogenesis and development: forebrain	Poster	Mon PM
280.	Differentiation, morphogenesis and development: glia	Poster	Wed AM
523.	Differentiation, morphogenesis and development: molecular correlates	Poster	Fri AM
237.	Differentiation, morphogenesis and development: neurogenesis and control of neuron number	Poster	Tue PM
153.	Differentiation, morphogenesis and development: neuromuscular development	Poster	Tue AM
46.	Differentiation, morphogenesis and development: position and form	Poster	Mon AM
524.	Differentiation, morphogenesis and development: tissue culture models	Poster	Fri AM
404.	Differentiation, morphogenesis and development: transmitters and enzymes	Poster	Thu AM
39.	Endocrine control and development I	Poster	Mon AM
42.	Endocrine control and development II	Poster	Mon AM
233.	Endocrine control and development III	Poster	Tue PM
234.	Endocrine control and development IV	Poster	Tue PM
68.	**From Early Embryogenesis to Circuits: Cellular and Molecular Strategies Applied to the Analysis of Vertebrate Brain Development**	**Symp.**	**Mon PM**
319.	**Hebbian Synapse: Learning Rules and Mechanisms**	**Symp.**	**Wed PM**
440.	**Inhibitory Influences on Growth Cones and Cells**	**Symp.**	**Thu PM**
378.	**Instructive Effects of Activity in the Developing Visual Pathway**	**Symp.**	**Thu AM**
391.	Limbic system I	Poster	Thu AM
38.	Long-term potentiation I	Poster	Mon AM
71.	Long-term potentiation II	Slide	Mon PM
162.	Long-term potentiation III	Poster	Tue AM
436.	Long-term potentiation IV	Poster	Thu AM
325.	Morphogenesis and differentiation	Slide	Wed PM
30.	Motor systems I	Poster	Mon AM
31.	Motor systems II	Poster	Mon AM
32.	Motor systems III	Poster	Mon AM
13.	Neural plasticity in adult animals I	Slide	Mon AM
383.	Neural plasticity in adult animals II	Slide	Thu AM
390.	Neural plasticity in adult animals: limbic system	Poster	Thu AM
311.	Neural plasticity in adult animals: monoamines and ACH	Poster	Wed AM
120.	Neural plasticity in adult animals: motor systems	Poster	Mon PM
481.	Neural plasticity in adult animals: sensory systems	Poster	Thu PM
177.	Neuronal death: lesion induced	Poster	Tue AM
175.	Neuronal death: models and mechanisms	Poster	Tue AM
279.	Neurotoxicity II	Poster	Wed AM
484.	Neurotoxicity: MPTP and excitotoxic	Poster	Thu PM
407.	Neurotoxicity: metals and organics	Poster	Thu AM

Session Number	Session Title	Type	Day and Time
353.	Nutrition and prenatal factors	Poster	Wed PM
408.	Nutrition and prenatal factors: substances of abuse	Poster	Thu AM
136.	Process outgrowth, growth cones and guidance mechanisms I	Slide	Tue AM
231.	Process outgrowth, growth cones and guidance mechanisms II	Poster	Tue PM
232.	Process outgrowth, growth cones and guidance mechanisms III	Poster	Tue PM
263.	Process outgrowth, growth cones and guidance mechanisms IV	Slide	Wed AM
348.	Process outgrowth, growth cones and guidance mechanisms V	Poster	Wed PM
349.	Process outgrowth, growth cones and guidance mechanisms VI	Poster	Wed PM
382.	Process outgrowth, growth cones and guidance mechanisms VII	Slide	Thu AM
410.	Process outgrowth, growth cones and guidance mechanisms VIII	Poster	Thu AM
413.	Process outgrowth, growth cones and guidance mechanisms IX	Poster	Thu AM
500.	Process outgrowth, growth cones and guidance mechanisms X	Slide	Fri AM
137.	Regeneration I	Slide	Tue AM
498.	Regeneration II	Slide	Fri AM
127.	Regeneration: CNS I	Poster	Mon PM
347.	Regeneration: CNS II	Poster	Wed PM
126.	Regeneration: GAP43	Poster	Mon PM
351.	Regeneration: PNS	Poster	Wed PM
350.	Regeneration: cell biology	Poster	Wed PM
125.	Regeneration: general	Poster	Mon PM
417.	Somatosensory system I	Poster	Thu AM
525.	Somatosensory system II	Poster	Fri AM
55.	Specificity of synaptic connections	Poster	Mon AM
40.	Sprouting and sprouting mechanisms I	Poster	Mon AM
41.	Sprouting and sprouting mechanisms II	Poster	Mon AM
70.	Synaptogenesis I	Slide	Mon PM
544.	Synaptogenesis II	Poster	Fri AM
532.	Synaptogenesis: neuromuscular junction	Poster	Fri AM
14.	The aging process	Slide	Mon AM
8.	Transplantation I	Slide	Mon AM
200.	Transplantation II	Slide	Tue PM
539.	Transplantation III	Poster	Fri AM
108.	Transplantation: cortex and brainstem	Poster	Mon PM
433.	Transplantation: hippocampus and basal forebrain	Poster	Thu AM
538.	Transplantation: retina	Poster	Fri AM
487.	Transplantation: spinal cord	Poster	Thu PM
533.	Transplantation: striatum I	Poster	Fri AM
534.	Transplantation: striatum II	Poster	Fri AM
12.	Trophic agents I	Slide	Mon AM
112.	Trophic agents II	Poster	Mon PM
286.	Trophic agents III	Poster	Wed AM
287.	Trophic agents IV	Poster	Wed AM
345.	Trophic agents V	Poster	Wed PM
346.	Trophic agents VI	Poster	Wed PM
379.	Trophic agents VII	Slide	Thu AM
499.	Trophic interactions I	Slide	Fri AM
535.	Trophic interactions II	Poster	Fri AM
536.	Trophic interactions III	Poster	Fri AM
537.	Trophic interactions IV	Poster	Fri AM
4.	Visual system: development and plasticity I	Slide	Mon AM
182.	Visual system: development and plasticity II	Poster	Tue AM
202.	Visual system: development and plasticity III	Slide	Tue PM
316.	Visual system: development and plasticity IV	Poster	Wed AM
477.	Visual system: development and plasticity V	Poster	Thu PM
527.	Visual system: development and plasticity VI	Poster	Fri AM

Session Number	Session Title	Type	Day and Time
Theme B: Cell Biology			
331.	Blood-brain barrier I	Slide	Wed PM
409.	Blood-brain barrier II	Poster	Thu AM
480.	Blood-brain barrier III	Poster	Thu PM
65.	Cytoskeleton, transport and membrane targeting	Poster	Mon AM
261.	Gene structure and function I	Slide	Wed AM
381.	Gene structure and function II	Slide	Thu AM
450.	Gene structure and function III	Poster	Thu PM
503.	Gene structure and function IV	Poster	Fri AM
230.	Membrane composition and cell surface macromolecules I	Poster	Tue PM
355.	Membrane composition and cell surface macromolecules II	Poster	Wed PM
142.	mRNA regulation I	Slide	Tue AM
205.	mRNA regulation II	Slide	Tue PM
339.	mRNA regulation III	Poster	Wed PM
393.	mRNA regulation IV	Poster	Thu AM
451.	mRNA regulation V	Poster	Thu PM
504.	mRNA regulation VI	Poster	Fri AM
10.	Neuroglia	Slide	Mon AM
209.	Neuroglia in disease	Poster	Tue PM
146.	Neuroglia: active membrane responses	Poster	Tue AM
145.	Neuroglia: myelin and myelin-forming cells	Poster	Tue AM
210.	Neuroglia: structure and biology	Poster	Tue PM
122.	Staining and tracing techniques	Poster	Mon PM
Theme C: Excitable Membranes and Synaptic Transmission			
11.	Calcium channels I	Slide	Mon AM
147.	Calcium channels II	Poster	Tue AM
264.	Calcium channels III	Slide	Wed AM
332.	Calcium channels IV	Poster	Wed PM
457.	Calcium channels: modulation and regulation	Poster	Thu PM
76.	Ion channel modulation and regulation I	Slide	Mon PM
514.	Ion channel modulation and regulation II	Poster	Fri AM
456.	Ion channels: cell function	Poster	Thu PM
36.	Ion channels: chloride and miscellaneous	Poster	Mon AM
387.	Ligand-gated ion channels I	Slide	Thu AM
458.	Ligand-gated ion channels II	Poster	Thu PM
333.	Ligand-gated ion channels: cholinergic	Poster	Wed PM
334.	Ligand-gated ion channels: glutamate	Poster	Wed PM
84.	Pharmacology of synaptic transmission	Poster	Mon PM
216.	Pharmacology of synaptic transmission: amino acids and calcium channels	Poster	Tue PM
79.	Postsynaptic mechanisms I	Slide	Mon PM
215.	Postsynaptic mechanisms II	Poster	Tue PM
516.	Postsynaptic mechanisms III	Poster	Fri AM
35.	Potassium channels I	Poster	Mon AM
139.	Potassium channels II	Slide	Tue AM
220.	Potassium channels III	Poster	Tue PM
395.	Potassium channels IV	Poster	Thu AM
396.	Potassium channels: modulation and regulation	Poster	Thu AM
83.	Presynaptic mechanisms I	Poster	Mon PM
197.	Presynaptic mechanisms II	Slide	Tue PM
189.	Presynaptic mechanisms: calcium	Poster	Tue AM
190.	Presynaptic mechanisms: facilitation and depression	Poster	Tue AM
85.	Sodium channels I	Poster	Mon PM
219.	Sodium channels II	Poster	Tue PM
105.	Synaptic structure and function I	Poster	Mon PM
276.	Synaptic structure and function II	Poster	Wed AM

Session Number	Session Title	Type	Day and Time
Theme D: Neurotransmitters, Modulators, and Receptors			
29.	Acetylcholine I	Poster	Mon AM
165.	Acetylcholine II	Poster	Tue AM
226.	Acetylcholine III	Poster	Tue PM
472.	Acetylcholine IV	Poster	Thu PM
102.	Behavioral pharmacology: analgesics and NMDA	Poster	Mon PM
252.	Behavioral pharmacology: benzodiazepines and stimulants	Poster	Tue PM
422.	Behavioral pharmacology: dopamine	Poster	Thu AM
466.	Behavioral pharmacology: other	Poster	Thu PM
73.	Catecholamine receptors	Slide	Mon PM
173.	Catecholamine receptors: adrenergic	Poster	Tue AM
172.	Catecholamine receptors: dopaminergic	Poster	Tue AM
171.	Catecholamine receptors: dopaminergic (D1)	Poster	Tue AM
170.	Catecholamine receptors: dopaminergic (D2)	Poster	Tue AM
330.	Catecholamines I	Slide	Wed PM
399.	Catecholamines II	Poster	Thu AM
403.	Catecholamines III	Poster	Thu AM
482.	Catecholamines IV	Poster	Thu PM
517.	Catecholamines V	Poster	Fri AM
518.	Catecholamines VI	Poster	Fri AM
483.	Catecholamines: anatomy	Poster	Thu PM
402.	Catecholamines: neurotoxicity	Poster	Thu AM
203.	Cholinergic receptors	Slide	Tue PM
260.	Excitatory amino acids: anatomy and physiology I	Slide	Wed AM
372.	Excitatory amino acids: anatomy and physiology II	Poster	Wed PM
373.	Excitatory amino acids: anatomy and physiology III	Poster	Wed PM
374.	Excitatory amino acids: anatomy and physiology IV	Poster	Wed PM
195.	Excitatory amino acids: excitotoxicity I	Slide	Tue PM
305.	Excitatory amino acids: excitotoxicity II	Poster	Wed AM
306.	Excitatory amino acids: excitotoxicity III	Poster	Wed AM
307.	Excitatory amino acids: excitotoxicity IV	Poster	Wed AM
86.	Excitatory amino acids: receptors I	Poster	Mon PM
87.	Excitatory amino acids: receptors II	Poster	Mon PM
133.	Excitatory amino acids: receptors III	Slide	Tue AM
217.	Excitatory amino acids: receptors IV	Poster	Tue PM
218.	Excitatory amino acids: receptors V	Poster	Tue PM
380.	Excitatory amino acids: receptors VI	Slide	Thu AM
461.	Excitatory amino acids: receptors VII	Poster	Thu PM
462.	Excitatory amino acids: receptors VIII	Poster	Thu PM
463.	Excitatory amino acids: receptors IX	Poster	Thu PM
259.	GABA and benzodiazepine receptors I	Slide	Wed AM
335.	GABA and benzodiazepine receptors II	Poster	Wed PM
397.	GABA and benzodiazepine receptors III	Poster	Thu AM
459.	GABA and benzodiazepine receptors IV	Poster	Thu PM
199.	GABA and benzodiazepines I	Slide	Tue PM
272.	GABA and benzodiazepines II	Poster	Wed AM
398.	GABA and benzodiazepines III	Poster	Thu AM
95.	Interactions between neurotransmitters I	Poster	Mon PM
235.	Interactions between neurotransmitters II	Poster	Tue PM
326.	Interactions between neurotransmitters III	Slide	Wed PM
491.	Interactions between neurotransmitters IV	Poster	Thu PM
318.	**Mesopontine Cholinergic Neurons: The Neuronal Substrate of the Ascending Reticular Activating System?**	**Symp.**	**Wed PM**
515.	Muscarinic receptors	Poster	Fri AM
275.	Nicotinic receptors	Poster	Wed AM
285.	Opiates, endorphins and enkephalins: anatomy and chemistry I	Poster	Wed AM
392.	Opiates, endorphins and enkephalins: anatomy and chemistry II	Poster	Thu AM
340.	Opiates, endorphins and enkephalins: behavioral effects	Poster	Wed PM

Session Number	Session Title	Type	Day and Time
62.	Opiates, endorphins and enkephalins: physiological effects I	Poster	Mon AM
221.	Opiates, endorphins and enkephalins: physiological effects II	Poster	Tue PM
270.	Opiates, endorphins and enkephalins: physiological effects III	Slide	Wed AM
61.	Opiates, endorphins and enkephalins: tolerance and dependence	Poster	Mon AM
97.	Other biogenic amines and purines: adenosine and histamine	Poster	Mon PM
81.	Peptides: anatomical localization I	Slide	Mon PM
228.	Peptides: anatomical localization II	Poster	Tue PM
298.	Peptides: anatomical localization III	Poster	Wed AM
337.	Peptides: anatomical localization IV	Poster	Wed PM
80.	Peptides: biosynthesis, metabolism and biochemical characterization I	Slide	Mon PM
151.	Peptides: biosynthesis, metabolism and biochemical characterization II	Poster	Tue AM
338.	Peptides: biosynthesis, metabolism and biochemical characterization III	Poster	Wed PM
506.	Peptides: biosynthesis, metabolism and biochemical characterization IV	Poster	Fri AM
91.	Peptides: physiological effects I	Poster	Mon PM
92.	Peptides: physiological effects II	Poster	Mon PM
388.	Peptides: physiological effects III	Slide	Thu AM
143.	Peptides: receptors I	Slide	Tue AM
229.	Peptides: receptors II	Poster	Tue PM
273.	Peptides: receptors III	Poster	Wed AM
394.	Peptides: receptors IV	Poster	Thu AM
256.	**Progress in Research on D1 Dopamine Receptors**	**Symp.**	**Wed AM**
389.	Receptor modulation: up and down regulation I	Slide	Thu AM
479.	Receptor modulation: up and down regulation II	Poster	Thu PM
519.	Receptor modulation: up and down regulation III	Poster	Fri AM
96.	Regional localization of receptors and neurotransmitters I	Poster	Mon PM
236.	Regional localization of receptors and neurotransmitters II	Poster	Tue PM
308.	Regional localization of receptors and neurotransmitters III	Poster	Wed AM
72.	Second messengers I	Slide	Mon PM
174.	Second messengers II	Poster	Tue AM
267.	Second messengers III	Slide	Wed AM
520.	Second messengers: adenylate cyclase	Poster	Fri AM
400.	Second messengers: calcium	Poster	Thu AM
401.	Second messengers: phosphoinositide turnover	Poster	Thu AM
336.	Second messengers: protein phosphorylation	Poster	Wed PM
6.	Serotonin I	Slide	Mon AM
94.	Serotonin II	Poster	Mon PM
168.	Serotonin III	Poster	Tue AM
225.	Serotonin IV	Poster	Tue PM
93.	Serotonin receptors I	Poster	Mon PM
169.	Serotonin receptors II	Poster	Tue AM
198.	Serotonin receptors III	Slide	Tue PM
274.	Serotonin receptors IV	Poster	Wed AM
2.	**The Neurobiology of Neuropeptide Y (NPY)**	**Symp.**	**Mon AM**
16.	Transmitters in invertebrates I	Slide	Mon AM
150.	Transmitters in invertebrates II	Poster	Tue AM
271.	Transmitters in invertebrates III	Slide	Wed AM
297.	Transmitters in invertebrates IV	Poster	Wed AM
48.	Uptake, storage, secretion and metabolism I	Poster	Mon AM
242.	Uptake, storage, secretion and metabolism II	Poster	Tue PM
328.	Uptake, storage, secretion and metabolism III	Slide	Wed PM
505.	Uptake, storage, secretion and metabolism IV	Poster	Fri AM

Theme E: Endocrine and Autonomic Regulation

277.	Adrenal medullary regulation	Poster	Wed AM
138.	Cardiovascular regulation I	Slide	Tue AM
239.	Cardiovascular regulation II	Poster	Tue PM
327.	Cardiovascular regulation III	Slide	Wed PM
385.	Cardiovascular regulation IV	Slide	Thu AM

Session Number	Session Title	Type	Day and Time
467.	Cardiovascular regulation V	Poster	Thu PM
468.	Cardiovascular regulation VI	Poster	Thu PM
240.	Cardiovascular regulation: hypertension	Poster	Tue PM
290.	Hypothalamic-pituitary-adrenal regulation I	Poster	Wed AM
321.	Hypothalamic-pituitary-adrenal regulation II	Slide	Wed PM
429.	Hypothalamic-pituitary-adrenal regulation III	Poster	Thu AM
58.	Hypothalamic-pituitary-adrenal regulation: CRH	Poster	Mon AM
250.	Hypothalamic-pituitary-gonadal regulation I	Poster	Tue PM
430.	Hypothalamic-pituitary-gonadal regulation II	Poster	Thu AM
528.	Hypothalamic-pituitary-gonadal regulation III	Poster	Fri AM
493.	**Melatonin: New Light on CNS Mechanisms of Action**	**Symp.**	**Fri AM**
7.	Neural-immune interactions I	Slide	Mon AM
156.	Neural-immune interactions II	Poster	Tue AM
289.	Neural-immune interactions III	Poster	Wed AM
291.	Neural-immune interactions IV	Poster	Wed AM
119.	Neural-immune interactions: stress	Poster	Mon PM
82.	Neuroendocrine regulation I	Slide	Mon PM
540.	Neuroendocrine regulation II	Poster	Fri AM
428.	Neuroendocrine regulation: neurohypophysial peptides	Poster	Thu AM
375.	Neuroendocrine regulation: photoperiod/pineal	Poster	Wed PM
292.	Neuroendocrine regulation: prolactin	Poster	Wed AM
439.	**Neuropeptide Regulation of Reproduction**	**Symp.**	**Thu PM**
107.	Regulation of autonomic functions I	Poster	Mon PM
251.	Regulation of autonomic functions II	Poster	Tue PM
269.	Regulation of autonomic functions III	Slide	Wed AM
357.	Regulation of autonomic functions IV	Poster	Wed PM
426.	Regulation of autonomic functions V	Poster	Thu AM
45.	Respiratory regulation I	Poster	Mon AM
471.	Respiratory regulation II	Poster	Thu PM

Theme F: Sensory Systems

445.	Auditory system	Slide	Thu PM
50.	Auditory system: avian connections and processing	Poster	Mon AM
300.	Auditory system: brainstem and higher nuclei	Poster	Wed AM
90.	Auditory system: cochlea	Poster	Mon PM
299.	Auditory system: cochlear nucleus	Poster	Wed AM
49.	Auditory system: cortex and integration	Poster	Mon AM
89.	Auditory system: hair cells	Poster	Mon PM
368.	Chemical senses: gustatory pathways	Poster	Wed PM
367.	Chemical senses: olfactory pathways and processing	Poster	Wed PM
301.	Chemical senses: peripheral olfaction	Poster	Wed AM
302.	Chemical senses: taste and carotid receptors	Poster	Wed AM
257.	**Computing Motion in Flies, Monkeys and Man: Linking Physiology with Psychophysics and Computational Theory**	**Symp.**	**Wed AM**
3.	**Functional Organization of the Thalamus**	**Symp.**	**Mon AM**
510.	Invertebrate sensory systems I	Poster	Fri AM
511.	Invertebrate sensory systems II	Poster	Fri AM
78.	Pain modulation	Slide	Mon PM
63.	Pain modulation: CNS pathways	Poster	Mon AM
341.	Pain modulation: afferent mechanisms	Poster	Wed PM
222.	Pain modulation: biogenic amines I	Poster	Tue PM
223.	Pain modulation: biogenic amines II	Poster	Tue PM
64.	Pain modulation: opioid mechanisms	Poster	Mon AM
152.	Pain modulation: spinal opioid mechanisms	Poster	Tue AM
502.	Pain pathways	Slide	Fri AM
470.	Pain pathways: CNS	Poster	Thu PM
188.	Pain pathways: primary afferents	Poster	Tue AM
187.	Pain pathways: spinal cord	Poster	Tue AM

Session Number	Session Title	Type	Day and Time
51.	Retina I	Poster	Mon AM
88.	Retina II	Poster	Mon PM
366.	Retina III	Poster	Wed PM
386.	Retina IV	Slide	Thu AM
476.	Retina V	Poster	Thu PM
546.	Retina VI	Poster	Fri AM
268.	Sensorimotor integration	Slide	Wed AM
47.	Somatic and visceral afferents I	Poster	Mon AM
176.	Somatic and visceral afferents II	Poster	Tue AM
329.	Somatic and visceral afferents III	Slide	Wed PM
123.	Somatosensory cortex and thalamocortical relationships I	Poster	Mon PM
124.	Somatosensory cortex and thalamocortical relationships II	Poster	Mon PM
418.	Somatosensory cortex and thalamocortical relationships III	Poster	Thu AM
303.	Spinal cord	Poster	Wed AM
157.	Subcortical somatosensory pathways I	Poster	Tue AM
158.	Subcortical somatosensory pathways II	Poster	Tue AM
75.	Subcortical visual pathways I	Slide	Mon PM
183.	Subcortical visual pathways II	Poster	Tue AM
315.	Subcortical visual pathways III	Poster	Wed AM
545.	Subcortical visual pathways IV	Poster	Fri AM
131.	**The Initial Events in Taste: Chemosensory Transduction in the Vertebrate Taste Bud**	**Symp.**	**Tue AM**
52.	Visual cortex I	Poster	Mon AM
69.	Visual cortex II	Slide	Mon PM
132.	Visual cortex III	Slide	Tue AM
320.	Visual cortex IV	Slide	Wed PM
419.	Visual cortex V	Poster	Thu AM
441.	Visual cortex VI	Slide	Thu PM
547.	Visual cortex VII	Poster	Fri AM
53.	Visual psychophysics and behavior I	Poster	Mon AM
249.	Visual psychophysics and behavior II	Poster	Tue PM
497.	Visual psychophysics and behavior III	Slide	Fri AM

Theme G: Motor Systems and Sensorimotor Integration

115.	Basal ganglia and thalamus I	Poster	Mon PM
116.	Basal ganglia and thalamus II	Poster	Mon PM
358.	Basal ganglia and thalamus III	Poster	Wed PM
359.	Basal ganglia and thalamus IV	Poster	Wed PM
360.	Basal ganglia and thalamus V	Poster	Wed PM
361.	Basal ganglia and thalamus VI	Poster	Wed PM
362.	Basal ganglia and thalamus VII	Poster	Wed PM
77.	Cerebellum I	Slide	Mon PM
164.	Cerebellum II	Poster	Tue AM
245.	Cerebellum III	Poster	Tue PM
447.	Circuitry and pattern generation	Slide	Thu PM
416.	Circuitry and pattern generation: invertebrates and models	Poster	Thu AM
415.	Circuitry and pattern generation: vertebrates	Poster	Thu AM
25.	Control of posture and movement I	Poster	Mon AM
26.	Control of posture and movement II	Poster	Mon AM
74.	Control of posture and movement III	Slide	Mon PM
160.	Control of posture and movement IV	Poster	Tue AM
161.	Control of posture and movement V	Poster	Tue AM
243.	Control of posture and movement VI	Poster	Tue PM
281.	Control of posture and movement VII	Poster	Wed AM
473.	Control of posture and movement VIII	Poster	Thu PM
474.	Control of posture and movement IX	Poster	Thu PM
113.	Cortex I	Poster	Mon PM
114.	Cortex II	Poster	Mon PM
313.	Cortex III	Poster	Wed AM

Session Number	Session Title	Type	Day and Time
314.	Cortex IV	Poster	Wed AM
513.	Invertebrate motor function	Poster	Fri AM
363.	Motor systems: reflex function I	Poster	Wed PM
364.	Motor systems: reflex function II	Poster	Wed PM
213.	Muscle I	Poster	Tue PM
214.	Muscle II	Poster	Tue PM
99.	Oculomotor system I	Poster	Mon PM
312.	Oculomotor system II	Poster	Wed AM
324.	Oculomotor system III	Slide	Wed PM
475.	Oculomotor system IV	Poster	Thu PM
268.	Sensorimotor integration	Slide	Wed AM
159.	Spinal cord and brainstem I	Poster	Tue AM
207.	Spinal cord and brainstem II	Slide	Tue PM
282.	Spinal cord and brainstem: cord anatomy	Poster	Wed AM
365.	Spinal cord and brainstem: cord physiology	Poster	Wed PM
377.	**The Basal Ganglia: Structure and Function**	**Symp.**	**Thu AM**
206.	Vestibular system	Slide	Tue PM
211.	Vestibular system: VOR and integration	Poster	Tue PM
212.	Vestibular system: receptor organs and vestibular nuclei	Poster	Tue PM

Theme H: Other Systems of the CNS

33.	Association cortex and thalamocortical relations I	Poster	Mon AM
34.	Association cortex and thalamocortical relations II	Poster	Mon AM
179.	Brain metabolism and blood flow I	Poster	Tue AM
343.	Brain metabolism and blood flow II	Poster	Wed PM
17.	Brain metabolism and blood flow	Slide	Mon AM
180.	Brainstem systems	Poster	Tue AM
19.	Comparative neuroanatomy I	Poster	Mon AM
154.	Comparative neuroanatomy II	Poster	Tue AM
163.	Hippocampus and amygdala I	Poster	Tue AM
489.	Hippocampus and amygdala II	Poster	Thu PM
490.	Hippocampus and amygdala III	Poster	Thu PM
431.	Hypothalamus	Poster	Thu AM
488.	Limbic system II	Poster	Thu PM

Theme I: Neural Basis of Behavior

21.	Alcohol, barbiturates, benzodiazepines I	Poster	Mon AM
28.	Alcohol, barbiturates, benzodiazepines II	Poster	Mon AM
167.	Alcohol, barbiturates, benzodiazepines III	Poster	Tue AM
224.	Alcohol, barbiturates, benzodiazepines IV	Poster	Tue PM
184.	Biological rhythms and sleep: invertebrates	Poster	Tue AM
15.	Biological rhythms and sleep: neuroregulators	Slide	Mon AM
201.	Biological rhythms and sleep: other I	Slide	Tue PM
293.	Biological rhythms and sleep: other II	Poster	Wed AM
420.	Biological rhythms and sleep: other III	Poster	Thu AM
522.	Biological rhythms and sleep: other IV	Poster	Fri AM
100.	Biological rhythms and sleep: sleep	Poster	Mon PM
253.	Drugs of abuse	Poster	Tue PM
434.	Drugs of abuse: CNS pathways	Poster	Thu AM
103.	Drugs of abuse: biogenic amines	Poster	Mon PM
104.	Drugs of abuse: cocaine I	Poster	Mon PM
322.	Drugs of abuse: cocaine II	Slide	Wed PM
432.	Drugs of abuse: dopamine mechanisms	Poster	Thu AM
469.	Drugs of abuse: stimulants	Poster	Thu PM
155.	Hormonal control of behavior I	Poster	Tue AM
304.	Hormonal control of behavior II	Poster	Wed AM
435.	Hormonal control of behavior III	Poster	Thu AM
130.	**Hormones, Neural Circuits and Communication**	**Symp.**	**Tue AM**

Session Number	Session Title	Type	Day and Time
191.	Human behavioral neurobiology: event related potentials	Poster	Tue AM
5.	Human behavioral neurobiology: memory	Slide	Mon AM
101.	Human behavioral neurobiology: memory and language	Poster	Mon PM
196.	Human behavioral neurobiology: other I	Slide	Tue PM
294.	Human behavioral neurobiology: other II	Poster	Wed AM
246.	Ingestive behaviors I	Poster	Tue PM
266.	Ingestive behaviors II	Slide	Wed AM
356.	Ingestive behaviors III	Poster	Wed PM
384.	Ingestive behaviors IV	Slide	Thu AM
452.	Ingestive behaviors V	Poster	Thu PM
507.	Ingestive behaviors VI	Poster	Fri AM
421.	Interhemispheric relations	Poster	Thu AM
446.	Invertebrate learning and behavior I	Slide	Thu PM
455.	Invertebrate learning and behavior II	Poster	Thu PM
501.	Invertebrate learning and behavior III	Slide	Fri AM
509.	Invertebrate learning and behavior IV	Poster	Fri AM
185.	Learning and memory—pharmacology: NMDA	Poster	Tue AM
186.	Learning and memory—pharmacology: monoamines	Poster	Tue AM
464.	Learning and memory—pharmacology: other I	Poster	Thu PM
465.	Learning and memory—pharmacology: other II	Poster	Thu PM
295.	Learning and memory—pharmacology: acetylcholine I	Poster	Wed AM
296.	Learning and memory—pharmacology: acetylcholine II	Poster	Wed AM
121.	Learning and memory: anatomy I	Poster	Mon PM
141.	Learning and memory: anatomy II	Slide	Tue AM
244.	Learning and memory: anatomy III	Poster	Tue PM
258.	Learning and memory: anatomy IV	Slide	Wed AM
354.	Learning and memory: anatomy V	Poster	Wed PM
437.	Learning and memory: anatomy VI	Poster	Thu AM
37.	Learning and memory: physiology I	Poster	Mon AM
208.	Learning and memory: physiology II	Slide	Tue PM
309.	Learning and memory: physiology III	Poster	Wed AM
166.	Monoamines and behavior I	Poster	Tue AM
227.	Monoamines and behavior II	Poster	Tue PM
453.	Monoamines and behavior III	Poster	Thu PM
460.	Monoamines and behavior IV	Poster	Thu PM
508.	Monoamines and behavior V	Poster	Fri AM
20.	Motivation and emotion I	Poster	Mon AM
27.	Motivation and emotion II	Poster	Mon AM
144.	Neuroethology I	Slide	Tue AM
247.	Neuroethology II	Poster	Tue PM
454.	Neuroethology III	Poster	Thu PM
512.	Neuroethology IV	Poster	Fri AM
288.	Neuropeptides and behavior	Poster	Wed AM
423.	Neuropeptides and behavior: CCK	Poster	Thu AM
424.	Neuropeptides and behavior: CRF	Poster	Thu AM
425.	Neuropeptides and behavior: oxytocin and vasopressin	Poster	Thu AM
485.	Psychotherapeutic drugs	Poster	Thu PM
342.	Psychotherapeutic drugs: antidepressants	Poster	Wed PM
109.	Psychotherapeutic drugs: dopamine and neuropeptics	Poster	Mon PM
110.	Stress, hormones and the autonomic nervous system	Poster	Mon PM

Theme J: Disorders of the Nervous System

135.	Alzheimer's disease I	Slide	Tue AM
443.	Alzheimer's disease II	Slide	Thu PM
262.	Alzheimer's disease: amyloid I	Slide	Wed AM
541.	Alzheimer's disease: amyloid II	Poster	Fri AM
414.	Alzheimer's disease: neuropathology	Poster	Thu AM
344.	Alzheimer's disease: transmitters and behavior	Poster	Wed PM

Session Number	Session Title	Type	Day and Time
486.	Clinical CNS neurophysiology	Poster	Thu PM
22.	Degenerative disease: Parkinson's I	Poster	Mon AM
54.	Degenerative disease: Parkinson's II	Poster	Mon AM
371.	Degenerative disease: Parkinson's III	Poster	Wed PM
369.	Degenerative disease: other I	Poster	Wed PM
370.	Degenerative disease: other II	Poster	Wed PM
278.	Developmental disorders: genetic and chemical models	Poster	Wed AM
526.	Developmental disorders: human diseases	Poster	Fri AM
248.	Epilepsy: anti-epileptic drugs	Poster	Tue PM
140.	Epilepsy: basic mechanisms I	Slide	Tue AM
284.	Epilepsy: basic mechanisms II	Poster	Wed AM
411.	Epilepsy: basic mechanisms III	Poster	Thu AM
427.	Epilepsy: benzodiazepines and inhibitory amino acids	Poster	Thu AM
478.	Epilepsy: excitatory amino acids	Poster	Thu PM
24.	Epilepsy: genetic models	Poster	Mon AM
98.	Epilepsy: human studies	Poster	Mon PM
181.	Epilepsy: kindling I	Poster	Tue AM
310.	Epilepsy: kindling II	Poster	Wed AM
43.	Genetic models of nervous disorders I	Poster	Mon AM
44.	Genetic models of nervous disorders II	Poster	Mon AM
265.	Genetic models of nervous disorders III	Slide	Wed AM
543.	Infectious disease	Poster	Fri AM
323.	Ischemia	Slide	Wed PM
529.	Ischemia: energy metabolism and ischemic models	Poster	Fri AM
148.	Ischemia: excitability and neurotransmission	Poster	Tue AM
149.	Ischemia: mediators of neuronal death	Poster	Tue AM
23.	Ischemia: pharmacological protection	Poster	Mon AM
449.	Mental illness	Slide	Thu PM
18.	Mental illness: affective disease	Poster	Mon AM
283.	Mental illness: schizophrenia	Poster	Wed AM
412.	Neuromuscular disease	Poster	Thu AM
60.	Neurotoxicity I	Poster	Mon AM
530.	Neurotoxicity III	Poster	Fri AM
531.	Neurotoxicity IV	Poster	Fri AM
59.	Neurotoxicity: dopamine	Poster	Mon AM
67.	**New Opportunities for Study of Mechanisms of Central Nervous System Ischemia**	**Symp.**	**Mon PM**
194.	**Recent Advances in the Biology of Affective Disorders**	**Symp.**	**Tue PM**
56.	Trauma I	Poster	Mon AM
57.	Trauma II	Poster	Mon AM
444.	Trauma III	Slide	Thu PM

Other

193.	**Optical Imaging of CNS Development, Organization and Function**	**Symp.**	**Tue PM**

2

SYMPOSIUM: THE NEUROBIOLOGY OF NEUROPEPTIDE Y (NPY). W.F. Colmers, Univ. of Alberta (Chairperson); J. M. Allen, Roy. Postgrad. Med. Sch.; S.H.C. Hendry, U.C. Irvine; C. Wahlestedt Fidia-Georgetown Inst. Neurosci.; T.A. Westfall, St. Louis Univ.; R.J. Miller, Univ. Chicago.

NPY, discovered in 1982, is one of the most abundant peptides in the nervous system, and is distributed broadly throughout phylogeny. This symposium will examine the present state of knowledge on NPY, its receptors and their physiological effects. cDNA sequences for rat and human NPY are remarkably well conserved; the deduced amino acid sequences are identical. These data also permit comparisons with sequences encoding other related peptides. NPY exists in several classes of neurons: 1) interneurons (NPY + amines or amino acids) 2) interneurons (NPY + peptide(s)); 3) projection neurons (NPY + classical transmitters). Actions of NPY in a given system may be predictable based on its co-transmitters. NPY receptors have effects by themselves, and mediate interactions with other receptors. In PNS, there are 2 types of NPY receptors: the postjunctional (Y_1) receptor mediates excitation in smooth muscle and (at lower concentrations) modulates excitatory effects of other transmitters, while the prejunctional (Y_2) receptor inhibits release of noradrenaline. NPY is involved in many physiological functions, e.g. cardiovascular control, reproduction, feeding, etc., and may be associated with major depression/anxiety in patients. Microinjection of NPY into posterior hypothalamus (which has interneurons 1) and 2) above), increases blood pressure by sympathetic excitation, but intrathecal injections reduce blood pressure by decreasing sympathetic outflow. In slices of hippocampus (interneurons: NPY + GABA), NPY presynaptically inhibits transmission at some, but not all, excitatory pathways, probably by reducing Ca^{++} influx at nerve terminals. This action does not involve a pertussis toxin (PTX)-sensitive G-protein. In cultured rat sensory neurons, NPY inhibits voltage-dependent Ca^{++}influx, in part possibly by activation of phosphatidyl inositide metabolism, an action which is sensitive to PTX.

3

SYMPOSIUM. FUNCTIONAL ORGANIZATION OF THE THALAMUS. S.M. Sherman, SUNY at Stony Brook (Chairperson); E.G. Jones, U.C. Irvine; D.A. Prince, Stanford Univ.; H.J. Ralston, U.C.S.F.

Advances in our understanding of the thalamus have been dramatic in recent years. We have learned much about its overall organization and connectivity. We also now appreciate its role as a variable gateway in the relay of information to cortex, and we are beginning to understand the cellular mechanisms underlying this role. The symposium will cover certain highlights of these advances. *E.G. Jones* will begin with an overview of thalamic organization and thalamocortical relationships. He will also discuss new findings relating morphological, functional, and neurochemical characteristics of thalamic neurons to differential thalamocortical projections. *D.A. Prince* will discuss properties of the low threshold calcium current (LTCC) which underlies burst generation in thalamocortical relay neurons and factors controlling it, including modulation of LTCC by changes in intracellular second messengers and by anticonvulsant drugs. *H.J. Ralston* will use the ventral posterolateral nucleus as a model to describe details of intrinsic thalamic organization, and *S.M. Sherman* will do likewise for the lateral geniculate nucleus. These latter two talks will emphasize the wealth and complexity of thalamic circuitry available to underlie gating functions in the thalamocortical transmission of sensory information.

VISUAL SYSTEM: DEVELOPMENT AND PLASTICITY I

4.1

TRANSIENT EXPRESSION OF A SUBPLATE-SPECIFIC ANTIGEN IN CAT VISUAL CORTEX. J.R. Naegele, C.J. Barnstable and P. Wahle, Dept of Ophthalmology and Visual Sciences, Yale University School of Medicine, New Haven, CT 06510

Transient neurons of the cerebral cortical subplate are thought to play a central role in establishing mature connections. No specific markers have yet been identified that distinguish subplate from cortical plate neurons. We used a novel immunlogical approach to generate monoclonal antibodies against subplate neurons. One of these antibodies stained cortical subplate neurons selectively. This marker, called **Subplate1**, has been characterized further using immunocytochemical double-labeling with antisera against various neuropeptides and GABA.

On the day of birth (P1) and until P21, Subplate1 stained neurons restricted to the cortical subplate. The strongest immunoreactivity appeared within cell bodies, dendrites, axons and growth cones. Several morphologically distinct types were labeled, including inverted pyramids and multipolar forms. Fainter immunoreactivity was also present throughout the subplate neuropil. The immunoreactive staining of subplate neurons was strongest between P1 and P12, diminished by P21 and was absent by adulthood. Subplate1+ neurons were biochemically heterogeneous; nearly half were Somatostatin+, but only a minority were Neuropeptide Y+ or GABA+. These findings indicate that Subplate1 identifies a developmentally-regulated antigen expressed by early-generated, transient cortical neurons. (Supported by NIH grants EY07119 and EY05206).

4.2

SUBPLATE CELLS IN VISUAL CORTEX FUNCTION IN TRANSIENT SYNAPTIC MICROCIRCUITS. E. Friauf, S. K. McConnell and C. J. Shatz, Dept. Neurobiology, Stanford Univ. Med. Sch., Stanford, CA 94305.

During cat fetal development, the immature white matter below the cortical plate is filled with waiting axons, synapses and a special class of transient neurons, the subplate (SP) cells. We investigated whether SP neurons receive synaptic input and function in neural circuits. Intracellular recordings of SP cells were performed in cortical slices *in vitro*. Responses were elicited by electrical stimulation of the white matter. Subsequently, biocytin was injected iontophoretically to reveal the location and morphology of recorded cells. At all ages tested (E50 - P4), short latency EPSP's (average latency: 4.1 ms, range: 2.5-6.0 ms) and orthodromic spikes could be elicited from SP cells. When depolarized by current pulses, most SP neurons displayed a regular spiking pattern. The majority of biocytin-injected SP neurons resembled inverted pyramids. Their axons branched within the subplate; many also entered the cortical plate and ascended to the marginal zone. At neonatal ages, some axons collateralized within the cortical plate as well. Thus SP cells receive functional inputs from the white matter and send outputs to the cortical plate.

To examine the spatial and temporal sequence of synaptic activation in the developing cortex we used current source density analysis in conjunction with white matter stimulation *in vitro*. At E55, a short latency (monosynaptic) response is present in the SP, most likely mediated by inputs from waiting axons to SP neurons. A longer latency (polysynaptic) response is present at the top of the cortical plate. Since at E55, waiting axons have just begun to invade the cortical plate at its base, the polysynaptic pathway is likely to be mediated by ascending axons of SP neurons. Thus SP neurons may participate in a complex circuit during fetal and neonatal life, a circuit that disappears when waiting axons finally reach their adult targets.

Supported by NIH grants EY02858, EY06028 and a DAAD-fellowship.

4.3

RELATION BETWEEN NEUROCHEMICAL PHENOTYPE AND PROJECTION PATTERNS OF SUBPLATE NEURONS.
A. Antonini *and C.J. Shatz. (SPON: C.J.Shatz) Dept. of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305

During fetal and early postnatal development, the earliest generated cells of the mammalian telencephalon reside in a region of the white matter beneath the cortical plate (CP) designated the subplate (SP). Previous studies in cat (Chun, J.J. et al., Nature, 6105: 617, 1987) have shown that, although present only transiently during development, SP cells have features typical of mature CP neurons. They have complex dendritic and axonal arborizations and can be involved in local circuits or long range projections. SP cells are also immunoreactive for putative neurotransmitters such as GABA and for one of several neuropeptides including neuropeptide Y (NPY) and somatostatin (SRIF). Here we have examined whether there is a correlation between the neurotransmitter phenotype of SP cells and the targets of their axonal projections. To do so we used a double-label technique, with colloidal gold WGA-HRP or Diamidino-Yellow as retrograde tracer in combination with immunohistochemical visualization of NPY, SRIF or calbindin (used as a marker of a subtype of GABAergic neurons). SP cells in neonatal cats and ferrets were retrogradely labeled from either the thalamus, CP or hemispheric white matter. SP cells immunoreactive for neuropeptides or calbindin could be double labeled with retrograde tracers injected into the cortex or white matter, but never by thalamic injections. On the other hand, SP cells projecting to the thalamus could be labeled by the retrograde transport of 3H-aspartate. Thus, during development, SP neurons, like CP neurons in the adult, can be divided into local circuit neurons that are peptidergic or GABAergic, and projection neurons some of which are likely to employ excitatory amino acids as their transmitter.

Supported by NIH Grant EY02858

4.4

SUBPLATE NEURONS TRANSIENTLY EXPRESS NGF RECEPTOR IMMUNOREACTIVITY. K.L. Allendoerfer,* D.L. Shelton, E.M. Shooter, and C.J. Shatz. Dept. of Neurobiology, Stanford Univ., Stanford, CA 94305.

NGF and its receptor (NGFR) are now known to be present in diverse embryonic and neonatal CNS tissues including the cerebral cortex. We have used immunolabeling coupled with thymidine autoradiography to determine the identity of the cortical cells expressing the NGFR in cat and ferret brain during development. The antibody used for this study was a polyclonal serum generated to a synthetic peptide corresponding to a conserved sequence of the NGFR. Western blots show that this antibody recognizes specific NGFR and precursor proteins. In both species, NGFR immunoreactivity is associated with the transient, early-generated subplate neuron population, as supported by the following evidence: the immunoreactive cells 1) are located directly beneath the developing cortical plate, 2) have the inverted pyramid shape characteristic of many subplate neurons, and 3) can be labeled by an injection of ^{3}H-thymidine at E28, a time when only subplate neurons are being generated. In cat, strong immunostaining is seen associated with the cell bodies of these neurons as early as E30, two days after their birth, and it remains high for about three weeks. The NGFR immunoreactivity begins to decline around E52 and has disappeared from the region altogether by E60. The cellular localization and timing of expression suggest that the NGFR may play a role in subplate neuron maintenance and its loss may be involved in the subsequent death of subplate neurons.

Supported by grants from NIH (EY 02858, NS 04270) and NSF.

*Indicates nonmember of the Society for Neuroscience

4.5

TRANSMITTER-INDUCED CHANGES IN INTRACELLULAR FREE CALCIUM IN BRAIN SLICES OF DEVELOPING NEOCORTEX. R.M. Yuste* and L.C. Katz (SPON: J. Sparrow). Lab. of Neurobiology, The Rockefeller University, 1230 York Ave. New York, NY 10021.

Calcium flux through the NMDA channel may be a critical signal for establishment of long-term synaptic changes in adult and developing cortices. We have investigated the effects of putative cortical neurotransmitters on the intracellular concentration of free calcium ($[Ca^{+2}]_i$) in slices of developing rat visual and somatosensory cortices at postnatal days 2-10 (P2-10). Slices were loaded with 1-10 μM Fura-2 AM, and the changes in $[Ca^{+2}]_i$ of individual neurons in layer 2/3 were monitored using either a photodiode or a SIT video camera.

Neurons at all ages, even those which had just completed migration, were highly sensitive to glutamate and its analogues. All neurons responded within 30 seconds of application of drugs to the perfusion medium with a dramatic increase in $[Ca^{+2}]_i$ in the cell body and proximal dendrites. Threshold for responses were about 30 μM for glutamate, 10 μM for NMDA and ibotenic acid, and 1 μM for quisqualate. After the agonist was removed, $[Ca^{+2}]_i$ returned to baseline in under 3 minutes. No persistent elevation of $[Ca^{+2}]_i$ in cell bodies was detected. Single electrical shocks of the underlying white matter caused transient increases in $[Ca^{+2}]_i$; trains of shocks prolonged the increase, but $[Ca^{+2}]_i$ still returned to baseline after a few seconds.No elevation in $[Ca^{+2}]_i$ was detected in response to norepinephrine (200 μM), substance P (10 μM), somatostatin (10 μM), phorbol esters (PdAc and PdBu) or caffeine. Furthermore, none of these clearly potentiated the increase in $[Ca^{+2}]_i$ elicited by glutamate or NMDA. However, acetylcholine (50 μM) markedly increased $[Ca^{+2}]_i$; this increase was blocked by atropine. By combining optical techniques with brain slices, it is possible to observe the responses of single cells, as well as modulatory interactions, while preserving much of the intrinsic organization of the cortex. Supported by the L.P. Markey Charitable Trust.

4.6

EARLY POSTNATAL DEVELOPMENT OF SYNAPTIC AND POTASSIUM CURRENTS IN THE VISUAL CORTEX OF THE RAT. J.J. LoTurco, M.G. Blanton, and A.R. Kriegstein, Dept. of Neurology and the Neurosciences Program, Stanford Univ. Sch. of Med., Stanford, CA 94305.

Whole-cell voltage-clamp recordings were made in slices of visual cortex from 0 to 20 day old rats. Recorded cells were labeled with either Lucifer Yellow CH or biocytin so that location and morphology could be determined for each neuron.

During the first postnatal week, spontaneous synaptic currents were present in neurons within the cortical plate . These spontaneous currents were infrequent, small, and reversed near the equilibrium potential for monovalent cations (4 mV). Stimulation within the cortical layers evoked small currents that resembled the spontaneous currents. The pyramidal cells labeled on these earliest days had descending axons with horizontal collaterals that may be the presynaptic elements of the observed synaptic currents. Through the second and third weeks the frequency of spontaneous synaptic currents increases as did the size of the evoked currents.

By the second week, spontaneous events appeared that reversed at the chloride equilibrium potential and were blocked by 5 uM bicuculline methiodide (BMI). At the same age that BMI-sensitive spontaneous events appeared stimulation of the white matter evoked currents that reversed between the equilibrium potentials for chloride and monovalent cations. In BMI the evoked current reversed near 4 mV and had a large d-APV-sensitive component.

Voltage-activated potassium currents were recorded at all ages. Both an inactivating and a non-inactivating outward current were present at the earliest ages examined, both reversed near the potassium equilibrium potential, and increased in conductance through the first two postnatal weeks.

4.7

NMDA RECEPTOR DEVELOPMENT IN THE VISUAL CORTEX OF CATS L.J. Reynolds and M.F. Bear, Dept. Pharmacology, Univ. Pittsburgh, Pittsburgh PA 15261, and Center for Neural Science, Brown Univ. Providence RI 02912.

Neurons in the visual cortex of the cat display an age dependent plasticity that can be demonstrated by modifying the visual environment of the animal. Between 3 and 12 postnatal weeks surgically closing one eye results in a shift in ocular dominance of neurons in area 17 of the visual cortex. These plastic events probably involve NMDA receptors, as the infusion of an NMDA antagonist into area 17 will prevent the shift in ocular dominance (Kleinschmidt et al. Science, 238:355, 1987). In this study we examined the development of NMDA receptors in visual cortex of developing cats through the critical period for visual plasticity.

NMDA receptor density was monitored using $[^3H]$ MK801 binding. Well washed membranes from area 17 or area 6 from cats of different ages were incubated with 0.6nM $[^3H]$ MK801, 100μM Glu and 30μM Gly for 2hr at 24°C. The binding parameters obtained are shown below (Mean±S.E.M. n=3-4).

	B_{max} (pMol/mg prot)		k_D (nM)	
Age, days	Area 17	Area 6	Area 17	Area 6
7	0.66±0.3		4.93±2.72	
36	1.54±0.17	1.49±0.17	1.11±0.12	1.53±0.14
57	1.49±0.18	1.85±0.38	1.16±0.26	1.60±0.25
84	1.34±0.06	1.59±0.16	0.98±0.06	1.46±0.13
Adult	1.24±0.27	1.41±0.03	1.01±0.15	1.45±0.08

There is clearly an abrupt increase in NMDA receptor density associated with the onset of the critical period. However, NMDA receptor density remains relatively high following the end of the critical period. Thus, although NMDA receptors play a critical role in visual plasticity, their presence does not necessarily predict the capability for plasticity.

Supported in part by NIH grant NS 06929 to M.F.B.

4.8

BLOCAKDE OF VISUAL CORTICAL NMDA RECEPTORS PREVENTS THE SHRINKAGE OF LATERAL GENICULATE NEURONS FOLLOWING MONOCULAR DEPRIVATION. H. Colman* and M.F. Bear. Center for Neural Science, Brown University, Providence, RI 02912.

Monocular deprivation of kittens during the second postnatal month leads to a shift in visual cortical ocular dominance such that few neurons remain responsive to stimulation of the deprived eye. One correlate of this physiological change is a shrinkage of the neurons in the lateral geniculate nucleus that receive input from the deprived eye. Because the morphological changes in the LGN are most prominent in the binocular segment, it has been proposed that they reflect a process of binocular competition. It has been hypothesized that this competition occurs among the converging geniculocortical axons in layer IV of visual cortex. Recent work has suggested that intracortical infusion of APV, an NMDA receptor antagonist, interferes with the physiological consequences of MD. We set out to examine the possibility that LGN cell size changes were also sensitive to cortical NMDA receptor blockade.

Kittens were implanted bilaterally with minipumps that delivered either 50 mM D,L-APV or sterile Ringer directly into area 17 at a rate of .1 μl per hour. At the same time, one eyelid was sutured closed. After 8 days, HRP was injected into striate cortex 6 mm anterior to the infusion site, where we calculate the extracellular APV concentration to be ~150 μM. 2 days later, the animals were perfused and processed for HRP histochemistry. The cross sectional areas of Nissl-stained LGN neurons were measured within the projection column defined by the retrograde label. In the Ringer treated group, lamina A1 neurons ipsilateral to the deprived eye were 18.2±1.1% smaller that those in lamina A. In contrast, in the APV-treated group, lamina A1 cells were 13.9±2.6% *larger* than those in lamina A. Thus, the effects of MD on LGN cell size clearly differ in the APV and control groups, and this difference is significant at P<.001 (t-test).

These data demonstrate that the site of binocular competition in the control of LGN cell size is the striate cortex and, because NMDA receptors are not presynaptic, that binocular competition requires postsynaptic activity. Further, the data suggest that the relevant component of postsynaptic activity depends specifically upon NMDA receptor activation. (Supported by ONR contract N00014-81-K0136 and NIH grant NS06929)

4.9

DEVELOPMENT OF LOCAL CONNECTIONS IN HUMAN VISUAL CORTEX. A.Burkhalter and K.L.Bernardo*, Department of Neurosurgery & McDonnell Center for Studies of Higher Brain Function, Washington University School of Medicine, St. Louis, MO 63110.

The human visual system is immature at birth. In primary visual cortex (V1), for example, the synaptic density doubles during the first year of life, indicating the formation of new connections. Because the visual system is extremely sensitive to deprivation during this period, it is important to determine the sequence in which cortical circuits develop.

We have used the fluorescent dye, dil, to trace axonal projections within V1 of post-mortem, fixed fetal (gestational age (GA) 168 and 203d) and 14d postnatal (P) human brains. At GA 168, injections into the cortical plate (CP) label mostly vertical fibers that enter the intermediate zone. Horizontal projections are confined to layer 1 and the subplate. At GA 203, the projection from layer 2/3 to layer 5 has formed within the CP. In addition, long (>2mm) horizontal fibers run along the layer 3/4 and 4/5 borders and short vertical collaterals are seen at regular intervals. Labeling in layer 1 persists. At P 14, the density of the projections in superficial layers of the CP is increased but their horizontal extent remains unchanged. These results suggest that connections within cortical columns are generated before those between columns which largely develop after birth. (Supported by NIH Grant EY05935).

4.10

DEVELOPMENT OF CLUSTERED HORIZONTAL CONNECTIONS IN CAT STRIATE CORTEX: PROGRESSIVE AND REGRESSIVE EVENTS. L. C. Katz and E. M. Callaway Laboratory of Neurobiology, The Rockefeller University, 1230 York Avenue, New York, NY 10021.

In the striate cortex of adult cats, horizontal axons of pyramidal cells from layers 2/3 and 5 specifically interconnect regions of similar orientation selectivity (Gilbert & Wiesel, J. Neurosci., in press). Cortical injections of retrograde neuronal tracers label clusters of cells up to 4mm from the injection site. We have examined the development of this intralaminar circuitry based on the pattern of retrograde label in tangential sections of flattened cortex following small (100-400 μm diam.) injections of flourescent latex microspheres in area 17. Following injections at 4-6 days postnatal (P4-6) the pattern of retrograde label was even, unclustered and extended up to 2mm from the injection site. Injections at day 8 resulted in a more extensive pattern of label (3-4mm radius) with clustering just discernable amongst the most distant cells. These results suggested that after P6 horizontal axon outgrowth was more directed. By P12-15 clustering was much more apparent both within and beyond the region labeled at 4-6 days. The changes observed between P6 and P15 appeared to result from two phenomena: a reduction in the number of cells making inappropriate connections to the injection site, and an increase in the number making appropriate connections. Early (P12-15) crude clusters were gradually refined during the third through sixth weeks: the proportion of labeled cells between clusters (those making inappropriate connections to the injection site) declined sharply, and the characteristic adult pattern of crisp clusters emerged. At all ages the pattern of labeling was similar in supragranular and infragranular layers, despite a clear difference in the developmental ages of the laminae. Thus, regulation of both the timing of axonal outgrowth and elaboration of clustered local connections is independent of the ages of the cells involved. These events may be initiated by other developmental influences common to both populations. Supported by NIH grants EY 07960 and EY 06128 and the L. P. Markey Charitable Trust.

4.11

DEVELOPMENT OF CLUSTERED HORIZONTAL CONNECTIONS IN CAT STRIATE CORTEX: ROLES OF PROCESS ELIMINATION AND CELL DEATH. E. M. Callaway and L. C. Katz. Laboratory of Neurobiology, The Rockefeller University, 1230 York Avenue, New York, NY 10021.

We have found that regressive events are an important component in the development of intralaminar horizontal connections. Reductions in the numbers of cells making inappropriate connections contribute to the appearance of early, crude clustering during the second postnatal week and to its subsequent refinement during the third through sixth weeks (see preceeding abstract). The potential contribution of cell death was assessed by experiments in which fluorescent latex microspheres were injected in area 17. In the first experiment, animals were injected at P6 and killed at P16; in the second, injections were made at P15 and the animals killed at P40. In the P6/P16 experiment, the pattern of labeling was unclustered, and identical to that of P6 animals killed on P7. Similarly, in the P15/P40 experiment, clusters were as unrefined as those characteristically observed in P15 animals. These results demonstrate that specific cell death did not contribute to either establishment or refinement of clusters. We therefore investigated specific elimination of axonal processes as a possible mechanism for cluster refinement. When an injection of red microspheres was made on P14, and an injection of green microspheres was made at precisely the same location on P29, the 2 injections resulted in overlapping clusters of retrogradely labeled cells. The double labeled cells were confined almost exclusively to the regions of densest labeling from the early injection. In one representative tangential section 33% (350 of 1076) of the cells labeled by the early injection were outside of clusters, while only 11% (16 of 146) of cells labeled by both early and late injections were outside clusters. Based on these results, and on observations of intracellularly stained cells, we conclude that cluster refinement entails specific elimination of inappropriately projecting axonal collaterals. Supported by NIH grants EY 07960 and EY 06128 and the L. P. Markey Charitable Trust.

HUMAN BEHAVIORAL NEUROBIOLOGY: MEMORY

5.1

WORD STEM COMPLETION PRIMING ACROSS THE LIFESPAN. H.P. Davis and M.G. Gandy*. Department of Psychology, University of Colorado at Colorado Springs, Colorado Springs, CO 80933.

Subjects in each decade of life from the twenties through the eighties, matched on WAIS-R IQ scores, were administered two verbal recall and recognition tests, three tests of frontal lobe functioning, and a word stem completion test of priming. Subjects from twenty through fifty-nine years of age performed similarly on the recall tests and tests of frontal lobe functioning. Sixty, seventy, and eighty year old subjects demonstrated impaired performance on the tests of recall and frontal lobe functioning. Subjects from all decades of life performed similarly on the recognition tests. Baseline guessing scores on the word stem completion task were similar across all decades of life. Priming scores were significantly higher than baseline scores for subjects in all decades of life ($p<.01$). However, the magnitude of priming was significantly less for subjects in their seventies and eighties than for all other age groups ($p<.01$). The findings reported here are relevant to previous suggestions that elderly subjects and amnesic patients have qualitatively similar memory deficits because both are impaired for explicit information, and yet, implicit memory remains intact in both groups as measured by priming. The present study shows that priming does decline with age when old-old subjects are tested, and these results are consistent with the view that amnesic patients and elderly subjects do not have qualitatively similar memory impairments.

5.2

A DISSOCIATION BETWEEN LEXICAL-SEMANTIC AND PERCEPTUAL-STRUCTURAL PRIMING IN ALZHEIMER'S DISEASE. M.M. Keane, J.D.E. Gabrieli, J.H. Growdon, and S. Corkin. Dept. of Brain and Cognitive Sciences and Clinical Research Center, MIT, Cambridge, MA 02139; Dept. of Psychology, Northwestern University, Evanston, IL 60208; and Dept. of Neurology, Massachusetts General Hospital, Boston, MA 02114.

In previous studies, we reported a dissociation in Alzheimer's disease (AD) between lexical-semantic priming (word completion) and perceptual-structural priming (identification of perceptually degraded pictures or words). In those studies, impaired lexical-semantic priming and intact perceptual-structural priming were demonstrated in different groups of AD patients. In the current study, we evaluated the status of both kinds of priming within a single group of AD patients (with dementia ranging from mild to severe). High and low-frequency words were presented one or three times in a study list prior to each of two priming tasks: a word completion task (in which subjects had to complete a 3-letter stem with the first word that came to mind) and a perceptual identification task (in which subjects had to identify briefly presented, masked words). In both tasks, half of the stimuli corresponded to words in the study list and half did not; the measure of priming was the difference between studied and unstudied stimuli in completion or identification performance. Priming in AD (relative to normal control subjects) was impaired in the word completion task and normal in the perceptual identification task. This within-subject dissociation in AD provides strong evidence for the separability of cognitive and neural mechanisms mediating lexical-semantic and perceptual-structural priming.

5.3

COMPUTATIONAL GOALS OF LEXICAL-SEMANTIC PRIMING: EVIDENCE FROM GLOBAL AMNESIA. J.D.E. Gabrieli, M.M. Keane and S. Corkin. Dept. of Brain and Cognitive Sciences and Clinical Research Center, MIT, Cambridge, MA 02139; and Dept. of Psychology, Northwestern Univ., Evanston, IL 60208.

The amnesic patient H.M. has demonstrated intact lexical-semantic repetition priming on word completion, fragment completion, and category exemplar production tasks despite severely impaired recall and recognition memory. We hypothesized that lexical-semantic priming processes reflect context-free memory of word frequencies that contributes to efficient word retrieval. In two experiments, we examined the influence of homophone presentation upon subsequent homophone spelling in H.M. and normal control subjects. In the first experiment, homophones (e.g., STEAK or STAKE) were presented auditorily in sentence contexts that disambiguated the meaning and spelling of the homophone. In the second experiment, homophones were presented visually, and without sentence context; the visual presentation precluded spelling ambiguity. In both experiments, the measure of priming was the number of homophones, relative to baseline, that subjects spelled in accordance with the prior presentation. H.M.'s level of priming was severely impaired after auditory (context-dependent) presentation, but normal after visual (context-free) presentation. H.M.'s recognition memory for the prior presentation of the homophones was severely impaired in both experiments. The sparing of lexical-semantic priming in global amnesia may reflect the contrasting computational goals of context-free, cortico-cortical learning mechanisms subserving word retrieval and context-dependent, cortico-limbic learning mechanisms subserving recall and recognition.

5.4

DETERIORATION OF H.M.'S MEMORY FOR REMOTE EVENTS. S. Corkin, E.V. Sullivan, J.D.E. Gabrieli, and J. Yucaitis*. Dept. of Brain and Cognitive Sciences and Clinical Research Center, MIT, Cambridge, MA 02139.

We used an updated version of Marslen-Wilson and Teuber's (1975) Famous Faces Test to study the time course of remote memory in the amnesic patient H.M., who was born in 1926, and received an operation for intractable epilepsy in 1953, which resulted in severe anterograde amnesia. The present study explored the effects of aging and passage of time on H.M.'s recognition of public figures from black and white newsphotographs. The photographs included 12 assigned to the 1920s, 17 to the 1930s, 17 to the 1940s, 19 to the 1950s, 20 to the 1960s, and 17 to the 1970s. H.M. was tested on five occasions, twice in 1974 and once in 1977, 1980, and 1988. The results show consistently poor unprompted memory for public figures from the 1970s, 1960s, and 1950s, in contrast to consistently good memory for those from the 1940s. H.M.'s memory for public figures from the 1930s and 1920s has become progressively worse with each successive testing. The time course of H.M.'s remote memory reflects impaired initial acquisition of ongoing events postoperatively in the 1970s, 1960s, and 1950s; robust initial acquisition of ongoing events in the 1940s (because at the corresponding ages of 14 to 23 years he was old enough to be interested in the news, and the wartime news had heightened importance); and patchy initial acquisition of ongoing events during his childhood in the 1930s and 1920s. The results indicate a selective vulnerability of memories from H.M.'s childhood relative to those from his adolescent and young adult years.

5.5

RECOGNITION MEMORY FOR TONES: THE IMPORTANCE OF THE LEFT POSTERIOR-SUPERIOR TEMPORAL REGION. J.M. Tonkonogy, M.D.* (SPON. D. Pollen). Dept. of Psychiatry, Univ. of Mass. Med. School, Worcester, MA 01655.

Patients with different types of aphasia were required to recognize a reference tone of 200 Hz interspersed among tones ranging between 205 Hz and 300 Hz. Interstimulus intervals (ISI) ranged between 2.0 sec. and 15.0 sec. While all aphasic patients were able to discriminate tone differences of 5 Hz, when presented with an ISI of 2.0 sec., only patients with temporal lobe aphasia showed deficits in recognition memory for tones. Patients with Wernicke's aphasia (left posterior-superior temporal lesion) were impaired on short term memory for tones and required at least 15 Hz tone difference for recognition. The transcortical sensory aphasics (left posterior temporal lesion outside the Wernicke's area) presented with less impairment in memory for tone recognition. Patients with Broca's aphasia (left fronto-central lesion) did not show any significant impairment in recognition for memory for tones. These results suggest that the left temporal lobe region is important for tone recognition memory.

5.6

ROLE OF HUMAN TEMPORAL LOBES IN MUSICAL AND VERBAL MEMORY. S. Samson* & R.J. Zatorre. (SPON:O Floody) Montreal Neurological Inst., McGill Univ. Canada H3A 2B4

The relative contribution of learning versus retention abilities in auditory memory was tested in two analogous tasks using unfamiliar tunes and nonsense words. Learning on five successive trials as well as delayed recognition was tested. The performance of patients with anterior right (RT) and left (LT) temporal lobectomy was compared with performance of normal control subjects. RT and LT patients were impaired in learning and delayed recognition of tunes, although they did show learning over successive trials. A similar pattern of results was obtained in the learning and retention of nonsense words. Recognition of tunes by LT subjects improved after 24 hours, but word recognition did not. Conversely, word recognition by RT subjects improved after the delay, but recognition of tunes did not. These findings suggest that the learning of auditory patterns difficult to associate with a semantic content requires the contribution of both temporal lobes. The role of RT and LT lobes in consolidation of such patterns seems to depend upon the musical or verbal nature of the material, respectively.

5.7

ROLE OF THE RIGHT TEMPORAL NEOCORTEX IN AUTOMATIC VISUAL-CUE LEARNING. J. Doyon and B. Milner. Montreal Neurological Institute, McGill University, Montreal, Canada, H3A 2B4.

The role of the right temporal neocortex in the learning of a cue-system during tasks requiring visual discrimination of relavant from irrelevant stimuli was investigated in 74 patients with unilateral temporal- or frontal-lobe lesions, the amnesic patient H.M., and 20 normal control subjects. Subjects were tested on both a letter- and an abstract-design version of Rabbitt's (1967) visual-cue learning task. The material for both versions comprised two packs of cards, each containing a different set of irrelevant letters or designs. The subject's task was to sort a pack of cards into two piles according to which of two targets was present on the card. Depending on the condition in which the subjects were to be tested, they were given either 3 or 6 learning trials with one pack of cards before they were transferred to the other pack that contained the new set of background letters or designs. The results showed that with letters, all groups and H.M. took longer to complete the task when the irrelevant information was changed after three learning trials. With abstract designs, only patients with right-temporal lobe lesions failed to show this interference after three learning trials, but did so after six. Hence, it is argued that the right temporal neocortex plays a role in automatic visual pattern-discrimination learning.

5.8

BRAIN MORPHOLOGY ON MR IMAGES IN KORSAKOFF'S SYNDROME. T.L. Jernigan, K. Schaffer*, N. Butters and L.S. Cermak. VA Medical Center, San Diego, CA 92161

Six patients with Korsakoff's Syndrome have been compared to age-matched groups of normal controls and chronic alcoholic patients using Magnetic Resonance Imaging (MRI). Quantitative image-analytic techniques were used to estimate volumes of ventricular, cortical, and cerebellar CSF, and cortical and subcortical grey matter structures. The results reveal CSF increases, but no significant decreases in grey matter volumes, in non-amnesic alcoholics. In contrast, patients with Korsakoff's syndrome when compared to normal controls showed reduced grey matter volumes in addition to CSF increases, with greatest reductions observed in diencephalic structures. Significant grey matter reductions in Korsakoff's Syndrome relative to alcoholics were observed only in subcortical structures, again most dramatically in the diencephalon. Significantly greater involvement of right than left diencephalic structures was also observed in this sample of amnesic Korsakoff's patients. Statistical evidence is presented supporting a specific role for diencephalic damage in the pathogenesis of Korsakoff's Syndrome.

5.9

MESIAL TEMPORAL LOBE SUBSTRATES OF LEXICAL RETRIEVAL. R.J.Caselli*,J.R.Duffy* and R.J.Ivnik* (SPON: A.J.Windebank). Dept. of Neurology, Mayo Clinic, Rochester, MN 55905.

Two nonepileptic adults with chronic mesial temporal lobe lesions were studied with linguistic, neuropsychological, and neuroimaging techniques to explore the hypothesis that neuroanatomical substrates of memory also subserve object naming (lexical retrieval). A 40 year old (yo) right-handed (RH) businessman with magnetic resonance imaging (MRI) documented post-anoxic bilateral hippocampal infarctions had a dense amnesic syndrome and impaired lexical encoding, but only minimal evidence of impaired lexical retrieval from auditory, visual, and somatosensory modalities. In contrast, a 60 yo RH nurse with MRI documented post-encephalitic left amygdala (and possibly temporal pole) damage had severe impaired lexical retrieval as well as impaired lexical encoding. She could recount episodic memories associated with a target object, but was unable to name the object, or in some instances, to either visually or aurally recognize the target word. Both hippocampal and amygdala lesions may impair lexical encoding, but anterior mesial temporal structures appear to be more important for lexical retrieval than the hippocampus.

6.1

DEMONSTRATION OF TWO MORPHOLOGIC CLASSES OF SEROTONERGIC FIBERS IN HUMAN CORTEX. B.E. Kosofsky and N.W. Kowall. Neurology Service, Mass General Hosp., Boston MA 02114.

We employed a monoclonal antibody against monkey phenylalanine hydroxylase (PH8, courtesy of Dr. R.G.H. Cotton) which has been shown to selectively label serotonergic neurons in postmortem human brain (Haan et al, Brain Res. 426:19). Cortical blocks from patients diagnosed with autism and other neurological diseases were immersion fixed in periodate-lysine-paraformaldehyde within 24 hours of death. Frozen 50μ sections were processed with an indirect immunoperoxidase method. The density and distribution of labeled fibers varied from one cortical area to another. Within each cortical field examined, fibers displayed a spectrum of morphological features. Two classes of fibers could be identified by virtue of the size and shape of their varicosities: fibers with small pleomorphic varicosities and fibers with large, spherical varicosities. In neocortex, the predominant fibers have small, fusiform varicosities; gradual expansions in axon diameter, arising at variable distances along the fiber length. Other fibers are interspersed, characterized by large, round, darkly staining varicosities which form abrupt axonal expansions. We performed control experiments to establish the specificity of PH8 antiserum to selectively label serotonergic fibers in postmortem human cortex. We conclude that there are morphologic features that distinguish two classes of serotonergic fibers in human cortex as in other species (Kosofsky and Molliver, 1987). As in rat, these distinct fiber populations may originate from different raphe nuclei providing parallel raphe cortical projections with different structural and functional patterns of organization.

6.2

EXCITATORY AND INHIBITORY ACTIONS OF SEROTONIN ON NEURONS OF THE RAT MEDIAL PONTINE RETICULAR FORMATION *IN VITRO*. D.R. Stevens, R.W. Greene, and R.W. McCarley, Harvard Med. Sch./VAMC, Brockton, Ma. 02401

The striking changes in activity of neurons of the medial pontine reticular formation (mPRF) during the sleep-wake cycle may be modulated by serotonin (5HT). The direct cellular actions of 5HT in the mPRF are not known. We have examined the action of 5HT on 34 mPRF neurons in slices.

5HT applied either by superfusion (1-30 uM) or pressure ejection (0.1-0.2 mM) depolarized (7.5 ±4 mV, n=19) or hyperpolarized (7.0 ±2 mV, n=11) or had no effect (n=4). Depolarizing responses were associated with a decrease in conductance (22 ±15%, n=11) and an inward current (n=5). Hyperpolarizing responses were accompanied by outward current (n=3) and were associated with a conductance increase (25 ±7%, n=7) or no conductance change (n=3). Both responses persisted in the presence of tetrodotoxin. The reversal potentials of both responses were insensitive to chloride loading and consistent with that expected for potassium ions (-76 to -88 mV, n=3, $[K^+_o]$= 5 mM).

We conclude that 5HT acts postsynaptically to either excite or inhibit neurons of the mPRF by opposing actions on potassium conductance(s).

6.3

ACTIVATION OF THE 5-HT ASCENDING FIBERS INHIBITS THE FIRING OF MEDIAL PREFRONTAL CORTICAL NEURONS. R. Godbout, J. Mantz*, J. Glowinski* and A.M. Thierry*. INSERM U. 114, Chaire de Neuropharmacologie, Collège de France, 75231 Paris Cedex 05, France.

The effects of median (MR) and dorsal (DR) raphé nuclei stimulation on the neuronal activity of medial prefrontal cortex (PFC) were studied in the rat. Stimulation of MR and DR respectively inhibited 52.9% (n=210) and 35.0% (n=60) of spontaneously active PFC neurons. Antidromic responses were induced by MR (n=39) and DR (n=5) stimulations and were followed by inhibitions of firing in most cases. Neurotoxic lesion of the ascending 5-HT pathways by microinjections of 5,7-DHT decreased the proportions of inhibited neurons to 8.8% for the MR (n=238) and to 5.5% for the DR (n=145). The inhibitory effect of MR stimulation was blocked reversibly by acute i.p. administrations of the 5-HT_2 receptors antagonists ketanserin (9/11 neurons) and ritanserin (4/5 neurons) at doses of 2.0 and 4.0 mg/kg respectively.

Excitatory responses could be evoked in most PFC neurons by stimulating the mediodorsal thalamic nucleus (MD) at 5 to 10 Hz. This effect was blocked by stimulation of the MR, 5 to 35 ms prior to MD pulses.

These results show that 5-HT midbrain raphé nuclei inhibit the spontaneous as well as evoked firing activity of PFC output neurons, and that the 5-HT_2 receptors are involved in this effect.

6.4

DIFFERENTIAL RESPONSIVENESS OF THE RAT DORSAL AND MEDIAN RAPHE 5-HT SYSTEMS TO 5-HT$_{1A}$ RECEPTOR AGONISTS AND p-CHLOROAMPHETAMINE (p-CA). P. Blier, A. Serrano* and B. Scatton. Synthélabo Recherche, Bagneux, 92220 France.

Dorsal and median raphe 5-HT neurons give rise to projections that differ in axon morphology and in vulnerability to certain amphetamines. In the present study, we studied the effects of 5-HT_{1A} or 5-$HT_{1A/1B}$ agonists and of p-CA on extracellular levels of indoleamines, using differential pulse voltammetry with carbon fiber electrodes, in cell body and nerve terminal regions of these two subsets of 5-HT neurons in anesthetized male Sprague-Dawley rats. The 5-HT_{1A} agonist 8-OH-DPAT (30 μg/kg, i.v.) produced a 60% decrease in the height of the 300 mV oxidation peak in the dorsal raphe and frontal cortex but was ineffective at this dose in the median raphe and dentate gyrus. At 150 μg/kg i.v., 8-OH-DPAT produced a 60% decrease in the 300 mV peak in the median raphe but only a 20% decrease in the dentate gyrus. The microiontophoretic application of 8-OH-DPAT was 3 times more potent in suppressing the firing rate of dorsal raphe 5-HT neurons than that of median raphe 5-HT neurons. 8-OH-DPAT and buspirone were 10 and 4 times, respectively, more potent in decreasing 5-HT synthesis in the cortex than in the hippocampus. The 5-$HT_{1A/1B}$ agonist RU 24969 (10 mg/kg, i.p.) decreased by 70% the 300 mV peak in both raphe nuclei and by 50% in the cortex and dentate gyrus. p-CA (5 mg/kg, i.p.) increased the 300 mV peak in the cortex but was ineffective in the dentate gyrus. In conclusion, the dorsal raphe-frontal cortex 5-HT system exhibits a greater responsiveness to 5-HT_{1A} agonists than the median raphe-dentate gyrus 5-HT system. The terminal 5-HT_{1B} autoreceptor seems to play a more important role in the control of 5-HT release in the dentate gyrus than in the frontal cortex. Finally, 5-HT terminals in the dentate gyrus appear to be insensitive to the 5-HT releasing effect of p-CA, consistent with the inability of this drug to destroy 5-HT terminals in that region of the brain.

6.5

THE β-ADRENOCEPTOR AGONIST FLEROBUTEROL ENHANCES 5-HT NEUROTRANSMISSION IN THE RAT BRAIN: AN ELECTROPHYSIOLOGICAL STUDY. A. Bouthillier*, P. Blier and C. de Montigny. (SPON: L. Vachon). Dept. of Psychiatry, McGill Univ., Montréal, Canada H3A 1A1.

Flerobuterol is a potent β-adrenoceptor agonist which readily crosses the blood-brain barrier and which has been reported to be an effective antidepressant. Acute administration of flerobuterol (up to 2 mg/kg, i.v.) to male Sprague-Dawley rats did not modify the firing activity of dorsal raphe 5-HT neurons. However, a two-day treatment with flerobuterol (0.5 mg/kg/day delivered s.c. by an Alza minipump) markedly reduced the firing activity of these neurons. That this reduction was attributable to an increased activation of somatodendritic 5-HT autoreceptors was suggested by its complete reversal following the administration of the 5-HT_{1A} antagonist spiperone (1 mg/kg, i.v.). After 7 days of treatment with flerobuterol, there was a partial recovery of the firing activity of 5-HT neurons and a complete one after 14 days of treatment. At this point in time, there was a conspicuous attenuation of the effect of i.v. LSD on the firing activity of 5-HT neurons, indicating a desensitization of somatodendritic 5-HT autoreceptors. The responsiveness of postsynaptic dorsal hippocampus pyramidal neurons to microiontophoretically-applied 5-HT was unaltered following a 14-day flerobuterol treatment. However, there was a large increase in the efficacy of the electrical stimulation of the ascending 5-HT pathway in suppressing the firing activity of these neurons.

It is concluded that long-term treatment with flerobuterol results in a marked enhancement of 5-HT neurotransmission. The mechanisms whereby this β-adrenoceptor produces this effect remains to be determined. The effect of flerobuterol on the 5-HT system might underlie its therapeutic efficacy in major depression.

6.6

POTASSIUM CHANNELS BLOCKERS AND 5-HT1A CONTROL OF SEROTONERGIC NEURONAL ACTIVITY IN THE DORSAL RAPHE NUCLEUS. L. Lanfumey, S. Hajj-Dahmane*, B. Franc* and M. Hamon*, INSERM U288. Faculté de Médecine Pitié-Salpétrière, 75634 Paris cedex 13, France

5-HT1A agonists are known to depress the activity of serotonin (5-HT) neurons in the dorsal raphe nucleus (DRN), by acting on somatodendritic autoreceptors. Stimulation of these receptors triggers an increased K^+ conductance through a channel which has yet to be characterized. Attempts to identify which type of K^+ channel is coupled to 5-HT1A autoreceptors were presently made by examining the effects of various K^+ channels blockers on 5-HT1A-evoked response of DRN neurons in rat brain slices. A single barrel pipette of 15MΩ, filled with NaCl 2M, was used for extracellular recording of 5-HT neurons, and drugs were added to the Krebs medium superfusing the slice. The 5-HT1A agonist ipsapirone induced a concentration-dependent inhibition of 5-HT spontaneous neuronal activity, with a IC50 of 60nM. 4-aminopyridine (4-AP, 1mM), which blocks both the A type and G protein-coupled K^+ channels, prevented the inhibitory effect of ipsapirone in low concentration range, and significantly reduced that due to higher concentrations of the 5-HT1A agonist (IC50=110nM). In contrast apamine (50nM), which blocks certain $K^+(Ca^{2+})$ conductances did not alter ipsapirone-induced inhibition of 5-HT neurons. These results indicate that somatodendritic 5-HT1A autoreceptors are coupled to 4-AP-sensitive K^+ channels, which may belong to those associated with G proteins.

6.7

DEPOLARIZING ACTIONS OF SEROTONIN ON NEURONS OF THE GUINEA-PIG ISOLATED INFERIOR MESENTERIC GANGLION. A.G. Meehan* and D.L. Kreulen (SPON: S.S. Hsiao). Department of Pharmacology, College of Medicine, University of Arizona, Tucson, Arizona 85724.

The effects of serotonin on neurons of the guinea-pig isolated inferior mesenteric ganglion (IMG) were examined. Intracellular recordings of membrane potential were made in principal IMG neurons. Serotonin was applied by pressure ejection from micropipettes. Responses to serotonin were dependent upon the concentration of the amine in the pipette. High concentrations (10 mM) evoked responses of 2-5 s duration, low concentrations (100 μM) evoked a slow depolarization in 70% of neurons tested. The slow depolarization was unaffected by: ICS 205,930 (5 μM), MDL 72222 (1 μM), ketanserin (1 μM), MDL 11,939 (5 μM), methiothepin (1 μM), methysergide (1 μM), and cyproheptadine (50 μM). The slow depolarization was associated with a 30% decrease in input resistance. In the presence of low Na^+ the serotonin-evoked slow depolarization was markedly reduced but was unaffected by low Ca^{2+}/ high Mg^{2+}, high K^+ or low K^+ medium. In addition, the slow depolarization was not affected by tetrodotoxin (3 μM) or amiloride (1 mM). Thus serotonin evokes a slow depolarization in a subpopulation of guinea-pig IMG neurons through the activation of an as yet unidentified receptor. Furthermore, the slow depolarization appears to be mediated in part by Na^+-influx. Support: DK36289, HL27781.

6.8

LSD ANTAGONIZES 5-HT_2-MEDIATED DEPOLARIZATIONS IN CORTICAL PYRAMIDAL NEURONS. P.A.Pierce and S.J.Peroutka. Department of Neurology, Stanford University School of Medicine, Stanford CA, 94305.

5-HT has been shown to produce both depolarizing and hyperpolarizing effects on cortical neurons. 5-HT produces an increase in K^+conductance and cell hyperpolarization via the 5-HT_{1A}receptor. Membrane depolarization with decrease in K^+conductance appears to be mediated by the 5-HT_2 receptor. Since lysergic acid diethylamide (d-LSD) interactions with 5-HT_2 receptors have been implicated in hallucinosis, we analyzed the effect of d-LSD on the 5-HT_2-mediated depolarization in rat cortical pyramidal neurons.

Intracellular recordings of rat cortical pyramidal neurons (n=65) were performed to determine the membrane effects of 5-HT, d-LSD and the 5-HT_{1A} agonist 8-OH-DPAT. 5-HT (10^{-4} M) produces an initial depolarization of 4.5$\pm$0.4 mV with a decrease in conductance of 24$\pm$9% (7/9 cells). The depolarization is immediately followed by a hyperpolarization of 3.9$\pm$0.5 mV below the original resting membrane potential, and a 16$\pm$5% increase in membrane conductance (6/9 cells). The 5-HT_{1A} agonist 8-OH-DPAT (10^{-7} - 10^{-6}M) produces only a hyperpolarizing response (5.9$\pm$1.1 mV) and increase in conductance of 14$\pm$3% (12/18 cells). Similarly, d-LSD (10^{-6} M) causes only a hyperpolarization (6.0$\pm$1.1 mV) and increase in conductance of 21$\pm$6% (7/9 cells). Application of 8-OH-DPAT does not affect the 5-HT-induced depolarization (4.2$\pm$0.6 mV; 9/13 cells) but slightly increases the 5-HT hyperpolarizing response (4.8$\pm$0.7 mV; 10/13 cells). In contrast, in 13 of 16 cells d-LSD partially inhibits (6 cells) or completely blocks (7 cells) the 5-HT depolarizing response (avg = 0.7$\pm$0.2 mV), in addition to increasing the 5-HT hyperpolarization (5.2$\pm$0.6 mV; 7/16 cells). These data suggest that while 8-OH-DPAT and d-LSD are both 5-HT_{1A} agonists, d-LSD is a potent antagonist of 5-HT_2 receptors mediating cortical neuronal depolarization.

6.9

CENTRAL AND PERIPHERAL EFFECTS OF DOI, A SELECTIVE 5-HT_2 AGONIST, ON CORTICOSTERONE SECRETION. R.H. Alper. Dept. of Pharmacology, Toxicology and Therapeutics, University of Kansas Medical Center, Kansas City, KS 66103.

The serotonin (5-HT) agonist DOI [(±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane] increases corticosterone secretion. The site of action was determined in conscious, unrestrained male rats implanted with arterial and venous catheters. Blood samples (0.25 ml) were obtained via the arterial catheter. Plasma corticosterone levels were increased as early as 5 minutes after DOI (500 μg/kg, iv), and 6-7 fold at 15 to 60 minutes. Pretreatment with the central 5-HT_2 antagonist LY 53857 (100 μg/kg, iv) blocked the effect of DOI on corticosterone secretion at all times. The peripheral 5-HT_2 antagonist xylamidine (100 μg/kg, iv) attenuated the corticosterone response elicited 15 minutes after DOI but did not alter the 60 minute response. In contrast, dexamethasone pretreatment (350 μg/kg, sc) attenuated the corticosterone response at 15 minutes, but abolished the response 60 minutes after DOI. These data suggest that DOI initially increases corticosterone secretion in part through a direct adrenal mechanism not dependent on ACTH, and at later times via a central, dexamethasone-suppressible mechanism. [Supported by the Kansas Affiliate of the American Heart Association]

6.10

THE 5-HT3 ANTAGONIST MDL 73,147 RESEMBLES CLOZAPINE, NOT HALOPERIDOL, IN ITS EFFECT ON DA AUTORECEPTOR SUPERSENSITIVITY IN RAT STRIATUM. M. Dudley, S. Sorensen and A. Ogden. Merrell Dow Research Institute, Cincinnati, OH 45215.

Chronic administration of MDL 73,147 (MDL) (1H-indole-3-carboxylic acid-octahydro-3-oxo-2,6-methano-2H-quinolizin-8-yl ester), like haloperidol (HAL), decreased the spontaneous electrical activity of A9 and A10 cells (Sorensen et al., Eur. J. Pharmacol., in press). This treatment resulted in significantly reduced striatal levels of the DA metabolites DOPAC and HVA 24 hr after the last dose. The effect of HAL can be ascribed to autoreceptor supersensitivity. MDL was tested in two models of striatal autoreceptor function to determine if DA receptor changes occur. HAL (1 mg/kg), MDL (5 mg/kg) and clozapine (CLZ, 10 mg/kg) were given ip daily for 21 days. A challenge dose of HAL (0.1 mg/kg, ip) given on day 22 to the HAL treated rats did not produce the 40% increase in DOPAC and HVA that it did in the saline controls. The challenge after MDL and CLZ gave significant increases in DOPAC (167 and 170%, respectively) and HVA (150 and 153%, respectively) above those produced by the challenge in saline controls. Chronic HAL enhanced the ability of apomorphine (APO) to decrease L-DOPA synthesis by activation of presynaptic receptors in the GBL/NSD treated rat. Chronic MDL and CLZ did not increase sensitivity to APO. The biochemical profile of MDL in these two models resembles CLZ, not HAL. MDL may have antipsychotic effects without the extrapyramidal liability of HAL.

6.11

SUMATRIPTAN (GR 43175): A SELECTIVE 5-HT_{1D} AGONIST WITH NOVEL ANTI-MIGRAINE PROPERTIES? S.J. Peroutka and B.G. McCarthy*. Dept. of Neurology, Stanford University, Stanford, CA 94305.

Ergot compounds are the most common class of agents used in the acute treatment of severe migraine. Dihydroergotamine (DHE), for example, has been shown to acutely relieve a migraine attack in approximately 70% of patients. Recently, a novel pharmacological agent, sumatriptan (formerly called GR 43175), has been reported to be extremely effective in the acute treatment of migraine (Doenicke et al., *Lancet*, 1309, 1988). Unlike DHE, sumatriptan appears to exhibit few side effects. In the current study, an attempt was made to assess if DHE and sumatriptan share a common site of action at the molecular level.

The potency of dihydroergotamine (DHE) was determined at 13 neurotransmitter receptors using radioligand binding techniques. DHE is a potent agent (i.e. K_i < 100 nM) at 5-hydroxytryptamine$_{1A}$ (5-HT_{1A}), 5-HT_{1C}, 5-HT_{1D}, 5-HT_2, dopamine$_2$, alpha$_1$- and alpha$_2$-adrenergic binding sites. DHE displays moderate affinity (K_i = 100 - 1000 nM) for beta-adrenergic and dopamine$_1$ sites and is completely inactive (K_i > 10,000 nM) at 5-HT_3, muscarinic and benzodiazepine receptors. These results were then compared to similar data on sumatriptan. Sumatriptan is essentially inactive at all of the above sites (K_i > 10,000 nM) except for 5-HT_{1D} (K_i = 17 nM) and 5-HT_{1A} (K_i = 100 nM) receptors. Therefore, these data suggest that 5-HT_{1D} and/or 5-HT_{1A} receptors may play an important role in the pathophysiology of migraine.

6.12

CHARACTERIZATION OF SEROTONIN RESPONSES IN NEURONS OF GUINEA PIG CELIAC AND INFERIOR MESENTERIC GANGLIA IN PRIMARY CULTURE SR Knoper, SG Matsumoto, AG Meehan, and DL Kreulen. Departments of Internal Medicine, Physiology and Pharmacology, University of Arizona. Neurons from adult celiac or inferior mesenteric ganglia were enzymatically dissociated (papain, collagenase-dispase) and maintained in primary culture for 3-30 days. Voltage responses to serotonin (10 μM) applied by pressure ejection were measured with intracellular microelectrodes (80-120 megohms; 3M KCl). Mean resting E_m was -61 mV. All 7 IMG and twenty-eight of 32 celiac cells measured responded with a 2-25 (mean=10.7) mV depolarization and 1-16 (mean=4) second duration to single ejection pulses. Some had superimposed action potentials. In 12 celiac and 5 IMG cells MDL 72,222 (5μM) antagonized the depolarizations (85% mean reduction). Neither hexamethonium nor atropine antagonized this response. Repetitive ejection pulses evoked a two phase response consisting of an initial fast phase blocked by MDL 72,222 and a later slow phase not blocked by MDL 72,222. Neither methysergide nor methiothepin antagonized slow serotonin responses in those cells tested (n=3). These findings suggest serotonin activated $5HT_3$ and non $5HT_3$ receptor mediated events on the same neuron. Supported by DK36289 and HL27781.

6.13

EXPERIMENTAL MODIFICATION OF SEROTONIN EXPRESSION IN CAUDAL VS. ROSTRAL RHOMBENCEPHALON CULTURES: IMMUNOCYTOCHEMICAL AND BIOCHEMICAL ANALYSIS. N. König, D. Becquet*, MJ. Drian* and F. Héry*. INSERM U 249 and CNRS UPR 41, Montpellier; and INSERM U 297, Marseille, France.

This study aimed at the analysis of genetic vs. epigenetic regulation of serotonin expression in neurons taken from different regions of the embryonic rat rhombencephalon (RE). The caudal basal-plate (BP) part of the RE (including groups B1-B3 or B1-B2 alone) and the rostral BP part (including B4-B9) were dissected from day 14 or 16 rat embryos. The cells were dissociated and plated either alone or in cultures where 25 % of BP cells were mixed with 75 % of caudal or rostral alar-plate (AP) cells. Since the embryonic AP does not contain serotonin expressing cells, the number of immunostained cells in the mixed cultures should be reduced to 25 % due to the dilution. In the rostral RE mixed cultures, the number of immunostained cells was close to the expected value; in the caudal RE mixed cultures however, it was significantly lower. Radio-isotopic assays also showed an overproportional decrease in newly synthesized serotonin, and this effect was stronger in the caudal than in the rostral RE mixed cultures. Pharmacological analysis showed that the B1-B3 as well as the B4-B9 cells were able to negatively autocontrol serotonin synthesis by type 1 5-HT autoreceptors. It is likely that additional mechanisms are implicated in the regulation of serotonin expression in RE subpopulations. GRANTS: INSERM, CNRS, IRME.

NEURAL-IMMUNE INTERACTIONS I

7.1

BRAIN INTERLEUKIN-1 INDUCED IMMUNOSUPPRESSION: DOSE RESPONSE INHIBITION BY ALPHA-MSH. J.M. Weiss, S.K. Sundar*, M.A. Cierpial and J. Ritchie. Dept. of Psychiatry, Duke Univ. Med. Ctr., Durham, NC 27710.

In recent studies showing that microquantities of IL-1 in brain suppress peripheral cellular immune responses, we found that alpha melanocyte stimulating hormone (a-MSH) introduced into the ventricular system blocked immunosuppressive effects of both exogenous IL-1 and endogenous IL-1 released in response to lipopolysaccharide infused into the lateral ventricle. The present study demonstrated that a-MSH blocks effects of intraventricular IL-1 in a dose-dependent manner.

Animals were infused intraventricularly with IL-1 (100 pg) together with ascending doses of a-MSH (0,0.1,1.0, or 10.0 ng). In comparison to vehicle-infused controls, IL-1 alone (i.e., 0 a-MSH) depressed natural killer cell (NK) activity; increasing concentrations of a-MSH progressively blocked this effect, with the highest dose (10.0 ng) completely blocking the suppressive effect of IL-1.

IL-1 in brain produces part of its immunosuppressive effects by stimulating the pituitary-adrenal axis and elevating plasma corticosteroids. Measurement of plasma steroids showed that the ability of intraventricular IL-1 to stimulate the pituitary-adrenal axis was also blocked by alpha-MSH in a dose-dependent manner, which may account, in part, for how alpha-MSH blocks the immunosuppressive effects of brain IL-1.

7.2

BRAIN INTERLEUKIN-1 (IL-1) INDUCED IMMUNOSUPPRESSION: MEDIATION BY CRF AND SYMPATHETIC ACTIVATION. S.K. Sundar*, M.A. Cierpial, C.D. Kilts & J.M. Weiss. Dept. of Psychiatry, Duke Univ. Med. Ctr., Durham, NC 27710.

We recently demonstrated that infusion of picogram quantities (i.e. 3.1 X 10^{-15} moles) of IL-1 into the lateral ventricle of rats results in a rapid decrease in peripheral cellular immune responses. This study investigated whether corticotrophin releasing factor (CRF) in brain and the sympathetic nervous system (SNS) mediates immunosuppression produced by IL-1 in brain.

Rats received either ventricular infusion of affinity purified anti-CRF IgG or i.p. injection of the ganglionic blocker chlorisondamine prior to central IL-1 infusion. Fifteen minutes after IL-1, in vitro immune responses of blood and splenic lymphocytes were analyzed. Immunoneutralization of brain CRF attenuated the immunosuppressive effects of IL-1 completely in blood lymphocytes but only partially in splenic lymphocytes. Conversely, chlorisondamine blockade of the SNS attenuated immunosuppression completely in splenic but not blood lymphocytes. These results indicate that brain IL-1 affects immunological cells via CRF pathways in brain and the SNS, and that these pathways differentially affect different populations of lymphocytes.

7.3

THE EFFECT OF DIFFERENT NUMBERS OF MICE PER CAGE ON IL-1 AND BONE MARROW STEM CELLS. B. S. Rabin and S. B. Salvin. Dept. of Pathology, Univ. Pittsburgh, Pittsburgh, PA 15261.

A number of studies have been conducted in our laboratories for the purpose of examining immunologic changes which occur when different numbers of animals are housed per cage. The studies indicate that an alteration of immune reactivity occurs when certain mouse strains are housed either 1 or 5 per cage. C3H/HeJ male mice and CD-1 mice of either sex, when housed individually, have an enhanced immune response to sheep erythrocytes, produce more IL-2 , and respond more vigorously to mitogenic stimulation. We now report that IL-1 production, macrophage phagocytosis, as well as changes occurring as early as bone marrow precursor cells, have an enhanced activity in individually housed C3H/HeJ male mice. Peritoneal exudate cells were collected and incubated with LPS. The supernatants and cell lysates were tested in a mouse thymocyte stimulation assay. The individually housed mice produced more IL-1 in the supernatant ($P<0.005$) and cell lysate ($P<0.02$). Phagocytic activity was increased 50% and colonies of macrophages developing from bone marrows of individually housed mice were 1.5 times more abundant than from group housed mice. The production of macrophage colony stimulating factor from spleen cells was twice that of individually housed mice. The data suggest that housing can influence multiple factors relating to resistance to infection. Environment appears to be an important modulator of immunity.

7.4

INTERLEUKIN-1 POTENTIATES THE SECRETION OF B-ENDORPHIN INDUCED BY SECRETAGOGUES VIA PROTEIN KINASES IN A MOUSE PITUITARY CELL LINE (AtT-20). M.O.Fagerasen[1], M.S.Rinaudo[2], J.F.Bishop[2], and J.Axelrod[3]. [1]Clinical Neuroscience Branch, NIMH and Peptide Design; [2]Experimental Therapeutic Branch, NINDS; [3]Laboratory of Cell Biology, NIMH.

Interleukin-1 (Il-1) enhances the release of B-endorphin induced by corticotropin releasing factor (CRF), vasoactive intestinal peptide, forskolin, norepinephrine, isoproterenol and phorbol ester in the AtT-20 mouse anterior pituitary cell line. The effect is apparent after 12 hours of pretreatment with Il-1, reaches a maximum at 24 hours and is dose dependent. Desensitization of protein kinase C abolishes the potentiation of the secretory action of phorbol esters by Il-1, but only partly reduces Il-1 enhancement of the secretory effects of CRF and other secretagogues. Treatment of the cells with staurosporin, an inhibitor of protein kinases, resulted in further decreases in the enhancement of CRF-induced B-endorphin release produced by Il-1.

To examine the role of protein phosphorylation in Il-1 receptor mediated events, AtT-20 cells were preloaded with ^{32}P and stimulated with IL1-a. Cytosolic phosphoproteins were identified using two dimensional gel electrophoresis with subsequent autoradiography. Il-1 increased the incorporation of ^{32}P into several proteins in a time-dependent manner.

After a 15 minute treatment of the cells with Il-1, an increase in the phosphorylation of a 19 kDa protein associated with secretory activity was observed. After abolishing protein kinase C activity by pretreating the cells with phorbol ester (10^{-7} M, 24 hours), the effect was still apparent indicating that this phosphorylation event does not occur via the protein kinase C pathway. Il-1 also increases early phosphorylation of a 60 kDa protein. After 1 hour of treatment with Il-1, an increased phosphorylation of the 87 kDa protein, which has been previously characterized as a protein kinase C specific substrate, appeared.

These results indicate that Il-1 potentiates the effects of secretagogues in AtT-20 cells possibly by acting on protein kinases in sequential fashion.

7.5

HYPEREXPRESSION OF INTERLEUKIN 1 mRNA BY PERIPHERAL MACROPHAGES OF CEREBELLAR MUTANT MICE STIMULATED IN VITRO BY LPS. B. Kopmels*, E.E. Wollman*, J.M. Guastavino*, N. Delhaye-Bouchaud*, D. Fradelizi* and J. Mariani. Lab. Immunologie, Institut Gustave Roussy, Villejuif (94) and Institut des Neurosciences, Univ. P. et M. Curie. 75005- Paris (France).

Several mutations in mice produce complex patterns of neuronal degeneration of the cerebellum and of its afferent pathways. In the staggerer (sg/sg) mutant, atrophy of the lymphoïd organs and immunological abnormalities have been recently described (Trenkner and Hoffman, 1986). To search for a possible link between the neurological and the immune disorders in this mutant, we studied the production by peripheral monocytes of interleukin 1 (IL-1), whose roles in both immune and nervous system are well established.

Suspensions of peritoneal and/or spleen monocytes from mutants and their controls were stimulated in vitro by LPS; total RNA was prepared and Northern and dot blots were performed by using murine recombinant IL-1 α and β probes. They revealed an up to tenfold expression of IL-1 α and mRNA in staggerer macrophages. A similar phenomenon was detected using bioassay of IL-1 activity. The hyperexpression was found in 3 week to one year old staggerer and also in heterozygous +/sg. A similar phenomenon existed in cerebellar mutants lurcher, pcd and to a lesser extent in reeler but was absent in weaver and jimpy.

Work is in progress to disclose a possible hyperexpression in brain microglia of these mutants and to unravel the mechanisms involved.

7.6

CORTICOTROPIN-RELEASING FACTOR (CRF) RECEPTORS ARE PRESENT ON MOUSE SPLENIC MACROPHAGES. E.L. Webster, D.E. Tracey*, S.A. Wolfe, Jr., M.A. Jutila* and E.B. De Souza. Neurobiology Laboratory, NIDA/ARC, Baltimore, MD 21224; The Upjohn Co., Kalamazoo, MI; Department of Pathology, Stanford University School of Medicine, VA Medical Center, Stanford, CA.

Previous studies in our laboratory have identified CRF binding sites in mouse spleen with kinetic and pharmacological characteristics comparable to the well-characterized CRF receptors in pituitary and brain. In addition, splenic CRF receptors have been demonstrated to be functionally coupled to a guanine nucleotide protein mediating stimulation of adenylate cyclase activity. In autoradiographic studies, ^{125}I-oCRF binding sites were localized to the red pulp and marginal zones of splenic follicles known to be enriched for macrophages. In the present study, the identity of spleen cells bearing ^{125}I-oCRF binding sites was elucidated. The autoradiographic distribution of ^{125}I-oCRF binding sites in spleen was similar, if not identical, to the histological distribution of phagocytic cells in spleen as indicated by uptake of India ink particles *in vivo*. Furthermore, when splenic cells were fractionated on the basis of density, adherence, and phagocytic activity, ^{125}I-oCRF binding significantly correlated with the percentage of cells phagocytosing latex beads and staining with a monoclonal antibody (MONTS-4) specific for resident splenic macrophages. The presence of CRF binding sites on adherent, phagocytic, light density cells, and the histological localization of CRF binding to areas of the spleen known to be enriched in macrophages strongly suggests that splenic macrophages are targets for direct immunomodulatory actions by CRF.

7.7

A DEFECT IN CORTICOTROPIN RELEASING HORMONE (CRH) BIOSYNTHESIS IS ASSOCIATED WITH SUSCEPTIBILITY TO STREPTOCOCCAL CELL WALL (SCW) ARTHRITIS IN LEWIS (LEW/N) RATS. E.M. Sternberg*, W.S. Young III, A.E. Calogero*, R. Bernardini*, J. Glowa, M. Smith, S. Aksentijevich*, C. Smith*, S. Listwak*, G.P. Chrousos, P.W. Gold* and R.L. Wilder*. (SPON:N.H.Spector) NIMH, NIH, Bethesda, MD, 20892.

A CNS-immune system feedback loop has recently been described in which immune mediators stimulate the hypothalamic-pituitary-adrenal (HPA) axis, and the resultant glucocorticoid release suppresses excessive inflammation. We have found that a defect in this feedback loop is associated with susceptibility to development of SCW arthritis in LEW/N rats, while resistance to SCW arthritis in histocompatible F344/N rats is related to intact HPA axis responses to the same inflammatory mediators (*Proc. Natl. Acad. Sci.*, 86:2374, 1989). We now report that the depressed LEW/N ACTH and corticosterone responses to both inflammatory mediators and behavioral stresses are related to a defect in CRH biosynthesis in the paraventricular nucleus (PVN) of the hypothalamus. In contrast to F344/N rats, there was no increase in LEW/N total hypothalamic CRH content in response to intra-peritoneal (i.p.) SCW or recombinant interleukin-1 alpha (rIL-1). CRH secretion from LEW/N hypothalamic explants increased 10% over baseline in response to rIL-1 compared to 150% from F344/N hypothalami. LEW/N rats also exhibited depressed expression of CRH mRNA in the PVN in response to i.p. SCW, as evidenced by *in situ* hybridization histochemistry. The additional finding of deficient expression in the PVN of the co-regulated enkephalin gene suggests that the primary defect is not in the CRH gene but is in its inappropriate regulation.

7.8

CLASS II MHC GENE EXPRESSION IN THE MOUSE BRAIN IS ELEVATED IN THE AUTOIMMUNE MRL/Mp-Lpr/Lpr STRAIN. K.R. McIntyre[1], C. Ayer-LeLievre[2], H. Persson[1](SPON: M. Schalling). [1]Dept. of Medical Chemistry, Lab. Molecular Neurobiology and [2]Dept. of Histology and Neurobiology, Karolinska Institute, Box 60400, S-10401, Stockholm, Sweden.

Class II MHC gene expression in the brain of several normal and autoimmune mouse strains has been quantified by RNA blot analysis using murine I-A and I-E genomic probes. We have detected Ia-A and B mRNA in the brain of every mouse strain examined, albeit at levels 30-50-fold lower than those found in the spleen, with the exception of the autoimmune MRL/l strain, in which Ia transcripts in the brain are elevated to a level comparable to that found in normal mouse spleen. The elevation of Ia mRNA in MRL/l as compared to control C3H mice also extends to other organs, being most pronounced in kidney and brain but not significant for thymus and lung. Evidence against a major role for blood cells as the source for Ia mRNA comes, among other things, from the finding that Ia mRNA in MRL/l as compared to control C3H mice is elevated to a greater extent in the poorly vascularized diencephalom than in the richly vascularized choroid plexus. Further experiments with normal rats demonstrated a large quantitative variation in Ia mRNA in different brain regions, with the highest levels being found in the olfactory bulb, followed by the cerebellum, the superior colliculi, and the pons/medulla. The findings are discussed in relation to an animal model for central nervous system (CNS) involvement in systemic lupus erythenatosus.

7.9

INTERLEUKIN-1 ADMINISTRATION PRODUCES A STRESS-LIKE REDUCTION OF EXPLORATORY BEHAVIOR IN MICE. F. Spadaro*, C.W. Berridge and A.J. Dunn (SPON: C.S. Sebastian). Department of Pharmacology, Louisiana State University Medical Center, Shreveport, LA 71130-3932.

The time for which rats and mice investigate novel stimuli in the multicompartment testing chamber (MCC) is reduced by various stressful treatments. This effect is mimicked by corticotropin-releasing factor (CRF) injected intracerebroventricularly (ICV). Because interleukin-1 has been shown to activate the hypothalamic-pituitary-adrenal (HPA) axis, an effect that is presumed to involve secretion of hypothalamic CRF, we have investigated whether IL-1 has any related behavioral activity.

Human recombinant IL-1α was injected ICV in doses of 100-100,000 Units. Each of these doses produced reductions in the time mice spend in contact with the stimuli in the MCC. At the higher doses, locomotor activity was also reduced, but this effect was not observed at the lower doses. Although IP administration of IL-1 activates the HPA axis, we only observed seemingly nonspecific reductions in all behaviors at doses of 10^5-10^6 Units. Similar responses were observed following IP administration of bacterial endotoxin (lipopolysaccharide), a known stimulator of endogenous IL-1 production. Thus our results suggest that central administration of IL-1 may elicit cerebral CRF secretion that elicits the behavioral response. This hypothesis is consistent with the reported role of CRF in ICV IL-1-induced fever.

Supported by grants from NIMH (MH 45270) and NINCDS (NS27283)

7.10

RELEASE OF INTERLEUKIN-6 FROM RAT HYPOTHALAMUS. B.L. Spangelo*, I.S. Login*, A.M. Judd*, P.C. Isakson* and R.M. MacLeod. Departments of Medicine, Neurology and Pharmacology and the Center for Cancer Research, University of Virginia Health Sciences Center, Charlottesville, Virginia 22908.

We have reported that the inflammatory cytokine, interleukin-6 (IL-6), stimulates anterior pituitary hormone release *in vitro*. We therefore hypothesized that IL-6 may be released from the hypothalamus, thus functioning as a pituitary hormone releasing factor *in vivo*. Female Sprague-Dawley rats were sacrificed and their brains rapidly removed and placed on an ice-cold surface. Medial basal hypothalami were removed to a depth of 3 mm, halved, weighed and placed in 1 ml RPMI-1640 containing 10% serum (complete medium) in a metabolic shaking water bath (1 half/well) under an atmosphere of 95% O_2/5% CO_2. After a 1-hr preincubation, the tissues were removed and placed in fresh complete medium (vehicle) or complete medium containing lipopolysaccharide (LPS) or IL-1. After 2 and 4 hr, aliquots of incubation medium were removed and assayed for IL-6 activity in the hybridoma 7TD1 growth factor assay.

Hypothalamic tissue released an IL-6 activity in a time-dependent manner (2-4 hr; 5.29-18.5 U/mg). LPS (10-100 ng/ml) significantly increased this release after 2 (2.7-fold) and 4 (3.1-fold) hr of incubation. IL-1 (200 U/ml) significantly reduced the basal release of IL-6 by 44%. Sensori-motor cortex released smaller amounts of IL-6 (2-4 hr; 2.38-12.1 U/mg), but this release was not stimulated by LPS.

These data suggest that IL-6 is released from the hypothalamus and cortex. In the presence of LPS, the release of this cytokine from the hypothalamus is much greater than that from the cortex. Hypothalamic production of IL-6 suggests that it could function as an anterior pituitary releasing factor *in vivo*. [Supported by NCI grants CA-07535 to R.M.M. and CA-38228 to I.S.L., and a new initiative grant from the University of Virginia DERC (AM22125) to P.C.I.]

7.11

DIFFERENTIATION OF TRANSPLANTED FETAL NEURAL CELLS AND IMMUNOLOGICAL RESPONSES IN THE HOST BRAIN. K.Shimizu*, M.Yamada*, Y.Matsui*, S.Moriuchi*, H.Mogami*. Dept.of Neurosurg., Osaka Univ., Sch.of Med., Osaka 553, Japan (SPON: E. Senba)

Attempts of neural transplantation have been done in patients with Parkinson disease, who become inexorably progressive disability regardless of administration of drugs. To examine whether or not some immunological responses are induced in the recipient's brain in which some kinds of neural tissues were transplanted, we established Parkinson models which were made the unilateral 6-OHDA lesions in the nigrostriatal pathway of C57BL/6 (H-2^b) mice. A complete recovery of methamphetamine-induced rotational response was shown about 60 days after transplantation of embryonic DA-rich cells from syngeneic or allogeneic (C3H/HeN, H-2^k) mice. Immunohistochemistry showed that TH-immunoreactive cells were deeply embedded in the ipsilateral striatum, with a dense network of neuronal processes, and that the NSE, NFP(200K), GFAP or MBP-positive cells mingled among these grafted cells. The FACS IV analysis revealed no H-2 (H-$2K^k$ and Ia^k) antigens on the embryonic cells of C3H/HeN mice. However, H-$2K^k$ antigens could be induced on these embryonic cells by mouse IFN-gamma. Now, we investigate H-2 antigens on the grafted cells survived in the host brain.

7.12

MOLECULAR CLONING AND CHARACTERIZATION OF NEUROIMMUNE GENES. A. Ericsson*, G. Barbany*, W. Friedman and H. Persson*. (SPON: M.E. Ehrlich). Lab. of Molec. Neurobiol., Dept. of Med. Chem., Karolinska Institute, Stockholm, Sweden.

Recent neuroimmunological research has detected the presence of common sets of signalling substances and receptor systems among the neuroendocrine and the immune systems, thus providing a possible mechanism for communication. In order to explore the neuroimmunological interplay, we have isolated several genes from rodent cDNA libraries that are expressed predominantly within the neuroendocrine and immune systems, using a combination of subtractive hybridization and cDNA cloning. Northern blot analysis and *in situ* hybridization revealed that these genes, encoding high molecular weight mRNAs, are expressed by neurons over the entire CNS as well as in various immune tissues from rodents and man. Other tissues examined showed a low or insignificant expression. One clone, termed NI-1, was analyzed in further detail and in CNS this gene was highly expressed in the pyriform cortex, various nuclei in the limbic sysem and in some hypothalamic nuclei. A tenfold increase in total brain mRNA expression was detected around birth. Mitogen stimulation of mouse spleen cells induced a massive mRNA expression. Also, the gene appears to be under negative control by adrenal steroids as adrenalectomy in rat resulted in a tenfold increase in CNS, thymic and splenic expression. The DNA sequence of this cDNA clone showed no significant homology to any other known genes.

TRANSPLANTATION I

8.1

NONINVASIVE LASER-LESIONING OF MOUSE NEOCORTICAL NEURONS IN VIVO. Madison, R.[1], and Macklis, J.D.[2]. [1] Division of Neurosurgery, Duke University, Durham, N.C. 27710, and [2] Dept. of Neurology, Program in Neuroscience, Harvard Medical School and Children's Hospital, 300 Longwood Ave., Boston, MA. 02115

The ability to selectively label neurons on the basis of their connections for subsequent lesioning would constitute a powerful tool for studying the roles of cell-cell interations in development and adult reorganization. Toward that aim, we have labeled neuronal subpopulations in vivo and subsequently lesioned the targeted neurons via noninvasive exposure to unfocused 650 nm laser illumination. Sixty-seven mice were used for these studies. Approximately 50-70% of the surface energy at 650 nm is transmitted through mouse skull and brain. Forty animals served as controls for laser exposure alone. Through intact skull, mice received fractionated unilateral exposures that totaled 12.5, 25, 50, or 160 Joules/cm^2. Animals were perfused 3 hr. - 7 days later and histologically processed, using a silver degeneration stain to demonstrate damaged neurons. Experimental animals received unilateral injections into motor cortex of latex nanospheres with an incorporated cytolytic chromophore (see Macklis and Madison Abst.). Following 2-3 months animals received laser exposure as described above. Variable numbers of damaged neurons were evident in layers II/III and V of lased cortex within the area of laser exposure. Control animals which received either laser exposure alone, or nanosphere injection alone, did not display equivalent damage. Whitaker Fdn., NS26311, HD00859, Alz. Fdn.

8.2

NONINVASIVE LESIONING OF MOUSE NEUROBLASTOMA CELLS BY DYE-TARGETED LASER PHOTOLYSIS AFTER GRAFTING INTO MOUSE NEOCORTEX IN VIVO. J.D.Macklis[1] and R.Madison*, [1]Dept. of Neurology, Prog. in Neuroscience, Harvard Medical School, Children's Hosp., Boston, MA and *Div. of Neurosurgery, Duke Univ., Durham, NC

We are developing methods by which neurons targeted by retrograde incorporation of photoactive chromophores can be noninvasively lesioned within the rodent CNS without injury to intermixed neurons, glia, axons, or vascular and connective tissue. At long wavelengths between 650 and 800 nm, laser energy can penetrate nervous system tissue several millimeters without absorption by unpigmented tissue. Illumination at these long wavelengths damages cells containing latex nanospheres with incorporated cytolytic chromophores (chlorin e6, e.g.). We studied damage by this process of laser photolysis (PL) to C1300 neuroblastoma (NB) cells grafted into mouse neocortex in vivo.

NB cells were cultured by standard methods, labeled in vitro by brief exposure to latex nanospheres containing rhodamine as a fluorescent label and chlorin e6, and repeatedly washed for 24 hours. Young adult mice were injected with labeled NB cells within deep layers of motor cortex bilaterally, and overlying skull was replaced. After 24 hours, mice were exposed unilaterally through intact skull to pulsed 650 nm wavelength laser energy to total doses of approximately 120 Joules/cm^2. Control mice had either labeled NB injections without laser exposure, unlabeled NB injections with laser exposure, or laser exposure alone. Serial sections of brains were examined histologically after 2, 7, and 14 days. Injected control mice and the unlased experimental hemispheres typically showed large NB tumors at 7 and 14 days with areas of necrosis and hemorrhage. Surrounding tissue was undamaged by these laser energies. Injected hemispheres exposed to laser energy showed absence or reduction of NB tumor volume with degenerating NB cells, lymphocytic and macrophage infiltrate, and normal surrounding cellular architecture. These results suggest that prelabeled neurons could be noninvasively removed following transplantation into rodent CNS. HD00859, NS26311, Alzheimer's Assn, Whitaker Fdn

8.3

RETROVIRUS-MEDIATED ONCOGENE TRANSFER INTO FETAL BRAIN TRANSPLANTS - A NOVEL APPROACH TO NEURO-ONCOGENESIS. O.D. Wiestler, P. Kleihues* and A. Aguzzi*. Laboratory of Neuropathology, University of Zürich, CH-8091 Zürich, Switzerland.

Using a neural transplantation model and retrovirus-mediated gene transfer, we have introduced activated oncogenes into the developing rat brain. With mock-infected donor cells, the transplants gave rise to highly differentiated neuroectodermal tissue containing all major CNS cell types. A high percentage of transplants exposed to the *polyoma medium T* oncogene developed endothelial hemangiomas which expressed factor VIII-related antigen. No abnormalities were seen in neurons and glial cells. By *in situ* hybridization, transcripts of the *medium T* oncogene were detectable in hemangiomas and in morphologically normal neuroectodermal cells. Medium T protein was also identified in hemangioma-derived cell lines by immunoprecipitation and *in vitro* kinase assay. In order to assess the cell-specificity of the oncogene, analogous experiments were carried out with retroviral constructs harboring the *v-src*, *c-src-Phe527*, *v-Ha-ras* and *v-myc* oncogenes, respectively. Transplants infected with the *v-src* construct developed astrocytic tumors and mesenchymal neoplasms, but no vascular abnormalities. in these grafts, *v-src* transcripts were detectable in the tumors, but also in non-transformed neuroectodermal and endothelial cells. Coexpression of *v-ras* and *v-myc* cDNAs yielded highly malignant, polyclonal lesions apparently derived from poorly differentiated precursor cells. These observations indicate that activated oncogenes are capable of transforming specific target cells in the developing brain. Experiments to uncover the molecular basis of cell-specific oncogene action and to identify target cell lineages are in progress.

8.4

GENE TRANSFER INTO NEURONS OF THE ADULT RAT BRAIN. B.A. Sabel, C. Martin*, C. Waldmann*, A. Freese and A. I. Geller. Inst. Med. Psych. U. Munich Med. Sch. F.R.Germany, Div. HST., MIT, Cambridge MA, and Div. Cell Growth & Regulation, Dana Farber Can. Inst. Boston, MA 02115.

Genes were delivered into neurons of the adult rat brain using a defective Herpes Simplex Virus (HSV-1) vector. This approach could be used to alter normal neuronal physiology to study learning and memory or perform gene therapy on neuronal diseases, such as Parkinson's Disease.

We have previously described a HSV-1 vector, pHSVlac, which stably expresses ß-galactosidase from the E. coli Lac Z gene in cultured neurons from throughout the nervous system (Science 241, 1667, 1988). We now demonstrate stable expression of ß-galactosidase in neurons following stereotaxic injection of purified pHSVlac virus into the adult rat brain. Following injection of pHSVlac virus into the anterior medial secondary visual area, dorsal hippocampus, stratum opticum of the superior colliculus, red nucleus, and substantia nigra, ß-galactosidase immunoreactive (ß-Gal-IR) cells were observed around the injection site and in neurons projecting to it, presumably due to retrograde transport of the virus. Furthermore, some ß-Gal-IR cells also contained neurofilament-IR. Expression of ß-galactosidase was stable; injection of pHSVlac virus into the superior colliculus resulted in expression of ß-galactosidase in layer 5 pyramidal neurons of primary visual cortex for at least 1.5 months. In contrast to wild type HSV-1, pHSVlac virus did not spread throughout the brain; persistent pHSVlac DNA was not reactivated. Therefore, the location of the injection site and amount of virus in the innoculum determines which neurons are infected. Finally, the Lac Z gene in pHSVlac can now be replaced by other coding sequences whose products affect neuronal physiology, such as second messenger enzymes or components of neurotransmitter release.

8.5

MYELIN FORMATION IN MYELIN-DEFICIENT RAT SPINAL CORD AFTER TRANSPLANTATION OF NORMAL FETAL SPINAL CORD CULTURES. J.Rosenbluth, M.Hasegawa*, N.Shirasaki*, C.Rosen & Z.Liu*. Depts. Physiol. & Rehab. Med., N.Y.U. School of Med., NY 10016.

Normal E15-18 d. rat CNS glial cells cultured 2 or 7 d. and injected into juvenile mdr spinal cord resulted in equal myelin formation in the host 11-14 d. later. In both cases, more myelin formed than after injection of spinal cord fragments, but the area myelinated was still largely restricted to the injection site. Immunostains showed no galactocerebroside in cells cultured 2 d., but after 7 d., ~10-15% of cells were GC+. At both times, ~25% were GFAP+, but none showed neurofilament staining. Injection of donor tissue that had been frozen and thawed, heated to 60°C, or fixed in formaldehyde resulted in no myelin formation. Nor did injection of fetal mouse spinal cord, adult rat cord or a solvent extract of adult rat cord. Thus, we were unable to induce host oligodendrocytes to form normal myelin by injecting killed or disrupted donor cells or tissue extracts. The results imply that the myelin formed after injection of cultured fetal glia is generated by donor glia rather than by host glia that have incorporated injected cell components. Supported by NIH & NMSS.

8.6

MYOBLAST TRANSFER THERAPY AND NUCLEAR TERRITORY IN DYSTROPHIC MICE. P.K. Law, H-J. Li*, T.G. Goodwin*, G. Ajamoughli*, X. Y. Zhang*, and M. Chen*. Depts. of Neurology, and Physiology/Biophysics, Univ. of Tennessee, Memphis, TN. 38163.

A therapy has been developed to treat hereditary muscle degeneration. Injection of histoincompatible normal myoblasts into dystrophic muscles improved the structure and function of the muscles to almost normal. Immunosuppression of the dy^{2J}/dy^{2J} hosts was induced by daily subcutaneous injection of cyclosporin-A at 50mg/kg body weight for 6 months. Eleven out of nineteen mice that received myoblast injections in their leg and intercostal muscles on both sides showed locomotive patterns similar to normal. Life spans of the myoblast-injected dystrophic mice were extended from nine to eighteen months. Using dimeric isozymes of glucosephosphate isomerase as genotype markers for host and donor cells, the demonstration of parental and hybrid isozymes inside the injected muscles substantiated the survival and development of donor myoblasts into normal myofibers, and the fusion of normal myoblasts with dystrophic satellite cells to form genetically mosaic myofibers. Immunocytochemical analyses of host and donor nuclei within mosaic myofibers indicated that normal-appearing fibers contained at least 20% of randomly-distributed normal nuclei. The therapy replenishes lost cells and repairs degenerating cells and is based on muscle developmental processes universal to all mammals. (Supported by USPHS NS-20251,26185)

8.7

TRANSPLANTATION OF RODS TO RODLESS RAT RETINA. P. Gouras, J. Du*, M. Gelanze*, R. Lopez* and H. Kjeldbye*. Columbia Univ. Dept. Ophthal. New York, N.Y. 10032

Photoreceptors are highly specialized neurons which can be easily identified in the retina. They can be dissociated from their contacts with other cells and maintained viable in vitro where they can form synapses with other neurons (MacLeish and Townes-Anderson, Neuron 1:751, 1988). We have dissociated rat rods and mixed them with previously dissociated retinal epithelial cells from normal pigmented rats from a strain congenic to an ocular albinotic mutant for retinal dystrophy. The dystrophic rats lose their photoreceptors at 2-3 months of age. We have transplanted the rods and retinal epithelial cells to the subretinal space of 4-5 month old dystrophic rats. The normal pigmented epithelium both corrects the phagocytic defect in the mutant strain and allows tracking of the rods by light microscopy. Electron microscopy reveals the presence of rods, with outer segment, inner segment and nucleus in close apposition to transplanted pigment retinal epithelial cells for at least 24 hours after transplantation. These are transplanted rods because none are found in retinas of contralateral control eyes.

8.8

NEURAL TRANSPLANTS IN AN EXPERIMENTAL MODEL OF AMYOTROPHIC LATERAL SCLEROSIS F. Nothias*, J.C. Horvat*, M. Pécot-Dechavassine*, J.C. Mira* and M. Peschanski (spon: B. Onténiente) INSERM U 161. Univ. Paris V. Inst. Neurosc. CNRS. Paris France.

Amyotrophic lateral sclerosis is a motoneuronal degenerative disease leading to paralysis and muscle atrophy. Deep intra-spinal injections of excitotoxic substances reproduce some aspects of the disease. The present study was undertaken to determine whether fetal spinal neurons transplanted into the lesion as a cell suspension may reconstruct parts of the disrupted circuitry.

Transplanted neurons developed and differentiated although few motoneurons were immunocytochemically identified. Afferent fibers from the host innervated the transplants: descending monoaminergic fibers grew into the transplants and formed a loose network: cortico- and rubro-spinal fibers were few in number and contacted neurons only in the peripheral areas of the grafts: primary CGRP-immunoreactive C-fiber afferents contacted transplanted neurons mostly in their usual localization in the dorsal horn.

Grafted neurons did not grow axons into the host ventral roots. In contrast, axonal outgrowth was obtained when a piece of sciatic nerve was implanted at one end into the neural graft.

These experimental results are presented as a first step in the search for a palliative therapy against motoneuronal degenerative diseases. Supported by APA grants TC88-01 and AI-88002-1

8.9

INTRAPARENCHYMAL GRAFTING OF CEREBELLAR CELL SUSPENSIONS TO THE DEEP CEREBELLAR NUCLEI OF pcd MUTANT MICE: RATIONALE AND HISTOCHEMICAL ORGANIZATION. L.C. Triarhou, W.C. Low and B. Ghetti. Dept. Pathol. (Neuropathol.) and Physiol. & Biophys., Med. Neurobiol. Pgm, Indiana Univ. Sch. of Med., Indianapolis, IN 46223

In transplanting embryonic cerebellar grafts to the cerebellar cortex of 'Purkinje cell degeneration' (pcd) mutant mice to replace missing Purkinje cells (Pc), donor Pc leave the graft and migrate to the molecular layer of the host. However, Pc axons do not reach the deep cerebellar nuclei (DCN) of the host, which would be a key element in providing the necessary inhibitory cortico-nuclear projection associated with cerebellar function in normality. Rather, grafted Pc often leave their axon inside the transplant, while the perikaryon migrates to the host molecular layer. In the present study, aiming at re-establishing a Pc innervation of the DCN, we implanted E12 cerebellar cell suspensions intraparenchymally to the DCN of the hosts. The development of grafted Pc was monitored with 28-kDa calcium-binding protein (CaBP) immunocytochemistry. Donor Pc were found in clusters both deeply in the DCN parenchyma and aligned along cortical folia. A CaBP immunoreactive fiber plexus innervated the host DCN. Physical continuity between Pc in the DCN and in the cortex was seen, indicating the possibility that donor Pc establish their axonal contacts in the DCN and then move to their final cortical locality, thus recapitulating a migratory path normally taken during embryonic development. It appears, therefore, that DCN may be an important site for Pc implantation both from the ontogenetic and pathophysiological standpoint.

8.10

ISOCHRONIC TRANSPLANTATION OF NEONATAL GRAFTS IN THE VISUAL CORTEX OF CATS: RESPONSIVENESS, OCULAR DOMINANCE AND SPECIFICITY OF THE CELLS TO VISUAL STIMULATION. U.Yinon and S. Gelerstein*. Physiol. Lab., Goldschleger Eye. Res. Inst., Tel-Aviv Univ. Fac. Med., Sheba Med. CTR, Tel-Hashomer, 52621, Israel.

Neonatal isochronic cortical transplants were studied physiologically in order to reveal possible functional integration within the host cortex. Extracellular unit recording was made in cortical areas 17 and 18 of 3 homograft cats with transplants between brothers (N=225 cells), 4 cats with reimplanted autografts (N=133), and pseudograft controls with analogous sectioning (3 operated kittens, N=173; 5 adults, N=204). Cellular activity was indicated by typical bursts of action potentials in the transplanted sites and in the host tissue. Visual responsiveness in the transplanted sites was the highest in the adult pseudografts (>60%) and the smallest in the homografts (<20%). Ocular dominance distribution of cells showed the highest performance (similarity to the host tissue) in the kitten autografts and the lowest in the homografts. Orientation nonspecificity was the least in the adult pseudografts (<5%) and the most in the kitten autografts (>35%) while directional specificity showed no consistency in the various cats. It is concluded that cells in the transplanted site of the visual cortex showed partial functional recovery; their exact proportions, however, will be known after determining anatomically the boundaries of the transplant.

8.11

VASOPRESSIN NEURONS IN TRANSPLANTED PARAVENTRICULAR (PVN) AND SUPRACHIASMATIC (SCN) NUCLEI ESTABLISH DISTINCT SETS OF EFFERENT CONNECTIONS WITH THE HOST BRAIN. S.J. Wiegand and D.M. Gash. Dept. of Neurobiology and Anatomy, University of Rochester Medical Center, Rochester, NY 14642.

Vasopressin-immunoreactive (VPir) neurons in transplanted fetal hypothalami exhibit several distinct morphological phenotypes. In particular, two types of parvicellular VPir neuron are often present in significant numbers, one of which develops from the anlage of the SCN and the other from the PVN. Previous work with grafts of whole anterior hypothalamus indicated that these morphologically distinct types of VPir neurons established discrete sets of efferent connections with the host brain. The present study was designed to confirm and extend these observations by separately transplanting microdissected PVN and SCN.

Donor tissue was obtained from normal Long Evans rat fetuses (E15-17), and the SCN or PVN from one donor was transplanted to the periventricular hypothalamus of an adult, VP-deficient Brattleboro host. Developing neocortex served as a tissue control. Host animals were sacrificed 6-12 weeks after transplantation; viable grafts were recovered in all cases. All SCN grafts contained VPir cells that resembled those of the endogenous SCN and which projected to areas of the host periventricular diencephalon that ordinarily are innervated by this nucleus. The subparaventricular zone, thalamic paraventricular nucleus and host SCN were especially likely to be densely innervated by SCN grafts. Conversely, transplanted PVN contained VPir neurons that resembled the parvicellular neurosecretory component of this nucleus. VPir neurons in PVN grafts projected to vasculature in the graft and host brain, and also to a well defined set of neural targets that were largely distinct from those innervated by SCN grafts. The medial and dorsal preoptic area, and the rostral paraventricular nucleus of the host brain were most consistently innervated by PVN grafts. Fewer VPir fibers were found in the periaqueductal gray, lateral hypothalamus and basal forebrain areas (especially the bed nucleus of the stria terminalis).

Supported by NIH grant NS 19900.

8.12

MAJOR HISTOCOMPATIBILITY ANTIGEN EXPRESSION AND CELLULAR RESPONSE IN SPONTANEOUS AND INDUCED XENOGRAFT REJECTION. I. F. Pollack*, R. D. Lund and K. Rao. (SPON: E. M. Nemoto). Department of Neurobiology, Anatomy, and Cell Science, Univ. of Pittsburgh, Sch. of Med., Pittsburgh, PA 15261

Embryonic mouse retinal transplants placed in the midbrains of neonatal rats generally survive for prolonged periods without immune suppression. Only 10 - 20 % of such grafts undergo "spontaneous" rejection, however, rejection can be induced by skin grafting, host eye removal (producing optic tract degeneration), and disruption of the blood-brain barrier with mannitol. In this study, the pattern and time course of major histocompatibility (MHC) antigen expression and the nature of the cellular response in each of these four rejection models were examined in detail histologically and immunohistochemically.

Embryonic CD-1 mouse retinal grafts (E13-14) were implanted in the dorsal mesencephalon of neonatal Sprague-Dawley rats. One month later, host rats received skin grafts, eye lesions, or intracarotid mannitol infusion. Control animals (for evaluation of spontaneous rejection) received none of these sensitizing stimuli. After appropriate survival times, fixation, and tissue preparation, adjacent sections were stained with cresyl violet, OX-6 (for MHC class II), OX-18 (for MHC class I), OX-42 (for microglia), W3/25 (for reactive microglia), anti-lymphocyte antibody, and GFAP (for astrocytes), and examined by light microscopy.

Spontaneous rejection (8 animals) was accompanied by diffuse expression of class I and II antigens (Ags), and widespread infiltration of inflammatory cells throughout the transplant and in the surrounding brain well rostral and caudal to the graft. Skin graft-induced rejection (16 animals) produced a much more localized expression of MHC Ags and infiltration of inflammatory cells that involved only the transplant itself and a small rim of surrounding host brain associated with a profound degree of graft necrosis. After eye removal (18 animals), prominent class I and II Ag expression was seen at 7 days in the optic terminal area of the superior colliculus, within the projections of the graft to the colliculus, and around the graft itself, well in advance of any lymphocytic infiltration. With longer survival times, widespread MHC Ag expression associated with astrocytosis, microgliosis, and perivascular cuffing by lymphocytes, were found within the graft, the optic terminal region, and the surrounding colliculus. Involvement of distant areas of the brainstem, typical of spontaneous rejection, was not seen. Following mannitol infusion (8 animals), widespread MHC Ag expression and cellular infiltration were seen, closely resembling spontaneous rejection. These results suggest that the pattern of MHC Ag expression and cellular infiltration associated with rejection is critically dependent on the mechanism by which graft rejection is induced.

8.13

AN ANALYSIS OF IMMUNE RESPONSE TO CELLULAR AND ACELLULAR NERVE ALLOGRAFTS AND THEIR USE IN NERVE REPAIR. A.K. Gulati* (Spon: T.A. Harrison). Department of Anatomy, Medical College of Georgia, Augusta, GA. 30912.

The present study was designed to determine the immunogenicity of acellular basal lamina allografts and their potential as bridging material for nerve gap repair. Inbred strains of Fischer and Buffalo rats were used. Acellular grafts were prepared by repeated freezing and thawing of 6 week in situ predegenerated nerve. Non-frozen predegenerted nerves were used as cellular grafts for comparison. Fischer rats served as hosts and received 2 cm long cellular or acellular isografts (genetically identical) and allografts (genetically different). Grafts were morphologically evaluated at 1,2,4 and 12 weeks after transplantation for rejection and their ability to support regeneration. The cellular isografts supported axonal regeneration best. The cellular allografts were invariably rejected. Acellular allografts, in spite of their mild immunogenicity were successful in supporting regeneration, as were the acellular isografts. The rate of host axonal regeneration and recovery of target muscle was reduced in acellular allografts and isografts as compared to cellular isografts. It is concluded that acellular allografts exhibit reduced immunogenicity and are suitable for supporting axonal regeneration and may be used to bridge gaps in injured peripheral nerves.

(Supported by NIH grant NS24834)

CELL LINEAGE AND DETERMINATION I

9.1

USE OF DiI TO TRACE THE MIGRATION OF NEURAL CREST-DERIVED CELLS TO THE BOWEL H.D. Pomeranz, T.P. Rothman, and M.D. Gershon. Department of Anatomy and Cell Biology, Columbia University, P & S, New York, NY 10032.

Although the enteric nervous system (ENS) has been shown in avian embryos to be formed by emigrés that migrate to the bowel from the neural crest, the axial levels from which these precursors originate is controversial. Experiments with chick-quail chimeras have suggested that both the vagal and sacral crest contribute precursors to the gut. In contrast, the use of morphological markers to study enteric neuronal development *in situ* and in explants of short segments of bowel has failed to confirm a sacral origin of enteric crest-derived cells. The non-deleterious fluorescent probe, DiI, was therefore employed to trace the migration of crest cells by means that do not involve the construction of chimeras. DiI (2.5% in 100% ethanol) was injected with pressure through a micropipette (tip diameter ≅ 10 µm; 1-2 pulses [100 msec] of N_2 at 8 psi) into the neural crest of chick embryos (10 to 28 somites). The embryos were incubated for 24-96 hrs post-injection. DiI-labeled cells were found in the region of the injection site and also in distant locations. Injections into the vagal crest led to the appearance of DiI-labeled cells in the bowel. Some of these cells were round or polygonal, while others had the appearance of neurons with varicose fluorescent nerve fibers. The fluorescent cells were located in the outer gut mesenchyme. Injections of thoracic or lumbar crest did not label cells in the bowel; however, DiI-labeled cells were found in the sympathetic and dorsal root ganglia, peripheral nerves, adrenal glands, and underneath the epidermis. These observations indicate that microinjections of DiI into the neural crest can be used to identify the specific levels of origin of crest-derived cells in target organs, such as the gut. Experiments involving the injection of DiI into the neural crest caudal to somite 28 are now in progress to test the hypothesis that the sacral crest contributes to the formation of the ENS.

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9.2

DEVELOPMENTAL POTENTIAL OF TRUNK NEURAL CREST CELLS IN SITU. Marianne Bronner-Fraser and Scott E. Fraser, Devel. Biol. Center, U.C. Irvine, Ca. 92717

Trunk neural crest cells migrate extensively and contribute to diverse cell types including cells of the sensory and autonomic nervous system. To analyze the developmental potential of individual neural crest cell precursors, we have microinjected a vital dye, lysinated rhodamine dextran (LRD), into the dorsal neural tube. Previously, we demonstrated that some trunk neural crest cells give rise to descendants in multiple derivatives (Bronner-Fraser and Fraser, 1988, Nature 335:6186). Here, we examine the phenotypes derived from LRD-labelled cells using neurofilament expression as a marker for neurons. By two days after injection, the LRD-labelled clones contained from 2 to 73 cells, which were distributed bilaterally in the majority of embryos, and extended over 20 to 530 microns in the rostro-caudal dimension. Individual labelled precursors gave rise to both sensory and sympathetic neurons (neurofilament positive), non-neuronal cells (neurofilament negative), and cells with the morphological characteristics of Schwann cells or pigment cells (neurofilament negative). Furthermore, in some cases, a single labelled precursor contributed to both neural crest- and neural tube-derived neurons. This suggests that the neural crest is not a presegregated population, but shares a common lineage with some neural tube cells. Several LRD-labelled clones, including three derived from emigrating neural crest cells, contributed only to neural crest derivatives; many of these clones were multipotent. Our data demonstrate that premigratory and emigrating trunk neural crest cells can give rise to multiple neural crest derivatives, and can contribute to both neuronal and non-neuronal elements within a given structure. (Supported by USPHS HD-25138 and BNS 8608356).

9.3

WITHDRAWN

9.4

RELATIONSHIP BETWEEN TRANSLOCATING PITUITARY PRECURSOR CELLS AND BRAIN CELLS IN THE EXPRESSION OF POMC mRNA IN EMBRYONIC FROG: W.P. Hayes and Y.P. Loh. Laboratory of Developmental Neurobiology, NICHD, Bethesda, MD 20892.

To determine the sequence of events involved in the activation of the proopiomelanocortin (POMC) gene in brain and pituitary, the cells that first express POMC were identified in Xenopus laevis embryos using *in situ* hybridization. Intronic and exonic 48-mer oligonucleotide probes encoding regions of the Xenopus POMC gene (Martens, G., Eur. J. Biochem. 165: 467, 1987) were used to differentiate primary transcript RNA from mRNA.

POMC gene expression was first seen at developmental day 1.3 (Stage 28) in cells of the embryonic stomodeal-hypophyseal plate. In 1.5 to 2 day old embryos (Stage 29/30 to 33/34), this entire structure, which is homologous to Rathke's pouch of amniotes, was labeled by exonic, but not intronic, probes. In frog, these cells undergo a poorly understood process translocating caudally from the oral ectoderm between the brain and foregut. By day 2.3 (Stage 37/38), they near the notochord, where they are presumed to form the anterior and intermediate lobes of the pituitary. It is now clear from these *in situ* findings that many pituitary precursor cells are already differentiated as they pass in proximity to the developing brain. Labeling in neural tube showed that cells are expressing POMC in the diencephalon by Stage 31. These cells were in the most ventral part of the tube just adjacent to the labeled hypophyseal plate. More labeled cells were seen in the dorsolateral part of the ventral diencephalon at Stages 32 and 33/34. These cells appeared to radiate from the point at which the hypophyseal plate made its closest pass with the embryonic brain.

The possibility that the onset of POMC gene expression in brain is somehow dependent on interactions with these hypophyseal cells is being explored. (W.P.H. is supported by an Associateship from the National Research Council.)

9.5

ENVIRONMENTAL CUES INFLUENCE DIFFERENTIATION OF RAT RETINAL GERMINAL NEUROEPITHELIAL CELLS. T.A. Reh, Dept. Medical Physiology, Univ. Calgary, Calgary, Alta, CANADA T2N 4N1.

Previous studies in amphibians and fish have shown that local environmental cues play a role in determining the types of neurons produced by the retinal germinal neuroepithelial cells. We have devised an in vitro strategy to demonstrate that the same processes are involved in regulating neurogenesis in the mammalian retina. By co-culturing E15 rat retinal cells, labelled with H3-thymidine, and retinal cells from various postnatal ages, we can determine whether the postnatal cells can influence the types of neurons that the E15 germinal cells differentiate into. We have found that the E15 germinal cells can be induced to form rods when co-cultured with P1 and P3 cells, but not when cultured alone or with P11 cells. Thus, we conclude that the local environment plays a role in determining the phenotypic expression of germinal neuroepithelial cells during rat retinal development. Supported by MRC and Retinitis Pigmentosa. T.A.R is a Sloan Fellow and an AHFMR Scholar.

9.6

Determination of total segment number in the leech. Katerina Markopoulou* and G.S. Stent. Dept. of Molecular and Cell Biology, University of California, Berkeley, CA 94720.

The ectodermal and mesodermal lineages in the leech derive from five bilateral pairs of blastomeres called teloblasts. Each teloblast divides asymmetrically giving rise to a bandlet composed of several dozen primary blast cells. The five bilaterally paired bandlets form the germinal bands, which later coalesce along the ventral midline to form the germinal plate. Each teloblast produces more blast cells than are needed to form the 32 segments of the mature leech. These supernumerary cells do not become incorporated into the germinal plate and degenerate. Previous work (Shankland, M. Nature, 307: 541-543, 1984) has shown that positional signals are probably necessary for the correct termination of blast cell incorporation into the germinal plate. One hypothesis regarding these signals maintains that a progressive disparity in relative developmental age between the various blast cell lineages serves as an indicator of position along the longitudinal embryonic axis, which determines total segment number. The results of preliminary experiments aimed at disturbing the relative developmental age between the bandlets by causing a delayed production of blast cells in one of the lineages appear to support this hypothesis. The injection of the polypeptide antibiotic mithramycin, a non-specific transcription inhibitor, into a teloblast retards blast cell production, which in turn results in the formation of an abnormal number of segments. A variety of other abnormalities are also observed, depending on the blast cell lineage whose production was delayed, such as transfating of elements normally derived from one lineage (o) into elements normally derived from another lineage (p), and changes in the number and position of identified neurons normally derived from a predominantly neural lineage (n). These findings suggest that determination of segment number may be associated with segment-specific deflection of a general metameric developmental pathway.

9.7

CONTROL OF NEURONAL FATE BY THE HOMEOTIC GENE *MAB-5* IN THE NEMATODE *C. ELEGANS* C. M. Loer and C. K. Kenyon*. Dept. of Biochem. and Biophysics, Univ. of California, San Francisco, CA 94143.

The gene *mab-5* is required to determine posterior-specific cell fates in neuronal, epidermal, and mesodermal cells in the posterior body of the nematode *C. elegans* (Kenyon, 1986, Cell 46: 477). For example, cells V5 and V6 divide postembryonically to produce neuronal sensilla called rays, whereas their anterior homologs, V1-V4, produce epidermal seam cells. Loss of *mab-5* activity causes V5 and V6 cells to divide and differentiate like V1-V4. Mutations in the gene *lin-22* cause the opposite changes in cell fate: V1-V4 produce rays instead of seam cells. Varying the dosage of wild-type *mab-5* and *lin-22* can shift the boundary between anterior and posterior cell fates, suggesting that these genes may mutually inhibit one another. The gene encoding *mab-5* has been cloned and partially sequenced (Costa et al., 1988, Cell 55: 747). The sequence suggests that *mab-5* may act by regulating other genes: it encodes a homeodomain, a putative DNA-binding motif, in which 44 of 60 amino acids are identical to those of the homeodomain of *Antennapedia* from *Drosophila*. *In situ* hybridization with *mab-5* cDNA probes reveals that expression of *mab-5* RNA is restricted to the posterior of larvae (Costa et al., *ibid.*) and embryos. We will test whether the expression of *mab-5* message is altered in *lin-22* mutants. If wild-type *lin-22* gene product acts to restrict *mab-5* to the posterior, then *mab-5* expression is predicted to extend anteriorly in *lin-22* mutant worms. We also plan to examine the expression of *mab-5* protein during development of wild-type and pattern-formation mutant worms. We have generated a *ß-gal/mab-5* fusion protein that will be used to produce antisera to the *mab-5* protein.

9.8

GENERATION OF ENTERIC NEURONS FROM AN EPITHELIAL PLACODE DURING INSECT EMBRYOGENESIS P.F. Copenhaver and P.H. Taghert. Department of Anatomy & Neurobiology, Box 8108, Washington University School of Medicine, St. Louis, MO 63110.

We have been examining the program of neurogenesis that gives rise to the Enteric Plexus, a discrete region of the Enteric Nervous System of the moth, Manduca sexta. The Enteric Plexus spans the foregut-midgut boundary and contains approximately 400 neurons (the EP cells) of diverse morphological and biochemical phenotypes, including one class that expresses peptides related to the molluscan peptide FMRF-amide. Previously we have shown that during embryogenesis, these neurons achieve their mature distributions by a stereotyped sequence of migration, and that the delayed expression of peptidergic phenotype is regulated by post-migratory cellular interactions. We have now characterized the neurogenic events that precede the onset of migration, and we have analyzed the mitotic relationships giving rise to this neuronal population. All of the EP cells arise from a common placode in the dorsal lip of the foregut epithelium; this placode first appears at 30% of embryogenesis and subsequently evaginates onto the dorsal foregut surface (between 30-40%) to form a discrete packet of several hundred premigratory cells. During this process, columnar epithelial cells cease to be mitotically active, lose their dye coupling with adjacent cells, and round out of the epithelium. Labels for DNA synthesis activity and intracellularly injected lineage tracers indicate that individual epithelial cells undergo a limited number of symmetric divisions prior to their extrusion onto the foregut surface. However, the vast majority of EP cells appear post-mitotic during their subsequent migration and differentiation. Experiments using an in vitro culture preparation have shown that these events can proceed even when the foregut epithelium has been isolated prior to the onset of placode evagination. We are currently studying the role of cell lineage in the differentiation of these placode-derived neurons. Supported by an NIH postdoctoral fellowship to PFC (F32NS07957) and NIH grant #NS21749.

9.9

MULTIPOTENT NEURAL CELL LINES, GENERATED VIA RETROVIRAL-MEDIATED GENE TRANSFER, INTEGRATE IN VIVO FOLLOWING TRANSPLANTATION INTO DEVELOPING MOUSE CEREBELLUM (CB). E.Y.Snyder*, D.L.Deitcher*, C.Walsh, C.L.Cepko*, Dept. of Genetics, Harvard Medical School, Boston, MA 02115.

We previously reported generation of immortalized neural cell lines via retroviral-mediated v-myc transfer into neonatal mouse CB cells (Soc.Neurosci.Abstr.13:700,1987). Infection established families of clonally-related lines with neuronal-glial multipotency, some lines evincing heterogeneity & plasticity of phenotype. We now report that, when transplanted into neonatal mice, these lines become integrated into the developing CB. Cells from a given line were marked by infection in vitro with virus containing the lacZ reporter gene. Concentrated helper-free cell suspensions were transplanted into P1 mice. After 9-22 mos., sections of CB, processed to locate labeled cells, revealed integration of transplanted cells in a cytoarchitectonically appropriate, nontumorigenic manner. In some animals, 5-10x10^3cells spanning 1.7mm were incorporated. Furthermore, lines which were multipotent in vitro retained their multipotential nature in vivo within the same animal. Some cells migrated to the internal granular layer & possessed neuronal morphology; others localized to the molecular layer & possessed glial morphology. These lines may have potential for repair or for gene transport into CNS. Future work will establish the functional integrity of these transplanted cells, the extent of their temporal & anatomic plasticity, & their ability to reconstitute deficits in mutant mice.

9.10

NEURONAL LINEAGES IN CHIMERIC FOREBRAIN ARE COMPARTMENTALLY SEGREGATED. G. Fishell, J. Rossant*§, and D. van der Kooy Neurobiology Research Group, Department of Anatomy and §Sinai Hospital Research Institute, Department of Medical Genetics, University of Toronto, Toronto, Ontario, Canada, M5G 1X5.

The mammalian forebrain is comprised of two major structures, the cortex and the striatum. These structures can be further subdivided developmentally and biochemically into compartments. The cortex can be subdivided into deep versus superficial layers and the striatum into patch versus matrix compartments. Interspecific chimeric *Mus musculus/ Mus caroli* mice were used to determine the contribution of lineage to cellular position within these forebrain compartments. Cells from either strain could be unambiguously identified in adults using *in situ* hybridization with a satellite DNA probe specific to the *Mus musculus* strain. Qualitative analysis did not reveal any obvious segregation of the ratio of genotypes among forebrain compartments. In contrast, a statistical analysis revealed evidence of both spatial and compartmental lineage segregation. A significant difference depending on chimeric specimen was observed between areas (regardless of compartment) that were separated by greater than 300 μm in the rostrocaudal plane. Perhaps most remarkably, differences were observed between early born (striatal patch and deep cortex) and late born (striatal matrix and superficial cortex) neurons, but not between cortex and striatum, as a whole. On a smaller scale of analysis, differences in genotype ratios were seen between radially aligned deep and superficial cortical areas, in both the neuronal and glial populations. This evidence supports the existence of early lineage restrictions to time of neurogenesis and thus to forebrain compartment.

9.11

EXPRESSION OF A NEURONAL PHENOTYPE BY PANCREATIC ISLET CELLS OF ADULT MOUSE *IN VITRO*. G. Teitelman and M. Moustakos, Neurobiology, Cornell U. Med. Coll., NY, NY 10021

Pancreatic islet cells of embryonic and adult mouse contain the catecholamine biosynthetic enzyme tyrosine hydroxylase (TH) and several other neuronal specific antigens. In this study we sought to determine whether endocrine cells of pancreas can express not only neuronal markers but also a neuronal morphology. Pancreatic islets from adult white outbred (CD-1) mice were dissociated into single cells and maintained *in vitro* in culture media [90% RPMI 1640, 10% fetal calf serum (Gibco), 20mM Hepes buffer, 16.7mM glucose and antibiotics]. Two weeks later cells were fixed and processed for immunocytochemical staining. Many cells were found to contain insulin (β cells), while others remained unstained. Some stained cells were round or elongated while others formed neuritic-like processes, did not fasciculate, were often branched, and extended long distances in the culture dish. Preliminary experiments indicate that these elongations also stained with neurofilament antisera (gift of P. Levitt). To test the role of cellular interactions on process formation, adult mouse pancreatic islets were isolated, maintained *in vitro* without dissociation and immunostained 2 weeks later. Transplanted islets contained all endocrine cell types characteristic of islets *in vivo* but did not extend processes. Other cells had a neuronal morphology. Presumably, these cells are the pancreatic parasympathetic neurons, since they were found outside the islets and did not contain pancreatic hormones. Our studies, therefore, indicate that β cells of adult mouse pancreas can form neurite-like processes and that this ability is inhibited by normal histotypical associations. Since islet cells arise from endoderm, this suggests that cells originating in non-ectodermal cell layers may express neuron-like biochemical and morphological traits. Supported by NIH #HL 18974 and DK 38171.

9.12

Stage-Specific Mechanisms Regulate Transmitter Phenotype in Developing Rat Sensory Ganglia. D.M. Katz and M.J. Erb*, Dept. Medicine and Center for Neuroscience, Case Western Reserve Univ., School of Medicine, Cleveland, OH 44106.

A subpopulation of petrosal ganglion (PG) sensory neurons express catecholaminergic properties, including functional tyrosine hydroxylase (TH). Most TH cells are also distinguished by innervation of a single peripheral target, the carotid body (Katz, D.M. and Black, I.B., J. Neurosci., 6:983, 1985). During development, however, two different populations of TH neurons appear in the PG; early cells, in which TH is transiently expressed between embryonic day (E) 11.5 - 15.5, and late cells in which expression begins on E16.5 and is subsequently maintained. To elucidate mechanisms of sensory neuron differentiation, immunocytochemical and tissue culture methods were used to determine whether TH expression is triggered by common mechanisms, including neuron-target interactions, at these two stages of development. In culture, TH expression by the transient population occurs in the absence of peripheral target tissues. In contrast, neuron-target interactions appear important for expression after E16.5. Our data suggest that distinct mechanisms may regulate expression of a single transmitter trait at different stages of sensory ganglion development. Grant PL653 (Am. Heart, OH) and Dysautonomia Fndtn., Inc.

NEUROGLIA

10.1

CULTURED HUMAN OLIGODENDROCYTES ARE SUSCEPTIBLE TO LYSIS BY MHC CLASS I-DIRECTED CYTOTOXIC LYMPHOCYTES. T.C.G. Ruijs*, A. Olivier*, M.S. Freedman*, J.P. Antel. Montreal Neurological Institute,McGill University, Montreal, Quebec, Canada H3A 2B4.

Adult human oligodendrocytes (OGC) in culture express major histocompatibility complex (MHC) class I antigens, suggesting a potential susceptibility to cytolysis by MHC class I restricted cytotoxic lymphocytes. This interaction could contribute to the tissue injury seen in multiple sclerosis, where abundance of activated T-lymphocytes of all phenotypes, and damage to OGC and myelin are found in CNS lesions. We have previously established a ^{51}Chromium release assay with OGC-enriched cultures of adult human glial cells, derived from surgically resected tissue. In this study, lymphocytes were allo-activated with peripheral blood-derived mononuclear cells from the glial cell donors. Activated lymphocytes exhibited a mean specific lysis of labelled OGC of 30% (n=4, range 19-42% at a 10:1 effector:target ratio). Lysis by the CD8+ (MHC class I restricted) subset exceeded lysis by CD4+ (MHC class II restricted) cells and could be partially blocked by the anti-MHC class I antibody W6/32. Lymphocytes activated against mononuclear cells bearing MHC class I epitopes discordant with the glial cell donor's MHC class I, showed significantly less cytotoxicity (mean 12%, range 8-15%, $p < .02$).

10.2

PROLIFERATION OF MATURE ASTROCYTES IN RESPONSE TO TUMOR NECROSIS FACTOR. K.W.Selmaj*, M.Farooq*, W.T.Norton, C.S.Raine and C.F.Brosnan*.(SPON: DC Miller) Dept. of Path., Albert Einstein Coll. Med. Bronx, NY 10461.

The effect of cytokines on primary cultures of mature bovine astrocytes was determined in chemically-defined medium with or without the addition of serum. The results show that in serum-free medium human tumor necrosis factor (TNF) and, to a lesser degree lymphotoxin (LT) and interleukin-6 (IL-6), are mitogenic for astrocytes. Uptake of [^{3}H]-thymidine could be detected within 36h in vitro. In contrast neither IL-1α nor IL-1β induced astrocyte proliferation in serum free medium. The proliferative effect of TNF and LT was also observed in serum-containing medium and was confirmed by autoradiography and cell counting. Analysis of mRNA for GFAP by Northern blotting demonstrated a complex response that was both dose- and time-dependent. None of the cytokines tested was toxic for astrocytes as measured by ^{51}chromium release. No mitogenic effect for oligodendroglia, purified from the same source, was detected. The results support a role for TNF and LT in the development of reactive gliosis such as that found in diseases like multiple sclerosis (MS). Supported by NS 11920 and NMSS 1089.

10.3

DISTINGUISHING CELLS FROM THE MAMMALIAN NERVOUS SYSTEM BY PROTON NMR SPECTROSCOPY. R.Small, D.Gadian*,P.Patel*, N.Van Bruggen* and S.Williams*. Institute of Neurology and Royal College of Surgeons of England, London WC1N 3BG, UK.

Proton NMR spectroscopy provides a sensitive non-invasive method for analysing localised regions of brain. Signals obtained include lactate, N-acetyl-aspartate(NAA), choline-containing compounds(Cho) and creatine+phosphocreatine(Cr). Abnormal ^1H spectra have been reported in patients with CNS disorders involving neuronal degeneration and/or proliferation of non-neuronal cells. To understand the processes underlying such alterations, we have examined the ^1H spectra of purified astrocytes, oligodendrocytes, meningeal cells and types of neurons. After assessing samples purity by immunostaining with specific antibodies, cells were washed in PBS and extracted into 12% perchloric acid. Each cell preparation showed a characteristic ^1H profile, marked either by the presence of unique peaks or by distinct concentration ratios between compounds. Cortical astrocytes, for example, showed a Cho/Cr ratio of 0.49±0.07 and no detectable signal from NAA. This ^1H spectra from astrocytes is remarkably similar to ^1H spectra recently reported from astrocytomas (Gadian et al, Soc. Magn. Reson. 1989) showing elevated Cho/Cr ratios, and no NAA signals. Different neuronal-types could also be distinguished by their NMR profiles. These data suggest that in vivo NMR offers a powerful technique for examing alterations in cellular constituents during CNS diseases.

10.4

MICROGLIAL CELLS IN THE RABBIT RETINA AND THEIR RESPONSES FOLLOWING GANGLION CELL DEGENERATION. Jutta Schnitzer* and Jürgen Scherer. MPI für Hirnforschung, Abt. Neuroanatomie, D-6000 Frankfurt/M. 71, F.R.G.

Microglial cells are thought to be involved in phagocytosis of cellular debris when cell death occurs during ontogenesis. The intension of this study was to see whether and how microglial cells would respond to transection of the optic nerve of adult rabbits, which is known to lead to retrograde degeneration of ganglion cells in the retina.

Microglial cells have been studied by using the enzyme-histochemical method for nucleoside diphosphatase (NDPase). In the normal retina, regularly scattered NDPase-positive microglial cells were found throughout the inner plexiform layer (IPL). Their radially oriented processes gave them a star-shaped appearance, processes of individual cells did not overlap. In the nerve fiber layer (NFL), microglial cell density was highest at and close to the medullary ray region, but low in the peripheral retina. Two days after transecting the optic nerve, the number of NDPase-labeled cells began to increase in the NFL as well as in the IPL. This increase continued with longer survival times. Microglial cell density was considerably higher after four weeks compared to controls. Although their density is higher in the IPL of the degenerating retina, each microglial cell still occupies its own territory, which is smaller compared to controls. It is concluded that microglia are involved in processes accompanying ganglion cell death, their particular role needs further studies.

10.5

INDUCTION OF MYELIN SCHWANN CELL PHENOTYPE IN VIVO AND IN VITRO. R. Mirsky*, K.R. Jessen* and L. Morgan*. (SPON: Brain Res. Assoc.) Dept. Anatomy, Univ. Coll. London

The two mature Schwann cell phenotypes seen in mature peripheral nerves, myelin-free and myelin Schwann cells, arise from a common early Schwann cell. Axonally induced developmental events occur in both pathways. The molecular differences between the two mature Schwann cell types all result, however, from axonally regulated events restricted to the myelination pathway. Thus the myelin-free pathway is constitutive. The development of the myelin Schwann cell requires not only induction of myelin proteins but also down-regulation of proteins e.g. N-CAM, A5E3, 217c(Ran-1) and GFAP, associated with the constitutive myelin-free pathway. Schwann cells in short term cultures which express proteins typical of the constitutive pathway, can be induced to up-regulate the myelin protein P_0 and down-regulate N-CAM, A5E3, 217c and GFAP after treatment with cAMP analogues or cholera toxin for a total of 3 days. In induced cells, P_0 is expressed in all areas of the Schwann cell membrane. Furthermore, cells expressing high levels of P_0 show down-regulation of the proteins GFAP, 217c, N-CAM and A5E3. Thus a series of molecular events related to induction of myelin Schwann cells in vivo can be reproduced in early Schwann cell cultures.

10.6

NEUROGLIAL CELL LINEAGE: IN VIVO AND IN VITRO DIFFERENCES. R.P. Skoff, P.E. Knapp and M.S. Ghandour. Dept. of Anatomy and Cell Biology, Wayne State University School of Medicine, Detroit, MI 48201.

Astroglial and oligodendroglial (OL) specific antibodies have been used to trace the lineage and differentiation of macroglia in vivo and in vitro. Our work has shown the following differences between the glial lineages in rodent CNS. Immunocytochemistry was combined with ^3H-thymidine autoradiography or bromodeoxyuridine (BdU) immunostaining to determine the origin of OLs in postnatal development. In vivo, one hour after an injection of ^3H-thymidine, galactocerebroside (GC) and sulfatide positive cells are thymidine labeled. The GC+ and sulfatide+, thymidine labeled cells are moderately immunostained, indicating recent expression of these markers. GC is a specific marker for OLs and our preliminary morphologic studies indicate that sulfatide is also specific for OLS in vivo. These findings indicate that cells expressing OL specific markers divide in vivo. In vitro, after a one hour pulse with the thymidine analogue BdU, sulfatide+, BdU+ cells are present but GC+ cells are postmitotic. The in vitro studies support the conclusion that OLs don't divide during development, however, the in vivo studies indicate that they do. Since newly formed OLs in vivo can arise from GC+ OLs, bipotential progenitor cells are not the only source for OLs in postnatal rodent CNS. Supported by NS 15338.

10.7

FURTHER EVIDENCE FOR THE EXISTENCE OF TYPE 2 ASTROCYTES IN VIVO. B.A. Barres, L.L.Y. Chun*, and D.P. Corey, Program in Neuroscience, Harvard Med School; Dept of Neurology, HHMI, Mass Gen Hosp. Boston, MA.

Two types of astrocytes have been described in cultures of rat optic nerve and other white matter; these are morphologically, antigenically, and developmentally distinct (Raff *et al.*, 1983), and also electrophysiologically distinct (Barres *et al.*, 1988; in preparation). In neuron-free cultures, type 1 astrocytes are polygonal and A2B5 negative, while type 2 astrocytes are stellate and A2B5 positive. Two classes of astrocytes are also found in Golgi impregnations and after HRP injections: one with radial processes terminating on blood vessels, the other with longitudinal processes terminating at nodes of Ranvier (Miller, Fulton, and Raff, 1989). They suggested that these longitudinal cells are type 2 astrocytes because they develop only after 2 weeks postnatal and because perinodal astrocyte processes *in vivo* are antigenically similar to type 2 astrocytes *in vitro*.

To determine whether a subclass of astrocytes exists *in vivo* with the electrophysiological properties of the type 2 astrocytes in culture, we recorded from acutely isolated cells in a new preparation called a tissue print. Cells were prepared by gently touching papain-treated optic nerve tissue to nitrocellulose-coated glass coverslips. A thin layer of viable cells--still bearing extensive processes--adhered tightly to the glass. Adherent cells were identified using indirect immunofluorescence. Ionic currents in labelled cells were studied using whole-cell patch recording.

These experiments have revealed the existence of a subclass of astrocytes, arising only after the 2nd week of postnatal life, that express the same complement of ion channels observed in type 2 astrocytes that develop in serum-free culture: Na_N, Ca_T, Ca_L, K_{IR}, K_A, K_{CA}. Consistent with the observations of ffrench-Constant and Raff (1986), these cells are not A2B5 surface positive, nor are any other astrocytes in the tissue prints. These results further confirm the existence of a distinct subclass of astrocytes in white matter.

10.8

GAP JUNCTIONS BETWEEN LEPTOMENINGEAL CELLS: IDENTIFICATION OF CONNEXIN PROTEINS AND mRNAs AND CHARACTERIZATION OF MACROSCOPIC AND SINGLE CHANNEL CURRENTS. D.C. Spray, R. Dermietzel, E.L. Hertzberg*, and J.A. Kessler (SPON: H. Buschke). Depts. Neuroscience & Neurology, Einstein Coll. Med., Bronx, NY 10461

Leptomeningeal (LM) cells form the boundary between the CSF and the extracerebral compartment; they are interconnected by abundant gap junctions, which could function in signal relay and in the regulation of osmotic or ionic balance. We identified immunoreactive gap junction proteins using antibodies specific for the 21 and 27 kDa liver gap junction proteins (connexins 26 and 32), the 43 kDa cardiac gap junction protein (connexin 43) and a 34 kDa antigen localized to junctional regions of brain. Indirect immunofluorescence on cultured LM cells indicated immunoreactive puncta at regions of cell contact as well as intracellularly with antibodies to the 21 and 43 kDa proteins; no binding of the other antibodies was detected. Western blots with anti-43 kDa antibodies demonstrated the presence of crossreacting proteins with mobilities similar to that observed in heart samples. At the EM level, the anti-21 and anti-43 antibodies stain junctional membranes. Freshly dissociated cell pairs from intact tissue or from cultures as old as two weeks were electrically coupled. Junctional conductance (g_j) averaged 8.6 ± 1.1 nS (n= 37) and was reversibly reduced by exposure to 2 mM halothane, 1 mM heptanol or 100% CO_2. Forskolin (10 uM) or 8 Br-cAMP (1 mM) increased, and 1 uM phorbol ester (PDBu) irreversibly decreased g_j. Single channel analysis revealed two classes of unitary events with conductances of about 50 and 80 pS. The 50 pS conductance is comparable to that we have observed from 43 kDa-containing junctions in heart cells; perhaps the 90 pS conductance is due to channels formed with the 21 kDa protein.

10.9

MODULATION OF INTRACELLULAR Ca^{++} IN ASTROCYTES: STUDIES USING FURA-2 AND INDO-1. B.A. MacVicar, S. Weiss and M. Delay, Neuroscience Research Group, University of Calgary, Calgary, Alberta T2N 4N1.

Astrocytes have voltage activated Ca^{++} channels that can be modulated by β-adrenergic stimulation. Primary cultures of mouse cortical astrocytes were loaded using the AM form of indo-1 or fura-2. Intracellular Ca^{++} levels were calculated from fluorescence images of stained cells obtained with a SIT camera and digitized for fura-2 studies or from fluorescence intensities measured using photomultiplier tubes for indo-1 studies. Intracellular Ca^{++} varied widely between cells but averaged 144nM. Perfusion of K^+ (55mM) alone decreased Ca^{++} in most cultures (1-2 weeks old), however perfusing Bay K-8644 (1μM) with K^+ increased Ca^{++}. The Bay K-induced increase was blocked by nifedipine (1μM) indicating that the Ca^{++} channels are of the L-type. Cells from cultures that were pretreated with dibutyryl-cAMP (0.5mM) demonstrated a K^+-induced increase without the addition of Bay K. Met-enkephalin (1μM), which has been reported to inhibit cAMP synthesis in astrocytes, induced a small decrease in Ca^{++} and inhibited the K^+-induced increase in Ca^{++}. These experiments support the hypothesis of an important role for cAMP in modulating Ca^{++} channels in astrocytes.

Supported by the Medical Research Council of Canada.

10.10

Anomalous stimulation of pH regulation by ethylisopropyl amiloride in cultured mammalian astrocytes. G. Boyarsky, B.R. Ransom*, W-R. Schlue, M. Davis, W.F. Boron. Depts. of Cell. and Mol. Physiol. and Neurology* Yale Univ. Sch. of Med., New Haven, CT 06510.

Intracellular pH (pH_i) regulation in the nominal absence of HCO_3^- was studied in single cultured rat astrocytes using the pH-sensitive fluorescent dye BCECF. In other cell types, application of 50μM ethylisopropyl amiloride (EIPA) in HCO_3^--free solution causes an inhibition of Na-H exchange, resulting in either no pH_i change or an abrupt decline. Application of 50μM EIPA to astrocytes caused a triphasic change in pH_i: (1) a rapid pH_i decrease of ~.1 within 20 sec., (2) an alkalinization of ~.6 within 2-3 min., and (3) a slow return to baseline over ~20 min. Similar results were observed with as little as 50 nM EIPA. Unexpectedly, qualitatively similar triphasic responses occured either following removal of external glucose (10.5 mM) or chloride, or following exposure to 100nM arginine vasopressin, 10 μM epinephrine or 10mM lactate, suggesting that some common cellular mechanism may underly this stereotypic pH_i response to such diverse perturbations. Removal of external Na^+, which causes a profound acidification, blocked the pH_i response to addition of EIPA, suggesting that the EIPA-induced alkalinization is Na^+-dependent. In the presence of EIPA, recovery from an acid load (pulsing with 20mM NH_4^+ for ~3min) was ~2-fold greater during the phase-2 alkalinization, suggesting that EIPA stimulated a Na^+-dependent acid-extrusion mechanism. Pulsing with NH_4^+ in the absence of external Na^+ revealed a Na^+-independent recovery which caused pH_i to recover to ~6.3. The subsequent readdition of Na^+ caused pH_i to recover to near the initial level of ~6.8. In the presence of 50 μM EIPA, the Na-independent recovery was only modestly stimulated, whereas the Na-dependent recovery was as much as 4-fold faster, driving pH_i to ~7.1. These results suggest that, in contrast to the effect of EIPA on other cell types, in astrocytes EIPA anomalously stimulates a Na-dependent acid-extrusion mechanism, perhaps Na-H exchange.

10.11

AXONAL MODULATION OF MYELIN PROTEIN mRNA LEVELS IN OPTIC NERVE. G.J.Kidd*, P.Hauer* and B.D.Trapp* (SPON: S.M.Logan) Neurology, Johns Hopkins Univ. Sch. of Med., Baltimore MD, 21205.

Previous studies from this lab have demonstrated that significant expression of myelin protein mRNA by myelinating Schwann cells is dependent on axonal influences (Trapp et al, J.Neurosci., 8:3515, 1988). In this study, we have investigated the effect of axonal transection on myelin protein mRNA levels in the CNS. Optic nerves of 21 day old rats were transected and levels of mRNA encoding proteins of CNS myelin were compared with levels from control nerves at 5,10, 20 and 40 days post transection. Specifically, total RNA was extracted from transected and age matched control optic nerves, slot blotted onto nitrocellulose and hybridized with ^{32}P labeled cDNA probes complementary to mRNA encoding proteolipid protein (PLP) and myelin basic protein (MBP). In transected nerves, PLP and MBP mRNA levels were approximately 90%, 75% and 10% of control values at 5, 20 and 40 days post transection. In situ hybridization studies using ^{35}S labeled cDNA probes detected PLP and MBP mRNA in control and transected nerves at all time points. Compared to control nerves, hybridization signal was significantly decreased in sections of 20 and 40 day transected nerves. The distribution of these mRNAs (PLP- in oligodendrocyte perinuclear regions, MBP- distributed diffusely) was similar in control and transected nerves. In contrast to Schwann cells, therefore, oligodendrocytes continue to express significant levels of myelin protein mRNA following loss of axonal contact.

10.12

STRUCTURE AND EXPRESSION OF PLP mRNA IN THE PNS. M. Sessa*, P.L. Baron*, T. Behrman*, D. Pleasure and J. Kamholz. University of Pennsylvania School of Medicine and Children's Hospital of Philadelphia, Philadelphia, PA 19104

PLP is expressed in Schwann cells (SC), but is not incorporated into PNS myelin. We have characterized two overlapping PNS PLP cDNA clones which contain the entire PLP coding region, and a portion of the 3' untranslated region. The sequence of the PNS PLP mRNA was identical to that previously determined from a rat CNS cDNA except for a 250 bp portion at the far 3' end of the non-coding region. Polymerase chain reaction (PCR), performed on sciatic nerve mRNA and genomic DNA with two sets of synthetic oligonucleotides primers specific for either the CNS or PNS sequence, suggests that alternative splicing within the 3' untranslated region of the PNS PLP mRNA can account for this sequence divergence. In situ hybridization studies, performed on adult rat sciatic nerve and spinal cord sections using PLP cRNA probes, demonstrate PLP transcripts diffusely distributed within the endonerium. This pattern is similar to that found for the extrinsic membrane protein MBP, which is synthesized on free ribosomes. In contrast, PLP mRNA's in spinal cord were found in clusters around oligodendrocyte nuclei. This pattern is similar to that found for the major PNS intrinsic membrane protein PO, which is synthesized on membrane bound ribosomes. These data suggest that PLP message is differentially distributed within myelinating glial cells in the PNS and CNS. Alternative splicing within the 3' untranslated region of the PLP mRNA may, in part, determine the different tissue distributions of the PLP message in Schwann cells and oligodendrocytes.

CALCIUM CHANNELS I

11.1

VOLTAGE-DEPENDENT EFFECTS OF PROSTAGLANDIN D_2 AND HISTAMINE ON CALCIUM CURRENTS IN ADULT RAT SENSORY NEURONES IN CULTURE.

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Prostaglandin D_2 (PGD_2) and histamine (HIS) are inflammatory mediators which increase excitability of sensory neurones - an effect thought to be mediated via an increase in intracellular cAMP levels[1]. We have studied the effect of HIS and PGD_2 on whole-cell voltage-clamped calcium currents (I_{Ca}) in freshly plated adult rat dorsal root ganglion neurones in culture. In about 50% (n=46) of cells either HIS (0.05-10μM) or PGD_2 (0.01-1μM) increased the size of I_{Ca} recorded at command potentials (V_c) near threshold ($\cong$ -40mV) but decreased I_{Ca} near the peak of the IV curve ($V_c \cong$ 0mV). The threshold for activation of I_{Ca} was shifted to more negative potentials. This effect was rapid in onset, not reversed by washing and mimicked by forskolin (1μM). Pretreatment of cells with pertussis (PTX) or cholera toxin (CTX) did not alter the proportion of cells sensitive to HIS or PGD2.

The results show that these inflammatory mediators modulate I_{Ca} in a potential-dependent manner which may be secondary to an increase in intracellular cAMP. This effect may be important in the regulation of transmitter release during neurogenic inflammation as well as in control of sensory neuronal excitability.

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1. Weinreich, D & Wonderlin, W.F. J.Physiol. 394 415-427 (1987)

11.2

BRADYKININ INHIBITS VOLTAGE-DEPENDENT BARIUM CURRENT IN A CLONAL POPULATION OF DORSAL ROOT GANGLION X NEUROBLASTOMA HYBRID CELLS.

L.M. Boland, A. Allen, and R. Dingledine, Curr. Neurobiology, Dept. Biochemistry, Dept. Pharmacology, University of North Carolina, Chapel Hill, NC, 27599.

Currents through voltage-dependent Ca channels in a rat DRG cell line, F-11, were studied using the whole-cell patch clamp technique. Inward currents were carried by 30 mM Ba. An internal Cs solution containing 100 μM GTP and Ca was buffered to 15 nM. F-11 cells have sustained and transient Ba currents, both of which have multiple components based on sensitivity to Cd, Ni, and omega-conotoxin. Both currents were larger when Ba rather than Ca carried the current. Since the F-11 cell line is heterogeneous, we isolated more homogeneous subclones for use in biochemical and electrophysiological experiments. The F11-B9 subclone does not express the sensory neuron antigens SSEA-3, SSEA-4, nor the antigen recognized by B23D8. Bradykinin (BK, 100 nM) reduced the whole-cell Ba current in 73% of the F11-B9 cells. In most cells, block of the sustained was more prominent (39 ± 4% block) than that of the transient current (12 ± 4% block). The block began within 5 sec. of BK application and was maximal within 10 sec. F11-B9 cells were prelabelled with 3H-arachidonic acid and then stimulated with 100 nM BK. An 8 sec. exposure to BK produced nearly a 3-fold increase in diacylglycerol but only a small increase in arachidonic acid release. Current studies are investigating the possible role of diacylglycerol in regulation of the Ba currents by BK.

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11.3

BRADYKININ MODULATION OF CALCIUM CURRENTS AND $[Ca^{2+}]_i$ IN RAT DORSAL ROOT GANGLION NEURONES IN VITRO. David Bleakman*, Stanley A. Thayer and Richard J. Miller. Department of Pharmacological and Physiological Sciences, University of Chicago, Chicago, IL 6063.

In dorsal rat ganglion (DRG) neurones, the nonapeptide bradykinin (BK) has been implicated as a modulator of nociceptive information. In the present study we have made simultaneous measurements of both calcium currents (I_{Ca}) and intracellular calcium concentrations ($[Ca^{2+}]_i$) from primary cultures of rat DRG neurones using a combined patch clamp/fura-2 microfluorimetry technique. BK (100nM) reduced both the I_{Ca} and the subsequent rise in $[Ca^{2+}]_i$ evoked at 0mV from a holding potential of -80mV in 22 of 32 cells examined (Whole-cell patch clamp with (in mM) 140 Cs, 1 Mg, 10 HEPES, 3.6 ATP and an ATP regenerating system in the pipette and 140 TEA , 2 Ca and 1 Mg bathing the neurones). BK was more effective in inhibiting I_{Ca} than the rise in $[Ca^{2+}]_i$ for longer depolarization steps. In 6 of 32 cells tested, BK (100nM) additionally produced a transient increase in basal $[Ca^{2+}]_i$ (1.7 +/- 0.4 fold, n=6) presumably due to BK-stimulated IP3 release.

In current clamp studies, repetitive action potentials were evoked in DRG cells by short duration injection of current and the concomitant rise in $[Ca^{2+}]_i$ measured (Whole-cell patch clamp with (in mM) 144 K, 1 Mg, 10 HEPES , 3.6 ATP and an ATP regenerating system in the pipette and 140 Na, 2 Ca in the bathing solution). BK (100nM) reduced the rise in $[Ca^{2+}]_i$, its effect being more pronounced for shorter spike trains. The resting membrane potential of the DRG cells ranged between -50 and -70 mV. With the resting membrane potential set to approximately -60mV we also observed that BK (100nM) depolarized 69% of cells tested from a membrane potential of -61.7 +/- 1.1 to 51.1 +/- 2.6 mV (n=13).

Thus the actions of BK on the peripheral processes of DRG neurones in situ would reduce $[Ca^{2+}]_i$ and the Ca^{2+} -activated K+ and Cl- currents which normally serve to limit the excitability of these cells. These effects, together with BK-induced depolarization may be responsible for BK-induced excitation in sensory neurones.

11.4

PHORBOL ESTERS INCREASE UNITARY CALCIUM CHANNEL ACTIVITY IN CULTURED HIPPOCAMPAL NEURONS. Daniel V. Madison, *Department of Molecular and Cellular Physiology, Stanford School of Medicine, Stanford, CA 94305-5426.*

Phorbol esters, activators of protein kinase C, modulate the activity of several ion conductances in hippocampal neurons and cause potentiation of excitatory synaptic transmission in hippocampal slices. We have been investigating mechanisms by which this potentiation might occur. In this study we show that application of phorbol esters causes an increase in the unitary activity of voltage-dependent calcium channels in cultured hippocampal CA3 neurons, recorded with cell-attached patch clamp methods. In preliminary analysis, this increase in activity appears to consist of an increase in both the number of openings and the average length of openings evoked by a depolarization. Phorbol ester appears to affect both the N- and L-types of calcium channel in similar ways. This effect is similar to that recorded in frog sympathetic neurons (Lipscombe et. al.). In these neurons, phorbol ester increases unitary calcium channel activity in cell-attached patches, but decreases I_{Ca} in dialyzed whole-cell recordings. Thus, is interesting that in hippocampal cell, phorbol ester also decreases I_{Ca} in whole-cell recordings (Doerner, et. al). This suggests that dialyzed cells may lack a cytosolic component necessary to express the phorbol ester-induced increase.

11.5

CHANGES IN RAPID GATING KINETICS UNDERLYING NOREPINEPHRINE INHIBITION OF UNITARY N-TYPE Ca CHANNEL ACTIVITY IN SYMPATHETIC NEURONS. R.W. Tsien & D. Lipscombe. Dept. Mol. Cell. Physiol., Stanford CA 94305.

Norepinephrine (NE) inhibits Ca channels and transmitter release in frog sympathetic neurons via interaction with an α_2-like receptor linked to the channel by a G-protein. Our previous recordings of whole cell Ca channel currents indicated that α-stimulation selectively inhibits N-type Ca channels. We now describe the inhibitory effect of NE at the single channel level. Cell-attached patch recordings (110 mM Ba) with 10-100 μM NE in the pipette were compared to drug-free patches. Activity of N-type and L-type Ca channels could be studied separately with appropriate voltage protocols: N-type channels were selectively activated by depolarizing from a holding potential (HP) of -80 mV to -10 mV; setting HP=-40 mV largely inactivated N-type channels, but left L-type channels available for opening with stronger pulses to +20 mV. With 30 μM (n=10) and 100 μM NE (n=5) in the pipette, average N currents fell to 45% and 14% of N current without drug (n=33). In contrast, the average L current in the same patches was not significantly affected by NE. Analysis of patches with few overlapping openings indicated that NE did not alter the unitary flux through N-type channels but markedly changed their gating kinetics. The mean open time decreased from 0.87 ms in control to 0.40 ms in 100 μM NE, while the percentage of blank sweeps increased from 8% to 24%. These changes largely account for the overall inhibition of averaged N current records. In contrast to its effects in the patch pipette, 100 μM NE did not affect the average N current in the patch (n=5) when applied to the bulk of the cell, arguing against involvement of a readily diffusible second messenger. The activity of N-type channels was neither increased by cyclic AMP nor decreased by PKC stimulation. A relatively direct G-protein-mediated coupling to the N-type Ca channel seems likely.

11.6

ANTIBODIES TO THE G-PROTEIN, G_o, ATTENUATE THE INHIBITION OF CALCIUM CURRENTS BY NORADRENALINE IN NG108-15 CELLS. I. McFadzean[1]*, I. Mullaney[2]*, D.A. Brown[1] and G. Milligan[2]*. [1]University College London, London WC1E 6BT and [2]University of Glasgow, Glasgow G12 8QQ, U.K.

Noradrenaline (NA) acting on α_2-adrenoreceptors inhibits the whole-cell calcium current in NG108-15 cells. The response is mediated by a pertussis toxin sensitive GTP-binding (G) protein. To identify this G-protein we have studied the response to NA in cells pre-injected with specific antibodies raised against the C-terminal decapeptides of the α-subunits of the G-proteins G_i (recognising both $G_{i}1$ and $G_{i}2$) and G_o. Control cells were injected with pre-immune rabbit serum. We found that the response to NA in cells pre-injected with the antibodies against G_o was attenuated by over 50%. The response in cells injected with the antibodies raised against G_i was unchanged despite the ability of the same antisera to inhibit the agonist-induced inhibition of adenylate cyclase in NG108-15 cells. We conclude that the NA-induced inhibition of the calcium current in NG108-15 cells is mediated by G_o.

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11.7

INTRACELLULAR $[Ca^{2+}]$ AND TRANSMITTER RELEASE FROM SYMPATHETIC NEURONS STIMULATED WITH K^+, CAFFEINE OR ISOPROTERENOL. S. Kongsamut, D.D. Friel, D. Lipscombe & R.W. Tsien. C & M Physiol., Yale and M & C Physiol., Stanford.

We asked how closely transmitter release is correlated with changes in $[Ca^{2+}]_i$ in frog sympathetic neurons. Transmitter release was measured from sympathetic ganglia (presumably cell bodies) preloaded with $[^3H]$-norepinephrine. K^+ depolarizations produced Ca entry through Ca channels and transmitter release that was (1) steeply dependent on $[K^+]_o$ over the range 30-80 mM, (2) dependent on $[Ca^{2+}]_o$ (EC50 ~ 0.5 mM), (3) completely inhibited by cadmium (IC50 ~10 μM). Release was dominated by Ca entry through N-type Ca channels: it was not affected by selective block of L-type channels with dihydropyridines, but it was strongly reduced by α-adrenergic agents which selectively inhibit N-type Ca channels. Evidently, some pathways for Ca delivery are more effective than others in producing release. Caffeine-evoked Ca release from internal stores represents another means of Ca delivery. In isolated neurons loaded with fura-2, caffeine (10 mM) produced a large $[Ca^{2+}]_i$ transient (even with 0 Ca), comparable to that produced by voltage-gated Ca entry. However, in contrast to the pronounced release evoked by high K^+, caffeine was unable to produce detectable transmitter release from ganglia. This provides further illustration that rises in bulk $[Ca^{2+}]_i$ do not always produce transmitter release.

Conversely, high concentrations (30-100 μM) of NE or isoproterenol were capable of evoking some transmitter release with little or no change in $[Ca^{2+}]_i$. The isoproterenol-induced release was not abolished by removal of external Ca. This suggests that cells with voltage-gated channels also have mechanisms for transmitter release that do not require Ca entry. Overall, transmitter release is not necessarily correlated with changes in bulk $[Ca^{2+}]_i$.

11.8

EFFECT OF RUTHENIUM RED AND RYANODINE ON THE CAFFEINE INDUCED RISE OF INTRACELLULAR CALCIUM IN VERTEBRATE SYMPATHETIC NEURONS. N.V. Marrion*, S.J. Marsh*#, B.J. Burbach*, D.A. Brown*# and P.R. Adams. (SPON: W. G. Van der Kloot) Howard Hughes Medical Institute, SUNY at Stony Brook, Stony Brook, NY 11794, USA. #Department of Pharmacology, University College London,London,WC1E 6BT,England.

Image analysis of intracellular calcium levels (Ca^{2+}_i) and simultaneous membrane current recording was performed on fura 2-loaded whole-cell voltage-clamped dissociated bullfrog sympathetic neurons. Bath application of caffeine (10 mM) evoked a large outward current which was associated with a rise in Ca^{2+}_i. This increase in Ca^{2+}_i was independent of extracellular calcium and was presumed to arise from intracellular stores. Both the outward current and Ca^{2+}_i increase subsided during caffeine's application although their time-courses differed. The transient outward current declined within 2-10 secs to a net inward current, which was associated with suppression of the voltage- and time-dependent M-current. The rise in Ca^{2+}_i decayed over a longer time than the outward current. Ca^{2+}_i increased to approximately 300 nM from a resting level of 60 nM. This increase declined leaving a Ca^{2+}_i near to 150 nM while the M-current was suppressed. Removal of caffeine produced an initially rapid fall in Ca^{2+}_i which slowed to transiently take Ca^{2+}_i below resting conditions, recovering within 5 minutes.

Inclusion of ruthenium red (30 μM) in the electrode solution increased the amplitude and greatly prolonged the duration of both the calcium transient and the outward current evoked by caffeine. Incubation with ryanodine (1-10 μM) was found to block caffeine's response in both control and ruthenium red-treated neurons, leaving a slowly rising residual increase in Ca^{2+}_i. This inhibition occurred with the second application of caffeine, the first being little affected, implying the presence of caffeine and/or raised Ca^{2+}_i was required for its effect. Ryanodine partially antagonized ruthenium red's effect, reducing the amplitude and prolonged duration of caffeine's response. These results suggest that the release of Ca^{2+}_i initiated by caffeine is inhibited by ryanodine and the sequestration of the raised Ca^{2+}_i is modulated by ruthenium red.

11.9

DYNAMICS OF CA-RELEASE FROM CAFFEINE-SENSITIVE INTRACELLULAR STORES IN AMPHIBIAN SYMPATHETIC NEURONS. A. Hernandez-Cruz*, F. Sala* and P. R. Adams. (SPON: J. Fenstermacher) Howard Hughes Medical Institute, SUNY at Stony Brook, NY 11794.

The dynamics of Ca^{2+} mobilization from internal stores were studied with confocal Ca-imaging and membrane current recording in isolated bullfrog sympathetic neurons. $[Ca^{2+}]_i$ was monitored by directly loading the cell with the membrane-impermeant indicators fluo-3 and rhod-2 (200 μM). Macroscopic Ca-activated K currents, IK(Ca), were simultaneously measured as an independent indicator of changes in submembrane $[Ca^{2+}]_i$. (Vh=-30 mV). Caffeine (10 mM), pressure-ejected from a puffer pipette, produced varied responses depending upon the duration of the pulse. A gradual, transient increase of IK(Ca) (50-200 pA) was produced with short (10-100 ms) applications. These responses were additive and showed no refractory period. Pulse durations about 100 ms or longer evoked, on top of the gradual response, a transient, large all-or-none activation of IK(Ca) (0.8-8 nA). These responses exhibit a refractory period of several minutes, suggesting depletion and subsequent replenishment of an internal store. The development of an inward current, lasting tens of seconds, contributed to the termination of this response (see Marrion et al., this volume). High-speed imaging during large caffeine responses revealed that the Ca signal starts earliest in the region where the caffeine first reaches the cell and then propagates throughout the cell, reaching the opposite site within 600 to 1,200 ms. This pattern is in marked contrast to the symmetrical, radial spread of Ca^{2+} seen following electrical stimulation). Changes in $[Ca^{2+}]_i$ appeared to be most prominent in the nucleus and other unidentified structures, suggesting regional variations in density of release sites. Bath application of Dantrolene Na (100 μM), an agent that bloks Ca-release from sarcoplasmic reticulum, produces a reversible, partial inhibition of caffeine-induced IK(Ca) responses. Procaine (2 mM), which inhibits Ca-induced Ca-release in skeletal muscle, suppresses completely and reversibly the all-or-none component of the caffeine response, leaving the gradual component less affected. These data suggest that the gradual component of the caffeine response may result from a direct effect on localized Ca-release site(s), whereas the all-or-none component may result from a generalized response involving a Ca-induced Ca release process.

11.10

SUSTAINED INCREASES IN CYTOPLASMIC Ca ION CONCENTRATION MEDIATED BY TRANSIENT Ca CHANNELS IN CULTURED EMBRYONIC AMPHIBIAN NEURONS. Michael E. Barish (Spon: W.J. Moody). Department of Physiology and Biophysics, University of California, Irvine, CA 92717.

Three components of Ca current corresponding to T-, N- and L-types are found in neurons that differentiate in cultures of dissociated Xenopus neural plate cells. T-type current is activated at voltages more positive than -45 mV and blocked by Ni. Its activation and steady-state inactivation curves overlap between -40 and -30 mV, and within this region a small sustained component of inward current can be recorded. Additional Ca currents are a more slowly inactivating component (N-type) reduced by met-enkephalin (met-ENK), and a nonrelaxing component (L-type) activated at voltages more positive than -10 mV.

Using the Ca-sensitive fluorescent dye fura-2 and a video-interfaced fluorescence microscope, I have investigated the changes in intracellular Ca ion concentration ($[Ca^{2+}]i$) that occur in these neurons during K^+-induced depolarizations. Images were acquired at 10 sec intervals. Typically, application of 50 mM K^+ (determined to depolarize these neurons to about -15 mV) resulted in an increase in $[Ca^{2+}]i$ that rose rapidly to a peak and then decayed over 10's of seconds. The peak amplitude of $[Ca^{2+}]i$ was reduced when 200 μM Ni or 17.5 μM met-ENK were added along with 50 mM K^+, and the peak was almost completely removed when both of these agents were added together. In contrast, depolarization to about -40 mV (using 20 mM K^+ external solution) elicited a slowly rising but sustained increase in $[Ca^{2+}]i$. This maintained increase in $[Ca^{2+}]i$ was sensitive to Ni but not met-ENK.

These observations suggest that sustained Ca^{2+} influx through the population of transient channels active in the voltage region where activation and inactivation curves overlap may be important in regulating resting levels of cytoplasmic Ca^{2+} at subthreshold voltages.

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11.11

TWO TYPES OF CALCIUM CHANNELS PROVIDE DIFFERENT TEMPORAL CONTRIBUTIONS TO Ca^{2+} ENTRY IN VERTEBRATE NERVE TERMINALS. Martha C. Nowycky, Dept. Anatomy, Med. Coll. Penn., Philadelphia, PA 19129.

Nerve terminals isolated from the neurohypophysis are 1-10 microns in diameter and can be studied with patch clamp techniques. Two types of Ca^{2+} channels coexist in these terminals (Lemos & Nowycky, Neuron, vol. 2, 1989), a typical, dihydropyridine-sensitive, non-inactivating current (L-type) and a second high-threshold transient current (N-like). Neurohypophysial terminals release the peptides oxytocin and vasopressin most effectively when stimulated with characteristic bursts of action potentials (APs). To test the contribution of the two Ca^{2+} channels to Ca^{2+} entry during such bursts, nerve terminals were voltage clamped and stimulated with various test patterns. Both current types have the same voltage range of activation and contribute to Ca^{2+} influx at the beginning of a burst of APs. After 16-32 simulated APs of 1 msec duration, N-current amplitude diminishes. Once inactivated, N-type channels recover very slowly, requiring about 1 to 2 sec at -90 mV to regain full amplitude (stimulation rate = 0.1 Hz). In contrast, the L-type current amplitude changes little even during 1 sec long depolarizing pulses, implying that its contribution will be constant even during hundreds of APs. Thus, different Ca^{2+} channel types may contribute to the temporal shaping of the Ca^{2+} transient which underlies neurosecretion.

11.12

A NEW APPROACH TO OPEN VOLTAGE SENSITIVE CALCIUM CHANNELS AND EVOKE TRANSMITTER RELEASE. D. Przywara*, T. Wakade*, S. Bhave*, J. Ram and Arun R. Wakade, Dept. of Pharm., Wayne State Univ., Detroit, MI 48202.

Sympathetic neurons (SN) cultured from chick embryos were loaded with ^{3}H-norepinephrine (^{3}H-NE) and washed for 1 h. After SN were bathed in Ca/Mg free medium for 15 min, release of ^{3}H-NE was determined in 2 min period ($1.78 \pm 0.1 \times 10^{-2}$). Switchover to Ca-containing medium caused massive release in next 2-min period ($17.1 \pm 4.4 \times 10^{-2}$); release remained elevated for 30 min. Release was related to Ca concentration and blocked by verapamil. Resting membrane potential (Em) of SN averaged -26 ± 1 mV in 2.5 mM Ca medium. In Ca/Mg free medium Em averaged +4 ± 2 mV and recovered to -26 ± 1 mV during 30 min in Ca medium. Intracellular Ca concentrations were determined in Indo-1-loaded SN by laser cytometry. Ca increased from 83 ± 13 nM in Ca/Mg free medium to 597 ± 72 nM following switchover to 2.5 mM Ca medium. Magnitude of Ca influx was similar in cell body and nerve terminal. Excess K (35 mM) produced less depolarization (Em=-13 ± 3 mV) and smaller increases in Ca and release of ^{3}H-NE in comparison to those seen after changeover of the media. We conclude that in addition to elevated K, electrical stimulation or drug application, the present method offers new approach to open voltage sensitive Ca channels and to stimulate transmitter release in cultured SN.

TROPHIC AGENTS I

12.1

NEUROTROPHIC ACTION OF PHORBOL ESTERS AND EXCESS K IS MEDIATED BY A MESSENGER OTHER THAN PROTEIN KINASE C. Arun R. Wakade*, S.V. Bhave*, R.K. Malhotra* and T. Wakade. Dept. of Pharm., Wayne State Univ., Detroit, MI

Phorbol esters have become the drugs-of-choice in studying intracellular signalling because of their stimulatory effect on protein kinase C (PKC). Phorbol 12, 13-dibutyrate (PDB) supported survival of sympathetic neurons (SN) in culture. Effect was related to concentration of PDB and activation of PKC. Excess KCl (17.5 to 75 mM) supported SN and produced concentration-dependent increase in PKC activity (Wakade, et al., J. Neurochem. 51:975, 1988). In this study, we have investigated effects of H_7 and sphingosine, on survival and PKC activity of SN of chick embryo supported in culture by NGF, PDB and 35 mM K. Incubation of SN for 15 min with H_7 (0.3 to 1 uM) and sphingosine (1 to 100 uM) caused dose-dependent inhibition of 100 nM PDB-induced activation of PKC. 1 uM H_7 or 100 uM sphingosine almost completely prevented the effect of PDB. Survival of SN in culture by 100 nM PDB, 40 ng/ml NGF or 35 mM K was unaffected by simultaneous presence of 1 to 30 uM H_7 for 3 to 7 days, although PKC activity of SN remained completely inhibited. Sphingosine proved to be very toxic and killed SN in a day. 1,2- oleoylacetylglycerol (1 to 100 uM) did not support the survival. These results indicate that phorbol esters and excess K may have targets other than PKC to affect survival of SN.

12.2

IN VITRO BIOLOGICAL EFFECT OF NERVE GROWTH FACTOR INHIBITED BY SYNTHETIC NGF PEPTIDES. F.M. Longo, T.H. Vu* and W.C. Mobley. Depts. of Neurology, Pediatrics and Neuroscience Program, UCSF School of Medicine, San Francisco, CA 94143.

Nerve growth factor (NGF) is a neurotrophic polypeptide which acts via specific receptors. The molecular domain(s) of NGF which triggers biological activity is as yet undefined. One strategy for identifying polypeptide regions which interact with their receptors consists of synthesizing short peptides with sequences corresponding to the potential active site. At relatively high concentrations (uM or mM), peptides containing the active site may block or mimic biological activities of the native polypeptide (Nature 309:30, 1984). We synthesized small peptides corresponding to sequences within three regions of NGF which are hydrophilic and highly conserved: Region A, mouse NGF sequence 64-76; Region B, 199-107 and Region C, 26-40. Peptides were synthesized in the C-terminal amide form by solid-phase methods, purified by reversed phase and cation-exchange HPLC and characterized by analytical HPLC, amino acid analysis and sequencing and FAB-mass spectrometry. We reasoned that inhibition of NGF effects may be more readily achieved than NGF agonist responses. Peptides were assayed for NGF antagonist activity by adding them to low density cultures of chick dorsal root ganglia neurons supported by NGF. Four peptides from Region A and 3 from Region B did not inhibit NGF activity. In contrast, 3 out of 8 peptides from Region C inhibited NGF-induced neurite outgrowth with an IC50 of approximately 1mM. Inhibition was overcome by increasing the concentration of NGF. Peptides did not block the neurotrophic activity of ciliary neuronotrophic factor or phorbol 12-myristate 13-acetate. Inhibitory activity was lost when the sequence of residues was randomized or a sterically nonconservative substitution was made. Inhibitory peptides did not decrease neuronal binding of radiolabelled NGF to DRG cells. These studies define one region of NGF which may be required for neurotrophic activity.

12.3

FUNCTIONAL EXPRESSION OF THE NGF RECEPTOR IN A NON-NEURONAL CELL LINE. B.-Y. Wu,* R.H. Edwards, W.J. Rutter,* Hormone Res. Inst., UCSF Sch. of Med., SF, CA 94143.

Nerve Growth Factor-responsive neurons express two apparent types of NGF receptors. The larger population binds NGF with relatively low affinity (Kd 2 nM), whereas the smaller population binds with high affinity (Kd 20 pM). Expression of the cloned receptor cDNA in a variety of neuronal cell lines has previously conferred high-affinity NGF binding and biological responsiveness. Expression of receptor in the non-neuronal mouse fibroblast L929 cells has conferred only low-affinity binding. Because the mechanism of signalling by the NGF receptor remains unclear (the sequence shows no substantial homology to know signalling elements) and may require the action of another, neuron-specific protein which confers both high-affinity binding and biological responsiveness, we have introduced the human NGF receptor into CHO fibroblasts. We have established three stably transformed CHO cell lines, with varying levels of NGF receptor mRNA. Preliminary characterization of the highest expressing cell line shows high affinity NGF binding and transient induction of c-fos. This suggests that the cellular apparatus involved in transducing the NGF signal is not neuron-specific. The appearance of cell lines with only low-affinity NGF receptors (e.g., L929, Schwann cells) may then be due to down-regulation of high-affinity receptors through simultaneous secretion of NGF by the same cell, or to low numbers of receptors which may make a small high-affinity component difficult to detect.

12.4

DETECTION OF mRNA HOMOLOGOUS TO NERVE GROWTH FACTOR RECEPTOR (NGFR) IN RAT DERMAL FIBROBLASTS. P.A Barker*, F. Miller, J. Haskins, R.J. Riopelle†, A. Acheson, R.A. Murphy, Dept of Anatomy and Cell Biology, University of Alberta, Edmonton, Alberta, Canada. †Division of Neurology, Queens University, Kingston, Ontario, Canada.

cDNA molecules representing both rat and human NGFR have been cloned (Nature.325.593. Cell.47.545). In the rat, NGFR mRNA (3.4 kb) encodes a 42.5 Kd protein that has an apparent molecular weight of 82 Kd on SDS-PAGE. NGFR mRNA has been detected in dorsal root ganglia, sympathetic ganglia, brain and Schwann cells.

In this study, riboprobes prepared from a cDNA encoding nucleotides 400 to 3200 of the rat NGFR have detected a mRNA of approximately 3.0 kb in cultured dermal fibroblasts prepared from neonatal rats. To characterize the protein product, we carried out immunocytochemistry using a monoclonal antibody against rat NGFR (192-IgG). Immunoreactivity was apparent throughout the cytoplasm and was visible only when the cells were permeabilized, suggesting that the immunoreactive protein is not located on the cell surface. Under identical conditions, cell surface staining of NGFR was apparent on Schwann cells. Equilibrium binding studies on intact cells with 125I-NGF revealed specific binding sites on Schwann cells but not on fibroblasts. EDAC-mediated crosslinking of 125I-NGF to cell surface receptors followed by immunoprecipitation with 192-IgG revealed a 95 kd complex in PC12 and Schwann cells. A similar complex was detectable when binding was done on detergent solubilized cell extracts. However, no crosslinked immunoprecipitate has so far been identified in fibroblast extracts.

These data suggest that fibroblasts express a 3.0 kb mRNA homologous to NGFR mRNA. Fibroblasts also express a 192-IgG immunoreactive protein that is distributed throughout the cytoplasm but not localized on the cell surface. Further characterization of this mRNA and this protein are underway.

12.5

INTERLEUKIN-1 INJECTED INTO NEOSTRIATUM OF ADULT RAT BRAIN STIMULATES SYNTHESIS OF NERVE GROWTH FACTOR

U. Otten[1], H.P. Lorez[*2], R. Gadient[*1] and C. Boeckh[*3]
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Interleukin-1(IL-1) has been shown to be a potent inducer of nerve growth factor (NGF) synthesis in peripheral tissues. Since IL-1 and NGF have been found to accumulate at sites of brain injury it was interesting to study whether IL-1 acts as a stimulator of NGF synthesis in rat brain. We found that stereotactic intracerebral injection of recombinant human IL-1ß into the neostriatum of adult rats elicited a transient increase in NGF synthesis in a dose-dependent fashion. Within 24 h IL-1ß (1.2 U) maximally increased NGF and NGF mRNA levels by 4- and 2-fold, respectively, as compared to vehicle-treated controls. 5 days after IL-1 treatment NGF and NGF mRNA returned to control levels. The IL-1 effect is specific, since other brain areas including neocortex, hippocampus and hypothalamus, showed only minor changes. Thus, our results suggest a role of IL-1 as a regulator of NGF synthesis in rat brain. Since IL-1 functions as a growth factor for astrocytes it is possible that activated astrocytes are the cellular sites of IL-1 stimulated NGF-synthesis.

12.6

STRIATAL HOMOGENATES TAKEN FROM RATS CHRONICALLY TREATED WITH HALOPERIDOL STIMULATE CELL GROWTH AND DA UPTAKE IN VENTRAL MESENCEPHALIC CULTURES. L.R. Ptak*, L.C. Kao, D.H. Lin*, T.J. Zhang, H.L. Klawans*, and P.M. Carvey. (SPON: R. Zimmerman). Rush University, Chicago, IL, 60612.

Pharmacologic denervation of striatal tissue induced by haloperidol (HAL), leads to DA receptor site hypersensitivity in a fashion analogous to that observed following denervation of muscle. However, denervation of muscle also leads to an increase in a muscle-derived neuronotrophic factor (NTF) capable of stimulating neuron growth. Using ventral mesencephalic cultures, we examined whether or not chronic pharmacologic denervation of rat striatum similarly leads to an increase in a target-derived NTF.

8 rats were rendered behaviorally hypersensitive to apomorphine challenge by 2 months chronic treatment with HAL (0.75 mg/kg). The striata and cerebella of these, and 8 saline treated animals, were homogenized in Hank's Balanced Salt solution. The supernatants from these samples were diluted to achieve equivalent protein content and 250 ul of each were incubated with E-13 ventral mesencephalic cultures for 6 days. Cultures incubated with HAL treated striatal homogenates exhibited an overt increase in cell number as well as process growth relative to cultures incubated with HAL treated cerebellar or saline treated homogenates. Cultures incubated with HAL treated striatal homogenates also exhibited a statistically significant elevation in DA uptake relative to all other culture conditions suggesting increased DA neuron growth. This data would imply that a target-derived, soluble, NTF exists in the adult striatum and further, that it is inducible by chronic pharmacologic denervation. This raises the possibility that alterations in behavioral responsiveness resulting from chronic DA antagonist treatment is the result of an increase in a DA target-derived NTF in addition to the established alterations in DA receptor sensitivity.

12.7

γ-INTERFERON PROMOTES MATURATION OF MAMMALIAN CENTRAL NEURONS IN CULTURE. S. Sherry Raissdana*, Michael E. Barish, Neil B. Mansdorf* and Jennifer Kerlin*. Department of Physiology and Biophysics, University of California, Irvine, CA 92717.

We have examined the effect of the lymphokine γ-interferon (IFN-γ) on the development of embryonic mouse brain cells. Cells isolated from the hippocampal region of embryonic day 15-16 CD-1 mice were cultured on poly-L-lysine-coated coverslips in defined medium. By three different measures of properties characteristic of differentiated neurons, recombinant IFN-γ (rat and mouse) promoted neural development.

I. Increase in the number of cells expressing the 160 kD neurofilament protein epitope recognized by monoclonal antibody NN18. Cells cultured in 1-10 U/ml (murine units) IFN-γ for 1-4 days showed increases of up to 250% over control.

II. Increase in the number of neurites per NN18(+) soma. NN-18(+) cells were photographed individually after 18-24 hr of growth, and the negatives were projected onto a digitizing tablet for analysis. IFN-γ at 10 U/ml increased the mean number of processes per soma from about 2.5 to about 4.0. The mean length of individual processes was not affected.

III. Increase in the number of cells expressing NMDA-type glutamate receptors. Cells were grown in the presence of up to 50 U/ml IFN-γ for 20-48 hr. NMDA receptor expression was quantified using a video-interfaced fluorescence microscope by stimulating fura-2-loaded cells with NMDA and measuring changes in cytoplasmic Ca ion concentration. More than 1000 cells were analyzed for each data point. IFN-γ increased the proportion of cells showing responses to NMDA by up to 50%.

These data indicate that lymphokines can promote the differentiation of embryonic brain cells, and add to evidence suggesting linkage in the development of fetal immune and nervous systems.

(Supported by grants from the N.I.H., March of Dimes, and Am. Heart Assoc.)

12.8

PREVENTION OF MOTONEURON DEATH IN VIVO BY A PUTATIVE CNS-DERIVED NEUROTROPHIC FACTOR. R. Oppenheim, U. Dohrmann*, D. Prevette* and Y. Qin-Wei*. Department of Anatomy, Wake Forest University Medical School, Winston-Salem, NC 27103 and Department of Neurochemistry, National Hospital, London, U.K.

Previous studies have shown that partially purified extracts from skeletal muscle promote motoneuron survival both in vitro and in vivo. Spinal cord and brain (CNS) extracts and conditioned media (CM) from CNS tissues also enhance motoneuron survival in vitro and these distinct CNS and muscle-derived factors interact synergistically on motoneuron survival (Dohrmann et al., Dev. Biol., 124: 145, 1987). Neither the muscle nor the CNS-derived activities appear to be mediated by NGF, BDNF or FGF. We have now shown that crude or partially purified extracts from embryonic day (E) 10 and E17 chick brain and spinal cord also prevents normal motoneuron death in vivo. Daily treatment from E6 to E9 resulted in 25-30% more surviving spinal motoneurons on E10. Similar effects on survival were found following in vivo treatment with CM from astrocyte cultures. Studies are in progress to determine whether the muscle- and CNS-derived factors interact synergistically in vivo to promote motoneuron survival. Normal motoneuron survival may require trophic support from both targets and CNS cells. Supported by NIH Grant NS20402.

12.9

TROPHIC SUPPORT OF SOMATOMEDIN C (IGF-I) AND MSA (IGF-II) ON CENTRAL NEURONAL CELLS IN VITRO. D.D. SVRZIC[1], L.F. CONGOTE[2], D. SCHUBERT[3] (SPON: D.M. SIMMONS) 1) MBVL, The Salk Institute, San Diego, CA 92138, 2) Endocrine Laboratory, Royal Victoria Hospital, 687 Avenue des Pins Ouest, Montreal, Quebec, Canada H3A 1A1, 3) SSL, The Salk Institute, San Diego, CA 92138.

Trophic support by bovine serum albumin (BSA) and ovalbumin (OA) of pure cultures of central neurons was observed. Our experimental model was monolayered cultures of cells obtained as dissociates of chick forebrain on embryonic day 8 and seeded in serum-free medium. All cells stained positive with monoclonal antibody to neurofilaments. Regardless of the substratum on which they were grown (untreated tissue culture (TC) plastic, polyornithin (PORN) or PORN-laminin pretreated TC plastic or glass) these cells showed not only an unusual longevity (34 days in culture without any condition medium changes), but also the normal maintenance with appropriate neuronal sprouting in the presence of BSA. When we fractionated BSA by HPLC, the activity copurified with somatomedin C (insulin-like growth factor, L.F. Congote et al, In Vitro Develop. Cell Biol. 45:245, 1986). To confirm this finding, the neurotrophic effect was blocked with a neutralizing monoclonal antibody to IGF-I (gift from Dr. Van Wyk, J.), and two batches of recombinant human IGF-I exerted the same effect as the BSA purified material. This trophic support on these particular cells has not been described before.

12.10

PARTIALLY PURIFIED MEMBRANE-ASSOCIATED FACTOR DIFFERENTIALLY REGULATES TRANSMITTER PHENOTYPIC EXPRESSION. J.-M. Lee, J.E. Adler, and I.B. Black, Division of Developmental Neurology, Cornell University Medical College.

Cell-cell contact appears to play a critical role in the expression of transmitter traits in developing neurons. We have previously shown that cell membrane contact induces the *de novo* appearance of choline acetyltransferase (CAT), the enzyme catalyzing acetylcholine synthesis, in virtually pure cultures of dissociated sympathetic neurons. This CAT-inducing activity has been purified 5000-fold from adult rat spinal cords and appears to be a membrane-associated protein factor.

To further characterize the scope of biological activities of this factor, we have examined its effect on a number of different transmitter traits in sympathetic neurons. After three days *in vitro*, cultures exposed to the factor showed 40-fold higher levels of the neuropeptide substance P than controls. The neuropeptide somatostatin was also dramatically elevated by the factor (270pg/well compared to undetectable levels in controls). In contrast, the factor produced the opposite effect on the neuropeptide leu-enkephalin, decreasing levels to half that found in controls. Finally, the specific activity of tyrosine hydroxylase, the rate limiting enzyme in catecholamine biosynthesis, was reduced to 10% of control activity in factor-treated cultures. These effects occurred in the absence of significant changes in cell number. Thus, it appears that cell contact via membrane-associated factors may exert differential effects on phenotypic expression. (Supported by NIH grants HD 22315, NS 10259, and a grant from the Bristol Myers Co. Inc.)

12.11

EPIDERMAL GROWTH FACTOR INDUCES A FOS-LIKE NUCLEAR ANTIGEN IN PRESUMPTIVE MULLER CELLS. S.M. Sagar and F.R. Sharp+, Departments of Neurology and +Physiology, Univ. of California, and VA Med. Ctr., San Francisco, CA 94121.

Epidermal growth factor (EGF) is a mitogen for astrocytes and may be a trophic factor for CNS neurons. To identify retinal cells that respond to EGF, 500 ng EGF was injected intravitreally into the eyes of light adapted adult rabbits; phosphate-buffered saline was injected into the opposite eye as a control. 1-3 hr later, the retinas were processed for Fos immunocytochemistry using a polyclonal antiserum raised to a synthetic peptide representing residues 132-154 of Fos.

Specific Fos immunostaining was seen in EGF-injected retinas in numerous nuclei at the middle of the inner nuclear layer. Because of their size, abundance and position, they are presumably nuclei of Muller cells.

We conclude that (1.) Muller cells respond to EGF in vivo, although the response may not be direct; and (2.) Fos immunocytochemistry may be useful for mapping responses to growth factors in a manner similar to its use in mapping neuronal responses to synaptic input.

12.12

Vasoactive Intestinal Peptide Regulates Mitosis, Neurite Outgrowth and Survival of Cultured Rat Sympathetic Neuroblasts D.W. Pincus, E. DiCicco-Bloom and I.B. Black. Div. Dev. Neurol., Cornell Univ. Med. Coll., New York, N.Y., 10021.

While the acute, msec to msec actions of neurotransmitters are well-recognized, diverse, longer-term effects have been discovered only recently. We now present evidence that a single, putative transmitter, vasoactive intestinal peptide (VIP), exerts multiple, long-term effects simultaneously. VIP stimulates mitosis, promotes neurite outgrowth and enhances survival of sympathetic neuron precursors in culture.

Dissociated embryonic rat sympathetic neuroblasts were cultured in fully defined medium and assayed for mitotic activity, neurite elaboration and long-term cell survival. Cells were identified immunocytochemically by detection of neurofilament and the catecholamine biosynthetic enzyme, tyrosine hydroxylase (TH). VIP treatment more than doubled the proportion of TH-positive cells entering the mitotic cycle, as indicated by autoradiography of ^{3}H-thymidine incorporation into nuclei. In addition to regulating mitosis, VIP markedly increased the number and complexity of neurites. The peptide evoked a 6-fold rise in the number of process-bearing cells 10 hours after plating. VIP also increased the percentage of neuroblasts exhibiting long processes at 24 hours. Finally, VIP influenced neuroblast survival: peptide treatment resulted in a 3-5 fold increase in cells per culture after 4 days. The above effects of VIP on sympathetic precursors are highly specific. Two closely related peptides, secretin and growth hormone releasing factor were devoid of effects on SCG cultures.

In sum, our observations suggest that a single neurotransmitter, VIP, influences a spectrum of processes composing early neuronal ontogeny. Since the peptide appears to be a normal presynaptic transmitter in the sympathetic system, synaptic transmission may exert heretofore unexpected effects. (supported by NIH grant HD 22315)

NEURAL PLASTICITY IN ADULT ANIMALS I

13.1

LONG-TERM RESCUE OF DAMAGED FRONTAL CORTEX NEURONS AND SPARING OF VISUALLY GUIDED BEHAVIOR BY ACUTE ADMINISTRATION OF CORTEX CONDITIONED MEDIUM FRACTION. F. Haun, R. Perry, T.J. Cunningham. Dept. of Anatomy, Medical College of Pennsylvania, Phila.,PA 19129.

This study tested both neurotrophic and behavior effects of a macromolecular fraction of conditioned medium (CM) derived from embryonic cortex cultures, compared to a similarly prepared fraction of unconditioned medium (UM). Osmotic pumps (2wk capacity) containing either CM or UM are implanted in adult Long-Evans rats immediately after making a discrete rostral visual cortex lesion unilaterally. At 3-4 mos post-lesion, UM animals show a 31% loss of 3-H thymidine labeled neurons in layers 2,3, and 5 of the caudal third of medial frontal cortex (area 8), a region containing cells of origin of the fronto-occipital pathway. These animals are also impaired in learning a visual discrimination that requires spatially discontiguous responses, a deficit attributable to a 250% increase in the frequency of incorrect responses to the side contralateral to the lesion, compared to normals. In contrast neuron loss is only 9% and visual learning is normal in animals with comparable lesions that received the CM fraction. These results demonstrate a long-term neurotrophic effect on damaged cortical neurons, coupled with a related behavioral improvement, after an acute dosage of a cortex-derived CM fraction, results suggestive of reorganization in the fronto-occipital pathway in response to the trophic agent. Supported by NINDS Grant NS16487 to TJC.

13.2

LESIONS DISRUPTING CORTICAL CHOLINERGIC INNERVATION PREVENT PLASTICITY OF SOMATOSENSORY MAPS IN CAT SOMATOSENSORY CORTEX. S.L. Juliano, D.E. Eslin*, W. Ma. Dept. of Anatomy, USUHS, Bethesda, MD 20814.

A number of recent studies have demonstrated that the organization of maps in the somatosensory cortex (SSC) undergo dramatic changes after manipulations to peripheral somatic input. The mechanisms that produce such changes are only beginning to be understood. Experiments in sensory systems suggest that acetylcholine (ACh) may play a significant role in mediating the capacity of the CNS to adapt to changes in the environment. Previous experiments in our lab demonstrated that disruption of cortical cholinergic innervation substantially reduced the pattern of stimulus-evoked activity in cat somatosensory cortex during a 2-deoxyglucose (2DG) experiment. In contrast to the reduction of metabolic activity, amputation of a single digit in a cat's forepaw causes dramatic expansion of the metabolic pattern evoked by stimulation to an adjacent digit. This change in metabolic pattern was determined using bilateral stimulation to digit 3 in cats that received a unilateral amputation of digit 2, either 2, 4, or 6 weeks prior to a 2DG experiment. The normal, reconstructed pattern of 2DG label produced by stimulation to digit 3 occurs as a broken strip of activity extending through the SSC. The metabolic label evoked in the hemisphere contralateral to the digit amputation was of greater areal extent than that in the normal hemisphere. The reconstructed pattern tended to fill in regions of low activity, thereby making the normally occurring broken strip of activity continuous. Additional strips of activity also occurred. Further experiments performed unilateral digit 2 amputations and concurrent NBM lesions in the same cat. In this situation, which depleted the SSC of ACh, the expansion of stimulus-evoked metabolic activity by stimulation to digit 3 did not occur. These findings suggest that ACh is an important component in mediating the plasticity of somatotopic maps.

13.3

NEONATAL NUCLEUS BASALIS LESION-INDUCED PERTURBATION OF CORTICAL DENDRITIC MORPHOLOGY IN THE ADULT RAT. D.E. Stearn*, R.F. Mervis, R. Dvorak*, and G.W.Arendash#. (SPON: L. Liss). Div. Neuropathology, The Ohio State Univ. College of Medicine, Columbus, OH. 43210,#Dept. Biol.,Univ. South Florida, Tampa, FL.

Neonatal lesions of the nucleus basalis magnocellularis (nBM) will prevent subsequent cholinergic innervation of the neocortex. Adults show prolonged cholinergic hypofunction and cognitive deficits. Two-day-old Sprague-Dawley rat pups had their nBM bilaterally lesioned using ibotenic acid. When 8 months-old, the rats were killed. The parietal cortex was stained using the rapid Golgi method. Controls were sham-lesioned. Analysis of the basilar tree of layer V pyramidal cells showed that nBM-lesioned subjects had significantly more dendritic material and more branching ($p<0.05$); however, dendritic fields retained substantially normal morphology. Long-term nBM lesions appear to result in dropout of cortical neurons. In neonates it produces a transient abnormal cortical cytoarchitecture. The present findings suggest that in the absence of normal extrinsic cholinergic innervation, both neuronal plasticity and a compensatory dendritic response (to neuronal loss) may be important factors influencing neuronal development.
[Supported in part by Sigma Kappa Sorority Foundation]

13.4

NUCLEUS BASALIS LESIONS IN THE AGING RAT: EFFECT ON DENDRITIC BRANCHING IN CORTICAL PYRAMIDAL CELLS. R.F. Mervis, M. Bedo-Wierdl*, R. Dvorak*, & G.W. Arendash#. Div. Neuropathology, The Ohio State University College of Medicine, Columbus, Ohio 43210, and #Dept. Biology, Univ. South Florida, Tampa, Florida 33620.

To help develop an animal model for Alzheimer's disease, the effect of long-term excitotoxic lesions of the nucleus basalis (NB) on neocortical branching of pyramidal cells was evaluated in Golgi-impregnated preparations. Sprague-Dawley rats (21 months-old) were infused unilaterally with ibotenic acid in the NB. The subjects were killed 5 months later. The Sholl method of concentric circles was used to evaluated extent of dendritic branching of the basilar tree of layer III pyramidal cells from the frontal cortex. There were two Control groups: neurons from the contralateral (unlesioned) hemisphere and from sham-lesioned rats. Results showed that there was a significant increase in the amount of dendritic branching in the neurons from the NB-lesioned rats compared to controls. There was also a significant increase in the radius of the dendritic domain in neurons from the lesioned hemisphere. Previously, it had been found that long-term NB lesions resulted in loss of cortical neurons. The present findings suggest that the surviving cortical neurons express compensatory dendritic hypertrophy.
(Supported in part by the Sigma Kappa Sorority Foundation)

13.5

RELATIONSHIP BETWEEN BRAIN CATECHOLAMINES AND BEHAVIOR FOLLOWING EXPERIMENTAL TRAUMATIC BRAIN INJURY. A.W.Deckel, A.Merians, B. Fennelly*, P.Freeswick*, and B.Levin. Depts. of Psychiatry, Physical Therapy and Neurology, UMDNJ—N.J. Medical School and Sch Allied Health, Newark, N.J., 07103; Dept. Neurology, E.Orange V.A. Med. Center, E. Orange, N.J.

Twenty-three adult male Sprague Dawley rats, including control (n=7), moderately lesioned (n=8) and severely lesioned (n=8) were analyzed on a variety of behaviors at 1 and 3 weeks post lesion. These measures included assessment of spontaneous locomotor activity in the horizontal and vertical plane and turning behavior following injection with apomorphine (0.2 mg/kg s.c.). At the completion of the 3 week measures, brains were removed and CNS catecholamine (CA) analysis was done via HPLC biochemistry. Severely lesioned animals turned 10 times more frequently to the left side than controls following apomorphine injection ($p<.017$), but less often to the right side ($p<.002$); moderately lesioned animals turned approximately 5 times more often to the left side than controls, while controls turned 3.2 times more often to the right. Controls showed twice as much vertical activity as did severely and moderately lesioned at 1 week post injury ($p<.05$). No statistically significant biochemical differences were seen between controls and lesioned. Nonetheless, left-hemisphere cortical NE "predicted" horizontal locomotor activity in controls when forward stepwise regression analysis was employed, while right-side brainstem DA "predicted" vertical locomotor activity. In contradistinction to controls, in lesioned animals right-sided anterior NE levels accounted for the majority of the variance in most measures of spontaneous locomotion. These results suggest that left cortical NE has a close association with locomotor activity in controls and that this relationship "shifts" following TBI.

13.6

DIFFERENTIAL EFFECT OF DOPAMINERGIC DEAFFERENTATION ON THE MORPHOLOGY OF STRIATAL TARGET NEURONS IN YOUNG AND OLD MICE. T.H. McNeill, S.A. Brown*, B. J. Davis and L.L.Koek*. Andrus Gerontology Center, Univ. of Southern California, Los Angles, CA 90089 and the Dept. of Neurology, Univ. of Rochester, Rochester, NY 14642.

A recent study from our laboratory has provided morphological evidence for the dendritic regression of striatal target neurons in Parkinson's disease (PD) (Br.Res. 455:148,1988). Since it is known that PD is characterized by a partial dopaminergic (DA) deafferentation of the striatum (ST) we hypothesized that the lack of an appropriate compensatory response of striatal target neurons in the aged brain to DA cell loss may underlie the regression of striatal dendrites found in PD. To test this hypothesis we examined by Golgi impregnation the morphology of medium spiny I (MSI) striatal neurons, a principle target population for DA fibers, following a unilateral lesion of the SN using the DA neurotoxin 6-OH-DA. For our study adult (6 mo.) and old (20 mo.) C57Bl/6N mice were given unilateral injections of 6-OH-DA (4.5ug). Mice were killed at 14 days postinjection and tissue blocks containing the ST were processed according to the Golgi Cox procedure of Van der Loos. DA lesions were confirmed by immunocyto-chemical staining. We found that there was a differential response of individual dendritic segments to DA cell loss and the response was age dependent. Specifically, there was a significant increase in the length and number of distal dendritic segments of MSI neurons in adult (6 mo.) but not old (20 mo.) mice following the 6-OH-DA. These data suggest that MSI neurons of aged mice lack the ability to compensate or modify their dendritic arbor in response to DA cell loss and that the absence of a similar compensatory response in the aged brain of man may contribute to the regression of MSI dendrites found in PD. Supported by AG00300, AG05445.

13.7

AUTOMATED COMPUTER RECOGNITION AND QUANTIFICATION OF NEURONS AND NEURITES IN HUMAN BRAIN. T. El Rashidy and J. Rogers. Institute for Biogerontology Research, Sun City, AZ 85351.

Neuron and neurite losses are one of the more likely bases for cognitive changes associated with aging and dementia. Unfortunately, techniques for quantifying perikaryal and, especially, neurite alterations remain extremely labor intensive. Currently available "computer assisted" methods often amount to little more than using the computer as a glorified adding machine, because the operator must still, in one way or another, help identify the quantified elements (i.e., by pointing or tracing each one or by setting parameters for thresholding). To the extent that these techniques rely on gray scale thresholding, they are also error prone: the gray levels of neurons, neurites, and background overlap too extensively for accurate segmentation based on thresholding alone. We have developed an **automated** approach that incorporates both gray scale information and information about spatial frequencies. In a first step, acquisition and preprocessing, adjacent fields of the entire sample (or restricted areas traced by the operator) are automatically digitized and optimized. In a second phase, two original algorithms are used to segment the fields into their different components: cell bodies, neurites, and field background. A specially constructed lowpass, local neighborhood filter preserves perikarya while eliminating high frequency components such as neurites and background debris. Neurites are then separated from background by a second spatial domain algorithm that, in tandem with a highpass filter, analyzes gray level variance along fixed length bars rotated in 32 directions. Conventional measurement programs are then used to quantify the results. Operator assistance is needed only to monitor performance. Advantages of the method include higher sampling rates, objectivity, and precision than currently available techniques for counting neurons and neurites.

13.8

IN VIVO OBSERVATION OF MOTOR NERVE TERMINAL REMODELLING IN REINNERVATED NEUROMUSCULAR JUNCTIONS OF THE FROG. A.A. Herrera, M.J. Werle, & N. Nagaya, Dept. of Biol. Sci., Univ. of Southern Calif., Los Angeles, CA 90089.

The polyneuronal innervation (PI) that forms at adult neuromuscular junctions during reinnervation is reduced by subsequent synapse elimination (SE). We are using electrophysiology, histology, and repeated in vivo observation to examine this process in the reinnervated sartorius of Rana pipiens. Although SE is extensive, PI remains higher than normal even after 2 years. Histology shows that reinnervated junctions are larger and have abnormally-shaped nerve terminals; they also have more axonal inputs, abandoned synaptic sites, and doubly innervated sites than normal junctions. We have posed two hypotheses to explain the persistence of higher PI and the apparent synaptic remodelling: 1) SE may cease after a certain time, freezing the pattern of innervation into a deceptively plastic state. 2) SE may be ongoing, but is balanced by enhanced synapse formation to maintain higher PI in a dynamic equilibrium. We are observing identified reinnervated junctions in vivo to test these hypotheses. Our goals are to explore the validity of this form of SE as a model for development, test the precision of reinnervation of synaptic sites, better define the sequelae of nerve injury, and characterize a convenient preparation for studying pre- and postsynaptic change during synaptic remodelling. Supported by NIH.

13.9

REGULATION OF CONNECTIVITY IN THE ADULT SPINAL CORD. By G.R.Lewin* and S.B.McMahon*, Dept. Physioogy, St Thomas' Hospital Medical School, London SE1 7EH. (SPON: BRA).

We have previously shown that some properties of unmyelinated muscle primary afferents change when they reinnervate a new target. We have now asked whether the connectivity of these afferents has changed in the spinal cord. In adult rats the nerve to the gastrocnemius muscle (GN) and the cutaneous sural nerve (SN) were self- and cross-anastomosed on left and right, so that they regenerated to either appropriate or inappropriate targets. Ten to 12 weeks later, symmetrical recording tracts were made with a tungsten microelectrode to map L4, L5 and L6 spinal segments in these animals under urethane anaesthesia. The microelectrode was advanced to a depth of 720 μm in 6μm steps at 0.5 Hz whilst repetitively stimulating the GN at C-fibre strength, every 2 seconds.

Comparisons between the control and experimental side were made on the basis of estimates of the number of cells activated at C-fibre latencies and quantitative counts of the number of C-evoked spikes in each track. By both these measures the GN now innervating skin showed an average increase of 200% over control which was seen in all 7 animals studied and was significantly different ($p<0.01$). One explanation for this increased connectivity may be the altered peptide content of muscle afferents innappropriately innervating skin (McMahon and Gibson, Neurosci. Lett. 73, 9-15).

13.10

RECOVERY OF MOTOR FUNCTION AND PLASTICITY OF DORSAL ROOTS FOLLOWING SPINAL CORD HEMISECTION IN ADULT CATS. M.E. McBride and M.E. Goldberger. Medical College of Pennsylvania, Philadelphia, PA. 19129.

Hemisection sparing the dorsal columns results in a loss of monopedal postural reflexes and locomotion, followed by partial recovery. To test the hypothesis that recovery is associated with increased dorsal root projections we used quantitative densitometry of immunocytochemically identified subpopulations of dorsal roots. Large fibers were labeled with monoclonal antibody Rat 102 (Martin & Hockfield, 1988 Soc.Neurosci.Abst.14:693) and small fibers with CGRP. Descending serotonergic (5-HT) and noradrenergic (DBH & TH) systems were similarly examined. On the hemisected side Rat 102 immunoreactivity increased 4 fold in the medial dorsal horn and in lateral lamina V and 3 fold in medial lamina VII and the medial ventral horn. CGRP increased in laminae I & II. 5-HT decreased 2 fold in laminae I & II and lamina V, 7 fold in the IML and 3 fold in the medial ventral horn. One interpretation of the increase in immunoreactivity of Rat 102 and CGRP is collateral sprouting of dorsal roots. Reactive reinnervation of spinal neurons deafferented by hemisection is consistent with enhanced reflex activity in chronically hemisected cats.

Supported by NIH grants NS24707,NS16629 & NSF grant NS8605441.

13.11

MECHANISM OF RECOVERY OF FUNCTION AFTER SPINAL CORD TRAUMA: NEW EVIDENCE J.B. Walker, B.Scroggins* Lab of Bio-electronics, Walker Inst. 881 Alma Real Drive, Pacific Palisades, CA. 90272

Recovery of function after brain or spinal cord injury as a function of administration of certain drugs or hormones, fetal transplants, or exposure to enriched environments or electric fields has been described in animals.

A proportion of human subjects with chronic spinal cord injury recover voluntary motor function, below the lesion (Walker,J. Neurosci. 22 S:1669, 1987).

In order to investigate the mechanism by which recovery occurs, we examined muscles that appeared paralyzed by conventional clinical examination using quantitative electromyography. Quantitative electromyography using the Biocomp 1000 system can detect myoelectric activity at a threshold at .5 microvolts. EMG examination of paralyzed muscles hopefully would differentiate between several logical alternatives that could explain recovery of function. These alternatives are:

(1) regeneration
(2) voluntary movement present but "masked" by spasticity
(3) the nerves to the muscle are intact but not functioning because of some reversible or irreversible metabolic deficiency
(4) voluntary muscle function is present but is too weak to be detected with clinical examination.

Electromyograph examination revealed that over 50% of "paralyzed muscles" showed "minipotentials" with recruitment patterns on voluntary effort. We therefore conclude that even in apparently paralyzed muscles, "minipotentials" are present and may represent a mechanism by which this apparent recovery of function occurs. The present data are inconsistent with the existence of regeneration, masking of voluntary function by spasticity as has been suggested by several authors, and any peripheral neuropathy. The present data are also consistent with autopsy data indicating that even with so called complete lesions, much of the spinal cord is spared.

THE AGING PROCESS

14.1

INCREASED NEUROTROPHIC ACTIVITY IN THE AGING BRAIN. B. A. Yankner and P. Dikkes*. Dept. of Neurology, Children's Hospital and Harvard Medical School, Boston, MA 02115.

The dynamics of aging in the CNS and the mechanisms by which the brain repairs itself are unknown. We have identified a substratum-associated neurotrophic activity in the murine CNS that increases adhesion, viability and neurite outgrowth in cultured cortical neurons. This activity is enriched in grey matter and present at high levels in the hippocampus, amygdala and associative regions of the cortex. Low levels are present in spinal cord and peripheral nerve. The highest levels of activity are present during fetal development with a subsequent decline postnatally. In the aging brain, there is a significant resurgence of neurotrophic activity througout the CNS but with considerable regional variation. The activity is sensitive to digestion with proteinase K and trypsin but resistant to heparinase. Neurite outgrowth was partially inhibited by NCAM antibody but was unaffected by laminin antibody or antibodies to the amyloid precursor protein. Neuronal cell death in the aging brain may trigger the release of neurotrophic factors resulting in compensatory process outgrowth from remaining viable neurons.

14.2

THE MICRENCEPHALIC RAT: AN ANIMAL MODEL FOR THE STUDY OF ACCELERATED AGING ASSOCIATED WITH DEVELOPMENTAL BRAIN DEFECTS. M.H. Lee, A. Rabe, P. Wang* and J. Currie. New York State Institute for Basic Research in Developmental Disabilities, Staten Island, NY 10314.

The rat with prenatally induced hypoplasia of the cerebrum is an animal model of congenital brain defects and mental retardation. Born with profound morphological and neurochemical changes in the brain, these rats show retarded development and impaired memory and spatial competence. All previous studies have focused on developing and young adult animals. In order to determine how such an altered brain would fare in old age, offspring of Long-Evans rats given 30 mg/kg of methylazoxymethanol acetate (MAM) on E15 were tested at old (24 mo), middle (15 mo) and young (6 mo) ages in the Morris maze, a task sensitive to aging. Although the MAM rats of all ages were impaired on all measures of acquisition and retention, the magnitude of the deficit increased linearly with age, the old MAM rats being significantly poorer than the young ones to learn and remember the position of a hidden escape platform. No such age difference was evident in the control group. Moreover, variability also increased as a direct function of age in the MAM group, suggesting individual differences in the aging process. Changes in neurotransmitter balance may be responsible for the accelerated aging in MAM rats. The results suggest the micrencephalic rat may provide a useful model for the study of aging.

14.3

IN VIVO DOPAMINE AND ACETYLCHOLINE RELEASE IS IMPAIRED IN THE BRAIN OF AGED RATS. EFFECTS OF ACETYL-L-CARNITINE. A. Imperato*, M.T. Ramacci*°, O. Ghirardi*° and L. Angelucci*. (SPON: G. Engel). Inst. of Medical Pharmacology, University "La Sapienza", I-00185 Rome. °Sigma Tau Biological Laboratories, I-00040 Pomezia (Rome).

Several in vitro findings have shown deficiency in the aged rat's brain of the neurotransmitter function at the level of synthesis, metabolism and release. We have studied in the conscious rat of different strains, the changes with aging from 3 up to 27-31 months in dopamine (DA) and acetylcholine (ACh) release from the striatum and hippocampus. While a clear decrease of both DA and ACh release is observed in the rat's aging from 18 (-25%) to 24-31 months (-80%), no significant changes in DA and ACh intraneuronal levels are observed. Moreover, starting from 18 month old rats, the stimulation of striatal DAergic and hippocampal cholinergic terminals by K+ 30 mM, enhances release much less than in the younger rats underlining that the release function is impaired in spite of the presence of normal intraneuronal stores. The effects of acetyl-l-carnitine in young and aged rats, will be described.

14.4

FUNCTIONAL DENDRITIC ELONGATION IN CA1 PYRAMIDAL NEURONS IN SENESCENT F344 RATS. D.A. Turner and D.L. Deupree. Neurosurgery, Univ. Minn. and VAMC, Minneapolis, MN 55417.

Dendritic lengthening during normal aging may compensate for a progressive loss of neurons and afferent synapses. However, impaired behavior (senescence) may result from ineffective compensatory mechanisms. A behavioral measure of hippocampal function *in vivo* was correlated with *in vitro* physiological data on CA1 cells, to identify neuronal alterations specific to senescent animals.

Aged F344 rats (>26 months) were divided into behaviorally normal and impaired groups using a water maze task. Subsequent *in vitro* recordings in CA1 neurons demonstrated no difference in intracellular parameters between young and aged groups, including resting potential, spike height and input resistance. Electrotonic length (L, in λ) was calculated from transient responses in young (n=44 cells) and aged (n=17 cells) rats. Neuronal L values from senescent aged rats ($L=0.60\pm0.15$ λ) showed significant elongation ($p<0.001$) compared to either young ($L=0.42\pm0.09$ λ) or normal aged rats ($L=0.38\pm0.05$ λ).

The specific correlation of increased electrotonic length in CA1 pyramidal neurons *in vitro* with behavioral impairment *in vivo* suggests that dendritic lengthening may be a critical element in abnormal dendritic signal processing in senescence. Supported by grants from the VA Research Service, the MMF, the B.S. Turner Foundation and the Alzheimer's Disease and Related Disorders Association.

14.5

INTRAVENTRICULAR PITUITARY GRAFTS STIMULATE THE IN SITU ACTIVITY OF TYROSINE HYDROXYLASE (TH) IN TUBEROINFUNDIBULAR DOPAMINERGIC (TIDA) NEURONS OF AGED RATS. N. Aquila-Mansilla*, W. Kedzierski*, and J.C. Porter. Dept. OB/Gyn and Physiology, U.T. Southwestern Med. Ctr., Dallas, TX 75235

Aging is associated with a decrease in TIDA neuronal function as judged by content, synthesis, and turnover of dopamine (DA) in median eminence (ME) and by the concentration of DA in hypophysial portal blood. Hyperprolactinemia can stimulate TIDA neurons. We investigated whether such stimulation is peculiar to TIDA neurons or occurs in other dopaminergic neurons. Anterior pituitary (AP) tissue was placed in the lateral ventricles of female rats and maintained there as tissue grafts. Liver tissue served as control. Seven days after implantation, the quantity of TH and its in situ activity, assayed by measuring the rate of accumulation of DOPA, were quantified in the ME, substantia nigra (SN) and corpus striatum (CS). TH mRNA was measured in the SN and hypothalamus. TH was quantified by an immunoblot analysis, using rat TH as a standard. DOPA was measured by HPLC with electrochemical detection. TH mRNA was quantified by an S1 nuclease protection assay using TH sense RNA as the standard. Compared to aged OVX animals with liver implants, the molar activity of TH in the ME of aged OVX rats with AP implants was significantly (P<.001) increased (39±3 vs 59±3 moles DOPA/mole TH/hr). The PRL concentration in the CSF of OVX rats with AP implants was 345± 83 ng/ml compared to 23±9 ng/ml in OVX rats with liver implants. In the CS, the activities of TH in OVX rats with AP grafts and liver grafts were 34±4 and 37±4 moles DOPA/mole TH/hr, respectively. In the SN, the in situ activity of TH was 30±2 moles DOPA/mole TH/hr in animals bearing AP grafts and 29±2 in rats with liver grafts. The quantity of TH mRNA was 34±2 attomoles in the hypothalamus of rats with AP grafts and 38±3 attomoles in animals with liver grafts. In the SN of rats with AP grafts and liver grafts, there were 705±63 and 755±47 attomoles of TH mRNA, respectively. In contrast to OVX rats, aged intact animals bearing AP or liver grafts showed no difference in the activity of TH, mass of TH, and TH mRNA levels. Although the PRL concentration in CSF of AP grafted animals was significantly higher (P<.01) than those with liver implants (729±180 vs 223±62 ng/ml), the latter still exhibited a marked elevation in the PRL concentration in CSF compared to OVX rats with liver grafts. These findings show that 1) the secretory activity of TIDA neurons of aged rats is reduced by ovariectomy, 2) the TIDA neurons of aged OVX animals are stimulated by intraventricular AP grafts, possibly due to PRL, and 3) the effect of AP grafts is specific for TIDA neurons and not for DAergic neurons of the SN. Lastly, the increase in TH activity is not attributable to an increase in the quantity of TH mRNA or TH. (Supported by NIH grants AG-04344 and AG-08173.)

14.6

LOSS OF SEXUAL DIMORPHISM IN RAT ARCUATE NUCLEUS (AN) NEURAL MEMBRANES WITH SENESCENT CONSTANT ESTRUS/DIESTRUS. L-M Garcia Segura*, E. Jones and F. Naftolin. Instituto Cajal*, Madrid, Spain and Department of Obstetrics and Gynecology, Yale University, New Haven, CT.

At all ages thus far studied, compared to males, female rats have more intramembranous protein particles (IMP) in their AN neural membranes. During the estrus cycle and as a result of administration of estradiol the number of IMP in female post-synaptic membranes decreases toward that found in males. Estrogen administration also can cause a loss of positive feedback (tonic reproduction/constant estrus), in which case the changes in IMP become fixed at male levels. Since senescent constant estrus is the natural outcome of aging in the cycling female rat, we proposed that cyclic preovulatory estrogen exposure could induce the senescent constant estrus of aging (J Ster Biochem 30:195, 1988). To test this hypothesis we examined AN membranes from aged females in senescent constant estrus or diestrus, comparing them to young cycling females and equally-aged males. EXPERIMENTAL: Following assessment of reproductive status by vaginal smears, female rats and equally-aged male rats were perfusion-fixed and the AN analyzed by freeze-fracture. Cycling females had higher numbers of IMP in post synaptic membranes than males (1265 ± 21 versus 877 ± 29 IMP/μM^2 [P-face], $p < 0.001$); but membranes from 15 month old females in senescent constant estrus (793 ± 32 IMP/μM^2) and 18 month old females in senescent constant diestrus (847 ± 23 IMP/μM^2) had the same IMP density as males. To control for aging, 15 and 18 month old males were compared with 3 month old males and found to have the same IMP density. CONCLUSIONS: Neuronal membranes from senescent female rats have lost their male/female IMP difference. Since estrogen causes both depletion of membrane IMPs and the development of tonic reproduction, the present results support the idea that senescent constant estrus may be the result of repeated synaptic remodelling driven by periodic estrogen surges during the ovarian cycle. (Supported by NIH-HD13587 and CSIC, Spain.)

14.7

STUDIES OF NEURON LOSS WITH AGE IN THREE DIFFERENT BRAIN REGIONS IN HUMANS. JB Lohr, DV Jeste. San Diego VA Medical Center and Univ of CA, San Diego, 92161.

Age-related neuron loss varies in different brain regions although there have been few studies of neuronal density with aging in different areas of the same brains. We examined the cerebellum, hippocampus, and locus ceruleus (l.c.) from 47 brains from the "normative" series of the Yakovlev Collection, Washington, D.C. We have reported the techniques of morphometric analysis previously (Biol Psychiat 21:865, 1986; Acta Psych Scand 77:689, 1988). Pearson's correlation coefficient of neuron density with age ranged from .03 for dentate nucleus to .91 for l.c. After correcting for age effects, there was a significant correlation (r=.67) of neuron density in the l.c. with Purkinje cell density in the anterior vermis. Also, areas in the hippocampus and cerebellum which had the greatest neuron loss with age were those that may receive greater noradrenergic input from the l.c. These results suggest an association between catecholamine content and age-related neuron loss in different brain regions.

(SUPPORTED BY VA MERIT REVIEW GRANT TO JBL)

14.8

REDUCED BRAIN GREY MATTER VOLUME IN ELDERLY VOLUNTEERS. K. O. Lim*, R. B. Zipursky*, M. C. Watts*, and A. Pfefferbaum*. (SPON: W. Faustman). Stanford University School of Medicine, Stanford, CA and Veterans Administration Medical Center (116A3), Palo Alto, CA 94304.

Magnetic Resonance (MR) imaging affords the potential for quantitative in vivo assessments of brain grey matter and white matter volumes. In order to study differences in grey and white matter in different disease states, it is critical to understand the changes that take place with normal aging.

Accordingly, 8 young men (mean=24yrs) and 7 elderly men (mean=73yrs) underwent a cardiac gated, spin echo, axial MR scan (effective TR≥2400msec; TE=20 and 80msec). Six 5mm thick sections per subject were analyzed. Quantification of cerebrospinal fluid (CSF) and tissue pixels was accomplished by segmenting each section into CSF, grey matter and white matter compartments (Lim and Pfefferbaum, *J. Comput. Assist. Tomogr.*, in press).

The young and the old subjects did not differ significantly in the total intracranial volume of the sections analyzed. Cortical sulcal volume was significantly greater in the elderly subjects ($P<0.001$) as was lateral ventricular volume ($P<0.01$). The elderly subjects had significantly reduced cortical grey matter volume ($P<0.001$) and subcortical grey matter volume ($P<0.05$) relative to the young subjects. The two groups did not differ significantly in cortical or subcortical white matter volumes.

Using quantitative computerized assessment of MR images, significant reductions in cortical and subcortical grey matter were found in elderly men. These results suggest that the age-related increases in ventricular and sulcal volume seen with current structural imaging techniques are due primarily to decreases in grey matter rather than white matter volume.

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14.9

HOX 1.3, AMYLOID &-PROTEIN AND AGING. D. Goldgaber, D.E. Schmechel, and W.F. Odenwald* (SPON: F. A. Henn). NINDS, NIH, Bethesda, MD 20892; Duke Univ. Durham, NC 22710.

While analyzing the promoter region of the APP gene that encodes amyloid β-protein found in brains of patients with Alzheimer's disease, we found five potential binding sites similar to the Hox 1.3 consensus sequence CPyPyNATTAT/GPy. Two of these sites located near positions -1500 and -2600 were recognized by the Hox 1.3 protein in a band shift assay. This suggests that the APP gene might be a target gene for Hox 1.3 protein. Both APP gene and Hox 1.3 gene are highly conserved in evolution and are expressed in neurons of adult mammalian brain in a very similar distinct pattern. We used antibody to the Hox 1.3 protein to immunostain sections of adult human temporal cortex and hippocampus. As expected, nuclei of most neurons were darkly stained with the antibody. However, this was true only for young adults. When brain sections of old individuals with or without Alzheimer's disease were stained with this antibody the number of stained neurons dramatically decreased.

The homeo box protein Hox 1.3 is a regulatory factor whose expression is regulated in space and time. The disappearance of Hox 1.3 in neurons of aged individuals might have a significant effect on expression of target genes including the APP gene. Our data suggests that aging might be a final stage of development with the Hox 1.3 gene turned off.

14.10

AMYLOID PRECURSOR PROTEIN (APP) UNDERGOES FAST ANTEROGRADE TRANSPORT. E.H. Koo, S.S. Sisodia*, D.R. Archer*, L.J. Martin*, K. Beyreuther*, A. Weidemann* and D.L. Price. Neuropathology Lab., The Johns Hopkins Univ. Sch. Med., Baltimore, MD 21205.

In senile plaques, abnormal nerve terminals (neurites) surround extracellular deposits of β-amyloid protein (BAP). BAP is derived from APP that may originate from the circulation or from brain elements. If BAP is derived from neuronal APP and is deposited at locations remote from where synthesis occurs, then APP must translocate from perikarya to distal terminals in proximity to sites of amyloid deposition. In this study, we determined that APP is rapidly transported in axons. Two ligatures (1 cm apart) were placed on sciatic nerves in adult rats; animals were sacrificed 6-48 hours later. Segments of nerve (5 mm) proximal to, within, and distal to the ligatures were analyzed by SDS-PAGE and immunoblotting. APP accumulated after six hours and increased up to 24 hours in segments proximal to the first ligature. In immunoprecipitation experiments, APP labeled by microinjecting sensory neurons was detected in sciatic nerve segments proximal to the ligation. Finally, immunocytochemistry showed APP immunoreactivity within swollen axons proximal to the ligation. These findings suggest that APP is delivered to nerve terminals where proteolysis of APP may liberate BAP into the neuropil, leading to deposits of amyloid.

14.11

BIOSYNTHESIS AND PROCESSING OF AMYLOID PRECURSOR PROTEIN (APP) *IN VITRO*. S.S. Sisodia*, E.H. Koo, L.J. Martin*, A.J. Unterbeck*, K. Beyreuther*, A. Weidemann* and D.L. Price (SPON: D.L. Price). Neuropathology Lab., The Johns Hopkins Univ. Sch. Med., Baltimore, MD 21205.

Amyloid deposits in plaques and cerebral blood vessels are hallmarks of Alzheimer's disease. Principally, amyloid is a 42 amino-acid peptide (BAP), a truncated form of a larger precursor, APP. Three APP transcripts code for APP-695, -751, and -770 isoforms that appear to be glycosylated cell-surface molecules; the two larger polypeptides also contain domains homologous to protease inhibitors. We have begun to examine the biochemical mechanisms by which the APP isoforms mature *in vitro*. Engineered APP genes were introduced into mammalian cell lines, and APP isoforms were analyzed by pulse-chase/immunoprecipitation and endoglycosydase cleavage assays, in conjunction with studies using sugar-precursor labeling and metabolic inhibitors. Using both biochemical and immunocytochemical approaches, we document that APP is transported rapidly to the cell surface; a large N-terminal portion of APP is secreted into the extracellular environment. APP does not appear to be cleaved at the membrane/extracellular interface but at a site near the N-terminal of BAP. Mutagenesis techniques and biochemical analyses of wild-type and modified APP polypeptides are being used to define sites that are necessary and/or sufficient for the initial cleavage of APP.

14.12

LOCALIZATION OF AMYLOID PRECURSOR PROTEIN (APP) IN BRAINS OF YOUNG AND AGED MONKEYS. L.J. Martin*, L.C. Cork*, E.H. Koo*, S.S. Sisodia*, A. Weidemann*, K. Beyreuther*, C. Masters* and D.L. Price. (SPON:A. Pestronk). Neuropathology Lab., The Johns Hopkins Univ. Sch. Med., Baltimore, MD 21205.

APP is a membrane-spanning glycoprotein that is processed to generate a 42 amino-acid β-amyloid protein (BAP) deposited extracellularly in senile plaques and blood vessels. In plaques, BAP is associated with enlarged neurites (i.e., abnormal axons/nerve terminals). Little is known of the distribution of APP in primate brain or how patterns change with age. We investigated APP-immunoreactive patterns in fresh-frozen cortices from monkeys (n = 11) ranging from 4-41 years of age. APP immunoreactivity was present in neuronal perikarya, proximal dendrites, and axons. APP and synaptophysin (a presynaptic marker) antibodies stained neurites in a subset of plaques containing BAP. Knob-shaped neurites appeared to be capped by BAP immunoreactivity. The presence of APP in axons is consistent with our findings in rats that APP is transported anterogradely. The accumulation of APP in neurites suggests that local alterations in the processing of APP to BAP contributes to amyloid deposition in plaques of aged nonhuman primates.

BIOLOGICAL RHYTHMS AND SLEEP: NEUROREGULATORS

15.1

NORADRENERGIC AND CHOLINERGIC REGULATION OF CANINE CATAPLEXY, A PATHOLOGICAL MODEL OF REM SLEEP ATONIA

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Human narcolepsy is usually considered as a REM sleep disorder and its symptoms as dissociated REM sleep manifestations. Using *in vivo* pharmacology and various neurochemical measurements (radioreceptor binding and LCEC measurements of brain monoamines) in a canine model of the human disorder, we suggest that cataplexy is under the dual control of a noradrenergic system involving the locus coeruleus and the amygdala, and a pontine cholinergic system starting from the pedunculopontine nucleus and projecting in the reticular formation. *In vivo* pharmacology clearly indicates that the noradrenergic system inhibits REM sleep and cataplexy whereas the pontine cholinergic system activates cataplexy (Baker T and Dement WC, In Brain Mechanims of Sleep, pp199-234. D. Mc Ginty et al. Eds, New-York , Raven Press, 1985; E. Mignot et al., J. Clin. Invest. 82: 885-894, 1988). The pontine cholinergic system seems to be the final commun pathway since all the effects of the noradrenergic compounds on cataplexy are dependant upon the integrity of the cholinergic system. We included the amygdala in our model because of our findings of abnormal content of catecholamines, metabolites and receptors reported in this brain region (Faull et al., Sleep 9: 107-110, 1986). The involvement of this structure would also explain the fact that cataplexy is precipitated by emotional stimuli since the role of the amygdala in emotional behavior is well documented. The possibility that a specific defect of one of these systems could be the primary cause of canine narcolepsy, an autosomal recessive disorder of REM sleep, will be discussed.

15.2

THE EFFECT OF GONADAL STEROIDS ON THE DIURNAL RHYTHM OF OXYTOCIN IN THE CEREBROSPINAL FLUID OF RHESUS MONKEYS. J.A. Amico and J.L. Cameron. Depts. of Medicine and Psychiatry, Univ. of Pittsburgh School of Medicine and Oakland VAMC, Pittsburgh, PA 15261.

The magnocellular oxytocin (OT) neurons that project to the posterior pituitary release OT into the periphery. Peripheral OT does not cross the blood brain barrier. Parvicellular OT neurons project to other central nervous system (CNS) areas and may be the source of OT in CSF. In primates, OT has a diurnal rhythm in CSF with peak and nadir concentrations occurring during light and dark hours, respectively. Factors influencing this rhythm have yet to be identified. Estradiol (E_2) stimulates OT transmission in the CNS and may alter the CSF OT rhythm. To determine the effect of E_2 and progesterone (P), ovariectomized rhesus monkeys (n = 7) bearing subarachnoid catheters were S.Q. implanted with two 4-cm silastic capsules containing 17β-E_2 for 6 days and one 4-cm P capsule for the last 3 days of E_2 treatment. Hourly samples of CSF were collected before, during and after gonadal steroid treatment and measured for OT by RIA and HPLC. Mean $\pm$ SEM serum E_2 concentrations before, during, and after implantation were 112 $\pm$ 12, 325 $\pm$ 11, and 99 $\pm$ 6, pg/ml. The daily phase, amplitude, peak, and nadir of the CSF OT rhythm were not altered by E_2 or E_2 + P (ANOVA). HPLC chromatograms of CSF before and during gonadal steroid treatment were identical. OT immunoreactivity eluted as a single peak in the position of synthetic OT. We conclude that the CSF OT rhythm in primates is not estrogen or progesterone dependent.

15.3

ULTRADIAN CYCLICITY: MORPHOLOGICAL SEX-LINKED INFLUENCES. J. Fang* and W. Fishbein. Neurocognition Program, CUNY, City College & Graduate School, N.Y. 10031

At the 1987 SN meeting we reported for the first time, in the mouse, that sex differences exist in the ultradian cyclicity of sleep. The findings were the first to indicate that sleep is sexually dimorphic. At the same time we also reported that the sleep of males could be completely sex-reversed by prenatally stressing them. The findings led us to conclude that sleep-cycle rhythmicity follows the same general rule as the genital system: the basic plan is inherently female. In the absence of male hormones the genetic male develops a female brain and female sleep-cycle rhythms. At the 1988 SN meeting we reported that we had extended our observations to the Sprague-Dawley rat.

A key finding in our research, both in mice and rats, is that the sexual dimorphism of sleep is solely accounted for by a sex-linked difference in the frequency of occurrence of paradoxical sleep (PS) bouts. It appears that the normal biological clock timing mechanism(s) that controls the interval between PS episodes is sexually dimorphic and runs at a different speed in male rodents than in females. As a result the total amount of PS is substantially different between the sexes.

Therefore, the pontine-cholinergically mediated sleep-cycle trigger zone must be regulated by extra-pontine, sex-linked influences.

The Locus Coeruleus and Suprachiasmatic Nuclei are sexually dimorphic structures that depend on the levels of gonadal steroids early after birth to influence their morphological architecture. Both structures are thought to be crucially involved in the regulation of circadian and ultradian timing. The gender differentiation of sleep, therefore, may result from sex-linked morphological differences in these structures.

15.4

SOLUBILIZATION OF MELATONIN RECEPTORS FROM LIZARD BRAIN. S.A. Rivkees, R.W. Conron*, S.M. Reppert. Laboratory of Developmental Chronobiology. Children's Service, Mass. Gen. Hosp., Boston, MA 02114

Melatonin receptors from lizard brain (Anolis carolensis), a highly enriched source of melatonin receptors, were identified with ^{125}I-labeled melatonin (I-MEL) after solubilization with digitonin. Receptors solubilized in the absence of Mg^{2+}, retained the essential characteristics of lower affinity receptors (non-G protein coupled) previously characterized in crude membrane preparations (PNAS 86:3882, 1989). Binding was saturable (Kd=347±89 pM, Bmax 86±15 fmol/mg; n=6), reversible, and inhibited in a characteristic manner by melatonin and closely related analogs. Binding was not affected by GTP-gamma-S (100 uM) suggesting that solubilized receptors were not coupled to G proteins. Based on gel filtration chromatography estimates, the molecular range of non-G protein coupled receptors was 110,000 to 150,000 (n=3). When Mg^{2+} was included during solubilization, subsequent specific I-MEL binding was reduced by GTP-gamma-S by 90% suggesting that receptors solubilized in this manner were coupled to G proteins. Gel filtration chromatography yielded an apparent molecular range of ca. 440,000 for G protein coupled ligand-receptor complexes (n=2). These results suggest that I-MEL binding sites in solubilized lizard brain membranes are melatonin receptors, with Mg^{2+}-dependent G protein coupling.

15.5

A D-2 DOPAMINE RECEPTOR AGONIST RESETS THE PHASE OF A CIRCADIAN CLOCK IN CULTURED EYECUPS FROM *XENOPUS LAEVIS*. G.M. Cahill and J.C. Besharse. Dept. of Anatomy and Cell Biology, Emory University School of Medicine, Atlanta, GA 30322.

Dopamine, acting through D-2 receptors, has been implicated in the regulation of rhythmic retinal processes by light and a circadian clock. In the present study, we have begun to explore the role of dopamine in the ocular pacemaker that controls retinal melatonin synthesis. Rhythmic release of melatonin by individually superfused *Xenopus* eyecups was used as an assay of pacemaker function. The defined culture medium includes 100 μM 5-hydroxytryptophan, which elevates melatonin production sufficiently that circadian rhythms of melatonin release can be measured from individual eyecups for up to 5 cycles in constant darkness. When the D-2 agonist quinpirole (10^{-7} M) was added to the superfusion medium, acute suppression of melatonin production, as well as entrainment and resetting of the rhythm were observed. The rhythms of the paired eyes from an individual could be entrained *in vitro* to 180° antiphase by two alternating 12-hr treatments of each eyecup with quinpirole. The phases produced by quinpirole cycles were maintained for over 2 cycles in constant conditions. Single 6-hr pulses of quinpirole caused phase-dependent phase shifts of the rhythms in test eyecups when compared to paired, untreated eyecups. Pulses in the early subjective night caused phase delays, and pulses in the late subjective night caused phase advances. These effects of quinpirole are similar to the expected effects of light, and suggest a role for dopamine receptors in the generation or entrainment of ocular rhythmicity.

15.6

CIRCADIAN ACTIVITY RHYTHMS IN HYPERTENSIVE (SHR) AND NORMOTENSIVE (WKY) RATS: STRAIN DIFFERENCES AND EFFECTS OF CLONIDINE. A.M. Rosenwasser, Dept. of Psychology, Univ. of Maine, Orono ME 04469.

Chronic clonidine treatment shortens the free-running period and reduces the level of circadian activity rhythms in rats. The present study extends these observations to spontaneously hypertensive SHR rats and their normotensive WKY controls. SHR rats display several neurochemical differences from WKYs, and the two strains respond differentially to clonidine in certain pharmacological tests. Circadian wheel-running rhythms were assessed before, during, and after clonidine treatment (5.0 ug/ml in the drinking water for 4 weeks), under constant light. SHR rats showed shorter periods and were hyperactive relative to WKYs during baseline conditions. Clonidine administration shortened free-running periods similarly in the two strains, but reduced activity more dramatically in the SHRs. These results suggest that central noradrenergic systems influence both the periodicity and the level of spontaneous activity, and that clonidine may act at different neural or neuronal loci to alter these two parameters.

15.7

NEUROPEPTIDE Y (NPY) PRODUCES CIRCADIAN PHASE CHANGES OF SUPRACHIASMATIC NUCLEUS (SCN) NEURON FIRING RATE RHYTHM IN VITRO. S. Shibata* and R.Y. Moore. Dept. Pharmacol., Kyushu University, Fukuoka, Japan and Depts. Neurology and Neurobiology, SUNY Stony Brook, NY 11794.

The geniculohypothalamic tract (GHT) contains NPY neurons projecting upon the SCN. Stimulation of the GHT (Johnson et al, 1989) or injection of NPY into the SCN (Albers and Ferris, 1984) produces changes in the phase of circadian activity rhythms with a phase response curve (PRC) that differs from that for light but is similar to that for triazolam (Turek and Losee-Olson, 1986), protein synthesis inhibition (Inouye et al, 1989) and social interaction (Mrosovsky, 1988). The present study was carried out to analyze the effects of NPY administration on the phase of the circadian rhythm in firing rate of SCN neurons in in vitro (Shibata et al, 1982) and compare this to potassium depolarization, protein synthesis inhibition (cycloheximide), optic chiasm stimulation and calmodulin inhibition. NPY, produces phase changes with a PRC with phase delays during early subjective day, phase advances in mid- to late subjective day and phase delays during subjective night. This PRC was essentially mimicked by cycloheximide and the calmodulin inhibitors. Potassium depolarization and optic chiasm stimulation produced phase changes with PRC's similar to light. These data suggest that there are two principal pathways, receptors and/or second messenger systems, by which entraiing influences interact with SCN circadian pacemakers. Supported by NIH grant NS-16304.

15.8

EFFECTS OF 5,7DHT LESIONS OF THE HAMSTER SEROTONERGIC SYSTEM ON CIRCADIAN ACTIVITY RHYTHMS. K.M. Michels, L. Smale, R.Y. Moore and L.P. Morin. Neurology and Psychiatry, SUNY Stony Brook, NY 11794.

The role of the serotonergic system in the regulation of hamster circadian rhythms was analyzed using intraventricular injections of the selective neurotoxin, 5,7-dihydroxytryptamine (5,7DHT). 5,7DHT depleted immunoreactive serotonin (5HT) in the forebrain, particularly the suprachiasmatic nuclei and intergeniculate leaflet. 5,7DHT also produced an immediate, sustained advance in running wheel activity onset relative to the 14:10 light-dark cycle of 0.71 ± 0.07 h prior to lights out for 5,7DHT treated animals compared to 0.18 ± 0.04 h after lights out for controls. This advanced phase angle was maintained after an 8 hour phase shift of the cycle. Wheel running offset was also delayed by 5,7DHT, but the period in constant darkness, the rate of reentrainment to an 8 h phase advance, and the daily amount of wheel running activity were not significantly affected. In addition, 5,7DHT did not affect the ability of triazolam to accelerate reentrainment to an 8 h phase advance. These data indicate that ascending serotonin projections of the midbrain raphe participate in the pattern of entrainment of the hamster activity rhythm, however, the mechanism of action remains to be elucidated. Supported by NIH grant NS 16304 to RYM and NIH grant NS 22168 to LPM.

15.9

THE INTERGENICULATE LEAFLET: DELINEATION BY RETINAL AFFERENTS AND NEURONAL MARKERS. R.Y. Moore and J.P. Card (Spon: K.L. Olsen) Depts. of Neurology and Neurobiology, SUNY, Stony Brook, NY 11794 and Medical Products Division, E.I. DuPont de Nemours, Wilmington, DL 19898

The intergeniculate leaflet (IGL) of the lateral geniculate complex is intercalated between the dorsal (DLG) and ventral (VLG) geniculate nuclei (Hickey and Spear, 1976). The IGL contains neuropeptide Y (NPY+)-and enkephalin (ENK+)-immunoreactive neurons (Card and Moore, 1982; Mantyh and Kemp, 1983) which project to the suprachiasmatic nucleus (SCN; NPY+) and contralateral IGL (ENK+), respectively (Card and Moore, 1989). The present study was directed to a precise delineation of the IGL using anterograde transport of cholera toxin-HRP and quantitative analysis of NPY+ and ENK+ neurons after retrograde transport of fluorogold (FG) from the SCN and IGL. The IGL is present rostrally as a thin lamina of neurons between the VLG and DLG. Caudally, it is a wedge of cells between the VLG and the medial geniculate and then swings ventrally lying between the medial geniculate and the lateral terminal nucleus of the accessory optic system. After FG injection into the SCN, the IGL contains 750+35 labeled cells of which 559+35 are NPY+. After FG injection into the IGL, the contralateral IGL contains 1259+112 neurons of which 1058+85 are ENK+. Thus, the IGL contains approximately 2000 neurons of which 35% project to the SCN and 65% project to the contralateral IGL. Supported by NIH grant NS16304 and the E.I. DuPont Co.

TRANSMITTERS IN INVERTEBRATES I

16.1

PROCTOLIN IS ASSOCIATED WITH CRAYFISH SWIMMERET COMMAND NEURON ACTIVITY. L.D. Acevedo and B. Mulloney. Zoology Dept., University of California, Davis, California 95616.

Five command neurons excite the crayfish swimmeret system. Their axons were mapped to specific locations in the interganglionic connectives [Wiersma and Ikeda, 1964]. Stimulation of any one of these axons will activate the swimmeret rhythm, the motor pattern that drives normal swimmeret beating. The neuropeptide proctolin will also activate the swimmeret rhythm [Mulloney et al., 1987].

To test the hypothesis that proctolin is the transmitter used in these command pathways, we studied the distribution of proctolin immunoreactivity and the stimulus-dependent release of proctolin. We demonstrated that proctolin-like immunoreactivity occurred in the same regions of the ganglion where command axons project. Affinity-purified antibodies to proctolin reveal 15-20 bilateral pairs of axons in each abdominal ganglion. Approximately half of these axons sent branches into the Lateral Neuropils, the region of each ganglion that contains most of the swimmeret pattern generating circuitry. Many of these axons also lie in longitudinal tracts predicted from the positions of command neurons given by Wiersma and Ikeda.

Small bundles of axons teased from the interganglionic connectives that contained one command neuron also contained immunoreactive axons. Stimulation of axon bundles that contained single command axons sometimes released proctolin into the superfusate. Proctolin released during command neuron activity and measured with a locust leg bioassay was blocked by preabsorption of the releasate to proctolin antibodies. These results are consistent with the hypothesis that proctolin is the transmitter used by these command neurons.

16.2

PROCTOLIN AND OCTOPAMINE PRODUCE DIFFERENT PATTERNS OF MUSCLE TENSION ENHANCEMENT AND CALCIUM CHANNEL ACTIVATION IN CRAYFISH SKELETAL MUSCLES. C.A. Bishop, M.E. Krouse* and J.J. Wine, Dept. of Psychology, Stanford Univ., Stanford, CA 94305.

Proctolin, a pentapeptide cotransmitter released by three identified tonic flexor motoneurons, greatly potentiates depolarization-induced tension, in part by increasing activity of voltage-dependent Ca^{2+} channels (Bishop, *Neuro. Abst.*, 1988). Octopamine, a biogenic amine that plays a neurohumoral role in crustacea, can also potentiate tension by direct action on some muscles (Kravitz, *Sci.* 241: 1775, 1988). We compared the effects of proctolin and octopamine on tension-voltage curves [T(V) curves] generated in individual tonic flexor muscle fibers by intracellularly injected currents. **Results:** 1. Neither modulator affected resting muscle tension or membrane potential. 2. Both enhanced induced tension, but in different ways: proctolin (at a maximal dose of 5×10^{-9} M) shifted the T(V) curve to the left with a variable and sometimes small increase in slope, while octopamine (10^{-7} M) caused only a small shift in threshold but a marked increase in slope. 3. Combined effects of the two modulators were at least additive. 4. The rate of tension relaxation was unaffected by proctolin but was increased 1.9-fold by octopamine.

Attempts to account for these findings with single-channel patch-clamp recordings have revealed a large Ca^{2+} channel (50 pS in 137 mM Ba^{2+}) which has an increased probability of opening when depolarized. The open probability of this channel in inside-out patches was greatly increased by proctolin but not by octopamine. We conclude from these results that proctolin and octopamine enhance crayfish muscle tension via different mechanisms and the actions of these two neuromodulators subserve different physiological roles. (Supported by NIH grant NS20557 and the Muscular Dystrophy Assn.)

16.3

MODULATION OF MUSCLE TARGET EXCITABILITY BY A DUAL TRANSMITTER MOTONEURON. I.R. Duce and M. E. Adams (SPON: P. Wilson) Dept. of Entomology, Univ. of California, Riverside, CA 92521

The neuropeptide proctolin is contained in a subset of skeletal motor units in insects and crustaceans, where it functions as a cotransmitter with L-glutamic acid. We have investigated the occurrence and possible functions of proctolin and glutamate in motor neurons innervating the longitudinal ventrolateral muscles 6A and 7A of the larval house fly, *Musca domestica*. Immunohistochemical staining with a glutamate-specific antiserum revealed the following three morphological types of motor axons on the surface of these muscles: Type A axons are short and thin (1-2 μm), make large nerve terminals (5-10 μm) and are found predominantly on the lateral muscle cell (6A) in each segment. Type B axons are long and thick (3-5 μm), contain large (5-10 μm) varicosities and arborize extensively over both muscles 6A and 7A. Type C axons are very thin (<1 μm), have small varicosities (1-2 μm) and, unlike Type A or B axons, extend to the outer edges of each muscle cell. Type A axons only are immunoreactive against both glutamate and proctolin antisera.

Muscles 6A and 7A are electrically inexcitable under normal conditions. However, muscles depolarized by current injection or nerve stimulation support calcium-dependent action potentials (APs) following exposure to nanomolar concentrations of proctolin. Proctolin-induced APs are cobalt-sensitive and persist for several minutes after removal of the peptide from the bath.

The effects of bath-applied proctolin on the EJP can be elicited by selective stimulation of one of the motor neurons innervating the muscles. Repetitive stimulation of a low threshold motor neuron results in EJPs which do not generate APs, but recruitment of this unit together with a higher threshold axon leads to the EJPs which generate APs. We hypothesize that a dual transmitter motor neuron containing both glutamate and proctolin modulates voltage-sensitive calcium channels in these muscles.

Supported by NIH grant NS24472 and USDA grant 86-CRCR-1-2097 (to MEA) and a Fulbright travel grant to IRD.

16.4

FMRFAMIDE-LIKE PEPTIDES MODULATE PHASIC NEUROMUSCULAR SYNAPSES IN CRUSTACEANS. A.J. Mercier, M. Schiebe, H. Bradacs and H.L. Atwood. Dept. of Physiol., Univ. of Troront, Toronto, Ont. M5S 1A8

Two FMRFamide-like peptides (TNRNFLRFamide and SDRNFLRFamide), found in lobster neurohaemal organs, are thought to modulate cardiac and tonic neuromuscular systems (Trimmer et al, J. Comp. Neurol. 266: 16, 1987). Both compounds increased nerve-evoked tension in phasic abdominal extensor muscles of crayfish by 5-fold or more. Threshold concentrations were below 10^{-9} M, and resting tension was unaffected. EPSP's increased by up to 90%, with thresholds for an observable effect between 10^{-9} and 10^{-8} M. Input resistance of muscle fibers increased by about 15%, suggesting that the effect on EPSP's was not solely due to postsynaptic changes. An increase in quantal content was observed with extracellular recording at exposed synaptic terminals. Similar changes in EPSP size also occurred in the homologous muscle of lobsters at 10^{-7} to 10^{-6} M. The effect on tension was mimicked by 10^{-7} M YGGFMRFamide and by 10^{-8} M met-enkephalin, but 10^{-6} M FMRFamide had no effect. These data provide the first evidence for modulation of a phasic neuromuscular system in crustaceans.

Supported by NSERC of Canada.

16.5

PHARMACOLOGICAL EVIDENCE THAT GLUTAMATE IS THE EXCITATORY NEUROTRANSMITTER TO AN INTERNEURON IN THE CRAYFISH MEDULLA. C.L. Pfeiffer-Linn and R.M. Glantz, Department of Biology, Rice University, Houston, TX. 77001.

Although glutamate is the most likely excitatory transmitter at arthropod neuromuscular junctions, little is known about its role in the CNS. We have examined the distribution and pharmacological actions of glutamate in the optic lobe of the crayfish, Procambarus clarkii. Distribution of glutamate was examined with an antisera to a glutamate-glutaryl-protein conjugate. Glutaraldehyde fixed tissue was sectioned in cryostat, incubated in primary antibody at 1:500 dilution and visualized with the PAP procedure. Glutamate-like reactivity was observed in the lamina's amacrine cell body layer, in the glia surrounding the lamina, in columnar projections of the first and second chiasm, in three layers of the medulla externa and in four layers of the medulla externa.

Pharmacological actions of glutamate were examined on the principle output cells of the medulla externa; the sustaining fibers (SFs). SFs respond to light with a sustained compound EPSP. Pressure injected glutamate (10^{-6} M), quisqualate (10^{-7} M) and kainate (10^{-7} M) all mimic the synaptic action, producing a depolarization associated with an increase in conductance and a reversal potential similar to that of the synaptic response. All of these actions persist in 20 mM cobalt. NMDA and known glutamate antagonists; DNQX, L-glutamic acid gamma methylester and piperidine, have no effect on this receptor. Glutamate has no effect on two other medullary interneurons; tangential and amacrine cells. These results support the hypothesis that glutamate is the excitatory neurotransmitter to the major output cells of the secondary neuropil. Supported by NSF Grant BNS 87-11141.

16.6

ARE TWO SUB-TYPES OF PRESYNAPTIC CALCIUM CHANNELS INVOLVED IN NEUROTRANSMITTER RELEASE AT THE INSECT NEUROMUSCULAR JUNCTION? V. P. Bindokas* and M. E. Adams, (SPON: R. E. Whalen) Div. Toxicol. & Physiol., Dept. of Entomology, Univ. of California, Riverside, CA 92521.

Venom of *Agelenopsis aperta* spiders contains a family of calcium channel antagonists (ω -agatoxins) which blocks transmitter release at the insect neuromuscular junction. The ω-agatoxins are distinguished as Type I and Type II toxins based on structural and biochemical criteria (Adams *et al.*; Pocock *et al*; this meeting). We present evidence showing that Type I (ω-Aga-IA, IB, IC) and Type II (ω-Aga-IIA, IIB) ω-agatoxins may have separate effects on transmitter release at the larval fly (*Musca domestica*) neuromuscular junction.

ω-Aga-IA, a Type I toxin, irreversibly blocks neuronal calcium channels in insects (Bindokas and Adams, *J. Neurobiol.*, 20, in press). Maximal block caused by ω-Aga-IA is 98% in low Ca (0.75 mM) saline, but is reduced to about 40% upon introduction of high Ca (5 mM) saline. This effect appears not to involve displacement of bound toxin, since the block returns to its original level when low Ca saline is re-introduced. Rather, elevated extracellular Ca may provide sufficient Ca flux through ω-Aga-IA-insensitive channels to permit partial recovery of transmitter release. Maximal block caused by ω-Aga-IA in high Ca saline is not increased upon addition of other Type I toxins, suggesting that Type I-sensitive binding sites are saturated. However, application of a Type II toxin (IIA or IIB) increases the partial block caused by ω-Aga-IA to 90%. Similarly, Type II toxins block only partially in high Ca saline, but 90% block is reached upon subsequent application of a Type I toxin.

We hypothesize that separate subtypes of presynaptic calcium channels mediate neurotransmitter release at the insect neuromuscular junction. These are distinguished by differential Type I and Type II ω-agatoxin sensitivity.

Supported by NIH grant NS24472 and USDA grant 86-CRCR-1-2097.

16.7

CHARACTERIZATION OF AN AFFERENT-INDUCED CHANGE IN THE EXCITABILITY OF AN INSECT MOTONEURON. B.A. Trimmer and J.C. Weeks. Institute of Neuroscience, University of Oregon, Eugene, OR 97403.

PPR, one of the motoneurons involved in retracting the prolegs of *Manduca sexta* larvae, receives direct cholinergic input from mechanosensory afferents at the tip of the proleg. Unitary EPSPs evoked by each afferent are mediated by nicotinic acetylcholine receptors (nAChRs). After these nAChRs are blocked with mecamylamine, a short train of stimuli to a group of afferents evokes a slowly developing, long-lasting depolarization of PPR (the post-train depolarization, **PTD**). During PTD, PPR is much more excitable, such that further afferent stimulation or depolarization of PPR by current injection cause prolonged spiking. PTD is accompanied by a decreased spike threshold which is not a result of the depolarization measured at the soma. No consistent changes in input resistance can be recorded. Both PTD and the change in spike threshold seem to be mediated by muscarinic receptors since they are blocked by very low concentrations (0.1μM) of scopolamine-methyl bromide and can be mimicked by iontophoresis or bath application of oxotremorine-M or muscarine. The M1 selective agonist McN-A-343 is without detectable effect at concentrations as high as 3μM. The M1 selective antagonist pirenzepine blocks PTD only at concentrations above 10μM. The M2 selective antagonists vary in their effectiveness. 4DAMP is without effect at 10μM but hexahydrosiladifenidol inhibits PTD at 1μM. Thus, we are not able to classify the receptors according to the vertebrate scheme. This is the first system in which a physiological role for insect muscarinic receptors has been established, and we are presently studying the ionic and intracellular mechanisms involved in their actions. Supported by a research grant from the Whitehall Foundation.

16.8

NEUROPEPTIDE EXPRESSION, MODULATION AND FUNCTION IN THE EMBRYO OF AN INSECT. K.S. Broadie*, C.M. Bate* and N.J. Tublitz. Inst. of Neurosci., U. Oregon, Eugene, OR 97403, & Dept. Zool., U. Cambridge, UK.

Little is known about the differentiation and function of peptidergic neurons during embryogenesis. We have addressed this issue in the embryonic CNS of the moth, Manduca sexta, which in post-embryonic stages contains two Cardioacceleratory Peptides (CAPs) that serve several modulatory functions. Using a highly specific anti-CAP monoclonal antibody, the temporal and spatial distribution of a CAP-like antigen was determined in the embryonic moth CNS. Two groups of CAP immunoreactive neurosecretory cells, medial and lateral, were identified in the abdominal ventral nerve cord during the final 30% of embryogenesis. Of these, the lateral cells exhibited a marked and transient drop in CAP-like immunoreactivity between 75 and 80% of embryonic development. This decline in CAP-like immunoreactivity was independently confirmed by measurements of CAP-like bioactivity in whole embryo extracts. Results from immunoprecipitation and chromatographic studies unequivocally identified the embryonic CAP-like factor as Cardioacceleratory Peptide$_2$ (CAP$_2$).

Since these results suggested that CAP$_2$ may be released at 75% embryonic development, a series of physiological studies were undertaken to identify a possible embryonic role for CAP$_2$. The embryonic gut responded in a dose-dependent manner to exogenous application of CAP$_2$. The gut became extremely sensitive to CAP$_2$ at 70-75% development, and in vivo measurements indicated that the previously quiescent gut produced its initial rhythmic contractures at this period. Developmental studies showed that ingestion of extraembryonic yolk occurs between 75% and 85% of development. Based on these observations, we postulate that CAP$_2$ is released during embryogenesis at 75% development to facilitate digestion of the extraembryonic yolk by stimulating gut activity.

This work is supported by NIH grants #NS-24613 and #NS-01258 and by the Sloan Foundation.

16.9

CHARACTERIZATION OF A NEW PEPTIDE IN THE FMRFAMIDE FAMILY FROM THE CNS OF THE HAWKMOTH, *MANDUCA SEXTA* T.G. Kingan[1], D.B. Teplow*[2], J.G. Hildebrand[1], E. H. Hanneman[1], K.R. Rao[3], A.E. Kammer[4], I. Jardine*[5], D.F. Hunt*[6] [1]ARLDN, U. of AZ, Tucson, AZ 85721; [2]Div. of Biol., Cal. Tech., Pasadena, CA 91125; [3]Dept. of Biol., U. of W. FL, Pensacola, FL 32514; [4]Dept. of Zool., ASU, Tempe, AZ 85287; [5]Finnigan-Mat, 355 River Oaks Pkwy, San Jose, CA 95134; [6]Dept. of Chem., U. of VA, Charlottesville, VA

FMRFamide was first identified as a cardioacceleratory peptide in extracts of molluscan ganglia. Since then, additional members of this peptide family, most of which are N-terminally extended, have been identified in four phyla of animals, yet little is known of their actions as hormones or neurotransmitters outside the molluscs. We have purified and determined the amino acid sequence of a new member of this family from the moth Manduca sexta. The purification was monitored by competitive ELISA and accomplished by gel chromatography and ion-exhange and reverse-phase HPLC. Peptides were purified separately from extracts of brain and corpora cardiaca, then sequenced by automated Edman degradation after deblocking with pyroglutaminase. Ambiguities in the second residue were resolved by mass spectrometry. The peptides purified from the two sources are identical. Synthetic analogues were prepared and shown to increase, by 50-80%, the force of the stimulus-evoked contraction in the major flight muscles, the dorsal longitudinals. Histological studies reveal extensive immuno-reactive (ir-FMRFa) patches on the heart, apparently arising from ncc I+II and ncc V; ir-FMRFa in the 55% acetone supernatant of hemolymph declines 2-3 fold with 20 min of forced flight. Thus, hormonal peptide may function in flight by action on muscle or their motorneurons.

16.10

A HISTAMINE RECEPTOR IN THE INSECT VISUAL SYSTEM IS A LIGAND-GATED CHLORIDE CHANNEL. R.C. Hardie* (SPON: P.D. Evans). Dept. of Zool. Cambridge Univ., Cambridge CB2 3EJ U.K.

Recent evidence strongly suggests that histamine is the neurotransmitter released by a variety of arthropod photoreceptors (Hardie, R.C. J. comp. Physiol. 161:201, 1987). To investigate the postsynaptic histamine receptors, patch-clamp recordings have been made from second-order neurones (LMCs) enzymatically dissociated from the first visual neuropile (lamina) of the blowfly, Calliphora vicina. Histamine-activated chloride channels were detected in the majority of inside- and outside-out patches. Open probability depended very steeply on histamine concentration (Hill coefficient ca. 4; KD = 60 μM histamine). The short mean open time of the channel (ca. 0.5 ms) coincides with estimates of the synaptic time constant. The long term stability of single channel activity in perfused, isolated patches and the negligible latency (<0.5ms) of responses to ionophoretically applied histamine support the conclusion that this novel histamine receptor represents a new ligand-gated ion channel.

16.11

AMINERGIC NEUROTRANSMITTERS REVERSE THE EFFECT OF RESERPINE ON BITING BEHAVIOR BUT NOT SWIMMING ACTIVITY IN THE LEECH *HIRUDO MEDICINALIS*. B.A. O'Gara, H. Chae*, and W.O. Friesen. Dept. of Biology, Univ. of Virginia, Charlottesville, VA 22901.

Reserpine injected into the crop (100 μg/leech) causes depletion of aminergic neurotransmitters and induces behavioral and physiological abnormalities (O'Gara and Friesen, Soc. Neurosci. Abstr. 14:383, 1988; Friesen et al., ibid. 14:384, 1988). Biting behavior, which is dependent on CNS serotonin levels (Lent, Brain Res. Bull. 14:643-655), is eliminated by reserpine. We found that biting can be restored by bathing the intact leech in serotonin, dopamine or octopamine. The degree of restoration is dependent on both the concentration and duration of exposure to each transmitter. With a 20 min exposure to serotonin or dopamine, thresholds are between 1 and 10 μM, while the maximal effects occur at 1 mM for serotonin and 9 mM for dopamine. Both serotonin and dopamine inhibit biting behavior when applied at high concentrations or for a long duration.

Swimming activity is enhanced by serotonin or octopamine (Hashemzadeh and Friesen, Soc. Neurosci. Abstr. 13:1060, 1987). The depletion of amines by reserpine abolishes swimming activity from isolated nerve cords. Unexpectedly, reserpine increases the duration of swimming activity that is induced by transferring an intact leech and its home container water to another container. This effect is not reversed by external exposure to serotonin, octopamine, dopamine, or histamine. However, the aminergic antagonist cyproheptadine eliminates all swimming activity. This suggests that the swimming activity of intact reserpine-treated leeches is dependent on the very low levels of amines still present in the nerve cord. We postulate that isolated nerve cords from reserpine-treated leeches do not swim because the small quantities of amines remaining in the nerve cord diffuse into the surrounding bath. These results suggest that biting behavior requires higher levels of amines in the CNS than swimming activity. Supported by NIH grants NS08263 (B.A.O.) and NS21778 (W.O.F.).

BRAIN METABOLISM AND BLOOD FLOW: BLOOD FLOW

17.1

THE EFFECTS OF ENDOTHELIN (ET-1) ON LOCAL CORTICAL MICROVASCULAR PERFUSION IN THE RAT. R.N. Willette, C. Sauermelch*, M. Ezekiel*, G. Feuerstein and E. Ohlstein*. Dept. of Pharmacology, Smith, Kline and French Laboratories, King of Prussia, PA, 19406.

Laser-Doppler flowmetry was used to study the effects of ET-1, a vasoactive endothelin isopeptide, on local cortical microvascular perfusion (CP) and resistance (CVR) in anesthetized rats. The i.v. administration of ET-1 (10-300 pmole) elicited a reduction in blood pressure (BP) and CVR and an increase in CP. A different response profile was obtained when ET-1 was administered at the origin of the internal carotid artery (i.c.). Low doses of ET-1 (10-100 pmole) by this route caused an increase in CP and a reduction in BP and CVR, whereas high doses (≥300 pmole) reduced CP and increased BP and CVR. Occaisionally, high dose/low flow patterns were associated with ischemic changes in the ECoG. The low dose/high flow and high dose/low flow response profile was not altered by blockade of muscarinic and alpha-adrenergic receptors. A mild metabolic acidosis developed following 300 pmole doses (i.c.), but this could not account for any of the cerebrovascular effects observed. Platelet aggregation also did not appear to play a role in the high dose/low flow response. In fact, ex vivo rat platelet aggregation, stimulated with ADP, was inhibited by ET-1 (300 pmole, i.c.). In conclusion, the cerebrovasculature exhibits differential sensitivity to vasodilator and vasoconstrictor effects of ET-1.

17.2

FOCAL ENCEPHALIC MICROVASCULAR ALTERATIONS FOLLOWING CARDIOPULMONARY BYPASS PUMP. D.M.Moody*,M.A.Bell and V.R.Challa*. Bowman Gray Sch. of Med., Winston-Salem, NC 27103.

In the course of a current study using the alkaline phosphatase endothelial stain (Bell and Scarrow, Microvasc Res 27:189-203) to demonstrate cerebral microvascular changes in hypertension, we have had the opportunity to assess four subjects that had recent surgery requiring support from cardiopulmonary bypass pumps.

Some phenomenon is markedly altering small vessels throughout the brain in a way not seen in other (twenty-nine) subjects. The smallest arterioles and capillary network have numerous symmetrical focal dilatations or "bubbles." At this stage we do not know whether these swellings mark the positions of emboli (fat or silicone?) or represent a response of the endothelium to ischemia, emboli, or toxic injury.

Attempts to produce these swellings experimentally and further histochemical procedures to characterize them are planned. Well-documented clinical evidence of subtly reduced mental capacity after surgery assisted by these pumps suggests that careful assessment of hitherto unsuspected cerebrovascular effects is warranted.

17.3

COGNITION-RELATED ALTERATIONS IN REGIONAL CEREBRAL BLOOD FLOW INVESTIGATED BY SINGLE PHOTON EMISSION TOMOGRAPHY. K.J. Shedlack*, R. Hunter*, D. Wyper*, R. McLuskie*, M.T. Hansen*, G. Fink & G.M. Goodwin*. MRC Brain Metabolism Unit, Edinburgh, UK, Wellcome Neuroscience Group, Inst. of Neurological Sciences, Glasgow, UK; McLean Hosp., Harvard Med. Sch., Belmont, MA 02178, USA.

Regional cerebral blood flow (rCBF) patterns were measured at rest and during a cognitively-activated state in which healthy subjects performed a verbal fluency test. As fluency is impaired following frontal lobe lesions, metabolic activation of frontal cortex was expected during this task.

Healthy volunteers and clinical controls were imaged during rest via i.v. ^{99m}Tc-HMPAO in a single photon emission tomography (SPET) split-dose technique for quantification of rCBF. Subsequently, controls were re-imaged at rest while volunteers were re-imaged during oral generation of words beginning with a particular letter.

Volunteers had significantly increased rCBF ($U = 0$, $p < .05$) in two areas of left frontal cortex during the fluency task. Frontal activation was accompanied by a trend towards decreased left posterior rCBF. Alterations in perfusion were not observed the right hemisphere. These data reveal dynamic changes in cortical metabolic demand within the left frontal lobe in association with the performance of a specific cognitive task that is known to depend upon that cortical region.
Support: Wellcome Trust & Ethel Dupont Warren Fellowship

17.4

HUMAN REGIONAL CEREBRAL BLOOD FLOW ALTERATIONS DURING NALOXONE-PRECIPIATED OPIATE WITHDRAWAL: J. Krystal, T. Kosten, S. Woods, J. Seibyl, C. McDougle, L. Price, G. Zubal, P. Hoffer, H. Kleber, D. Charney. Yale Univ. Sch. of Med., CMHC, 34 Park St., New Haven, CT 06508

Regional cerebral blood flow (rCBF) alterations suggestive of changes in regional brain activity were assessed using Single Photon Emission Computerized Tomography (SPECT). **METHODS:** Patients maintained on methadone (20-30 mg./day) and opiate-naive healthy subjects participated in two SPECT scans separated by 1 day. On scan days patients received either placebo or naloxone 0.8 mg., s.c. under randomized double-blind conditions. After symptoms emerged or within 15 min., withdrawal severity was rated and patients were administered 20 mCi of Tc-99m HM-PAO as an rCBF agent. Patients remained at rest in a light- and sound-attenuated room for an additional 5 min., after which, oral clonidine was administered to suppress withdrawal. Approximately 45 minutes later, patients were imaged using a Strichman neuro-dedicated multicrystal camera. **RESULTS:** In the first 6 opiate-dependent patients, naloxone elicited moderate to severe withdrawal. Most patients showed a decrease in frontal and parietal cortical rCBF and an increase in brainstem (pontine) and cingulate rCBF during opiate withdrawal. **COMMENTS:** Pontine activation and frontal inhibition are consistent with the effects of locus coeruleus stimulation on rCBF in animals. These preliminary data are consistent with neuroendocrine data implicating central noradrenergic systems in the human opiate withdrawal syndrome.

17.5

BASAL FOREBRAIN (BF)-ELICITED INCREASES IN CORTICAL CEREBRAL BLOOD FLOW (CBF): SELECTIVE AGE-RELATED IMPAIRMENTS. D. Linville* and S.P. Arneric (SPON: J. Couch), Department of Pharmacology, Southern IL University School of Medicine, Springfield, IL 62702

Electrical stimulation of BF elicited up to 150% increases in cortical CBF via nicotinic receptors (Neurosci. Abstr. 14:488.2 1988), whereas destruction of BF neurons reduced CBF topographically corresponding with loss of cholinergic innervation (Neurosci. Abstr. 13:288.12, 1987). We sought to determine: 1) Is there an age-related impairment of increases in cortical CBF elicited by BF-stimulation? 2) If so, are these impairments regionally selective? Sprague-Dawley rats (3-5 or 24-26 months) were anesthetized (chloralose), paralyzed, artificially ventilated and arterial blood gases controlled. CBF was measured concurrently in parietal cortex by laser-doppler flowmetry (LDF) and the ^{14}C-iodoantipyrine (^{14}C-IAP) technique with tissue dissection. In addition, CBF was quantified bilaterally with ^{14}C-IAP in 12 regions throughout the brain. Unilateral electrical stimulation (100 μA; 50 Hz) of BF elicited ipsilateral increases in CBF (ml/100g/min; % increase above contralateral control) predominantly in frontal (175±22; 106%), parietal (238±63; 162%) and occipital (136±25; 56%) cortices of young rats (N=6). Other regions were relatively unaffected. BF-elicited increases were differentially attenuated in aged rats (N=3):frontal (221±22; 95%), parietal (144±24; 58%) and occipital (106±25, - 8%); resting CBF did not differ between ages (range: 85-115). Frequency and current intensity-response curves measured by LDF indicated that efficacy of the response, but not threshold, was decreased ($p < 0.05$). CONCLUSION: Increases in cortical CBF following electrical activation of neurons originating in or passing through the BF are spared in frontal cortex and markedly impaired in parietal and occipital cortex of aged rats. (Supported by the American Health Assistance Foundation for Alzheimer Disease Research).

17.6

DIMINISHED CBF RESPONSE AND CORTICAL FUNCTION IN DIABETIC VERSUS NON-DIABETIC RATS DURING ACUTE HYPOGLYCEMIA. D.A. Pelligrino,* W.E. Hoffman, A. Sharp,* Dept. of Anesthesiology, Michael Reese Hospital, Chicago, IL

A cerebral microangiopathy (CMA) has been reported in diabetic humans and rats. We investigated the possibility that a CMA in diabetic (D) rats could diminish the compensatory CBF increase normally observed in non-diabetics (ND) in response to hypoglycemia (H) and the possibility that this could affect cerebral cortical function as determined via somatosensory evoked response (SER) analysis. The D rats were used at 6-8 weeks post-streptozotocin (plasma glucose [PG] >25 umol/ml). All rats were studied under conditions of N_2O sedation, paralysis and mechanical ventilation. CBF (microspheres) and SER measurements were made in fasted D rats, prior to and following i.v. insulin, at PG levels of 20, 6.8, and 1.8 umol/ml. Fasted ND rats were studied at 7.7 and 1.7 umol/ml. The D rats showed a >50% attenuation of the CBF response to H in cortical and subcortical tissue when compared to ND rats. This analysis is based on the relative CBF change occuring when going from normoglycemia (N) to H in both groups. SER amplitudes in ND rats during H decreased to 60% of the N level. In D rats, during H, the SER amplitude fell to <15% of the N value and <10% of the value observed prior to insulin. We would conclude that D rats have a diminished cerebrovascular reactivity to H. This can produce a greater limit on cerebral glucose supply during H and a greater impairment of brain function.

17.7

REGIONAL CEREBRAL BLOOD FLOW (rCBF) FOLLOWING BRAIN MISSILE WOUNDING (BMW) IN SURVIVING AND NON-SURVIVING ANESTHETIZED CATS. D. Torbati, A.F. Jacks,* J.F. Davidson,* J.B. Farrell* and M.E. Carey* Dept. of Neurosurg. Louisiana State University Medical Center New Orleans, LA 70112.

Extreme reductions in rCBF may be responsible for acute brain death following BMW. we measured rCBF in 15 structures before and after BMW in 12 pentobarbital anesthetized cats using microspheres technique. 4 cats survived up to termination of the experiments at 90 min post-BMW. 8 non-surviving cats developed permanent apnea between 6 to 42 min post-BMW. Arterial blood pressure (BP), intracranial pressure (ICP), EEG, ECG and respiratory frequency (f) were monitored. In surviving cats 10/15 structures had significant reductions in rCBF 90 min post-BMW. However, residual rCBF were relatively normal (22-54 ml/100g/min). Residual rCBF values found in non-surviving cats a few min before death, were 20-56 ml/100g/min at 5 and 20 min post-BMW. In surviving cats BMW-induced increases in ICP, BP, and decreases in heart rate and f were stabilized within 5-10 min, whereas in non-survivors they were unstable with a continuous reduction in f, indicating brain stem damage. The present data do not support the hypothesis that severe reduction in rCBF is primarily responsible for acute brain death following BMW. However, gradual and significant reductions in rCBF may become a late complicating factor in survivors. Supported by contract No. DAMD17-86-C-6098 LAIR, USMRDC.

17.8

VASOMOTOR REACTION OF CEREBRAL INTRAPARENCHYMAL BLOOD VESSELS FOLLOWING A TREATMENT OF VASOACTIVE AGENTS: CORROSION CAST AND BLOOD FLOW STUDY. K.Nakai,H.Imai*, M.Nakai*,H.Yokote*,S.Hayashi*,N.Komai*. Dept. Neurol. Surg.,Wakayama Med.Col.,Wakayama 640 Japan.

Morphlogical changes of the actual luminal surface of the intraparenchymal blood vessels in the brain were examined following a treatment of potent vasoactive agents such as neuropeptide Y(NPY) and endothelin(ET) both of which have strong vasoconstrictive effect on the pial arteries in the brain. NPY($1x10^{-5}$M) or ET($1x10^{-6}$M) was locally injected into the rat parietal cortex(1 ul,over 10 minutes) through the glass capillary. Monitoring the local blood flow over the injection point of those agents using a laser flow meter(Advance),animals were perfused with aldehyde mixture. Luminal surface of blood vessels in the parietal cortex were observed either by the corrosion cast technic under scanning electron microscope or the dark field light microscope method (Nakai et al.Stroke 12:653 1981). In the NPY injected cortex where the blood flow was lowered to 50-60% of control, apparently smaller diameter of the proximal portions of perforating arteries was noted. Whereas the injection site of ET showed incresed blood flow for 20-40 minutes (30-80%), when marked dilation of the blood vessels were observed around the injection site ,especially in the perforating veins.

These results suggested a selective role of cortical perforating arteries and veins in the regulation of the cerebral cortical blood flow.

17.9

CEREBRAL BLOOD FLOW (CBF) HETEROGENEITY: SPATIAL AND TEMPORAL CORRELATION. S.C. Jones, M. Shea*, J.R. Little*, A.J. Furlan*. Cleveland Clinic Foundation, Cleveland, Ohio, 44195-5070, U.S.A.

A new hypothesis of CBF regulation, vascular cycling, is supported by the following observations: 1) spatial (~400 μm) and temporal (~6/min) heterogeneity of CBF; 2) the vascular architecture of penetrating arteries; 3) the columnar neuroarchitecture of the cortex; and 4) evidence of capillary recruitment. This hypothesis, that intermittently opening and closing vascular units control CBF, is tested by combining morphological (vessel position) and physiological (blood flow) evidence.

In 7 pentobarbital anesthetized, Sprague-Dawley rats with normal physiological variables (MABP 120± 7; $PaCO_2$ 35± 0.4; PaO_2 137± 5; and pH 7.41± 0.01), vessel position was determined *in vivo* with the intravenously administered fluorochrome, thioflavine S (TS). The spatial distribution of CBF was measured using the 10 sec indicator fractionation autoradiographic technique with ^{14}C-iodoantipyrine (^{14}C-IAP). Vessel positions were mapped and aligned with a digitized CBF image or enlarged autoradiogram that indicated high and low areas of CBF. Two time dependent protocols were used: one involved the simultaneous administration of ^{14}C-IAP and TS 10 sec before decapitation; in the other, TS was administered 45 seconds before, and the ^{14}C-IAP 10 sec before, decapitation. The ratios of the number of vessels in the high CBF region over the total number of vessels were compared.

In the protocol using sequential administration, vessels were found in the low CBF areas, indicating that they had been perfused before the CBF study epoch and the high/total ratio± SEM was 54%± 4% (n=4). In the simultaneous protocol, the high/total ratio was 68% ± 3% (n=3) (t-test, $p<0.01$). This supports the concept that the high CBF areas correspond to open vascular units and the low CBF areas correspond to closed or turned-off vascular units.

These observations confirm the hypothesis that one aspect of CBF regulation involves the intermittent opening and closing of vascular units. Further questions concern the dysfunction of this proposed cycling process in focal cerebral ischemia, especially in border zone areas that are at cerebral vascular risk.

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17.10

DISTRIBUTION OF CEREBRAL INFARCTION FOLLOWING MIDDLE CEREBRAL ARTERY OCCLUSION AND FASTIGIAL NUCLEUS STIMULATION IN RAT. M.D. Underwood, S.B. Berger, M. Khayata*, N. Zaiens*, M. Springston* and D.J. Reis, Div. of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021.

Stimulation of the cerebellar fastigial nucleus (FN) reduces the volume of the cerebral infarct produced by occluding the middle cerebral artery (MCA) in spontaneously hypertensive rats (SHRs) (Neurosci. Abstr. 14:1065, 1988). We sought to determine whether the reduction in infarct volume with MCA occlusion and FN stimulation has a specific topography or is randomly distributed. SHRs were anesthetized and the MCA occluded. The FN was stimulated electrically for 1 hour (1 sec on/1 sec off, 50 Hz, 50 μA) following MCA occlusion. 24h later the brains were removed, infarcts reconstructed and the volume computed. In controls (n=6) MCA occlusion resulted in infarction extending from the frontal and parietal sensory-motor cortex rostrally, lateral occipital cortex (OCx) caudally, piriform/insular cortex ventrally and was medially bounded by the lateral half of the caudate-putamen, lateral hippocampus and corpus callosum. The volume of the infarct was 203 ± 11 mm^3, greater than sham-operated controls (1 ± 0.7 mm^3; $p<0.05$; n=4). Electrical stimulation of FN (n=7), but not lateral cerebellar nuclei (n=6), resulted in a 38% reduction in infarct volume to 126 ± 10 mm^3 ($p<0.05$). The area of preservation consistently rimmed the rostral, dorsal and caudal borders of the infarct observed following MCA alone and included frontal, parietal and OCx. We conclude: 1) FN stimulation reduces infarct volume following MCA occlusion and 2) the preserved areas correspond to border zones between different vascular territories. The topography of rescued cerebral cortex following MCA occlusion and FN stimulation suggests that the salvage is mediated by increased local cerebral blood flow to areas with a collateral vascular supply.

17.11

RAT BRAIN ATP RECOVERS AFTER PROLONGED CBF ARREST
J.C. de la Torre, J.K. Saunders,* T. Fortin,* K. Butler,* J. MacTavish,* Div. of Neurosurgery, Univ. Ottawa and National Research Center, Ottawa.

We examined the loss and reappearance of ATP/PCr, in rats subjected to cerebral blood flow (CBF) arrest. Rats were anesthetized, intubated, mechanically ventilated and prepared for bilateral subclavian-carotid artery occlusion (Soc. Neurosci. Abs. 14:1211,88). Both subclavian arteries were tied off and loose snares were placed on each common carotid artery (CCA). A surface coil was fixed to the skull and rats were placed in a Bruker Biospec 4.7 T/30 magnet. Both CCA were then occluded for 5,8,15,25,30 and 70 minutes. P-31 spectra showed energy phosphates and ß-ATP peaks disappeared 8-10 minutes after global CBF arrest. The ATP metabolite Pi rose abnormally as it chemically shifted. Following release of both CCA to reperfuse brain, ß-ATP peak returned to normal levels after a delay of 25 min in rats subjected to 5-8 minutes of CBF arrest. Data from rats subjected to 15-70 minutes of CBF arrest indicated that ß-ATP returned after CCA perfusion but delay took several hours. Spectral findings were supported by EEG and CBF analysis. Results suggest that rat brain neurons can idle metabolically during prolonged absence of cerebral blood flow.

17.12

GLUTATHIONE MEDIATES THE HYDROPHILIC DEGRADATION OF (99mTc) d,l-HEXAMETHYL-PROPYLENEAMINE OXIME (HM-PAO) IN CULTURED BRAIN CELLS.
S.Huck and E.Suess*, Department of Neuropharmacology and Clinic of Neurology, University of Vienna. A-1090 Vienna, Austria.

Following i.v.-injection, (99mTc)d,l-hexamethyl-propyleneamine oxime (HM-PAO) is rapidly taken up by the human brain, with negligible redistribution after 3-5 min. Although cerebral blood flow is the predominant factor determining the accumulation of radioactivity, additional mechanisms regulate the conversion of HM-PAO into hydrophilic, non-diffusible derivatives.

We have studied this phenomenon using cell cultures 4-6 days *in vitro* from dissociated postnatal rat cerebellum. The basic techniques for the determination of uptake kinetics have been presented previously. Briefly, the uptake was determined after a 5 min incubation period with 2.85pM HM-PAO (Ceretec, Amersham) at room temperature. After several washes with PBS cultures were lysed in distilled water. The released radioactivity was counted in a γ-counter.

In this study we analyzed the chemical composition of the radioactivity accumulated in the cultures by HPLC. After filtering through a 10,000mW cutoff filter unit the lysate was injected into a Hamilton PRP1/5µ 4x150mm column. The filtrate yielded a single peak of free (99m)TcO_4 in the HPLC. However, a substantial amount of radioactivity was bound to cellular fragments or high molecular weight compounds of the retentate and thus not accessible to HPLC-analysis.

The uptake of radioactivity was reduced in dose-dependent manner when cultures were pre-treated with ≤5µM $HgCl_2$ or ≤10mM diethyl maleate (DEM). DEM selectively depletes tissue glutathione by a glutathione transferase-mediated reaction, whereas $HgCl_2$ nonspecifically blocks thiol residues. We have measured 3H-2-deoxyglucose (2-DG)-uptake as an indicator of metabolic requirements of the cultures. The 2-DG-uptake was severely impaired by $HgCl_2$ but unaffected after treating the cultures with DEM.

These observations strongly support the hypothesis of a glutathione-dependent reaction crucial for the conversion of HM-PAO into non-diffusible derivatives trapped within the cells, making HM-PAO a feasible agent for SPECT-supported diagnosis.

MENTAL ILLNESS: AFFECTIVE DISEASE

18.1

PLASMA HOMOVANILLIC ACID IN GERIATRIC MAJOR DEPRESSION.
RC Young, GS Alexopoulos*, T Finver*. New York Hospital-Cornell Medical Center, Westchester Division, White Plains, NY 10605

Changes in central dopaminergic turnover have been implicated in both major depression and in normal aging. Differences in plasma homovanillic acid (HVA) between elderly depressed patients and controls were hypothesized.

Inpatients with major depressive episode (DSM III-R) (n=32) and controls without psychiatric disorder (n=11) were studied. All were aged >60 yrs; the average age of the patients was 74.2 yrs ± 6.7 yrs (S.D.) and that of the controls was 77.0 yrs ± 3.8 yrs. Plasma samples obtained in the morning were assayed by high performance liquid chromatography.

Plasma HVA in the patients before treatment averaged 49.2 pmol/ml ± 26.9 pmol/ml and was lower than in controls (79.8 pmol/ml ± 32.5 pmol/ml; t=3.08; p<.005). A subset of 7 patients was sampled again during treatment; plasma HVA increased in 5.

These preliminary findings are consistent with reports of decreased cerebrospinal fluid HVA in some mixed-age depressed patients.

Supported by funds from the Xerox Foundation.

18.2

TWO BEHAVIORAL PARADIGMS FOR STUDYING CORRELATES OF ANXIETY OR DEPRESSION IN RATS: ACTIVITY IN A LIGHT/DARK COMPARTMENT AND THE STARTLE RESPONSE OF OLFACTORY BULBECTOMIZED RATS.
J.V.Cassella+, J.Alexander*, and W.Call*. Neuroscience Program, Oberlin College, Oberlin OH 44074 and +Neurogen Corporation, Branford CT 06495.

Animal models of psychiatric disorders have been instrumental in developing new treatment strategies and compounds as well as gaining an understanding of the underlying physiological mechanisms of the disorders. Using a light/dark chamber, increased crossings between the lighted and dark side and more time spent on the lighted side correlated with anxiolytic treatment while decreases on these measures correlated with anxiety-provoking manipulation. Results obtained in this apparatus were correlated with the Elevated Plus Maze. In olfactory bulbectomized rats, acoustic startle was significantly greater than controls and this hyperreactivity was reversed by chronic, but not acute, imipramine treatment. The change in startle was compared to passive avoidance deficits in these animals. Therefore, activity in a light/dark chamber in rats and the startle response of bulbectomized rats are useful behavioral models for studying anxiety and depression, respectively.

18.3

INTERACTION BETWEEN MONOAMINE OXIDASE-INHIBITORS (MAO-I) AND FLUOXETINE COMPARED TO NON-SELECTIVE UPTAKE-INHIBITORS.
David Calligaro* and David Helton* (Spon. Ilene Cohen). Toxicol. Div., Eli Lilly & Comp., Greenfield, IN 46140.
Studies were conducted to investigate the interactions between fluoxetine (Flu) and MAO-I in the rat. Rats were pretreated with either tranylcypromine or phenelzine (1 to 60 mg/kg p.o.) 30 min prior to Flu or Imipramine (Imi) (0.1 to 10 mg/kg p.o.) administration. A significant potentiation in the median lethal dose of tranylcypromine required 10 mg/kg for Flu or Imi. The potentiation to the interaction was not increased after multiple 5 day dosing of either Flu or Imi (10 mg/kg). While serotonergic behavior was present at lower doses, a general malaise masked this behavior at higher doses. Although the behaviors were unique for each drug combination, all combinations produced a rise in body temperature which correlated with onset of behavioral symptoms. The non-selective 5-HT antagonist cyproheptadine prevented fatal interaction, whereas more selective serotonergic and noradrenergic receptor antagonists did not (e.g. mianserin, ketanserin, piperazine, propranolol, pindolol, phenoxybenzamine). The protective effects correlated with preventing the rise in body temperature. Furthermore, diazepam alleviated the behavioral symptoms, but did not prevent mortality. In conclusion, the non-selective uptake inhibitor Imi produced an interaction with MAO-I at comparable doses to Flu. Also, both interactions result in hyperpyrexia which cyproheptadine was able to reverse.

18.4

PLASMA GABA AND MOOD DISORDERS.
F.Petty and G.L.Kramer*. Dept. of Psychiatry. Univ.Tex.Southwest Med.Cent.Dallas, 4500 S. Lancaster Rd.,Dallas, TX 75216.

During the last 10 years evidence has accumulated to suggest a role for GABA in mood disorders. Briefly, low levels of GABA in CSF have been reported by several investigators and increased density of GABA-benzodiazepine receptor binding sites was found in cortex of depressed suicide victims. Drugs which facilitate GABA transmission such as progabide and valproic acid are useful therapeutic agents for mood disorders. We have previously reported plasma levels of GABA to be low in patients with primary unipolar depression. We have also reported that plasma GABA levels in control subjects are not influenced by diet, exercise, menstrual cycle, or gender. We now report the results of a larger study using high performance liquid chromatography with post-column fluorescence detection. Levels of GABA were significantly low in patients with mood disorders compared to control. Levels of plasma GABA were equally low in bipolar patients whether depressed or manic and in secondary depressives as well as in primary depressives. Plasma GABA levels did not correlate with severity of depression nor did they change with treatment up to 12 months. Taken together, these findings suggest that plasma GABA may represent a stable trait-like marker of mood disorder.

18.5

INCREASED PLASMA α_1-ACID GLYCOPROTEIN IN DEPRESSION: BIOLOGIC, CLINICAL AND DEMOGRAPHIC CORRELATIONS. D.L. Knight, L.R. Meyerson, D. Benjamin*, K.R.R. Krishnan*, D.G. Blazer* and C.B. Nemeroff. Depts. of Psychiat. & Pharmacol., Duke Univ. Med. Ctr., Durham, NC 27710 and Med. Res. Div., Amer. Cyanamid Co., Mahwah, NJ 07430.

Several investigators have reported a decrease in the number of [^{3}H]-imipramine binding sites in platelets of drug-free depressed patients. The plasma concentrations of α_1-acid glycoprotein (AGP), a putative endogenous inhibitor of the platelet receptor site labeled by [^{3}H]-imipramine, is significantly elevated in depressed patients (n=51) as compared to age- and sex-matched controls (n=36). There was a significant positive correlation between plasma AGP concentration and the maximal post-dexamethasone plasma cortisol concentration. There was a positive correlation between AGP concentrations and two measures of depression severity- the Montgomery-Asberg Rating Scale for Depression and the Center for Epidemiological Studies-Depression Scale. Within the depressed population there was a slight decrease in AGP concentration with increasing age. The relationship between AGP concentrations in plasma and the density of platelet [^{3}H]-imipramine binding sites will also be discussed. These data provide evidence of alterations of an endogenous inhibitor of the [^{3}H]-imipramine binding site/serotonin transporter in depressed patients. (Supported by NIMH MH-40159).

18.6

OLFACTORY BULBECTOMY ALTERS PLASMA α-1 ACID GLYCOPROTEIN LEVELS IN RATS. F.J. Arnold and L.R. Meyerson. American Cyanamid Co., Med. Res. Div., Ramapo College, Mahwah, NJ 07430.

In a previous collaborative effort we have shown that α-1 acid glycoprotein (AGP), an endogenous modulator for the tricyclic binding-serotonin transport complex, is increased 29% in plasma of depressed patients compared to controls (Neurosci. Abs. 14:372,1988). To extend this research to animals, a radial immunodiffusion assay to measure plasma AGP levels in rat tissues was developed. Turpentine induced AGP in rats was purified from plasma to homogeneity by chromatographic techniques. Antisera to purified rat AGP was produced in New Zealand white rabbits. Immunodiffusion plates containing the rabbit antisera in a 1% agarose gel solution were prepared. The AGP concentration in ZML:(SD) rats is approximately 6 times lower compared to normal human values of 617 ± 27 μg/ml. In five week olfactory bulbectomized ZML:(SD) rats, an animal model of depression, a 36% increase in plasma AGP levels was observed compared to sham operated animals (140 ± 8 and 104 ± 4 μg/ml, respectively). Evidence mounts from animal and human studies suggesting that increased plasma AGP may be a biological marker in depression.

18.7

CAUDATE NUCLEUS METABOLISM RELATIVE TO PREFRONTAL CORTEX SPECIFICALLY DISTINGUISHES BIPOLAR FROM UNIPOLAR DEPRESSION. J.M.Schwartz*,L.R.Baxter*, M.Phelps,J.Mazziotta,B.Guze* (SPON:D.X.Freedman) UCLA Sch. of Med., Los Angeles, CA 90024.

Prior work by us using PET demonstrated unipolar depressed (UD) patients show a decreased metabolic rate (MR) in head of caudate nuc. (Cd) relative to ipsilateral hemisphere (Hem),compared with bipolar depressed (BD)patients, and the MR of Cd relative to prefrontal cortex (PFC) mainly accounts for these differences (Schwartz, et. al. JAMA,258:1368, 1987). We extended this work in 10 UD and 10 BD, matched for age, sex,handedness and severity, and 12 matched normal controls (NC).

Cd/hem ratios were decreased in UD compared to BD and NC (Left ANOVA:F=7.85;df=2,29;P=.002; Rt: F=7.92;df=2,29;P=.002). Anatomical analysis calculating Cd MR relative to mid. and inf. frontal gyr.(MFG, IFG), orbital gyri (OG), ant. cingulate gyr (ACG), parahippocampal gyr (PHG),and thalamus (T) demonstrated that only Cd/PFC (PFC=MFG,IFG,OG) ratios sig.($p<.05$) distinguish BD and UD, with UD lower in all cases. Cd MR relative to ACG, PHG, and T did not show sig. differences between UD and BD. Cd/PHG values for UD and BD were identical, and were decreased ($p<.05$) from NC.

These data may indicate relevance of Cd-PFC relations to differing pathophysiology in UD and BD.

18.8

SLEEP DEPRIVATION CHANGES ARDEN RATIOS IN DEPRESSED PATIENTS. K. N. Sokolski*, C. Reist* and E. M. DeMet. Dept. Psychiatry, Long Beach VA Med. Center, Long Beach, CA 90822

One night of sleep deprivation produces significant, but temporary, mood improvements in about two out of three depressed patients. While the mechanism of this improvement is unknown, some evidence suggests a possible dopaminergic role in this action. The Arden ratio (eye potential in bright light divided by eye potential with dark adaptation) is a putative non-invasive measure which may reflect dopaminergic sensitivity. A previous study has shown decreased Arden ratios in depressed patients. The present study examined Arden ratios of depressed patients prior to and following a night of sleep deprivation. Patients were divided into two groups on the basis of symptom severity. A high group had Hamilton depression scores of $\geq$25; and a low group had scores of $\leq$10. Baseline Arden ratios of the high group were significantly lower than either the low group or normal controls. Sleep deprivation significantly improved depressive symptoms and increased Arden ratios of the high group. An inverse relation between clinical response and Arden ratios was also evident in low group responders. In contrast, no change in the Arden ratio was found in non-responders. The results support a possible dopaminergic role in the mechanism of sleep deprivation and underscore the possible usefulness of Arden ratios as a diagnostic marker of depressive illness.

18.9

DEPRESSION LEVELS AMONG HIGH SCHOOL STUDENTS J. W. Jones,* L. C. Parsons, and L. J. Crosby* (spon: L. L. Rankin). College of Nursing, Univ. of Arizona, Tucson, AZ 85721

Suicide is one of the second leading causes of death among young people between the ages of 15 and 24 years. Thus, identification of depression should be very important to teachers, school administrators and families. The purpose of this study was to assess depression levels in a randomized stratified sample of middle class high school students. The sample was composed of 249 high school students between the ages of 14 and 18 years. The depression level of the sample was assessed using the Beck Depression Inventory (BDI). Demographic variables, factors which caused disturbed feelings and self identified priorities were also measured. Frequency counts and descriptive statistics were used to analyze the data. Findings: BDI scores for the total sample revealed that 191 (76.7%) of the subjects were within the range of "no depression", 22 (8.8%) subjects were within the "middle" range of depression and 17 (6.9%) were within the "high" range of depression. Nineteen subjects were eliminated since they did not answer all of the BDI questions. Black subjects were significantly more depressed than white subjects. Orientals were also more depressed than white but the difference was not significant at the $p = .05$ level. Also girls were more depressed than boys but not at significant levels. "Family life" was identified as the factor which caused the most disturbed feelings (40.2%) while "friends" were identified as the highest priority in the total sample. When the sample was split according to depression levels, and as depression levels increased, "family life" was more frequently identified as a disturbing factor. Peer acceptance was not selected by a single subject within the severely depressed group indicating, perhaps, a withdrawal pattern which should be noted by teachers and families. School assumed greater importance as the subject became more depressed as did boyfriend/girlfriend relationships. In summary, of this sample of 249 high school students, 15.65% were moderately to severely depressed. These findings suggested that numerous students could have profited from emotional support and/or counseling.

1. Study supported by NINDS NS24169

18.10

TRAIT AND STATE CEREBRAL BLOOD FLOW ABNORMALITIES IN DEPRESSION. WC Drevets, ME Raichle, PT Fox, SH Preskorn, TO Videen Washington Univer.St. Louis MO 63110

The neuroanatomical correlates of depression were investigated with PET measurements of regional cerebral blood flow (rCBF). Two unmedicated study groups were selected: 1) (n=7) with unipolar major depression - melancholic subtype in the depressed phase (UPD) and, 2) (n=8) with a history of the same diagnosis, in the euthymic phase (UPE) Appropriate controls were selected for each study group (n=13 and n=14, respectively). PET studies were performed using intraveneously administered $H_2{}^{15}O$, a 40 second emission scan, and the eyes closed rest state. For omnibus testing, primary tomographic images were converted to anatomically standardized 3-dimensional images. rCBF Differences were identified using intra-group image averaging and inter-group subtraction; these were subjected to post hoc statistical analysis. No global CBF differences were found between groups. The UPD subjects demonstrated increased rCBF in the inferior frontal gyrus pars orbitalis (f_3-orb) bilaterally, (left>right) and in the left frontal polar cortex, (LFPC) and decreased rCBF in the left caudate in the UPD group. The UPE subjects demonstrated the same rCBF increase in LFPC and decrease in the left caudate, but the rCBF in f_3-orb did not differ from controls. In addition, the UPE group demonstrated increased rCBF in the left anterior cingulate gyrus(LACG). Our results suggest that persons susceptible to unipolar depression have a trait abnormality manifested by decreased rCBF in the left striatum,and increased rCBF in LFPC and LACG. In the depressed state, they additionally demonstrate increased rCBF in f_3-orb. This suggests that the frontal cortico-striato-pallidal loop may be involved in the pathophysiology of unipolar depression.

19.1

ASCENDING THALAMIC PROJECTIONS IN THE SILVER LAMPREY, ICHTHYOMYZON UNICUSPIS. Helmut Wicht* and R. Glenn Northcutt, SIO Neurobiology Unit, Univ. Calif. San Diego, La Jolla, CA, 92093

Attempts to identify telencephalic homologues in lampreys have a long tradition in neurobiology but experimental studies are limited to secondary olfactory projections (Northcutt and Puzdrowski, Brain Behav. Evol. 32: 96-107). This study suggested that the lateral pallium (lp) dominates the cerebral hemispheres and that the dorsal (dp) and medial pallium (mp) occupy an uneverted portion of the telencephalon. This hypothesis can be tested in part by the examination of the ascending projections of the dorsal thalamus (dth). Injections of the flourescent tracer DiI into the dth of the fixed brains of 4 lampreys revealed extensive terminals in the ipsilateral mp and a few fibers and terminals in the contralateral mp. Numerous varicose fibers were seen in the neuropil lateral to the ipsilateral striatum (st) and among the cells of the septum. A number of fine varicose fibers were labelled throughout the ipsilateral lp and dp. A few retrogradely labelled cells occurred in the ipsilateral lp, dp, and st, whereas many labelled cells were present in the ipsilateral mp. Injections in the mp and lp confirm that the dth projects to these telencephalic areas. The reciprocal connection between the mp and dth supports the hypothesis that there is a medial pallial homologue in lampreys but the sparse thalamic projection to other pallial areas as well as the absence of a distinct terminal field adjacent to the st require further attention.

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19.2

CENTRAL REPRESENTATION OF THE VII, IX AND X CRANIAL NERVES IN GNATHONEMUS PETERSII. G. Lazar*, T. Szabo, S. Libouban*, M. Ravaille-Veron* (SPON: C. Oyster). Dept. of Sensory Neurophysiology, CNRS, F-91198 Gif/Yvette, Cedex and (G.L.) Anat. Inst., Medical School, Pécs, Hungary.

The three cranial nerves were investigated by axonal transport of HRP and Cobaltous-Lysine in order to determine their origins and their projections into the CNS. Retrograde labelling shows that the motoneurons of the IX and X nerves form a continuous column located ventrally to the sensory vagal lobe; the columns of both sides fuse in a single nucleus at the level of the apex of the IVth ventricle. The VII motor nucleus forms a separate nucleus located more ventrolaterally in the reticular formation between the glossopharyngeal and trigeminal motor nuclei. The sensory lobe, into which the three nerves project, forms a continuous volume located below the floor of the IVth ventricle. The X sensory fibers originating from five branches (4 from the gills and one from the oesophagus) terminate in the caudal two thirds of the sensory lobe. The IX sensory fibers arise from the first gill nerve and project to the anterior pole of the sensory lobe,whereas those of the VII nerve run with the trigeminal root and end cranial to the glossopharyngeal terminal area.

19.3

NEURAL SYSTEMS FOR CLASPER CONTROL IN STINGRAYS. L.S.Demski, L.Qin* and H.J.Wynder*. School of Biological Sci. Univ. of Kentucky, Lexington, KY 40506.

The innervation of the clasper has been studied in several rays with emphasis on Urolophus halleri. Several large myelinated nerves (ca. 10 at diameters approx. .7mm; #56-65 counting from the vagus) innervate the clasper muscles and skin. Although the same number of nerves are present (from vagus to level of posterior pelvic fin) in females they appear to be smaller in cross sectional area. In males, low level electrical stimulation (<150μA) of the nerves evokes clasper movements including: rotation, elevation, medial and lateral extension and opening. Stimuli as low as 20uA were effective in triggering opening from nerves 61-63. The responses are abolished by xylocaine. Stimulation of the spinal cord in the area of the roots of the latter nerves also evoked the movements (<100μA with best freq. 3-10 Hz). Cobalt filling of nerves 62 & 63 confirmed that motor neurons and sensory components of the nerves are at the levels indicated by stimulation.

19.4

LOCATION OF SEROTONIN-LIKE IMMUNOREACTIVE NEURONS IN TWO BATOID ELASMOBRANCH FISHES: MYLIOBATIS CALIFORNICUS AND PLATYRHINOIDIS TRISERIATA. S.L. Stuesse, W.L.R. Cruce, and R.G. Northcutt. Neurobiology Dept., Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272 and Dept. of Neuroscience, University of California at San Diego, La Jolla, CA 92093

The reticular formation is a phylogenetically old and conservative portion of the brain. Variations in reticular nuclei, when they occur, may be used to test hypotheses of relationships among vertebrates. We are studying elasmobranchs as a group of fish which diverged from other vertebrates about 350-400 million years ago. There is considerable brain variation among batoid elasmobranchs: the thornback guitarfish, Platyrhinoidis triseriata has a relatively smaller cerebellum and telencephalon than the bat ray, Myliobatis californicus. Brain sections were incubated with antisera made in rabbit against serotonin. The avidin-biotin or PAP method was used to visualize cells. In both fish, a presumptive raphe pallidus, obscurus, magnus, and centralis superior could be identified. In Myliobatis, however, raphe pallidus was much smaller than in Platyrhinoidis. Myliobatis had much heavier labeling in the posterior thalamus and the nucleus interpeduncularis. These 5-HT+ cells extended into the ventral tegmental area ventral and lateral to the red nucleus. In both fish, there were numerous 5-HT+ fusiform cells paralleling the ventral surface of the metencephalon and mylencephalon. These were located in several reticular nuclei but were especially prominent in reticularis magnocellularis and reticularis paragigantocellularis lateralis. The homologue of raphe pontis, if present, is very small and no raphe dorsalis was identified. Supported in part by N.I.H. grants NS25895, NS24669, and NS24869.

19.5

IMMUNOCYTOCHEMICAL AND HISTOCHEMICAL EVIDENCE FOR TWO NEUROTRANSMITTER SYSTEMS IN THE ELASMOBRANCH NERVUS TERMINALIS GANGLION. J. White* (SPON: M. Meredith). Dept. of Biological Sciences, Florida State University, Tallahassee, FL 32306-3050.

Previous electrophysiological data from this laboratory suggest that acetylcholine and norepinephrine suppress the activity of elasmobranch nervus terminalis (NT) ganglion cells. We are conducting anatomical experiments to further investigate the neurotransmitters and neuronal circuitry of the elasmobranch NT ganglion. Bonnethead sharks (*Sphyrna tiburo*) were perfused with 1% paraformaldehyde in phosphate buffer for 30-45 min. This light fixation was necessary for successful acetylcholinesterase (AChE) histochemical staining. Cryostat sections were first processed for double-label peptide immunocytochemistry (ICC) using 1:500 dilutions of rabbit anti-LPLRFamide (gift of Dr. G. Dockray) and guinea pig anti-LHRH (gift of Dr. L. Jennes) antisera, followed by a secondary antiserum mixture of goat anti-rabbit (Texas Red) and goat anti-guinea pig (FITC). Sections were then processed for AChE histochemistry (Karnovsky & Roots, 1964). Separate populations of LPLRFamide-immunoreactive (ir) and LHRH-ir ganglion cells were seen. Although both ganglion cell types were AChE-positive, the LPLRFamide-ir cells stained more darkly for AChE. In some experiments with long ICC incubation times, the LHRH-ir cells did not stain for AChE. Staining with anti-tyrosine hydroxylase (TH) antiserum (Eugene Tech) revealed fibers and terminal-like puncta in the NT ganglion but no cell bodies, suggesting a catecholaminergic innervation. Experiments are underway to determine which of the cell types in the ganglion are contacted by TH-ir terminals. The anatomical data presented here therefore support the electrophysiological data, suggesting that two neurotransmitter systems are present in the NT ganglion.

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19.6

SECONDARY OLFACTORY PROJECTIONS IN THE ATLANTIC STINGRAY. R.L. Puzdrowski and R.B. Leonard. Marine Biomed. Inst., Univ. of Tex. Med. Branch, Galveston, TX 77550.

In the classic descriptions of the forebrain, the secondary olfactory tracts were said to distribute fibers to most regions of the telencephalon. In batoids, more recent studies utilizing degeneration techniques have revealed the secondary olfactory projections to be restricted to the lateral pallium. To test these more recent observations horseradish peroxidase was injected unilaterally into the olfactory bulbs of five adult Atlantic stingray, Dasyatis sabina. After survival times of 5 to 15 days, the brains were removed, sectioned at 40μm in either the transverse or horizontal plane, and processed according to Mesulam's ('78) TMB protocol. The distribution of the olfactory tract fibers is entirely ipsilateral to the injected bulb, the majority terminating in the rostral portion of the lateral pallium. Distinct medial and lateral olfactory tracts could not be distinguished. However, a small number of fibers were observed exiting the dorsomedial portion of the olfactory tract and coursing caudomedially into area SP4 of the subpallium (Smeets et al., '83). These results suggest the secondary olfactory projections in rays may be less restricted than studies utilizing degeneration methods have indicated. Supported by NS11255-14 and NS07185-07.

19.7

THE DEVELOPMENT OF PURKINJE CELLS AND THEIR EXTRACEREBELLAR PROJECTION TO THE ACOUSTICO-LATERALIS AREA IN THE WEAKLY ELECTRIC TELEOST *EIGENMANNIA* (GYMNOTIFORMES), REVEALED BY THE MONOCLONAL ANTIBODY ZEBRIN II. (SPON: P. Jackson) M. Lannoo[1]*, H. Vischer[2]*, L. Maler[3], G. Brochu[1], R. Hawkes*[1], 1)Lab. Neurobiology, Laval Univ., Quebec, P.Q. Canada G1K 7P4, 2) Scripps, La Jolla, CA 92093; 3) Univ. Ottawa, Ont. K1H 8M5.

The teleost cerebellum consists of three parts, the corpus (CCB), valvula (VA), and eminentia granularis (EG). The monoclonal antibody zebrin II recognizes a single polypeptide antigen of apparent molecular weight 36kD, and selectively reacts with Purkinje cells in CCb, lateral VA, and EG anterior in many teleost species. We use zebrin II to identify Purkinje cells and their neurites within these cerebellar divisions in the developing brain of *Eigenmannia*. We also describe the development of the descending projections of EGa and ventral CCb Purkinje cells to the mechanosensory medial nucleus and the dorsal octavolateral nucleus within the brainstem. Purkinje cells in the dorsolateral portion (probably EGa) of the rostral hindbrain first become immunoreactive at day six; about four cells are stained. Cells in the dorsal CCb first stain at day eight. Axons of the extracerebellar projection appear at day 10 from a cell population that includes CCb and EGa cells. Axons are visible caudally in the acousticolateralis area of the brainstem at day 12. Lateral VA cells do not immunoreact until day 29. These data suggest the presence of separate Purkinje cell growth modes for the three cerebellar divisions. The development of zebrin II expression in this teleost does not follow the pattern of antigenicity in the developing rat cerebellum. Unlike rats, in *Eigenmannia* only cells that are zebrin II$^+$ in adults react during development.

19.8

DIFFERENT ELECTROSENSORY PATHWAYS TO THE TELENCEPHALON IN SILURIFORM TELEOSTS, MORMYRID TELEOSTS AND CARTILAGINOUS FISHES. Georg F. Striedter, Dept. of Neurosciences, University of California, San Diego; La Jolla, CA 92093.

Electroreception has evolved independently in cartilaginous fishes, mormyrid teleosts, and siluriform teleosts. The central nervous pathways for electroreception are similar up to the midbrain in all three taxa. An electrosensory pathway to the telencephalon is known for cartilaginous fishes, but not for any of the teleost taxa. This study anatomically describes a telencephalic electrosensory pathway in two siluriform species (a catfish, Ictalurus punctatus, and a gymnotoid, Apteronotus leptorhynchus). This pathway involves two diencephalic nuclei, versus one in cartilaginous fishes, and is not found in the mormyrid teleost examined (Gnathonemus petersii). This suggests that, while there may be stringent constraints on how to construct an electrosensory system up to the midbrain, there are at least three different ways of doing so at forebrain levels.

In siluriform teleosts the lateral preglomerular nucleus was injected in fixed brains (4% paraformaldehyde) with the carbocyanine dye DiI. After 6-45 days the brains were cut on a vibratome and counterstained with m-phenylenediamine (see Quinn and Weber, '88, Soc. Neurosci. Abstr.). Retrogradely labeled were cell groups in the diencephalon which receive an input from the electrosensory midbrain. Anterogradely labeled were two areas (Dm and Dd+Dld) in the telencephalon (see also International Congress for Neuroethology, '89, abstract in press). In mormyrids DiI was injected into the portion of the lateral preglomerular nucleus which projects to the telencephalon (Wullimann and Northcutt, Soc. Neurosci. Abstr., '87). There were no cells retrogradely labeled in the diencephalic nuclei which receive an input from the electrosensory midbrain in mormyrids.

This work was supported by an NIH training grant fellowship (GM08107) to G.F.S. and an NIH research grant NS24669 to R.G. Northcutt.

19.9

IMMUNOHISTOCHEMICAL ASPECTS OF NUCLEUS OLFACTORETINALIS (TERMINAL NERVE) IN MORMYRID AND GYMNOTID FISH.

T.SZABO, J.P.DENIZOT*, S.BLAHSER*, M.RAVAILLE-VERON*, D.ROUILLY*. Dept. Neurophysiol. Sens., Lab. Physiol. Nerv., CNRS, F-91198 Gif/Yvette Cedex.-Anatomie und Zytobiol. Univ. Giessen GFR.

The nucleus olfactoretinalis (nor) was identified with immunohistochemical methods in the mormyrid *Gnathonemus petersii* and in several gymnotid species, *Hypopomus* sp., *Gymnotus carapo*, *Ramphychthys rostratus*, *Apteronotus leptorhynchus*, *Eigenmannia* sp. and *Sternopygus* sp. Application of anti-substance P (sP), anti-FMRF and anti-LH antisera showed that, among the seven species, *Hypopomus* and *Sternopygus* have a defined nor whereas in the others, individual neurons scattered along the olfactory nerve and the ventro-medial telencephalic surface, constitued the nor. In all species the nor somata as well as their processes were FMRF immunoreactive(IR). The nor in *Hypopomus* was LH-IR but neither in *Sternopygus* or in *Eigenmannia*. The nor was not sP-IR in any of the species. However, besides, the FMRF-IR elements, a particular sP-IR structure was found in the olfactory nerve and bulbe of *Eigenmannia*, which extends from the olfactory epithelium to the dorsomedial pole of the olfactory bulbe.

19.10

SEGMENTAL TEMPLATE FOR RETICULOSPINAL ORGANIZATION. R.K.K. Lee and R.C. Eaton. Neuroscience Group, Univ. of Colorado, Boulder, CO 80309-0334.

The organization of the reticulospinal (RS) system has been conserved over the course of vertebrate evolution. Furthermore, Kimmel et al. '82 and Metcalfe et al. '86 (J. Comp. Neurol. 205:112 and 251:146) have demonstrated that the larval zebrafish contains at least 27 different types of RS neurons organized within 9 serially repeated segments. We have asked whether discrete types of larval RS neurons are retained and distinguishable in the mature brain. Our study, based on retrograde transport of HRP from the spinal cord in adult zebrafish and goldfish, shows 7 segments. In addition, at least 8 of the larval cell types have been found: RoR1, RoL1, RoV, RoM, MiD2cm, MiM1 and CaD (as well as two other midbrain types). We suggest that the preservation of this organization demonstrates a template which may underlie the organization of the adult RS system across a wide range of vertebrates. [Supported by NIH grant NS22621].

19.11

NON-PERCOMORPH TELEOSTS POSSESS A HOMOLOGUE OF NUCLEUS GLOMERULOSUS. M. F. Wullimann and R. G. Northcutt. UCSD, Dept. of Neurosciences A-001, La Jolla, CA 92093.

In derived percomorph teleosts a complex nucleus glomerulosus (NG) receives input from two visually related pretectal nuclei (nucleus corticalis, NC; nucleus intermedius, PSi) and projects to the hypothalamic inferior lobes (LI); PSi receives afferents from the retinofugal nucleus parvocellularis (PSp). More primitive teleosts lack NG but have a more simply organized posterior prectectal nucleus (PO). The carbocyanine dye DiI was applied to specific sites in paraformaldehyde fixed brains of the non-percomorph teleost Osteoglossum bicirrhosum in order to study the connections of PO. After 10-15 days of incubation at 40°C the brains were sectioned with a vibrotome at 50µm. Injections into PO (4 cases) showed that it receives ipsilateral input from NC and projects heavily to the ispilateral LI. After injections into LI (2 cases) neurons in PO were labeled. Injections into PSp (2 cases) revealed a bilateral terminal field in the rostral portion of PO. These results suggest that the rostral portion of PO is homologous to PSi and the caudal portion of PO is homologous to NG of percomorph teleosts. Supported by the Swiss National Science Foundation and NIH grants NS24869 and NS24669.

19.12

PHYLOGENY OF SOME PRETECTAL VISUAL NUCLEI IN TELEOSTS. Ann B. Butler, Mario Wullimann and R. Glenn Northcutt. I.T.N.I., 4433 N. 33rd St., Arlington, Va. 22207; Georg-August-Univ., Zentrum Anat., Kreuzbergring 36, D-3400 Göttingen; Scripps Inst. Oceanog. and Dept. Neurosci., A-001, Univ. Ca. San Diego, La Jolla, Ca. 92093.

In many derived euteleosts 3 prominent pretectal nuclei are present: pretectalis superficialis pars intermedius (PSi), glomerulosus (NG) and corticalis (NC). Their phylogeny has been uncertain. Recent work in Osteoglossum (O.) on a large nucleus--posterior pretectal (PO), to which NC projects--suggests that PO is homologous to both PSi and NG (Wullimann and Northcutt, '89). A cladistic analysis of cytoarchitecture in various teleosts was done. The large PO in O. has a rostral neuropil with cells. We conclude that an L-shaped PO with distal portions connected by neuropil in elopids, clupeids and primitive euteleosts is homologous to the PO in O. and to PSi plus NG, and that a PO nucleus with subdivisions is the plesiomorphic condition for teleosts. Whether the relatively enlarged PO in O. is plesiomorphic or apomorphic cannot presently be resolved. The separate nuclei PSi and NG are apomorphic. Further, in primitive euteleosts NC cells lie within the ventral quadrant of the central tectal zone; the NC cell plate may thus be a specialized, migrated sub-population of tectal neurons.

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20.1

NUCLEI WHOSE FIBERS CO-LOCALIZE WITH THE SUBSTRATE FOR MEDIAL FOREBRAIN BUNDLE (MFB) SELF-STIMULATION. C.R. Gallistel, P.W. Glimcher and R.R. Miselis. Institute of Neurological Sciences, University of Pennsylvania, Philadelphia, PA 19104.

Our earlier work has identified two MFB self-stimulation sites in rats connected by continuous reward-relevant fibers (*Soc Nsci Abstr. 18:443.7*). In this study nuclei which contribute fibers to both of these sites were identified as candidate nuclei of origin for the MFB self-stimulation system. True Blue, a retrogradely transported tracer taken up by fibers of passage, was injected into either the medial lateral hypothalamus or the dorsal anterior ventral tegmental area (VTA). Only nuclei labeled by injections into both sites could give rise to the continuous reward relevant axons which connect these sites. The vertical limb of the diagonal band, the bed nucleus of stria terminalis, the lateral preoptic area, the paraventricular nucleus of the hypothalamus, the dorsomedial hypothalamic area, the arcuate/VTA region, the parabrachial nuclei and the dorsal and median raphe, ipsilateral to the site of injection, all contribute fibers to both of these self-stimulation sites. We conclude that one of these nuclei may contain the cells of origin for the directly stimulated substrate for self-stimulation

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20.2

DORSOMEDIAL HYPOTHALAMIC NEURONS GIVE RISE TO MOST OR ALL OF THE SUBSTRATE FOR MEDIAL FOREBRAIN BUNDLE (MFB) SELF-STIMULATION. P.W. Glimcher and C.R. Gallistel. Institute of Neurological Sciences, University of Pennsylvania, Philadelphia, PA 19104.

Work presented in the preceding abstract identified 8 areas likely to give rise to the substrate for MFB self-stimulation. Other investigators have lesioned five of these nuclei without attenuating the rewarding efficacy of MFB self-stimulation. The effect of lesions of the three remaining nuclei on self-stimulation were examined. We report here that electrolytic or ibotenic acid lesions of the dorsomedial hypothalamic area (DMHA) of the rat permanently attenuates the rewarding efficacy of dorsal anterior VTA stimulating electrodes by up to 90%. Electrolytic lesions of the paraventricular nucleus or the posterior arcuate/anterior ventral VTA border have no effect on the rewarding efficacy of stimulation. We conclude that a substantial fraction of the substrate for MFB self-stimulation has its origin in the cell bodies of the DMHA.

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20.3

AFFERENTS AND EFFERENTS OF THE DORSOMEDIAL HYPOTHALAMIC AREA (DMHA) IDENTIFIED USING CT-HRP, AN EXAMINATION WITH REGARD TO SUBSTRATES FOR SELF-STIMULATION. R.R. Miselis and P.W. Glimcher. Institute for Neurological Sciences, University of Pennsylvania, Philadelphia, PA 19104.

Rats received 20nl injections of cholera toxin conjugated horseradish peroxidase into the DMHA. Afferents were identified in the medial prefrontal cortex, lateral septum, bed nucleus of stria terminalis, medial preoptic area, hypothalamic paraventricular nucleus, amygdala, subiculum, parabrachial nuclei, central gray and periventricular thalamus. Efferents include the lateral septum, bed nucleus of stria terminalis, lateral habenula, amygdala and central gray. These findings confirm and extend earlier work (Ter Horst & Luiten, *Brain Res Bull.* 16: 231-248).

Two principal descending pathways terminate in the central gray. The more ventral of these pathways precisely overlaps the lowest threshold self-stimulation sites of the midbrain. The anatomical circuit(s) in which the DMHA participates are colocalized with many, if not most, of the behaviorally identified sites for self-stimulation. These data support the conclusion that some of these anatomically identified circuits subserve self-stimulation reward processing. Historical data reevaluated in light of this hypothesis further support this conclusion.

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20.4

MAPPING THE SUBSTRATE FOR BRAIN STIMULATION REWARD IN THE RAT BY MEANS OF CURRENT-NUMBER TRADE-OFF FUNCTIONS. M.L. Forgie and P. Shizgal. CSBN, Dept. of Psychology, Concordia University, Montreal, Quebec H3G 1M8.

In previous studies, the spatial distribution of reward-related neurons has been estimated from across-site changes in the current or number threshold for intracranial self-stimulation (ICSS). We combined these two methods by deriving, at each stimulation site, the trade-off between the current and number of pulses required to sustain a constant level of performance. Thus, we could assess the dependence of depth profiles derived from current or number thresholds on the arbitrary choice of the second parameter.

Using moveable electrodes aimed at the ventral tegmental area, we found some sets of current-number trade-off functions that were roughly parallel in log-log space. Hence, the shape of depth profiles obtained by sectioning these functions was independent of the axis or location of the plane of section. In contrast, trade-off functions for other subjects were non-parallel and sometimes intersecting. Depth profiles derived from these functions depended both on the axis of the plane of section and on its location.

These results suggest that trade-off functions should be substituted for single-parameter threshold estimates in order to map the sensitivity of ICSS sites in a manner that is less dependent on arbitrarily-chosen stimulation parameters.

20.5

REFRACTORY PERIODS OF SINGLE NEURONS INCREASE WITH PULSE DURATION: IMPLICATIONS FOR INTERPRETING PSYCHOPHYSICALLY-DERIVED ESTIMATES. K.L. Conover*, D.C. Schindler*, P.-P. Rompré and P. Shizgal. CSBN, Dept. of Psychology, Concordia University, Montreal, Quebec H3G 1M8.

The slope of behaviorally-derived curves describing the time course of recovery from refractoriness in neurons mediating intracranial self-stimulation (ICSS) decreases with the pulse duration. Bielajew et al. (Behav. Brain Res., 1987, 24, 233) attributed this to recruitment of small fibers by the longer duration pulses. Alternatively, accommodation during long-duration pulses and/or a delay in post-firing repolarization might delay recovery in a fixed population of fibers.

Extracellular recordings of action potentials were obtained in rats from forebrain neurons antidromically driven by stimulation of the MFB. In all 21 neurons tested at currents 1.2 times threshold, refractory period (RP) estimates increased (mean = 66%) as the duration of the pulses was lengthened from 0.1 to 2.0 msec. Ten of these cells were also tested at twice-threshold currents. The RP estimates again increased with the pulse duration, but the magnitude of this increase was smaller (mean = 56%).

Given that the RP of each neuron increased with pulse duration, the decrease in slope of psychophysically-derived RP curves as a function of increasing pulse duration cannot be unambiguously attributed to the recruitment of an additional population of neurons.

20.6

THE EFFECTS OF SEROTONERGIC ANTAGONISTS ON REWARD INDUCED BY DORSAL RAPHE ELECTRICAL STIMULATION. P. BAUCO* and P.P. Rompré (SPON: J.Y. Montplaisir). Psychology Dept., Concordia University, Montreal, Quebec, H3G 1M8.

The present experiment was aimed at investigating the contribution of the serotonergic neurotransmission to the rewarding effect of dorsal raphe electrical stimulation. Changes in the function relating the rate of responses to the stimulation frequency and in the asymptotic rate of responses were measured after systemic administrations of mianserin (0.1, 1 and 10 mg/kg), pirenperone (0.032, 0.1 and 0.32 mg/kg), ketanserin (1, 3.2, 10, 16.4, 24.3 and 36 mg/kg) and ICS 205930 (0.1, 1 and 10 mg/kg). Both the 5-HT3 (ICS-205930) and the mixed 5-HT1/5-HT2 (mianserin) antagonists produced no change in the rate-frequency function and in the asymptotic rate of responding. However pirenperone and ketanserin, the more selective 5-HT2 antagonists, shifted the rate-frequency function by 0.2 log units and decreased the asymptotic responses by less than 20%. The 0.15 log units shift produced by 16.4 mg/kg of ketanserin was not significantly reduced by a pretreatment with 5 mg/kg of the 5-HT2 agonist, DOI; at this dose, DOI alone had the same effect as ketanserin and shifted the function towards higher stimulation frequencies. These results suggest that serotonin, through the 5-HT2 but not the 5-HT1 and 5-HT3 receptors, can modulate the rewarding effect of dorsal raphe stimulation.

20.7

SINGLE UNIT ACTIVITY IN THE NUCLEUS ACCUMBENS OF THE FREELY MOVING RAT DURING MEDIAL FOREBRAIN BUNDLE SELF-STIMULATION. M. Wolske, P-P. Rompre, R.A. Wise, and M.O. West Dept. of Psychology, Rutgers Univ., New Brunswick, NJ 08903 and Dept. of Psychology, Concordia Univ., Montreal, Quebec, Canada.

Previous studies have suggested an involvement of the mesocorticolimbic dopamine system in the reinforcing effects of medial forebrain bundle (MFB) self-stimulation. The present study used extracellular single unit recordings in the nucleus accumbens of the awake, freely moving rat to determine the effects of MFB stimulation on accumbens neurons. Male Long-Evans rats (300g) were permanently implanted with microwires (25 micron diam.) in the nucleus accumbens and ipsilateral stimulating electrodes in the MFB and the fimbria. Verification that recorded units were accumbens neurons was achieved by stimulating the fimbria to evoke unit discharges at monosynaptic latencies (4-11 msec). Rats were trained to lever-press using a train (200-400 msec duration) of MFB pulses (0.1-0.5 mA, 0.1-1.0 msec) as the reinforcer. Intermittent schedules of reinforcement were used, enabling a comparison of reinforced to non-reinforced responses. Interpulse intervals (20-30 msec) were chosen to allow analysis of unit activity evoked by each pulse in the train. Preliminary results (N=73 units) suggest that firing rates decreased (x = -12%) during the MFB train, compared with a period of comparable duration immediately preceding the train. Conversely, in the same time frame following non-reinforced lever presses, firing rates increased (x = 29%). This difference could not be accounted for by factors such as changes in motor behavior, sensory cues, or behavioral state, but rather was apparently due to mono- or polysynaptic neural activity evoked by the MFB train. This study demonstrates the potential usefulness of this technique in elucidating neural activity patterns underlying MFB self-stimulation. Supported by BNS-8708523, DA 04551 and RR 07058-21.

20.8

NUCLEUS ACCUMBENS INJECTIONS OF MU AND DELTA BUT NOT KAPPA OPIOIDS FACILITATE HYPOTHALAMIC BRAIN STIMULATION REWARD. T. E. G. West* and R. A. Wise (SPON: Z. Amit). Dept. Psychol., Concordia U., Montreal, Canada, H3G 1M8

Nucleus accumbens injections of morphine (2.5 and 10 ug), the mu agonist DAGO (0.8 and 3.2 ug), and the delta agonist DPDPE (0.8 and 3.2 ug) potentiated the rewarding effects of lateral hypothalamic stimulation, shifting the rate-frequency function to the left with no change in asymptote. Injections in medial and lateral portions of the nucleus seemed equally effective; injections in the rostral portion of the nucleus were less effective and acted with longer latencies than mid-level and caudal injections. The kappa agonist U-50,488H (0.05, 0.2, 0.8, and 3.2 ug) had no effect; it shifted the rate-frequency function neither to the right nor to the left and it had no effect on asymptote. The effects of morphine, DAGO, and DPDPE were blocked by naltrexone (1 and 2.5 mg/kg). These data suggest that mu and delta receptors mediate the reward-facilitating effects of nucleus accumbens opioids; they suggest no role for nucleus accumbens kappa receptors.

20.9

EFFECT OF FOOD DEPRIVATION ON SELF-STIMULATION RATE-FREQUENCY FUNCTIONS. S.A. Frutiger. Univ. Wisconsin-River Falls, River Falls WI 54022.

Although food deprivation has been reported to facilitate lateral hypothalamic (LH) self-stimulation on CRF schedules (Blundell & Herberg, 1968) it has been reported to have no effect on rate-frequency functions (Giovono & Wise, 1986). Since significant facilitation of self-stimulation is noted only at current intensities close to threshold (Blundell & Herberg, 1968), it is possible that the effect of food deprivation is intensity dependent. If so, the relatively high current levels which would be necessary to support self-stimulation at 28-33 Hz may have obscured any facilitation by food deprivation in the Giovono & Wise (1986) study. The present study was designed to test this hypothesis.

Male rats were implanted bilaterally with LH electrodes, tested for stimulation-induced eating, drinking, and/or gnawing, and trained to self-stimulate for a 0.5 sec current train. Rate-intensity functions were established at 100 Hz and used to derive the current which supported 50% or 75% maximal response rate. Baseline rate-frequency functions were established at these current values and the effects of 24 or 48 hr food deprivation on rate-frequency functions evaluated. Food deprivation produced no significant change in either the frequency threshold or asymptotic response rate at either current level tested.

20.10

ATROPINE FAILS TO REVERSE THE EFFECTS OF PIMOZIDE ON BRAIN STIMULATION REWARD RATE-FREQUENCY FUNCTIONS. S.M. Boye, P.P. Rompré and R.A. Wise. Psychology Dept., Concordia University, Montreal, Quebec H3G 1M8.

Previous reports showed that anticholinergic drugs reverse neuroleptic-induced attenuation of rate of responding for electrical brain stimulation. It is not clear, however, whether the anticholinergic drugs reversed neuroleptic-induced reward attenuation or neuroleptic-induced decrease in asymptotic rate of responding. In this experiment, we studied the effects of atropine on brain stimulation reward threshold and on asymptotic rate of responding as measured with the curve-shift method, in pimozide-naive and pimozide treated animals. Rats were trained to press a lever to obtain intracranial electrical stimulation and were tested first with tartaric acid followed by saline. They were then divided into different groups. Animals in groups 1, 2 and 3 were treated with tartaric acid or pimozide (0.35 mg/kg) followed by atropine sulphate (0.8, 1.6 and 3.2 mg/kg, respectively) and animals in groups 4, 5 and 6 were treated with tartaric acid or pimozide followed by atropine methylnitrate (0.42, 0.84 and 1.68 mg/kg, respectively). Atropine sulphate failed to reverse the attenuation of reward and the decrease in asymptotic rate of responding produced by pimozide. On the contrary, it shifted the curve further to the right without changing the asymptotic rate of responding. Atropine methylnitrate produced a similar shift to the right but further decreased the asymptotic rate of responding.

20.11

POTENTIATION OF BRAIN STIMULATION REWARD BY MICROINJECTION OF NEUROTENSIN IN THE VENTRAL MESENCEPHALON: A COMPARISON WITH THE EFFECTS OF MORPHINE. P.P. Rompré[1], P. Bauco* and A. Gratton[2] Psychology Dept[1], Concordia University, Montreal, Quebec H3G 1M8. Douglas Hosp. Res. Ctr.[2], McGill University, Verdun, Quebec H3H 1R3.

Brain stimulation reward was found to be sensitive to pharmacological treatments that modify central dopamine neurotransmission. Morphine infusions in the ventral mesencephalon, for instance, increase dopamine impulse flow, potentiate brain stimulation reward and reverse the effects of dopamine receptor blockade. In this experiment, we studied the effects on brain stimulation reward, of ventral mesencephalic microinjection of neurotensin, a peptide suspected to regulate the activity of the mesocorticolimbic dopamine system. We compared the effects of neurotensin to the effects of morphine injected at the same sites. Changes in the function relating the rate of responses to the stimulation frequency were measured after central injections of 1) neurotensin (vehicle, 2.5, 5.0, 10 and 20 ug/0.5 ul) and 2) morphine (5.0 ug/0.5 ul). At 5 to 20 ug, neurotensin, like morphine, potentiated the rewarding effect of the stimulation and shifted the curve (by 0.03 to 0.1 log unit) towards lower stimulation frequency. In two cases, neurotensin attenuated brain stimulation reward (0.1 log unit shift) while morphine, injected at the same sites, produced strong facilitations (0.12 and 0.25 log unit shift).

20.12

Effects of dopamine antagonists injected into the ventral tegmental area on lateral hypothalamic self-stimulation. S. Nakajima, Department of Psychology, Dalhousie University, Halifax, Nova Scotia, Canada, B3H 4J1.

In earlier studies, either SCH 23390 (D1 specific antagonist) or raclopride (D2 specific antagonist), injected IP, reduced operant responding for stimulation of the medial forebrain bundle. When injected into the nucleus accumbens (NAc), SCH 23390 attenuated the reinforcing effect of brain stimulation but raclopride did not. Now these antagonists were injected into the ventral tegmental area (VT), and their effects on lateral hypothalamic (LH) self-stimulation examined.

Rats were implanted with a bipolar electrode into LH and a cannula into VT. Injection of SCH 23390 into VT ipsilateral to the electrode reduced responding with a dose range of 1-3µg. Contralateral injection had either no effect or a delayed effect. Raclopride had no effect up to 5µg; 10µg reduced responding regardless of the side of injection.

These results are similar to the results of injection into NAc. D1 receptors in both NAc and VT seem to be involved in the mechanism of reinforcement, but the participation of D2 receptors in these areas was not confirmed.

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20.13

REDUCTION OF HYPOTHALAMIC STIMULATION REWARD BY MEDIAL FOREBRAIN BUNDLE LESIONS: AN AUTOTITRATION ANALYSIS. A. M. McDonald* and D. B. Neill. Dept. of Psychology, Emory University, Atlanta, GA 30322.

Responding for hypothalamic stimulation is reduced by lesions within the medial forebrain bundle (MFB), but it is difficult to separate reward from performance changes. We examined the effect of MFB lesions using the less rate-dependent autotitration ICSS. In our procedure, the stimulation current is reduced 3 uA every 5th lever press. The rat is free to reset the current to the maximum by responding on a separate reset lever. We found that electrolytic lesions anterior and posterior and ipsilateral to the stimulation electrode resulted in decreased response rate and resetting at higher intensities than previously, i.e., decreased reward. Lesions contralateral to the electrode were ineffective. Lesions placed in the posterior midbrain, posterior to the dopaminergic cell fields, also decreased reward. Our results support the idea of decreased reward following these lesions and the ability of autotitration ICSS to detect this reward change. The results also suggest that the reward pathway may continue posteriorly in midbrain past the dopaminergic cell bodies.

20.14

THE EFFECTS OF LATERAL HYPOTHALAMIC SELF-STIMULATION ON SOME IMMUNE MEASUREMENTS. **J. D. Butler and D. B. Neill. Dept. of Psychology, Emory University, Atlanta, Ga. 30322**

Intracranial self-stimulation (ICSS) of the lateral hypothalamus (LH), while highly rewarding, appears to be a stressor in rats. Blood levels of corticosteroids, ACTH, and beta-endorphin have been reported to increase. We report here immunological effects consistent with this hypothesis. Male Lewis rats were implanted with an electrode into the LH and trained to self-stimulate. In experiment 1 the rats were allowed to self-stimulate for 35 days and then sacrificed. On day 19, experimental and operated home-cage control rats were injected with sheep red blood cells (SRBCs) I.P. The ICSS rats had significantly smaller thymuses and SRBC antibody titer. In experiment 2 SRBCs were injected into experimental, unoperated, and operated home-cage control rats on the day ICSS testing began. These rats were sacrificed after 7 days of ICSS. These ICSS rats had significantly smaller thymuses but the SRBC titer was not different from controls.

20.15

SEX STEROID HORMONE ADMINISTRATION DOES NOT INFLUENCE AUTOTITRATION SELF-STIMULATION OF THE PARS-COMPACTA SUBSTANTIA NIGRA OR THE MEDIAL FOREBRAIN BUNDLE IN THE FEMALE RAT. P.R. Hartley and D.B. Neill. Department of Psychology, Emory University, Atlanta, GA 30322.

Steiner et al., (Psychoneuroendocrinology, 6:81, 1981) reported that intracranial self-stimulation (ICSS) in female rats varied with the estrous cycle for electrodes in substantia nigra pars compacta (PC-SN) but not the lateral hypothalamus (LH).

To determine if the above changes were a function of altered motoric or hedonic functioning we studied the effects of exogenous administration of estradiol benzoate (E_2) and progesterone (P) on a rate-independent measure of self-stimulation known as autotitration. In autotitration, the rat presses a bar for electrical brain stimulation on one lever. Every fifth response results in a decrease in the current by 3uA. On the other side of a Plexiglas partition in the chamber there is a lever that, when pressed, resets the current to a predetermined intensity.

Ovariectomized female rats were implanted with electrodes in either the LH or the PC-SN. The first simulated cycle using E_2 and P to produce a state of behavioral estrus produced no differences in mean reset intensity (MRI), or total number of responses (TR) as compared to baseline in either LH or PC-SN implanted rats. The second replacement cycle revealed no differences in any of the response measures in rats with electrode implants in the LH but a trend towards a decrease in MRI was observed in rats with electrodes in the PC-SN.

20.16

EFFECTS OF PERFORMANCE AND REWARD MANIPULATIONS ON CURRENT-INTENSITY THRESHOLDS AND OTHER MEASURES DERIVED FROM A DISCRETE-TRIAL SELF-STIMULATION PROCEDURE IN RATS. A. Markou, S.J. Hanley*, A.K. Chehade* and G.F. Koob. Research Institute of Scripps Clinic and UCSD Dept. of Psychology, La Jolla, CA 92037.

Intracranial self-stimulation (ICSS) thresholds are presumed to provide a measure of reward and are used in the study of the anatomical and neurochemical substrates of reward. The purpose of the present experiments was to validate the four measures derived from a discrete-trial current-intsnsity ICSS procedure (modified from Kornetsky and Esposito, Fed Proc, 38, 2473-2476, 1979). The effects of adding weight on the manipulandum (performance manipulation) and changing train duration (reward manipulation) on thresholds, extra responses, time-out responses and reaction time were investigated. It was found that the weight manipulation affected reaction time and both extra and time-out responses, ($p < .05$), with very little effect on thresholds. In contrast, the train duration manipulation primarily influenced thresholds ($p < .05$). Two other experiments examined the effects of acute cocaine injections (reward manipulation) and a muscle relaxant (performance manipulation) on the same measures. These validation studies will allow the detection and discrimination of reward and performance effects of future manipulations (e.g., drugs, lesions).

(Supported by NIDA grants DA 05365 and DA 04398)

20.17

PERTUSSIS TOXIN BLOCKS MORPHINE SELF-ADMINISTRATION IN THE CA3 REGION OF THE HIPPOCAMPUS. D. W. Self and L. Stein. Dept. of Pharmacology, College of Medicine, Univ. of California, Irvine, CA 92717.

Rats rapidly learn to self-administer the selective μ-opioid agonist morphine directly in the CA3 region of the hippocampus. The reinforcing action of morphine suggests that μ receptors in this region may be involved in opioid reward. The μ-opioid receptor belongs to a family of receptors that can initiate cellular responses via activation of the pertussis toxin-sensitive G_i protein. Pertussis toxin irreversibly blocks the effects of these receptors by catalyzing the ADP ribosylation of the α subunit of G_i. In this study, naive rats were injected with two 500 nl microinjections of pertussis toxin (0.1 or 1.0 μg/injection) or the Ringer's vehicle, through cannula aimed at the CA3 region of dorsal hippocampus 2 days prior to self-administration testing. Untreated rats or rats pretreated with vehicle self-administered morphine (10 pmoles/100 nl injection) through the cannula at rates significantly higher than controls self-administering saline. Rats pretreated with 1 μg of pertussis toxin failed to groom, exhibited seizures, and in half the cases died 6 or 7 days later. In contrast, rats pretreated with 0.1 μg of pertussis toxin exhibited no obvious behavioral deficits or sickness, but they failed to self-administer morphine. These results suggest that pertussis toxin may block acquisition of morphine self-administration behavior by preventing μ-opioid receptor activation of G_i. If so, the reinforcing properties of opioid drugs may depend on the activation of G_i in target neurons possessing the μ-opioid receptors.

20.18

HIPPOCAMPAL SELF-ADMINISTRATION OF MORPHINE AND COCAINE . E. Grauer*, D.L. Lutz* and L. Stein. Dept. of Pharmacology, Coll. of Medicine, Univ. of California, Irvine CA. 92717.

Although the hippocampus has been principally associated with learning and memory functions, this region contains both opioid and dopaminergic neurotransmitters implicated in motivation and reward. Previous work has shown that dynorphin and dopamine are self-administered into the CA3 field of dorsal hippocampus, suggesting that the hippocampus might be an important action site for drugs of abuse. Rats were trained to self-administer morphine or cocaine through a permanently implanted cannula aimed at the CA3 field of the hippocampus. Both drugs were tested at doses of 1, 10 or 100 pmole/100 nl injection using the EMIT self-administration system. Morphine and cocaine supported significant self-administration behavior but only at the 10 pmole/100 nl dose. Rates associated with the higher and lower doses of each drug did not differ from saline control rate. Since the effective 10 pmole/100 nl doses found here is an order of magnitude lower than doses of morphine and cocaine reported effective for other self-administration sites, the hippocampus may be a particularly important site of action for opiate and psychostimulant drugs of abuse.

20.19

MU RECEPTORS MAY MEDIATE HIPPOCAMPAL ENDOGENOUS OPIOID REINFORCEMENT. K.E. Stevens and L. Stein. Department of Pharmacology, University of California, Irvine, CA 92717

Previously, we showed that animals would work to self-administer dynorphin A directly into the CA3 region of hippocampus. Furthermore, mediation of dynorphin A reinforcement via an opiate receptor was demonstrated by a dose-related attenuation of self-administration rates by naloxone co-administered with the opiate. In the current studies, we show that this reinforcement is largely mediated by the mu opiate receptor.

Rats were unilaterally implanted with a chronic indwelling guide-cannula aimed at the hippocampal CA3 region. Self-administration testing using the EMIT self-administration system was conducted every third day for a total of three tests. Rats were allowed access to a 1 pmol/100 nl dynorphin A solution that also contained a "selective" opioid receptor antagonist. Selective kappa receptor blockade was provided by nor-binaltorphimine (5 pmol/100 nl), delta blockade by ICI 174,864 (125 pmol/100 nl) and mu blockade by beta-funaltrexamine (415 pmol/100 nl). The dose of each of antagonist was selected on the basis of its relative binding affinity as compared to naloxone, and was calculated to produce, at the preferred receptor, the same blockade as that produced by 500 pmol/100 nl naloxone. Neither kappa nor delta receptor blockade significantly reduced dynorphin A self-administration rates. However, mu receptor antagonism produced a complete blockade of dynorphin self-administration; in fact rates in the beta-funaltrexamine group were below vehicle control and almost identical to those of a non-injected control group. Taken in conjunction with other work in this laboratory showing that the mu-selective agonist morphine produces high rates of hippocampal self-administration, these results strongly suggest that mu receptors in the hippocampal CA3 region play an important role in opioid reinforcement.

20.20

GUSTATORY IMPAIRMENT CONSECUTIVE TO THE LESION OF THE LATERAL HYPOTHALAMIC NEURONS BY IBOTENIC ACID.
Velley,L. and Touzani*,K., Lab. Psychophysiologie, CNRS URA 339, Univ. Bordeaux I, Av. Facultés 33405 TALENCE FRANCE.

Our previous studies showed that neurons of the lateral hypothalamus modulate both self-stimulation in the parabrachial area and taste preference or aversion. The present study further analyzes this gustatory impairment. Four groups of rats were used: bilaterally,simultaneously or successively lesioned, vehicle injected and intact controls. The effect of these different treatments on body weight, fluid intake and preference-aversion ratio for 6 concentrations of saccharin was compared. No significant difference was observed either between the 2 lesioned or the 2 non-lesioned groups. Lesioned rats displayed neophobia for saccharin, an increase in appetitive and aversive thresholds and a similar decrease in body weight and daily fluid intake. The comparison of the effect of morphine and naloxone on saccharin preference of the same groups of rats showed an altered reactivity of lesioned rats to opiate agonists and antagonists. Animals were then tested for conditioned taste aversion (CTA) with threshold values of saccharin solution as conditioning stimulus. No difference was observed in CTA learning between lesioned and non lesioned groups with a saccharin concentration apparently neutral for lesioned and appetitive for non lesioned rats, suggesting that the gustatory impairment observed in the choice task is not related to a sensory deficit.
(Supported by INSERM grant 856024).

ALCOHOL, BARBITURATES, BENZODIAZEPINES I

21.1

COMBINED EFFECTS OF ETHANOL AND NICOTINE ON INTRACRANIAL SELF-STIMULATION AND LOCOMOTOR ACTIVITY IN RATS. G.J. Schaefer and R.P. Michael, Department of Psychiatry, Emory University, School of Medicine, Georgia Mental Health Institute, 1256 Briarcliff Rd., Atlanta, GA 30306.

Two groups of rats were implanted with electrodes in the lateral hypothalamus, and trained to lever-press for intracranial self-stimulation (ICSS) on either a fixed interval 15-second (FI:15 sec) (N = 10), or a fixed ratio 15 (FR:15) (N = 11) schedule of reinforcement. Animals were then tested with graded doses of ethanol (0.1-1.0 g/kg), nicotine (0.01-0.3 mg/kg) and combinations of ethanol and nicotine. By itself, ethanol decreased response rates at 1.0 g/kg in the FI:15 sec paradigm, while nicotine alone increased rates at 0.1-0.3 mg/kg. These doses of nicotine, when combined with 0.1 and 0.3 g/kg alcohol, also increased response rates. In the FR:15, alcohol decreased rates at 0.56 & 1.0 g/kg, while nicotine increased rates at 0.03-0.17 mg/kg. No combination of ethanol and nicotine increased response rates in the FR schedule. A third, non-implanted group (N = 10) was tested in a locomotor activity procedure; ethanol (0.1-1.0 g/kg) did not alter activity, but nicotine increased it at 0.1-0.3 mg/kg. When combined, 0.17 mg/kg nicotine increased activity at all ethanol doses. These preliminary results demonstrated that under certain conditions, combinations of ethanol and nicotine can produce behavioral stimulation.
(Supported by Georgia Department of Human Resources.)

21.2

ALCOHOL PRODUCES RATE-DEPENDENT FACILITATION OF BRAIN STIMULATION REWARD. M.J.Lewis, J.R.Andrade and S.Reynolds* Dept. of Psychology, Howard Univ.,Washington, DC 20059.

Intraperitoneal injection of ethanol (E) has been found to produce mixed effects on brain stimulation reward (BSR) response rate. No effect or reduction in response rate have frequently been found. Increases have also been found, although less frequently. In the present experiment, analysis of E effects on BSR lever-press response rate in animals with differing baseline response rates indicates that facilitation or depression appears to be rate dependent

Randomly bred albino rats (Charles River) were surgically implanted with stainless steel electrodes in the lateral hypothalamic area. All were shaped to lever-press for electrical stimulation (100 Hz biphasic rectangular wave, 1.0 msec duration, 0.2 sec train; continuous reinforcement) during a 15 min operant session. Animals were injected with saline and then twice with E (0.25 g/kg,ip). Injections were administered 5 to 10 min prior to the operant sessin.

E increased responding in all animals with low to moderate response rates (20 to 50 responses/min) over saline injections. In those with higher response rates (50 responses/min), E generally decreased responding. Animals with very low response rates (20 responses/min) also a general reduction in response rate was found.

These data show that E's effects on lever-press responding are rate-dependent and may account in part for the conflicting data concerning E's effects in this task.

21.3

TASTE REACTIVITY IN ALCOHOL PREFERRING (P) AND NON-PREFERRING (NP) RATS. P.J. Bice* and S.W. Kiefer Department of Psychology, Kansas State University, Manhattan, Kansas 66506.

The taste reactivity test was used to examine the orofacial responses of naive alcohol preferring (P) and alcohol non-preferring (NP) rats to the taste of alcohol. In the initial exposure, rats were tested for reactivity to five concentrations of alcohol (5%, 10%, 20%, 30%, and 40% v/v) and a sucrose and a quinine solution. A two bottle consumption test then was given for a three week period to allow the animals access to 10% alcohol. A second reactivity test was used to examine the taste responses of experienced P and NP rats to the same solutions that were used in the initial reactivity test. The results indicated no significant differences between the P and NP rats during the initial reactivity measures. Taste reactivity following alcohol access indicated that P rats showed more ingestive responses and fewer aversive responses to all alcohol concentrations relative to the NP rats. There were no significant differences between rats in their response to sucrose and quinine. These results suggest that P and NP rats taste response to alcohol differs only after experience with alcohol.
(Supported by NIAAA AA07185)

21.4

CYCLOOXYGENASE INHIBITORS ANTAGONIZE ETHANOLS' DEPRESSANT EFFECTS: POTENCY CORRELATIONS WITH SCHEDULE CONTROLLED BEHAVIOR. (SPON. N. Khazan), G.I. Elmer* and F.R. George*. NIDA Addiction Research Center, Balt., MD, 21224 and Dept. of Pharmacology and Toxicology, Univ. of MD, 21201.

Ethanol-induced increases in the production of cyclooxygenase (CO) metabolites are proposed to be important in ethanols' mechanism of action. If CO metabolites are important there must be a significant correlation between in vitro anti-enzyme activity and in vivo therapeutic potency. The potency correlation for the ability of PGSIs to antagonize the rate depressant effects of 1.5 g/kg ethanol was investigated in C57Bl/6J mice using operant fixed ratio responding for water. Ethanol decreased responding to 20% of saline baseline levels. Indomethacin, flufenamate, ibuprofen and aspirin antagonized up to 50% of the rate depressant effects of ethanol in a dose dependent manner. The potency of these PGSI's to antagonize ethanols' effects were significantly correlated with their in vitro IC_{50} and in vivo ED_{50} anti-inflammatory values. Pharmacological correlations between enzyme inhibition and antagonism of ethanols' effects may add support to the hypothesis that CO metabolites are important in mediating ethanols' CNS effects.

21.5

200 mg/kg Ethanol Enhances Appetitive Pavlovian Conditioning of Motor Activity. James D. Valentine* and Linda L. Hernandez. VA Medical Center and University of South Carolina, Columbia, South Carolina 29201.

A low dose of ethanol, 200 mg/kg, enhances aversively motivated Pavlovian conditioning (Psychopharmac. (1989) 97:476-480). We now report that this dose also enhances appetitively motivated Pavlovian conditioning.

Male Sprague-Dawley rats were reduced to 90% of free-feeding body weight and given 2 daily sessions of pellet-feeder training before being returned to ad lib food. This was followed by 4 days of habituation to a 20 sec tone (Sonalert) that later served as the conditional stimulus (CS). Separate groups then received either 200 mg/kg ethanol or saline, i.p., 15 min before each of 6 Pavlovian conditioning sessions; CS termination was immediately followed by the delivery of 5 food-pellets, the unconditioned stimulus. One week after the completion of conditioning, both groups received 3 days of extinction, followed 5 weeks later by 3 additional days of extinction; no injections preceded the extinction sessions. All sessions were 30 min long, and included 5 trials with a 5 min intertrial interval. Motor activity was measured during all sessions using an ergometric activity monitor (Colbourn).

The conditioned response (CR) was a large increase in motor activity. The ethanol group acquired the CR far more rapidly than did controls, and the duration of their CR may have been increased, as well. During extinction, the CR of the ethanol group showed greater recovery at the beginning of each session, and almost complete recovery during the 5 week period between the 3rd and 4th extinction sessions.

These results show that 200 mg/kg of ethanol enhances appetitive, as well as aversive, Pavlovian conditioning in rats, and that the appetitive model may be more sensitive to these effects.

(Supported by VACO and USPHS #R23-AA06817)

21.6

ETHANOL, DIAZEPAM AND GEPIRONE EFFECTS ON REACTIONS TO DISTANT PREDATORS AND PREDATORY LOCI. R.J. Blanchard, D. C. Blanchard, B.B. Young, and R.J. Rodgers[1]
Békésy Laboratory of Neurobiology, University of Hawaii, 96822 and [1]University of Bradford, United Kingdom.

Ethanol, the serotonin 1A agonist gepirone, and diazepam were compared in a battery of tests designed to measure reactions to the sight and odor of an inescapable distant predator, and to the place where the predator was seen. Three sequential defensive reactions occur to these stimuli: Initial movement arrest (MA); followed by active risk assessment (RA) behaviors; with concommittant inhibition of non-defensive behaviors (INB) (Blanchard & Blanchard, Comp. Psychol., 1989). Measures of these patterns, plus proxemics, the S's spatial location with reference to the predatory stimulus, will be presented.

Diazepam (0, 2 and 4 mg/kg) selectively altered RA in a rate-dependent fashion, with minimal effects on INB. Ethanol (0, 0.6, 1.2 g/kg) influenced MA, RA, and INB evenly, producing moderate reductions in each of these defensive behaviors. Gepirone (0, 5, 10 mg/kg) reduced RA at the higher dose but also reduced most active behaviors.

Each anxiolytic thus reduced some defense measures, but each produced a unique profile of changes in the anxiety-related defense pattern.

This research was supported by NIH Grant NIAA 06220, and RCMI Grants RR03061 and RCMI RR01825.

21.7

DRUG DISCRIMINATION LEARNING WITH RHESUS MONKEYS: AN ASSESSMENT WITHIN THE CONDITIONED TASTE AVERSION DESIGN R. Jeffreys, A. Riley and J. Glowa, Psychopharmacology Laboratory, The American University, Washington, DC 20016 and Clinical Neuroendocrinology Branch, NIMH, Bethesda, MD 20892.

Drug discrimination learning within a conditioned taste aversion paradigm has been reported recently in rats. Under these conditions, rats drink less saccharin-flavored water paired with 1.8 mEq LiCl when the access to this solution is preceded by a drug injection than the same solution that is not paired with LiCl and preceded by distilled water injections. In order to begin to assess the possibility that similar effects could be obtained in primates, two rhesus monkeys were trained under a FR 30 schedule of sucrose pellet delivery and injected with low doses (0.03-0.1 mg/kg) of alprazolam, 60 min prior to sessions which were immediately followed by the adminitration of 1.8 mEq LiCl. On the intervening days, subjects were injected with saline in a similar manner (both 60 min before and immediately after the session). Although these doses of alprazolam originally had no overall effect on FR responding (complete dose-effect determinations from 0.03-3.0 mg/kg were obtained previously using banana pellets), with repeated conditioning trials responding decreased relative to both baseline and saline control. Although overall baseline rates decreased, comparisons between the rates on the day before conditioning trials and the day of the trial revealed a marked decrease in one of the monkeys and smaller effects in the other. These findings are consistent with the notion that drug discrimination can be rapidly acquired in the primate using a conditioned taste aversion design.

21.8

LOW LEVEL HYPERBARIC EXPOSURE DOES NOT AFFECT PICROTOXIN INDUCED SEIZURE LATENCIES IN C57 MICE. P.J. Syapin, L.S. Kobayashi,* B.L. Jones,* D.A. Finn* and R.L. Alkana, Alcohol and Brain Research Lab., Schls. of Pharmacy and Medicine, U. of Southern California, Los Angeles, CA 90033

Exposure to 12 atmospheres absolute (ATA) helium-oxygen (HEOX) gas mixtures antagonizes the acute and chronic behavioral effects of ethanol in animals. To assess the contribution of a pressure-induced increase in general CNS excitabiity in mediating the antagonism, we tested the effects of 12 ATA HEOX on picrotoxin-induced seizure latencies. Male, drug naive C57BL/6J mice were injected with a low dose of picrotoxin (2 mg/kg s.c.), placed 3/chamber and immediately exposed to 1 ATA air, 1 ATA HEOX or 12 ATA HEOX. The subjects were recorded on video tape and scored later for time to onset of drug action (Straub-like tail) and time to first convulsion. Exposure to 12 ATA HEOX did not significantly alter latencies compared to the 1 ATA HEOX controls. In contrast, 1 HEOX significantly altered the onset of picrotoxin's effects, but the effect was not consistent. These results with picrotoxin suggest that 12 ATA HEOX does not antagonize ethanol via increasing CNS excitability. Further studies are in progress to determine the generality of these preliminary results to other convulsant drugs. (Supported by NIAAA research grant AA03972).

21.9

ACTION OF ICV EGTA OR VERAPAMIL ON SOME CENTRAL ACUTE ETHANOL EFFECTS IN RATS. X. Paez* and R.D. Myers, Department of Pharmacology, School of Medicine, East Carolina University, Greenville, NC 27858

Ethanol (ETOH)-induced hypothermia can be prevented by EGTA in the third ventricle and attenuated by verapamil in the lateral ventricle of rats. In order to determine the possible involvement of Ca^{++} imbalance in other acute ETOH effects (sleep, loss of righting reflex and motor incoordination), 25 adult rats were prepared for icv EGTA (12.5, 25, 50 ug/15 ul) or verapamil (25, 50 ug/10 ul) injections in the right ventricle. The drugs were given simultaneously with ETOH 4 g/kg ip (to prevent effects) or when the maximum hypothermic ETOH effect was reached (to reverse effects). The colonic temperature was monitored continuously from 1 hour prior to 5-7 hours after injections; sleep, righting reflex and motor coordination were recorded periodically. In a dose-dependent manner, EGTA prevented and reversed the ETOH hypothermia and accelerated the recovery of the other monitored effects. Verapamil was less effective than EGTA on ETOH-induced hypothermia, but accelerated the recovery of righting reflex and motor coordination. These dose-dependent results with a Ca^{++} chelator and a Ca^{++} channel blocker indicated that both the ETOH hypothermia and acute behavioral effects could be prevented or reversed. An imbalance in Ca^{++} may be in part responsible for acute effects of ETOH, but further investigation is necessary.

21.10

ETHANOL DIFFERENTIALLY AFFECTS ORIENTING AND RIGHTING REFLEXES IN WEANLING, BUT NOT PREWEANLING OR ADULT, RATS. J.A. Saiers*[1], D.J. Knapp[2], L.A. Pohorecky*[2] and B.A. Campbell[1]. [1]Dept. Psychology, Princeton Univ., Princeton, NJ 08544-1010; [2]Center for Alcohol Studies, Rutgers Univ., New Brunswick, NJ 08855-0969.

The effects of ethanol on orienting and righting reflexes in preweanling (16-17 day-old), weanling (30-35 day-old), and adult (60-70 day-old) rats were assessed in this research. Both cardiac and behavioral measures were used to index the occurrence of the orienting reflex: in response to an auditory stimulus, rats exhibit a bradycardic (40-50 BPM) heart rate (HR) orienting response accompanied by a quick, lateral head jerk. In preweanling, weanling, and adults rats, ethanol (injected i.p. at dosages of 0, .5, 1, 2, or 3 g/kg) blocked these two components of the orienting reflex to a pulsating auditory stimulus in a dose-dependent manner, with prevention of both behavioral and HR responses occurring at a dosage of 3 g/kg of the drug. The righting reflex was also prevented in a dose-dependent manner in preweanling and adult rats 15 min after ethanol administration. In these animals, magnitude of righting and orienting reflex disruption was similar at any given dosage of ethanol. In weanling rats, in contrast, ethanol failed to affect the righting reflex at any dosage. Measures of locomotor activity taken during the 20 min following ethanol injection revealed that ethanol produced behavioral activation in weanling animals but sedation in preweanling and adult animals. Implications of this dissociation between ethanol's effects on the righting reflex and behavioral activation versus its effects on the orienting reflex in weanling animals will be discussed.

21.11

EFFECT OF ETHANOL AND GABA ANTAGONISTS IN MICE WITH DIFFERENT SENSITIVITY TO ETHANOL. S. Liljequist. Dept. of Psychiatry and Psychology, Karolinska Institute, S-10401 Stockholm, Sweden.

Various behaviors and the effect of ethanol and GABA antagonists were studied in NMRI, CBA, and C57 mice. Inbred C57 mice habituated rapidly in a locomotor activity test situation, whereas inbred CBA mice showed no habituation. There was no difference between the mouse strains in a passive avoidance learning test. CBA mice were most sensitive to the convulsive effects of picrotoxin, whereas C57 mice were most resistant. No difference between the mice to the convulsive effects of the specific GABA receptor antagonist bicuculline was noted. CBA mice were significantly more sensitive to the muscle relaxant and hypnotic effects produced by acute ethanol administration as compared to NMRI and C57 mice. The ethanol-produced hypothermia was similar in all mice tested. When the mice were given daily injections of ethanol during five consecutive days, C57 developed tolerance to the hypnotic effects of ethanol, whereas no tolerance to ethanol was observed in CBA mice. All animals developed tolerance to the ethanol-produced hypothermia. These results suggest that genetic differences in the regulation of the GABA receptor-coupled chloride channel may be of importance for the observed differences in ethanol sensitivity and in the rate of development of ethanol tolerance.

21.12

RO 15-4513 AND ETOH EFFECTS ON OPERANT BEHAVIOR OF MALE AND FEMALE C57 MICE. K. Bao*, H.C. Becker and L.D. Middaugh. (SPON: W. Boggan) Dept Psychiat Behav Science, Med. Univ. So. Car., Charleston, SC 29425.

Previous reports suggest that the behavioral effects of Ro 15-4513 and ETOH may be influenced by gender. The present study investigated the effects of Ro (1,2 or 4 mg/Kg), ETOH (.25 to 3.0 g/Kg), and combinations of the two on lever-responding of male and female C57BL/6 mice maintained on an FR20 schedule of food reinforcement. ETOH disrupted lever responding; however, the only sex difference was a slightly more prolonged disruption for female mice. Ro also produced a transient disruption of lever-responding at all doses examined with no evidence of a sex difference in response to the drug. The disruptive effect of ETOH on lever-responding was not altered by Ro injections given just prior to testing. The results confirm previous work indicating that Ro disrupts behavior under the control of reinforcing stimuli but does not alter the effects of ETOH on this behavior. Since both sexes responded similarly to the two drugs, gender is not accountable for the reported differences in the effects of Ro on ETOH discrimination using operant paradigms. (Supported by NIAAA Grant #AA06611 and MUSC Grant #22620-GR29.)

21.13

ETOH DISCRIMINATION BY C57 MICE IS NOT ALTERED BY Ro 15-4513. L.D. Middaugh, K. Bao,* S.J. Sergent* and H.C. Becker. Dept Psychiat Behav Sci, Med. Univ. So. Car., Charleston, SC 29425.

Of the two reported studies, one indicated that Ro 15-4513 (Ro), the partial benzodiazepine agonist, given to male mice after ETOH injections attenuated its discriminability. When Ro was given to female rats prior to ETOH injection, no attenuation of ETOH discrimination was observed. In the present study we used an operant drug discrimination paradigm and 6 female C57BL/6 mice to determine if the order of injecting Ro relative to ETOH might account for the above difference. After first assessing ETOH discrimination at doses of .5, .75, & 1.0 g/Kg, we determined the effects of Ro (.37, .75, & 1.5 mg/Kg) on responding and ETOH discrimination when it was given 5 min before ETOH or vehicle and 10 min prior to testing. Finally, we assessed the effects of Ro (.15 or .37 mg/Kg) when it was injected 10 min after ETOH and 10 min prior to testing. Although Ro disrupted the lever-response, hence was biologically active, it did not interact with the disruptive effects of ETOH on this measure nor did it attenuate the discriminability of ETOH when given either before or after ETOH injection. (Supported by Grant #AA06611).

21.14

THE BENZODIAZEPINE INVERSE AGONIST RO15-4513 ANTAGONIZES THE ANXIOLYTIC ACTION OF ETHANOL IN A NONSHOCK CONFLICT TASK. H.C. Becker. VA Medical Center and Department of Psychiatry, Medical University of South Carolina, Charleston, SC 29403.

The purpose of this study was to examine the effects of RO15-4513 on the anxiolytic action of ethanol (EtOH) in a consummatory (nonshock) conflict test referred to as negative contrast. The negative contrast task involves quantification of how animals respond to an abrupt, unexpected reduction in reward (sucrose solution). The depressed behavior engendered by reward reduction is antagonized by anxiolytics such as EtOH and benzodiazepine (BDZ) agonists. The BDZ inverse agonist RO15-4513 (0.1875-3.0 mg/kg) dose-dependently antagonized the anxiolytic effect of EtOH (0.75 g/kg) without influencing general consumption ($p<.01$). At the highest dose (3 mg/kg) RO15-4513 alone produced an anxiogenic effect (further response suppression). However, at lower doses this intrinsic activity of RO15-4513 was not apparent. Thus, these data suggest that in the negative contrast task RO15-4513 can antagonize the anxiolytic action of EtOH at doses that do not produce the intrinsic inverse agonist effects of the drug.

Supported by VA Medical Research and NIAAA.

21.15

PATERNAL ALCOHOL EXPOSURE AFFECTS OFFSPRING ACQUISITION OF WORKING MEMORY TASK IN RADIAL ARM MAZE IN RATS. D.F. Wozniak, T.J. Cicero, L.C. Kettinger III*, E.R. Meyer*. Dept. of Psychiatry, Washington University School of Medicine, St. Louis, MO 63110.

Male rats were given ad libitum access to an alcohol liquid diet containing 35% ethanol-derived calories (ALC) or were pair-fed an isocaloric control diet (PF) for 39 days. The concentration of alcohol in the diet was gradually increased to permit adaptation, then stabilized and then gradually tapered to prevent an alcohol withdrawal syndrome which could confound the results. Following treatment, all males were mated with nontreated females. A behavioral teratologic approach was used to study the offspring throughout development and into adulthood. The offspring sired by the ALC males did not differ from the offspring of PF males on several parameters including: litter size; body weight measured on postnatal days (PNDs) 0 or 1, 4, 7, 14, 21, 30, 60, 90, 120; when various developmental landmarks (incisor eruption, eye opening, testes descent, vaginal opening) appeared; tests of sensorimotor development (negative geotaxis and ascent on an inclined wire screen); or on 1-hr activity tests conducted on PNDs 21 and 60. As adults (beginning on PND 75), the male offspring were trained on the working memory protocol in the radial arm maze and the ALC offspring (n=15) required significantly more days to reach an acquisition criterion (8/9 correct arm choices for 4 consecutive days) than the PF offspring (n=15). After acquisition, rats were tested on a protocol which included a 15 min delay between the first four correct (reinforced) arm choices and subsequent choices and the two groups did not differ according to a post-delay performance criterion (4 consecutive days of 4/4 correct on post-delay choices). The results suggest that high levels of paternal alcohol consumption may lead to relatively selective learning deficits in their male adult offspring. Supported by grants AA-07144, AA-03539, DA-03833 and DA-00095.

21.16

PERSISTENCE OF ACUTE TOLERANCE TO ETHANOL IN THE P LINE OF RATS DOES NOT REQUIRE PERFORMANCE WHILE INTOXICATED. G.J. Gatto*, J.M. Murphy, W.J. McBride, L. Lumeng* and T.-K. Li* Indiana Univ. Sch. Med. & VAMC; Psychology Dept., Purdue Univ. Sch. Science, Indianapolis, IN 46223

Selectively bred, alcohol-preferring P rats develop acute ethanol (E) tolerance faster than nonpreferring NP rats, and P rats maintain E tolerance longer (Pharmacol Biochem Behav 28:105, 1987). This study tested whether performance while intoxicated contributes to the persistence of tolerance. P rats were trained to jump 50 cm to a descending platform to avoid shock. Three groups were injected ip on day 0 as follows: Group E/J (n=8) was given 2.5g E/kg body wt and was tested on the jump task until recovery to criterion (37.5 cm); group S/J (n=21) received saline and testing yoked to an E/J rat; group E/NJ (n=19) received E but no testing. Seven days later, all rats received 2.5g E/kg and were tested to criterion. Recovery times for the E/J (141±11 min) and E/NJ (145±6 min) rats were shorter ($p<0.05$) than the S/J group (169±7 min). Blood E levels at recovery for the E/J group were higher on day 7 than day 0 (259±3 vs 245±4 mg%; $p<0.05$). Blood E levels for the E/NJ group were higher than the S/J group on day 7 and higher than the E/J group on day 0 ($p<0.05$). The findings show that the persistence of tolerance manifested by the P rats does not require performance while intoxicated. (AA-03243, AA-07462 & AA-07611)

21.17

EFFECTS OF *IN UTERO* ADMINISTRATION OF ALCOHOL ON SLEEP TIME IN ADULT RATS. E. Reyes, J. Wolfe*, M. E. Marquez*, E. Duran* and S.H. Switzer*. Univ. New Mexico Sch. of Med., Albuquerque, NM 87131.

It has been shown that the *in-utero* administration of alcohol effects tolerance to ethanol in adult rats. This study was designed to demonstrate that prenatal exposure to ethanol also effects sleep times. On day one of gestation female Sprague-Dawley rats received one of five types of food. These types included: lab chow, liquid Bioserv diet containing 6.7% ETOH, 4% ETOH, 2% ETOH, and 0% ETOH. Four percent, 2%, and 0% mothers were pair-fed against 6.7% mothers. At day 45, pups were injected with 3.4 g/kg of a 50% ethanol solution ip. Sleep time was recorded from the loss of righting reflex to the time at which it was regained. Long sleep (LS) was defined as the mean sleep time of control animals plus two standard deviations. The mean sleep time of control animals minus two standard deviations was considered short sleep (SS). Fifty percent of the rats whose mothers were treated with 6.7% ETOH were classified as LS. The animals whose mothers were pair-fed 4%, 2%, and 0% ETOH demonstrated 20, 36, and 11% LS characteristics respectively . Of pups born to mothers fed lab chow, 3.2% were SS and 19% were LS. In addition, the rate of elimination of a 3.0 g/kg dose of alcohol was determined. Blood alcohol levels were determined by the method previously described by Lundquist (Meth. Biochem. Anal.1:217, 1959). The rates of elimination for control and 6.7% ETOH treated animals were 52.57 and 50.66mg%/hr, respectively. It appears that the significant differences in sleep time between the animal groups cannot be attributed to differences in the rates of ETOH elimination.

21.18

TEMPERATURE DEPENDENCE OF ETHANOL SENSITIVITY IN HOT AND COLD SELECTED LINES OF MICE. D.A. Finn*, B.L. Jones*, L.S. Kobayashi*, J.C. Crabbe and R.L. Alkana. Alcohol and Brain Research Laboratory, School of Pharmacy, Univ. of Southern California, Los Angeles, CA 90033.

The purpose of the present study was to determine whether manipulation of body temperature during intoxication would affect ethanol sensitivity in mice that were selectively bred for their sensitivity (COLD) and resistance (HOT) to ethanol-induced hypothermia. Replicate lines of HOT and COLD mice were injected with 4.0 g/kg ethanol (20% w/v) and exposed to 22 or 34°C. Exposure to 34°C offset ethanol-induced hypothermia and increased ethanol sensitivity, measured by loss of the righting reflex (LORR) duration and ethanol concentration at return of the righting reflex (RORR) in HOT1, HOT2 and COLD1 animals. The 34°C exposed COLD2 animals became hyperthermic following ethanol injection and died. Overall, the results are consistent with previous work in C57, BALB, DBA, A/He and SS mice. In contrast, 129 and LS mice became less sensitive to ethanol as intoxicated body temperature was increased in previous studies. Taken together, these results indicate that body temperature during intoxication influences sensitivity to ethanol-induced LORR and that there are genetically determined differences in the direction of the response. (Supported by USPHS research grant AA05234, AA03972 and AA05828, NIAAA).

21.19

ALCOHOL AND SLEEP DEPRIVATION EFFECTS ON REACTION TIME PERFORMANCE AND EVENT-RELATED POTENTIALS. R.Sinha*, L.T. Smith* and H.L.Williams* (SPON:J.Holloway). Alcohol Research Center, Univ. Of Okla. Health Scs. Ctr., Oklahoma City, OK 73104.

The effects of low and moderate doses of alcohol (.07 mg/kg; .10mg/kg) and 30 hrs. of sleep deprivation were investigated using a reaction time task with a checker-board reversal for the stimulus. There were 18 subjects each in placebo, low and moderate dose groups. Reaction times showed additive treatment effects at the low dose. Sleep deprivation appeared to ameliorate the effects of the moderate dose of alcohol. Forward and backward averaging of the concurrent event-related potentials (ERPS) associated with the reaction process resolved components of the waveform that may have been related to stimulus processing and response execution functions. The forward averaged ERP recorded from Oz, Pz and Cz locations showed significant increases in P2 and P3 component latencies as a function of alcohol dose, and similar increases with sleep deprivation. The P1 and N1' components of the backward averaged motor potential showed similar results. These findings suggest that alcohol, at moderate doses, and sleep deprivation may have independent effects on the reaction process.

DEGENERATIVE DISEASE: PARKINSON'S I

22.1

IN VIVO CHANGES IN STRIATAL BINDING OF DOPAMINE UPTAKE INHIBITOR [^{18}F]GBR 13119 AND MUSCARINIC RECEPTOR LIGAND [^{11}C]-2α-TROPANYL BENZILATE IN MPTP LESIONED C57BL/6 MICE: DUAL TRACER INJECTION STUDIES. G.K. Mulholland,* M.R. Kilbourn,* P.S. Sherman* and T.L. Pisani*. Division of Nuclear Medicine, Univ. of Michigan, Ann Arbor, MI 48109. (Spons: G. W. Dauth).

This study examined whether previously reported in vitro decreases in striatal dopamine reuptake and muscarinic acetylcholinergic receptor levels in the MPTP lesioned C57BL/6 mouse Parkinsons model could be shown in vivo using positron-labeled ligands [^{18}F]GBR 13119 (GBR, DA uptake inhibitor) and [^{11}C]tropanyl benzilate (TRB: mChR antagonist) using dual tracer injection. Male 16 wk old mice were lesioned (Ricaurte, Brain Res. 403, 43, 1987) and studied 2 and 6 wk later. Mice were co-injected with TRB and GBR and sacrificed at 60 min. Brain regions were excised and counted twice for quantification of ^{11}C ($t_{1/2}$ = 20 min) and ^{18}F ($t_{1/2}$ = 110 min) in samples. GBR striatum/cerebellum (str/cer) ratios were reduced by 45% (p=.0 03) at 2 wk post MPTP and remained decreased (40%) at 6 wk post MPTP, as compared to controls (str/cer = 3.2). In contrast, str/cer ratios for TRB in the 2 wk post MPTP mice did not differ from controls (str/cer = 14). However in 6 wk post MPTP mice there was a 35% drop (p = .007) in str/cer ratio for TRB. Thus, in vivo tracer techniques can be used to demonstrate changes in both DA uptake sites and mChR sites after MPTP treatment; the time course of loss of these binding sites, however, appear different. These results suggest that both ligands for DA uptake sites and mChR should be examined for utility in PET studies of Parkinson's disease.

22.2

DOPAMINE (DA) METABOLITES IN THE FRONTAL CORTEX AND STRIATUM OF NORMAL AND MPTP-TREATED RHESUS MONKEYS MEASURED BY BRAIN DIALYSIS. H. Miyake*, D.J. Doudet, T.G. Aigner & R.M. Cohen*. SCBI/LCM & Lab Neuropsychology NIMH, Bethesda, MD, 20892.

MPTP is known as a neurotoxin that selectively destroys the DA nigro-striatal pathway while sparing the mesocortical system. DA metabolites were evaluated in the dorso-lateral prefrontal cortex, caudate nucleus and putamen of 4 MPTP-treated (2-5 mg/kg, i.v., 3 years before the present study) and 4 age matched normal rhesus. The treated animals acutely showed rigidity and akinesia but spontaneously recovered and were evaluated as neurologically normal at the time of the study by investigators unaware of their previous treatment. The 3 microdialysis probes were stereotaxically implanted in the 3 areas, in the anesthetized animals. Data collection lasted 2 hrs. The DA metabolites levels were reduced in the prefrontal cortex, caudate and putamen in the MPTP-treated monkeys compared to controls by respectively, 60, 75 and 40%

These data suggest that 1) deficits in striatal DA may precede the appearance of clinical symptoms and 2) MPTP may affect mesocortical as well as nigro-striatal neurons.

22.3

^{3}H-MPP$^+$ BINDING SITES: RELEVANCE FOR MPTP NEUROTOXICITY. M.Del Zompo, S.Ruiu*, F.Bernardi*, A.Bocchetta*, M.P.Piccardi*, G.U.Corsini*^, Dept.Neurosciences, Univ.of Cagliari,Via Porcell 4,09124 Cagliari, and ^Inst. Pharmacology,Univ.of Pisa,Via Roma 55, 56100 Pisa, Italy.

The present study was designed to clarify the biological significance of ^{3}H-MPP$^+$ binding sites and their relevance for MPTP neurotoxicity. We used 6-OH-DA-lesioned rats and MPTP-lesioned mice in attempts to localize the ^{3}H-MPP$^+$ binding site. Unilateral 6-OH-DA lesions of the nigrostriatal DA neurons reduced by 30% the B_{max} of the ^{3}H-MPP$^+$ binding in the homogenate of lesioned caudate. The localization of specific ^{3}H-MPP$^+$ site and its modifications in the nucleus caudate will be evaluated by quantitative autoradiography. Inhibition experiments showed that debrisoquin displaces ^{3}H-MPP$^+$ from its sites (IC_{50}: 0.015 μM). Since MPP$^+$ is thought to be the actual toxic metabolite of MPTP, we have investigated whether debrisoquin might be in itself a nigrostriatal toxin. Striatal DA levels were unchanged in mice injected bilaterally intracerebroventri cularly with amounts of debrisoquin up to 50 μg, compared with saline injected animals. A single dose of MPTP (30 mg/kg, i.p.) significantly decreased DA levels to 40-55% of control values. Debrisoquin (up to 100 μM) had no effect on MAO-B activity or MPP$^+$ uptake, whereas it inhibited MAO-A with an IC_{50} of 2,5 μM. We will investigate whether debrisoquin might interact with MPTP toxicity.

22.4

MPTP-INDUCED HEMIPARKINSONIAN MONKEYS EXHIBIT ABNORMALLY HIGH METABOLIC ACTIVITY RATES WHEN EXPERIENCING PASSIVE MANIPULATION. H.H. Holcomb, G.E. Alexander, M.R. DeLong and H.L. Loats, Johns Hopkins Medical Institutes, Dept. of Neurology; U. MD Psych. Res. Ctr.; Baltimore, MD; and Loats Associates, Westminster, MD.

MPTP-induced hemiparkinsonism produces unilateral rigidity, bradykinesia, tremor and visual neglect. Electrophysiological studies have documented marked changes in the internal and external pallidum following MPTP treatment, especially when the subject is manipulated. Using a modification of the double-label quantitative 2-deoxyglucose method we have studied one normal and one MPTP treated primate in resting and passively manipulated conditions, right forearm, to assess the impact of movement on neural metabolic activity patterns. Trained to accept passive, repetitive flexion-extension of the forearm, elbow fixed, one hemiparkinsonian monkey was first given [14C]-2DG, 100 microCuries/kg, immediately following the onset of passive manipulation, 20 minutes. 45 minutes after the first injection, 8 milliCi/kg of [3H]-2DG was given prior to a 45 minute rest phase. Quantitative autoradiographic image subtraction and correction for isotope contamination of the second condition by the first tracer permitted accurate assessment of the movement phase metabolism. In the MPTP primate, during rest the striatum exhibited increased metabolism, external pallidum increased, internal pallidum normal, subthalamus reduced, ventrolateral thalamus normal, and motor cortex normal. During passive manipulation all regions exhibited only modest elevations in the striatum and cortex during passive manipulation. This increase in neural metabolic activity is consistent with the large elevations in electrical activity associated with hemiparkinsonism.

22.5

POTENTIATION OF THE ANTIPARKINSONIAN EFFECT OF BROMOCRIPTINE BY D_1 DOPAMINE AGONISTS IN MPTP MONKEYS. B. Gomez Mancilla*, P.J. Bédard, C. Rouillard, C. Gagnon* and T. Di Paolo. Lab. of Neurobiology, Hôp. Enfant-Jésus and Dept. of Molecular Endocrinology, Laval University, Medical Center, Québec, Canada.

We have previously reported that SKF 38393, a dopamine D_1 agonist had little effect by itself against the MPTP-induced parkinsonian syndrome in monkeys (Falardeau et al. Eur. J. Pharmacol. 150, 59, 1988) thus confirming earlier reports by Nomoto et al. Neurosci. Lett. 57, 37, 1985. Preliminary results of the combination of bromocriptine and SKF 38393 disclosed little additive effect (Rouillard et al. Soc. Neurosci. Abstr., 18, 389, 1988). We now report that after full analysis of the data SKF 38393 5 mg/kg given orally seems to increase the locomotor effect of bromocriptine 5 mg/kg orally. The new putative D_1 agonist CY 208243 also potentiates the action of smaller doses of bromocriptine (0.16 mg/kg) orally in a dose-dependent manner. There was no difference in [^{3}H] Spiperone binding or [^{3}H]-SCH 23390 in the striatum of animals treated with bromocriptine alone or bromocriptine plus SKF 38393. Similar determinations are currently being performed on the brains of animals treated with bromocriptine alone or in combination with CY 208243 and will be reported. Supported by MRC of Canada and the Parkinson Foundation of Canada.

22.6

ALTERATIONS IN OPIATE RECEPTOR BINDING IN MPTP-INDUCED HEMIPARKINSONIAN MONKEYS. L.J. Porrino[1], J.J. Viola[2,3], F.E. Pontieri[3], A.M. Crane[3], K.S. Bankiewicz[1], I.J. Kopin[1]. [1]NINDS, [2]HHMI, [3]NIMH, Bethesda, MD 20892.

The neurotoxin, MPTP, when infused directly into one internal carotid artery of primates, causes selective destruction of the dopaminergic neurons of the substantia nigra pars compacta ipsilateral to the infusion, resulting in a hemiparkinsonian (HP) syndrome (Bankiewicz et al., 1986). Such lesions of the nigrostriatal system cause alterations in the properties of striatal dopaminergic receptors (Joyce et al., 1985). In this study, because of the close link between dopamine and opiate systems, we investigated possible accompanying alterations in the distribution of opiate receptors in the striatum of HP monkeys. In vitro binding with [^{3}H]naloxone (conditions appropriate for the μ receptor subtype) was determined autoradiographically in four HP and two normal monkeys. In normal monkey and on the non-lesioned side of HP monkeys, striatal [^{3}H]naloxone binding was generally arranged in faint patches which were most evident in caudate and anterior putamen. Striatal patches corresponded to acetylcholinesterase-poor zones, assessed in adjacent sections. In regions outside the patches (matrix) the density of opiate binding sites was highest in the nucleus accumbens, and ventral caudate and putamen. Density was also higher rostrally than caudally. In the MPTP-lesioned hemisphere, although the regional distribution of opiate binding remained similar, the differentiation of patch from matrix was more apparent. The density of binding in the patches was increased by 25-50%, whereas a more moderate increase (+13-22%) was observed in the matrix. These data are a further demonstration of dopamine/opiate interactions in striatum, and suggest opiate receptor upregulation in response to dopaminergic denervation by MPTP in the primate.

22.7

CONTINUED GM1 GANGLIOSIDE ADMINISTRATION IS REQUIRED TO MAINTAIN BIOCHEMICAL AND BEHAVIORAL RECOVERY IN MICE EXPOSED TO MPTP. **F.B. Weihmuller*, M. Hadjiconstantinou, J.P. Bruno, and N.H. Neff. College of Medicine, Depts. of Pharmacology, Psychiatry, and Dept. of Psychology, The Ohio State University, Columbus, OH 43210-1222.**

Administration of a low dose of haloperidol, one that has no sensorimotor effects in intact mice, produces akinesia, catalepsy, and somatosensory neglect in MPTP-treated mice. Recently, we demonstrated that chronic daily injections of GM1 ganglioside return striatal DA and DOPAC content to near-normal levels and eliminate neuroleptic-induced sensorimotor impairments in MPTP-treated mice (Weihmuller et al., Neurosci. Lett. 92:207, 1988). To determine the extent of GM1-induced recovery, we examined striatal DA and DOPAC content, receptor density, and haloperidol-induced sensorimotor effects at time points up to 30 days after terminating an initial 23 days of GM1 treatments. Discontinuing GM1 therapy reinstated the neurochemical and behavioral profile of mice given MPTP alone. Striatal DA and DOPAC content fell to 50% of control values, striatal D2 receptor density increased, and neuroleptic-induced sensorimotor deficits returned. These results suggest that continued GM1 administration is required to maintain the biochemical and behavioral recovery seen in MPTP-treated mice.

22.8

NEUROPATHOLOGY OF MPTP-TREATED SQUIRREL MONKEYS. LONG TERM STUDIES. L.S.Forno, J.W.Langston, L.E. DeLanney and I.Irwin, VA Medical Center, Palo Alto,CA 94304 and Institute for Medical Research, San Jose, CA 95128.

Experimental MPTP-induced parkinsonism in non-human primates has shown great similarities to human Parkinson's disease. Previous studies have dealt mainly with animals living less than a year after treatment. We have examined the brain in 10 squirrel monkeys with 1 to 4 years survival after treatment. The animals were young or middle-aged except for one old monkey. Five of the monkeys received only a brief series (5 days or less) of i.p. injections. Three were given injections over several months. We found considerable nerve cell loss and glial scarring in the substantia nigra in all except one monkey. In contrast to monkeys with acute lesions, the pathology was most pronounced in the ventral and lateral cell groups. There were only questionable changes in the locus coeruleus. No inclusion bodies and no convincing abnormality were seen in other parts of the CNS. The distribution of lesions in MPTP-treated monkeys with long survival closely resembles that seen in Parkinson's disease, but the disease process appears to be relatively inactive.

22.9

CALCIUM CHANNEL AGENTS DIFFERENTIALLY ALTER MPTP INDUCED CHANGES IN MOUSE BRAIN SLICE DOPAMINE. J.A. Wilson, J.S. Boyle*, & Y.-S. Lau. Creighton University School of Medicine, Omaha, NE 68178

A Parkinson's disease like syndrome can be produced by administration of the neurotoxin MPTP. The mechanism by which MPTP exerts its toxic effect is unknown, and while studies are underway to elucidate this mechanism, the structural similarity between MPTP's toxic dihydropyridinium (DHP) metabolite MPDP* and the DHP calcium channel blockers provided an impetus for us to investigate the ability of these drugs to alter dopamine levels.

Mouse brain slices were used to examine the effect of calcium channel agonists and antagonists on MPTP's ability to alter dopamine levels in the slices. Each slice contained the nigro-striatal dopamine system and was maintained in artificial CSF for at least 1 hour before treatments were started. Slices were first pre-treated with the agent acting on the calcium channel and then MPTP was added to the experimental slices. We found that the non-DHP calcium channel blocker diltiazem caused an increase in basal levels of dopamine. The DHP calcium channel blocker nifedipine did not have an effect on basal levels or an effect on MPTP's ability to deplete dopamine whereas the DHP calcium channel agonist BAY-K-8644 decreased the MPTP induced reduction in dopamine concentrations. In light of the earlier physiological finding that low calcium - high magnesium CSF prevented MPTP's ability to decrease the amplitude of synaptic transmission, further studies are now planned to better define the interaction between MPTP and synaptic transmission.

22.10

MPTP OR METHAMPHETAMINE INDUCES GLIOSIS AND AXONAL NEUROPIL CHANGES IN THE CAUDATE NUCLEUS. W. G. McAuliffe*, C. Desiderio* and A. Hess (SPON:S. Rosner). Dept. of Anatomy, UMDNJ, R. W. Johnson Med. Sch., Piscataway, NJ 08854.

MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) and methamphetamine, both able to produce a parkinsonian syndrome, cause similar pathological changes in the caudate nucleus of mice. Two s.c. injections of 50mg/kg MPTP, one per day, or three i.p. injections of 10mg/kg methamphetamine at two hour intervals were given. Three to six days after the last injection, brains were immunostained for tyrosine hydroxylase (TH) and glial fibrillary acidic protein. After either drug, the TH content of the caudate nucleus is obviously reduced. In the neuropil, normally occurring densely packed punctate terminals can no longer be detected, but now appearing are long, branching, varicose pre-terminal (sprouting?) fibers. Normally sparse glial cells are not only dramatically increased in number, but are grossly hypertrophied compared with unaffected glia. Gliosis ensues during or soon after degeneration of dopaminergic synaptic terminals. The structural alterations in the axonal neuropil and gliosis are acute pathological manifestations of the dopamine depletion induced by these toxic drugs. Studies are under way to ascertain how long these degenerative effects can still be detected and if there is recovery in the caudate nucleus.
Supported by the National Parkinson Foundation

22.11

ASTROGLIAL INVOLVEMENT IN MPTP NEUROTOXICITY:ANATOMICAL ASSESSMENT. M. Takada, K.J. Campbell, M.S. Nishihama* and T. Hattori. Dept. of Anatomy, Univ. of Toronto, Toronto, Ontario M5S 1A8, Canada.

The oxidative conversion of MPTP to MPP^+, the major active metabolite, is mediated by the action of monoamine oxidase B, which is localized in astroglia. Various combinations of intranigral injections of MPTP (50 μg) and astroglia-specific toxin, L-alpha-aminoadipic acid (L-alpha-AA, 20-100 μg), were employed to examine the effects of selective astroglial ablation on MPTP-induced nigrostriatal cell death. Varying cell loss was assessed by the aid of fluorescent retrograde tracing. Treatment with MPTP alone caused tremendous nigrostriatal cell loss, while intranigral co-injections of MPTP and L-alpha-AA produced protection against MPTP neurotoxicity in a dose-dependent fashion. Similar protection was seen in the case of gliotoxin pretreatment just prior to or 1 day before MPTP administration. Intraventricular injections of ^{3}H-MPTP (50 μCi, 82.3 Ci/mmol) resulted in perikaryal labeling in various motor-related regions below the midbrain, out of which only the substantia nigra pars compacta and dorsal raphe were accompanied by extremely heavy radiolabeling to astroglia. The present findings thus provide anatomical evidence for the significance of astroglial involvement in the onset of MPTP neurotoxicity. Supported by the MRC of Canada.

22.12

MPTP EFECTS IN THE MOUSE BRAIN AND VITAMIN E. Ifeoma N. Odunze*, Lori K. Klaidman* and James D. Adams, Jr. School of Pharmacy, University of Southern California, Los Angeles, CA 90033.

MPTP is known to affect the midbrain levels of the antioxidant, glutathione. Therefore, the relation between another antioxidant, vitamin E, and MPTP toxicity was investigated.

Vitamin E in extracts from various brain regions was assayed by HPLC with fluorescence detection. Catecholamine levels in the substantia nigra and striatum were analyzed by HPLC with electrochemical detection.

MPTP (4 doses at 2 h intervals, 25 mg/kg, ip) was found to alter levels of vitamin E in all brain regions examined. These alterations occurred within 2 days of MPTP administration and may have been reversible.

Vitamin E deficient mice were produced by raising mice on a vitamin E deficient diet for 16 weeks at which time brain levels are about 25% of control vitamin E levels. MPTP was more lethal to vitamin E deficient mice than to control mice, which made using lower MPTP doses necessary (4 X 15 mg/kg). Catecholamine levels in the substantia nigra were depleted to lower levels by MPTP in vitamin E deficient mice than in control mice. This depletion was significantly different for DOPAC and the ratio DOPAC/dopamine. Catecholamines in the striatum were not more depleted in vitamin E deficient mice than in control mice.

Vitamin E may play a role in protection against MPTP toxicity, especially in the substantia nigra. More research is needed to see if vitamin E is important in Parkinson's disease.

22.13

EFFECT OF CHRONIC DOPAMINERGIC TREATMENT ON D-1 AND D-2 RECEPTORS IN PARKINSONIAN MPTP-TREATED MONKEYS. C. Gagnon[1]*, C. Rouillard[1], P.J. Bédard[2] and T. Di Paolo[1] (SPON: S. Radouco-Thomas). [1]School of Pharmacy, Laval Univ. and Dept. of Molecular Endocrinology, CHUL, Québec G1V 4G2 and [2]Dept. of Anatomy, Fac. Med., Laval University, Québec G1K 7P4, Canada

We have compared the effect of chronic treatment (at least 4 weeks) of MPTP monkeys (Macaca Fascicularis) with different DA agonists (Sinemet, D-1 and D-2 agonist; bromocriptine, D-2 agonist or SKF 38393, D-1 agonist). D-1 and D-2 receptors were quantified by autoradiography of [^{3}H]SCH 23390 and [^{3}H]spiperone binding in the striatum of these animals. All MPTP monkeys had a decrease of DA of more than 95%, vs control as measured by HPLC. MPTP increased specific binding of D-1 and D-2 receptors vs intact control by 66% and 55%, respectively Chronic treatment with Sinemet, bromocriptine or SKF 38393 caused respectively, a decrease of 26% ($p<0.01$), 128% ($p<0.01$) and 52% ($p<0.01$) of D-2 receptors and a decrease of 46% ($p<0.01$), 55% ($p<0.01$) and 10% (n.s.) of D-1 receptors, vs MPTP. No dyskinesia was seen in monkeys on bromocriptine or SKF as opposed to the Sinemet-treated animals. The DA receptor changes observed could be implicated in the loss of efficacy and side effects following these different treatments. Supported by the Parkinson Foundation of Canada and the MRC.

22.14

CHRONIC EXPOSURE TO MPTP CAUSES COGNITIVE DISTURBANCES WITHOUT PARKINSONIAN MOTOR DEFICITS IN PRIMATES. C.J. Kovelowski,II* and J.S. Schneider (SPON: E.L. Mancall) Dept. of Neurology, Hahnemann Univ., Philadelphia, PA. 19102.

The present study investigates the cognitive status of monkeys chronically exposed to low doses of MPTP. Four Nemistrina monkeys were trained to perform delayed response (DR), delayed alternation (DA), and visual discrimination (VD) tasks. After training, monkeys were given intravenous MPTP injections 3 times/wk. in doses of 0.010 - 0.175 mg/kg over periods ranging from 3 - 11 mos. Total cumulative MPTP doses ranged from 17.68 to 56.07 mg. No animal developed a parkinsonian motor disorder. In contrast, all animals developed great difficulty in performing DR (especially at longer delay intervals) and DA, while VD performance remained intact. Some attentional problems were also noted. The dopamine D-2 agonist Quinpirole enhanced DA performance at low doses, while the D-1 agonist SKF-38393 had no positive effect on behavior. Clonidine, an alpha-2 agonist, enhanced DR performance, especially on trials with long delays. Neurochemical analysis revealed both cortical and subcortical catecholamine deficits. Immunocytochemical findings will be discussed. These results show that specific cognitive disturbances can be produced in monkeys exposed chronically to small amounts of MPTP without an accompanying motor disorder, despite extensive damage to the dopamine system. We propose this as a new model for "early" parkinsonism. (Supported by the Alzheimer's Disease and Related Disorders Association, Inc).

23.1

PRETREATMENT WITH A MIXED CYCLOOXYGENASE-LIPOXYGENASE INHIBITOR IMPROVES BEHAVIORAL AND HISTOLOGICAL OUTCOME FOLLOWING SPINAL CORD ISCHEMIA-REPERFUSION. S.H. Graham*, P.D. Demediuk and A.I. Faden (SPON: M. Lemke). Dept. of Neurology, U.C.S.F. and Center for Neural Injury, V.A. Med. Center, San Francisco, CA 94121.

Prostaglandins, hydroxy-eicosatetraenoic acids and leukotrienes, the cyclooxygenase and lipoxygenase products of arachidonic acid metabolism, have potent pathophysiologic effects and have been shown to accumulate in tissues after ischemic injury. In the present study, spinal cord ischemia was induced in the rabbit by temporary occlusion of the abdominal aorta via a femoral balloon-tipped catheter. BW-755C (10mg/kg iv), a mixed cyclooxygenase and lipoxygenase inhibitor, or saline was given 10 minutes prior to induction of 16 minutes of ischemia. BW-755C treated animals (n=12) had better motor performance scores ($P<.05$) and a greater number of surviving anterior horn cells ($P<.05$) at 48 hours after ischemia than saline controls (n=14). There was no detectable thromboxane B2 (TxB2) in BW-755C treated animals 4 hours after ischemia compared to 32.6±14.0 p.g. TxB2 /mg. protein in control spinal cord ($P<.05$). These data support the hypothesis that cyclooxygenase and lipoxygenase products mediate secondary injury in spinal cord ischemia-reperfusion and suggest that cyclooxygenase-lipoxgenase inhibition may be a potential therapy for spinal cord ischemia.

23.2

THE RELATIONSHIP BETWEEN FREE FATTY ACIDS PRODUCTION, GLUTAMATE RELEASE AND HISTOPATHOLOGICAL OUTCOME AFTER TRANSIENT ISCHEMIA. R. Busto, M.Y.-T. Globus, W.D. Dietrich, I. Valdes*, M. Santiso*, and M.D. Ginsberg. Cerebral Vascular Disease Research Center, University of Miami, School of Medicine, Miami, FL, 33101.

Moderate reduction in intra-ischemic brain temperature inhibits glutamate release and confers a marked protective effect on histopathological outcome. We evaluated the relationship between these findings and ischemia-induced free fatty acids accumulation. Rats whose intra-ischemic brain temperature was maintained at 36°C or 30°C were subjected to 20 min of 4-vessel occlusion plus hypotension. Cortical and striatal free fatty acids were measured at the end of ischemia by gas chromatography. In the 36°C animal group, all cortical free fatty acids were significantly elevated, with a preferential rise in arachidonic and stearic acid levels. A similar increase was observed in striatal levels of the palmitic, stearic and arachidonic acids, while oleic acid remained unchanged. In the 30°C, a similar increase was observed in cortical free fatty acids, while even a greater increase in arachidonic and stearic acid accumulation was found in the striatum. These results suggest that reduction in intra-ischemic brain temperature does not attenuate free fatty acid accumulation. Taken together with our previous findings we conclude that 1. ischemia-induced release of glutamate is not associated with free fatty acids production; 2. free fatty acids accumulation is not a indicator of irreversible ischemic brain injury.

23.3

ADENOSINE AND ITS ROLE IN ISCHEMIA/HYPOXIA. J.C. Fowler, Life Sciences Division, Los Alamos National Lab., Los Alamos, NM 87545

Adenosine is an inhibitory neuromodulator that has been hypothesized to influence neuronal excitability during periods of cerebral metabolic stress such as hypoxia or ischemia. The effect of adenosine antagonists on hypoxia- and in vitro ischemia-induced depression of neuronal activity was investigated in the isolated rat hippocampal slice

The Schaffer collateral pathway was stimulated (1/30 sec, suprathreshold stimulation) while the population spike and small fiber volley were recorded in CA1 pyramidal cell region in the submerged hippocampal slice (400 μm). Slices were superfused with normoxic physiological medium (33-34°C, 2 ml/min) equilibrated with 95% O_2/ 5% CO_2. Hypoxia was initiated by superfusing with medium equilibrated with 95% N_2/ 5% CO_2. Under hypoxic conditions, the population spike was completely blocked within 10 minutes. The addition of the adenosine antagonist theophylline (100 μM) resulted in a population spike 30% of control after 15 minutes in hypoxic medium while 8-phenyltheophylline (8-PT) exerted a dose-dependent protection from hypoxia with 10 μM essentially preventing depression at 15 minutes.

In vitro ischemia (superfusion with hypoxic medium lacking glucose) resulted in an initial depression of the population spike followed by a transient return of the response and finally a depression of both the population spike and the fiber volley. In the presence of 8-PT (10 μM) the transient return of synaptic transmission did not occur prior to a complete depression of the population spike and the fiber volley. These results suggest that the initial depression of synaptic activity in CA1 during hypoxia or in vitro ischemia is due to the release of the inhibitory modulator adenosine.

23.4

ADENOSINE AGONISTS INDUCE HYPERGYLCEMIA; IMPLICATIONS FOR ADENOSINERGIC STROKE THERAPEUTIC STRATEGIES. T.M.Loftus* and P.J. Marangos. Gensia Pharmaceuticals, San Diego, CA 92121

Adenosine agonists induce a dramatic hyperglycemia approaching four fold higher than normoglycemic levels. Recently we have shown that the adenosine agonist, N6 cyclohexyladenosine (CHA) is neuroprotective in cerebral ischemia. Blood glucose levels have also been found to affect the outcome of ischemic insult. Hypoglycemia has been shown to be beneficial, (LeMay, D.R. et al., Stroke, 1988; 19:1411-1419) while hyperglycemia has been demonstrably detrimental (Duverges, D. and Mackenzie, E.T., J. Cerebral Blood Flow and Metabolism, 1988; 8:449-461).

Agonists were administered intraperitaneally to mice and rats, and glucose levels were analyzed in plasma by the hexokinase/glucose 6-phosphate dehydrogenase method. Direct addition of agonists to the assay mixture had no effect on the accuracy of determination of solutions of known concentration. Elevations in glucose levels were observed in response to CHA, 5'N-ethylcarboxamidoadenosine (NECA), and the D and L isomers of N6-phenylisopropyladenosine (PIA). These elevations were evident by fifteen minutes and persisted beyond four hours post injection at doses not greater than 1 mg/kg. It was possible to reverse the effect by the coadministration of the peripheral adenosine antagonist 8(p-sulfophenyl) theophylline which blocks the peripheral effects of the agonists while perserving the central effects. These findings suggest that previous results with adenosine agonists in stroke, although impressive, may have been partially masked due to the coincident hyperglycemia. Furthermore, blocking of this hyperglycemia with a peripheral adenosine receptor antagonist might substantially improve the therapeutic efficacy of adenosine agonists in various animal models of cerebral ischemia .

23.5

THE ADENOSINE ANTAGONIST 8-CYCLOPENTYLTHEOPHYLLINE REDUCES THE HYPOXIC DEPRESSION OF SYNAPTIC RESPONSES IN SLICES OF RAT HIPPOCAMPUS. V.K. Gribkoff, L.A. Bauman, and C.P. VanderMaelen. CNS Biology, Bristol-Myers Co., 5 Research Parkway, Wallingford, CT 06492.

The actions of adenosine on hippocampal neurons provide evidence for a purinergic neuroregulatory function in this structure, and suggest a potential therapeutic role in conditions such as neuronal ischemia. In the present experiments we have examined the possibility that the depression of hippocampal synaptic responses during hypoxia may result from an endogenous action of adenosine.

Slices from young rats were bathed in either normal medium, 100nM 8-cyclopentyltheophylline (8-CPT, an A_1 receptor antagonist), 250nM 8-CPT, or 1.0µM 8-CPT. Infusion of 250nM or 1.0µM 8-CPT, but not 100nM 8-CPT produced a small but consistent increase in synaptic responses in CA_1. In normal medium, 15' hypoxic episodes produced rapid and severe depression of all synaptic responses. In 250nM and 1.0µM, but not 100nM 8-CPT, the depression was decreased in amplitude and delayed in onset. Recovery of the responses occured in all conditions. Similar experiments were performed on aged rat slices (Fisher 344, 25-27mo.), using 1.0µM 8-CPT vs. normal medium only. The adenosine antagonist again delayed hypoxia-induced synaptic depression, and reduced its magnitude.

These results suggest that adenosine may protect hippocampal neurons during periods of hypoxia by reducing transmission.

23.6

THE ADENOSINE ANALOGS 2-CHLOROADENOSINE (2CA) AND 5'-(N-ETHYL)CARBOXAMIDOADENOSINE (NECA) PROTECT AGAINST DYNORPHIN A (DYN)-INDUCED RAT SPINAL CORD INJURY. J.B. Long, D.D. Rigamonti, and A. Martinez-Arizala, Dept. of Med. Neurosci., Walter Reed Army Inst. of Res., Washington, D.C. 20307.

Lumbar subarachnoid injection of DYN causes decreased blood flow, ischemic neuronal degeneration and persistent hindlimb (HL) paralysis in rats. Excitatory amino acids have been implicated as mediators of DYN-induced rat spinal cord injury due to protective effects of selective noncompetitive NMDA receptor antagonists. Adenosine analogs, in addition to having potent vasodilatory effects, have also been shown to inhibit presynaptic release of the putative excitotoxin glutamate and to protect against ischemia-induced neuronal cell loss. Therefore, we examined potential protective effects of 2-CA and NECA on the blood flow reductions, ischemia, and HL motor deficits caused by L4-L5 spinal subarachnoid injections of 20 nmoles of DYN. Immediate preinjection of 2CA and NECA (62-250 nmoles, i.t.) failed to block the initial HL paralysis induced by DYN; however both compounds caused significant dose-dependent improvements in motor scores by 24 hr postinjection. In contrast, the spinal cord blood flow reductions (measured by radiolabeled microspheres) and ischemia (reflected by three-fold elevations in CSF lactic acid concentrations 20 minutes after injection of 20 nmoles of DYN) were both unchanged by these compounds. Thus, through mechanisms independent of their vascular effects, these adenosine analogs protect against DYN-induced spinal cord injury.

23.7

THE COMBINATION OF DEXTROMETHORPHAN AND PHENYTOIN HAS POTENT ANTI-ISCHEMIC PROPERTIES. C. Yang*, P.H. Schwartz, H. Hattori*, D.G. Fujikawa, A.M. Morin and C.G. Wasterlain. Epilepsy Res. Lab., V.A. Med. Ctr., Sepulveda, CA. 91343 and Dept. of Neurology, UCLA Sch. of Med., Los Angeles, CA 90024.

Dextromethorphan (DM) and phenytoin (PHT) potentiate each others binding, and both have some neuroprotective properties, yet the anti-ischemic effects of their combination have never been studied. We treated 4 groups of 10 Wistar rats aged 7 days by bilateral carotid ligation under methoxyflurane anesthesia, followed by exposure to an 8% O_2 atmosphere for 1 hour. Three days later the brains were perfused-fixed and sectioned. The surface of infarcted neocortex was measured on a computerized image analysis system and the severity of neuronal necrosis was graded (0-5+ scale). Rats were pre-injected with saline (group 1), DM 20mg/kg (group 2), PHT 50 mg/kg (group 3) or DM & PHT (group 4). The mean surface of neocortical infarct was 9.6 mm^2 ± 3.9 (gr. 1), 6.7 ± 3.9 (gr. 2), 3.5 ± 3.5 (gr. 3), and 0 (gr. 4). Necrosis was graded 2.8 ± 0.6 (gr. 1), 2.2 ± 0.4 (gr. 2), 0.5 ± 0.3 (gr. 3, $p<.01$) and 0 (gr. 4, $p<.01$). These data suggest that the combination of dextromethorphan & phenytoin has powerful anti-ischemic properties in newborn rat neocortex.

23.8

EFFECT OF THE NMDA ANTAGONIST DEXTRORPHAN ON NEUROLOGICAL FUNCTION 24 HOURS AFTER FOCAL CEREBRAL ISCHEMIA. D. Kunis, G. Steinberg, R. DeLaPaz*, and A. Poljak*

The non-competitive NMDA antagonist dextrorphan (DX) has been shown to protect against focal cerebral ischemia when ischemic neuronal damage or ischemic edema is measured at 5 1/2 hours post-ischemia (Steinberg, Stroke, 1989, in press). We tested the ability of DX to protect against clinical stroke, as well as infarct and edema at 24 hours after cerebral ischemia in a rabbit model. Anesthetized rabbits underwent occlusion of the left internal carotid and anterior cerebral arteries with induced hypotension for one hour, followed by 23 hours of reperfusion. One hour prior to occlusion they were loaded with either i.v. DX (15 mg/kg) followed by 15 mg/kg/hr for the next 5 hours or an equivalent volume of normal saline. Anesthesia was terminated with the start of reperfusion. Animals were scored for neurological deficits at 24 hours post-ischemia (scale 0-13), sacrificed and their brains analyzed.

Compared with the controls, DX treated rabbits had a better clinical grade (1.4 vs. 3.8, p=.2), less infarct (13.1% vs. 27.1%, p=.08) and reduction in ischemic edema (14.7% vs. 26.5%, p=.24), but none of these differences reached statistical significance. One DX animal died at 22 hours and one control died at 5 hours post-ischemia. DX treated rabbits were slower to recover from anesthesia than the controls. Despite the lack of statistical significance between groups, our results suggest a trend towards DX protection against neurological deficit and cerebral injury after focal ischemia. Failure to attain significance may be due to 1) adverse systemic effects of DX; 2) discontinuation of DX infusion after 5 hours; 3) severity of the ischemic insult; or 4) large variance within groups.

23.9

DEXTROMETHORPHAN FAILS TO IMPROVE OUTCOME FOLLOWING SPINAL ISCHEMIA IN THE RABBIT. A. Martinez-Arizala, D. D. Rigamonti, J.B. Long, E.E. Echevarria*, J.M. Kraimer*, and J. W. Holaday. Dept. of Med. Neurosciences, Walter Reed Army Institute of Research, Washington, D.C. 20307

Excitatory amino acid transmission, specifically at the NMDA receptor, has been implicated in the pathophysiology of ischemic CNS injury. Recent evidence suggests that the D-opioid dextromethorphan (DM), which can block the NMDA receptor ion channel, is neuroprotective in in-vitro and in-vivo models of CNS ischemia. In the present study, we examined the effects of DM in a model of spinal ischemia in the rabbit. Anesthesized male N-Z rabbits were implanted with a ligature around the infrarenal aorta. The following day, the conscious animals were subjected to 25 min of spinal ischemia, and five minutes after reperfusion they were randomly treated with either DM (n=11; 5 mg/kg i.v. - bolus and infusion), DM (n=10; 10 mg/kg i.v. - bolus and infusion) or saline (n=10). Hindlimb motor function was graded on a 0-5 scale. By six hours post-reperfusion, all groups exhibited similar recovery of motor function. However, this initial recovery was progressively lost such that, at 48 hours, the neurological scores were not different among the groups. In addition, mortality was higher in the DM 10 mg/kg group (6/10 died), when compared to the DM 5 mg/kg group (2/11) or the saline group (3/10). These results indicate that when administered after completion of the ischemic insult in the doses used for these experiments, DM failed to protect against the secondary deterioration of function associated with this model of spinal ischemia.

23.10

ADMINISTRATION OF GLYCINE ANTAGONISTS, HA-966 AND 7-CHLOROKYNURENIC ACID REDUCE ISCHEMIC BRAIN DAMAGE IN GERBILS. J.B. Patel, L.E. Ross*, B. Duncan*, M. Asif*, A.I. Salama and M. Valerio*. Dept. of Pharmacology, ICI Pharmaceuticals Group, ICI Americas Inc., Wilmington, DE 19897.

HA-966 (HA) and 7-chlorokynurenic (7-cl) acid have been shown to inhibit N-methyl-D-aspartate (NMDA) responses via selective antagonism of the modulatory glycine site on the NMDA receptor/ion channel complex. Several NMDA antagonists (e.g., AP-7, CPP, MK-801) have been reported to reduce ischemic brain damage in experimental models. Here we examined the effects of HA and 7-cl on cerebral ischemia induced by bilateral carotid occlusion (10 min) in female gerbils with reference to neuropathological and behavioral (motor activity) changes. Motor activity was assessed 24 hrs and 4 days post ischemia; brains were removed 4 days post ischemia. Drugs were administered intraperitoneally 45 min prior to and immediately post occlusion. As expected, MK-801 (2.0 mg/kg) exhibited greater than 70% protection of damage in hippocampal area. Likewise, HA (10.0 mg/kg) and 7-cl (20.0 mg/kg) produced approximately 60% protection. The ischemia-induced increase in motor activity was also attenuated by these agents, suggesting a restoration of function. These results suggest that a glycine antagonist may be useful in protecting against ischemia-induced damage.

23.11

MK-801, A NONCOMPETITIVE NMDA RECEPTOR ANTAGONIST, DOES NOT PROTECT AGAINST NEURONAL DAMAGE IN THE RAT BRAIN FOLLOWING CEREBRAL ISCHEMIA. B. Nellgård*, J. Gustafson*, A. Hansen*, M. Lauritzen, T.Wieloch* (SPON: M. Lauritzen), Laboratory for Experimental Brain Research, Research Dept. 4, Lund University Hospital, S-221 85 Lund, Sweden. Dept. of Physiol, Panum Institute, University of Copenhagen, Denmark.

Glutamate has been proposed to induce neuronal damage in the hippocampal CA1 region following transient periods of cerebral ischemia, and in some ischemia models competitive and noncompetitive NMDA receptor antagonist have proven cerebro-protective. Ten to 15 min of transient cerebral ischemia induced by bilateral common carotid occlusion combined with hypotension (2-VO), leads to neuronal damage in the hippocampus, neocortex and striatum. Using this model, the effects of MK-801 a noncompetitive NMDA receptor antagonist on ischemic damage was studied. Since MK-801 inhibited spreading depression in the range 0.1-10 mg/kg i.v., a dose of MK-801 was chosen in this range and given either postischemia (post) or before ischemia (pre). In the study 120 animals were used, in the following experimental groups (n=6):

10 min 2-VO isch: 0.1mg/kg i.v. post; 0.1mg/kg i.v.+0.4mg/kg diazepam post; 0.3mg/kg i.p. pre; 1mg/kg i.p. pre; 1mg/kg i.p.+0.4 mg/kg diazepam pre; 3mg/kg i.p. pre; 3mg/kg i.p. pre+1mg/kg/h i.v. for 6h post.

15 min 2-VO isch: 5 mg/kg i.p. pre; 5mg/kg i.p. post; 10 mg/kg i.p. pre.

Cardiac arrest isch: 0.3mg/kg pre; 0.6mg/kg post. Control: 8 groups.

In no experiment was there any significant difference in the extent of neuronal necrosis between a treated group and the appropriate control group.

Conclusion: Glutamate antagonists are not effective in ischemia where complete energy failure has ensured, and where no postischemic complications are evident (hypotension, neuronal hyperactivity). In focal ischemia, and hypoglycemia where some energy production prevail the antagonists may be cerebro-protective.

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23.12

POSTISCHEMIC TREATMENT WITH OLIGO-PROSTAGLANDIN B_1 (DI- AND TRI- CALCIPHOR) IN GERBILS. D.v. Lubitz, A.E. Kalenak, T.M. Devlin, (Spon. M.C. Kennedy) Dept. of Biol. Chem., Hahnemann University, School of Med., Phila., PA 19102

Injection of the trimer of 16,16'-dimethylPGB$_1$ (Tricalciphor) at 10 mg/kg improves survival of brain ischemia in gerbils. Different concentrations of Tricalciphor and Dicalciphor (dimer) administered 5 min plus 24 hrs postischemia were compared. Brain ischemia was induced in 225 gerbils by bilateral carotid occlusion for 20 min. Body temp., MABP, BPM and blood 02 were monitored during ischemia. Treated animals were injected i.p. with either drug at 2,5,10 or 15 mg/kg at 5 min and 24 hrs postischemia. Controls received the vehicle. Survival was monitored for 14 days.

For both Dicalciphor and Tricalciphor, the 10 mg/kg injections were most effective (68% and 65% survivors respectively vs. 38% for controls, $p > 0.03$). Other concentrations were beneficial up to 4 days post-ischemia; thereafter, the statistical reliability of survival declined. The chemically simpler and better defined Dicalciphor is as effective as Tricalciphor and we believe that Dicalciphor is more advantageous in postischemic interventions. (Supp. by ONR).

23.13

GM1 GANGLIOSIDE REDUCES Ca++ LOADING & BEHAVIORAL DYSFUNCTION IN FOCAL CORTICAL ISCHEMIA. C.G. Wakade, V.A. Bharucha*, A. Tagliavia*, S.P.Mahadik and S.E. Karpiak. Div Neurosci NYS Psychiat Inst, Depts Psychiat, Biochem & Mol Biophys, P&S, Columbia U, NY, NY 10032.

We are studying the protective effects of GM1 ganglioside on biochemical & behavioral dysfunctions/losses associated with focal cortical ischemia. Na+, K+ & Ca++ levels have been studied in primary & peri-infarct cortical areas after ischemia insult. Also, rats were monitored for activity levels, and, behavior on pole grasping/balancing and inclined plane tasks. Rats received GM1 injections immediately after the MCAo+CCAo procedure and daily for the experiment's duration. After 3 days the saline treated rats had Ca++ levels of 250±40 mmoles/gr in the primary infarct area. In GM1 rats (30mg/kg/day i.m.) these Ca++ levels were reduced by 50%. In sham controls Ca++ levels = 10mmoles/gr.

One day after ischemia GM1 & saline treated rats became hyperactive, and had significantly reduced performance scores on the pole and plane tasks. However 2-3 days after ischemia, GM1 treated rats showed significantly reduced levels of hyperactivity and markedly improved performance on the pole & plane tasks as compared to saline controls.Three days after ischemia the activity levels of GM1 rats were almost identical to those of sham controls. GM1 rats continued to show significantly improved performance on the pole and plane tasks as compared to saline treated animals. [NINCDS NS-2525856].

23.14

EFFECTS OF ANESTHETIC AGENTS AND ARTERIAL PCO2 IN FOCAL CEREBRAL ISCHEMIA. J.L. Browning, M.L. Heizer* and D.S. Baskin*. Dept. of Neurosurgery, Baylor College of Medicine, Houston, TX 77030.

We studied the effects of halothane (Hal), α-chloralose (α-C) and arterial pCO2 on infarct size and CSF β-endorphin (β-E) levels in a model of focal cerebral ischemia. Cats were anesthetized with either 1.15% Hal or 3% α-C. A cannula was inserted into the cisterna magna for withdrawal of CSF. Arterial pCO2 was maintained at 28-35 mm Hg (LO) or 55-65 mm Hg (HI). Unilateral focal cerebral ischemia was induced by transorbital occlusion of the middle cerebral, anterior cerebral and internal carotid arteries. Samples of CSF were withdrawn prior to ischemia and at 2,4 and 6 hours afterwards. At six hours post-occlusion the animal was sacrificed. The brain was removed, sliced and stained with 2,3,5 triphenyltetrazolium chloride. Increased pCO2 caused a significant increase in infarct size irrespective of type of anesthetic. Hal anesthesia resulted in ipsilateral hemisphere infarct sizes of 32±7% (LO) and 45±7% (HI) while α-C yielded infarct sizes of 31±8% (LO) and 48±3% (HI). Thus, arterial pCO2 should be controlled in order to produce consistent infarct size. The effects of anesthetic, infarct size and time post occlusion on β-E levels will be presented. These results suggest that anesthetic technique may have a significant effect in focal cerebral ischemia.

23.15

COMBINED TREATMENT WITH DOPAMINE AND NMDA RECEPTOR ANTAGONISTS PROTECTS AGAINST ISCHEMIC DAMAGE. M.Y.-T. Globus, W.D. Dietrich, R. Busto, I. Valdes*, and M.D. Ginsberg. Cerebral Vascular Disease Research Center, University of Miami, School of Medicine, Miami, FL, 33101.

The release of both dopamine and glutamate is involved in ischemic neuronal death. In this study we evaluated whether combined antagonist therapy aimed at both the NMDA and the dopamine sites would attenuate postischemic damage. Rats were pre-treated with MK-801 (5 mg/kg), SCH-23390 (2.5 mg/kg), sulpiride (10 mg/kg), MK-801 + SCH-23390 or MK-801 + sulpiride. They were subjected to 10 min of bilateral carotid occlusion plus systemic hypotension. Three days later, normal neurons were counted in the medial, middle and lateral sub-regions of the CA1 hippocampus. Dopamine antagonists alone failed to confer protection, and MK-801 produced a moderate effect in lateral sub-region. No additive protection was demonstrated with the combined treatment of MK-801 and D-2 antagonist (sulpiride). However, the combined treatment with MK-801 and D-1 antagonist (SCH-23390) was markedly protective in all CA1 regions. The numbers (mean ± SE) of preserved neurons in the medial, middle and lateral areas were 84 ± 23 (normal = 131 ± 5), 124 ± 15 (normal = 126 ± 6) and 137 ± 8 (normal = 137 ± 7), respectively. This results support the hypothesis that both dopamine (through D-1 receptors) and glutamate (through NMDA receptors) are detrimental in ischemia, and combined pharmacotherapy may have potential in the clinical management of cerebrovascular disorders.

23.16

RS-8359, A REVERSIBLE AND SELECTIVE INHIBITOR OF MAO-A, AMELIORATES ISCHEMIA-INDUCED BRAIN DAMAGES. N. Iwata*, K. Kobayashi*, M. Kozuka*, K. Kato*, T. Tonohiro*, K. Yoshimi* and Y. Kubo* (SPON: T. Kaneko) Biol. Res. Labs., Sankyo Co., Tokyo 140, Japan.

Since monoamines are reduced in the ischemic brain, effects of RS-8359((±)-4-(4-cyanoanilino)-7-hydroxycyclopenta(3,2-e)pyrimidine), a new selective and reversible inhibitor of MAO-A (Ki= 2.9×10^{-7}M) and an inhibitor of c-AMP phosphodiesterase (PDE) whose IC50 value was the same as that of theophylline, were studied upon ischemia-induced brain damages. Brain ischemia was made by occlusion of 4-vessels (rat) or bilateral common carotid arteries (gerbil) for a short term. In these animals, RS-8359 ameliorated passive avoidance tasks impaired by the ischemia at doses which elevated monoamine levels in the brain (10-30 mg/kg, PO), accompanied with mitigation of delayed neuronal death in the hippocampus. RS-8359 also improved erythrocyte deformability like some other inhibitors of c-AMP PDE, and improved hypoperfusion of the local cerebral blood flow (LCBF) after the ischemia. Thus RS-8359 ameliorated ischemia-induced some brain damages probably due to restoration of monoamines in the brain and increase of the LCBF.

23.17

PROTECTION AGAINST ISCHEMIC DAMAGE IN THE RAT BRAIN BY AN ALPHA-2-ANTAGONIST GIVEN POSTISCHEMIA. T. Wieloch*, I. Gustafson*, E. Westerberg* (SPON: J. Storm-Mathisen), Laboratory for Experimental Brain Research, Research Dept. 4, Lund University Hospital, S-221 85 Lund, Sweden

Ten minutes of transient cerebral ischemia induced by bilateral common carotid occlusion combined with hypotension, induces 85 % neuronal necrosis in the CA1 region of the hippocampus and 7 % damage in the cortex, as determined by histological examination one week following the insult. Lesions of the nc. locus coeruleus increase ischemic neuronal damage in the hippocampus.

Idazoxan is an alpha-2-adrenoceptor antagonist that enhances the activity of the LC neurons and increases the release of noradrenaline (NA). When given i.v. as a bolus of 0.1mg/kg immediately after a transient 10 min ischemia period and then continuously for 6 h as an infusion 10ug/kg/min, idazoxan decreased the damage in the hippocampus to 22 %. No significant protective effect was seen when idazoxan was given as a bolus dose 0.1 mg/kg immediately following ischemia. When given 30 min after the insult and for an additional 24 h the protective effects was less marked and the damage decreased from 82 % to 54 %. Idazoxan did not displace [^{3}H]glutamate or [^{3}H]MK-801 from 6um brain slices, and is thus not a glutamate antagonist.

Conclusions:

1. Idazoxan may be protective by enhancing the release of NA thereby stimulating the inhibitory effect of NA at postsynaptic sites.

2. Dispite the delayed neuronal damage observed by light microscopy 48h after the ischemic insult, cellular processes occuring during first hours postischemia are crucial for induction of the necrotic processes.

3. Alpha-2-adrenoceptor antagonists may be a new class of cerebro-protective drugs that are approved for clinical use.

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23.18

SYNAPTOSOMAL UPTAKE AND METABOLISM OF TRYPTOPHAN IN CEREBRAL ISCHEMIA. C. Chang*, K. Kumami* and M. Spatz Lab. of Neuropathology and Neuroanatomical Sciences, NINDS, NIH, Bethesda, MD 20892

The postischemic persistent disturbance of 5-HT in the brain is associated with an increased content of free tryptophan. To further evaluate the mechanism responsible for these observations we investigated the effect of ischemia on synaptosomal uptake of tryptophan and its conversion to 5-HT and 5-HIAA.

Cerebral ischemia was induced in gerbils by bilateral carotid artery occlusion for 15 min with and without 1 hr release. Tryptophan, 5-HT and 5-HIAA content were simultaneously measured (HPLC) in cerebrocortical synaptosomes prior to and after tryptophan addition 5μM/mg protein) according to Wolf and Kahn method (J. Neurochem. 46: 61, 1986).

Ischemia alone had no effect on the intrasynaptosomal uptake of tryptophan and its conversion to 5-HT and 5-HIAA. The 5-HT and 5-HIAA remained significantly decreased (33%) as compared to controls (100%). In postischemia, intrasynaptosomal uptake and the increase in the content of 5-HT and 5-HIAA were significantly lower (50%) than in controls. However, the percentage of indolamines formed from the exogenous added tryptophan was the same in both groups. These findings indicate that the attenuated intrasynaptosomal content of 5-HT is not due to inability of tryptophan to be converted to 5-HT.

23.19

FLUMAZENIL PREVENTS MIDAZOLAM INDUCED PROTECTION AGAINST ANOXIC DAMAGE IN THE RAT HIPPOCAMPAL SLICE. A.E. Abramowicz*, I.S. Kass, G. Chambers*, and J.E. Cottrell*, Department of Anesthesiology, SUNY Health Science Ctr., @ Brooklyn, Brooklyn, NY 11203.

We are examining the mechanism of benzodiazepine protection of neurons against anoxic damage.

The evoked population spike in the CA1 region of the rat hippocampal slice recovers little from 5 min. of anoxia (10 ± 6% of preanoxic amplitude). When midazolam 100 uM is present, the response in CA1 recovers to 63 ± 13% (p <.001). Flumazenil 10 mg/L, a competitive benzodiazepine receptor antagonist, has no protective effect on the evoked response (5 ± 5%). When added with midazolam, flumazenil abolishes the protection observed with midazolam alone; these slices recover only 5 ± 3% of the predrug, preanoxic amplitude.

Ca^{45} influx during 5 min. of anoxia was measured in normoxic and anoxic slices with and without midazolam 100 uM. The CA1 Ca^{45} uptake increases during anoxia from 5.82 ± .11 to 6.56 ± .18 nM/mg dry tissue weight (p <.001) when no drug is present. Midazolam prevents the increase in CA1 Ca^{45} uptake during 5 min. of anoxia: 4.9 ± .13 (p <.001 versus normoxic and anoxic slices).

Our results suggest that the protective effect of midazolam on CA1 pyramidal cells in vitro is benzodiazepine receptor mediated and that the electrophysiologic recovery observed correlates with reduced Ca^{45} influx during anoxia.

23.20

NICARDIPINE DOES NOT PROTECT NORMOTHERMIC GERBILS AGAINST ISCHEMIC INJURY IN HIPPOCAMPUS. V.A.Harris*, F.A.Welsh, E.S.Flamm. Div. of Neurosurg., Univ. of Penna., Phila., PA 19104.

Administration of nicardipine, an antagonist of voltage-sensitive calcium channels of the "L" type, has been reported to reduce ischemic injury in CA_1 hippocampus of the gerbil (Br. J. Pharmacol., 93:877, 1988). However, even minor degrees of hypothermia induced by drug treatment may contribute significantly to the protective effect. Therefore, we regulated brain temperature at 37°C during 5 min ischemia (bilateral carotid occlusion) and for 2 hr reperfusion in nicardipine- (1 mg/kg, i.p.) and saline-pretreated gerbils. Following recovery for 1 week, the brains were quick-frozen, and alternate thin slices were stained for histologic scoring of CA_1 injury or freeze-dried for microassay of ATP in the CA_1 zone. Both groups of gerbils exhibited maximal histologic change in CA_1 hippocampus. Further, the reduction of ATP content in the CA_1 zone was the same in nicardipine- and saline-pretreated animals. Thus, pretreatment with nicardipine did not reduce ischemic injury in normothermic animals.

23.21

INHIBITION OF NMDA RECEPTOR ACTIVATION IN HIPPOCAMPAL SLICES FACILITATES THE RECOVERY OF PROTEIN SYNTHESIS FROM DAMAGE CAUSED BY ANOXIA. A. J. Carter and R. E. Müller (SPON: C.Wehrhahn) Dept. of Pharmacology, Boehringer Ingelheim KG, Ingelheim, West Germany.

We have investigated the relationship between anoxic damage and NMDA receptor activation. Anoxic damage was assessed by measuring protein synthesis (PS) in rat hippocampal slices ([^{14}C]-lysine incorporation for 30 min in normal medium). PS was reduced after anoxia. The extent of anoxic damage and the ability to recover from it was related to the duration of anoxia. Activation of the NMDA receptor by removing Mg^{2+} potentiated anoxic damage. Removal of Mg^{2+} in the absence of anoxia also caused inhibition of PS; this effect was enhanced by adding NMDA. Although the NMDA antagonists, MK-801, PCP and N-allylnormetazocine, did not prevent the inhibition of PS measured immediately after anoxia, they did facilitate the later recovery of PS. Furthermore, they prevented the damaging effects of removing Mg^{2+} and adding NMDA in the absence of anoxia. These results indicate that activation of the NMDA receptor results in a reduction in PS, and that inhibition of NMDA receptor activation facilitates the recovery of PS after anoxic damage.

23.22

COMBINED TREATMENT WITH MK-801 AND CNQX PREVENTS ISCHEMIC NEURONAL DEGENERATION IN THE *IN VIVO* RAT RETINA. J. L. Mosinger and J. W. Olney, Washington University School of Medicine, St. Louis, MO.

Anoxia and ischemia are common causes of irreversible neuronal damage in the CNS. We have previously described a non-invasive method of producing vascular occlusion and ischemic neuronal degeneration in the adult rat retina (Expt. Neurol., in press). This method utilizes the ability of rose bengal dye to initiate clot formation in blood vessels when stimulated by intense light. We consider this a promising model for testing the ability of various drugs to block acute ischemic neuronal degeneration in the *in vivo* adult mammalian CNS.

A reproducible pattern of neuronal degeneration was induced in the rat retina using 5 min exposure to an intense light following i.v. injection of rose bengal (40 mg/kg). The typical reaction, after 1 hr, included conspicuous dendritic swelling in both the outer and inner plexiform layers and similar swelling of numerous neurons in the inner nuclear and ganglion cell layers, with early pyknotic changes in the nuclei of these cells. The acute neurodegenerative reaction was judged identical in pattern, cytopathological appearance and time course to the excitotoxic reaction typically seen in the retina following exposure to exogenous glutamate or related excitatory amino acids (EAA).

MK-801 is a powerful antagonist of the N-methyl-D-aspartate (NMDA) subtype of EAA receptor, and CNQX preferentially blocks other (non-NMDA) EAA receptors. Both MK-801 (up to 800 nmol) and CNQX (up to 100 nmol) resulted in inconsistent protection when injected intravitreally prior to induction of ischemia. However, when the two drugs were combined (320 nmol MK-801 and 80 nmol CNQX), little or no swelling or other ischemic changes occurred in either the plexiform or nuclear layers. These results are consistent with the hypothesis that ischemic neuronal degeneration in the adult rat retina is mediated by endogenous glutamate or related excitotoxins acting at both NMDA and non-NMDA receptors, and that neurons possessing multiple EAA receptor subtypes may not be protected from ischemic degeneration by an antagonist that acts at only one EAA receptor subclass. Since EAA receptor heterogeneity probably characterizes many neurons throughout the adult CNS, the relevance of our findings may extend to the rest of the CNS. Supported by RSA MH 38894 (JWO) and DA 05072.

23.23

PROTECTION AGAINST CEREBRAL ISCHEMIA BY THE NMDA ANTAGONIST DEXTRORPHAN IS DOSE DEPENDENT AND CORRELATED WITH PLASMA AND BRAIN LEVELS. G.K. Steinberg, J. Saleh, D. Kunis and R. DeLaPaz*, Div. of Neurosurgery, Stanford Med. Center, Stanford, CA 94305

Dextrorphan (DX), a non-competitive NMDA antagonist attenuates hypoxic neuronal injury in culture and reduces cerebral damage after focal cerebral ischemia. We tested the efficacy of post-ischemic treatment with different doses of DX. Thirty-three anesthetized rabbits underwent occlusion of the left internal carotid and anterior cerebral arteries for one hour, followed by 4 1/2 hours of reperfusion. One hour after the onset of ischemia they were treated i.v. with an infusion of varying DX doses or normal saline. Plasma DX levels were measured. After sacrifice the brains were analyzed for ischemic edema and ischemic neuronal damage (IND). A separate group of unanesthetized rabbits received similar i.v. doses of DX, measuring plasma and brain DX levels at sacrifice.

Compared with controls, the DX 15 mg/kg/hr group had significantly less neocortical IND (5.3% vs. 33.2%, p=.01) and a reduction in cortical edema (9.1% vs. 41.2%, p=.02). The DX 10 mg/kg/hr rabbits showed partial protection and DX 5 mg/kg/hr had no benefit. The protective effect of DX was correlated with plasma DX levels (r=-0.50, p<.02 for IND; r=-0.66, p<.001 for ischemic edema). Rabbits with levels >2,000 ng/ml had <12% cortical IND and <34% edema. Rabbits with levels >3,000 ng/ml showed <7% cortical IND and <11% edema. Plasma levels of approximately 2,500 ng/ml correlated with loss of the righting reflex and with brain DX levels of approximately 7,000 ng/g. These results suggest that systemic treatment with DX after the onset of focal ischemia can significantly protect against cerebral damage if adequate plasma levels of DX are achieved. The brain levels necessary to attain in vivo protection are similar to the concentrations that prevent hypoxic or NMDA-induced injury in neuronal culture.

24.1

INCREASED GABA OR DECREASED GLUTAMATE (GLU) LEVELS IN INFERIOR COLLICULUS (IC) BLOCK AUDIOGENIC SEIZURE (AGS) SUSCEPTIBILITY IN GENETICALLY EPILEPSY-PRONE RATS (GEPRs).
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Reduced effectiveness of GABA and increased release of excitant amino acids (EAAs) in the IC are suggested to be important in initiation of AGS in the GEPR (Faingold, C.L. **Gen. Pharmacol.** 19:331, 1988). Microinjection of GABA agonists or EAA antagonists into the IC of the GEPR blocks AGS. Microinjection of NMDA or bicuculline into the IC of normal rats induces AGS susceptibility (Millan, M.H. et al., **Exp. Neurol.** 91:634, 1986). Exogenously-applied GABA and GABA-mediated acoustically evoked inhibitory responses are less effective in IC neurons of the GEPR (Faingold, C.L. et al., **Exp. Neurol.** 93:145, 1986). This study examined the effects of microinjection into the IC of gabaculine (GBC), which blocks GABA degradation or L-canaline (CAN), which blocks GLU synthesis, on AGS severity and amino acid levels in the GEPR IC. AGS were evoked using a bell at 122 dB SPL. AGS severity was reduced after bilateral microinjections (0.5-1 μl) of GBC (0.01- 100 μg/side) or CAN (6-50 μg) into IC. The 2 h required for onset of AGS blockade was longer than that needed with EAA antagonists or muscimol (15 min). The duration of AGS blockade was also greater, and AGS susceptibility returned by 24-48 h. Endogenous amino acids levels in IC were compared with contralateral IC micropunches (1.6 mm) taken 90 min after unilateral GBC or CAN microinjection, using HPLC methods. After microinjection of GBC (10 μg) GABA levels rose significantly ($p < .05$) to 202% of control levels [47.4±5.8 (SEM) nmol-/mg protein]. After microinjection of CAN (50 μg) GLU levels decreased below control levels (177.6±21.6), but this change was not significant, probably because of the large metabolic pool of GLU. Slices (0.5 mm thick) were taken from IC, and release of GLU was examined in the presence of 10^{-4} M CAN. Potassium-evoked release of endogenous GLU fell significantly ($p < .05$) to 43% of control (538±36 pmol/mg protein/5 min). **CONCLUSION:** These findings further support an important role of reduced effectiveness of GABA and increased EAA transmission in the IC in the initiation of AGS in GEPRs. (Support: NIH NS 21281, NS 13849)

24.2

INFUSIONS OF GABA AGONISTS OR 2-APH INTO AMYGDALA (AMY) OR MEDIAL GENICULATE (MGB) REVERSIBLY REDUCE SEIZURE DURATION AND CLONUS AFTER REPEATED AUDIOGENIC SEIZURES (AGS) IN THE GENETICALLY EPILEPSY-PRONE RAT (GEPR-9).
D.K. Narituku, L.B. Meçozzi,* M.E. Randall,* C.L. Faingold.
Southern Illinois University Sch Med, Springfield, IL, USA.
GEPR-9s display AGS with tonic seizures and hindlimb extension (HLE). After repeated AGS, seizure duration increases with appearance of cortical polyspike and spike-wave activity. Prolongation of seizures occurs after HLE during clonic phase, which is absent before repeated AGS (Naritoku, DK, et al, **Soc. Neurosci. Abs.**, 14:252, 1988). Bilateral microinfusions of muscimol (MUS) (.03 μg), baclofen (BAC) (.001 μg) or 2-amino-7-phosphonoheptanoate (2-APH) (.90 μg) were performed in AMY or MGB after repeated AGS. There was a >50% reduction of mean clonus duration with 2-APH infusions into MGB and nearly total reduction after BAC or MUS. In addition, spike-wave activity was markedly reduced in duration or totally absent. The cortical epileptiform abnormalities did not persist after the end of seizure behavior. With AMY infusions there was an approximate 50% reduction in the mean duration of clonus ($p \leq .01$) following BAC or 2-APH infusions, with almost complete suppression after MUS infusion. The duration of cortical spike activity was reduced by about 50% ($p \leq .01$) with MUS, BAC or 2-APH infusions. Unlike MGB infusions, polyspike (without spike-wave) activity persisted after the end of behavioral seizure following AMY infusions. After AMY or MGB infusions, the seizures resembled those seen before repeated AGS. There were no changes in HLE duration with infusions at either site. Clonus and epileptiform EEG activity returned to the preinfusion patterns by 24 hours after microinfusions. **CONCLUSION:** The data suggest that AMY and MGB are involved in the progressive seizure severity increase in GEPR-9s during repeated AGS, and may participate in the neuronal network subserving the clonic phase following HLE. MGB appears to be crucial for seizure severity increases produced by repeated AGS, whereas the AMY may be critical for behavioral but not EEG severity increases. The differential effects of infusions into AMY and MGB on the cortical epileptiform activity suggest that discrete seizure components involved in increases in seizure severity may be identified. (Support NIH NS 21281, SIU-CRC)

24.3

AUDITORY BRAINSTEM RESPONSES (ABRs) AND EFFECTS OF MICRO-INJECTION INTO INFERIOR COLLICULUS (IC) OF AN EXCITANT AMINO ACID (EAA) ANTAGONIST OR GABA AGONIST ON AUDIOGENIC SEIZURES (AGS) IN THYROID DEFICIENT (THX) OR GENETICALLY EPILEPSY-PRONE RATS (GEPRs).
D.L. Patrick* and C.L. Faingold. (SPON: H. R. KONRAD) Dept. Pharmacol. So. IL. Univ. Sch. Med., Springfield, IL 62794.
Neonatally THX rats exhibit cochlear damage and are susceptible to AGS (Van Middlesworth,L. and Norris, C.H. **Endocrinology** 106: 1686, 1980). GEPRs also display AGS and cochlear damage and are THX during development (Penny, J.E., et al., **Life Sci.** 39:887, 1986; Mills, S.A. and Savage, D.D., **Epilepsy Res.** 2:102, 1988). EAAs in IC are critically involved in AGS initiation in GEPRs (Millan M.H. et al., **Exp. Neurol.** 91:634, 1986; Faingold, C.L. **Gen. Pharmacol.** 19:331, 1988). This study examined ABRs and effects of microinjection into IC of a competitive EAA (NMDA) antagonist, 2-amino-7-phosphonoheptanoate (2-APH), or a GABA agonist, muscimol, in GEPRs and THX rats. AGS severity was tested using a bell (122 dB SPL, for 60 sec or until AGS onset). ABRs were examined under ketamine [1024 clicks (0.1 msec) at 8/sec, vertex and mastoid electrodes (nuchal ground)]. The dosage of propylthiouracil (PTU) (0.0075% day 0-19) that produced 100% AGS (with clonus) resulted in a mean ABR threshold of 81 ± 5.5 dB. Higher doses of PTU increased ABR thresholds to a greater extent but decreased AGS severity. Bilateral infusion of 2-APH (in 0.5 μl) into IC completely blocked AGS in THX rats at 25 nmol. The dose required to suppress AGS in GEPRs was 1 nmol (Faingold C.L. et al., **Exp. Neurol. 99:678, 1988**). Microinjection of muscimol (0.12 μg) did not block AGS in THX rats, but 1/4 of this dose produced a significant reduction of AGS severity in GEPRs (Browning, R.A. et al., **Epilepsy Res.** 3: 1989 in press). **CONCLUSIONS:** These data suggest that only a restricted range of ABR threshold elevations results in maximal AGS in THX rats, which is similar to observations in GEPRs (Faingold C.L. et al., **Epilepsia** 28:583, 1987). Since, the sensitivity for blockade of AGS in IC is considerably less in THX rats, cochlear damage may be more important in AGS of THX rats as compared to GEPRs wherein IC may be more critical in AGS control. (Support: NIH NS 13849, 21281)

24.4

DEVELOPMENTAL HEARING IMPAIRMENT AND AUDIOGENIC SEIZURE (AGS) SUSCEPTIBILITY IN THE GENETICALLY EPILEPSY-PRONE RAT (GEPR).
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Studies in mice have suggested that AGS susceptibility may be related to a hearing deficit at a critical developmental period. Recently, Faingold et al. (Epilepsia 28:583, 1987) reported that adult AGS susceptible GEPRs possess elevated auditory brainstem response (ABR) thresholds. However, hearing impairment in adult GEPRs could have developed long after the appearance of AGS susceptibility and thus could be unrelated to AGS susceptibility. The present study examined whether hearing deficits co-exist developmentally with AGS susceptibility in immature GEPRs. ABR thresholds were determined in both moderate seizure (GEPR-3) and severe seizure (GEPR-9) pups at 14, 21 and 30 days of age. Day 14 just precedes the initial appearance of AGS susceptibility in the GEPR. GEPRs first exhibit 100 percent incidence of AGS at 21 days and near adult patterns of AGS at 30 days of age. Animals were anesthetized with pentobarbital and electrodes were placed subdermally at vertex and mastoid (nuchal ground). Clicks (0.1 ms duration, 1024 presentations) were presented at 8/sec. ABR thresholds were elevated in both GEPR-3s and GEPR-9s over controls at 14 days of age (prior to AGS susceptibility). At 21 and 30 days of age, ABR thresholds remained elevated in GEPR-3s and GEPR-9s, however, ABR thresholds were more elevated in GEPR-3s than in GEPR-9s, consistent with the findings of Faingold et al. (1987) in adult GEPRs. GEPR-9 progeny that were not susceptible to AGS exhibited normal ABR thresholds at 21 and 30 days of age. Collectively, these results support the hypothesis that a developmental hearing deficit contributes to AGS susceptibility in the GEPR. (Supported by NIH grant NS 13849.)

24.5

DECREASE IN LOCUS COERULEUS 3H-IDAZOXAN BINDING SITES IN GENETICALLY EPILEPSY-PRONE RAT.
S Razani-Boroujerdi, DY Tso-Olivas*, TJ Hoffman*, GK Weiss*, and DD Savage. Depts. Pharmacology and Physiology, Univ. New Mexico Sch. Med., Albuquerque, NM, 87131.
Widespread deficits in a variety of neurochemical markers for the noradrenergic (NE) system have been reported in Genetically Epilepsy-Prone (GEPR) rats. These NE system deficits may be a consequence of neonatal hypothyroidism (NH). NH, which is profoundly detrimental to the development of monoamine neurotransmitter systems, has been reported in both GEPR-3 and GEPR-9 rats. We have observed that propylthiouracil (PTU)-induced NH causes a significant reduction in 3H-Idazoxan (RX) binding to $\alpha 2$ adrenergic receptors in the locus coereleus (LC). Given the effect of NH on LC 3H-RX binding, we investigated the possibility that LC 3H-RX binding would be decreased in GEPR-3 and GEPR-9 rats.

Coronal sections of brainstem containing the LC were collected from 31-day-old non-epileptic Sprague-Dawley control, GEPR-3 and GEPR-9 rats. The sections were incubated with 20 nM 3H-RX in the absence and presence of 10 μM phentolamine and specific 3H-RX binding determined by quantitative autoradiography. LC specific 3H-RX binding was decreased significantly in both GEPR-3 and GEPR-9 rats compared to controls. Saturation of binding studies indicated that the total number of binding sites was decreased by 23% and 17% in GEPR-3 and GEPR-9 rats respectively. No changes in the affinity constant were observed. The decrease in total number of LC 3H-RX binding sites in GEPR rats was not as great as that observed in PTU-treated rats. This difference may be due in part to the fact that GEPR rats experience a degree of hypothyroidism intermediate to that produced by PTU treatment. Given evidence that LC $\alpha 2$ receptors are located on NE cell soma and nerve terminals, these results would suggest a possible decrease in the number of LC neurons and/or nerve terminal density in GEPR rats. Alternatively, this alteration could decrease presynaptic $\alpha 2$-mediated inhibition of NE release, a mechanism that might compensate for other presynaptic NE deficits. (Supported by NS23262 and RR08139).

24.6

EFFECTS OF EXCITANT AMINO ACID (EAA) ANTAGONISTS ON AUDIOGENIC SEIZURES (AGS) DURING ETHANOL WITHDRAWAL (ETX) AND IN GENETICALLY EPILEPSY-PRONE RATS (GEPRs).
A. Riaz and C.L. Faingold. Dept. Pharmacol. Southern Illinois Univ. Sch. Med. Springfield, IL 62794
Alterations in EAA action are observed following chronic administration of ethanol (Yool, A.J.and Groul, D.L., **Brain Res.** 420:205, 1987). Enhanced EAA neurotransmission in the inferior colliculus (IC) has been suggested to play a crucial role in the initiation of AGS (Faingold, C.L. **Gen. Pharmacol.** 19:331, 1988). Microinjection of NMDA into the IC in normal rats induces AGS susceptibility, and infusion of the competitive NMDA antagonist, 2-amino-7-phosphonoheptanoate (2-APH), into the IC or pontine and mesencephalic reticular formation (RF) blocks AGS in the GEPR (Millan, M.H. et al., **Exp. Neurol.** 91:634, 1986; Faingold, C.L. et al., **Exp. Neurol.** 99:678, 1988). In the present study guide cannulae were implanted bilaterally over the IC or RF (gigantocellular nucleus) stereotaxically. Drugs were infused (0.25 μl/min) for 2 min, and AGS testing involved presentation of a bell (122 dB SPL) for 60 sec or until onset of AGS. Effects of a quinoxalinedione non-NMDA antagonist (CNQX) into the IC were compared with those of 2-APH on AGS in GEPRs. CNQX (20 nMol/side) completely blocked AGS in GEPRs at 15 min post-infusion (with recovery by 24 h), while 10 nMol was ineffective. In the ETX study Sprague-Dawley rats were implanted with guide cannulae over the IC or RF. Ethanol was given intragastrically (9-15 g/kg/day) in divided doses given every 8 h. At the end of day 4, ethanol was withdrawn. Animals exhibiting AGS at 10 h received infusions of vehicle (phosphate buffer or dimethylsulfoxide) into the IC. No effects on AGS incidence or severity was observed with vehicle infusions. This was followed by infusion of 2-APH. The incidence of all behavioral components of AGS were significantly reduced with 2-APH (1 nMol) in IC and in RF (15 nMol). These findings support a role of enhanced EAA neurotransmission in the IC and RF in determining the susceptibility to AGS during ETX and in the GEPR. NMDA antagonists exerted more potent anticonvulsant effects in both AGS models. (Support NIH NS 13849, NS 21281)

24.7

INFERIOR COLLICULUS (IC) NEURONAL RESPONSES DURING AUDIOGENIC SEIZURE (AGS) ONSET IN BEHAVING GENETICALLY EPILEPSY PRONE RATS (GEPRs). C.A. Boersma Anderson* and C.L. Faingold. Dept. Pharmacol., So. IL. Univ. School Medicine Springfield, IL 62794
The IC is critically involved in AGS initiation in GEPRs (Browning, R.A., **Life Sci.** 39:856, 1986; Faingold, C.L. **Metab. Brain Dis.** 2:81, 1987). Preictally, IC neurons in the GEPR display abnormal response patterns to acoustic stimuli with evidence of reduced acoustically-evoked inhibition (Faingold et al., **Exp. Neurol.** 93:145, 1986; Faingold, C.L. and Boersma Anderson, C.A. **Soc. Neurosci. Abs.** 14:253, 1988). The convulsions during AGS in the GEPR begin with wild running and progress to tonic hindlimb extension followed by post-ictal depression. The changes in IC neuronal firing that occur during the abrupt and violent transition to the behavioral seizure have not previously been able to be examined. In the present study microwire electrodes were chronically implanted into the IC under ketamine anesthesia, and unit activity was examined at least one week later in freely moving GEPRs during AGS onset in the absence of drugs. Responses were quantified using poststimulus time and interspike interval (ISI) histograms. The AGS-inducing stimulus consisted of 12 kHz tone bursts (60-100 msec duration, 75-105 dB SPL) presented at 2/sec. Use of the chronic microwire technique allowed recording of units during the behavioral seizure in freely moving GEPRs. Just prior to the onset of AGS, the number of neuronal responses evoked by the stimulus increased. However, at the transition to AGS, **evoked** neuronal responses fell abruptly in 61% of units (N=18) to a mean discharge rate of 1/3 of the rate observed immediately prior to AGS onset. At this time, **non**-acoustically-evoked neuronal firing (in 83.3%, of units, N=18) was greatly increased (mean, 30-fold) and large (mean, 16-fold) increases in ISIs at $\leq$ 5 msec, associated with burst firing, occurred in 89% of units. The response to the stimulus returned after AGS in all units that were held throughout the entire seizure. **CONCLUSIONS:** These data indicate that once AGS begins the firing pattern of IC units changes to reflect seizure discharge, and the time-locked response to the acoustic stimulus that evoked the seizure is temporarily lost. (Support NIH NS 13849; NS 21281)

24.8

EFFECTS OF CARBAMAZEPINE AND ANTIEPILEPSERINE ON DIALYZABLE NOREPINEPHRINE AND SEROTONIN FROM HIPPOCAMPUS OF GENETICALLY EPILEPSY-PRONE RATS. **Q-S. Yan*, P.K. Mishra, R. Burger*, P.C. Jobe and J.W. Dailey. Department of Basic Sciences, University of Illinois College of Medicine at Peoria, Peoria, Illinois 61656.**

Substantial evidence suggests that previously documented widespread deficits in norepinephrine and serotonin are integral neurochemical components of the seizure predisposition characteristic of Genetically Epilepsy-Prone Rats [GEPRs](Epil Res 3: 3, 1989). Both carbamazepine and antiepilepserine are effective anticonvulsants in GEPRs. It was of interest to determine if these drugs produce part of their anticonvulsant effects through actions on norepinephrine or serotonin. Guide cannulae for dialysis probes were stereotaxically implanted over right hippocampi of moderate seizure GEPRs (GEPR-3) and after recovery from surgery the dialysis probes were inserted. Dialysis sites were confirmed by histology. The animal was placed into a plexiglass chamber and allowed to move freely. Artificial CSF was perfused at 1 ul/min and was collected in microvials containing L-cysteine and HCl to stabilize the dialysates. Norepinephrine and serotonin were analyzed by HPLC with electrochemical detection. Either carbamazepine (45 mg/kg) or antiepilepserine (100 mg/kg) [about an ED_{90} dose of each] was given i.p after establishing basal release and dialysis was continued for 5 more hrs. Significant increases in serotonin, but not norepinephrine, were seen at the approximate time to peak anticonvulsant effect for each drug. This result is consistent with a role for serotonergic neurons in the anticonvulsant effects of both carbamazepine and antiepilepserine in GEPRs. (Supported in part by a grant from Tsumura Juntendo Fdn.)

24.9

ASPARTAME FAILS TO FACILITATE BICUCULLINE-INDUCED SEIZURES IN DBA/2 MICE. **A.F. Bettendorf*, J.W. Dailey, S.M. Lasley and P.C. Jobe.**Dept of Basic Sci, Univ of IL Col of Med, Peoria, IL 61656.

It has been proposed that aspartame facilitates seizures in man and animals because its major metabolite (phenylalanine) interferes with brain transport of neurotransmitter precursors and synthesis of monoamine transmitters (norepinephrine, dopamine and/or serotonin). This facilitation is purportedly more likely in subjects predisposed to seizures although we have been unable to detect seizure facilitation in a variety of rodent seizure models including the Genetically Epilepsy-Prone Rat. In the present study, we used DBA/2N Hsd mice because they are genetically predisposed to seizures. The convulsant dose of bicuculline in 50% of the animals (CD_{50}) was determined by giving six doses of the drug i.p. to groups of 20 mice. The CD_{50} was 3.49 mg/kg (confidence interval, 3.14-3.89). Following CD_{50} calculation, aspartame (0, 10, 30, 300 and 1000 mg/kg) or vehicle (0.5% methylcellulose & 0.1% polysorbate-80) was given by gavage to groups (25 each) of 27 day old mice. Four hours later each mouse received 3.5 mg/kg of bicuculline i.p. and was observed for 30 minutes. Incidence of convulsions with loss of righting reflex was recorded. The 10 mg/kg aspartame dose was associated with a statistically significant increase in convulsion incidence. On replication, with 58 mice per group, this increased incidence could not be confirmed. Therefore, no doses of aspartame were associated with an incidence of convulsions different from control. Thus, a wide range of acute oral doses of aspartame did not alter the severity of bicuculline-induced seizures in seizure prone subjects. (Supported by a grant from the NutraSweet Co.)

24.10

AUDIOGENIC SEIZURES IN DBA/2 MICE ARE NOT FACILITATED BY ASPARTAME. **P.C. Jobe, A.F. Bettendorf*, S.M. Lasley and J.W. Dailey.** (SPON: J.D. Lane) Dept of Basic Sciences, Univ of IL Col of Med, Peoria, Illinois 61656.

It has been suggested that aspartame might facilitate seizures because of hypothesized effects of its major metabolite (phenylalanine) on brain transport of amino acids and synthesis of biogenic amine neurotransmitters (e.g.,norepinephrine, dopamine & serotonin). The alleged facilitation is presumed to be more likely in subjects predisposed to seizure disorders. We have previously been unable to detect seizure facilitation in a variety of rodent seizure models. In a further attempt to uncover seizure facilitation, we used DBA/2N Hsd mice because they are genetically predisposed to audiogenic seizures. In these experiments, groups of 27 day old DBA/2 mice were given aspartame (doses from 5 to 2000 mg/kg) or vehicle (0.5% methylcellulose & 0.1% polysorbate-80) by gavage and then subjected to a seizure-provoking audiogenic stimulus (bells at approximately 105 dB for 30 seconds). Convulsion intensity was evaluated on a 5 point scale (0 = no response, 5 = tonus). Approximately 76% (277/366) of the mice had a score greater than 0. In three separate experiments audiogenic seizures were evaluated at 0.5, 2 or 4 hrs after aspartame or vehicle gavage. No dose of aspartame was associated with a significant alteration in convulsion intensity at any of the time points. Thus, a wide range of acute oral aspartame doses did not alter the severity of audiogenic convulsions in DBA/2 mice. (Supported by a grant from the NutraSweet Co.).

24.11

GRAFTING OF FETAL LOCUS COERULEUS (LC) TISSUE INTO THE HIPPOCAMPUS (HP) OF GENETICALLY EPILEPSY-PRONE RATS. **R.W. Clough, R.A. Browning, M.L. Lanker, P.C. Jobe, Depts. of Anatomy and Physiology, Southern Illinois Univ. Sch. of Med., Carbondale, IL 62901 and Dept. of Basic Science, Univ. of Illinois-Peoria, Peoria, IL 61656.**

Previous studies have demonstrated that the central norepinephrine (NE) system inhibits seizure behavior in genetically epilepsy-prone rats (GEPRs). Additionally, several brain regions of the GEPR brain including the HP have reduced content and uptake of NE. Furthermore, administration of NE agonists reduces the severity of seizures in GEPRs (Jobe et al., Life Sci. 39:1986). We have attempted to replace NE in the GEPR HP by transplanting tissue from the dorsal pons including the LC of 16 day PC fetal rat donors. Tissues were lowered stereotaxically into the HP using coordinates AP=-4.3 mm,V=3mm and L=3mm. At 1, 4, and 12 weeks post-surgery, rats were tested for audiogenic-induced seizures and were subsequently sacrificed for neurohistology. Seizure response scores were not altered by transplantation of LC or cortical tissue in this experiment. In each of 3 post-surgery tests, the seizure response scores of each rat was 9. Histological studies demonstrated that transplants were present in or immediately subjacent to the HP. Transplanted tissues showed no signs of encapsulation and numerous neuron profiles were present within the grafts. In each case, tissue appeared to be integrated with the HP of the host. In partial support of the present findings, Stevens et al., (Epilepsia 79:1988) have shown that adrenal medula implants into the lateral ventricle also do not alter seizure behavior in GEPR-9s. The present study suggests that the presence of NE within the HP, as delivered by transplanted locus coeruleus does not alter the expression of seizure behavior in the GEPR. Other brain areas are currently under investigation. Supported by HD23209 and the Deafness Research Foundation.

24.12

THE EPILEPTIC MUTANT *TOTTERING* SHOWS A VOLTAGE-DEPENDENT PROLONGATION OF DEPOLARIZING SHIFTS IN BURSTING CA3 PYRAMIDAL NEURONS *IN VITRO*.

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The mutant mouse *tottering* (*tg/tg*) is a single locus mutation showing a gene-linked noradrenergic hyperinnervation by LC axons, and spontaneous 6 Hz electrocorticographic spike and wave seizures. In isolated mutant hippocampal slices, high extracellular $[K^+]$ (10 mM) induced field bursts in the CA3 region are significantly longer in duration than in control (+/+) slices. To study the cellular mechanisms involved in this prolonged network response, intracellular recording was carried out in CA3 pyramidal neurons under current clamp in *tg/tg* and +/+ mouse hippocampal slices. No obvious qualitative abnormality in spontaneous synaptic and spiking activity was noted under non-epileptic conditions (3 - 5 mM $[K^+]_o$). In contrast, at high $[K^+]_o$ (10 mM) using KCl filled microelectrodes, a 60 % prolongation in the mean duration of paroxysmal depolarizing shifts (PDS) was observed at membrane potentials of -40 - -50 mV in the mutant (393.43 $\pm$ 49.80 msec, n=6) as compared to the control (245.77 $\pm$ 24.17 msec, n=5, $p < 0.05$) but not at -70 - -80 mV. The voltage-dependent prolongation was also observed with K-acetate filled microelectrodes, suggesting that an artificial shift in the chloride equilibrium potential is unlikely to be a factor. In the latter recordings, 6 out of 8 mutant cells showed no post-PDS afterhyperpolarization (AHP) at -40 - -60 mV, as compared to only 1 of 6 control cells. In conclusion, a distinct abnormality in excitability is present in *tg/tg* CA3 pyramidal neurons under epileptogenic conditions. This voltage-dependent prolongation of the PDS may be produced by enhanced release of norepinephrine in the mutant. *(Supported by NIH 11595 and Basil O'Connor grant (JLN)).*

24.13

ABNORMAL DISTRIBUTION OF HIPPOCAMPAL MOSSY FIBERS IN THE EPILEPTIC (EL) MOUSE. J.E. Miyashiro* and C.R. Houser, Dept. of Anatomy and Brain Research Institute, UCLA, and VA Medical Center, Los Angeles, California 90024.

The El mouse is a genetic model of epilepsy with apparent involvement of the hippocampus. Timm's staining and dynorphin immunohistochemistry have been used to determine if there are morphological alterations of the mossy fiber path. In control, C57BL/6 mice, mossy fibers end at the junction of CA3 and CA2, but, in adult El mice that had experienced many seizures, mossy fiber staining extended beyond this border. In coronal sections of the rostral hippocampus, a narrow band of staining was present in the suprapyramidal zone of the putative CA2 and CA1 fields. At the most rostral levels, the aberrant staining was continuous with that of CA3; at slightly more caudal levels, the staining became discontinuous but persisted in the medial part of CA1. After 300-400μm, a normal pattern of Timm's staining was present. No intra- or supragranular staining has been observed. Studies are in progress to determine if the aberrant staining is unique to the El mouse or is also present in the ddY (mother) strain. Supported by NS 21908 and VA Medical Research Funds.

24.14

THE C57BL10 sps/sps MOUSE; A MODEL OF ABSENCE SEIZURES. J.G. Ortiz, A.E. Negrón*, J.E. Rosado* M.T. García*, C.S. Maldonado*, A.P. Thomas*, J. A. Moreira* and H. Heimer*; Department of Pharmacology, University of Puerto Rico Medical School, San Juan, Puerto Rico 00936

In contrast to other types of epilepsy, there are relative few models of absence seizures. C57BL10 sps/sps mouse mutants have similar behavioral and electroencephalographic characteristics to those observed in generalized absence seizures in humans. (Maxson et. al., 1983)

These animals have reduced glutamate decarboxylase activity (GAD) in cortex, midbrain and hippocampus. ^{3}H-flunitrazepam (^{3}H-Flu) binding is reduced in these areas, as well as, in the cerebellum and brainstem. In the hippocampus of sps/sps mice, the uptake and release of ^{3}H-GABA are significantly reduced when compared to that of the parent strain, SPS/SPS or to their heterozygote littermates.

These results suggest the presence of GABAergic deficits in this animal model, although how these deficits are mediate the absence seizure-like behavior remains to be clarified. (Supported partially by the Epilepsy Foundation of America, the institutional NIH/MBRS program and the Center for Animal Care)

24.15

STARGAZER: A NEUROLOGICAL MUTANT WITH A COMPLEX PATTERN OF INHERITED SPIKE-WAVE SEIZURES. X. Qiao*, J.L. Noebels, R.T. Bronson[1], M.T. Davisson[1], (Spon: D. Shine) Developmental Neurogenetics Laboratory, Section of Neurophysiology, Dept. of Neurology and Institute of Molecular Genetics, Baylor College of Medicine, Houston, TX 77030, and [1]The Jackson Laboratory, Bar Harbor, ME, 04609.

A new spontaneous recessive mutation, *stargazer* (stg), arising in the A/J inbred mouse strain (now on C3B6Fe F1) has been mapped to chromosome 15 between the Bld and bt loci. The neurological phenotype in the adult homozygous mutant includes mild ataxia and periodic spells of sustained neck retroflexion. Initial histopathological survey reveals no cerebellar or inner ear lesions. The electrocorticographic phenotype features frequent and prolonged (10-30 secs) episodes of 6 Hz generalized spike and spike-wave discharges (500-1000 μv) accompanied by behavioral arrest. While these seizures are virtually identical to, but more frequent than those found in the *tottering* (tg, chr. 8) mutant, a second pattern distinguishes *stargazer* from other known seizure mutants. At either the initial or terminal stage of some seizures, the spike discharge frequency accelerates to 9-11 Hz. During this period, the mouse reinitiates motor activity, resulting in a mixed electroclinical seizure type. HPLC-EC assays of eight mutant brain regions (n=11) compared with age-matched +/+ mice showed no gene-linked elevation in NE or MHPG levels as found in the tg mutant. These data illustrate a frequency-specific correlation of generalized synchronous spike discharges with ictal motor behavior, and underline the genetic and neurochemical heterogeneity of inherited diseases expressing absence seizure phenotypes.
Supported by BSR 8418828 (MTD) and NIH 11535 (JLN).

24.16

MEGALENCEPHALY IN THE EPILEPTIC CHICKEN IS REGION-SPECIFIC AND IS ASSOCIATED WITH DECREASED NEURONAL DENSITY. D.George*, T.McConnell*, D.G.Munoz, R.D.Crawford*, (SPON:J. S.Richardson). Dept.of Pathology,University of Saskatchewan Saskatoon,Saskatchewan,S7N 0W0

The epileptic chicken is a genetic model of generalized epilepsy (Crawford RD,1983). The epileptic trait is inherited as an autosomal recessive (epi gene) in association with megalencephaly; heterozygous carriers are normal. The objective of this study is to determine the basis of the megalencephaly, and potentially of the epilepsy, which are both the result of a single abnormal gene. Bouins perfused brains of 5 epileptic and 5 carrier adult hens were compared by standard morphometric methods. We found that: 1) megalencephaly is not uniform: there is significant enlargement of some regions (telencephalon ($p<0.01$), hippocampus ($p<0.02$), archistriatum ($p<0.01$) and cerebellar cortex ($p<0.02$)),but not of others (optic tectum and nucleus rotundus); 2) in the enlarged regions that we have studied, neuron density is significantly decreased (hippocampus ($p<0.01$) and archistriatum ($p<0.05$)); 3) glial cell density in the hippocampus and archistriatum is not significantly different. These findings show regional variation in megalencephaly in the epileptic chicken; and they suggest that increased neuron size contributes to the megalencephaly, and may underlie the epilepsy.

CONTROL OF POSTURE AND MOVEMENT I

25.1

TIMING AND MAGNITUDE OF EMG ACTIVITY AT THE SHOULDER AND ELBOW FOR INITIATION OF PLANAR ARM MOVEMENTS. G.M. Karst and Z. Hasan. Dept. of Physiology, University of Arizona, Tucson, AZ 85724.

We have previously demonstrated that during the initiation of planar, reaching movements involving the shoulder and elbow joints, the sign (i.e. flexor or extensor) of the initial muscle activity at each joint is related to the target direction with respect to the forearm (ψ). Further analysis of the initial EMG activity at the shoulder and elbow has revealed that: 1) for most values of ψ, EMG activity at the shoulder precedes that at the elbow; 2) the latency between shoulder and elbow EMG onset varies systematically with ψ; and 3) this latency is positively correlated with the ratio of initial EMG magnitudes at the two joints (shoulder/elbow).

These findings suggest that the temporal aspects of the motor command may be stereotyped at the pre-motoneuronal level, while the systematic variation in EMG latency may result from an inverse relationship between the excitatory drive to a motoneuron pool and the time required to reach motoneuronal threshold.

Supported by NIH grants 2R01-NS19407 and 5T32-NS07309.

25.2

EFFECT OF CONTEXT ON GOAL-DIRECTED ARM MOVEMENTS. J. Nativ* and J.H. Abbs (SPON: T. Allard). Speech and Motor Control Laboratories, Waisman Center, Univ. Wisconsin, Madison, WI 53706.

While many studies of human arm movements have highlighted invariant movement parameters, it appears that the context in which tasks are performed influences these relations substantially (Marteniuk et al. 1987). This study attempted to investigate systematically the role of context in simple goal-directed reaching movements by manipulating the inherent meaning, content, and surface characteristics of the object to be moved.

Four subjects performed a self-initiated rapid movement which included reaching, grasping and transporting a test object to a target position. Movement of the forearm and hand were recorded using the WATSMART 3-d system. Inherent task meaning was manipulated using both a cup and an equal sized cylinder as test objects. The content (empty versus full) and surface characteristics (natural versus slippery) of the test object also were manipulated.

The inherent object meaning (cup versus cylinder) and content influenced the time to contact and the proportion of that time associated with movement deceleration. For example, when reaching for the cup, movement time was longer than that for the cylinder ($F=16.73$ $df=1,3$ $p<.05$). Further, the addition of liquid slowed down the reaching and transport movements with the cup but not with the cylinder. Object surface characteristics influenced the transport time depending on object content; whereas a slippery surface slowed down the transport of an empty cup, it actually shortened the transport time of the full cup.

These data indicate that searches for invariant movement parameters may be premature until a richer variety of functional motor tasks are carefully investigated. (Supported in part by NIH Grants NS-16373, NS-13274 and HD-03352)

25.3

PLANNING AND CONTROL OF PREHENSION: GRASPING SCREWDRIVERS. C.L. MacKenzie, B. Sivak, and T. Iberall. Dept. of Kinesiology, University of Waterloo, Canada and Dept. of Computer Science, University of Southern California, Los Angeles.

Prehension studies examined how transport of the hand and finger posturing (grasp) depend on object characteristics and the intent of the individual (Jeannerod, 1981; Marteniuk et al.,1987). An important question in grasp planning for hand held tools such as a screwdriver is how object and task properties affect movement kinematics. Subjects were instructed to make several turns on a screw with 1 of 4 different sized screwdrivers. The screwdriver handle was available from a holder (spatially accessible) or resting on the table surface (obstacle avoidance). Videotape and 3D kinematic analyses of the initial grasp phase showed that as screwdriver size changed, so did the transport component, the posture for grasping the screwdriver (type of opposition) and how the fingers grouped together (virtual fingers). Distinctive finger posturing and wrist transport depended on whether or not grasping the handle required obstacle avoidance. Precision aspects of screwdriver weight and size modified these results. This reflects the functional coordination of grasp and transport components in prehension. (NSERC)

25.4

THE EFFECTS OF OBJECT WEIGHT ON THE KINEMATICS OF PREHENSION. P.L. Weir*, S.L. Cargoe*, R.G. Marteniuk and C.L. MacKenzie. (SPON: E. Roy). Department of Kinesiology, University of Waterloo Waterloo, Ontario, Canada, N2L 3G1.

Several intrinsic object properties affect the planning and control of transport, grasp, or both components of prehension, including: object fragility (Marteniuk et al., 1987), object size (Marteniuk et al., 1989) and object shape (Jeannerod, 1981). This study examined object weight. Subjects grasped visibly similar metal dowels (20,55,150,410 grams) in both a blocked (weights known) and random (weights unknown) order.

Three dimensional kinematic analyses of infrared emitting diodes placed on the thumb and index finger (grasp component), and the wrist (transport component) revealed that: peak aperature, peak velocity and peak deceleration occurred earlier for heavier weights; and peak aperture occurred earlier when weights were unknown than for known weights. These changes in both grasp and transport components indicate a functional linkage in achieving the goal.

Supported by NSERC.

25.5

PRECISION EFFECTS AND GRASP TYPES. B. Sivak and C.L. MacKenzie. Department of Kinesiology, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1.

Preliminary kinematic analyses revealed differences in transport and grasp components of prehension depending on the type of grasp: time spent after peak deceleration was relatively shorter and maximum aperture was larger for a collective than for an independent finger grasp. This study examined the effect when when precision is equated between the two grasp types. Subjects grasped dowels 2.5, 4.0 and 7.0 cm in diameter and 11.5 cm in height using a collective grasp that required no precision (CNP), a collective grasp that required precision (CP) and an independent grasp (I). For the transport component, there were no differences. For the grasp component, maximum aperture for the 2.5 cm dowel was wider both for CNP and CP compared to I. The results suggest (1) when precision is equated, the transport component does not depend on the type of grasp used (2) organization of the grasp component may reflect inherent differences in the motor system for collective versus independent grasps (small sized objects only).

(Supported by NSERC)

25.6

THE COUPLING OF ARM AND FINGER MOVEMENTS DURING PREHENSION: PERTURBATION OF OBJECT LOCATION. Y. Paulignan*, C.L. MacKenzie, R.G. Marteniuk, and M. Jeannerod*. Laboratoire de Neuropsychologie Experimentale, INSERM U94, Bron, France and Dept. of Kinesiology, University of Waterloo, Canada.

To investigate the relation between two components of prehension, we used a visual perturbation paradigm to change the location of an object at movement onset. If transport and grasp components are independent, then changing object location should affect only transport, not grasp. Kinematic analyses of markers placed on the wrist (transport), index finger and thumb tips (grasp aperture) indicated rapid modifications for both transport and grasp components. Speed changes on the trajectory path indicated an interruption about 100 ms after the object changed location; the spatial path and aperture were modified accordingly. Subjects were unaware of these adjustments at the time they were effected. Moreover, different location changes resulted in differential relative contributions of the thumb, finger and wrist, demonstrating motor equivalence. This covariation of transport and grasp components suggest a functional synergy between the proximal and distal segments, to meet the task requirements. (Supported by INSERM, NSERC, and MRC)

25.7

IS THERE A PREFERRED COORDINATE SYSTEM FOR REPRESENTATION OF HAND ORIENTATION IN 3-DIMENSIONAL SPACE? W.G. Darling, L. Gilchrist* and T. Edholm*, Dept. of Exercise Science, University of Iowa, Iowa City, IA, 52242.

Traditionally, perception of location of the limbs in space has been attributed to information regarding joint angles provided by joint, cutaneous and muscle receptors. Recent studies have provided strong evidence that the preferred coordinate system for perception of upper limb orientation in space is in terms of angles of the limb segments relative to the line of gravity and in the horizontal plane (extrinsic coordinate system), rather than relative to the proximal body part (joint angles - intrinsic coordinate system). We have examined this same issue in terms of perception of hand orientation.

Subjects performed a perceptual matching paradigm in which the left hand and forearm were placed in position by the experimenter and the right forearm was also placed by the experimenter. With the eyes closed, the subject then matched a criterion hand or wrist angle (joint angles - flexion, abduction, pronation; hand angles - pitch, yaw, roll) by moving only the right hand. Segment positions in 3-dimensional space were recorded using a two camera WATSMART system with 3 infrared LEDs placed to represent the plane of the palm, forearm and arm (to measure pronation angles only). An overall preference for an extrinsic or intrinsic coordinate system for perception of hand position in 3-dimensional space was not observed.

25.8

ROLE OF THE RETINAL AND EXTRARETINAL FACTORS IN THE DIRECTIONAL CODING OF REACHING. V.Delreux*, S.Vanden Abeele*, M.Crommelinck* and A.Roucoux. Lab. de Neurophysiologie, Louvain Univ. Sch. of Med., B-1200 Brussels, Belgium.

The relative contribution of the retinal signal, documenting the position of the objects in visual space, and of the extraretinal factors, related to the eye and head position, to the elaboration of an internal representation of a body-centered space and to the generation of oriented goal-directed movements remains unclear. The aim of this study was to determine the respective role of the retinal and extraretinal signals in the localization of the objects in space.

Human subjects sat, facing a hemispheric screen, and were asked to move a handle in the direction of LED's presented at several positions along the horizontal meridian. The head and trunk were not allowed to move. The subjects had no visual feedback about the arm displacement. In one series of experiments, the eyes were oriented in the primary position before the movement started; in another, the eyes were deviated (15 deg) from this position, so that a given LED would yield two different retinal errors. In each of the two series, the subjects were either asked to orient their eyes towards the peripheral LED's or to keep them in the initial position. The final arm position was found to be a function of the LED's localization on the screen, when the eyes were allowed to move, and a function of the retinal error, in the immobile eyes condition.

These observations suggest that, when the eyes are not moving, a retinal frame of reference is used, whereas when the eyes can foveate the target, a head or body frame of reference is used. Foveating the target thus changes the spatial frame of reference used to generate the reaching arm movement.

25.9

EYE, HEAD AND HAND COORDINATION WHILE POINTING TO PERTURBED VISUAL TARGETS EITHER AS FAST OR AS ACCURATELY AS POSSIBLE. H. Carnahan, and R.G. Marteniuk. Dept. of Kinesiology, Univ. of Waterloo, Ontario, Canada, N2L 3G1.

The reorganization of eye, head and limb movements to perturbed target lights was studied. Subjects were required to touch with their right index finger, targets consisting of recessed 3mm lights located on a table top 15, 30 and 45 degrees to the left of the subject's midline either as fast or as accurately as possible. Twenty percent of the time, upon finger movement initiation to the 30 degree target, the target light was extinguished and either the 15 degree or 45 degree light was then illuminated. The response of the eyes to this perturbation was measured by EOG and the WATSMART 3-D system was used to monitor the movements of the head and hand. Results showed that not only did the experimental conditions affect the order of initiation of these body parts, but the body parts also responded to the target perturbations very differently. These differences were concerned with whether movement amendments were made and and if so, the latency of these amendments. These results are discussed in terms of the control of multimovement systems which are characterized by flexible and rapid reorganization of their constituent parts.

25.10

IS THERE A LEFT-HAND ADVANTAGE FOR AIMING MOVEMENTS MADE WITHOUT VISUAL FEEDBACK? D. P. Carey and M. A. Goodale, University of Western Ontario, London, Canada, N6A 5C2.

A much-cited study by Guiard et al. (*Neuropsychologia, 21(1), 1983*) claimed that when visual feedback was not available in an aiming task, right-handed subjects showed smaller constant errors with their left than with their right hand. The claim that this advantage was due to a right-hemisphere superiority for processing spatial relationships was further supported by a left hemispatial advantage in performance (as measured by constant error). The present study re-examined this claim by studying the aiming movements made by 17 right-handed males with their left and right hands in hand-visible and hand-invisible conditions. The subjects were required to reach out and place their index finger on a single target (0.25^{0} LED) or on the midpoint between two such targets, the location of which varied randomly from trial to trial. The position of the moving finger was monitored via a WATSMART system. In contrast to the results of Guiard et al., but consistent with a number of other studies (*e.g. Fisk and Goodale, Exp. Brain Res., 60, 1985*), subjects made larger constant and larger variable errors using their left hand rather than their right. Moreover, the magnitude of these differences increased rather than decreased when visual feedback about limb position was not available. Some evidence for a left hemispatial advantage was found, however, since constant errors were larger for targets on the right side of space. These findings suggest that the right hand/left hemisphere system may be better able to utilize non-visual as well as visual sources of feedback for producing accurate aiming movements. *Supported by MRCC grant #MA-7269 to M.A.G.*

25.11

LEFT/RIGHT HAND DIFFERENCES AND THE ROLE OF VISION IN HUMAN PREHENSION. L. S. Jakobson* and M. A. Goodale (SPON: R. Steenhuis), Dept. of Psychology, U. of Western Ontario, London, Canada, N6A 5C2.

Jeannerod (*J. Mot. Behav., 16, 1984*) has suggested that the relative timing of the two components of human prehension, the reach and the grasp, is achieved through a feedback-independent central motor program. This proposal was tested in the present experiment. Right-handed subjects were asked to pick up objects located at various distances in front of them. For half the session, vision of the hand and target was not available after movement onset. The performance of the left and right hands under each of these conditions was also compared. The kinematics were obtained via a WATSMART system.

Maximum grip aperture was achieved well before contact with the object and was scaled for object size in both viewing conditions. The absence of visual feedback, however, reduced the precision of the grasp; not only did the hand open wider when vision was not available, but the size of that opening showed much more variability from trial to trial. Contrary to Jeannerod's prediction, the relative timing of the reach and grasp components was also affected. When vision was not available, the relative timing of the grasp with respect to the reach was much more variable and maximum aperture occurred earlier. The two hands also differed. The right hand opened 2 cm less than the left, on average, and was less variable in its maximum aperture. This effect did not interact with feedback condition. The greater precision of the right hand, which enjoys privileged access to the left hemisphere, supports the idea that praxis systems within this hemisphere may be specialized for the control of prehension. *Supported by MRCC grant MA-7269 to M.A.G. and an MRCC Studentship to L.S.J.*

25.12

THE EFFECTS OF OBJECT SIZE AND DISTANCE ON VISUALLY GUIDED PREHENSION. M. A. Goodale and L. S. Jakobson*, Dept. of Psychology, U. of Western Ontario, London, Canada, N6A 5C2.

The act of prehension involves not only spatially accurate placement of the arm (the reach), but also appropriate posturing of the hand (the grasp). The present experiment examined the relationship between these two components of human prehension. Right-handed subjects were asked to pick up a small block that varied in size and distance from the subject. The reaching and grasping movements were reconstructed with a 3D-optoelectronic recording system (WATSMART). The manipulations of object size and distance had predictable effects on the kinematics of the reach and the grasp. Thus, reaches to distant objects reached a higher peak velocity than reaches to near objects, and maximum grip aperture was scaled for object size. In addition, maximum grip aperture was delayed in reaches to more distant targets of any size. Since these reaches were also of longer duration, the result was that maximum grip aperture was consistently achieved approximately two-thirds of the way through each reach, regardless of movement amplitude. This finding lends strong support to Jeannerod's contention (*J. Mot. Behav., 16, 1984*) that there is a temporal coupling of the reach and grasp components of prehension. However, the fact that reaches to larger objects (which reached a higher peak velocity) were of longer duration than reaches to smaller objects shows that factors normally thought to influence primarily grip formation can also influence aspects of the reach itself. This would suggest that the reach and grasp components are more than simply temporally coupled and that their normal integration is affected by visually-based estimates of object size and distance. *Supported by MRCC grant MA-7269 to M.A.G. and an MRCC Studentship to L.S.J.*

25.13

DIFFERENCES IN PROGRAMMING OF AMPLITUDE AND DIRECTION IN A TWO DIMENSIONAL FORCE AIMING TASK. R. Bermejo*, S. Pullman and C. Ghez. Ctr. for Neurobiol & Behav, Columbia Univ and NYS Psych Inst, New York, NY, 10032.

We have previously shown that when subjects aim impulses of elbow force to unpredictable flexion and extension targets, direction and amplitude are specified in parallel. Prior to target presentation, subjects prepare a default amplitude at the center of the expected range and select a default direction arbitrarily between the two possibilities. We now examine whether, when direction can vary in a continuous range and alternative directions are close together, subjects prepare a single default direction in the center of the range.

Subjects produced single uncorrected impulses of isometric wrist force in synchrony with a predictable tone and aimed to one of four visual targets appearing at unpredictable times prior to the tone. In each block of trials. The array of targets required flexion combined with either ulnar or radial deviation of the wrist. Target forces had one of two amplitudes. In different blocks of trials, the angle subtended by possible targets was 90° or 22°.

At short S-R intervals (<100ms), subjects' default responses were directed towards one or the other pair of possible targets regardless of their angular separation, never in the center of the range. In contrast, default amplitudes were at the center of the expected range of target amplitudes. As S-R interval increased, the proportion of wrong direction responses decreased progressively while the amplitudes of both right and wrong direction responses gradually converged on the targets. The preparation of response direction thus derives from a selection made among discrete alternatives while amplitude derives from a single average default value. Specification of direction expresses itself as a progressive change in the probability of a correct choice while amplitude specification is reflected in the gradual adjustment of response metrics. (Supported by NS 22715)

25.14

INDEPENDENCE OF DIRECTION AND AMPLITUDE ERRORS IN PLANAR ARM MOVEMENTS. J. Gordon and C. Ghez. Ctr for Neurobiol & Behav, Columbia Univ and NYS Psych Inst, New York, NY 10032.

This study examined the programming and accuracy of limb trajectories in two–dimensional arm and wrist movements. Subjects moved a hand–held cursor on a digitizing tablet in arm movements, or pointed a pen cursor taped to their index finger in wrist movements, to targets at different distances and in different directions. Targets and cursor were displayed on a computer screen. The subjects' limb was covered, and the screen cursor was blanked after an auditory signal to move. Final cursor position was displayed as knowledge of results. Subjects were instructed to make single uncorrected movements to the target either at a comfortable speed or as fast as possible.

Movements in a given direction had similar trajectories in which bell–shaped velocity profiles were scaled in amplitude and time. However, the relative symmetry of the profiles as well as the scaling factors differed for different directions of movement. The spatial distributions of end points were elliptical in shape for both arm and wrist movements. The long axes of the ellipses were oriented in the direction of movement such that amplitude errors were 2 to 3 times greater than direction errors. The eccentricity and size of the dispersion ellipses varied systematically with movement amplitude and direction whereas the velocity instruction had little effect. Eccentricity decreased with increasing movement amplitude; directional error was independent of movement amplitude, while relative amplitude error decreased. The relationship between eccentricity and movement direction was more complex. Differences in error distributions and trajectory profiles with different directions reflected variations in the contribution of different joints to the movement and resultant differences in the inertial load at the hand. The independence of direction and amplitude errors implies that separate processes contribute to the programming of direction and amplitude of two–dimensional movements. (Supported by NS 22713)

25.15

PATTERNS OF MUSCLE ACTIVITY IN ISOMETRIC ARM POSTURES. M. Flanders and J.F. Soechting. Department of Physiology, University of Minnesota, Minneapolis, MN 55455.

The goal of this study is to understand how the nervous system produces the complex patterns of muscle activity that move the human arm through three-dimensional space. We have begun by measuring the activity of several shoulder and elbow muscles during the production of static forces at the wrist.

Human subjects stood with their right arms in one of several isometric postures. We used a pulley system to require each subject to produce force at the wrist in various directions in three-dimensional space. We measured the electromyographic (EMG) activity of the latissimus dorsi, posterior, medial and anterior deltoid, pectoralis, biceps, brachioradialis, and the long and medial heads of the triceps. We analyzed the EMG data to determine the force direction for which each muscle was maximally active, and the breadth of the range of directions around this "best" direction. We defined coactivity as activity in the direction opposite to the best direction, and determined the extent and breadth of coactivity.

Directions and ranges of activity and coactivity will be compared between different isometric postures and between static and dynamic modes of force production.

25.16

Neuromuscular control of a reversal maneuver during post-contraction movements. B.R. Etnyre. Human Performance and Health Sciences Dept. Rice University, Houston, Texas 77251

A previous study of post-contraction movement accuracy reported undershooting of a target was primarily dependent upon reduced motor output of the agonist muscle with no change in antagonist EMG activity or in the fundamental frequencies of either muscle. The effect may be caused by proprioceptive biasing or by a perceptual distortion. A reversal movement may reset the muscle spindle, which could restore proprioceptive biasing to normal. The purpose of the present study was to compare post-contraction positioning accuracy with EMG quantity and frequency characteristics between biceps and triceps brachii muscles during elbow extension with reversal movements to a target. Following a learning session 20 blindfolded subjects performed 20 control trials in which each attempted to rapidly move past the target (elbow extension) and then return to the target position (elbow flexion). Experimental trials were identical to control trials except the subject preceded each movement with a 3 second isometric contraction of the triceps brachii muscle. Significant undershooting occurred in the post-contraction condition, $t(19) = 4.10$, $p < .001$. Integrated EMG (IEMG) of the triceps muscle was significantly less in the experimental condition than the control condition, $t(19) = 2.1$, $p < .05$, while no difference was observed in biceps IEMG between conditions. Frequency spectrum analysis revealed nearly identical mean peak frequencies between conditions in both muscles. It was concluded the undershooting following isometric contraction was dependent on the motor output of the triceps brachii muscle for reversal of an elbow extension movement with only slight changes in motor frequencies.

25.17

RECONSTRUCTION OF SHIFTING JOINT COMPLIANT CHARACTERISTICS DURING SLOW MOVEMENTS. M.L.Latash and G.L.Gottlieb. Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL 60612

The equilibrium-point (EP) hypothesis of motor control is based on a notion of joint compliant characteristics (CCs) which are controlled by a central motor planning system. CCs have until now been recorded only in "static" experiments in which a subject was asked to activate his muscles against a torque bias and "not to intervene voluntarily" with unexpected external torque changes. Recording CCs during voluntary changes in motor command has never been described.

In our experiments, the subjects (N = 7) first learned a simple relatively slow elbow flexion movement (movement time 600-1000 ms) against a torque bias and were asked to reproduce the learned command irrespective of possible external torque changes. Slow torque changes (loadings or unloadings over 800 ms) took place in half of the trials starting 100 ms after the tone signal to initiate the movement. The individual trials were later aligned according to the moment of visible deflection of the acceleration trace corresponding to the beginning of the voluntary movement (t_0). Angle and torque values were measured at different times Δt after t_0. Data for each Δt were plotted in the torque-angle plane and linear regression equations were computed (coefficients of linear correlation over 0.9 for more than 50% of the sets). The regression equations let one assess changes in the CC threshold and slope during a movement and provide for a "virtual trajectory" which was somewhat ahead of the "actual trajectory" (based on averaged unperturbed trials). Similar analysis was used for the static CCs recorded using the classical paradigm.

The method used allows measurement of shifts in CCs during movements. It may also be used for analysis of fast movements if one takes into account inertial torques. Our preliminary data provide direct support for using notions of the EP-hypothesis such as shifts of CCs for movement analysis.

The study was supported by NIH grants AR 33189 and NS 15630.

25.18

MEMORY-SCANNING OF MOVEMENT DIRECTIONS: EFFECT OF SET SIZE ON CONTEXT-RECALL. A. Georgopoulos, J.T. Lurito*, and T. Georgakopoulos*. Bard Labs., Dept. of Neuroscience, The Johns Hopkins Univ. School of Medicine, Baltimore, MD 21205.

Fourteen human subjects performed in a modified Sternberg memory-scanning task. First, they made a series of 2-6 movements in different directions from a central point towards peripheral lights on a planar working surface ("list trials"). Then, after a beep, one of the previous stimuli in the list was presented again ("test trial"). Subjects were instructed to move in the direction of the stimulus which was presented next in sequence in the list. The reaction time (RT) in the test trials was a strong linear function of the number of movements (k) in the list: RT (ms) = 159 + 195k (values are means of 14 subjects; the median Y-intercept and slope were 199 ms and 170 ms/item recalled, respectively). The mean r^2 (N = 14) was 0.932 (median = 0.978). In contrast, the RT did not vary with the serial position of the test movement in the list trials. These results indicate that successful performance in this task involves a recall of the list sequence that is more time consuming the higher the number of movements in the list.

25.19

ISOMETRIC FORCES EXERTED BY HUMAN SUBJECTS WITH AND WITHOUT VISUAL FEEDBACK OF POINT OF ORIGIN. R.A. Drake, J.T. Massey, and A.P. Georgopoulos. Bard Labs., Dept. of Neuroscience, The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

Twenty human subjects grasped an isometric handle and exerted an X-Y force which moved a cursor in a visual display. Subjects were instructed to exert forces on the handle such that the cursor moved toward a target in a reaction time task. The instruction for the direction of the vector of the upcoming force was defined visually by the location of the cursor and the location of the target on the display. In Experiment 1 the cursor remained visible after target onset, but did not move in response to the force exerted. This resulted in performance similar to the condition in which the cursor did move. In Experiment 2 the cursor disappeared simultaneously with the target onset. This resulted in significantly slower mean reaction times (513 vs. 417 ms) and less information transmitted (2.84 vs. 3.10 bits) than when the cursor remained visible. These results suggest that the generation of force in a particular direction depends on the availability of the visually defined instruction vector during the reaction time.

26.1

BILATERAL MOTOR CONTROL DEFICITS IN FRONTAL HEMINEGLECT IN MONKEYS. R.K. Deuel. Washington University School of Medicine, St. Louis, MO 63110

Hemineglect symptoms are generally attributed to abnormal processing in the damaged hemisphere. To test timing and force deployment of hand movements, monkeys were taught to grip handles without exerting force until a lateralized cue light signalled one hand to pull. Before a unilateral periarcuate lesion the pulling hand, whether L or R, exerted up to 6K while the gripping hand exerted <200 gms.. Postop, in monkeys with hemineglect the distribution and timing of forces changed, with the hand contralateral to lesion (Contra hand) pulling 40% less than preop, and the hand ipsilateral (Ipsi hand) pulling 7% more. When Contra hand was cued to pull, the ipsi hand pulled too, and vice-versa. Latency from lateralized cue to reward was also increased for both contra and ipsi hands. This data shows that the motor control of both hands is altered by a unilateral lesion, and that whether or not the undamaged hemisphere has assumed control of both hands, its ability to adequately suppress exertion of force by the hand it usually controls is severely compromised. Thus, processing in the undamaged hemisphere is abnormal in hemineglect. Supported by NIH NS16790.

26.2

AIR-STEPPING IN NEONATAL VERVET MONKEYS. J.A. Vilensky*, P. Wilson-Callmyer* and E. Gankiewicz*. (SPON: M.L. Dyken). Dept. of Anatomy, Indiana Univ. Sch. of Med., Fort Wayne, IN 46805

Limb movements during air-stepping were filmed and analyzed for three neonatal vervet monkeys over a three week period. The analysis showed the movements to have similar temporal organization both across animals and across time. For example, both hind and forelimb cycle durations tended to equal about 500 ms with the hind limb return strokes being much longer than power strokes. Forelimb return strokes were, however, shorter than the hind limb return strokes. In addition, there were clear indications of both intra- and interlimb coordination. Specifically, all the joints of a limb tended to flex and extend simultaneously, and contralateral and ipsilateral limb pairs had an average phase relationship of approximately 50% of cycle duration. Despite a qualitative similarity in limb movements between air-stepping in the neonates and overground locomotion in older animals, there were notable differences both in temporal relationships and joint displacement patterns. For example, hind limb power strokes showed no correlation with cycle duration and all of the hind limb joints exhibited a flexion bias. Finally, there appeared to be similarities between air-stepping in these monkeys and newborn stepping in humans. Most notably, both tend to disappear after a limited period.

26.3

MEDIAN NERVE RECORDING DURING GRASPING. T.E. Milner, C. Dugas, N. Picard* and A.M. Smith, Centre de recherche en sciences neurologiques, University of Montreal, Montreal, Québec, H3C 3J7.

A recording nerve cuff was implanted around the median nerve of a monkey for the purpose of detecting compound neural activity related to grasping. Distal to the level of the cuff the nerve innervated several thenar and lumbrical muscles; sensory innervation included the volar surfaces and nail beds of the first three digits, part of the fourth digit and the skin of the medial palm.

The neural signal was filtered using a high-pass filter with a sharp cutoff at 1500 Hz to remove EMG. By comparing the power spectrum of a signal generated by brushing the skin when the muscles were relaxed with one generated when the monkey was actively gripping and lifting an object, we determined that the filter eliminated almost all of the EMG leaving only a neural signal.

The monkey was trained to grasp, lift and hold an object whose weight and surface texture could be changed. There was an initial dynamic response consisting of a burst of activity which began as the digits contacted the surface of the object. The intensity of this burst rose sharply, reaching a peak well before peak grip force. Activity then declined in an exponential fashion to a steady level which was achieved much later than the onset of steady grip force during holding. This static response was greater for larger forces and rougher textures. When the grasp was relaxed neural activity dropped rapidly to its baseline level. As the object dropped from the hand there was a small symmetrical burst of activity probably caused by the object brushing the skin.

In some blocks of trials a pulse perturbation was applied during holding. It produced a sharp increase in activity coincident with the perturbation. This response preceded the increase in grip force associated with resistance to the perturbation. The amplitude and duration of this neural burst were similar to that observed during the initial lifting of the object. (This work was supported by the MRC and NSERC of Canada and le Fonds FCAR du Québec.)

26.4

A BIOMECHANICAL MODEL OF THE MONKEY'S ARM. M.Dornay*, T.Consi*, J.McIntyre*, F.A.Mussa-Ivaldi* and E.Bizzi. Dept. of Brain and Cognitive Sciences, M.I.T., Cambridge, MA 02139.

Several investigations of motor behavior have emphasized the role of muscle spring-like properties in the control of posture. Single-joint experiments have demonstrated that posture of the limb is determined by the equilibrium between length-tension curves of opposing muscles. This finding has been extended to the behavior of the multi-joint arm. It has been shown that if the hand is displaced by some external perturbation, the muscle elastic properties generate a restoring force which depends upon the amplitude as well as on the direction of the perturbation.

In this investigation we analyze the role of specific biomechanical factors in the postural behavior of the multi-joint arm. We have developed a computer model of the arm of the rhesus monkey. The model simulates the postural behavior of the arm in the horizontal plane. Each muscle is represented as an elastic element whose rest-length and stiffness is regulated by a control input. The ranges of stiffness and rest-length values have been estimated on the basis of in-vivo physiological data for the triceps muscle, combined with cross-sectional and length data measured on the corresponding muscles of rhesus monkeys. Other anatomical data concerning the origin and insertion and moment arm of the muscles were obtained by dissection and radiographic techniques. A total of seventeen muscles including shoulder, elbow and two-joint flexors and extensors have been included in the model. Simulation programs that have been developed in LISP on a Symbolics 3600 series computer, were used to determine the effect of a given input pattern on a) the equilibrium posture of the arm b) the restoring force following a displacement of the hand and c) the stiffness of the arm. Inputs to individual muscles in the model were derived by a backdriving technique (Mussa-Ivaldi, McIntyre, Bizzi, 1988). Given a desired posture and/or force output at the end-point of the arm, this approach makes it possible to derive the inputs to all the muscles consistent with a minimum potential energy constraint. Supp. by NIH NS09343, 1-F05-TWO4042-01; ONR N00014-88-K-0372.

26.5

INDIVIDUATED FINGER MOVEMENTS OF RHESUS MONKEYS: TO WHAT DEGREE IS MOVEMENT OF EACH DIGIT INDEPENDENT OF THE OTHERS? M.H.Schieber. Dept. of Neurol. & Neurosurg., Washington University Sch. of Med., St. Louis, MO, 63110.

In their natural behavior, monkeys use a variety of individuated finger movements, such as precision pinch. Observation suggests that monkeys, like humans, move their thumb and index finger more independently than digits 3 through 5. In the present study, the degree to which flexion and extension of each digit is independent of the other digits has been quantitated.

Monkeys were trained to perform individuated flexion and extension movements of each digit of the right hand or of the wrist (Soc. Neurosci. Abstr. 14:821). In each successful trial, the instructed digit had the greatest excursion. But the other digits also moved to some extent. To quantitate the motion of the other digits, linear regression was performed using the motion of the instructed digit as the independent variable and the motion of another digit as the dependent variable. The slope of the best fit line then is a measure of the dependence of the other digit's movement on the instructed digit, approaching 0 if they are independent, 1 if highly dependent. An **Individuation Index** then was calculated for each instructed movement by averaging the slopes for all the other digits and subtracting the average from 1. If all other digits moved with the instructed digit, this Individuation Index approaches 0; if the other digits were stationary while the instructed digit moved, the Index approaches 1.

Results support the hypothesis that the thumb is the digit moved most independently (Indexes range from .83 to .97), the index finger next (.64 to .92), and digits 3 through 5 the least independent (.50 to .78). Flexion movements generally were more individuated than extensions. Wrist movements were highly independent of the digits (.87 to .96), and vice versa.

This analysis provides i) a normal baseline for studies of the effects of cortical inactivation on individuated finger movements, and ii) a behavioral reference for comparison with muscle and neuron activity.

Support: K08-NS01150 to M.H. Schieber; R01-NS12777 to W.T. Thach.

26.6

INCOORDINATION IN ATTEMPTED REACHING AND PINCHING AFTER INACTIVATION OF CEREBELLAR DENTATE NUCLEUS S.A. Kane, H.P. Goodkin*, J.G. Keating*, and W.T. Thach. Dept. of Anatomy, Washington Univ. Sch. Med., St. Louis, MO 63110.

Rhesus monkeys were filmed or video taped while standing, sitting, walking, reaching and grasping, and using thumb and forefinger to pick food bits from deep, narrow food wells. These activities, along with trained wrist movements, were studied before and after injections of muscimol to produce temporary inactivation and kainic acid to produce a permanent lesion of the dentate nucleus.

Bits of food were normally removed from the well by the coordinated action of the thumb and forefinger. After inactivation, the monkey retained the ability to make individuated movements of the one finger, such as raking and stabbing during the food well task, but no longer coordinated the movements of thumb and forefinger in precision pinching. In reaching for a food bit, the normal pinch was replaced by 1) clawing: simultaneous flexion of all fingers about the food bit, 2) false pinch: holding the food bit between the extensor surface of the flexed thumb and the forefinger, or 3) spider hand: grasping with fingers abducted and independently flexed without effective coordination. Inactivation deficits were not confined to the fingers. During reaching, the shoulder and elbow overextended, sending the hand as much as 3 cm past the target (overshoot). After the permanent lesion, these deficits remained until sacrifice (7 weeks). Dentate inactivation had no effect on standing, sitting, or walking; no tremor was observed; and trained wrist movements were only slightly delayed. Injections aimed at the interpositus and fastigius had no effect on finger grasping during precision pinch and produced no overshoot during reaching.

In contrast to the relative normalcy of single joint movements (20–50 msec delay in initiation) following dentate inactivation, these results demonstrate an inability to make effective volitional movements involving more than one joint. These results suggest that the dentate mediates a type of control concerned primarily with multiple rather than single joints. (NIH grants NS12777 and NS15070; the McDonnell Center).

26.7

CHARACTERIZATION OF RESPIRATORY AND LARYNGEAL MUSCLE ACTIVITY ASSOCIATED WITH VOCALIZATION IN M NEMESTRINA. R.A. West, C.R. Larson, Dept. of Communication Sciences and Disorders and Neurobiology and Physiology, Northwestern University. Evanston, IL 60208.

In an attempt to determine the neuromuscular control of vocalization, electromyographic (EMG) activity of laryngeal and respiratory muscles was chronically recorded from five monkeys trained to vocalize for a fruit juice reward. Measured parameters included mean EMG, duration of EMG bursts, and latency from EMG onset to vocal onset for coos and barks.

Of the respiratory muscles recorded, intercostal (IC), rectus abdominus (RA) and external oblique (EO) had latencies that were relatively constant across individuals and call types. The diaphragm (D) generally had an earlier onset time for barks than coos and had reduced activity near vocal onset. This reduction was less frequently observed for longer duration calls. Other parameters of activity were somewhat variable across monkeys and call types.

Of the laryngeal muscles recorded, the posterior cricoarytenoid (PCA) was similar to the D in onset time and reduced activity near vocal onset. Thyroarytenoid (TA) and cricothyroid (CT) were extremely variable on all parameters across monkeys and call types, but were consistent for a monkey giving a specific call.

Overall, vocalizations across monkeys and call types were quite variable in duration and loudness. Latencies of respiratory muscles to vocal onset were much more consistent than those of laryngeal muscles. These data suggest that respiratory muscles may determine vocal onset time, whereas laryngeal muscles contribute to other measures of vocalization.

26.8

THE MOTOR EFFECTS OF UNILATERALLY STIMULATING THE CAUDATE NUCLEUS SIMULTANEOUSLY WITH THE CONTRALATERAL SUBSTANTIA NIGRA IN THE RAT: S.I. Lentz * and D. Asdourian, Department of Psychology, Wayne State University, Detroit, MI 48202

Unilateral electrical stimulation of the head of the caudate nucleus (Cd) elicits activity in the contralateral muscles of the neck and shoulder (15 twin pulse/sec, duration 0.5 msec, interpulse interval 1.0 msec, current 0.2- 0.6 ma, anesthetic sodium pentobarbital 60 mg/kg). If the Cd is stimulated with subthreshold current, the addition of threshold stimulation of the contralateral substantia nigra (SN) at roughly the same parameters as listed above, augments the effects of the subthreshold Cd stimulation so that Cd-elicited activity is seen in the trapezius muscle. In contrast, activity driven in the rectus capitis muscle with suprathreshold stimulation of Cd is almost always completely inhibited by threshold nigral stimulation. This nigrally elicited augmentation and inhibition is unaffected following large kainic acid lesions of the stimulated SN, leading to the conclusion that these results are due to stimulation of the caudally directed fibers of passage that run through that area rather than to outputs of nigral cells.

26.9

NMDA AND GABA: OPPOSITE EFFECTS ON ROTATION BEHAVIOR AFTER INJECTION IN UNILATERAL SUBSTANTIA NIGRA. Y.F.Jacquet. Nathan Kline Institute, Orangeburg, NY 10962.

Separate groups (n = 4-6) of drug-naive rats (previously implanted with 30-gauge cannula) were injected (using a 35-gauge needle) in the unilateral substantia nigra with the following drugs: N-methyl-D-aspartate (NMDA)(10 nmol/0.5 uL); muscimol (a GABA agonist)(0.85 nmol/0.5 uL); the NMDA competitive antagonist, (-)-2-amino-7-phosphonoheptanoate (D-AP7)(2.6 nmol/0.5 uL or 5.2 nmol/1 uL); or D-AP7 (5.2 nmol) + Bicuculline Methiodide (BM)(a potent GABA antagonist)(0.5 or 1 nmol) combined in a total volume of 0.5 uL.

NMDA resulted in ipsiversive rotation (5/6 rats). Muscimol resulted in contraversive rotation (4/4 rats), consistent with previous findings by other workers. The NMDA antagonist, D-AP7, however, not only blocked the ipsiversive rotation, but resulted in a dose-dependent contraversive rotation (5/5 rats). When the NMDA antagonist, D-AP7 was combined with the GABA antagonist, BM, ipsiversive rotation resulted (3/4 rats).

These results suggest that both excitatory (NMDAergic) and inhibitory (GABAergic) influences exert a tonic neuromodulation of the nigrostriatal output affecting posture and rotation behavior. When an imbalance occurs in the two (e.g., blockade of NMDA by D-AP7), the influence of the unopposed neurotransmitter (in this case, GABA) becomes evident in the direction of rotation behavior.

26.10

SCALING OF CHEWING FREQUENCY AND BODY WEIGHT IN MAMMALS. R.E. Druzinsky*.(SPON: C.H. Anderson). Depts. of Anatomy and Oral Anatomy, Univ. of Illinois at Chicago, Chicago, IL 60680.

T.A. McMahon (J. appl. Physiol., 186:1112, 1975) argued that single joint and multiple joint mechanical systems have "natural frequencies" of oscillation. Using his "elastic similarity" model of scaling, McMahon predicted that stride frequencies are proportional to (BODY WT)$^{-1/8}$. He compared animals running at the trot-gallop transition and found that: STRIDE FREQ $\propto$ (BODY WT)$^{-0.14}$ similar to the value of -1/8 he predicted.

In the present study, frequency of chewing cycles for 17 species of mammals was regressed against body weight. A significant correlation was found:
CHEW FREQ = 2.589 (BODY WT)$^{-0.101}$ with chewing frequency in cycles/minute and body weight in kilograms. This result is reasonably close to the predicted -1/8 exponent, and it is also similar to the exponent of -0.13 found for the relationship of STRIDE FREQ to BODY WT at the preferred rate of trotting (Heglund, N.C., and Taylor, C.R., J. exp. Biol., 138:301, 1988).
Supported by Sigma Xi and NIDR DE06279.

26.11

LOCOMOTOR HYPERACTIVITY IN CASTRATED MALE RATS WITH TESTOSTERONE IMPLANTS: BIOLOGICAL CORRELATES IN SUBCORTICAL STRUCTURES S.E.Starkstein, T.H.Moran,E.Nelson*, J.B.Bowersox*, R.G.Robinson Department of Psychiatry, Johns Hopkins University School of Medicine, Baltimore, MD 21205

We have reported that right but not left frontocortical suction lesions in the presence of testosterone produce locomotor hyperactivity as well as bilateral increments in dopamine (DA) turnover in the nucleus accumbens (NAS) in male rats. Whether this increase in turnover was a cause or a result of the hyperactivity is the subject of this study. Thirty-two prepuberally castrated male rats were divided into 4 groups and were given sham or testosterone-filled implants of different sizes (10, 20, and 30mm). Activity was monitored in running-wheel cages for 30 days. Rats with larger implants showed significantly more hyperactivity (% from baseline: sham 87%, 10mm: 118%, 20mm: 139%, 30mm: 179%). Plasma testosterone levels were significantly higher in rats with larger implants (sham: 1.6 ug/ml, 10mm: 7.8 ug/ml, 20mm: 10.4 ug/ml, 30mm: 13.8 ug/ml). Despite this hyperactivity, no significant elevations in either NAS or caudate DOPAC/DA ratios were evident in the testosterone-treated rats. This study showed that increments in NAS DA turnover are not necessary for the production of locomotor hyperactivity and suggests that the increments in NAS DA turnover after a right frontocortical suction lesion are not a result of the hyperactivity and may play a causative role in this behavioral response.

26.12

MONOAMINE METABOLISM IN THE BRAIN AND SPINAL CORD OF RATS DURING SWIMMING EXERCISE. T.Yamamoto*, A.Yamatodani*# and H.Wada*# (Spon: T.Watanabe) Faculty of Liberal Arts, Tezukayama University, Nara 631, and #Department of Pharmacology II , Faculty of Medicine, Osaka University, Osaka 530, Japan.

We studied the effects of swimming exercise on monoamine metabolism in several regions of the brain of female S.D. rats after long-term swimming training (120 min a day, 6 days a week for 10 weeks). On the final day, trained and untrained rats (used as controls to examine the effect of non-specific stress) were sacrificed 30, 60, and 120 min after the start of swimming session. Norepinephrine (NE), dopamine (DA), serotonin (5-HT) and their metabolites, and histamine (HA) in the brain tissue were determined by HPLCs. In the untrained rats, forced swimming caused a significant decrease of NE in the hypothalamus and cortex at 30 min, but no significant changes in the contents of DA, 5-HT and their metabolites and HA in any of the regions. In contrast, trained rats showed increases of 5-HT, 5-HIAA, DA and HA in the cortex at 120 min and of 5-HT in the striatum at 30 min, and decreases of DOPAC and HVA in the striatum at 30 and 60 min. The contents of NE, MHPG, DA, DOPAC, 5-HT and 5-HIAA in the thoracic spinal cord of trained rats were also significantly increased at 30, 60 and 120 min. These results suggest that not only the dopaminergic system, but also the noradrenergic, serotonergic and histaminergic systems participate in control of swimming exercise.

26.13

ANIMAL MOTILITY: SIGNAL SEGMENTATION AND CHARACTERIZATION. C.M. Arnaldo,* J.F. Czachura,* K.W. Brewer,* and L.P. Gonzalez. (SPON: J.T. Braggio) Department of Psychiatry and Behavioral Sciences, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73190-3000.

Our laboratory is investigating the use of an automated method to assess changes in motor behavior in small laboratory animals. This method involves the use of a radio-frequency capacitance field transducer that provides an output signal that varies with movements within the field of the transducer. Analysis of motor behavior requires segmentation of the motility signal into portions which contain information related to individual behaviors and characterization of the segment such that individual behaviors can be distinguished from one another. Results are reported comparing several segmentation techniques (arbitrary, fixed-length segments; segments of fixed cycle length; and, segments delimited by autoregression of the motility signal) and two methods of segment characterization (Fourier Analysis and Period Analysis). These methods permitted significant discrimination of motility signals recorded during several types of motor behavior, including spontaneous grooming behaviors and drug-induced stereotyped movements, and provide a useful technique for quantifying fine motor behaviors.

This work was supported in part by the Oklahoma Center for the Advancement of Science and Technology (Contract #1676).

26.14

YOHIMBINE-INDUCED CATALEPSY/AKINESIA: FORM AND PARTIAL REVERSAL BY HALOPERIDOL. R.M. Chesire. Psych. Dept., Univ. Hawaii, Honolulu, HI 96822.

Yohimbine HCl (YO), a potent MAOI (1), can prevent a form of catalepsy (CA; vertical rod apparatus) produced by haloperidol (HA; 2). In rats, 5 mg/kg systemic HA can produce a temporary form of catalepsy/akinesia (CA/AK) during which the defense of stable equilibrium is dramatically exaggerated (3). Preserved responses include postural support, tonic grasp, righting from a supine to a prone position, and defense against passive displacement by rigid bracing reactions (3). This report describes a form of YO-induced CA/AK, and a partial instatement of limb rigidity and tonic grasp by haloperidol.

11 male Long-Evans hooded rats were pretested for the defensive reactions mentioned above. They were then treated i.p. with 10 mg/kg YO HCl (retested at 20-30 min.) followed by 5 mg/kg HA (retested at 30-40 min.). YO abolished all normal equilibrium responses and produced AK (to be described). HA did not reinstate support, righting, or effective bracing, but limb rigidity was quite evident, and tonic grasp was reinstated.

YO produces a distinctive form of CA/AK which can be partially reversed by HA. The results suggest that the MAOI YO cannot prevent some HA-induced symptoms.

26.15

FROG ORIENTING BEHAVIOR: THE DESCENDING DISTANCE SIGNAL. P. Grobstein and V. Staradub*. Dept. Biol., Bryn Mawr Col., Bryn Mawr, PA 19010.

Visually triggered orienting in Rana pipiens depends on at least two differently organized descending tectofugal pathways, each carrying a distinct component of a signal related to stimulus location in a head or body centered coordinate frame. Information about the horizontal eccentricity (HE) of a stimulus apparently descends unilaterally while information about stimulus distance descends bilaterally. To determine whether the two pathways are also anatomically distinct, and to learn more about the character and location of the distance pathway, we have been studying the behavior of frogs following bilateral lesions at the midbrain/medulla junction.

Following large dorsal lesions, responses to prey items continued to vary appropriately with both the horizontal angle and the distance of stimuli. Descending pathways related to stimulus distance, as earlier shown for those related to HE, must be located in the more ventral white tracts. Following large ventral lesions, frogs responded to stimuli with a small forward movement which varied neither with the HE nor with the distance of the stimulus. This is consistent with a ventral trajectory for the distance as well as the HE pathways. It further suggests that information about the existence of a stimulus can reach the spinal cord in the absence of pathways which carry signals specifying its location.

Smaller bilateral ventral lesions yielded responses which varied more or less normally with HE but not with stimulus distance. The behavior of each of three frogs studied to date indicates that they were, for all stimulus distances, mislocating the stimuli to greater distances. The findings are consistent with the hypothesis of a ventrally located path carrying distance information which is anatomically distinct from the paths carrying HE information. The observations also suggest that distance is coded in terms of total activity in the distance pathway, with reduced total activity corresponding to greater rather than to lesser distance. Supported by NIH 1 R15 NS 24968 and the Whitehall Foundation.

26.16

THE ROLE OF THE CEREBELLUM AND BRAINSTEM IN ADAPTABILITY OF FROG WIPING MOTOR PROGRAMS M. Nitabach., S. F. Giszter*. Dept. Brain and Cognitive Sciences, M. I. T., Cambridge MA 02139.

Previous work has shown that the spinal frog can execute succesful wiping motions to the back and to the other limbs. This wiping can be adjusted on the basis of cutaneous information in spinal frogs but the spinal frog incorporates kinaesthetic information in a relatively coarse grained or discrete fashion. Specifically, spinal hindlimb-hindlimb wiping is restricted to a small area of workspace lying along the rostro-caudal body axis. If the leg is perturbed far from this area the spinal frog misses the target stimulus. Spinal frogs do not modify the wiping strategy in unsuccessful or blocked wipes.

To examine the level of the nervous system at which (1) kinaesthetic sensory motor transformations occur and (2) levels from which information is incorporated into the wiping motor program we have examined the kinematics of wiping frogs with only cerebellum, brainstem and spinal cord.

In the preparation with cerebellum we observe (i) that flexible wiping patterns occur using a larger area of workspace (ii) that the frog can incorporate kinaesthetic information to modify the hindlimb-hindlimb wiping behavior (iii) that the frog has an ability to recognize the location of mechanical restraints and to push against them with the other limbs in a persistent and probably motor equivalent fashion. These types of adjustments are not seen in the spinal cord alone.

These results suggest a layered control of wiping in which the spinal wiping circuits (which are able to use cutaneous information adaptively) are able to operate more flexibly using kinaesthetic sensory motor information mediated via the cerebellum and brainstem.

This work supported by NIH grant NS09343, ONR grant N00014/88/K/0372, and the Sloan Foundation.

26.17

ELECTROMYOGRAPHIC AND KINEMATIC ANALYSIS OF WIPE AND FLEXION REFLEXES OF THE SPINAL FROG. J.L. Schotland and W.Z. Rymer. Neuroscience Program and Dept. of Physiology, Northwestern University Medical School, Chicago, IL 60611.

The wipe reflex of the spinal frog is a complex, target-directed, rhythmical movement. The animal uses the toes of the hindlimb to remove an irritating stimulus located at any point on its body. This paper extends our previous report analyzing the premovement EMGs of wipe and flexion withdrawal (FWD) reflexes (Schotland and Rymer, 1988) to include an analysis of EMGs during these movements while simultaneously recording joint angles.

Frogs were spinalized and bipolar stainless steel wire EMG electrodes implanted in seven hindlimb muscles. Infrared emitters were attached to six positions on the animals midline and monitored hindlimb. EMG signals were collected on computer, as was an analog signal representing stimulus application and onset of WATSMART strobing (this was to accommodate a variable delay in onset of WATSMART strobing when externally triggered by the computer). Wipes were elicited with 1N HCl and flexion withdrawal with a pinch applied via forceps. A randomly ordered stimulus sequence was delivered and 5 (flexion) or 10 (wipe) second trials were collected.

FWD consists of flexion of all three joints - hip, knee, and ankle. During the forelimb wipe (FW) there is rapid extension of the knee while the ankle is at peak flexion and the hip is nearing peak flexion. The hindlimb wipe (HW) is characterized by alternating flexion and extension of knee and ankle joints superimposed on hip extension. In contrast to the rapid extension and slow flexion observed in FW, both phases are rapid in HW.

Ankle flexors and extensors are coactivated during ankle extension for both forms of the wipe, and precede extension during FWD. All other muscles are coactivated during the flexion phase of FWD. Two-joint muscles generally exert their extensor action at all joints during HW, while hip flexor activity of one- and two-joint muscles acting at the hip predominate during FW. These results suggest that different central circuits coordinate the three movements.

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26.18

MEASUREMENT OF FROG SEMITENDINOSUS MUSCLE FORCE AND TENDON LOAD-DEFORMATION AND LOAD-STRAIN PROPERTIES. Richard L. Lieber and Margot E. Leonard*. Division of Orthopaedic Surgery, University of California and V.A. Medical Center, San Diego, California 92161

INTRODUCTION AND METHODS: We previously modelled the relationship between sarcomere length and hip and knee joint angle during frog hopping assuming zero tendon compliance (Mai and Lieber, J. Biomech., in press). We found that, during the hopping motion, the ST first shortened and then lengthened . In order to determine the influence of tendon compliance on this finding, we measured the load-deformation and load-strain properties of the distal midportion of the frog semitendinosus (ST) using a dual-mode motor and video dimensional analyzer. Tendon load and strain were then related to the maximum tetanic tension of the isolated ST.

RESULTS AND DISCUSSION: Maximum tetanic tension of the ST averaged 0.3 ± 0.05 N for all samples (mean ± SD). Tendon strain at maximum tension corresponded to about 2%. These data suggest that during an isometric contraction at optimal sarcomere length, the ST muscle fiber would shorten from about 2.2 μm to 2.0 μm with the tendon slack at 2.2 μm. In this case, muscle force would not change. However, at shorter or longer sarcomere lengths muscle force would be altered due to external tendon compliance and altered filament overlap.

26.19

SPEED-RELATED CHANGES IN KINEMATIC OUTPUT AND MOTOR PATTERNS OF THE AVIAN HIND LIMB. S. M. Gatesy* (SPON: R. C. Holland). Museum of Comparative Zoology, Harvard Univ., Cambridge, MA 02138.

Walking birds, particularly chicks, have been used extensively as models for investigating the neural control of locomotion. To sample the avian locomotor repertoire more completely, the hind limb of adult helmeted guineafowl (*Numida meleagris*) was studied during walking and running at speeds from 0.2-3.0 m/sec. Simultaneous cineradiography and electromyography were employed to correlate the activity of 16 hip and knee muscles with skeletal movements.

Results show that guineafowl, unlike human bipeds, display a remarkably smooth transition from walking to running. As speed increases, step length is augmented by hip and knee extension late in the stance phase. Several muscles inactive during slow walking are recruited with speed, while others exhibit alteration of motor pattern. The overall pattern of muscle firing at high speeds resembles the reported pattern of motor output for walking in deafferented chicks (Bekoff et al., *J. Neurosci.*, 7:2320, 1987), where flexors and extensors have relatively equivalent burst durations. These results imply that comparisons between locomotor patterns should not be done exclusively with walking, where the central pattern generator (CPG) is being driven at a very low frequency. Study of motor output at different speeds may give clues to the interplay between sensory modulation and the locomotor CPG.

Supported by the Chapman Fund of the AMNH.

26.20

FIXED PATTERNS OF ACTIVITY OF MOTONEURONS AND INTERNEURONS IN LOAD COMPENSATORY REACTIONS OF LOCUSTS. S.N. Zill, S. F. Frazier* and S.E. Fish. Dept. Anat., Marshall Univ. Sch. Med., Huntington, WV 25704

Many animals generate compensatory contractions of limb muscles when the substrate upon which they are standing is displaced. We have reported that such reactions occur in tibial muscles of the locust hindleg when animals stand in a cage that is repeatedly swayed. We here report that fixed patterns of activity also occur in homologous flexor muscles of the middle legs and that motoneurons burst during the phase of movement (mean=.45 std .06) when animals are being pushed away from the surface upon which they stand. Thus, locusts use a number of limb muscles in load compensation to pull themselves toward the substrate. Using these data, we have developed a preparation permitting study of similar reactions during intracellular recordings. Animals are mounted on a beam and grasp a screen, to which forces equal to the animal's weight and additional displacements are applied. Locusts stably generate forces (up to 30 grams) greatly in excess of their weight to resist these displacements and show bursting in flexor muscles during the same phases of movement. A number of local circuit spiking interneurons also fire during this phase in these reactions. This preparation will be useful in determining which parameters are controlled in load compensation. Support by NIH grant NS 22682 and the Whitehall foundation.

MOTIVATION AND EMOTION II

27.1

EFFECTS OF HALOPERIDOL AND AMPHETAMINE ON ATTENTION-RELATED TASK PERFORMANCE IN THE RAT. M. La Cerra, R. Javid*, D. Weiner*, and A. Ettenberg. University of California, Santa Barbara, CA 93106.

Pharmacological antagonism of dopamine transmission produces reductions in operant response rates that are only partially accounted for by induced motor deficits. In the present study, the effect of DA antagonism with haloperidol on attention-related task performance was investigated in an analogue of Leonard's 5-choice serial reaction time task. At a dose that did not affect general locomotor activity, haloperidol (.075 mg/kg, i.p.) selectively diminished the number of correct responses animals emitted in the attention-related task session. In contrast, treatment with the dopamine agonist, d-amphetamine (1 mg/kg) produced both an increase in general locomotor activity and a selective increase in the number of inter-trial interval responses emitted in the attention-related task session. These findings are consistent with the view that pharmacologic manipulations of dopamine transmission alter attentional-reward processes subtending operant performance.

27.2

PROGRESSIVE WITHIN-SESSION DECREMENTS IN RESPONSE DURATION OBSERVED IN RATS RESPONDING FOR FOOD REINFORCEMENT UNDER NEUROLEPTIC CHALLENGE. A.Ettenberg, K.Gonzales* and S.C.Fowler Depts Psychology, Univ. California, Santa Barbara, CA 93106 and Univ. Mississippi, University, MS 38677.

Male albino rats were trained to reach through a small rectangular opening in their operant chamber and press an 18mm dia disk for a reward of either sucrose solution or sweetened condensed milk. The disk was attached to a sensitive isometric force transducer the output of which was monitored by computer to provide detailed information on the magnitude of force emitted during each response (sampled every 0.0025 s), the duration of each response and the number of responses over time. IP injections of both haloperidol (.04, .08, .16 mg/kg) and cis-flupenthixol (.15, .3 mg/kg) produced within-session decrements in response rates and increases in "peak" force similar to those observed when reinforcement was removed. However, only the drug manipulations resulted in reliable increases in response durations. Closer analysis revealed that while "rise times" (from response initiation to maximum or "peak" force) were relatively unaffected by neuroleptic treatment, "fall times" (from "peak" force to paw removal from the disk) greatly increased as the test session progressed. These data demonstrate a motoric deficit (i.e. progressive inability to terminate operant responses) that can account for the "extinction-like" response patterns observed in animals responding during neuroleptic challenge.

27.3

Differential Effects of Haloperidol, Pentobarbital, and Dantrolene on Operant Response Initiation but Not Spontaneous Locomotion.

E. O'S. Hammond, M. L. Torok*, and A. Ettenberg.
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Intraperitoneal injections of haloperidol (HAL; 0.00, 0.075, 0.15, 0.30 mg/kg), pentobarbital (PEN; 0.00, 4.5, 9, 12 mg/kg), and dantrolene (DAN; 0.00, 5, 7.5, 10 mg/kg) produced similar profiles of within-session decreases in spontaneous locomotion. In contrast, the same drugs produced three distinctly different patterns of response initiation over 10 trials in an operant lever-press task. HAL-treated animals' initiation latencies increased over trials whereas PEN produced latencies that tended to decrease over trials. DAN latencies were elevated throughout the session. Thus, application of food reinforcement in an operant setting revealed differences among drugs that in a spontaneous locomotor setting produce strikingly similar motor impairments. These results suggest that the drugs may in some fashion differentially affect the experience of reward.

27.4

OPPOSITE EFFECTS OF INTRA-ACCUMBENS AND INTRA-PREFRONTAL CORTEX APPLICATIONS OF CIS-FLUPENTHIXOL ON CONDITIONED PLACE PREFERENCES INDUCED BY REWARDING VTA STIMULATION. C.L. Duvauchelle*, M.Levitin* and A. Ettenberg. (Spon. C.W. Scouten). Dept Psychology, Univ California, Santa Barbara, CA 93106

Animals develop preferences for distinct environments in which they experienced sessions of rewarding brain stimulation. These conditioned place preferences (CPPs) are attenuated by systemic injection of the dopamine (DA) antagonist, haloperidol (Ettenberg & Duvauchelle, Behav Neurosci 102:687, 1988). In the present study, CPPs were produced for the side of a two-compartment apparatus in which the rats experienced four 5min exposures to experimenter-administered rewarding VTA stimulation. Pretreatment with bilateral applications of 0, 1, 5 or 10 ug/rat/side of the DA antagonist, cis-flupenthixol into the nucleus accumbens produced a dose-dependent attenuation in the size of the resulting preferences. This result is, therefore, consistent with previous reports that intra-accumbens injections of neuroleptic drugs can reduce the rewarding properties of intracranial stimulation. Conversely, similar applications of cis-flupenthixol into the medial prefrontal cortex resulted in dose-dependent increases in the size of the VTA induced preferences. These data suggest that DA sensitive elements in the prefrontal cortex may provide a modulatory influence on the functional output of DA cells within the nucleus accumbens.

27.5

THE EFFECT OF PIMOZIDE ON WATER-REINFORCED RUNNING AND UNCONDITIONED DRINKING. J.C. Horvitz and A. Ettenberg. Dept. of Psych., Univ. of Calif., Santa Barbara, CA 93106.

Thirsty male albino rats were trained to traverse a straight-arm runway for 100 licks of water reward. The duration of each lick, and interval between licks were recorded. Single daily trials were administered until animals traversed the runway in less than 20 seconds. After animals had acquired the running behavior, they received single daily nonrewarded trials until running speeds had slowed substantially. In the midst of this extinction phase, animals recieved another water-rewarded trial either drug-free or under the influence of dopamine receptor-blocking agent pimozide (PIM) (0.5 or 1.0 mg/kg). Running speeds were recorded 24 hours later, during a drug-free test trial. Animals nondrugged during the water-reinforced trial showed a reinstatement of operant running the following day. PIM (1.0 mg/kg) blocked this response-reinstating effect of water reward. In addition both PIM and non-reward conditions produced a decrease in the duration of individual licks.

27.6

THE KINETICS OF THE MOTIVATIONAL PROPERTY OF SACCHARIN: MEASUREMENT OF REWARDING AND REINFORCING EFFECTS. T.L. Stefurak*, D. van der Kooy (SPON: N. Mrosovsky), Neurobiological Research Group, Dept. of Anatomy, University of Toronto, Toronto, Canada M5S 1A8

We asked if a single motivational property of saccharin can explain its actions in different paradigms.Saccharin preferences measured in the two bottle tests comparing consumption of water against various saccharin concentrations (0.025 to 3.2%) revealed decreasing preferences (rewarding effects) with increasing concentrations. However, the reinforcing effects of saccharin, as measured by the conditioned place preferences produced by consumption of these same concentrations of saccharin, revealed increasing conditioned place preferences with increasing concentration. In fact, any combination of saccharin concentration and volume consumption that resulted in a pairing intake of more than 20 mg of saccharin produced conditioned place preferences. We suggest that high concentrations of saccharin have aversive taste properties that limit their volume consumption, but that the absolute amount of saccharin consumed in mg is still reinforcing. The ability of consumption tests to measure the rewarding effects of low concentrations of saccharin, those measured as non-reinforcing by the conditioned place test, may be accounted for by a greater sensitivity of taste versus place stimuli to assess the motivational properties of saccharin. We suggest that there is only a single positive motivational property of saccharin.

27.7

LACK OF TOLERANCE TO THE INCENTIVE MOTIVATIONAL PROPERTIES OF OPIATES: APPARENT TOLERANCE IS OVERSHADOWING. A.Bechara and D. van der Kooy, Neurobiology Research Group, Dept. of Anatomy, Univ. of Toronto, Toronto,Ont.,Canada,M5S 1A8.

There has been a long controversy as to whether physically dependent subjects become tolerant to the incentive motivational effects of opiates. This question can now be addressed more explicitly because the incentive and the withdrawal motivational properties of opiates can be measured separately in a modified place conditioning paradigm, and because lesions of the tegmental pedunculopontine nucleus(TPP) abolish specifically the incentive motivational effects of opiates. To measure the incentive motivational properties of opiates, rats were placed in a distinctive environment immediately after being injected with a dose of morphine. The incentive effects of morphine were assessed by the conditioned preference rats showed for this morphine associated environment over an unfamiliar neutral one. Apparent tolerance was revealed when naive, but not physically dependent (60 mg/kg/day for a minimum of 14 days prior to conditioning), rats conditioned with 2 mg/kg doses of morphine (i.p.) expressed morphine conditioned place preferences. Higher doses of morphine (20 mg/kg) were effective in producing incentive effects in both the naive and physically dependent rats.TPP lesions blocked the incentive motivational effects of morphine in the naive, but not in the dependent, groups. Most interestingly, when physically dependent animals were administered withdrawal alleviating doses of morphine (20 mg/kg) one hour prior to conditioning with morphine(2 mg/kg), they now showed morphine place preferences equal to those seen in naive rats at 2 mg/kg. Most importantly, these incentive effects of morphine in both naive and dependent groups were abolished by TPP lesions. Thus, the alleviation of withdrawal allowed the unmasking of the unaltered neural mechanisms underlying the incentive motivational effects of opiates. We suggest that the apparent tolerance to the incentive effects of opiates results from overshadowing of these incentive effects by withdrawal mechanisms associated with opiate dependence.

27.8

DIFFERENTIAL DISCRIMINATIVE CONTROL BY MORPHINE AS A DANGER VERSUS SAFETY SIGNAL. G.M. Martin*, A. Pridgar* and D. van der Kooy (Spon: R. Adamec). Dept. of Psychology Memorial University, Newfoundland, A1B 3X9, Dept.of Anatomy, University of Toronto, Toronto M5S 1A8

The conditional control exerted by a morphine induced drug state over consumption was examined. Half the rats received injections of 5mg/kg of morphine, as a danger signal, followed by a saccharin solution, which was followed by the injection of lithium chloride on training days (Morph-Sac-LiCl). Saline injections were substituted for morphine on safe days (Saline-Sac-Saline) in this group. The remaining rats had the roles of the first morphine and first saline injections interchanged so that morphine served as a safety signal.

The control over consumption exerted by morphine, as a danger signal, transferred to novel and familiar foods. Morphine, as a danger signal, was even learned with a delay of one minute between its offset (reversed by naloxone) and the presentation of saccharin. When morphine served as a safety signal, learning did not occur with delays after its offset, nor did transfer occur even when learning had occurred without any delays. It appears that when morphine is a safety signal rats learn that a single configuration of stimuli (morphine/saccharin) predict a motivational event. In contrast, when morphine is a danger signal rats learn that morphine is a conditional cue that is independent of the specific configuration of stimuli used.

27.9

BIPHASIC ALTERATION OF 3-MT LEVELS IN MOUSE NUCLEUS ACCUMBENS DURING STRESS S. Puglisi-Allegra and S. Cabib* Istituto di Psicobiologia e Psicofarmacologia (C.N.R.), Roma I-00198, Italy

Stressful stimulations are known to alter dopamine (DA) metabolism in various brain areas. We have recently presented results suggesting that 30-120 minutes of immobilization stress reduce DA release in the nucleus accumbens (NAS) and, less markedly, in the caudatus putamen of the mouse (Cabib et al. Brain Res., 441:153, 1988).

We now report that immobilization as well as unavoidable footshock induce biphasic alterations of 3-methoxytyramine (3-MT) levels in the NAS of mice. An initial dramatic increase of this metabolism was, in fact, evident within the first 10 minutes of exposure to both type of stressors; followed by a decrease at 30-60 min. An increase of the acid metabolites (DOPAC and HVA) was evident at all times during stressful stimulation.

These results suggest that stressful experiences induce a biphasic alteration of DA transmission in the NAS characterized by an initial increase of DA release, possibly related to an arousal state, followed by by a decrease possibly related to coping failure.

27.10

PREOPTIC STIMULATION SUPPRESSES AND NON-PREOPTIC STIMULATION ENHANCES MATERNAL BEHAVIORS IN POSTPARTURIENT RATS. M.A. Steuer* and M.B. Kristal (SPON: M.A. Bozarth) Dept. of Psychology, SUNY at Buffalo, Buffalo, N.Y. 14260.

In an effort to determine how the preoptic area (POA) modulates the expression of maternal behaviors, the POA of postparturient rats was stimulated using either electrical or glutaminergic stimulation.

During electrical stimulation (100 cps, 0.2 msec separated by 0.1 msec, 2-20 μA) in and around the POA, retrieval was unaffected, but crouching, pup-licking, and nestbuilding were suppressed due to the emergence of arousal-induced autogrooming/locomotor activity which was incompatible with the expression of maternal responsiveness. In contrast, during electrical stimulation outside of the POA (neocortex, caudate, or even of the tail of the rat) retrieval and nestbuilding were not affected, but crouching and pup-licking were enhanced.

Infusion of glutamate (0.25 μL, 0.5 M) into the POA resulted in a strong suppression of all aspects of maternal behavior due to the activation of a thermosensitive motivational system which resulted in the emergence of incompatible responses (sprawling, autogrooming). Infusions into a control site (cortex) did not interfere with any aspect of maternal behavior.

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27.11

SPECIFIC BUT NOT GENERAL DAMAGE TO HIPPOCAMPAL SUBFIELDS DISRUPTS GROOMING IN RATS. R. L. Cannon*, D. J. Paul*, R. H. Baisden, and M. L. Woodruff. Dept. of Anatomy, Col. of Med., East Tenn. St. Univ., Johnson City, TN 37614.

The purposes of the present investigation were (1) to examine the effects of exposure to TMT on an unlearned, species-typical behavior and (2) to determine whether destruction of specific hippocampal subfields are responsible for behavioral deficits. Adult male Long-Evans rats were exposed to either TMT, kainic acid (KA), which destroys subfield CA3, or colchicine (COL), which destroys the dentate granule cells. Four months later grooming was observed under two conditions. First, rats were viewed in a large open field 15 min per day for 3 days. Next the rats were sprayed with a 16% sucrose solution to elicit excessive grooming and then observed in a transparent Plexiglas cage 15 min per day for 3 days. Normal rats were observed under the same conditions. The sequence of behavior and the efficacy of movement were examined. The TMT-exposed rats did not differ from the normal rats, but both KA- and COL-injected rats showed significant variations from the normal grooming pattern. These results indicate that the damage to the hippocampus induced by TMT does not inevitably correlate with all of the behavioral changes produced by restricted subfield damage. (Supported by NIH grant ES 04070-03 to MLW).

27.12

INFLUENCES OF SCHEDULE-INDUCED POLYDIPSIA ON BODY TEMPERATURE IN RATS. G. Mittleman, H. Verdoux, M. Le Moal and R. Dantzer. INSERM U-259, Domaine de Carriere, Rue Camille Saint Saens, Bordeaux, 33077, France.

Schedule-induced polydipsia (SIP) is an example of the larger category of adjunctive behaviors that occur in situations where a strongly motivated appetitive or consummatory behavior is interrupted or prevented. It has been suggested that SIP as well as other adjunctive behaviors arise from the excitement accompanying the delivery of a food reward. Thus, hungry animals exposed to intermittent presentations of small amounts of food show increased arousal as measured by either endocrine or behavioral indices (Brett, L.P. and Levine, S., JCPP, 93:946, 1979; Kileen,P., Hanson, S.J. and Osbourne, S.R., Psychol. Rev., 85:571, 1978) as well as other physiological changes consistent with increased metabolism. In this experiment, body temperature (Tb) and activity of rats food deprived to 85% of their baseline weight was continuously monitored during tests for schedule-induced drinking or forced wheel running. It was found that rats which showed polydipsia (SIP-pos) had a smaller increase in Tb than rats that did not drink (SIP-neg). This difference was related to baseline Tb and food motivation but was independent of water consumption. Forced wheel running induced an increase in Tb in SIP-pos rats similar to that observed during SIP testing, but little or no change in SIP-neg animals, in spite of large differences in activity levels. These results agree with previous demonstrations suggesting that SIP-pos and -neg rats differ in terms of energy metabolism.

27.13

DIFFERENTIAL EFFECTS OF PERIPEDUNCULAR AREA LESIONS ON MATERNAL AGGRESSION IN LACTATING RATS. E. M. Factor, A. D. Mayer* and J. S. Rosenblatt* Inst. Anim. Beh., Rutgers Univ., Newark NJ 07102.

Electrolytic lesions of the lateral midbrain which include the peripeduncular area (PPN) eliminate maternal aggression and the milk-ejection reflex in lactating rats when such lesions are made one week postpartum (Hansen,S. and Ferreira,A., Behav.Neurosci. 100:64, 1986). These data support the hypothesis that postpartum aggression in rodents is maintained by suckling stimulation, and suggest the PPN as a locus which integrates somatosensory (e.g. suckling) and motivational impulses. This study examined the behavioral effects of PPN lesions made at specific points in gestation or lactation. Primiparous rats received bilateral PPN lesions (RF current 10 mA/6 sec/side) at one of four reproductive stages: on gestation day 18, or lactation days 2 (24 hours postpartum), 5, or 8. Sham lesions were made without current. Another group received axon-sparing lesions with the cytotoxin N-methyl-d,l-aspartic acid (NMA, 0.4 ug/side) or saline vehicle. Data were recorded for nursing, nestbuilding, pup retrieving, aggression, hoarding and daily litter weight gain. Dramatic reductions in maternal aggression and litter weight gain and longer retrieving latencies developed in rats that received PPN lesions on lactation day 8. Other behaviors were not changed. RF lesions made prepartum or during early lactation did not affect litter weight gain or any of the observed behaviors. The functional significance of PPN neurons may increase with the duration of maternal experience in primiparous lactating rats.

27.14

ELECTROLYTIC LESIONS OF THE HABENULA OR DORSAL HIPPOCAMPUS REDUCE ANXIETY-ASSOCIATED BEHAVIORS IN RATS. B.L.Kendis* and J.V.Cassella+ (SPON: C.M.Sinton). Neuroscience Program, Oberlin College, Oberlin,OH 44074 and +Neurogen Corporation, Branford,CT 06405.

Anatomical evidence suggests that the habenula is an important relay between limbic forebrain and midbrain areas. The role of the habenula, as well as the dorsal hippocampus, in anxiety in two behavioral models was assessed. Male albino rats were tested initially in a shuttle-type box in which one side was lighted and the other side was dark (the LDC) and were then tested on an Elevated Plus-Maze (EPM). Testing was conducted at least 1 day before and seven days after surgery : sham, medial and lateral habenula and/or dorsal hippocampus.

Lesions in either the habenula or dorsal hippocampus increased specific activities in the LDC and EPM indicative of anxiety reduction without increasing independently-assessed general locomotor activity. Combined habenula and hippocampal lesions did not produce additive behavioral effects. The data suggest that these structures are independent components of a neural circuitry involved in the expression of anxiety.

27.15

THE INFLUENCE OF EARLY HANDLING AND PRIOR PLAY EXPERIENCE ON OPEN FIELD & ELEVATED-PLUS MAZE EXPLORATION, AND ON BRAIN & ADRENAL WEIGHTS IN JUVENILE RATS. L. Crepeau & J. Panksepp, Dept. of Psychology, Bowling Green State University, Bowling Green, OH 43403

Juvenile rats that received early handling (EH) during the first two weeks of life and that were subsequently tested in an open field holeboard exhibited reliably higher levels of grid crossings ($p < .05$) and rearings ($p < .01$), compared to unhandled controls. Rats that were provided with 15 min of daily prior play experience (PPE) for five days beginning at 22 days of age exhibited marginally higher levels of investigatory nose pokes when tested in the holeboard ($p < .07$).

When tested on an elevated-plus maze (Pellow, et al., *J. Neurosci. Meth., 14*, 149-167, 1985), PPE group rats explored onto and spent more time on the open arms of the apparatus, compared to play-naive controls ($p's < .01$). PPE group animals also explored marginally less onto the closed arms ($p < .10$), and spent less time on the closed arms than controls ($p < .01$). Additionally, EH group rats that received PPE exhibited reliably higher levels of closed arm entries, compared to play-naive EH rats ($p < .01$). PPE had no effect on closed arm entries among unhandled controls. This manipulation did not affect open arm exploration.

Brain/body weight ratios were reliably higher in female rats, compared to males ($p < .01$). Additionally, EH reliably increased the brain/body ratios among the males ($p < .01$), but not among the females.

Adrenal/body weight ratios were also reliably higher in female rats, compared to males ($p < .01$). Additionally, EH reliably increased the brain/body ratios in both males and females ($p's < .05$).

27.16

TEMPORAL SPECIFICITY OF FEAR CONDITIONING: EFFECTS OF DIFFERENT CS-US INTERVALS ON THE FEAR-POTENTIATED STARTLE EFFECT. M. Davis, L.S. Schlesinger* and C.A. Sorenson. Dept. of Psychiatry, Yale University School of Medicine, 34 Park St., New Haven, Conn. 06508

Separate groups of rats were given 30 pairings of a light (CS) and a 500-msec shock (US) at CS-US intervals of either 0, 25, 50, 100, 200, 800, 3,200, 12,800 or 51,200 msec. Other groups had lights and shocks inconsistently paired. 2-4 days later the startle reflex was elicited with a noise burst alone or 25-51,200 msec after light onset. Following CS-US pairing over a wide range of intervals (25-51,200 msec), startle was potentiated in testing, sometimes as rapidly as 50 msec after light onset. Magnitude of potentiation and resistance to extinction were generally greater with longer CS-US intervals. In several groups potentiation was maximal at a test interval that matched the CS-US interval used in training. This temporal specificity sharpened with increasing numbers of training trials but even occurred with a single training trial when a 51,200-msec CS-US interval was employed. The data indicate that even during "simple fear conditioning" animals rapidly learn a temporal CS-US relationship, which has important implications for understanding the neural mechanisms of fear conditioning.

27.17

ACTH EFFECTS ON ISOLATION-INDUCED DISTRESS CALLING IN CHICKS. L. Normansell and J. Panksepp. Dept. of Psychology, Muskingum College, New Concord, OH 43762, and Bowling Green State University, Bowling Green, OH 43403.

Previous work has implicated corticotropin releasing factor (CRF) in the brain control of separation distress (Panksepp, Clynes, & Crepeau, Soc Neurosci Abstr 13: 1320, 1987). As well, the pituitary hormone a-MSH has been shown to affect the number of vocalizations emitted by isolated chicks, producing a transient increase followed by a prolonged decrease (Abbott & Panksepp, Soc Neurosci Abstr 14: 287, 1988). In this series of experiments, the effects of adrenocorticotropic hormone (ACTH 1-24 and ACTH 4-10) on isolation- induced distress vocalizations (DVs) was assessed. Initially, a dose response determination was conducted on both peptides. ACTH 1-24 at doses of .5nM and above increased DVs relative to control when the animals were tested in a mirrored environment which generally serves to suppress calling. This effect, however, was short-lived. When tested 1 and 20 hours after injection, the treated animals did not differ from controls. ACTH 4-10 had no effect on vocalization when the animals were tested immediately after injection, but produced an increase in calling when the animals were tested an hour later. In addition to vocalization changes, ACTH 1-24 induced yawning, head shaking, and wing flapping. ACTH-treated chicks also preened themselves more than controls, and had longer latencies to close their eyes when they were held in the cupped hands of the experimenter. Taken together the results suggest that ACTH induces a central state of arousal in chicks that resembles fear/anxiety.

27.18

BEHAVIOURAL IMPAIRMENTS IN DEMANDING LEARNING PARADIGMS FOLLOWING HABENULAR LESIONS.
E.W. Thornton*, Dept. of Psychology, Liverpool Univ. Liverpool, England. (SPON: A.J. Goudie)
Habenular lesioned and control rats were tested for acquisition of either active avoidance or simple discrimination response under more, or less, demanding conditions. In the former, rats had to jump to a platform at heights of either 4 or 17 cm to avoid or escape from shock presented 20 sec after a warning CS. Intensity of shock and inter-trial-intervals (ITI) were also altered to increase the level of demand. Discrimination learning was carried out in conventional Y-maze. More demanding discrimination was carried out in a water tank in which the rat was required to swim to a platform on the non-preferred side of a bimodal spatial location with the correct choice confounded by random presentation of a salient, but irrelevant, visual cue (see Lee, K.K. & Maier, S.F. J. Exp.Psychol, 1988, 14, 302).

Results showed no lesion induced deficit in avoidance learning with non-demanding contingencies but impaired avoidance responding with short ITIs and higher shock intensity and more demanding response without effect on escape latencies. There was no impairment of simple discrimination learning but lesioned rats were impaired in the more difficult swim test. The deficits are indirect evidence consistent with the habenula regulating dopaminergic function.

27.19

BETA-ENDORPHIN WITHDRAWAL EFFECTS IN PREMENSTRUAL SYNDROME.
R.H. Loiselle, A.J. Giannini, D.J. Folts, B.S. Sullivan. Northeast Ohio Medical College, P. O. Box 2169, Youngstown, Ohio 44504.

Sixty women with premenstrual syndrome (PMS) were studied. All were between ages 21 and 34. β-endorphin levels were measured on the first, fifth, tenth, fifteenth, twentieth, and twenty-fifth day of each of two menstrual cycles. Responses were measured with the Brief Psychiatric Rating Scale (BPRS).

Twenty-two women had a significant decline in PMS from the fifteenth to twenty-fifth day ($p < .02$). When the BPRS scores of women with β-endorphin decline were compared to those without decline, there were found to be significant increases in anxiety ($p < .01$), depression ($p < .01$), and discomfort ($p < .01$). Concentration was decreased ($p < .05$). Also, women with β-endorphin decline had significantly increased caloric consumption ($p < .02$).

β-endorphin decline may be physiologically similar to morphine withdrawal. Symptoms in the PMS-β-endorphin group are similar to those of morphine withdrawal. A specific β-endorphin withdrawal syndrome may be a distinct subset of PMS.

ALCOHOL, BARBITURATES, BENZODIAZEPINES II

28.1

MICRODRINKING PATTERNS OF RATS DURING ACQUISITION AND MAINTENANCE OF FREE CHOICE ETHANOL. E.M. Sellers, H.L. Kaplan*, M.O. Lawrin* and B. Brands*, Psychopharmacology Program, Addiction Research Foundation and Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada M5S 1A1.

Regulation of rats' free choice ethanol (E) and water (W) drinking was studied on a minute-by-minute basis, using pressure transducers mounted in the bases of custom-built drinking tubes, continuously monitored by a laboratory computer during the 12-hour dark period. Male Wistar rats (n = 21) were given a free choice of E 2% or W until day 4 (D4) and then divided into 3 groups: n = 4 continued at E 2%, n = 7 were raised by 1% every 2 days to E 4% by D7, and n = 10 were similarly raised to E 6% by D11. All groups continued until D21. On D21, combining the groups, time to consume 50% of each fluid's daily volume (VT50) differed (E = 387 min, W = 204 min, $p = 0.0008$), as did the number of discrete drinks (NDK) (E = 60, W = 18, $p = 0.0001$) and the mean drink size (MDS) (E = 0.75 ml, W = 0.52 ml, $p = 0.04$), though the difference in the volume-weighted median drink size (VWD50) was smaller (E = 1.4 ml, W = 1.2 ml, $p = 0.44$). Considering days D5 and D21, E tube volume (D5 = 25 ml, D21 = 43 ml, $p < 0.0001$), E preference ratio (D5=61%, D21 = 76%, $p = 0.004$), E MDS (D5 = 0.66 ml, D21 = 0.83 ml, $p = 0.03$), and E NDK (D5 = 43; D21 = 60, $p < 0.0001$) all increased; W NDK, W MDS, W VT50, E VT50, and E VWD50 were unchanged; W VWD50 increased only in the E 2% group. Other group differences on these statistics were not statistically significant. E drinks are typically later in the session, more numerous, and somewhat larger than W drinks. If drink number, size, and time of occurrence are under different neuroregulatory control, they should show different susceptibility to drugs.

28.2

SUPPRESSION OF ALCOHOL AND FOOD INTAKE BY LY264453, A NEW INHIBITOR OF SEROTONIN (5HT) AND NOREPINEPHRINE (NE) UPTAKE. J.M. Murphy, W.J. McBride, L. Lumeng*, T.-K. Li* and D.T. Wong. Indiana Univ. Sch. Med. and VAMC; Purdue Univ. Sch. Science, Indianapolis, IN 46223; and Lilly Research Labs, Eli Lilly and Co., Indianapolis, IN 46285

Selective 5HT or NE uptake inhibitors decrease food and alcohol ingestion in rats (Pharmacol Biochem Behav 30: 1045, 1988). This study tested a new agent, LY264453, an inhibitor of both 5HT and NE uptake. Male HAD (high alcohol drinking) rats received ad lib food and water, but ethanol (10%, v/v) access was limited to 4 h/day. Rats were injected ip 10 min before the 4 h ethanol access with either the 5HT uptake inhibitor fluoxetine, the NE uptake inhibitor nisoxetine, LY264453 or saline. Food, water and ethanol intakes were recorded hourly over the 4 h period. LY264453 caused the most potent reduction of ethanol drinking (ED_{50}=3.3 mg/kg), followed by nisoxetine (ED_{50}=6 mg/kg) and fluoxetine (ED_{50}=13 mg/kg). Nisoxetine was 3 times more effective in reducing food (ED_{50}=2 mg/kg) than alcohol intake, while LY264453 was about equally effective in decreasing food (ED_{50}=3 mg/kg) and alcohol intake. Previously, we showed that fluoxetine decreased alcohol drinking more than food intake. Water intake was unaltered by most doses of all three drugs. Both the NE and 5HT transmitter systems may regulate alcohol and food intake of HAD rats, but NE may be less important in control of alcohol intake than 5HT systems. (AA-03243 & AA-07611)

28.3

THE 5-HT REUPTAKE INHIBITOR FLUOXETINE BUT NOT THE 5-HT3 ANTAGONIST ZACOPRIDE DECREASES ETHANOL CONSUMPTION DURING RESTRICTED ACCESS IN RATS. D.J. Knapp* and L.A. Pohorecky* (SPON: C. Vander Wende). Center of Alcohol Studies, Rutgers University, Piscataway, NJ 08855-0969.

One of the most consistent findings in clinical and experimental research on ethanol (ET) consumption is inhibition of drinking by serotonin reuptake inhibition. Serotonergic manipulations are currently an active area of research on ET intake. Male Sprague-Dawley rats (490-640 g) with a 50% free choice preference for 6% v/v ET received fluoxetine (FL) (5 mg/kg) and zacopride (1 and 10 mg/kg) sequentially at 4-5 day intervals, 15 or 45 minutes respectively before scheduled access to ET. FL (which is anxiolytic in rats and has no effect on motor function at this dose), increased latency to drinking and decreased number of bouts of drinking in a one hour restricted access period which began at the onset of the dark cycle. ET consumption was reduced by 52%. Drinking under these conditions followed a two-peaked pattern with a large peak during the first 8 minutes and an attenuated peak during the last 20 minutes. 100% of consumption by FL-treated rats occurred during the second 30 minutes of the hour while 77% of consumption by the saline-treated rats occurred during the first 8 minutes. Lick rate, licks/bout, and time/bout were not altered by FL. Similarly anxiolytic and non-motor impairing doses of zacopride did not significantly affect these measures.

28.4

MICROINJECTION OF THE SEROTONIN AGONIST TFMPP INTO THE NUCLEUS ACCUMBENS INCREASES ETHANOL DRINKING IN P RATS. A.D. Levy, J.M. Murphy, W.J. McBride, L. Lumeng*, & T.-K. Li* Indiana Univ Sch Med & VA, Indianapolis IN 46223

It has been proposed that serotonin (5-HT) systems in brain have an important role in mediating ethanol consumption. We are particularly interested in the role of 5-HT in the nucleus accumbens; studies from this lab on P and NP rats (selectively bred for high and low ethanol preference, respectively), have found lower levels of 5-HT in the accumbens of the P compared to the NP line (PBB (1987) 26:389). In preliminary studies, it was determined that intra-accumbens 5-HT injections decreased ethanol intake in P rats. The effect of intra-accumbens injections of TFMPP (5-HT 1b agonist) on ethanol drinking in P rats was therefore examined.

P female rats (N=9) were allowed access to 10% ethanol for 4 hr/day (food and water were available ad lib). Immediately prior to ethanol access, animals were microinjected with TFMPP (0.5-5 ug) or vehicle. Fluid intake was then monitored over the 4 hr testing period. Low (0.5 and 1 ug) doses of TFMPP substantially increased ethanol drinking (by approximately 60% over the first 30 min), while at higher doses this increase was lessened. The data suggest that the presynaptic 5-HT 1b autoreceptor in the nucleus accumbens is important in the mediation of ethanol intake. (AA03245, AA07472, AA07611)

28.5

EFFECTS OF CENTRALLY INJECTED l-AROMATIC AMINO ACID DECARBOXYLASE INHIBITORS ON ALCOHOL CONSUMPTION BY RATS. B.A. McMillen, F.J. Minano* and R.D. Myers, Dept. of Pharmacol. Sch. of Med., East Carolina Univ., Greenville, NC 27858

The central substrates for induction of alcohol (ETOH) consumption and reversal of this phenomena are unclear. Rats were allowed free choice between water or a concentration of ETOH solution such that the ETOH solution comprised 50% of fluid intake: the bottles were rotated each morning and the amount and proportion of ETOH consumed recorded. Inhibitors of l-AAAD were chosen as a means of manipulating central monoaminergic function. One group's ETOH consumption was increased by i.c.v. 50 ng tetrahydropapaveroline (THP), then the rats received 4 days of i.c.v. artificial CSF, followed by 3 days of benserazide (50 or 100 ug) with or without additional THP and then a 4 day postdrug period was recorded as well. Both doses of benserazide reduced ETOH consumption, which remained suppressed during the postdrug period. Another group had their ETOH intake increased by 15 mg/kg s.c. cyanamide for 4 days. Infusions of 5-1000 ng NSD-1015 (3-hydroxybenzylhydrazine) inhibited ETOH consumption, but there was no dose-response effect as seen with benserazide. Analyses of striata from NSD-1015 treated rats did not show accumulation of l-DOPA, which indicates that inhibition of l-AAAD may not be occuring at these low doses of drugs. The exact mechanism for these effects are unclear. (Supported by USPHS grants DA-04895 and AA-04200)

28.6

OPIOIDS' ENHANCEMENT OF INTAKE OF ETHANOL IS RELATED TO THEIR INDUCEMENT OF PLACE PREFERENCES (REWARD) BUT NOT OF THEIR NOCICEPTION (ANALGESIA). L.D. Reid, K.D Wild*, E.J.Bilsky* and C.L. Hubbell*. Dept. of Psychol., Rensselaer Polytechnic Institute, Troy, NY 12180.

Small doses of morphine, methadone, and fentanyl enhance rats' intake of alcoholic beverages. They also produce analgesia and can be used to establish a preference for the place of the drug-experience. Data will be summarized here, however, showing that some opioids that are agonists with respect to place preference and intake of ethanol are antagonists with respect to analgesia. One of these is WIN 44,441-3 (WIN), an agent that blocks ethylketocyclazocine's analgesia as well as that of other opioids.

Rats were given daily opportunities to take a sweetened alcoholic beverage and, across days, increased intake until they took considerable amounts a day. Then, prior to some sessions, rats were given one of 5 doses of WIN or placebo. Doses of 0.3, 1.0 and 10.0 mg/kg reliably incremented intake, i.e., WIN acted like morphine and not like naloxone. The most effective dose of WIN, 1.0 mg/kg, was also given to other rats in a standard conditioned place preference procedure. WIN produced a place-preference similar to morphine's, but unlike that of naloxone's.

The ability of opioids to enhance intake of ethanol is not due solely to their ability to be analgesic since certain opioids not having that ability increase intakes. When rats are tolerant to morphine's analgesia, morphine increments intake of ethanol. Taken together, the data support the idea that excessive alcohol-intake could be related to opioidergic processes of reward.

28.7

OPIOID EFFECTS ON ALCOHOL CONSUMPTION BY RATS ARE CENTRALLY MEDIATED. M.A. Linseman and S. Harding*. Addiction Research Foundation, Toronto, Canada, M5S 2S1.

Systemic administration of low doses of opioid agonists have been shown to increase subsequent alcohol consumption by rats, and of opioid antagonists to decrease it, but the mechanism(s) and locus(i) of these effects are still unknown. In an initial study, the effects of morphine and naltrexone were compared to those of analogous drugs (loperamide and methylnaltrexone, respectively) that do not cross the blood brain barrier. Drugs were administered i.p. to free-feeding rats 30 min. prior to their one-hour daily drinking sessions. Morphine (1, 3 and 10 mg/kg) increased alcohol consumption but loperamide (0.3, 1 and 3 mg/kg) did not; naltrexone (1, 3 and 10 mg/kg) decreased alcohol consumption but methylnaltrexone (3, 10 and 30 mg/kg) did not; and naltrexone (3 mg/kg) blocked the increased alcohol consumption seen following morphine (3 mg/kg) administration but methylnaltrexone (10 mg/kg) did not. In addition, chronic methylnaltrexone treatment also increased alcohol consumption dose-dependently perhaps through an upregulation of the endogenous opioid system. In a second experiment, intracerebroventricular administration of 10 micrograms of morphine 30 min. prior to access to alcohol, also increased alcohol consumption in a manner similar to that seen following systemic morphine. We conclude that opioid effects on alcohol consumption are mediated within the central nervous system.

28.8

SUBFORNICAL ORGAN LESIONS BLOCK THE ANGIOTENSION II-INDUCED SUPPRESSION OF ALCOHOL INTAKE. L.A. Grupp, E. Perlanski* and R.B. Stewart*. Dept. of Pharmacology, Univ. of Toronto, Toronto, Ont., Canada M5S 1A8.

The subfornical organ (SFO) is known to mediate a number of effects of blood borne angiotensin II (ANG II) including the increase in both water intake and blood pressure as well as the release of vasopressin. Recently, systemically administered ANG II has also been shown to reduce voluntary alcohol intake in rats. In order to assess the role of the SFO in the regulation of alcohol intake, two groups of rats were anesthetized with pentobarbital, lesioned or sham lesioned in the area of the SFO and following a one week recovery period offered a choice between alcohol and water on a daily basis for 6 weeks. While the SFO lesion and sham groups did not differ in the amount of alcohol per se that each consumed, SFO lesions did significantly attenuate the ability of ANG II to suppress alcohol intake. This attenuation was not due to a lesion-induced alteration in the pharmacokinetics of alcohol and was seen only in those lesioned animals which also displayed a verified functional deficit, i.e., a failure of ANG II to increase water intake. These findings suggest that the SFO plays a role in the suppressive effect of systemically administered ANG II on alcohol intake but does not otherwise influence alcohol consumption. Supported by the Addiction Research Foundation of Ontario.

28.9

STRAIN DIFFERENCES IN VOLUNTARY ETHANOL INTAKE USING THE "SAMSON"-SUCROSE FADING PROCEDURE. D.V. Gauvin*, K.M. Moore*, and F.A. Holloway. (SPON: J. Wood) Department of Psychiatry and Behavioral Sciences, University of Oklahoma Health Sciences Ctr., Oklahoma City, OK 73190-3000.

Two groups of rats were exposed to half-hour sessions of home-cage voluntary ethanol access using a modified Samson-sucrose fading procedure. Long-Evans (LE) and Wistar (WI) rats (n=9 per group) were provided a range of ethanol concentrations (5 to 40% w/v). Tail blood ethanol levels (BAL) were determined by gas chromatography at each concentration after stable volumetric intakes were achieved. With respect to the 10, 30, and 40% concentrations, the group average ethanol intake was a direct relationship to concentration; however, peak BALs demonstrated an inverse relationship to concentration. 20% w/v ethanol resulted in the greatest volumetric intake and peak BALs in both groups. A 20% w/v ethanol maintenance concentration produced consistently greater intake in the WI group compared to LE (WI = 2.67 g/kg; LE = .96 g/kg). Water substitution tests resulted in extremely low intakes in both groups (WI = .77 $\pm$ .13 ml; LE = .33 $\pm$ .11 ml). Intraperitoneal ethanol preloading produced a biphasic voluntary intake with low doses increasing intake. Both naltrexone and naloxone produced dose-dependent decreases in voluntary intake in both groups. However, haloperidol pretreatments increased ethanol intake. These data suggest a reciprocal modulation of a simple behavioral operant for ethanol self-administration between opiate suppression and dopaminergic facilitation of voluntary intake.

Supported by National Institute on Alcohol Abuse and Alcoholism grants 5 RO1 AA06351 and 5 T32 AA07222.

28.10

ALPHA-TOCOPHEROL MAY ALTER RATE BUT NOT MAGNITUDE OF ETHANOL TOLERANCE. J.R. Criado, D.V. Gauvin*, D. Brackett*, M.F. Wilson*, and F.A. Holloway. University of Oklahoma Health Sciences Center and Veterans Administration Medical Center, Oklahoma City, OK 73190-3000.

Tolerance development to ethanol's motor-disrupting effects was assessed in Sprague-Dawley rats using a computerized activity chamber before and after chronic treatment. After baseline activity measures were assessed, an initial ethanol dose-effect function was generated. Two groups of rats (n=8 per group) received ethanol (2 g/kg, i.g., q 6 hours) for 18 days. One group received dl-alpha-tocopherol chronically from five days prior to the first ethanol dose; the other group received vegetable oil. After one week of chronic ethanol exposure, half of each group was tested after a 3 g/kg ethanol dose. All animals were tested on Days 19 and 20 of ethanol exposure with 2 and 3 g/kg. Equivalent tolerance developed in both groups to both ambulatory and non-ambulatory activity. Post-chronic blood alcohol levels did not differ between groups. However, the tocopherol-treated group exhibited greater activity measures after one week of chronic exposure, and displayed parallel shifts in the ambulatory activity dose-effect curve. The vegetable oil control group activity measures increased at only the 3 g/kg dose after chronic exposure. These data suggest that the rate of tolerance development to ethanol's disruptive effects was altered by tocopherol treatment, but after equivalent chronic exposure, the magnitude of tolerance was similar in both groups.

Supported by National Institute on Alcohol Abuse and Alcoholism Grants 5 RO1 AA06351 and 5 T32 AA07222.

28.11

THE PHARMACOLOGY OF ALCOHOL SELF-ADMINISTRATION IN WISTAR AND ALCOHOL PREFERRING RATS. G.F.Koob, M.Mitchiner*, F.E. Bloom, and F. Weiss (SPON:T. Melnechuk). Research Institute of Scripps Clinic. La Jolla, CA. 92037.

The role of opioid, dopaminergic and serotonergic systems in ethanol reward was investigated in Alcohol-Preferring (P) and genetically heterogenous Wistar rats. The animals were trained to respond for ethanol (10% v/v) vs. water under a two-lever, free- choice contingency in the absence of food or water deprivation. Rats of both strains developed stable response rates and consumed ethanol at quantities sufficient to produce pharmacologically relevant blood alcohol concentrations ranging from 25-230 mg% (P-Rats) and 27-75% (unselected Wistars). Free-choice responding for water and ethanol was differentially altered by systemic injections of naloxone, bromocriptine and methysergide. Naloxone (0.125, 0.25 and 0.5 mg/kg) pretreatment resulted in a dose-dependent suppression of both ethanol and water intake, but did not alter ethanol preference when expressed as the ratio of ethanol to total fluid intake. In contrast, bromocriptine (1.0, 2.0 and 4.0 mg/kg) produced a significant, dose-dependent shift in preference from ethanol toward water by inhibiting responding for ethanol while enhancing water consumption. No changes in ethanol preference and water or ethanol intake were observed with methysergide (2.5, 5.0, 10.0 mg/kg) in either strain of rats. While all drug treatments produced similar behavioral changes in P- and Wistar rats, these effects were smaller and not consistently dose-dependent in the genetically unselected line of rats. The results suggest a specific involvement of dopaminergic mechanisms in the reinforcing properties of ethanol, but question the behavioral specificity of opioid systems in these ethanol actions. (Supported by NIAAA grant AA06420 and SANDOZ LTD.)

28.12

ETHANOL-INDUCED CONDITIONED PLACE PREFERENCE IN MICE SELECTIVELY BRED FOR INSENSITIVITY TO ETHANOL HYPOTHERMIA. C.L. Cunningham and D.R. Niehus*. Dept. of Medical Psychology, Oregon Health Sci. Univ., Portland, OR 97201.

Recent studies of EtOH-induced conditioned taste aversion and oral self-administration of EtOH in rats have suggested that EtOH's hedonic properties may be related to its thermal consequences. The present study provided a further test of this relation using a genetic animal model in the conditioned place preference paradigm. Male mice selectively bred for their sensitivity (COLD line) or insensitivity (HOT line) to EtOH hypothermia were used (Crabbe, J.C., et al. Alcohol Drug Res., 7:163, 1987). In a counterbalanced, differential conditioning procedure, mice from both lines received four pairings of a distinctive floor stimulus with injection of EtOH (2.25 g/kg). A different floor stimulus was paired with saline. EtOH increased general activity in both lines, but to a significantly greater extent in HOT mice. During the final choice test, HOT mice showed a significant difference in preference for the EtOH-paired vs saline-paired floor, whereas COLD mice showed no difference. This outcome supports the notion that EtOH's hedonic properties may be influenced by its thermal effect. Further, it suggests that the genes governing EtOH's effect on the thermoregulatory system may overlap with those determining EtOH's reward value.

28.13

ALCOHOL PREFERRING AND NON-PREFERRING RATS SHOW DIFFERENTIAL SENSITIVITY TO ETHANOL ADMINISTRATION IN A CONFLICT TEST. [1]H.A.Baldwin* [1]G.F.Koob [2]M.A.Schuckit* [3]L.Lumeng* and [3]T.-K.Li*. [1]Research Institute of Scripps Clinic, La Jolla, CA 92037. [2]V.A. Medical Center, La Jolla, CA 92161. [3]Indiana University School of Medicine, Indianapolis, IN 46223 (SPON: E.L.F.Battenberg).

The effect of acute ethanol administration in a modified Geller Seifter conflict test was investigated in Alcohol Preferring (P), Alcohol Non-Preferring (NP) and genetically heterogeneous Wistar rats. Rats were trained to lever-press for food reward where an unpunished RI-30s schedule rotated with an FR-1 incremental shock conflict schedule. Once a stable baseline rate of responding was achieved each rat received 5 doses of ethanol (0,0.25,0.5,0.75 & 1 g/kg i.p.), in a latin square design, with at least 3 drug-free days between each dose. Rats were tested in the conflict test 15 min after each dose of ethanol. ANOVA showed that ethanol significantly ($p<0.05$) and dose dependently increased the rate of punished responding and decreased the rate of unpunished responding in the control Wistar rats. NP rats displayed increased sensitivity to the effects of ethanol on punished and unpunished responding compared with controls. High doses of ethanol reduced punished responding in the NP rats presumably because of increased sedation. P rats showed a reduced sensitivity to ethanol in the punished component whilst ethanol did not significantly reduce unpunished responding compared with baseline scores. Thus, NP rats were more sensitive, and P rats were less sensitive, to the effects of ethanol in this test when compared with control Wistar rats. This work was supported by a grant from NIAAA (AA 06420).

28.14

EFFECTS OF STRESS ON ETHANOL INTAKE IN RATS THAT DIFFER GENETICALLY IN ETHANOL PREFERENCE. J.C. Froehlich,* M. Zweifel,* L. Lumeng* and T.-K. Li* SPON: (J. Nurnberger, Jr.) Department of Medicine, Indiana University School of Medicine and VAMC, Indianapolis, Indiana 46223

It has been postulated that stress increases ethanol intake and contributes to chronic ethanol drinking. The present study examined the effect of an intermittent schedule of unpredictable/uncontrollable restraint stress on ethanol intake in rats genetically selected for high (P line) or low (NP line) ethanol drinking. Fifteen male rats from each line were given nine weeks of free-choice between water and a 10% (v/v) ethanol solution with food freely available. Half of the rats from each line were stressed on days 16-25 and the remainder received no stress or handling. Stress consisted of immobilization in a nylon restraint sleeve for 30-120 min per day. Ethanol and water intakes were monitored on days prior to, during, and following stress. Rats of the P line consumed 92-106 mls of 10% ethanol/kg BW per day prior to stress. In contrast, rats of the NP line consumed less than 10 mls of 10% ethanol/kg BW per day prior to stress. In the P line immobilization stress significantly reduced ethanol intake during the first 5 days of stress application and significantly increased ethanol intake only on days 1-5 and 11-20 following stress termination. In contrast, no change in ethanol intake was seen in the NP line during stress application, but ethanol intake was significantly increased on days 16-60 following stress termination. Post-stress increases in ethanol intake were quite small in the P line (12.2%) compared with the NP line (52.1%). The data indicate that stress elevates ethanol intake only when chronic intake is low prior to stress application. (AA03243; AA07611 and AA08312)

28.15

DIFFERENCES IN PERFORMANCE ON ANXIETY TESTS IN RATS GENETICALLY SELECTED FOR ETHANOL PREFERENCE R.B. Stewart*, J.M. Murphy, L. Lumeng* and T.-K. Li* (SPON: S. Morzorati). Indiana Univ. Sch. Med. & VAMC; Purdue Univ. Sch. Science, Indianapolis, IN 46223

Anxiety-reduction is often cited as a motivation for alcohol use and abuse. The relationship between anxiety and ethanol intake was examined in rats selectively bred for high (P line) and low (NP line) ethanol preference. Two methods were used to measure anxiety in each line: 1) time spent exploring the open arms of an elevated plus-maze and 2) suppression of operant responding in an approach-avoidance conflict procedure. P rats spent significantly less time in the open arms of the plus-maze than NP rats during a 5-min test (P:27.9 $\pm$ 8.8 sec. NP:75.2 $\pm$ 9.6 sec.; $p<.01$), suggesting that P rats exhibit greater anxiety than NP rats. Separate groups of P and NP rats were trained to bar-press for food on a VI 60" reinforcement schedule during 60-min daily sessions. For two 3-min periods within each session, bar-presses were reinforced with food and punished with electric footshock at intensities of 0.125, 0.2 or 0.35 mA. The P rats showed greater response-suppression than the NP rats at each shock intensity ($p<.05$), indicating that the P rats are more sensitive to shock and/or more emotionally reactive. These rat lines represent a useful model for further examination of the relationship between anxiety and ethanol intake. (Supported by AA-03243 & AA-07611)

28.16

BLOOD-BRAIN BARRIER DYSFUNCTION MAY PERSIST FOLLOWING PROLONGED ABSTINENCE IN A RAT MODEL FOR CHRONIC ETHANOL CONSUMPTION EFFECTS. M. A. King, B. E. Hunter, and D. W. Walker, VA Medical Ctr. and Department of Neuroscience, University of Florida, Gainesville, FL 32610.

Adult male Long-Evans rats were treated for 210 days (30 weeks) with a nutrition-yoked liquid diet administration paradigm containing either ethanol or isocalorically substituted sucrose as previously described. Following the liquid diet treatment rats were maintained on lab pellet/water diets for approximately 300 days. Under pentobarbital anesthesia, horseradish peroxidase (HRP) was injected into the femoral vein and allowed to circulate for 20 min prior to cardiac perfusion with buffered mixed aldehyde fixatives. Vibratome sections were reacted with the Hanker-Yates method. The number and area of HRP leaks were quantified in coded tissue. Preliminary results indicate that alcohol-treated rats had 28% more HRP leaks than sucrose control rats, which accounted for a 37% increase in the total leak area in the alcohol group. The average number and area per tissue section were also increased in the alcohol group, suggesting that the increase in total number was not due to brain volume differences. These data suggest that long-term ethanol consumption may result in a dysfunction of the blood-brain barrier that persists long after ethanol intake ceases.

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28.17

BASAL GANGLIA AND THALAMOCORTICAL CIRCUIT INVOLVEMENT IN CRAVING AND LOSS OF CONTROL IN ALCOHOL DEPENDENCE. J. G. Modell.* J.M. Mountz (SPON: D. Kuhl). University of Michigan Departments of Psychiatry and Nuclear Medicine; Ann Arbor, MI 48109-0116

We recently proposed a model of neurophysiologic dysfunction in basal ganglia/limbic striatal and thalamocortical circuits as a pathogenetic mechanism of obsessive-compulsive disorder (OCD) (Modell JG, Mountz JM, et. al., J Neuropsychiatry, 1:27-36, 1989). OC symptoms were proposed to result from the development of a positive feedback loop in the reciprocally excitatory frontothalamic neuronal circuit which is inadequately integrated or inhibited by the limbic portions of the caudate nucleus (CN), n. accumbens (NA), and ventromedial pallidum (VP). This neuronal circuitry was collectively referred to as the fronto-striato-pallido-thalamo-frontal loop (FSPTFL). In OCD, the affected individual must struggle with the control of impulses or driven behaviors known to be maladaptive or potentially destructive, a struggle that bears resemblance to that which the alcoholic must endure between "craving" and "loss of control."

To determine whether FSPTFL function may play a role in the behavioral components of alcohol abuse and dependence, we reviewed the literature spanning the neurophysiologic mechanisms operative in alcohol intoxication and dependence; and the neuronal circuitry, neurotransmission, and pathologic processes of the FSPTFL. This review disclosed a wide body of evidence that acute intoxication suppresses the neuronal output of the NA and CN to the VP – probably by dopaminergic and serotonergic mechanisms – and that the effects of this inhibition within the FSPTFL may lead to loss of control and compulsive drinking behavior. The alcoholic patient may also have a defect in normal inhibitory mechanisms within the FSPTFL which may underlie the phenomenon of craving, and also make these individuals particularly vulnerable to the disruptive effects of alcohol on this circuitry. This model provides a novel and specific hypothesis for craving and loss of control in alcoholism which lends itself well to testing by neuropharmacologic and functional imaging techniques.

28.18

ORAL SELF-ADMINISTRATION OF ETHANOL VERSUS EXPERIMENTALLY ADMINISTERED ETHANOL FACILITATES REWARDING ELECTRICAL BRAIN STIMULATION. M. Moolten, G.T. Bain* and C. Kornetsky. Boston University School of Medicine, Boston, MA 02118.

We found ethanol will increase the sensitivity of animals to rewarding brain stimulation. Increased sensitivity was found only after oral self-administration but not when ethanol was given intraperitoneally (ip). Because the stress of ip or gavage administration of ethanol might preclude such an increase in sensitivity, we compared the effects of self-administered ethanol to those in yoked animals receiving ethanol noncontingently through a surgically implanted gastric cannula. Electrodes were stereotaxically implanted in the medial forebrain bundle of male F-344 rats and thresholds for brain-stimulation reward were determined using a rate free measure. Animals were water deprived, and then allowed to either drink or were infused with a sucrose or ethanol-sucrose solution prior to testing. After approximately 24 ethanol-sucrose experimental days over a 2 month period, significant threshold lowering effects were observed in the group of animals self-administering ethanol. Animals receiving ethanol noncontingently failed to show this threshold lowering effect. These findings suggest that learning and/or other non-pharmacologic factors play a role in the threshold lowering effects of ethanol on brain-stimulation reward.

(Supported in part by NIAAA grant AA05950 and Research Scientist Award DA00099 to CK).

28.19

REBOUND PENTYLENETETRAZOLE-SUBSTITUTION BY RATS AFTER A SINGLE LARGE-DOSE OF MIDAZOLAM. D.A. Mathis* and M.W. Emmett-Oglesby. Department of Pharmacology, Texas College of Osteopathic Medicine, Fort Worth, Texas, 76107-2690.

For rats trained to discriminate between the anxiogenic compound, pentylenetetrazole (PTZ), and saline, withdrawal from chronic diazepam treatment substitutes for the PTZ stimulus (Emmett-Oglesby *et al., J. Pharmacol. Exp. Ther.*, 244:892, 1988). In addition, for rats trained to discriminate between chlordiazepoxide (CDP) and PTZ, then injected with a large dose of CDP, detection of a PTZ-like stimulus occurs 12 and 18 h after CDP administration (Michaelis *et al., Psychopharmacology* 96:15, 1988). In the present study, the time-course of PTZ substitution and return to baseline after a single administration of midazolam (MDZ; 4 mg/kg) was assessed. Rats were trained to discriminate between MDZ (0.5 mg/kg), PTZ (16 mg/kg) and saline on a three-lever operant task under an FR10 schedule of food reinforcement. Substitution was tested at 1, 3, 6, 12, 18 and 24 h post MDZ injection. At 1 h post injection, rats primarily selected the MDZ lever; by 12 h post, rats primarily selected the PTZ lever. Saline-lever selection was obtained 24 h post MDZ administration. Changes in lever selection were orderly across time. Thus, after a single large-dose administration of MDZ, rats initially detected the MDZ stimulus, then they detected the PTZ cue. These data support the hypothesis that rebound withdrawal after MDZ administration produces a stimulus that is similar to the stimulus produced by the anxiogenic drug PTZ, and that a discrimination between a benzodiazepine, PTZ and saline is useful for assessing onset and recovery from benzodiazepine withdrawal.

This work was supported by grant DA 3521.

29.1

DIFFERENTIAL EFFECTS OF DENERVATION ON ACETYLCHOLINESTERASE ACTIVITY IN FROG PARASYMPATHETIC AND SYMPATHETIC GANGLIA.

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Innervation is known to regulate a number of molecules important in synaptic transmission. The influence of the presynaptic nerve on the enzyme acetylcholinesterase (AChE) was determined by comparing AChE activity in normal and denervated autonomic ganglia in the frog *Rana pipiens*. The effects of denervation upon AChE activity were distinct in the parasympathetic cardiac ganglion and the sympathetic lumbar ganglia. Upon denervation, specific activity was found to *increase* significantly in the cardiac ganglion while it *decreased* significantly in the lumbar ganglia. The contribution of cytoplasmic activity to total activity was determined by incubating the ganglia in echothiophate, a poorly lipid-soluble AChE inhibitor. In the cardiac ganglion, denervation resulted in a proportional increase in both cytoplasmic and extracellular AChE activity; in the lumbar ganglia, the majority of AChE activity lost was attributed to extracellular AChE. Therefore, presynaptic innervation appears to differentially regulate AChE activity in parasympathetic and sympathetic ganglia within a single species. These results suggest that denervation produces selective effects on AChE in cholinergic and adrenergic neurons. (Supported by NIH NS24207).

29.2

ELECTRIC STIMULATION OF THE LUMBAR COLONIC NERVE RELEASES ACETYLCHOLINE IN THE GUINEA PIG INFERIOR MESENTERIC GANGLION.

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Cholinergic mechanosensory neurons in the colon project to sympathetic ganglia. The aim of this study was to determine in vitro if acetylcholine (ACh) is released in the guinea pig inferior mesenteric ganglion (IMG) during lumbar colonic nerve (LCN) stimulation. Isolated ganglia with attached LCNs were loaded with ^{3}H-choline (750 nM, 80 Ci/mmole) followed by washing with 50 μM eserine and 10 μM hemicholinium-3. The amount of ^{3}H-ACh and ^{3}H-choline released during 5 minute periods was determined by radio-thin layer chromatography. Stimulation of the LCN increased the fractional release of ^{3}H-ACh from 0.08 ± 0.03 to 0.75 ± 0.15% ($p < 0.025$). Stimulation of the LCN during the loading period increased the amount of ^{3}H-ACh present in the IMG by 35% ($p < 0.05$), and resulted in a similar amount of ^{3}H-ACh released during three successive LCN stimulation periods. When the LCN was not stimulated during the loading period, there was a significant ($p < 0.05$) decline in the amount of ^{3}H-ACh released. These results provide direct evidence for the hypothesis that the LCN contains cholinergic afferent nerves that project to the guinea pig inferior mesenteric ganglion. (DK 17632, DK 07198, CA 37343)

29.3

A MAJOR, MUSCARINIC COMPONENT IN TRANSMISSION IN THE SUPERIOR CERVICAL GANGLION OF THE RAT.

C. Zhang* and J.W. Commissiong. (SPON: G. Melvill Jones). Dept. of Physiol., McGill Univ., 3655 Drummond St., Montreal, Quebec, Canada H3G 1Y6.

Male Sprague Dawley rats (300-325 g) were anesthetized with urethane (1.5 g/kg i.p.). The left, cervical, sympathetic trunk was cleared, and stimulated with a concentric, bipolar, stimulating electrode (SNEX-100, Korf, N.Y.), using a Grass S48 stimulator. The postganglionic, compound action potential (CAP) was recorded with hook, bipolar Ag/AgCl electrodes from the ventral, postganglionic, nerve trunk. Atropine (2.5 - 20 µmol/kg i.v.), caused a dose- and time-dependent suppression of transmission in the SCG. The CAP, measured at 10 sec after the injection of atropine, was decreased by 28%-97%. The second of the main four components of the CAP, was most resistant to the inhibitory effect of atropine, which varied from 5-10 min. The inhibitory effect of atropine was similar to that of hexamethonium (C6), at equimolar doses. Neither atropine, nor C6 affected conduction in fibres of passage in the SCG. The results suggest that a muscarinic mechanism may be equally as important as the traditional, nicotinic receptor-mediated mechanism, in the initiation of action potentials in the post-ganglionic neurons of the SCG.

Supported by the MRC of Canada.

29.4

EXCITATORY AMINO ACIDS STIMULATE ACETYLCHOLINE SECRETION BY LA-N-2 NEUROBLASTOMA CELLS.

U.I. Richardson* and R.J. Wurtman (SPON: B.E. Slack). Laboratory of Neuroendocrine Regulation, Massachusetts Inst. of Technology, Cambridge, MA 02139.

LA-N-2 are clonal human neuroblastoma cells which have retained cholinergic function during more than 10 years in continuous cell culture. Acetylcholine synthesis and secretion in these cells is regulated by the concentration of choline in the culture medium and can be stimulated up to 3-fold by depolarizing concentrations of extracellular K^+ and by veratridine (Richardson,U.I. et al., Brain Research, 476:323, 1989). We now report that neurotransmitter secretion in LA-N-2 cells is also stimulated by the excitatory amino acids L-glutamic acid (L-Glu) and N-methyl-D-aspartic acid (NMDA) but not by kainic acid. Half-maximal stimulation occurs at 3 mM L-Glu and 0.75 mM NMDA, respectively. The effect of NMDA is potentiated by glycine and by concurrent depolarization with elevated extracellular K^+. NMDA stimulation is inhibited by Mg^{+2} and by the NMDA antagonists 5-APV and MK801. Incubation of monolayer cultures with ^{3}H-L-Glu results in a time-, temperature- and Na^+-dependent association of ligand with the cells. A fraction (25%) of the cell-associated radioligand is displaceable by increasing concentrations of NMDA. This may represent specific membrane binding sites for NMDA which transmit the biological effects of the amino acid.

Supported by NIMH grant MH28783.

29.5

CLONING THE CHOLINE ACETYLTRANSFERASE STRUCTURAL GENE FROM THE NEMATODE C. elegans.

A. Alfonso-Pizarro*, K. Grundahl* and J. B. Rand. Dept. of Zoology, Univ. of Wisconsin, Madison, WI 53706; and Oklahoma Medical Research Foundation, Oklahoma City, OK 73104.

In C. elegans, cha-1 is the structural gene for choline acetyltransferase (ChAT). Severe cha-1 mutants have greatly reduced (>95%) ChAT activity; they are also uncoordinated, slow-growing, and resistant to inhibitors of acetylcholinesterase. Extremely severe mutations lead to complete developmental arrest. cha-1 is a complex gene which includes the previously identified unc-17 locus.

We have now cloned part of the cha-1 gene using the method of transposon tagging with the mobile element Tc1. We have taken advantage of the drug-resistance phenotype and examined more than 400 independent spontaneous resistance mutations isolated from strains with high rates of spontaneous Tc1 transposition. Ten mutations in the cha-1 complex were identified, and at least four of them were shown to be associated with Tc1 insertions. Using the DNA flanking one transposon insertion site, we have cloned a 4.9 kb genomic BamH1 fragment. This fragment represents part of the cha-1 complex because six independent cha-1 mutations and one unc-17 mutation are associated with alterations in this fragment, and three of these seven mutations have been shown genetically to map to the center of the cha-1 complex.

29.6

DROSOPHILA MELANOGASTER CHOLINE ACETYLTRANSFERASE USES GTG CODON AS AN INITIATION CODON.

V. Andrisani*, H. Sugihara* and P. Salvaterra. Division of Neurosciences, Beckman Research Institute of the City of Hope, Duarte, CA 91010.

We have previously reported that cRNA produced from Drosophila melanogaster choline acetyltransferase (ChAT) cDNA can be translated into active enzyme following injection into Xenopus oocytes or by rabbit reticulocyte lysates. We have also demonstrated the possibility of ChAT using a non AUG initiation codon by constructing frame shift deletion mutants. We have now constructed two different point mutations to more precisely identify the initiation codon of Drosophila ChAT. A GTG codon at position 33 of the clone is apparently used as the initiation codon. Conversion of this GTG to a GCG codon resulted in a drastic decrease of active enzyme translation. In contrast, when the same codon was changed to ATG (the normal initiation codon), increased efficiency of translation was observed. The ATG mutant produces a major protein with a size of 75 kD and is indistinguishable from the wild-type in vitro translation product or in vivo ChAT. In contrast, a smaller protein is produced by translating the GCG mutant RNA. Our results may indicate an important role for translation regulation of ChAT RNA.

29.7

EVIDENCE FOR PRECURSOR PROCESSING OF CHOLINE ACETYLTRANSFERASE IN *DROSOPHILA MELANOGASTER*.

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Choline acetyltransferase (ChAT) is generally acknowledged to have a molecular weight of *ca.* 67 kD in *Drosophila melanogaster* (Slemmon,J.R., *et. al., J.Biol.Chem.* 257:3847-52, 1982), as well as in mammals. However, a partial cDNA for *Drosophila* ChAT indicated an open reading frame that would produce a much larger protein, i.e. 50-100 extra amino acids on the amino terminus (Itoh, N., *et.al. PNAS* 83:4081-5, 1986).

We have synthesized a 26-amino acid peptide specific for this amino-terminal presequence, and produced polyclonal antibodies against the peptide, as well as other polyclonal antibodies against a fusion-free plasmid-expressed ChAT from the cDNA indicated above. Western blot analysis of *Drosophila* protein using these antibodies indicated specificity to a *ca.* 75 kD protein for the antisera against the presequence, whereas antisera against the entire ChAT sequence recognized the 67 kD,54kD, and75 kD proteins.

Immunocytochemistry of *Drosophila* brains with these antibodies localized staining specific for the larger form of the protein to neuronal cell bodies, whereas staining against the expressed ChAT molecule was localized to neuronal cell bodies and neuropil regions, which consists of axonal and synaptic processes.

Our data presents evidence that *Drosophila* ChAT is translated as a 75kD (or possibly larger) protein containing an amino terminal sequence that is subsequently cleaved to yield the smaller 67kD protein. We propose that this amino terminal sequence may be involved in the translocation of ChAT from the cell body, where it is synthesized, to presynaptic terminals, where it is utilized.

Supported by PHS grant NS24761 and by NSF grant BNS-8715047.

29.8

ISOLATION OF A GENOMIC CLONE OF HUMAN CHOLINE ACETYLTRANSFERASE.

L. B. Hersh, C. F. Kong*, S. Dyer*, W. Strauss, M. Lorenzi*, and D. Hilt. Dept. of Biochemistry, UT Southwestern Medical Center, Dallas, Tx 75235, Dept. of Pharmacology, Un. of Miami, Miami, Fl. 33101, and Dept. of Neurology, Un. Maryland School of Medicine, Baltimore, Md. 21201.

Oligonucleotides directed toward different regions of the choline acetyltransferase (ChAT) protein were synthesized on the basis of the porcine cDNA and human peptide sequences. These oligonucleotides were used to screen a Charon 28 human genomic library from which a putative human ChAT gene was isolated. On the basis of hybridization profiles it was estimated that this clone contains exons which account for approximately one third of the coding region. A BamH I fragment derived from this clone was sequenced and found to contain a 60 base sequence which showed 88% identity to porcine ChAT. Amongst the 12% non-identical nucleotides was a change from the AUG start codon of the porcine enzyme to an ACG in the human sequence. This indicates that the N-terminal sequence of the porcine and human enzymes differ.

29.9

ChAT mRNA EXPRESSION IN THE MAMMALIAN CNS.

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In order to study the regulation of ChAT gene expression in rat CNS, a nucleotide probe of 270 bp, derived from the porcine ChAT mRNA sequence (Berrard S. et al., PNAS, 84:9280, 1987) has been synthesized. Total RNA from cholinergic regions (septum, striatum and spinal cord) and also from other tissues (skeletal and cardiac muscles) or cell lines (glioma C6 or PC12 cells) was used as template to generate, by reverse transcriptase (RT) reaction, copies of ChAT and P1B15 mRNAs. The RT products were then amplified by means of a polymerase chain reaction, for up to 30 cycles. Results obtained show 1) occurrence of ChAT mRNA in discrete CNS areas also possessing ChAT enzymatic activity; 2) presence of ChAT mRNA, although in a very low level, in PC12 cells, but not in glioma C6 cells nor in cardiac or skeletal muscular tissues. In addition, 3) comparison of the relative amount of ChAT mRNA vs p1B15 mRNA in the various CNS areas indicate that ChAT gene transcript is expressed in extremely low abundance, with septum and spinal cord possessing relatively higher levels with respect to striatum. In view of the above results, we are currently evaluating, in different experimental conditions, ChAT gene expression in both developing and adult CNS, in particular forebrain cholinergic neurons.

29.10

TRANSCRIPTION OF NEURONAL NICOTINIC ACETYLCHOLINE RECEPTORS IN YEAST.

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Recently, expression of the four subunits of the nicotinic acetylcholine receptor (AchR) from Torpedo has been obtained in yeast (Jansen et al., J. Biol Chem., in press). We initiated the present work with the alpha 4 and beta 2 subunits which were first isolated in c-DNA libraries from PC-12 cells and rat brain and shown to be subunits for a neuronal AchR (Boulter et al., Proc. Natl. Acad. Sci. 84:7763, 1987). Because of the high GC content of the beta 2 5' untranslated leader sequence, and the preference for A rich leader sequences in yeast, we removed the native leader sequence by partial digestion with the endonuclease BssHII, followed by treatment with Mung Bean nuclease. Two constructs for alpha 4 DNA were used. One was the native rat form, and the second was one in which the native leader and signal sequence was removed and replaced with the Torpedo alpha 1 leader and signal sequence. Subsequently alpha 4 and beta 2 DNA were inserted into a yeast expression vector and used to transform yeast. m-RNA purified from transformed yeast was injected into frog oocytes, and functional AchRs were detected using the newly developed photolysible analog of carbamylcholine and laser flash photolysis (Milburn, et al. Biochem. 28:49, 1989). Functional AchRs were present which resulted in the depolarization of the oocyte membrane. We have established, therefore, that yeast cells are capable of synthesizing m-RNA which can direct the synthesis and assembly of functional rat neuronal AchRs in frog oocytes.

TRP was a Visiting Scientist in J. Patrick's laboratory, Salk Institute.

29.11

FURTHER CHARACTERIZATION OF A SUBSTANCE FROM TORPEDO ELECTROPLAQUE WHICH MODULATES NICOTINIC ACETYLCHOLINE RECEPTOR ACTIVITY.

B.A. Coleman, G.A.Weiland, and R.E. Oswald. Dept. of Pharmacology, Cornell Univ., Ithaca, NY 14853.

A substance isolated from *Torpedo* electroplaque which interacts with the nicotinic acetylcholine receptor (nAChR) has properties similar to tachykinins, but may be distinct from this class of peptides. Homogenization of *Torpedo* electroplaque in 6 M guanidine HCl and 0.1% trifluoroacetic acid followed by concentration on Waters' SepPak C-18 columns yielded a reconstituted fraction capable of inhibiting αbungarotoxin (αBgt) and carbamylcholine-stimulated PCP binding, increasing the rate of nAChR desensitization, and inhibiting agonist-stimulated ion flux through the ion channel. Under similar conditions, Substance P has shown the same effects. Consistent with the inhibitory activity being due to an acid-stable proteinaceous component, inhibition of αBgt binding is not affected by prolonged exposure of the extract to low pH but is reduced by prior incubation with proteinase K. As detected by TLC, a protein component of the extract is dialyzable through 6,000-8,000 MW dialysis tubing. Inhbition of PCP binding is also removed by dialysis. Further chromatography on a Beckman Ultrasphere ODS C-18 column using HPLC shows several peaks eluting between 30-40% acetonitrile. One of these peaks has been found to retain activity which inhibits αBgt binding, though at reduced levels. This peak can be detected at 214 nm but not at 280 nm, similar to many tachykinins which lack tyrosine residues. Incubation of the *Torpedo* extract with commercially prepared anti-Substance P antibodies removed the inhibitory activity. However, antibodies known to bind to a variety of tachykinins have not shown reactivity with the *Torpedo* extract. The reason for this discrepancy is unknown at present. Further purification and investigation of the exact nature of this substance is currently underway.

29.12

PROBING THE BINDING SITE FOR OPEN-CHANNEL BLOCKERS IN THE MUSCLE NICOTINIC ACETYLCHOLINE RECEPTOR.

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We are using site-directed mutagenesis and oocyte expression to probe the binding site for open-channel blockers at muscle nicotinic acetylcholine receptors. We employ QX-222, the model quaternary ammonium analog of lidocaine. Single channel and macroscopic relaxation recording give information about the residence time in the channel and the binding affinity of the blocker. Our previous studies on position 6' of the second transmembrane segment (M2) of each subunit showed that replacement of serine by alanine (α 248, δ 262) decreases, while the replacement of phenylalanine by serine (β 259) increases the residence time of the blocker. These observations suggest that the hydroxyl groups of the M2 helices line the pore of the channel, and that this site interacts with the positively charged end of the blocker. We now report that the same type of mutation at position 10' produces the opposite effect : replacing serine (α 252) or threonine (β 263) by alanine increases the residence time, while replacing alanine (γ 261) by serine decreases it. These data, taken together, suggest that binding of QX-222 depends on two differents types of interaction: the charged moiety of the molecule binds to the polar OH groups of position 6' whereas its nonpolar moiety binds to position 10', and would prefer a non polar environment. The distance (5.2 A) between the centers of the two moieties (common to most local anesthetics) matches well the distance (5.2 A) between positions 6' and 10' of the M2 segment. Supported by NS-11756.

29.13

SOLUTION STRUCTURE OF NEURONAL BUNGAROTOXIN DETERMINED BY TWO-DIMENSIONAL ^{1}H-NMR SPECTROSCOPY. R.E. Oswald[§†*], M. Bamberger[§†], M.J. Sutcliffe[†], R.H. Loring[α*], R.E. Zigmond[*α], C.M. Dobson[†]. [§]Dept. Pharmacology, NYSCVM, Cornell Univ., Ithaca, NY 14853 USA; [†]Inorganic Chemistry Laboratory, Univ. Oxford, Oxford OX1 3QR UK; [α]Dept. Pharmacology, Northeastern Univ. Boston MA 02115 USA; [*]Center for Neurosciences, Case Western Reserve, Cleveland OH 44106 USA.

Neuronal bungarotoxin (nBgt) is a minor protein component of the venom of *Bungarus multicinctus* which binds specifically and with high affinity to neuronal nicotinic acetylcholine receptors (nAChRs) in the peripheral nervous system. In contrast, the major neurotoxic component of α-bungarotoxin (αBgt) binds quasi-irreversibly to skeletal muscle nAChRs but with low affinity (or not at all) to peripheral nervous system nAChRs. These two toxins exhibit considerable sequence homology (~50%); but the sequence differs at several potentially important positions. We hope to determine the 3-D solution structure of nBgt and compare it with the known solution and crystal structures of αBgt and the structures of homologous neurotoxins specific for skeletal muscle nAChRs in an attempt to explain the unique specificity of nBgt. This should illuminate the diversity of nAChR subtypes. Assignment of ^{1}H NMR resonances for 60 of the 66 residues of nBgt have been obtained by sequence specific methods which enabled assignment of over 70% of the crosspeaks observed in the NOESY spectrum, defining distances between specific atoms in the protein. This provided ~550 distance constraints for use in structure determination. Coupling constants (NH-αH) and (αH-βH) were determined from COSY and E.COSY spectra which have in turn provided dihedral angle constraints. Hydrogen exchange work suggested the probable position of hydrogen bonds. The dihedral constraints from the COSY spectra and the known disulfide bonding pattern are being used to calculate the 3-D structure of the protein. Preliminary evidence suggests (1) that the C-terminal portion of the protein probably exhibits more structure than αBgt; and (2) that the triple stranded antiparallel β sheet in long and short neurotoxins may be considerably distorted in nBgt. Efforts are underway to refine the structure and to assign the remainder of the NOESY spectrum in an effort to provide additional distance constraints. Although additional resolution will be required, differences in the 3-D structures of nBgt and αBgt seem to exist and may be related to the differences in specificity of the toxins.

29.14

BINDING OF [^{3}H]MECAMYLAMINE TO THE NICOTINIC RECEPTOR COMPLEX IN THE RAT BRAIN: MODULATION BY ATP. E.D. London and M.D. Majewska, Neuropharmacology Lab., Addiction Res. Ctr., NIDA, Baltimore, MD 21224.

Mecamylamine (MEC), an allosteric antagonist of the ACh nicotinic receptor (nAChR), is believed to interact with the cationic channel associated with this receptor. We examined the interaction of MEC with the nAChRs in the rat brain. MEC, at μM concentrations, decreased the Bmax of [^{3}H]methylcarbamylcholine (MCC) (a ligand for nAChR) binding. [^{3}H]MEC interacted with at least two populations of sites: the higher affinity site (Kd = 48 nM, Bmax = 184 fmol/mg protein) and the low affinity site with Kd > 5 μM. Allosteric nAChR antagonists (tetracaine, PCP and its analogs, and chlorpromazine), inhibited [^{3}H]MEC (50 nM) binding, at low μM concentrations. Incubation with *l*-nicotine (Nic) for 30 min (equilibrium) did not alter [^{3}H]MEC binding, but brief exposure to Nic (1-2 min) markedly potentiated binding; the effect of Nic decayed with time. Our data suggest that the high affinity [^{3}H]MEC binding site may be localized in the cationic channel of the nAChRs, and brief activation of nAChRs may facilitate [^{3}H]MEC binding by opening the channels. ATP, which interacts with nAChRs (Takayama, H., et al., Soc. Neurosci. Abstr. 1989), also increased specific [^{3}H]MEC binding, suggesting that ATP modulates nAChRs in the brain, possibly by opening the ion channels.

29.15

ATP: AN ALLOSTERIC MODULATOR OF THE NICOTINIC RECEPTOR IN BRAIN. H. Takayama, M.D. Majewska and E.D. London. Neuropharmacology Lab., Addiction Res. Ctr., NIDA, Baltimore, MD, 21224.

ATP is co-released with ACh from Torpedo electric organs and the mammalian neuromuscular junction. We, therefore, assessed whether ATP interacts with nicotinic acetylcholine receptors (nAChRs) in the brain. ATP had a biphasic effect on [^{3}H]methylcarbamylcholine (MCC) binding to nicotinic receptors in the rat brain, increasing the binding affinity at concentrations < 1 mM and decreasing it at > 1 mM. To determine if actions of ATP at nAChRs corresponded to changes in the nAChR-gated cationic channel, we examined ATP effects on the binding of [^{3}H]chlorpromazine (CPZ), presumably to a site in the nAChR-associated channel (Changeux, J.-P., et al, TIPS 8:459, 1987). ATP, but not ACh or nicotine, enhanced [^{3}H]CPZ binding to brain membranes at equilibrium (30 min incubation). Inhibition of [^{3}H]CPZ binding to dopamine receptors by apomorphine did not abolish the effect of ATP, but even enhanced it, suggesting that the effect is not related to dopamine receptors. The interaction of ATP with MCC and CPZ binding sites suggest that ATP may be an agonistic type modulator of nAChR function. It is possible that ATP opens the cationic channels associated with nAChRs, thus facilitating [^{3}H]CPZ binding.

29.16

INTERACTIONS OF SUBSTANCE P WITH NICOTINIC ACETYLCHOLINE RECEPTOR-ENRICHED MEMBRANES OF *TORPEDO* ELECTROPLAQUE. C. Min*, J. Owens*, B. Howard*, and G.A. Weiland. Dept. of Pharmacology, NYSCVM, Cornell University, Ithaca, NY 14853.

The neuropeptide substance P (SP) noncompetitively inhibits activation of nicotinic receptors of both the neuronal and muscle subtypes. Inhibition does not appear to reflect an interaction with the high affinity local anesthetic binding site (*Molec. Pharmacol.* 32:625).

The effect of substance P on carbamylcholine (CC) binding was determined by measuring the inhibition of the initial rate of [^{125}I]αbungarotoxin (αBgt) binding. In the presence of 140 mM NaCl, SP did not inhibit αBgt binding at the concentrations used. SP increased the rate and extent of the CC-induced increase in apparent agonist affinity. In the absence of CC, SP did not induce an increase in CC affinity, indicating that the peptide does not stabilize the desensitized state in the absence of agonist. The kinetics of recovery after exposure to CC plus SP were complex and more rapid than after exposure to CC alone. Although it has been shown that SP induces an increase in apparent agonist affinity, the mechanism involves more than allosteric stabilization of the desensitized state.

The interaction of substance P with the nicotinic receptor was studied directly by measuring the binding of [^{3}H]SP to *Torpedo* membranes. In the absence of agonist [^{3}H]SP bound with low affinity (K_d > 40 μM); however, in the presence of CC the affinity was significantly increased (K_d ≈ 5 μM). CC had no effect on the number of [^{3}H]SP binding sites and there was one SP binding site per αBgt site. The CC-induced increase in [^{3}H]SP affinity was blocked by αBgt and d-tubocurarine but not by atropine. The EC_{50} of CC for increasing SP binding was ~1 mM, which implicates the involvement of the recently described low affinity agonist inhibitory site. Displacement of [^{3}H]SP binding by related peptides was consistent with their inhibition of receptor activation. Specific [^{3}H]SP binding dissociated exponentially with a $t_{1/2}$ of ~3 min. These results demonstrate [^{3}H]SP binding to a specific site on the nicotinic receptor which may mediate SP inhibition of receptor activation.

(Supported by the NSF and the Cornell Biotechnology Program)

29.17

CHARACTERIZATION OF MUSCARINIC RECEPTOR SUBTYPE-SPECIFIC ANTIBODIES. A.I. Levey*, W.F. Simonds, A.M. Spiegel, and M.R. Brann. NIH, Bethesda, MD, 20892 and JHMI, Baltimore, MD, 21205.

Five muscarinic receptor subtypes have been genetically defined (Bonner et al, Neuron, 1, 403), with complex pharmacological differences that do not reliably distinguish between them. In order to develop antibody probes that specifically recognize each of the receptor protein subtypes, we have prepared site-directed antibodies raised in rabbits against oligopeptide sequences specific for each receptor. Antisera were tested for their ability to bind the peptides and 3H-PrBC mustard-labelled receptors solubilized from either cell lines transfected with each of the receptors cDNAs (Buckley et al, Mol Pharmacol 35, 469) or brain or atrial membranes. An immunoprecipitation assay was developed to screen for antibody reactivity in which the amount of label was directly counted in extensively washed precipitates, and in which normal rabbit sera was used for controls. Antisera to the N- and C-terminal regions of the m1, m2, m3, and m4 receptor proteins each specifically bound the peptides derived from the appropriate receptor, but not the opposite terminal region of the same receptor or peptides of the other receptors. Antisera against the C-terminal peptides for the m2, m3, and m4 receptors each precipitated up to 40% of the radiolabeled receptor protein with no significant cross-reactivity to the other receptor subtypes. Preliminary immunoblotting experiments showed that affinity purified antisera to the m3 receptor labelled a band that co-migrated with the 3H-PrBC mustard-labelled receptor subunit identified by autoradiography.

29.18

[^{3}H]QUINUCLIDINYL BENZILATE (QNB) HAS DIFFERENT AFFINITIES FOR EACH OF TWO BINDING SITES IN HUMAN BRAIN:THE DENSITY OF THE LOWER AFFINITY SITE (M_1) DECREASES WITH AGE. A.C.Andorn, J.A.Vittorio* and E.S.Miller*. F.D.R. Vet. Adm. Hosp. and Depts. of Psychiat. and Pharmacol. N.Y. Med. Coll., Valhalla, NY 10548.

[^{3}H]QNB labels M_1 and M_2 receptor types but it had been thought that the affinity of [^{3}H]QNB was the same at each receptor. We examined [^{3}H]QNB binding in human brain. The results of saturation studies in prefrontal cortex (PFC) showed that [^{3}H]QNB labeled two affinity states with K_A of 1.4 ± 0.5 x $10^{11}M^{-1}$ and 1.70± 0.6 x $10^{10}M^{-1}$ for K_D of 7 and 59 pM (N=3). The densities of these affinity states were 8.6 ± 3.8 and 26 ± 3.4 pM respectively. In aged PFC the K_D were unchanged but the densities were 14 ± 1.1 and 15 ± 2.3 pM (N=3). The difference in the density of the highest affinity state was not significant, but the decline in the density of the lowest affinity state with aging was significant at $p < 0.01$.

Saturation studies in cerebellum showed only one binding site for [^{3}H]QNB and the K_D of that site was the same as the highest affinity state in PFC. Further, pirenzapine had nearly the same affinity for the cerebellar site (K_i of 80 nM) as it had for the highest affinity state in PFC (K_i of 21 nM). Therefore, the lowest affinity state in PFC most likely represents M_1 binding while the higher affinity state represents M_2.

30.1

HORMONAL REGULATION OF AXON TERMINAL REORGANIZATION DURING METAMORPHOSIS IN THE MOTH *MANDUCA SEXTA*. J.W.Truman. Dept. of Zoology, Univ. of Washington, Seattle, WA 98195.

Loss and regrowth of axon arbors during metamorphosis have been followed for the motoneuron, MN-12. Its larval muscle target (deo1) consists of 5 oblique fibers and begins to degenerate by d 2 after pupation. The lateral 4 fibers degenerate completely, but remains of the medial fiber persist to provide support for the growth of the adult muscle (de5). This remnant reorients on the segment by d 8, and regrows into the adult muscle starting about d 12-14. The adult emerges on d 20.

MN-12's distal axon undergoes a stereotyped series of changes: d 2, loss of larval endplates; d 3, retraction of axon from lateral fibers, maintains rostral and caudal attachments on medial remnant; d 4, localized sprouting at the two sites; d 8, sprouts grow longitudinally along remnant; d 10-12, establishment of higher order axon branches; d 14, establishment of nascent adult endplates.

Axon sprouting is triggered by steroids, the ecdysteroids, in the absence of juvenile hormone (JH). Depending on the time of application, JH blocks outgrowth itself, or just the longitudinal expansion while allowing the production of endplates.

30.2

SPONTANEOUS INNERVATION OF THE SUPERIOR OBLIQUE MUSCLE BY MOTONEURONS OF THE OCULOMOTOR (N III) NERVE AFTER TROCHLEAR (N IV) SURGERY. R. Sonntag* and B. Fritzsch. Univ. Bielefeld, Fac. Biol., 4800 Bielefeld, FRG.

In *Xenopus laevis*, the first trochlear motoneuron axons reach their sole target, the superior oblique muscle (SOM), on the contralateral side at stage 35. Proliferation of motoneurons than continues in a caudal to rostral manner until stage 47 (Sonntag, R. and Fritzsch, B., Progr. Zool. 37:in press, 1989) have tested the possibility that the earliest, most caudal motoneurons behave like pioneer neurons by cutting the trochlear nerve in the velum medullare anterior of embryonic (st.34-36) and larval (st.40-48) *Xenopus*. The pattern of reinnervation of the SOM was studied in 36 larvae, using anterograde and retrograde tracing techniques. Embryonic surgery reduces the total number of trochlear motoneurons, whereas ipsilateral motoneurons are increased in number. In contrast, cutting of the trochlear nerve after onset of ocular movement (st.42), leads to a reduced reinnervation of the SOM by trochlear motoneurons. In addition, the SOM is innervated by oculomotor motoneurons. After surgery in later stages (st.48) the trochlear nerve may not reinnervate the SOM. Instead the SOM is then innervated either by superior rectus or, more frequently, by inferior oblique motoneurons. In cases with successful reinnervation by the trochlear motoneurons, the most caudal, oldest motoneurons always reach the SOM. These data are compatible with the idea that the first trochlear motoneurons are pioneer neurons. That oculomotor motoneurons of adjacent ocular muscles can spontaneously innervate the denervated SOM argues also for a crucial role for pattern formation played by the early innervation. Supported by the DFG (SFB 223).

30.3

PATTERNS OF MUSCLE REINNERVATION BY REDUCED NUMBERS OF MOTONEURONES. V. Rafuse*, T. Gordon, S. Erdebil* and T. Martin*. (Spon: J. Jhamandas) Div. of Neuroscience, Univ. of Alberta, Edmonton, Canada T6G 2S2.

To test the possibility that regenerating motor axons follow the branch points of the distal nerve stump to supply muscle fibres in different fascicles, we studied the distribution of muscle fibres in identified motor units in the cat medial gastrocnemius muscle after reinnervation by restricted numbers of regenerating motoneurons. The MG nerve was crushed or cut and sutured either to the nerve stump or to the denervated muscle simultaneously with L7 or S1 root ligation. Four to 12 months later, muscle force was recorded bilaterally, 2-50 single motor units were characterized and a single unit depleted of glycogen for later histological recognition. When the number of motoneurones was close to normal, unit and non-unit fibres showed a normal mosaic distribution. However, as the numbers fell, the distribution became progressively more restricted. Fewer than 10%, muscle fibre units were localized in tight clumps, muscle force was dramatically reduced and extensive fibre degeneration was present. These findings suggest that 1) regenerating motor axons follow the branch points of the distal nerve stump to restore the mosaic pattern and that 2) terminal branching predominants only when the number of regenerating axons is drastically reduced. (Supported by MDAC and AHFMR).

30.4

REDISTRIBUTION OF MUSCLE UNITS IN THE REINNERVATED TIBIALIS ANTERIOR OF THE RAT. J. Totosy de Zepetnek*, S. Erdebil* and T. Gordon. Div. of Neurosci., U. of Alberta, Edmonton, Canada T6G 2S2.

To determine whether regenerating nerve fibres show preference for their former muscle compartments, the distribution of oxidative and glycolytic fibres was examined in reinnervated muscles, 4-10 months after common peroneal nerve section and resuture. Slow oxidative fibres are normally scattered within the deep compartment only, but were found in all regions of reinnervated muscles. Nevertheless, the incidence of slow fibres in the most lateral aspect and superficial regions was low with the fibres being distributed most commonly in small clumps in the deeper zones of the muscle. Identified glycogen depleted single unit fibres rarely occupied an entire clump and were distributed in as many as 3 different ones. These findings suggest that regenerating motor nerves may favor branch points in the distal nerve stump which lead them towards their former compartments. However, the marked clumping of fibres in single units indicates that the nerve branching at the level of the denervated muscle fibres is likely to be substantially different from normal. (Supported by MDAC and AHFMR)

30.5

NEUROMUSCULAR COMPARTMENTS: CENTRAL AND PERIPHERAL RESPONSE TO NERVE CRUSH. O.I. Weeks, C. Shum*, A.M. Colls*, and D. Bello*. Dept. of Biological Sciences. Florida International Univ. Miami, Florida 33199.

Our long range goal is to determine whether there is selectivity and/or specificity in the reestablishment of neuromuscular compartments following injury. The study model is the mouse (C576J) lateral gastrocnemius, a compartmentalized muscle and its motor nucleus.

The proximalmost end of the lateral gastrocnemius-soleus nerve was unilaterally crushed with #5 forceps. The contralateral, unoperated side, served as the control. Additionally, unoperated siblings of experimental animals also served as controls. The long term (5-13 months) responses as well as 21-30 day responses following nerve crush in 21 day old animals were analysed. In the present study tracing (HRP, Fluoro-gold), histo- (staining for SDH, myosin ATPase, glycogen), immunohistochemical (parvalbumin) methods were used to compare neuromuscular compartment boundaries, fiber type and motoneuron spatial, numerical and size distribution.

While there is not 100% specificity in reinnervation, neuromuscular compartments are selectively reestablished in that the overall integrity of compartments is maintained. NIH MBRS Suppt.

30.6

AGE-RELATED DENERVATION ATROPHY IN FIBER TYPES OF THE RAT NASOLABIALIS MUSCLE. K.K. White and D.W. Vaughan*. Anatomy Dept., Boston Univ. Sch. of Med., Boston, MA 02118.

Temporal patterns of denervation atrophy in red, white, and intermediate fibers of the nasolabialis muscle were compared in young adult (3-mo) and middle-aged (15-mo) male Sprague Dawley rats. Nasolabialis is responsible for the whisking movement of vibrissae. The facial nerve was crushed and at designated post crush days (dpc), frozen sections of the muscle were reacted for cytochrome oxidase activity to delineate the three fiber types. Cross-sectional fiber areas were traced and measured to determine the extent of atrophy.

The mean areas for all three fiber types are greater in the older animals than in the younger. Patterns of atrophy, therefore, are expressed both as absolute area difference and percent difference relative to control. In both age groups: 1) At 10, 16, 21, and 28 dpc the white and intermediate fibers atrophy to a greater extent than the red fibers. 2) By 28 dpc the red fibers have recovered the closest to normal fiber area.

The nasolabialis muscle is reinnervated by 14 dpc in the 3-mo and by 20 dpc in the 15-mo animals. In each age group, for each fiber type, a significant increase in fiber area occurs during the 7 days after the nasolabialis muscle is reinnervated.

Supported by grants #AG06154 and #AG00115 from NIH.

30.7

THE EFFECT OF ELECTRICAL STIMULATION ON NERVE ATROPHY AFTER AXOTOMY Gillespie, M.J. & Gordon T. Div. of Neuroscience, Heritage Medical Research Bldg., Univ. of Alberta, Edmonton, Can. T6G 2S2

To determine whether chronic electrical stimulation of axotomized nerves could retard or prevent atrophy, either the common peroneal (CP) or tibial nerve was axotomized in both legs in adult rabbits, and the nerve in one limb was stimulated at 10 or 100 Hz for 8 hours per day for up to 227 days. Cross-sections of stimulated and unstimulated nerves were compared.

An early reduction in the proportion of large fibres was greater in unstimulated than stimulated axotomized nerves. However, at longer times, an overall shift to smaller fibres was greater in the stimulated nerves than in the unstimulated nerves. By 227 days no large fibres remained in the stimulated nerves. Myelin thickness correlated directly to fibre diameter, (coefficient of 0.22 to 0.26), and was unaffected by stimulation.

Chronic electrical stimulation may initially protect axotomized nerves from atrophy, but is detrimental at longer periods. Fibres in the stimulated nerves became smaller than in the unstimulated nerves. (Supported by MRC of Canada).

30.8

SMALL CONDUCTANCE ACETYLCHOLINE RECEPTOR (ACHR) CHANNELS AT SNAKE (THAMNOPHIS SP.) TONIC MUSCLE FIBER ENDPLATES ARE IDENTICAL TO EXTRAJUNCTIONAL CHANNELS ON DENERVATED TWITCH FIBERS. R.L. Ruff, Neurology Dept. & Ctr. for Neurosci., Dept. Vet. Affairs Med. Ctr., & CWRU, Cleveland, OH 44106.

Tonic fiber endplates of the inferior costocutaneous muscle of the garter snake have 33pS and 51pS ACHR channels. The 51pS channel is electrically identical to the twitch fiber endplate ACHR. Segments of snake muscle were denervated by cutting serveral consecutive nerve roots. Twitch fibers were identified by their appearance with Nomarski optics. Endplate regions could be identified by degenerated nerve terminals. The extrajunctional membrane of denervated twitch fibers had ACHR channels that were similar in conductance and kinetic properties to the 33pS channel found at tonic endplates. Thus, adult tonic endplates appear to contain both twitch adult-type and fetal-type (extrajunctional) ACHR. Perhaps the development of tonic muscle fibers is altered in comparison to twitch fibers so that the adult tonic fibers support polyneuronal and multiterminal innervation and continue to express fetal-type ACHR.

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30.9

METABOLIC AND SIZE PROPERTIES OF SOLEUS MUSCLE FIBERS OF ADULT SPINALIZED CATS . B. Jiang *, R. R. Roy, D. J. Pierotti*, and V. R. Edgerton. (SPON: D.S. Maxwell) Brain Research Institute and Kinesiology Dept., UCLA, LA, CA, 90024.

This study was designed to determine the effects of reduced neuromuscular activity on the metabolic and size properties of single muscle fibers in the cat soleus. Adult cats were spinalized (Sp) at T12-T13 and maintained in a healthy condition for 6 months. Some of the cats were trained to weight-support (Sp-WS) for 30 min per day beginning one month post-transection. Cross-sectional area (CSA), succinate dehydrogenase (SDH), glycerophosphate dehydrogenase (GPD), and myosin ATPase activities were determined in a population of single fibers identified in serial frozen cross-sections. Each fiber was categorized as either light or dark based on its staining density for qualitative myosin ATPase, (pH=8.75). The Sp (44%) and Sp-WS (31%) groups had significantly higher percentages of dark fibers than control (<1%). Overall mean fiber CSA were significantly smaller (~25%) than control in both Sp groups. In the Sp-WS, but not the Sp cats, the dark fibers were larger than the light fibers ($p<0.05$). There were no significant differences among the three groups in any of the mean enzyme activities. However, there was a tendency for the Sp cats to have elevated GPD and ATPase activities and this appeared to be directly related to the percentage of fibers staining darkly for myosin ATPase. These data indicate that 6 months after spinalization some of the fibers of the slow soleus atrophy and develop myosin staining patterns and glycolytic capacities that are normally exhibited in fast muscles. Periods of daily weight-support appear to ameliorate some of these adaptations. Further, the observation that SDH activities are maintained at control values in spinalized adult cats, as well as in spinalized kittens (unpublished observations), suggest that, at least in the soleus, skeletal muscle fibers can maintain their oxidative potential even though there is a marked reduction in neuromuscular activity for 6 months. SUPPORTED BY NIH GRANT NS 16333.

30.10

SPATIAL DISTRIBUTION OF DARK ATPase FIBERS IN THE ADULT CAT SOLEUS MUSCLE SIX MONTHS AFTER SPINAL TRANSECTION. A. Garfinkel*, R.R. Roy, D.O. Walter*, G. Penaflor*, B. Jiang*, A. Fuchs* and V.R. Edgerton. (SPON: J.L. Feldman) Department of Kinesiology and Brain Research Institute, UCLA, Los Angeles CA, 90024

In the adult cat soleus muscle, nearly 100% of the fibers stain light for myosin ATPase (alkaline preincubation). Six months after spinal transection at a low thoracic level, a substantial proportion of these convert to dark. In this study, the spatial arrangements of these dark fibers were determined in 9 cats. We found that: (1) the dark fibers tended to be found disproportionately at fascicle boundaries, i.e., in 8 out of 9 animals, there was a statistically significant association (using a chi-squared analysis) between the properties of being adjacent to a fascicle boundary and being a dark fiber; (2) based on Monte Carlo simulations of random distributions, dark and light fibers are distributed spatially in a non-random manner with regard to each other, i.e., in 5 out of 8 animals there were significantly fewer dark/dark adjacencies and/or significantly more dark/light adjacencies than would be expected at random, and (3) there was also a significant tendency for dark fibers to be spread evenly across the region analyzed. These non-random spatial patterns imply that myogenic spatial factors influence the ATPase conversion of these fibers.

Supported by NIH Grant NS16333.

MOTOR SYSTEMS II

31.1

MECHANICAL PROPERTIES OF SELF-REINNERVATED CAT TIBIALIS ANTERIOR MOTOR UNITS. R.R. Roy, G.A. Unguez*, D.J. Pierotti*, S. Bodine-Fowler and V.R. Edgerton. Brain Research Institute and Kinesiology Department, UCLA, Los Angeles, CA 90024.

The reestablishment of neuromuscular connectivity was evaluated 6 months after cutting and reattaching the nerve branches innervating the anterior compartment of the cat tibialis anterior (TA) muscle. One motor unit (MU) in each limb of 7 adult female cats was functionally isolated via ventral root teasing, physiologically tested and glycogen depleted (Unguez et al. *Soc. Neurosci. Abstr.* ,15:, 1989). Based on the criteria established by Burke et al. (*J. Physiol.* ,234:723, 1973), 2 MUs were classified as slow (S), 1 as fast fatigue-intermediate (FI) and 12 as fast fatigue-resistant (FR). The absence of fast-fatigable (FF) units was somewhat surprising considering that the TA in normal cats has over 30% FF units (Dum and Kennedy, *J. Neurophysiol.*, 43:1615, 1980). Contraction and half-relaxation times, frequency-tension responses and fatigue indices were within the range of normal values for each unit type (*Ibid*; Bodine et al., *J. Neurophysiol.*,57:1730, 1987). The maximum tetanic tension was within the range of normal values (1.0-5.7g) in both S units tested (2.7 and 5.4g). In contrast, the maximum tetanic tensions of the FI (103g) and 7 of the FR (19.2-71.9g, mean = 29g) units were outside the range reported for normal FI (4.6-26.9g) and FR (1.5-18.3g) units, respectively. Further, all FR units had maximum tension values that were larger than the mean control value (6.7g). These data suggest that during the process of reestablishing neuromuscular connectivity, the target area (total fiber number and/or cross-sectional area) innervated by a motoneuron increases, while the variables regulating twitch speed and fatigability are unaffected. Further, it appears that the fast units in the TA were more capable of expanding their target area than the slow units after self-reinnervation. SUPPORTED BY NIH GRANT NS16333.

31.2

SPATIAL DISTRIBUTION OF MOTOR UNIT FIBERS IN THE SELF-REINNERVATED TIBIALIS ANTERIOR MUSCLE OF ADULT CATS. G.A. Unguez*, S. Bodine-Fowler, R.R. Roy, D.J. Pierotti*, and V.R. Edgerton. Dept. Kinesiology and BRI, UCLA, Los Angeles, CA. 90024

The spatial distribution of muscle fibers belonging to 6 motor units (MU) was studied in the cat tibialis anterior (TA) muscle 6 months after self-reinnervation (Roy et al., *Soc. Neurosci. Abstr.* 15: 1989). MU fibers were depleted of their glycogen through repetitive stimulation of an isolated ventral root filament. Subsequently, several cross-sections were cut from each tissue block along the length of each muscle and stained for glycogen using a periodic acid-Schiff reaction. For each unit, the location of each MU fiber was digitized in that cross-section which appeared to have the most depleted fibers. The spatial distribution patterns existing between MU fibers were analyzed by calculating the distribution of interfiber distances between all fibers within a unit. These distributions were compared to interfiber distance distributions generated using Monte Carlo simulation techniques. In 5 fast-twitch MUs there was a greater incidence of short and long interfiber distances suggesting the existence of multiple groups of fibers within the MU territory. Moreover, within the individual fiber groups there tended to be an excess of short distances suggesting that MU fibers were more clustered than dispersed. This clustering, however, did not appear to be due to a large increase in the number of adjacencies between MU fibers. For example, in 3 of the 5 fast units 52-63% of the fibers occurred alone which is similar to that observed in normal TA units. In the other 2 units, however, only 21 and 29% occurred alone. These units tended to have larger group sizes. However, entire fascicles were never observed to be depleted. In contrast, the spatial arrangement of fibers belonging to the slow unit was similar to the distributions observed in normal slow units. In general, the innervation patterns of the fast reinnervated units differed from those observed in normal fast TA units showing a greater tendency for clustering. SUPPORTED BY NIH GRANT NS 16333.

31.3

MOTOR UNITS OF THE CAT TIBIALIS ANTERIOR 6 MONTHS AFTER SPINAL ISOLATION. D.J. Pierotti*, R.R. Roy, J.A. Hodgson*, S. Bodine-Fowler, and V.R. Edgerton, Brain Research Institute and Kinesiology Dept., UCLA, LA, CA, 90024.

To study the effects of electrical inactivity on the motor units (MU) of the adult cat tibialis anterior (TA) muscle, the lumbar spinal cord was functionally isolated (SI) in 10 cats by transecting the cord at levels T12-T13 and L7-S1 and cutting all dorsal roots bilaterally between these two cord segments. Two 24-hour EMG recording sessions were used to verify that muscles in the lower limb were virtually silent. The cats were maintained in excellent health for 6 months. One MU from the TA of each hindlimb was functionally isolated using ventral root isolation techniques. Each unit was characterized physiologically as either fast fatigable (FF, n=2), fast fatigue resistant (FR, n=3) or slow (S, n=1) based on the classification scheme of Burke et al. (*J Physiol.* 234: 723, 1973), and repetitively stimulated to deplete the muscle unit of glycogen. Spatial distribution, as measured by interfiber distances of the MU fibers, within SI units was found to be similar to that reported for normal TA units. Mean muscle weights were 12% lower than control. Maximum tensions (Po) of the FR and FF units were lower than normal, while the S unit remained within the normal range. All other isometric contractile properties were within normal ranges for each of the unit types. The mean fiber cross sectional areas (CSA) of the fibers within the fast units were 25-50% smaller than normal, while the mean fiber CSA of the fibers within the S unit was normal. Thus, it appears that the decreased Po of the fast units was primarily related to the reduction in fiber size. Correction of Po based on the percentage of fiber atrophy suggests that the relationship between Po and innervation ratio is similar to that observed for normal TA units (Bodine et al., *J. Neurophysiol.*, 57: 1730 1987). These data demonstrate that the normal range of speed related and fatigue properties persists in a mixed muscle after 6 months of virtual silence. SUPPORTED BY NIH GRANT NS 16333

31.4

CHANGES IN SYNAPTIC INPUTS AND CELLULAR PROPERTIES OF AN IDENTIFIED INTERNEURON DURING INSECT METAMORPHOSIS. B.Waldrop and R.B.Levine, ARL Division of Neurobiology, University of Arizona, Tucson, AZ 85721

Metamorphosis is accompanied by striking changes in the organization of the nervous system in the hawkmoth *Manduca sexta*. For example, tactile stimulation of mechanosensory neurons innervating the abdominal body wall of the larva causes a general bending reflex. In the pupa, the same sensory neurons, motor neurons, and muscles mediate the gin trap reflex, during which the motor neurons discharge at a higher rate and in a different pattern than seen in the larva. We have been studying interneurons interposed between sensory and motor elements, attempting to determine how the different motor patterns are generated.

One intersegmental interneuron, designated IN 703, is retained during the larval-pupal transition and maintains the same basic dendritic structure, but its response to sensory inputs changes rather dramatically. In the larva, a train of electrical shocks to the sensory axons produces a brief depolarization and only 1 or 2 spikes in IN 703. In the pupa, IN 703 responds to the same stimulus with a prolonged depolarization and a strong burst of action potentials. We have found that the relative spike threshold is lower in pupal IN 703, which may account for some of the difference in responsiveness. Also, there is a class of synaptic input to IN 703, apparently from other interneurons, which is expressed in the pupa but not in the larva. Low frequency stimulation of sensory axons produces a short latency EPSP in IN 703 that is similar in larvae and pupae, but in the pupa there is also a delayed, asynchronous depolarization. The increase in the sensory response of IN 703 may partially explain the larger pupal motor response.

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31.5

EVIDENCE FOR DENDRITIC COMPETITION IN THE DEVELOPMENT OF MOTONEURON MORPHOLOGY IN THE RAT SPINAL CORD. E.M. Kurz, L.A. Goldstein, and D.R. Sengelaub. Program in Neural Science, Dept. of Psychology, Indiana University, Bloomington, IN 47405.

The spinal nucleus of the bulbocavernosus (SNB), located in the lumbar spinal cord of rats, contains motoneurons that innervate axial musculature involved in penile reflexes. The dendritic arbor of SNB motoneurons is bilaterally distributed, and overlaps extensively with the arbor of the contralateral nucleus, suggesting that the two halves of the SNB share common afferents. During development, SNB dendrites grow exuberantly to almost twice their adult length and then retract. To determine if the availability of afferents might play a role in regulating the retraction of SNB dendrites, we eliminated motoneurons from one side of the SNB early in development in an attempt to decrease potential dendritic competition.

On the day of birth, the right lateral bulbocavernosus muscle (BC), a major target of SNB motoneurons, was extracted from male rat pups (n=6). This removal results in the loss of BC-projecting motoneurons in the ipsilateral SNB. At seven weeks of age, when SNB dendritic morphology is normally mature, rats received injections of cholera toxin-HRP (.2%; 0.5 µl) into the remaining (left) BC. A group of intact males received similar injections (n=5). After processing with TMB, the length and spatial distribution of all HRP-labeled SNB dendrites were measured.

Following unilateral muscle extirpation, dendritic length of the remaining SNB motoneurons was 52% longer than that of normal males at this age (6493 µm *vs.* 4272 µm; $p < .025$). Despite this increased dendritic length, the spatial distribution of the arbor was unchanged from that of normal animals. These results suggest that an interaction between dendrites from the two halves of the SNB and their afferents may be involved in their retraction during normal development. (Supported by NIH NS 24877)

31.6

MUSCARINIC CHOLINERGIC RECEPTOR-BINDING SITES DURING MOTOR RECOVERY AFTER SPINAL CORD HEMISECTION IN RATS. A. P. Thomas*, J. W. Little, R. M. Harris and J. Leik*. Department of Biological Structure, University of Washington School of Medicine, Seattle, Washington 98195.

Hemisection of the thoracic spinal cord (T6) in the rat produces an initial paralysis of the hind limb ipsilateral to the lesion, followed by an early and then a late phase of motor recovery. The early recovery could be mediated by denervation supersensitivity (DS, increases in post-synaptic receptor-binding sites). To test this hypothesis, changes in muscarinic cholinergic receptor (MChR) -binding sites were examined in the ventral horn and intermediate gray matter of the lumbar spinal cord of T6 hemisected adult rats, using in vitro quantitative autoradiography with ^{3}H quinuclidinyl benzilate (QNB). Pre- and post-hemisection, the motor behaviors of the rats were evaluated, and the animals were sacrificed at 5 days postlesion. Cryostat sections (10 µm) were incubated in ^{3}H QNB with or without unlabeled atropine, and then exposed to ^{3}H-sensitive film (LKB) for 28 days. The autoradiographic images thus generated were quantified on a Drexel-DUMAS image-analysis system, with reference to co-exposed ^{3}H-brain mash standards. Specific binding for ^{3}H-QNB was determined in various areas of the cord. The lumbar cord ipsilateral to the lesion shows a significant increase in the densities of ligand-binding sites, compared to the contralateral side. The paralyzed hind limb shows partial motor recovery within 5 days postlesion. These findings may indicate the occurrence of up-regulation of receptor-binding sites resulting in DS in the lumbar cord after partial denervation by T6 hemisection. This DS may allow spared reflex or contralateral, descending pathways to mediate early motor recovery.

31.7

A MONOCLONAL ANTIBODY DISTINGUISHES SOMATIC MOTOR NEURONS FROM OTHER NEURONAL POPULATIONS IN THE RAT NERVOUS SYSTEM. H.Urakami* and A.Y.Chiu. Div. of Neurosciences, City of Hope, Duarte, CA 91010

In order to obtain markers selective for motor neurons, an *in vitro* immunization was carried out using a homogenate of embryonic rat ventral hemicords. We report a monoclonal antibody, MO-1, that is strongly immunoreactive for all somatic motor neuronal populations examined in the adult rat brain and spinal cord. MO-1 does not bind to other cholinergic neurons, such as those in the basal forebrain, nor to non-somatic motor neurons including preganglionic neurons within the spinal cord and post-ganglionic neurons in the ciliary ganglion and superior cervical ganglion. In fresh frozen cryostat sections of spinal cord, antibody binding appears to be concentrated within the cell body and proximal regions of axons. Preliminary studies show that immunoreactivity is detectable in the spinal cord by the end of the first week after birth. MO-1 may thus mark mature motor neurons in the final stages of synapse consolidation. (Supported by NIH grant #PO1 NS18858-08)

31.8

MORPHOLOGY OF PHRENIC MOTONEURONS IN NEONATAL RAT AS REVEALED BY RETROGRADE LABELING WITH HRP. A. D. LINDSAY* & J. L. FELDMAN (SPON: H. H. Ellenberger), Systems Neurobiology Laboratory, Dept. of Kinesiology, UCLA, L.A., CA, 90024-1568

Application of horseradish peroxidase (HRP) to cut muscle nerves in neonatal and fetal rats can result in Golgi-like staining of motoneuron cell bodies and dendrites in the spinal cord (Smith & Hollyday, J. Comp. Neurol. 220:16-28, 1983). We used this technique to reveal the morphology of phrenic motoneurons in rats ranging in age from 0 to 6 days (6.4 to 15.0 gms). The animals were anesthetized with chloral hydrate (35mg/100g, I.P.), and artificially ventilated. The phrenic nerve was isolated and cut as it traverses the brachial plexus. HRP crystals were placed on the cut nerve, allowed to dissolve for 15 min and then the wound was closed. The animal remained anesthetized for the duration of the incubation period (6-11 hours), at the end of which the animal was perfused with 4% paraformaldehyde. The tissue was cut into 30µm sections and processed with diaminobenzidine. Darkly labeled cell bodies and dendrites were seen in clusters in the ventral most portion of the ventral horn at intermediate laterality in segments C3 to C5. In animals as young as neonatal day 0, intensely labeled, tightly bundled and complexly branching dendrites were present, extending longitudinally as far as 300µm and projecting as far as the medial and lateral borders of the ipsilateral hemi-cord. Dendrites also projected dorsolaterally as far as the level of the central canal and up to 270µm across the midline into the contralateral ventral horn. Many dendrites were clearly studded with spines and appendages. In short, phrenic motoneurons in the neonatal rat have many of the characteristics of those studied in the adult rat (Goshgarian & Rafols, J. Comp. Neurol. 201:441-56, 1981). This is in marked contrast to the morphology of lumbar motoneurons, which do not attain the adult dendritic morphology until neonatal day 10-15 (Bellinger and Anderson, Dev. Brain Res. 35:55-67, 1987). This work was supported by NIH Grants NS24742 and NS08530.

31.9

ULTRASTRUCTURAL IDENTIFICATION OF TRANSIENT CEREBROCEREBELLAR TERMINALS IN NEONATAL CATS. D.L. Tolbert & D.S. ZAHM. Dept. of Anat. & Neurobiol., St. Louis Univ. Sch. of Med., St. Louis, MO 63104.

There are transient projections from the cerebral cortex to the cerebellar cortex and deep nuclei in neonatal cats. EM autoradiographic demonstration of orthogradely transported [^{3}H]-amino acids was carried out to determine if these transient axons form synaptic contacts with neurons in the cerebellar nuclei. Analysis of EM autoradiographs revealed numerous labeled cerebrocerebellar axons in the cerebellar white matter. These axons were frequently myelinated. Some labeled axonal profiles were vacuolated and appeared to be degenerating. Others contained large vesicles and had filiopodia and these were identified as growth cones. Labeled axon terminals containing predominantely clear, round vesicles were also observed in autoradiographs of sections through the cerebellar nuclei. Some labeled terminals contacted dendrites at asymmetric synaptic specializations. Other labeled terminals, which were not apparently associated with membrane specializations, were also present. These data represent morphological evidence that transient cerebrocerebellar axons form synaptic contact with cerebellar neurons. Preliminary electrophysiological data suggest that these synapses are functional. Supported by NIH grants NS-20227 and NS-23805.

31.10

THE ROLE OF CEREBELLAR CORTICAL POSTSYNAPTIC ACTIVITY ON THE DEVELOPMENT OF SPINOCEREBELLAR TOPOGRAPHY. T. Pittman* and D.L. Tolbert. (SPONSOR: H. Cantor) Depts. of Anat. & Neurobiol., and Surgery (Neurosurg.), St. Louis Univ. Sch. of Med., St. Louis, MO 63104.

The development of topographic maps may be dependent upon conjoint pre- and postsynaptic activity. In adult rats spinocerebellar (Sp-CB) projections terminate in 5 sharply demarcated, sagittally-oriented stripes in the anterior lobe. Initally these projections terminate diffusely, but assume an adult pattern of termination by 7PND. Elvax implants containing muscimol, an GABA agonist, were made onto the surface of the cerebellum of 2PND rats. Sp-CB projections were mapped using orthograde intraaxonal labeling techniques. Compared to controls, the chronic application of muscimol altered the development of Sp-CB projections in two ways. First, Sp-CB axons did not form sharply delineated stripes in the anterior lobe. Distinct columns of labeled Sp-CB terminals were present in the internal granule cell layer, but considerable intercolumnar label was still present at 8-9PND. Second, Sp-CB projections were not restricted to the internal granule cell layer; rather, Sp-CB axons also projected aberrantly to the molecular and external granule (germinal) cell layers. These findings indicate that the initial development of Sp-CB topography is not dependent upon postsynaptic activity, but that the elimination of exuberant interstripe projections and the targeting of some Sp-CB axons is dependent upon cortical neuronal activity. Supported by NIH grant NS20227.

MOTOR SYSTEMS III

32.1

DEVELOPMENT OF KITTEN PRECRUCIATE NEOCORTICAL NEURONS IN BRAIN SLICES: RESPONSE TO ACETYLCHOLINE. K. Uchida*, N.A. Buchwald and M.S. Levine (SPON: D. Birt). MRRC, UCLA, Los Angeles, California, 90024.

The present study assessed the ability of acetylcholine (ACh) to alter electrophysiological parameters of developing precruciate cortical (Cx) neurons. Sixteen kittens (2-90 days of age) were anesthetized with ketamine, sacrificed and coronal brain slices (400-500 μm) were obtained. Effects of ACh were assessed by adding a series of ascending concentrations (10, 20, 30 and 50 μM ACh and 10 μM eserine) to the bath. To date we have recorded intracellularly from 104 neurons, 28 of which were tested with Ach. Animals were divided into 2 age groups (2-19 and 20-90 days). There were no differences in average resting membrane potential (RMP) and average action potential (AP) amplitude between the two groups (-61.4±8.0 (S.D.) and -54.1±6.9 mV for RMP and 71.8±17.2 and 66.7±12.6 mV for AP for younger and older kittens, respectively). Average input resistance was also not different (37.4±15.4 MΩ and 32.8±18.9 MΩ for younger and older kittens, respectively). However, there was a tendency for neurons in kittens under 1 week of age to have higher input resistances. Ach increased excitability and/or input resistance of 75% (21/28) of the Cx cells. The proportion of cells that showed an increase in excitability and/or input resistance was greater in older (84%, 16/19 cells) than in younger kittens (56%, 5/9 cells). Excitability was measured by assessing changes in action potential frequency during depolarizing current pulses (500 msec duration, 0.1-2.0 nA intensity). Increases in input resistance were greater in cells from older kittens (42.9±34.9% (n=7) versus 27±9.8% (n=4)). This study demonstrates that developing Cx neurons are capable of responding to Ach. The magnitude and the type of response appear to mature over the first postnatal month. Supported by USPHS Grant HD 5958.

32.2

MOTOR DEVELOPMENT OF KITTENS WITH SPINAL CORD LESIONS AND TRANSPLANTS. D.R.Howland*, Y.Itoh, A. Tessler, M.E.Goldberger. Department of Anatomy, The Medical College of Pennsylvania, Philadelphia, PA 19129.

Large spinal cord lesions were made in newborn kittens and homotypic embryonic transplants were placed in the lesion. Motor development and adult motor patterns were observed. Unlike normal kittens, newborn operates demonstrated bipedal locomotion on a treadmill within 24 hours. This bipedal locomotion is atypical in time of onset and pattern in comparison to normal development. Long-latency, hypometric proprioceptive placing without weight support can be elicited inconsistently within 24 hours. This placing pattern also differs from that of normal kittens. A transient loss of bipedal locomotion and placing is then seen which lasts from days to weeks. Re-emergence of these behaviors appears to coincide closely with each other and loosely with the acquisition of quadrupedal treadmill and overground locomotion. In adulthood, differences and similarities are observed between bipedal and quadrupedal treadmill and overground locomotion. Thus, motor patterns may not develop as continuous additions and refinements of behavior after neonatal spinal cord injury. This suggests that previously observed motor behavior may be suppressed and emerge again later in development.

Supported by grants NS24707 and NS16629.

32.3

THE EFFECT OF GM1 AND BRIDGING WITH FETAL SPINAL CORD ON RECOVERY OF HINDLIMB MOTOR FUNCTION IN THE SPINALIZED RAT. J.W. Commissiong and G. Toffano. Dept. of Physiol., McGill Univ., Montreal, Canada H3G 1Y6 and Fidia Res. Lab. 35031 Abano Terme, Italy.

Rats recover hindlimb motor coordination spontaneously if they are spinalized at postnatal day (PN) 7, but not at PN14 (Commissiong & Toffano, 1989). Five groups of animals were studied to determine whether bridging the spinal cavity with embryonic day (E) 17 spinal cord tissue, and/or treatment with GM1 ganglioside could promote recovery in animals spinalized at PN14. Ten weeks after surgery, the rats were scored (N=19) for four-limb coordination on a scale of 0 (worst) to 10 (best). The results are shown below.

GROUP	MEAN ± SD
UOC	9.26±1.48
PN14,B$^-$,S	3.18±2.91
PN14,B+,S	2.77±2.01
PN14,B+,G	5.00±1.97
PN 7,B$^-$,S	6.13±2.31

An ANOVA test, followed by a multi-comparison test showed that the PN14,B$^-$,S vs PN14,B+,S; and PN 14,B+,G vs PN7,B$^-$,S groups were statistically identical. UOC: unoperated control; B+: with bridging; B$^-$: no bridging; G: treated with GM1; S: treated with saline. Therefore, bridging plus treatment with GM1 caused a significant recovery in hindlimb motor coordination.

Commissiong, J. & Toffano, G. (1989). Ex. Brain Res., In press. (Supported by the MRC of Canada).

32.4

GROWTH, ELIMINATION AND MYELINATION OF CORTICOSPINAL AXONS IN THE POSTNATAL RAT. M.T.S. Cox, D.L. O'Donoghue and D.R. Humphrey. Laboratory of Neurophysiology, Emory University., Atlanta, GA 30322.

Studies have shown that corticospinal axons innervate the cord by the 2nd wk. We now extend these observations by describing the patterns of axon growth, elimination and myelination in the tract from the 1st to the 8th postnatal wks. Rats were perfused at 1, 2, 3, 4, 6, or 8 wks of age. The 5th cervical spinal cord segment was removed and processed for electron microscopy. Using 17 micrographs from each of 4 tracts per age, measurements were made of: (a) the numbers of axons within the areas sampled, and from this an estimate of the total number in the tract; (b) axon diameter; and (c) the percent myelinated. The results support the following conclusions: (1) During wks 1-2, almost all axons are unmyelinated, with a modal diameter of 0.2 um. The modal diameter of unmyelinated axons did not change between wks 1 and 8. (2) During wks 2-3, myelinated axons, 0.65 um in modal diameter, appear. Thus, radial growth of an axon occurs during its myelination. From wks 3-8, the percentage of myelinated axons increases from 19 to 53. (3) From wks 3-4, the number of axons decreases from 115,000 per tract to approximately 80,000. The appearance of myelinated axons in wk 3 may account for the development of functional excitability in the motor cortex-to-muscle system described previously (Cox and Humphrey, Neurosci. Abstr., 12:1119)(Supported by NIH Grant NS 20146).

32.5

POSTNATAL CHANGES IN THE NUMBER, DENSITY AND DISTRIBUTION OF CORTICOSPINAL NEURONS IN THE RAT. D.R. Humphrey and D.L. O'Donoghue. Laboratory of Neurophysiology, Emory University, Atlanta, Georgia, 30322.

During the first two postnatal weeks, corticospinal (CS) neurons are distributed as a continuous 'continent' of cells over the fronto-parietal cortex. In adults, CS cells form 3 discrete islands. Changes in distribution may result from 3 factors: 1. the death of CS cells in 'gap' regions; 2. unequal decreases in CS cell density with cortical growth; and/or 3. the elimination of spinally projecting axons from cells, particularly those located in 'gap' regions. These three factors were studied using a retrogradely transported dye (Fast Blue). To assess factor '1', dye soaked gelfoam was placed into the CS tract at C5 on postnatal day (PND) 6 (N=24). Groups of animals were then sacrificed on PND 10, 15, 20 or 30 and counts of labeled cells were obtained. The number and distribution of labeled cells did not change with age, indicating that cell death plays no role in the postnatal changes. These observations also ruled out factor '2.' Factor '3' was assessed by placing dye into the CS tract at different ages (PND 2,6,10,15,22 or 29; N=33), and quantifying the number and distributions of cells after a survival of one week. These experiments showed that: (a) the number of CS cells declines by PND 17 to 1/2 that seen on PND 7; and (b) axon elimination occurs in all areas, although regional differences can account for the formation of the adult pattern. (Supported by NIH Grant NS20146)

32.6

THE DEVELOPMENT OF CORTICOSPINAL PROJECTIONS IN NEONATAL CATS. T.D. Swink* and D.L. Tolbert (SPON: A. Harolan) Depts. of Anat. & Neurobiol. and Surg.(Neurosurg.), St. Louis Univ. Sch. of Med., St. Louis, MO 63104.

The development of corticospinal (CS) projections was studied in neonatal kittens following unilateral injections of WGA-HRP into the sensorimotor cortex. At 4PND labeled axons were in the lateral corticospinal tracts (LCST) bilaterally through all spinal cord levels. Rostrally, axons entered lamina V-VII bilaterally and spread dorsally to lamina I-III, and ventrally to VII and IX. Label in the ipsilateral LCST was less dense in lumbar segments than in cervical levels, and labeled axons projected only in lamina III-VI. At 7PND lamina V-VIII were filled with diffuse HRP reaction product indicating labeled terminals. Compared to younger animals, labeling in the ipsilateral LCST and spinal gray was markedly reduced in animals 7PND and older. Although the ipsilateral LCST is present in animals as old as 10PND, it does not appear to contribute any input to the gray matter. The lumbar segments of older animals (16PND) showed a pattern of HRP labeling similar to that seen in younger animals (7PND) with extensive labeling of individual axons and diffuse labeling of terminal endings. These data indicate that CS projections appear first to extend to all levels of the spinal cord and then proceed to innervate the gray matter in a rostrocaudal sequence. Supported by: NIH grant NS20227, March of Dimes Fellowship, Southern Med. Assoc. Grant.

32.7

MATURATION OF CENTRAL CONDUCTION TIME IN CORTICOSPINAL TRACTS PREDICTS DEVELOPMENT OF FASTEST VOLUNTARY MOVEMENTS IN CHILDREN

V. Hömberg*,1, K. Müller*,2 (SPON: ENA). 1:Neurological Therapy Center and 2:Department of Pediatrics, Heinrich Heine University of Duesseldorf, West Germany

Magnetic stimulation of motor cortex provides a non-invasive way to look at the maturation of fastest efferent corticospinal projections to upper and lower extremity muscles. This for the first time allows a direct comparison between the maturational profiles of fastest efferent corticospinal path ways with the maturation of voluntary motor activities in children.

In 70 children between the age of 2 and 10 years fastest stylus tapping movements (TAP), fastest aimed movements using a stylus to touch onto 20 consecutive targets separated by 1 cm (AIM) and fastest peg board transportation rates needed to transport pegs (0.2 mm diameter, 25 mm length) into 25 target positions consecutively (PEG) were measured. The maturational profiles of these motor activities were compared with central conduction times to thenar muscles obtained by magnetoelectrical stimulation (Cadwell MES 10 stimulator). The coil was positioned either over the vertex or over the mid cervical roots. The latency difference between cortical and cervical stimulation served as an estimate of central conduction time (CCT).

A comparison between the age-related slopes of the motor variables and the CCTs demonstrated a marked similarity in the maturational profiles of all motor activities with the maturation of CCTs. This was reflected in very similar exponents after approximations by power functions.

It can be concluded that maturation of fast hand movements is primarily determined by the structure bound maturation of the fastest corticospinal efferents and not by effects of motor learning.

32.8

EFFECTS OF KETAMINE, HALOTHANE, AND PENTOBARBITAL ON LOCOMOTOR RECOVERY AND CELL LOSS FOLLOWING SENSORIMOTOR CORTEX INJURY. M.P. Weisend & D.M. Feeney, Depts. of Psychol. and Physiol., UNM, Albuquerque, N.M. 87131.

This study examined some histopathological sequalae (15-20 days post injury) remote from the area of primary necrosis and beam walking (BW) recovery rate following sensorimotor cortex contusion (SMCx) under four conditions: 1) pentobarbital (PENT-45mg/kg, i.p.); 2) halothane (HALO 2% at 1.5 l/min @ 15 min); 3) PENT with ketamine pretreatment (KET/PENT 60mg/kg ketamine hydrocloride i.m., followed after 10 min by 21mg/kg PENT, i.p.); 4) HALO/KET (contusion under HALO then 5 min post surgery 150mg/kg KET i.p.). Anesthetic conditions had no effect on the rate of BW recovery. SMCx contusion or ablation produced a marked loss of ipsilateral thalamic ventral basal complex and ventralis lateralis neurons accompanied by gliosis. The extent of thalamic reaction was highly correlated with BW recovery but unaffected by treatment. Under PENT contusion produced an extensive ipsilateral loss of hippocampal CA3 pyramidal cells with little effect on CA1 or granule cells. The CA3 cell loss was lessened when contused under HALO and for KET/PENT was severe only in CA3a whereas HALO/KET greatly reduced this cell loss. Hippocampal cell loss was uncorrelated with BW recovery and not observed after SMCx ablation. Supported by DHHS RO1-NS20220-03 AND US Army Contract DAMD17-86-C-6144.

32.9

The Role of Experience on Amphetamine-Facilitated Recovery of Beam-Walking in the Rat. L.B. Goldstein and J.N. Davis. V.A. & Duke Medical Centers, Durham, N.C. 27705

Treatment with amphetamine increases the rate of recovery of beam-walking in rats after a unilateral suction-ablation lesion of the sensory-motor cortex. The amphetamine effect is not found if the rats are physically restrained following drug administration (Feeney et al., Science 217, 1982). However, the impact of practice on the beam following drug administration has not been directly tested. Therefore, we carried out an experiment in which four groups of rats were compared: 1. Amphetamine/Practice (n=19), 2. Amphetamine/No Practice (n=20), 3. Saline/Practice (n=18), 4. Saline/No Practice (n=20). Amphetamine was given as a single dose 24 hours after lesioning. Practice was six trials on the beam at one hour intervals beginning one hour after drug/saline administration. "No Practice" rats were handled and allowed to walk in their home cages. Final ratings were performed by a blinded observer 24 hours following drug administration. Group 1 rats had greater improvements in beam-walking scores when compared to rats in the other three groups (Kruskal-Wallis H=15.592, DF=3, p<.01; Fisher LSD, p<.05). Amphetamine treatment alone (Group 2) or practice alone (Group 3) did not enhance recovery (Fisher LSD, P>.05). There were no differences in lesion sizes among the four groups (ANOVA, p=.73). Thus, practice on the beam is required for amphetamine-facilitated recovery of beam-walking.

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32.10

RECOVERY OF FORELIMB-PLACING BEHAVIOR IN RATS WHICH RECEIVED UNILATERAL CORTICAL DAMAGE AS NEONATES IS MEDIATED BY THE REMAINING HEMISPHERE T.M Barth and B. B. Stanfield. Lab. Neurophysiol. and Lab. Clin. Sci, NIMH, Poolesville, MD 20837.

Unilateral lesions in the sensorimotor cortex (SMC) of adult rats produce placing deficits with the forelimb contralateral to the damage. However, this deficit is temporary and following complete recovery, a second lesion in the remaining SMC produces deficits only with the forelimb contralateral to the second lesion. This suggests that recovery from unilateral SMC lesions in adult rats does not involve the contralateral homotopic cortex. In contrast, in rats lesioned as neonates (who also recover), there is anatomical evidence consistent with a role for the contralateral hemisphere in the recovery. For example, in adult animals which had been unilaterally lesioned as neonates there is an increase in an ipsilateral corticospinal projection which is not seen following adult lesions. This, as well as other aberrant projections, provides a potential substrate for the mediation of the behavioral recovery. The present study was designed to evaluate the contribution of the contralateral hemisphere in recovery of tactile forelimb-placing behavior following unilateral neonatal SMC lesions.

On the day of birth, rats received a unilateral SMC lesion and were tested for impairments of tactile forelimb-placing on days 30 and 42. There was no evidence of placing deficits with either forelimb. On day 42, some of these rats received a second lesion in the remaining SMC. In contrast to the results with adult rats, the second lesion produced tactile placing deficits with both forelimbs. This suggests that following unilateral SMC lesions in neonates, the contralateral hemisphere mediates the recovery of forelimb-placing. Finally, three days after the second SMC lesion, the rats were perfused and the brains and spinal cords were processed for the presence of degenerating fibers with the Fink-Heimer stain. Analysis revealed axon degeneration in the cord both ipsilateral and contralateral to the second lesion. We conclude that this aberrant ipsilateral corticospinal projection is a potential substrate for recovery of forelimb-placing which follows neonatal SMC lesions.

32.11

EXTRAUTERINE MOTOR DEVELOPMENT OF "FETAL RAT" DELIVERED BY C-SECTION. C. Robert Almli and Martina Dyer.* (SPON: J. DUBINSKY). Depts. of Anat. & Neurobiol., Psychol., Pgms. in Neural Science, Occup. Therapy, Washington Univ. Sch. of Med., St. Louis, MO 63110.

Studies of early motor development have revealed "cyclic, spontaneous movement" patterns during embryonic, fetal and neonatal periods for a wide variety of species. This investigation extends the study of early movement to "fetal rats" delivered prematurely to the extra-uterine environment. "Fetal rat pups" were studied at 20 and 21 days gestational age, and compared to fullterm, vaginally delivered rats. The 3 age groups were videotaped in an incubator at 2-3, 5-6 and 24 hours "postnatal". Tapes were analyzed (computer) for movements of mouth, head, limbs and trunk, complex movements (face brush, supine-prone, ambulation), and cycles. Although delivered prematurely, "rat fetuses" at 20 or 21 days gestation displayed movement of all body segments including complex movements, and cyclic, spontaneous movements. However, the groups differed quantitatively on a number of measures, e.g., total number of movements. Overall the results indicate "rat fetuses" are capable of displaying a wide variety of movements in the extra-uterine environment. The major differences between groups were quantitative. (Conducted under NIH Guide for the Care and Use of Laboratory Animals.

ASSOCIATION CORTEX AND THALAMOCORTICAL RELATIONS I

33.1

COMPARATIVE ANALYSES OF THE CHOLINERGIC AND SEROTONERGIC INNERVATIONS OF MONKEY (*MACACA FASCICULARIS*) PREFRONTAL CORTEX. D.A. Lewis and S. Slovenec*. Depts. of Psychiatry and Behav. Neurosci., University of Pittsburgh, Pittsburgh, PA 15213.

Immunohistochemical methods were used to visualize fibers immunoreactive (IR) for choline acetyltransferase (ChAT), a marker of the cholinergic system, or serotonin (5HT) in cynomolgus monkey prefrontal cortex. The majority of ChAT-IR fibers were extremely fine and studded with small varicosities. Smooth fibers with a larger diameter were also present, especially in the white matter. The density of ChAT-IR fibers was relatively uniform across cytoarchitectonic regions, although some differences were observed. Layers I-II had the greatest density of labeled fibers, layer VI the lowest, and layers III-V had a range of intermediate densities of ChAT-IR fibers. Compared to ChAT-IR axons, 5HT-IR fibers were morphologically more heterogeneous. However, 5HT-IR fibers had a more homogeneous distribution on both a regional and laminar basis than did ChAT-IR fibers. The patterns of distribution of these afferent systems are distinctly different from those of dopaminergic and noradrenergic axons, suggesting that these four neural systems differ substantially in their regulation of prefrontal cortical function.

33.2

ORGANIZATION OF CHOLECYSTOKININ (CCK)-IMMUNOREACTIVE (IR) STRUCTURES IN MONKEY PREFRONTAL CORTEX. K.M. Oeth and D.A. Lewis. Depts. of Behavioral Neuroscience and Psychiatry, Univ. of Pittsburgh, Pittsburgh, PA 15213

CCK-IR structures were analyzed in prefrontal cortical regions of monkeys (*Macaca fascicularis* and *Macaca mulatta*) using antibodies which selectively recognize either the terminal octapeptide (CCK8) or larger forms (CCK58, 39, and 33) of CCK. Large forms of CCK are confined to discrete portions of the soma whereas CCK8-IR is present in fibers as well as in the soma. Common neuronal morphologies include a vertically oval cell with a primary ascending and descending dendrite from which an axon vertically descends, and a multipolar cell with a descending axon. Total CCK8-IR cell density does not differ among cortical regions and is highest in layers II-superficial III. Within a region, CCK8-IR cells have a discontinuous distribution; in serial tangential sections through layers II-superficial III, bands of cells alternate with cell-poor regions. Prominent CCK8-IR terminal fields exist in layers II, IV, and especially VI, while radially oriented fibers traverse the sparsely innervated layers III and V. These radial fibers have a distribution which parallels the location of apical dendrites of pyramidal cells and may descend from the CCK8-IR neurons of layer II-III. Both labeled fibers and neurons are present in high density in layer II, whereas in layers IV and VI, the densities of CCK8-IR fibers far exceed that of labeled neurons. CCK-containing neurons are present in the mediodorsal thalamic nucleus and substantia nigra-ventral tegmental area; axons from these structures terminate in layers IV and VI, respectively, and may account for the CCK8-IR terminal fields in these layers. These data indicate that CCK may influence prefrontal cortical function via multiple neuronal circuits. Supported in part by the Scottish Rite Schizophrenia Research Foundation, N.M.J.,U.S.A.

33.3

DISTRIBUTION OF TYROSINE HYDROXYLASE (TH)-IMMUNOREACTIVE (IR) STRUCTURES IN HUMAN PREFRONTAL CORTEX. L.V. Le* and D.A. Lewis (SPON: J. Puig-Antich) Depts.of Psychiatry and Behav. Neurosci., University of Pittsburgh, Pittsburgh, PA 15213.

The distribution of TH-IR structures was characterized immunohistochemically in post-mortem human prefrontal cortex. The density of TH-IR fibers was heterogeneous across cortical regions. Fiber density was greatest in the cingulate gyrus. The superior frontal gyrus, especially the medial portion, also had a high density of labeled fibers. Fiber density decreased substantially across the middle and inferior frontal gyri. The orbital gyri had intermediate densities of TH-IR fibers. Regional differences were also present in the laminar distribution of fibers, although fiber density was consistently greatest in layers I-II. In addition, a subpopulation of nonpyramidal neurons was labeled with several different anti-TH antibodies, including 2 monoclonals. Labeled neurons were present in every layer with the highest density in layer VI. The density of TH-IR neurons also differed substantially (up to 3-fold) across prefrontal regions. In general, the regional distribution of TH-IR neurons paralleled that of TH-IR fibers. These findings demonstrate that the innervation of human prefrontal cortex by TH-IR fibers is quite similar to that of monkey (Br Res 449:225, 1988). They also suggest that intrinsic neurons may contribute to the catecholaminergic innervation of human prefrontal cortex. Supported in part by MH00519 and the Scottish Rite Schizophrenia Research Program, N.M.J.,USA.

33.4

CORTICOCORTICAL AND OTHER TELENCEPHALIC CONNECTIONS OF THE ORBITAL AND MEDIAL PREFRONTAL CORTEX IN THE MONKEY. S. T. Carmichael* and J. L. Price (Spon. H. Burton), Dept. of Anatomy and Neurobiology, Washington Univ. Sch. of Med. St. Louis, MO 63110.

The ventral and orbital prefrontal cortex (PFC) of the primate participate in many connections with other brain regions. We examined the connections of these areas in 10 Macaca fascicularis monkeys with the use of the retrograde tracers fast blue and diamidino yellow. Analysis of intrinsic prefrontal corticocortical connections revealed two regions with extensive internal connections but with few interconnections linking them: a medial region (Med) consisting of areas 32, 24 a,b, and part of area 10 and an orbital region (Orb) including the agranular insula (Ia), and areas 13, 13a, and caudal parts of 12, 11 and 14. Cells giving rise to projections in these systems formed radial columns or patches and complex, interlocking strips across cortical areas.

Input to these systems is segregated in other telencephalic areas. Temporal cortical cells projecting to Orb are located primarily in the ventral temporal pole and in the extreme rostral portion of the Superior Temporal Sulcus, while cells projecting to Med were fewer in number and located in the dorsal temporal pole and the rostral Superior Temporal Gyrus. Cells in the entorhinal cortex projecting to Med lie lateral and caudal to those projecting to Orb. Within the amygdala cells projecting to Med were primarily caudal and lateral to Orb-projecting cells in the basal nucleus; cells of the accessory basal nucleus projected to both regions. Finally, cells in the claustrum projecting to both PFC regions lie in the rostral ventral claustrum, but Med-projecting cells are clustered lateral and dorsal relative to Orb-projecting claustral cells.

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33.5

EFFECT OF SPATIAL AND COLOR CUES ON DELAY-RELATED NEURONAL RESPONSES IN PREFRONTAL CORTEX. F.A.W. Wilson & P.S. Goldman-Rakic, Yale Medical School, New Haven, CT

Neurons (n =180) were recorded in area 9 and superior area 46 during the performance of two tasks requiring spatially directed responses: a spatial delayed response (SDR) task in which the monkey moved a joystick left or right after a 1s delay following presentation of a left or right cue; in a color-cued delayed response task (CDR), the left/right responses were indicated by one of two different colored cues presented in the center of the monitor. SDR and CDR trials were presented quasi-randomly within each session and eye postion was monitored. Of the 151 neurons tested on at least 8 trials of each stimulus, a total of 39 neurons responded differentially during the delay period in one or both tasks. The majority of neurons appeared to reflect the nature of the cue rather than the response because they showed an increase in firing rate in the SDR task (n = 20); or in the CDR task (n = 12) but not both. A smaller fraction (n = 7) responded on both tasks, always for the same response direction whether signalled by the color or location of the cue. One neuron that responded differentially during the delay of the SDR task was driven by thalamic stimulation (lat. = 3.8 ms; conduction vel. = 6.6 m/sec) with following of 200 Hz pulse trains, indicating that neurons which participate in memory are connected with the thalamus. None of the 180 neurons recorded responded to the sight of foods, novel and familiar stimuli, or faces. In line with Fuster et al. (Brain Research 474; 1988), these data provide evidence that some prefrontal neurons code direction of the impending response and can do so either for spatial or color information while others represent separate spatial and non-spatial memory channels.

33.6

EVIDENCE FOR A SELECTIVE PROJECTION FROM THE HIPPOCAMPAL FORMATION TO THE PRELIMBIC AREA OF THE PREFRONTAL CORTEX IN THE RAT. T.M. Jay, J. Glowinski*, A.M. Thierry*. INSERM U114, Collège de France, 75231 Paris Cedex 05, France.

Connections from the hippocampal formation to the prefrontal cortex (PFC) were studied using both retrograde and anterograde tracers injected iontophoretically. The retrograde tracer Fluoro-Gold was placed into different subdivisions of the PFC. Only the injections located in the prelimbic area revealed the presence of labelled hippocampal cells in the ipsilateral CA1 temporal field and caudal subiculum. Injections of the anterograde tracer Phaseolus Vulgaris Leucoagglutinin (PHA-L) into CA1 revealed a projection to the PFC which was restricted to the prelimbic area, mainly in the deep layers. PHA-L injections into the caudal subiculum also resulted in labelling in the prelimbic area diffusely distributed over all the layers. All these fibers are branched and highly varicosed. These results demonstrate that the projection from the hippocampal formation to the PFC in the rat is unilateral and restricted to the prelimbic area. On the basis of these anatomical data, the functional interaction between these two structures is currently studied (S. Laroche et al., this meeting).

33.7

Prefrontal Projections to the Medial Nuclei of the Dorsal Thalamus in the Rabbit. R.H. Thompson* and S.L. Buchanan. VA Medical Center and University of South Carolina, Columbia, SC 29201.

Rabbits were injected with horseradish peroxidase (HRP) to investigate the efferent projections from the prefrontal cortex (PFC) to the medial nuclei of the dorsal thalamus. Injections limited to the mediodorsal nucleus resulted in labeling of both the agranular midline and insular regions of the PFC. Injections restricted to the ventromedial nucleus labeled the dorsolateral PFC above the forceps minor and a medial region in the granular insular cortex. An injection contained within the intralaminar nuclei, including both the centromedial and paracentral nuclei, labeled cells in both the more dorsal aspect of the agranular precentral PFC, and the dorsolateral PFC. Injections limited to the ventroposterior nucleus resulted in labeling of the dorsolateral portions of PFC with some overlap of the area labeled by VM injections. All labeled cells were observed in the deeper cortical layers (either V or VI), regardless of the architectonic region injected. Injections of WGA-HRP in the PFC confirmed that the observed retrograde labeling was not due to damage to thalamic fibers of passage. Thus, it was shown that there is a differential topographical projection to the various nuclei of the dorsal thalamus from the PFC in the rabbit.

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33.8

THE CLASSIFICATION AND ORIGIN OF AXON TERMINALS IN THE MEDIODORSAL THALAMIC NUCLEUS (MD) OF THE RAT. M. Kuroda* and J. L. Price, Dept. Anat. & Neurobiol., Washington Univ. Sch. Med., St. Louis, MO 63110.

Electron microscopy and the tracer WGA-HRP have been used to investigate the types and origin of pre-synaptic terminals in MD. At least four types of terminals have been identified on the basis of size, synaptic vesicle morphology and synaptic membrane specialization: 1) small axon terminals (<1 μm in diameter) with round vesicles, which make asymmetric synaptic contacts with small dendrites; 2) large axon terminals (>3 μm) with round vesicles, which make asymmetric contacts with large dendrites; 3) small to medium axon terminals (<2 μm) with pleomorphic vesicles, which make symmetric contacts with soma; 4) large axon terminals (>3 μm) with pleomorphic vesicles, which form symmetric contacts with large dendrites.

In order to label the terminals by anterograde transport, 50-100 nl of 1% WGA-HRP was injected into areas known to project to MD. Ultrastructural examination of the material prepared to date indicates that the first type of axon terminal arises from prefrontal cortical areas such as the prelimbic and lateral orbital cortex. The second type of terminal arises from ventral forebrain structures such as the basal amygdaloid nucleus. However, the ventral pallidum gives rise to the fourth type of axon terminal. On morphological grounds, therefore, the ventral pallidal projection may be inhibitory, while other ventral forebrain afferents may be excitatory. It is presumed that the third type of terminals arises either from interneurons or from the thalamic reticular nucleus.

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33.9

THREE-DIMENSIONAL ANALYSIS OF THALAMOCORTICAL PROJECTIONS AND VENTRAL FOREBRAIN AFFERENTS OF THE MEDIODORSAL NUCLEUS OF THE THALAMUS IN THE RAT. J. P. Ray and J. L. Price, Dept. of Anatomy and Neurobiology, Washington Univ. Sch. Med., St. Louis, MO 63110.

Recent studies have documented direct projections from the amygdaloid nuclei and the piriform (olfactory) cortex to the prefrontal and agranular insular cortices, and to the thalamic nucleus associated with these cortical areas, the medial and central subdivisions of the mediodorsal nucleus (MD_m and MD_c). Hence, there are both direct and transthalamic pathways between the ventral forebrain and prefrontal cortex. We have studied the organization of this system by injecting anterograde tracers into ventral forebrain structures and, in the same rats, retrograde tracers into connected frontal cortical areas, in order to directly compare cells labeled in MD with afferents from the ventral forebrain. 3-D reconstructions of sections through MD have been used to correlate the distibution of cells projecting to amygdaloceptive cortical areas (prelimbic, dorsal agranular insular and dorsolateral orbital cortices (DLO)) or to olfactory-related areas (ventral agranular insula (AI_v) or lateral orbital cortex) with the distribution of afferents in MD from the amygdala or piriform cortex, respectively. **Results indicate that the degree of overlap of thalamocortical cells in MD and ventral forebrain afferents varies, depending upon the cortical area.** For example, cells in MD_c projecting to AI_v are in the same part of MD_c that receives afferents from the piriform cortex, but cells in MD projecting to DLO are distant from basal amygdaloid afferents in MD_m, even though DLO receives direct basal amygdalocortical fibers.

Supported by NIH research grant NS09518.

34.1

CHANDELIER CLASS INTERNEURONS CONTAINING CORTICOTROPIN RELEASING FACTOR IN PRIMATE PREFRONTAL CORTEX. J.S. Lund and D.A. Lewis. Dept. of Psychiatry and Center for Neuroscience, University of Pittsburgh, PA 15261.

In prefrontal, parietal and temporal granular association areas of squirrel monkey cortex corticotropin releasing factor (CRF) immunoreactivity is present in small, vertically oriented, rod-like structures (Lewis et al., '89). These structures are uniquely located in layers 4-base 3. Golgi impregnations of macaque show layer 4 in prefrontal areas 46 and 9 to have a population of 'chandelier' interneurons whose axon cartridges strongly resemble the CRF positive 'rods.' Chandelier neurons have been shown (Somogyi, '77) to be GABAergic and have axon cartridges encasing pyramidal neuron axon initial segments. In prefrontal cortex we find the cartridges to surround the axon initial segments of pyramidal neurons within layer 4 and at the base of layer 3. Chandelier neurons in other locations do not appear to be CRF immunoreactive, suggesting that this neuron class has subgroups with different cotransmitters. These CRF interneurons lie in a strategic position for controlling the influence of inputs to layer 4 within prefrontal cortex. Supported in part by EY05282, MH00519, MH43784.

34.2

LOCALIZATION OF SOMATOSTATIN (SS) IMMUNOREACTIVITY (IR) IN HUMAN AND MONKEY NEOCORTEX: ARE THERE SPECIES DIFFERENCES? T.L. Hayes and D.A. Lewis, Departments of Behavioral Neuroscience and Psychiatry, University of Pittsburgh, Pittsburgh, PA 15216.

SS28- and $SS28_{1-12}$-IR were analyzed immunohistochemically in the anterior cingulate, inferior temporal and primary visual regions of perfused monkey, unperfused monkey of varying post-mortem (PM) intervals (.5, 1, 2, 4, 8 and 12 hrs) and PM human brains. The regional and laminar distributions of $SS28_{1-12}$-IR fibers were similar across species. For example, in both species anterior cingulate cortex had the greatest density of labeled fibers, and primary visual cortex had the least. However, perfused monkey and .5 hr PM monkey had the greatest overall fiber density, as well as prominent radial fibers in layers III-V that were not labeled after longer PM intervals or in humans. Furthermore, although SS28-IR was clearly evident in perfused monkey, it was not detected in human or PM monkey. In contrast, both perfused and PM monkeys showed a similar density of $SS28_{1-12}$-IR neurons in cortex and white matter, whereas the percentage of $SS28_{1-12}$-containing cells located in the white matter was 3-5 times greater in human than in monkey. Labeled neurons in the white matter were also larger in human than in perfused or PM monkey. These findings indicate that (1) the decrease in $SS28_{1-12}$-IR in humans compared to monkeys is probably an effect of the PM interval; (2) the lack of SS28-IR in humans may be due to the lability of SS28, and does not reflect a species difference; and (3) differences in the number of $SS28_{1-12}$-IR cells and their size do indicate a species difference between humans and monkeys.

34.3

MONOAMINERGIC AND PEPTIDERGIC INNERVATION OF MONKEY TEMPORAL POLE. M.L. Voytko, C.A. Kitt and D.L. Price. Neuropathology Lab., The Johns Hopkins Univ. Sch. Med., Baltimore, MD 21205.

The distributions of dopaminergic, serotoninergic, and peptidergic nerve fibers are not well documented in temporal polar cortex. Immunocytochemistry was used to demonstrate regional and laminar innervation patterns of immunoreactive fibers for tyrosine hydroxylase (TH), serotonin (5HT), substance P (SP), and neuropeptide Y (NPY) in the temporal poles of three rhesus monkeys. TH fibers formed a dense continuous band in the deep portion of layer I and throughout layer II. 5HT-positive fibers were also concentrated within layer I. NPY-immunoreactive fibers were conspicuous in layers I-III. Distinct bands of SP-positive fibers were observed in layer I, layers II-III, and layer V of temporal pole.

These findings indicate that temporal polar cortex receives a rich supply of dopaminergic, serotoninergic, and peptidergic afferents, with the highest density of innervation in supragranular layers I and II. These laminae are also primary targets of cholinergic innervation from the nucleus basalis of Meynert [Voytko et al., *Soc. Neurosci. Abstr.* 14:631, 1988]. These results suggest that inputs from diverse neurotransmitter/peptidergic sources have converging influences upon neurons in temporal polar cortex.

34.4

A SUBSET OF PRIMATE CORTICOCORTICAL NEURONS ARE NEUROFILAMENT PROTEIN (NFP) IMMUNOREACTIVE (ir): A COMBINED RETROGRADE IMMUNOHISTOCHEMICAL STUDY. M.J. Campbell, P.R. Hof, K. Cox*, T.A. Kimber*, W.G. Young and J.H. Morrison, Research Institute of Scripps Clinic, La Jolla, CA

In previous primate neocortical studies we observed that SMI-32-ir pyramidal neurons exhibited a regionally heterogeneous distribution and hypothesized that this resulted from shifts in the representation of SMI-32-ir neurons furnishing long corticocortical projections (JCN(1989) 282:191-205). SMI-32 is a Sternberger-Meyer monoclonal antibody that recognizes a non-phosphorylated epitope on NFP. We have initiated combined retrograde tracer and immunohistochemical studies in the *Macaca fascicularis* to test this hypothesis. Fast blue was injected into the cortical region rostral to the arcuate sulcus and ventral to the principal sulcus (PS) and we determined the number of fast blue retrogradely labeled neurons (FB) and double labeled neurons (DL) (those containing fast blue and SMI-32-ir) in 6-10, 1 mm cortical traverses from each source cortical region. Results are presented as a ratio of DL over FB for layers III and V-VI for several regions. Ipsilateral PS (III, 22±3/114±21, 18%; V-VI, 27±5/98±15, 27%); Contralateral PS (III, 23±1/88±5, 26%; V-VI, 10±1/32±2, 31%). Ipsilateral anterior cingulate (III, 0/1, 0%; V-VI, 10±1/20±1, 50%). Contralateral anterior cingulate (III, 0/1, 0%; V-VI, 7±1/14±2, 50%). Intraparietal sulcus (III, 17±2/29±3, 58%; V-VI, 12±1/20±2, 60%). Superior temporal sulcus (III, 11±1/13±2, 85%; V-VI, 9±1/11±1, 82%). These data illustrate: 1) The potential to define distinct subsets of corticocortically projecting neurons based on differences in their molecular constituents. 2) The degree to which specific corticocortical projections of an area vary in their proportional representation of neuronal subsets so defined.

34.5

CORTICOTHALAMIC CONNECTIONS OF THE SUPERIOR TEMPORAL SULCUS IN RHESUS MONKEYS. E.H. Yeterian and D.N. Pandya. Dept. of Psychology, Colby College, Waterville, ME 04901, ENRM Veterans Hospital, Bedford, MA 01730, and Boston Univ. Sch. of Medicine.

The cortex of the superior temporal sulcus (STS) contains both unimodal and multimodal association areas. These areas have distinctive patterns of thalamic afferents (Yeterian and Pandya, 1989). In the present study, the corticothalamic connections of the STS were examined in 20 monkeys using the autoradiographic technique. The results show that the rostral portion of the upper bank projects to the medial and caudal region of the medial pulvinar nucleus (PM), to the medial part of the medial dorsal nucleus (MD), and to the suprageniculate nucleus. The mid-portion of the upper bank projects to central and ventral PM and to the oral pulvinar nucleus (PO), to central MD, and to the magnocellular division of the medial geniculate nucleus (GM). The caudal portion of the upper bank projects to ventral and lateral PM and to PO, to central and lateral MD, and to the parvocellular portion of GM. Regions of the upper bank also have projections to intralaminar nuclei: paracentral, central lateral, central lateral superior, and limitans. In contrast to the upper bank, the lower bank projects to the inferior and lateral pulvinar nuclei, as well as to PM.

Thus the upper bank of STS, which contains multimodal area TPO, has widespread projections throughout the rostral-caudal extent of the thalamus, whereas the lower bank, which contains unimodal visual areas, projects mainly to the pulvinar. Supported by ENRM Veterans Hospital, Bedford, MA, NIH Grant 16481, and Colby College Social Science Grants 01 2202 and 01 2216.

34.6

THALAMOCORTICAL CONNECTIONS OF CORTICAL AREAS PROJECTING TO MULTIMODAL AREAS OF SUPERIOR TEMPORAL SULCUS IN THE MONKEY. C.L. Barnes and D.N. Pandya, Bedford Veterans Hospital, Bedford, MA 01730 and Depts. of Anatomy and Neurology, Boston Univ. Sch. of Med., Boston, MA 02118.

Anatomical (Jones and Powell, 1970; Seltzer and Pandya, 1978) and physiological (Desimone et al., 1984; Bayliss et al., 1987) studies have identified a multimodal region along the upper bank of the superior temporal sulcus (STS). This area has been designated as area TPO. This multimodal area TPO is preferentially related to distal parasensory association areas: area Opt in the inferior parietal lobule, area Ts1 and Ts2 in the superior temporal gyrus, and area TF in the parahippocampal gyrus (Barnes and Pandya, 1986). These areas are themselves in part multimodal in nature. Area TPO has been shown to receive its main thalamic projections from the central part of the medial pulvinar (PM) (Yeterian and Pandya, 1989). The aim of the present study was to see if these distant parasensory association areas have similar thalamic afferents as area TPO. Three Fluorescent Retrograde Tracers (FRT) were injected in parasensory association areas Opt, Ts1-Ts2 and TF in a single hemisphere of three monkeys. The results show that area Opt receives projections from lateral PM, areas Ts1-Ts2 from ventral and medial PM and area TF from dorsal PM. The location of the retrogradely labeled cells surrounds the central sector of PM that projects to area TPO. Thus it seems that medial pulvinar projects to area TPO and to related parasensory association areas. The thalamic projections, however, are differentially organized, with area TPO being related to the central region of PM whereas the parasensory association areas Opt, Ts1-Ts2, and TF are related to peripheral regions of PM. (Supported in part by Veterans Administration, ENRM VA Hospital, Bedford, MA, NIH Grant #16841 and Scottish Rite Schizophrenia Program, NMJ, USA.).

34.7

PROJECTIONS TO THE BASIS PONTIS FROM SUPERIOR TEMPORAL SULCUS (STS) IN THE RHESUS MONKEY. J.D. Schmahmann and D.N. Pandya. Departments of Neurology and Anatomy, Boston University School of Medicine, Boston, MA 02118, and ENRM Veterans Hospital, Bedford, MA 01730.

The projections to the pons from the parietal lobe are derived not only from sensory and parasensory association areas (Nyby and Jansen '51, Brodal '78, May and Anderson '86), but also from the multimodal caudal parietal cortex (Schmahmann and Pandya '89). In the present study the aim was to determine whether there are pontine projections from area TPO in the upper bank of the superior temporal sulcus (STS) which has been shown to be multimodal (Jones and Powell '70; Seltzer and Pandya '78; Desimone et al '84; Bayliss et al '87). The pontine projections were studied after the injection of tritiated amino acids in rostral to caudal regions in the upper bank of the STS in six rhesus monkeys, and in the lower bank in another five animals. Pontine projections were observed from the cases with injections in the upper bank, but not from those with lower bank injections. The projections from the upper bank are observed predominantly in the lateral, extreme dorsolateral, dorsolateral and peripeduncular nuclei.

The presence of projections to the pons from the multimodal area TPO in the upper bank of the STS contrasted with the absence of projections from the unimodal lower bank would seem to indicate that the cerebellum has access to higher order information via the corticopontocerebellar pathway. This would support our hypothesis that apart from its role in motor control, the cerebellum may be involved in the modulation of cognitive function. (Supported in part by the Veterans Administration, ENRM VA Hospital, Bedford MA, and NIH grant #16841).

34.8

PHYLOGENETIC CHANGES IN DENSITY OF GABA-A RECEPTORS IN SENSORY AND ASSOCIATION NUCLEI OF THALAMUS. R. B. Masterton, B. N. Baker* and K. K. Glendenning. Dept. Psychology, Florida State Univ., Tallahassee, FL 32306.

Conventional procedures for the quantitative labeling of high-affinity GABA-A receptors with tritiated muscimol in 3 sensory nuclei (DGL, GM, VB) and two association nuclei (LP, MD) in the dorsal thalamus were compared among 8 species of vertebrates. The species themselves were specifically selected on the basis of the time since their last common ancestor with Anthropoids -- allowing inferences regarding the sequence of changes that probably took place over the reptilian - pre-Eutherian - Eutherian - prosimian Primate segment of the Anthropoid lineage.

The results show that the average density of GABA-A receptors in the dorsal thalamus probably increased with the origin of mammals and after remaining nearly constant through the appearance and early radiations of marsupial and placental mammals, increased once again with the origin of Primates. Both the sensory nuclei and association nuclei considered collectively show this same 2-step increase. Among the individual nuclei, however, the medial geniculate nucleus shows a different pattern. MG appears to have had a single-step increase (at the origin of mammals) and remained nearly constant at least through the origin of true Primates.
Supported by NIH-NINCDS # NS7726.

34.9

CONTRALATERAL CORTICAL PROJECTIONS OF THE SUPERIOR TEMPORAL SULCUS IN THE RHESUS MONKEY. Benjamin Seltzer and Timothy Murphy*. Dept. of Psychiatr. & Neurol., Tulane Univ. Schl. of Med., V.A. Med. Ctr., New Orleans, LA 70112.

The superior temporal sulcus (STS) of the rhesus monkey contains unimodal and multimodal zones (Seltzer and Pandya, '78). Their contralateral cortical projections have not previously been explored.

Five monkeys received discrete injections of radio-labelled amino acids in each of four rostral-to-caudal divisions of multimodal area TPO in the upper bank of the STS, and in area IPa, in the depth. Following processing for autoradiography, each sulcal area was found to project to a restricted portion of the homotopical zone in the opposite STS. Each area also projects to immediately rostral and caudal zones within the contralateral STS as well as to certain post-Rolandic areas (insula, cingulate gyrus, parahippocampal gyrus, caudal superior temporal gyrus, and posterior parietal lobe). Thus the contralateral connections of areas TPO and IPa resemble their ipsilateral projections to the STS and post-Rolandic cortex (Seltzer and Pandya, '84;'87). Most contralateral projections terminate in and around layer IV, but those to caudal heterotopical areas in the STS terminate over layer I. Rostral regions of the STS send contralateral fibers by way of the anterior commissure; more caudal regions through the body of the corpus callosum.
Supported by the V.A. and Tulane University.

34.10

THE DEVELOPMENT OF CORTICAL FOLDING IN RHESUS AND HUMAN BRAINS. E. Armstrong, M. Curtis*. Yakovlev Collection, A.F.I.P., Washington DC. 20306 and Dept. Anatomy, U.S.U.H.S., Bethesda, MD.

Differences in the degree of cortical folding between adult human and rhesus brains are a function of brain weight. The rhesus attains adult levels of gyrification before its brain weighs 50 g. At this weight the human brain is lissencephalic. To analyze the roles of maturation and brain weight, the gyrification indices (GI) of fetal and perinatal rhesus and human brains were compared. The GI's are the ratios of the total to the superficially exposed cortical contours and were measured in brains from the Yakovlev Collection.

The GI's are low until the 17th gestational week in rhesus monkeys and the 20th in humans. Following this both species exponentially increase their GI's. The exponential increase in cortical buckling has a longer duration in human than in rhesus brains. When the GI values were plotted from the frontal to occipital poles, the middle and caudal portions of the adult human brain resembled those portions of fetal monkeys. This pattern is expected if delayed maturation (neoteny) produced the enlarged human brain. On the other hand, the human brain has a high prefrontal GI which is never observed in the rhesus cortex. This suggests that the developmental strategy here follows separate maturational events.
Supported by NSF 8820485.

34.11

CORTICAL AFFERENTS TO CAUDAL AREA 24C (THE CINGULATE MOTOR AREA) AND ROSTRAL AREA 23C. R.J. Morecraft*, G.W. Van Hoesen and J.A. Maynard* (SPON: W.W. Kaelber). Depts. of Anatomy and Neurology, Univ. of Iowa, Iowa City, IA 52242

Epicortical and intracortical stimulation studies have shown that motor responses can be elicited from the anterior cingulate area. Cytoarchitectural studies have suggested that the anterior cingulate cortex is the evolutionary precursor of M2 and may be a primordial motor area. These efforts have prompted recent neuroanatomical investigations to focus on the cingulate gyrus for additional motor representations. For example, connectional studies in the monkey have demonstrated that select portions of areas 24c and 23c project topographically to M1, M2 and the spinal cord. Thus, a study was initiated to examine the cortical input to areas 24c and 23c. Four rhesus monkeys were injected with the retrograde tracer diamidino yellow, fast blue or both into areas 24c and 23c and their cortical afferents were identified. Several cortical areas were found to project to both 24c and 23c. Both areas received afferents from: frontal areas 10, 11, 12, 14, 46, 9, 8B, 6DR, 6DC, M2 and M1; parietal areas 7 (PG), PGm, PGop and S2; limbic areas 29, 30, 31 and 35; and occipital areas OA. Powerful intracingulate and insular afferents were observed. Areas 13, 25, 6VB, 38 and 28 projected only to area 24c, while areas 1, 2, 5 (PE), and POa projected to area 23c. These afferents originated from layers III and V, and were most extensive on the medial and dorsal lateral convexity of the hemisphere. Labeling within frontal and parietal cytoarchitectural subdivisions was widespread, while labeling within the temporal and limbic subdivisions was well represented but more focal in its overall distribution. If additional motor representations are present in the cingulate cortex, the diversity of cortical input is impressive and appears to exceed that of M1 and M2. Our connectional data suggest that the anterior cingulate is the pivotal link between limbic and motor cortices, and may play an important role in complex motor functions subserved by the cerebral cortex such as emotional expression, attentional direction and motor memory. (Support: NS 14944 and PO 19632.)

34.12

CORTICAL INPUTS TO VISUAL (area 7a) AND TACTUAL (areas 7b & 5) SUBDIVISIONS OF POSTERIOR PARIETAL CORTEX IN RHESUS MONKEYS. J. Chmielowska, T.P. Pons and R.C. Saunders Lab Neuropsych. NIMH Bethesda, MD 20892.

Posterior parietal cortex (PPC) has traditionally been thought of as "association" cortex, where information from different sensory modalities is integrated. To determine the anatomical basis for such integration we injected fluorescent tracers, fast blue (FB), diamidino yellow (DY), and latex microspheres (LM) into electrophysiologically defined portions of visually and tactually responsive regions of PPC. A total of 16 injections were made into 8 hemispheres of 4 animals with a callosal commisurotomy. Cells projecting to 7a were found in the frontal eye field, in the depths of the superior temporal sulcus, and at the border region between visual areas PO and 18. Labeled cells were also observed in area 23 of cingulate cortex and areas TF\TH of the parahippocampal gyrus. Tactile sensory areas projecting to 7b included areas 1 & 2, SII, area 5, and the insula. Other cortical inputs to 7b included primary (area 4) and non-primary (areas 6 and 9) motor regions. In one case with an injection site located between areas 7a & 7b we observed connections characteristic of both areas. Finally, following injections of tracers into a tactually responsive region of area 5, labeled cells were seen in areas 1 & 2, 7b, and cingulate area 23. Our findings support recent electrophysiological studies which suggest that much of the PPC is modality specific.

34.13

SOME THALAMOCORTICAL CONNECTIONS OF AREAS 5, 7a AND 7b OF POSTERIOR PARIETAL CORTEX IN RHESUS MONKEYS. J. Duda, T.P. Pons, and R.C. Saunders. Lab Neuropsych. NIMH, Bethesda, MD 20892.

We compared the pattern of thalamic connections between visually and tactually responsive regions of the posterior parietal cortex (PPC) by injecting fluorescent tracers and tritiated amino acids into 5 monkeys (see prior abstract). Our results indicate that the major thalamic input to a tactually responsive region of area 5 is from the lateral posterior nucleus (LP). By contrast, another somatically responsive region of the PPC, area 7b, receives its primary input from the anterior pulvinar (Pa) and the rostral portion of the medial pulvinar (Pm) with a less dense input from the ventroposterior inferior nucleus (VPI). Major thalamic inputs to visual area 7a include Pm and caudal Pa, with less dense input from the suprageniculate and limitans nuclei. We did not observe label in LP following injections of 7a. These results indicate that at least two separate regions of PPC that are responsive to tactile stimulation receive inputs from different thalamic nuclei. Finally, since the border between Pa and Pm is not well defined cytoarchitectonically, our findings suggest that the rostral portion of Pm as clasically defined might better be redefined as the caudal portion of Pa. In this view, area 7b would receive its major thalamic input from Pa and none from Pm, while 7a would receive its major input from Pm, the suprageniculate and limitans nuclei, and none from Pa.

34.14

PATTERN OF CORTICAL LABELING FOLLOWING TRACER INJECTIONS INTO THE PULVINAR COMPLEX OF RHESUS MONKEYS. T.R.S. Johnston, T.P. Pons and R.C. Saunders. (SPON: R. Michaelis) Laboratory Neuropsychology, NIMH, Bethesda, MD 20892.

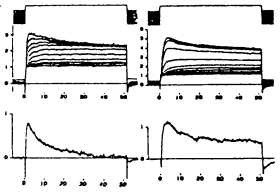
The pulvinar complex (PC) in rhesus monkeys can be divided into 4 classically recognized nuclei, the inferior (Pi), lateral (Pl), medial (Pm) and anterior or oral (Pa). While there has been a modest effort to study connections of visually responsive portions of the PC (Pi & Pl), there has been virtually no effort to study connections of more rostral portions (Pa & Pm). We have begun such a study by placing injections of different fluorescent tracers (see prior abstract) or tritiated amino acids into Pa and Pm of monkeys. Following injections of tracers into Pa dense cortical label was observed in several somatosensory related areas including SII, the insula, areas 2, 5, and 7b. Other densely labeled regions included the depths of the superior temporal sulcus (STS), rostral pole of the temporal lobe, the frontal eye field, cingulate cortex and TF\TH of the parahippocampal gyrus. Injections of tracers into Pm resulted in heavy labeling of visual areas 7a, the frontal eye field, and area 18. Dense label was also seen in areas TF\TH of the parahippocampal gyrus, cingulate cortex and the fundus and upper bank of the STS. These findings suggest that different nuclei of the PC have a distinct pattern of connections with modality specific cortical regions, as well as overlapping connections with multimodal cortical areas.

POTASSIUM CHANNELS I

35.1

POTASSIUM CURRENTS CHANGE IN CULTURE AND WITH DEVELOPMENT IN CHICK DORSAL ROOT GANGLION (DRG) NEURONS. C.D.Westbrook* and J.L.Kenyon* (SPON: C.ORT) Dept. of Physiol., Univ. of Nevada School of Med. Reno, NV 89577

We used the whole cell voltage-clamp to study K currents in DRG neurons isolated from 10, 14, and 18 day embryonic chickens. Pipettes contained K aspartate, ATP, creatine phosphate, and EGTA. The bath solution contained NaCl, TEA, and TTX but no added Ca. K currents during depolarizations from the resting potential showed little inactivation over 50 to 100 ms but did inactivate over 2 s. The top left panel of the figure shows currents (nA) from a 10 day neuron elicited after 2 s steps in the range -100 to 0mV. As conditioning voltage was made less negative the early current during the test pulse was reduced before the late current. The bottom left panel shows the difference between currents elicited from -80 and -100 mV. In cells from older embryos (right panels, 18 day) and in cultured 10 day cells, both the early and late currents were inactivated at the most negative conditioning voltages. These data suggest that the kinds of channels or the gating of K channels change with time in DRG neurons.



AHA 87648

35.2

NON-INACTIVATING K^+ CHANNELS ON GROWTH CONES, NEURITES, AND SOMATA OF LEECH RETZIUS CELLS IN CULTURE. S.R. Young, R.R. Stewart, Y. Liu , K.J. Muller and W.N. Ross. Dept. of Physiol., New York Med. Col., Valhalla, NY 10595; Dept. of Pharmac., Biocenter, Univ. of Basel, Basel, Switzerland CH-4056; Dept. of Physiol. & Biophys., Univ. Miami, Miami, FL 33101.

In order to examine the role of membrane ion channels in development and synapse formation, we have begun to apply standard techniques to record single channel activity in isolated leech neurons in culture (Hamill et al., Pfluegers Arch. 391:85, 1981; Bookman and Dagan, J. Physiol. 390:76P, 1987; Dietzel et al., J. Physiol. 372:191, 1986). At least four channel types were seen under steady-state voltage conditions with 125 to 140 mM KCl in the pipette. Two small channels of 10-15 and 20-30 pS were present in nearly all cell-attached patches. An intermediate channel with an inward conductance of about 30 pS and an outward conductance of 60 pS was also common in cell-attached patches. A larger channel of about 60 pS inward and 100 pS outward conductance was less common until the patches were excised, when it became dominant. The large channel was not permeable to Cl^- in inside-out patches. It was selective for K^+ over Na^+, and was only partially blocked by 50 mM internal TEA. Neither voltage changes nor 5 μM Ca^{++} internal markedly activated the large channel. All four channel types were seen on actively extending growth cones; none appear to be excluded from any other part of the cell.

Supported in part by the NIH and the Whitaker Foundation.

35.3

K-CHANNELS IN DISSOCIATED MUSCLE CELLS OF S. MANSONI (PLATYHELMINTHES). K. Blair, M. Lewis*, J. Bennett*, T. Day*, and R. Pax. Michigan State University, E. Lansing, MI 48824.

Muscle cells were isolated from adult S. mansoni by enzymatic and mechanical disruption of whole worms. Spindle-shaped muscle cells exclude trypan blue and contract when touched or exposed to high $[K^+]$. Cell-free (inside out) patches of sarcolemma contain multiple, non-inactivating K^+ conductances. Slope conductances of 20, 100, and 270 pS were observed in symmetrical KCl (130 mM)(0.4 mM Ca^{2+}). The 100 pS conductance is selective for K^+ over Na^+, as indicated by a shift in E_{rev} from 0 mV to -50 mV when bath (cytosolic side) K^+ was exchanged for Na^+. The probable open time (p_o) is sensitive to voltage (p_o=0.05 at -60 mV pipette potential, p_o= 0.98 for $\geq$0 mV). TEA (4 mM) in the bath reduced conductance at negative, but not positive, pipette potentials. Ba^{2+} (5 mM), and apamin applied to the cytosolic side reduced p_o. EGTA (EGTA:Ca^{2+}, 11:1) added to the cytosolic side decreased p_o from 0.95 to 0.05. The role of K^+ and other ion conductances in the primitive in muscle of S. mansoni is under study.

35.4

A LARGE-CONDUCTANCE ION CHANNEL IN THE TEGUMENT OF S. MANSONI (PLATYHELMINTHES). T. Day*, K. Blair, J. Bennett*, M. Lewis*, and R. Pax. Michigan State University, East Lansing, MI 48824.

The tegument of the parasite S. mansoni is a unique syncytial epithelium essential for nutrient transport, ion regulation and evasion of the host's immune system. Cell-free (inside-out) patches have been used to examine ionic conductances in the outer membrane of this tegument. A large-conductance ion channel (>230pS) was observed with 130mM KCl in the pipet and culture medium (125mM NaCl, 5mM KCl, 0.4mM Ca^{++}) in the bath. Channel activity was observed only when pipet potentials were more negative than -10mV. At those potentials, channel activity could be initiated or increased by +70mV pulses. An extrapolation of the I vs. V relationship predicted an E_{rev} of 0mV. E_{rev} and slope-conductance did not change when the culture medium in the bath was replaced with 130mM NaCl, 130mM N-methyl-glucamine chloride, or 130mM Na-gluconate.

35.5

SINGLE-CHANNEL RECORDING OF A POTASSIUM CURRENT ACTIVATED BY INTRACELLULAR SODIUM IN SENSORY NEURONS

C. Haimann*, and C.R. Bader, Département de Physiologie, Centre Médical Universitaire, 1211 Geneva 4 Switzerland.

Whole-cell recording in avian ganglionic neurons suggested the existence of a potassium current activated by intracellular sodium (Bader et al. Nature, 317:540-542). Here we describe the single-channel activity induced by sodium applied to the intracellular face of inside-out patches excised from quail trigeminal neurons. In the presence of sodium, channels of high conductance were seen (170 pS, with K_{in}=50 mM and K_{out}=150 mM), which showed no rectification between -75 and +50 mV. With physiological concentrations of potassium the conductance was 50 pS. Ion substitution experiments indicated that potassium ions were the major charge carriers of this current. Channel activity could be evoked in the presence of 12 mM sodium. Openings occurred in bursts and the burst frequency increased with Na concentration. Calcium, lithium and choline did not induce K_{Na} single-channel activity.

In neurons, whole-cell recording suggested that K_{Na} could be activated by a single action potential. Since this current can be activated already at low sodium concentrations, it is possible that K_{Na} could also contribute to the resting potential of neurons.

35.6

Voltage-Dependence of Fast and Slowly Inactivating A-Channels on Mammalian Sensory Neurons in Tissue Culture. *Sarah McFarlane*and Ellis Cooper. Dept. of Physiol. McGill Univ. Montreal, P.Q. H3G 1Y6*

We have recently found that A-channels on cultured neonatal rat nodose neurons inactivate in 3 different modes: a rapid mode where the channel opens once and then closes to an inactivated state, an intermediate mode where the channel opens and closes 3-4 times before inactivating, and a slow mode where the channel opens and closes several hundred times. Single channels gating in these 3 modes give rise to ensemble averages with time constants of 10-30 msec, 100-300 msec and 1-3 sec respectively. We find that the proportion of A-channels inactivating in any one mode varies from neuron to neuron, with the majority expressing channels of more than one type. While the single channel conductance for channels inactivating in the 3 modes is the same, the channels differ in their voltage-dependence. The I-V relationships for activation and the h_∞ curves for inactivation of the different A current components were fit by a Boltzmann distribution. Activation curves for the rapidly inactivating component had a $V_{1/2}$=-25mV and k=12, and $V_{1/2}$=-73mV and k=-6 for steady-state inactivation. The voltage-dependence of the slower inactivating components, however, was shifted approximately 25mV more positive and had values of $V_{1/2}$=-1mV and k=14 for activation, and $V_{1/2}$=-44mV and k=-14 for inactivation. Our results are consistent with the idea that nodose neurons express different subtypes of the same basic A channel. (Funded by MRC & FRSQ)

35.7

A STUDY OF ANOMALOUS (INWARD) RECTIFICATION IN FROG MYELINATED AXON. A.L. Padjen and M.O.Poulter. Dept. of Pharmacology and Therapeutics McGill University. Montreal, Canada, H3G 1Y6.

Anomalous or inward rectification (AR) was studied in intact large frog myelinated axons by single microelectrode current clamp. In response to a negative current step, which hyperpolarized the membrane 15-40 mV below resting membrane potential, all fibres showed a characteristic attenuation of the voltage response or a "sag" after an initial peak voltage deflection (400 to 600 ms after the start of the pulse; cf. Padjen & Hashiguchi, 1983, Can J Physiol Pharm 61, 626). In addition, an afterdepolarizing potential (ADP; 1-5 mV in amplitude; 2-10 s in duration) was evident following a current pulse in which inward rectification had been activated. The "sag" and ADP was abolished by external application of cesium ions (3 mM) but not by barium ions (2 - 10 mM). Reducing the potassium concentration in the perfusate increased the slope resistance calculated from the peak voltage response and at steady state; replacing sodium ions with choline in the perfusate increased the steady state resistance and decreased or abolished the ADP, suggesting that the AR is a mixed potassium and sodium conductance.

The experimental results were incorporated in a computational model of myelinated axon membrane based on Hodgkin-Huxley expressions. The model required that the AR activates near -100 mV, with both a short and a long time constant (4-7 and 450-600 ms, respectively) and a reversal potential near -55 mV.

Supported by the MRC of Canada.

35.8

THE SLOW-ACTIVATING SLOW-INACTIVATING POTASSIUM CURRENT IN MELANOTROPHS OF THE RAT PITUITARY. S.J. Kehl, Department of Physiology, University of British Columbia, Vancouver, Canada, V6T 1W5

Whole cell recordings were made from cells in which I_{Na} and I_{Ca} were blocked with TTX (1 micromolar) and Cd (300 micromolar). Under these conditions the outward current consisted of two components: a fast-activating fast-inactivating I_K ($I_K(f)$) (Kehl, S.J., J. Physiol., 411: 457, 1989) and a slow-activating slow-inactivating I_K ($I_K(s)$). The activation threshold for $I_K(s)$ was normally between -10 to 0 mV and it showed voltage-dependent sigmoidal activation kinetics in cells where its onset was not obscured by $I_K(f)$. The current-voltage relationship for $I_K(s)$ was monotonic with a maximal slope conductance of 26±6 nS (n=8). The reversal potential estimated from tail currents was near -75 mV in cells where the calculated value for E_K was near -90 mV; in symmetrical K concentrations the reversal potential was near 0 mV. $I_K(s)$ showed little or no inactivation during depolarizing commands lasting for up to 200 ms and it was reversibly blocked by quinidine sulphate (50 micromolar), TEA (20 mM) and Ba (10 mM).

Supported by a grant from the BCHCRF.

35.9

TWO A-CURRENTS IN HYPOTHALAMIC HISTAMINE NEURONS Peter B. Reiner, Robert W. Greene and Helmut L. Haas*. Kinsmen Laboratory of Neurological Research, Univ. of British Columbia, Vancouver, BC, Canada; Dept. Psychiatry, Harvard Medical School & VA Medical Center, Brockton, MA; Dept. Physiol., Univ. of Mainz, FRG.

Histamine neurons of the rat tuberomammillary nucleus exhibit remarkably long-lasting transient outward rectification. The present study was undertaken to characterize this phenomenon using the single-electrode voltage clamp technique applied to an *in vitro* slice preparation of the rat hypothalamus. The transient outward current was inactive at the resting potential, and removal of inactivation was both time- and voltage-dependent. The decay of the current was best fitted by two exponentials with time constants of 107±41 and 559±134 ms. 1 mM 4-AP blocked only the rapidly decaying component. 10 mM TEA reduced the slow component slightly, and blocked the non-inactivating outward current(s) seen with depolarizing steps from -50 mV. Removal of calcium from the medium reduced the amplitude of the slow component slightly, and addition of nimodipine further depressed the slow current. The reversal potential of the fast component was -110 mV in 2.5 mM external K^+, while the slow component reversed at -92 mV. Activation of the slow component occurred some 5-10 mV positive to that of the fast component, while removal of inactivation of the slowly decaying current was shifted 5-10 mV negative. It is concluded that histamine neurons possess two transient outward currents which can be distinguished on the basis of their inactivation kinetics, 4-AP sensitivity, calcium dependence, reversal potentials, and voltage-dependence of inactivation.

35.10

MULTIPLE COMPONENTS OF VOLTAGE-DEPENDENT POTASSIUM CURRENT IN RAT ANTERIOR PITUITARY CELLS. J. Herrington* and C. Lingle. Dept. of Anesthesiology, Wash. Univ. Sch. of Med., St. Louis, MO 63110.

Voltage-dependent potassium (K^+) current was studied using whole-cell clamp techniques on enriched populations of lactotrophs isolated from lactating rats. Pipette and bath salines were chosen to exclude inward currents and Ca^{+2}-dependent K^+ and Cl^- currents.

Total Ca^{+2}-independent K^+ current could be clearly separated into three components based on pharmacological sensitivities and the differential effects of conditioning pulse level and duration on potassium tail currents. The 3 currents are: 1) a lower threshold, rapidly inactivating current completely blocked by 5 mM 4-aminopyridine (4-AP) but insensitive to TEA (30 mM); 2) a slowly inactivating current sensitive to TEA and 4-AP; and 3) a sustained current sensitive to TEA but not to 4-AP. The rapidly inactivating current activates above about -40 mV, is half-inactivated at -97.9 mV (+ /- 8.6; n= 5), and inactivates with τ = 20-30 msec at -30 mV. Deactivation is fast with τ = 1-2 msec at -50 mV. Holding at -40 mV completely inactivates the current and removes the fast component of tail current. Another inactivating current is activated above about -20 mV and inactivates over hundreds of msec at + 60 mV. Deactivation of this current is also distinct from the lower threshold current with τ = 6-8 msec at -50 mV. The 3rd component of current is also activated above about -20 mV and inactivates over seconds. Deactivation is also very slow with τ = 30-40 msec at -50 mV.

All three currents were also found in prolactin-secreting cells identified by the reverse hemolytic plaque assay. The differential expression of these currents among lactotrophs may contribute to the variation in responses evoked by secretagogue application to single cells. Supported by NIH-DK-37109.

35.11

ANOMOLOUS COUPLING BETWEEN I_A AND I_J IN LOBSTER STOMATOGASTRIC NEURONS. B.R. Jones and D.K. Hartline. Békésy Laboratory of Neurobiology, University of Hawaii, Honolulu, HI 96822.

Many stomatogastric somata exhibit two outward currents (Graubard, K. and Hartline, D.K., Soc. Neurosci. Abstr. 10:1073, 1984): one is transient, voltage-activated, and 4AP-sensitive (I_A); the other is transient, Ca^{++}-dependent, and Cd^{++}- and TEA-sensitive (I_J). Last year (Hartline, D.K., et al., Soc. Neurosci. Abstr. 14:946, 1988) we reported that I_J tail currents show strong outward rectification, with a reversal potential dependence on $[K^+]_o$ that is only about half that expected for a pure K^+ current. We now report that:

1) I_A tail currents in these cells also show strong outward rectification, with a reversal potential dependence on $[K^+]_o$ that is less than 58 mV per decade.

2) I_A magnitude at a given voltage (measured by curve subtraction) is dependent on I_J. When I_J is reduced with Cd^{++} or TEA the apparent magnitude of I_A increases. The increase in I_A is directly proportional to the reduction in I_J magnitude. The constant of proportionality varies from cell to cell (range = 0.2 to 0.7).

3) Magnitudes of both I_A and I_J are reduced by replacing 90% of the external NaCl with mannose.

These properties seem consistent with a model which includes a shared, local, rectifying series resistance for A and J channels, and a shared external space having a higher $[K^+]$ than the bathing medium.

Supported by NIH grant NS15314 to DHK and a fellowship from the Ida Russell Cades Fund to BRJ.

35.12

CELL-SPECIFIC TEMPERATURE ACCLIMATION OF I_A CURRENTS IN IDENTIFIED NEURONS IN APLYSIA CALIFORNICA. A.J. Grant and S.N. Treistman. The Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

I_A is important in regulating action potential production, and exhibits temperature-sensitive kinetics and amplitude. We examined the effects of whole animal temperature acclimation on the subsequent temperature sensitivity of I_A in identified Aplysia californica neurons. Sibling cohorts of Aplysia were reared following metamorphosis at two different temperatures (10 and 20 °C). When animals were approximately two months old, I_A was measured from two sets of identified cells; B_1 or B_2 in the buccal ganglion and L_2, L_3, L_4, or L_6 (left upper quadrant cells) in the abdominal ganglion. In buccal cells, I_A amplitude at any given temperature was significantly greater in animals reared at the colder temperatures, compared with those raised in warmer conditions, conforming with classical acclimation. Cell size was also larger in the cold-reared animals, and when current was normalized to cell size, the resulting computed current density was the same in the two groups of buccal cells. Kinetics of I_A were not affected by rearing temperature in the buccal cells. In the LUQC, neither I_A amplitude nor cell size was dependent on rearing temperature. Thus, acclimation of I_A is cell-specific and occurs by changes in cell size rather than alteration of individual channel properties.

35.13

CHARACTERIZATION OF POTASSIUM CHANNELS OF *HERMISSENDA* PHOTORECEPTORS. R. Etcheberrigaray*, P.L. Huddie and D.L. Alkon. LMCN-NINDS, NIH, Bldg 9, Rm 1w125, Bethesda, MD 20892.

Reduction of potassium conductances of the type B photoreceptors of the nudibranch mollusc *Hermissenda crassicornis* contributes to the expression of the behavioral modification induced by associative conditioning. The K^+ currents of the soma membrane that are modified by conditioning include a Ba^{2+}-sensitive Ca^{2+}-dependent K^+ current (I_c), and a fast transient K^+ current (I_A). We have recorded several classes of steady state single K^+ channels in membrane patches from protease treated *Hermissenda* photoreceptors *in vitro*. One or more types of channel was usually seen in each patch; a ubiquitous small channel (γ < 10 pS), a medium conductance Ba^{2+}-sensitive channel (γ = 44 ± 2.9 pS; n = 6), and a large conductance channel (γ = 79 ± 5.8 pS; n = 11); a very large conductance channel was seen infrequently ($\gamma \approx$ 300 pS; n = 3). The 79 pS channel had mean open time 6.6 ± 33 ms, mean closed time = 56 ± 241 ms (means ± std. deviations); 2 open and 2 closed states were apparent from the open and closed time histograms. When $[Ca^{2+}]_i$ = 10 mM the Ba^{2+}-sensitive channel had an open probability (p_o) of 0.35; mean open time of outward currents was 8.6 ± 35 ms, and mean closed time was 15.5 ± 64.5 ms . Probability density functions derived from > 3300 events, reveal 2 open and 2 closed states; for the open state τ_1 = 1.8 ms, and τ_2 = 16.5 ms; for the closed state τ_1 = 6.5 ms, and τ_2 = 835 ms. With 4mM Ba^{2+} the mean open time was 4.9 ± 21 ms, and mean closed time was 27 ± 125 ms: Ba^{2+} appears to block the open state since τ_1 = 0.1 ms and τ_2 = 5.6 ms; for the closed state τ_1 = 8.9 ms, and τ_2 = 1335 ms. Ba^{2+} reduced p_o to 15% and reduced event frequency from 16 s^{-1} to 12 s^{-1}; this implies Ba^{2+} also inhibits the closed state of I_c. These single channel properties can now be assessed as a function of conditioning.

35.14

FAST, TRANSIENT K^+ CURRENT IN NEUROHYPOPHYSIAL NERVE TERMINALS. J.R. Lemos and P.J. Thorn*. Worcester Found. Expt. Biol., Shrewsbury, MA 01545.

Nerve terminals of the rat posterior pituitary were acutely dissociated and studied under whole-cell patch clamp. In physiological solutions depolarizing steps from a holding potential (H.P.) of -80 mV elicited a fast inward followed by a fast, transient outward current. The outward current reached a peak and then decayed to a steady but non-zero level. The decay was best fit by two exponentials with time constants 21.1 ±2.85 and 143.02 ±36 ms. At the above H.P. the threshold of activation of the outward current was -60 mV. The time to peak of this current decreased and the amplitude increased with increasing depolarized potential steps. The transient outward current showed steady-state inactivation at more depolarized H.P. (1/2 maximal at -50 mV). The time course of recovery from inactivation was complex, full recovery taking more than 16 s. The outward current was blocked by the substitution of K^+ with Cs^+ and its reversal potential was consistent with a potassium current. 4-AP (1-6 mM) blocked the transient K^+ current but charybdotoxin (4 μg/ml) had no effect. Low $[Ca^{++}]_o$ had no effect on the outward current, nor did the calcium channel blocker Cd^{++}. The neurohypophysial terminal transient outward K^+ current thus appears to be I_A and is different from the outward currents found in the corresponding cell bodies. (Supported by grant NS22542)

35.15

CALCIUM SENSITIVITY OF TWO LARGE CONDUCTANCE POTASSIUM CHANNELS IN CEREBELLAR PURKINJE NEURONS. D.L. Gruol and T. Jacquin*, Res. Inst. Scripps Clinic, La Jolla, CA 92037.

Cerebellar Purkinje neurons (PNs) in culture express two large conductance voltage-sensitive K+ channel types (Gruol et al, Soc. Neurosci. Abst. 14, 1988). Mean single channel conductances are 90 and 70 pS under physiological conditions and 220 and 134 under symmetrical K+ conditions. Both channel types are activated by depolarizations from a holding potential of -60 mV. The 70 pS channel type is active at depolarizations of 20 to 80 mV; the 90 pS channel is active at stronger depolarizations. Both channel types show similar sensitivity to K+ channel blockers. In the present series of experiments, the activity of these channel types was compared in cell-attached (CA; n=69), outside-out (O/O; n=59) and inside-out (I/O; n=25) patches. The 90 pS channel type was observed in 67% of the CA, 44 % of the O/O and 84% of the I/O patches. In contrast, the 70 pS channel type was observed in 58% of the O/O patches but only 19% of the CA and 19% of the I/O patches. In O/O patches, the frequency of detecting each channel type varied with the internal level of free Ca++. The 70 pS channel type was observed in 38% of the patches with 50 nM internal Ca++ (n=21) compared to 87% of the patches with 500 nM internal Ca++ (n=15). The 90 pS channel type was observed in 67% of the patches with 50 nM internal Ca++ compared to 27% of the patches with 500 nM internal Ca++. In I/O patches, the activity of the 90 pS channel dramatically increased as internal Ca++ was raised from 10 to 200 nM. These data indicate that the activity of both channel types is influenced by the level of intracellular Ca++. Supported by NS 21777 and the French Ministry.

35.16

PROPERTIES OF UNITARY POTASSIUM CHANNELS IN CULTURED ADULT HUMAN OLIGODENDROCYTES. J. G. McLarnon and S. U. Kim. Dept. of Pharmacology & Therapeutics and Div. of Neurology, Dept. of Medicine, The University of British Columbia, Vancouver, B.C. V6T 1W5, Canada.

In this study the properties of a potassium inward rectifier channel have been studied in cultured oligodendrocytes obtained at autopsy from adult human brain. With the cell-attached patch clamp configuration and a K^+ concentration of 140 mM in the pipette, inward K^+ currents were recorded with amplitudes increasing with patch hyperpolarization. Over the voltage range studied, the current-voltage relation was linear and the conductance of the channel was 21 pS. The mean channel open time showed an exponential dependence on potential with a hyperpolarization of 20 mV associated with an e-fold decrease in the mean open time. The probability of channel opening showed a sigmoid dependence on patch potential and was decreased with hyperpolarization. The voltage-dependent properties of the channel gating would be consistent with a role for the inward rectifier channel in the human oligodendrocytes as part of a regulatory mechanism in the maintenance of K^+ concentrations in the intercellular cleft. The channels, which passed inward but not outward current, were open for relatively long times at the normal cell resting potentials (mean open times of about 75 ms at the resting level) and the mean open times were increased with depolarization. In particular, the latter property could be important during periods of high neuronal activity to limit the accumulation of extracellular K^+.

35.17

EVIDENCE THAT A BARIUM-SENSITIVE POTASSIUM CURRENT CONTRIBUTES TO THE RESTING POTENTIAL AND SPIKE REPOLARIZATION IN RAT HIPPOCAMPAL NEURONS.

J.F. Storm and M. Helliesen*. Institute of Neurophysiology, Karl Johans gate 47, N-0162 Oslo 1, Norway.

Little is known about the ion channels generating the resting potential in vertebrate central neurons. Here we report evidence that a Ba-sensitive Ca-independent K current ($I_{K,rest}$) mediates at least 50-75 % of the resting conductance in CA1 neurones, and also contributes to the repolarization of the action potential. $I_{K,rest}$ seems to be distinct from seven K currents already postulated in the hippocampus (I_M, I_A, I_K, I_D, I_C, I_{AHP}, and the muscarine-sensitive "leak" K current), as well as the mixed Q-current.

CA1 pyramidal cells (n=14) in rat hippocampal slices were impaled with KCl-filled micro-electrodes, at 28-32°C. The input resistance (R_{inp}) was measured by injecting current pulses (200-400 ms duration), and action potentials were elicited by brief depolarizing pulses (2-10ms). Barium (0.2 - 1 mM) produced depolarization, increased R_{inp}, and spike-broadening, even when I_{Ca}, I_C, I_{AHP}, I_M, I_D, and/or I_Q had been eliminated prior to Ba, by adding 0.2-0.6 mM Cd, 100 µM carbachol, 40 µM 4-amino-pyridine and/or 3mM Cs to the bath. In the presence of Cd and carbachol, Ba induced spontaneous repetitive bursting. Current/voltage (I/V) plots indicated that $I_{K,rest}$ is active at least from -110 to -40 mV, and reverses near -95 mV in Cl-loaded cells. Thus, the activation range of $I_{K,rest}$ differs from those of I_M (> -60 mV), I_K (> -40 mV), I_A (> -60 mV), I_D (> -70 mV) and I_Q (< -80 mV); I_K, I_D and I_Q are also less sensitive to Ba (Halliwell & Adams 1982, Brain Res. 250, 71-92; Storm 1988, Nature 336, 379-381).

(Supported by the Norwegian Research Council and Nycomed. The effects of $I_{K,rest}$ were first noticed by JFS in P.R.Adams' laboratory, at SUNY, Stony Brook in April 1987. Thanks to P.Andersen, Oslo, for lab. space and support).

35.18

THE EFFECTS OF CESIUM ON THE SYNAPTIC AND INTRINSIC PROPERTIES OF NEOCORTICAL NEURONES MAINTAINED "IN VITRO".

G.G.C.Hwa & M.Avoli. MNI, McGill Univ., Montreal, Que., Canada.

We have observed that Cs^+ potentiates epileptiform discharges in rat neocortical slices (Avoli & Hwa, this meeting). Here, we recorded intracellularly from neurons in layers I-III to examine the mechanisms responsible for this increase in excitability. Bath application of Cs^+ (2-3mM) usually depolarized the cell by 5-15 mV, and disclosed spontaneous EPSP-IPSP. I-V plots indicated that Cs^+ increased the input resistance measured by injecting hyperpolarizing pulses. However, a sag which resembled the anomalous rectifier was still discernible (13/15 cells). This suggests that in these superficial cells, there is a component of rectification activated by membrane hyperpolarization that is insensitive to Cs^+. Other effects of Cs^+ include a prolongation of the action potential duration evoked by brief pulses of intracellular current, and a reduction of the IPSPs elicited by white matter stimulation. While the Cs^+ effects on the intrinsic and synaptic conductances may account for an increase in excitability, the significance of these changes in relation to epileptogenesis remains to be determined.

35.19

AXONAL CONDUCTION AND EXTRACELLULAR POTASSIUM ACTIVITY IN SPINAL CORD OF THE MYELIN-DEFICIENT MUTANT RAT.

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The myelin-deficient (md) mutant rat has a gross deficincy of central myelin. We recorded somatosensory evoked potentials (SEPs), extracellular K^+ ion activity ($[K^+]_e$), and K^+ clearance rates in the spinal cords of 14 md rats and 16 normal siblings (ns). Under pentobarbital anesthesia (25 mg/kg i.p.) and hypothermia (32-34°C), both groups had delayed cortical SEP latencies: 67±20 msec in md rats vs. 48±15 msec in ns rats ($p<0.05$). Resting $[K^+]_e$ levels were 2.6±0.8 mM and 2.6±0.5 in the md and ns groups. K^+ clearance rates were similar in both groups. Sciatic nerve stimulation (2-20 Hz, 2-6 second trains) produced 1-3 mM transient $[K^+]_e$ rises in the md spinal cords and no or very small rises in the normal cords. The $[K^+]_e$ rises were largest in dorsal horn at 200-500 µm depth. In md rats, injections of 1 mM 4-aminopyridine (4-AP) solution into the spinal cord completely suppressed the $[K^+]_e$ responses and increased spinal and cortical SEP amplitudes for 2-3 hours. In ns rats, 4-AP injections blocked spinal conduction for 20-30 minutes but thereafter enhanced cortical SEP amplitudes for 2-3 hours. We conclude that sciatic nerve stimulation produce larger spinal cord $[K^+]_e$ responses in md rats than in ns rats, that the $[K^+]_e$ rises result from greater K^+ release rather than impaired K^+ clearance, and that the K^+ ions come from 4-AP blockable sources.

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35.20

SODIUM DEPENDENT ACTION POTENTIALS ELICIT A POTASSIUM DEPENDENT AFTERHYPERPOLARIZATION IN FROG MYELINATED AXON.

M.O. Poulter and A.L. Padjen. Dept. of Pharmacology and Therapeutics, McGill University. Montreal, Canada, H3G 1Y6.

Blockade of voltage dependent potassium conductances in myelinated axons results in an increase in excitability, observed as trains of action potentials lasting a maximum of 100 ms in response to depolarizing constant current steps. We have observed that these pulses are followed by a 50-100 ms long afterhyperpolarizing potential (AHP). We are reporting some of its properties.

Intracellular recordings were made from the large myelinated axons impaled at the entry zone of dorsal roots in isolated hemisected frog spinal cord continuously superfused with Ringer solution (at 14°C). Microelectrodes contained either 4-aminopyridine (4-AP, 5-10 mM) or 4-AP and tetraethylammonium (TEA, 5-25 mM).

The AHP was absent when stimulation was below the threshold for the generation of one spike. The magnitude of the AHP increased proportionally with the number of spikes, up to a maximum of some 4 mV. No AHP was observed after blockade of action potential generation with 250 nM tetrodotoxin. The AHP was unaffected by reducing extracellular calcium and adding 10 mM magnesium or 2 mM manganese to the perfusing solution. The AHP reversal was around -95 mV at resting membrane potential. Increasing the external potassium concentration from 2 to 10 mM shifted the reversal potential 10-15mV in positive direction. Similar AHP was observed in experiments on isolated dorsal roots.

These results are consistent with the presence of a sodium-activated potassium conductance in frog myelinated axons, insensitive to intracellular 4-AP and/or TEA, which may be important for the regulation of repetitive activity in myelinated axon. Supported by the MRC of Canada.

35.21

ELECTROGENIC PUMP ACTIVITY IN RAT OPTIC NERVE.

T.R. Gordon, J.D. Kocsis, S.G. Waxman, Dept. of Neurology, Yale Med. Sch. and VA Med. Ctr., West Haven, CT. 06516.

Electrogenic (Na^+/K^+) pump activity can hyperpolarize sites of demyelination; therefore its block may improve conduction (Bostock and Grafe J Physiol 365:239-257, 1985). We investigated electrogenic pump activity in rat optic nerve. An afterhyperpolarization (AHP) up to 4.8 mV, >30 sec., was elicited by repetitive whole nerve stimulation in a sucrose gap chamber. The K^+ channel blocker tetraethylammonium (TEA) and the Ca^{2+} channel blocker $CoCl_2$ did not alter the AHP amplitude, but the K^+ channel blocker 4-aminopyridine alone and in combination with TEA increased AHP amplitude. Passage of hyperpolarizing and depolarizing constant current during stimulation did not alter the AHP amplitude, indicating that activation of K^+ channels is not responsible for the AHP. The AHP varied directly with temperature and was blocked by strophanthidin and by Li^+ substituted for Na^+. The response to a submaximal stimulus during the AHP was smaller than that at rest, an effect reversed by strophanthidin, which blocks Na^+/K^+ ATPase. The results indicate that the post-tetanic AHP in rat CNS myelinated axons is due to electrogenic pump activity that can increase threshold, mediated by Na^+/K^+ ATPase; this might lead to conduction block in demyelinated fibers with decreased safety factor and may be reversed by cardiac glycosides. Supported by NMSS, NIH, VA.

35.22

POTASSIUM CHANNEL FAMILY IN THE JELLYFISH, AGLANTHA.

R.W. Meech*, S.A. Arkett, G.O. Mackie*, & N.J. Maitland*. Departments of Pathology & Physiology, University of Bristol, UK. and Department of Biology, University of Victoria, Canada.

Jellyfish are the simplest animals to have evolved a nervous system and Aglantha is particularly interesting as it has an escape mechanism which is absent in other jellyfish. Both slow and fast ("escape") swimming arise from contractions in a single sheet of muscle innervated by a single set of motor axons. Differences in the force of contraction arise from differences in the electrical events generated postsynaptically by two different propagating impulses. Slow swimming depends on a low amplitude Ca++ spike; fast swimming depends on an overshooting Na+ dependent action potential (Mackie & Meech, 1985).

The Ca++ spike is prevented from reaching the threshold of the Na+ spike by a rapidly activating outward current. Patch clamp analysis of the K+ selective channels that form the basis of this current shows them to be steeply voltage dependent. Four classes of K+ channel have been identified. Each channel has different kinetics but they have the same conductance (43pS in 250mM KCl) and may be considered to be members of the same family (Mackie & Meech 1989). We have identified several fragments of Aglantha gDNA that hybridize with Shaker (ShA1, kindly supplied by Drs. Y&L Jan, Univ. of California).

36.1

CULTURED POSTNATAL RAT HIPPOCAMPAL NEURONS HAVE A Ca^{2+} INDEPENDENT AND VOLTAGE DEPENDENT Cl CURRENT. J. Yang, G.D. Clark, L.L. Thio, D.B. Clifford, and C.F. Zorumski, Dept. of Psychiatry, Washington Univ. Sch. Med., St. Louis MO 63110

Adult rat hippocampal pyramidal neurons in slices possess a time and voltage dependent Cl conductance largely localized on the dendrite (Madison, D.V., et al., Nature, 321:695, 1986). Using the whole cell mode of the patch clamp technique, we have found a similar conductance in postnatal rat hippocampal neurons cultured for 3 hours to 7 days. When E_{Cl} is 0mV, voltage steps to potentials more negative than -50mV from holding potentials more positive than -10mV evoke slow inward relaxations. Current activation is slow requiring 3-7s to reach a peak amplitude. At voltages more negative than -100mV, the relaxation sometimes declines in amplitude after reaching its peak. A tail current having a duration of several seconds occurs at the end of the voltage step. Although the current is inhibited by 400μM Cd^{2+}, the current is observed in cells simultaneously bathed with a solution containing no added Ca^{2+} and intracellularly dialyzed with a strong Ca^{2+} buffer. This result indicates that the conductance is not Ca^{2+} dependent. The properties of this Cl conductance suggest that it is located on the somas of cultured postnatal rat hippocampal neurons and may contribute to the resting membrane potential of these neurons.

36.2

ETHYLENEDIAMINETETRAACETIC ACID (EDTA) STIMULATES A Ca^{2+}-DEPENDENT, PICROTOXIN-INSENSITIVE Cl^- UPTAKE IN RAT BRAIN VESICLES J. Mathew* & G.D. Frye (SPON: B.Rohde) Dept. Med. Pharmacol., Texas A&M Col. of Med., College Station, TX 77483

During studies of Ca^{2+}, ethanol and sialic acid modulation of $GABA_A$-mediated Cl^- uptake, the cation chelator EDTA showed marked stimulation of Cl^- uptake into brain vesicles. The present report characterizes EDTA-stimulated Cl^- uptake and compares it with that induced by muscimol. Incubation of EDTA (0.5-2.5mM) with vesicles and $^{36}Cl^-$ for 10s induced dose-related increases in net Cl^-uptake with a maximum uptake of 33.9±0.6 nmoles/mg protein above basal levels. Maximal net Cl^- influx stimulated by EDTA was similar to that stimulated by a maximal concentration of muscimol (25uM); the effects of EDTA and muscimol were additive. Neither picrotoxin (100uM) nor strychnine (100uM) altered uptake induced by EDTA but picrotoxin completely blocked that induced by muscimol. Removal of Ca^{2+} completely blocked EDTA-stimulated Cl^- uptake but not that induced by muscimol. Readdition of Ca^{2+} (0.01-10mM) caused a graded reinstatement of EDTA (1mM) induced Cl^- uptake which was maximal at Ca^{2+} (1mM) and then decreased with higher Ca^{2+} (2.5-10mM). Cd^{2+} and La^{3+}, but not Ni^{2+}, Co^{2+} or Mn^{2+} blocked EDTA-stimulated Cl^- uptake. Of the more Ca^{2+} selective chelators, EGTA (0.5-2.5mM) and BAPTA (0.5-10mM), only EGTA induced Ca^{2+}-dependent Cl^-uptake. These results suggest that EDTA stimulates a Ca^{2+}-dependent, picrotoxin-insensitive influx of Cl^- that may not depend on external chelation of Ca^{2+}. (Supported by AA06322 & AA00101)

36.3

EFFECTS OF K^+ ON ANION AND CATION TRANSPORT IN ASTROCYTES AND NEURONS. S.Y. Chow*, Y.C. Yen-Chow*, H.S.White* and D.M. Woodbury Depts of Physiology and Pharmacology, Univ. of Utah, Salt Lake City, UT 84108.

Effects of K^+ concentration in medium ($[K^+]_o$) on the intracellular concentration of Cl^-, I^- ($[Cl^-]_i$ and $[I^-]_i$, derived from $^{36}Cl^-$ and $^{125}I^-$ studies), HCO_3^- (derived from measurements with ^{14}C-DMO), Na^+ and K^+ were studied in primary cultures of mouse astrocytes and neurons. In astrocytes, as $[K^+]_o$ was increased ($[Na^+]_o$ was decreased to maintain the same osmotic pressure), $[Cl^-]_i$, $[I^-]_i$, $[HCO_3^-]_i$ and $[K^+]_i$ all increased, but $[Na^+]_i$ decreased. Changes in $[Cl^-]_i$, $[I^-]_i$ and $[HCO_3^-]_i$ paralleled those with $[K^+]_i$ and were inversely related to $[Na^+]_i$. Changes of $[Cl^-]_i$, $[Na^+]_i$ and $[K^+]_i$ were much greater than those of $[I^-]_i$ and $[HCO_3^-]_i$. The changes in intracellular concentration of ions were more prominent in $[K^+]_o$ between 1.2 and 4.2 mM and between 60 and 122 mM. In neurons, altering $[K^+]_o$ produced biphasic changes in $[Cl^-]_i$ and $[I^-]_i$ and triphasic changes in $[Na^+]_i$. In lower $[K^+]_o$ (1.2 - 5.4 mM), $[Cl^-]_i$ and $[I^-]_i$ decreased as the $[K^+]_o$ increased whereas in higher $[K^+]_o$ (5.4 - 122 mM), $[Cl^-]_i$ and $[I^-]_i$ were directly proportional to the $[K^+]_o$. In addition, in lower $[K^+]_o$ (1.2 - 10 mM) changes in $[Cl^-]_i$ and $[I^-]_i$ paralleled those with $[Na^+]_i$, whereas in higher $[K^+]_o$ (10 - 122 mM) changes in these two anions paralleled those with $[K^+]_i$. Changes in $[HCO_3^-]_i$ paralleled those with $[K^+]_i$ at all $[K^+]_o$. These studies demonstrate that anion and cation transport in astrocytes and neurons are different. (NIH Grant #5R01 NS 21834).

36.4

BRADYKININ AUGMENTS CHLORIDE CURRENT IN T-LYMPHOCYTES VIA A RISE IN INTRACELLULAR CALCIUM. M.A. Schumann*, D.B. Maldonado* and P. Gardner. Dept. of Medicine, Stanford Univ. Sch. of Medicine, Stanford, CA 94305.

Bradykinin (BK), a potent vasoactive and pain impulse-involved peptide, has been known to be involved in inflammation and cellular immune response. Contrary to its well defined action on some neurons, its action on T-lymphocytes is unknown. We have used a tight seal-whole cell recording to investigate the mechanism by which BK affects a chloride current in Jurkat lymphocytes. Perfusion of the bathing solution with BK induced, with an onset of 0.5 to 1 minute, a dose- and time-dependent increase in current peak amplitude. The peak response, with an increase of 43.5%±7.5 (n=7), was attained in 6 minutes by $2x10^{-5}$M. In addition, the time constant of the current decay was prolonged 1.5 to 12 times. The effect of BK was mimicked by bath application of $1x10^{-7}$M Ca^{2+} ionophore (A23187). Diisothiocyanostilbene-2,2'-disulfonic acid (DIDS, $5x10^{-5}$M), a blocker of anion transport included in the pipette solution, blocked the action of BK and of A23187. Current enhancement by BK was dependent on Ca^{2+} since bathing the cells in a solution containing 5 to $25x10^{-6}$M bis-(o-aminophenoxy)-ethane-N,N,N',N' tetra-acetic acid, acetoxymethyl ester (BAPTA, AM, a Ca^{2+} chelator), resulted in complete elimination of the response to $4x10^{-5}$M BK or to $1x10^{-6}$M A23187. Furthermore, BK (0.01 to $1x10^{-4}$M) increased intracellular $[Ca^{2+}]$ up to two-fold as measured by the mean violet:blue fluorescence ratio of Indo-1-loaded Jurkat cells. The data are consistent with the mediation of intracellular Ca^{2+} in augmenting the chloride current in T-lymphocytes by BK.

36.5

REGULATION OF A RECTIFYING CL^- CHANNEL FROM JURKAT LYMPHOCYTES BY TEMPERATURE AND 8Br-cAMP. S. S. Garber and R.W. Aldrich, Dept. of Neurobiology, Stanford Univ., Stanford, CA 94305-5401

We have studied a rectifying Cl^- channel found in human Jurkat T-lymphocytes. This Cl^- channel can be activated by excising a patch of membrane using the inside-out (i/o) patch-clamp configuration and holding at depolarized voltages for prolonged periods of time (1-6 min at +80 mV, 20°C). The single channel current at +80 mV is 4.5±0.3 pA. The single channel amplitude is complicated by the presence of subconductance states. The ratio of current at +80 mV to -80 mV 4.3±0.7. This rectifying I/V relationship was used as a marker for Cl^- channel activity. 20% of attempts made at 20°C resulted in Cl^- channel activity. Patches usually showed only a single active channel. In contrast, 50% of i/o-excised patches showed multiple Cl^- channel activity when the temperature was increased to 30°C. There was no marked change in the single channel conductance or in appearance of substates at 30°C. Once active, the probability of being open (P_o) is voltage-independent but mean open and closed times are voltage-dependent such that P_o is constant. These observations suggest that the # of channels available to be activated depends on temperature. Channels observed in the cell-attached, or i/o configuration in cells treated with 100 µM 8Br-cAMP (20°C) exhibit shorter mean open and closed times. The gating kinetics observed in 8Br-cAMP-treated cells are distinct from excised, voltage-activated Cl^- channels suggesting 8Br-cAMP may regulate the gating of the channel or the recruitment of a different channel type. Supported by NS23294, National Cystic Fibrosis Foundation RDP and NCF fellowship to SSG.

36.6

ELECTROPHYSIOLOGICAL BEHAVIOR OF PLASMA CELLS FROM CHICKEN LACRIMAL GLANDS. P.R.Brink, E.J.Roemer*, S.Fan* and B. Walcott. Dept. of Anat. Sci., SUNY, Stony Brook, NY 11794.

Whole cell, detached and attached patch clamp modes were used to investigate the membrane channels of plasma cells isolated from chicken Harderian glands. Whole cell recordings show no inward current, but there is a large, slowly inactivating outward current, carried primarily by K ions, which is increased by perfusion with uM carbachol. In whole cell mode, with 100 mM Ba^{++} and 10 mM TEA, there is a small "inward current", which is reduced by verapamil, indicating the presence of Ca^{++} channels. In current clamp mode, the cell resting potential hyperpolarizes within 60s of exposure to 1 uM carbachol. Single channel records obtained from inside-out patches have revealed a dominant maxi-K channel with a 230 pS unitary conductance. Application of voltage steps to multichannel inside-in patches reveals activity which decays with the same time course as whole cell currents. The maxi-K channels appear to be largely responsible for the whole cell currents. Alteration of Ca^{++} concentration from 1-100 nM dramatically influences open time probability. These data suggest that carbachol increases cytoplasmic calcium, enhancing the activity of maxi-K channels, resulting in hyperpolarization of plasma cell membrane. Modulation of membrane potential may be an important aspect in the regulation of plasma cell function.

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36.7

EPITHELIAL mRNA-INDUCED ION CHANNEL EXPRESSION IN *XENOPUS* OOCYTES. D.H. Feldman, R.S. Schackmann*, M.S. Urnes*, and D. Carroll*. Dept. of Biochemistry, Univ. of Utah Med. Sch., Salt Lake City, UT 84132.

Secretory epithelia of the airway and elsewhere express various ion channels, including a cAMP-kinase regulated Cl^- channel whose function is disrupted in cystic fibrosis (CF). This defect affects either the Cl^- channel itself, or a regulatory component necessary for its function. We are using the *Xenopus* oocyte expression system to aid in the identification and isolation of the "CF gene" and genes for other constituents of epithelial transport. Ionic currents induced by injection of mRNA from canine or human epithelia are monitored by voltage clamp.

Oocytes injected with canine tracheal mRNA have 2-3 fold elevated steady Cl^- current, indicated by Cl^- replacement, compared to controls injected with water or liver mRNA. It is not yet established whether this represents expression of the Cl^- channel affected by CF. A slow-onset (4-5 min. to reach steady-state), voltage-dependent (-50 to >50 mV) K^+ current also appears in oocytes injected with either canine or human tracheal mRNA. The current resembles that observed in oocytes injected with mRNA transcribed from a rat kidney cDNA (Takumi *et al.* 1988, *Science 242*:1042); thus, we tested by means of a hybrid-arrest procedure whether the tracheal mRNA-induced K^+ current is due to a gene product related to the rat kidney cDNA. An anti-sense 45-base oligonucleotide constructed from the sequence of the rat cDNA, when mixed with tracheal mRNA prior to injection, prevented the appearance of the slow K^+ current. In contrast, the oligonucleotide did not affect the expression of TTX-sensitive Na^+, and A-type and delayed rectifier K^+ currents directed by rat brain mRNA.

Thus, it appears that canine and human tracheal epithelia express a transcript that is closely related to one cloned from rat kidney, and is unrelated to common brain ion channel genes. It is not yet known whether this transcript codes for a K^+ channel, or for a regulatory protein that alters the expression of an endogenous oocyte channel.

36.8

WHOLE CELL PATCH CLAMP STUDIES OF CULTURED EPITHELIAL CELLS. C. Pappas*, A. Oyler* and B. Koeppen* (SPON: E.M. Ostapoff). Depts. Med. and Physiol., Univ. Conn. Hlth. Ctr., Farmington, CT 06032.

The medullary collecting duct (OMCD) of the kidney secretes H^+ by an electrogenic ATPase. We have attempted to develop a model system for the electrophysiological study of this transporter. As a first step, we report the baseline electrical properties of these cells.

OMCD cells were isolated from the rabbit kidney, and grown in primary culture on collagen-coated coverslips. The input resistance of the cells averaged 686 ± 134 MΩ immediately after patch rupture, and 872 ± 181 MΩ after 20-30 min. of recording. The membrane voltage (Vm), as measured under current clamp conditions, was -36 ± 4 mV. The IV plot was nonlinear, with a chord conductance at Vm -40 to -80 mV of 1.94 ± 0.36 nS, and at Vm = +40 to +80 mV of 2.47 ± 0.41 nS. Removal of Cl^- from the bathing medium reduced outward current, and decreased the chord conductance at Vm = +40 to +80 mV from 3.68 ± 0.65 to 2.10 ± 0.41 nS. The chord conductance at Vm = -40 to -80 mV was reduced, but not significantly (1.95 ± 0.47 vs 1.74 ± 0.39 nS). Barium (1mM) did not have an effect when added to the bath. However, 10 mM barium reduced outward current, and decreased the chord conductance at Vm = +40 to +80 mV from 4.38 ± 1.37 to 3.01 ± 1.20 nS. The chord conductance at Vm = -40 to -80 was also reduced from 2.72 ± 1.14 to 1.45 ± 0.42 nS.

Conclusion: Cultured medullary collecting duct cells contain both Cl^- and barium-sensitive conductances. The electrical properties of the cultured OMCD cells are similar to those reported for the native cells measured with voltage-recording microelectrodes (Am. J. Physiol. 248: F500, 1985).

36.9

EFFECTS OF DEUTERIUM OXIDE ON THE GAP JUNCTION CHANNEL ACTIVITY OF EARTHWORM GIANT NERVE FIBER SEPTUM L. Barr[1], S.F. Fan*[2], and P.R. Brink[2]. [1]Dept. Physiol. Biophys., Univ. Illinois, Urbana, IL 61801, [2]Dept. Anat. Sci., HSC, SUNY at Stony Brook, NY 11794

With the double voltage clamp technique it had been shown that the reduction of junctional conductance of the earthworm giant nerve fiber septum with the exchange of H_2O with D_2O has two phases (Verselis, V. and Brink, P.R., Biophys. J., 50:1003, 1986). The reduction occurs within three minutes can be explained by the solvent isotope effects. The further reduction, which reaches steady state about 15-20 minutes after exchange, could only be explained by a primary solvent effect. The reduction of conductance could be the results of a decrease of the unitary conductance of individual channel or of a decrease of the open probability of the channel openings or both. With tight seal patch clamp technique we showed that after prolonged D_2O treatment, both the unitary conductance and the open probability of individual channels decrease. (supported by NIH Grant HL31299A to P.R.B.)

36.10

CONNEXIN 32, A MAJOR GAP JUNCTION PROTEIN OF LIVER AND NEURONS, FORMS VOLTAGE DEPENDENT INTERCELLULAR CHANNELS IN CELLS STABLY TRANSFECTED WITH ITS cDNA. A.P. Moreno*, B. Eghbali*, J.A. Kessler and D.C. Spray (SPON: J.A. Umbach). Department of Neuroscience, A. Einstein Coll. Med., Bronx, NY 10461

Coexpression of two or more gap junction proteins in a single cell type is common and has complicated the study of physiological properties of connexin 32 (Cx32), a major gap junction protein in rat liver and between neurons. We have stably introduced cDNA encoding Cx32 into a communication incompetent cell line (see Abstract by Eghbali et al), enabling direct study of channel properties. In control cells, junctional conductance (g_j, measured under dual voltage clamp) was generally undetectable (48/52 pairs in one series; 16/22 in another; in the 10 coupled pairs, g_j was 60-300 pS). More than 70% of the transfected pairs displayed g_j > 1 nS (in one series of 23 cell pairs, all were coupled with mean g_j 5.19±5.7 nS). g_j was reduced by heptanol and halothane, agents that gate g_j in tissues expressing Cx32. Unitary conductances of channel events in untransfected and transfected cells were compared; in control cells where g_j was detectable (2 cases, > 100 events in each), sizes averaged about 20-30 pS, and may represent a novel connexin. In transfected cells (7 pairs, some of which showed hundreds of analyzable events), a larger channel type was present, with a unitary conductance of about 120 pS; the small channel seen in control cells was also usually present. The open time of the channel corresponding to transfection with Cx32 was markedly voltage dependent. Since this gap junction protein is also expressed between neurons and between oligodendrocytes, we hypothesize that voltage dependent junctional conductance may play a role in electrotonic coupling in the nervous system.

36.11

GAP JUNCTIONS DO NOT AFFECT GROWTH RATE: STUDIES ON COMMUNICATION-INCOMPETENT CELLS STABLY TRANSFECTED WITH cDNA ENCODING CONNEXIN 32, A MAJOR GAP JUNCTION PROTEIN OF LIVER AND NEURONS. B. Eghbali*, J.A. Kessler, and D.C. Spray. Dept. of Neuroscience, A. Einstein College of Medicine, Bronx, NY 10461

A role for gap junctions in normal tissue growth and differentiation has long been postulated. As a direct test for a role of gap junctions in growth control, we have compared the growth rates of a noncommunicating, highly metastatic cell line (SKHep1) with those of clones in which we have introduced the cDNA encoding the major liver gap junction protein, connexin 32. SKHep1 cells are normally not coupled with respect to the dye Lucifer Yellow, do not express mRNAs detectable by low stringency hybridization with cDNA encoding connexin 32, and do not express junctional proteins as detected immunocytochemically. Following stable transfection and selection, first with selectable marker and then using intracellular injection of Lucifer Yellow to choose clones with high degree of dye spread, connexin 32 mRNA and punctate connexin-32 immunoreactive membrane contacts became abundant, and cells became strongly coupled with regard to dye and electrical current (macroscopic and unitary junctional conductances of about 10 nS and 120-150 pS were recorded in cell pairs). Growth rates during the rapid proliferation of the cells (1-5 days after plating at low density) were not altered after transfection; maximum cell density achieved in confluent dishes (7-10 days in culture) was slightly lower in transfectants than in controls. These observations suggest that the reduction in functional gap junctions often associated with tumorigenesis is not responsible for the increased growth rate of tumor cells.

36.12

A GENERAL METHOD FOR FUSING RECONSTITUTED PROTEOLIPOSOMES WITH PLANAR MEMBRANES. D. J. Woodbury*, A. G. Goldberg* and C. Miller* (SPON: T. Egan). Graduate Department of Biochemistry; Brandeis University; Waltham, MA 02254.

We are attempting to purify and characterize the voltage-dependent Cl^- channel from the electric organ of *Torpedo Californica*. Passive flux measurements on liposomes reconstituted with detergent-solubilized protein indicate the presence of a Cl^- channel. However, ardent attempts to fuse these liposomes with planar membranes have been unsuccessful.

We now report a novel method for fusion of liposomes. The liposome membrane is doped with nystatin-ergosterol channels that promote fusion and cause a transient increase or spike in bilayer conductance. This spike is due to active nystatin-ergosterol channels that close within seconds as ergosterol is diluted into the lipid bilayer. Spikes signal that individual fusion events have occurred without permanently altering bilayer conductance. Presumably, nystatin and ergosterol facilitate fusion by forming channels throughout the vesicle population (Woodbury and Hall, *Biophys. J.*, 54:1053-1063, 1988; Cohen et al., *J. Gen. Physiol.*, 93:201-210, 1989).

Using this method, we have observed the voltage-dependent Cl^- channel following fusion of liposomes as indicated by conductance spikes. Preliminary results indicate that only a subset of the vesicles contain the Cl^- channel, in agreement with flux measurements. This research was carried out during the tenure of a Postdoctoral Fellowship from Muscular Dystrophy Association, and was additionally supported by NIH grant #AR-19826.

37.1

EFFECT OF IBOTENATE AND QUISQUALATE LESIONS OF THE nBM ON WATER MAZE PERFORMANCE. D.J. Connor, L.J. Thal, Dept. Neurosciences, UCSD, La Jolla, CA 92093; Neurology Ser., VAMC, San Diego, CA 92161

Injection of ibotenic acid (IBO) and quisqualic acid (Quis) into the nucleus basalis magnocellularis (nBM) of rats resulted in depletion of cortical choline acetyltransferase (ChAT) in F-344 rats of 45.5% and 58.9% respectively. Acquisition of the Morris water maze task was determined using a dark maze with digital tracking. Acquisition over a 10-day period (2 trials/day) was significantly different between groups (ANOVA with repeated measures, $p < .01$), and post-hoc analysis showed both lesioned groups were significantly different from sham-operated controls (NK, $p = .01$). The acquisition deficit was greater for the IBO group than the Quis group. No change in performance was seen after a 10-day retention interval for any of the groups ($p > .05$). Spatial probe analysis indicated a significant difference on annulus swimming (ANOVA, $p < .01$) with post-hoc testing revealing a significant difference between all three groups (NK, $p = .05$). The 72-hour retention of a passive avoidance task (600 sec/max) was not significantly different between groups (ANOVA, $p > .05$). Biochemical analysis of cortical tissue from the three groups revealed no differences in catecholamines or indolamines but a significant decrease in the IBO group was seen with amino acid analysis. The greater impairment seen in the IBO group may be due to the lesioning of noncholinergic cell populations in addition to the known cholinergic lesion.

37.2

ENHANCEMENT OF BOTH EXCITATION AND INHIBITION IN THE SAME POPULATION OF HIPPOCAMPAL NEURONS BY AN α_2 ANTAGONIST. S.J.Sara & O.Bergis*, LPN2, CNRS, Gif/Yvette, France.

The α_2 adrenoceptor antagonist idazoxan (IDA) facilitates cognitive performance in the rat (Sara & Devauges, Behav. Neural Biol., 1989). The effective dose (2 mg/kg) increases firing rate of cells in the locus coeruleus (LC) and release of noradrenaline (NE) in forebrain. The effect of IDA on excitability of neurons of the dentate gyrus (DG) in awake and freely moving rats is examined here. 8 rats were implanted with stimulating electrodes in the perforant pathway and recording electrodes in the DG. The awake freely-moving rat was stimulated (single pulse, 0.1 ms duration at variable intensity, 100-800 μamp). Amplitude of the population spike and slope of the EPSP were measured as a function of intensity of stimulation. In 7/8 rats, IDA induced a large (±100%) increase spike in amplitude, with no effect of the EPSP. Paired pulses at 300 μamp were administered with interpulse intervals (IPI) varying from 15-80 ms. An IPI of less than 30 ms produced a reduction in the response to the 2nd pulse. IDA significantly increased this inhibition for the maximal IPIs. Thus, NE can not only increase the excitatory population response in the DG but can increase the efficacy of inhibitory circuits in the same population of neurons at the same time. Such a dual action could serve to hone and sharpen information input to the hippocampus and thus contribute to the cognitive enhancing effects of LC stimulation and IDA seen in behavioral experiments.

37.3

INVESTIGATIONS INTO THE ROLE OF SEPTAL α-NORADRENERGIC AND NMDA RECEPTORS IN THE INDUCTION AND MAINTENANCE OF SEPTO-HIPPOCAMPAL CHOLINERGIC ACTIVATION INDUCED BY SPATIAL MEMORY TESTING IN THE MOUSE.

DURKIN, T., MARIGHETTO, A., LEBRUN, C., TOUMANE, A. and JAFFARD, R., Laboratoire Psychophysiologie, CNRS URA 339, Université de Bordeaux I, 33405 TALENCE FRANCE.

Previous studies showed that spatial memory testing of mice in an 8-arm radial maze, induced long-term activation (measured by sodium-dependent high affinity choline uptake) of the cholinergic septo-hippocampal pathway and whose duration progressively decreased during repeated daily testing (TOUMANE et al., Behav. Neural Biol., 1989, in press). We have tested the hypotheses that 1) the initial cholinergic activation may be mediated by phasic excitatory noradrenergic septal afferents via α receptors and 2) the duration of the activation may be controlled by a feedback system mediated by glutamatergic hippocampal pyramidal neurones via septal NMDA receptors. Intraseptal injection of phenoxybenzamine (500 ng/0.2 μl bilateral) 20 min before working memory testing blocks the cholinergic activation induced by testing (while having no effect on quiet control mice) and produces a selective interference-related working memory deficit. Intraseptal injection of +MK801 (500ng/0.2μl bilateral) 30 sec after an acquisition session of a reference memory test suppressed the maintenance of the cholinergic activation and produced a deficit in retention in a subsequent session 24 hours later.

* This research was supported by the C.N.R.S. URA 339.

37.4

INTERACTION OF GABAERGIC AND CHOLINERGIC DRUGS ON MEMORY AND UNIT CELL HIPPOCAMPAL FUNCTION. D.J. CRITCHETT*, R.M. MANGANO, B. BEER AND D.E. CLODY*. Medical Research Division, American Cyanamid Co., Pearl River, NY 10965.

The present studies tested for the potential interaction of effects of cholinergic and GABAergic systems on passive avoidance behavior and single cell hippocampal function. When given to rats prior to the training trial, both scopolamine (SCOP) and diazepam (DZP) produced dose related amnestic-like effects on the performance of passive avoidance behavior. Ro15-1788(Ro15), a specific benzodiazepine receptor antagonist, completely reversed the amnestic effects of DZP, but was without effects on the passive avoidance deficits induced by SCOP. However, the cholinomimetic oxotremorine was able to antagonize the passive avoidance deficits of both SCOP and DZP. The basal and acetylcholine (ACh) induced changes in the electrical activity of hippocampal pyramidal cells was recorded from rats *in vivo*. After intravenous (iv) administration of DZP (1 mg/kg), ACh responding (microiontophoretic ejection of ACh at 50nA) and basal firing rate were reduced to < 50 % of control. This DZP induced reduction of basal rate and ACh stimulated firing was reversed by iv injection of 1 mg/kg of Ro15. In fact both basal and ACh rate were increased 25% over control levels following the combined Ro15 and DZP treatment. Ro15 or vehicle alone produced no change in basal rate nor ACh induced acceleration of firing rate. The behavioral and electrophysiological data together suggest that the amnestic effects of SCOP and DZP may be mediated by a common cholinergic pathway, and these effects may be related to hippocampal function.

37.5

MODULATION OF MEDIAL SEPTAL AREA OUTPUT ALTERS WORKING MEMORY. B.S. Givens and D.S. Olton, Department of Psychology, The Johns Hopkins University Baltimore, MD 21218

The role of the septohippocampal pathway in working memory was investigated by examining the behavioral and electrophysiological effects of compounds microinfused into the medial septal area. Saline, tetracaine, muscimol or carbachol was microinfused into the medial septal area. Behavior and hippocampal EEG were assessed 0.5 and 1.5 hr after infusion. Behavior was measured by performance in a continouous spatial alternation task conducted in a T maze. Choice accuracy was the number of correct choices in 20 trials; performance time was the amount of time required to complete 20 trials. Hippocampal EEG was measured by FFT analysis (1-20 Hz). Saline had no effect on choice accuracy, performance time or hippocampal EEG. Both muscimol (20 ng) and tetracaine (10 μg) decreased choice accuracy, and the amount and the power of the 7-8 hertz theta frequency range. All measures of behavioral performance and hippocampal physiology were normal 1.5 hr after infusion. Carbachol (0.5 μg) did not affect choice accuracy but decreased performance time. These data support a role for the septohippocampal projection in working memory.

37.6

EFFECTS OF AGING ON ^{3}H-NIMODIPINE BINDING IN RAT BRAIN. P.W. Landfield, D.G. Fleenor*, J.C. Eldridge*, and B.S. McEwen. Dept. Physiol. & Pharmacol., Bowman Gray School of Medicine, Wake Forest Univ, Winston-Salem, NC 27103, and Rockefeller Univ., New York, N.Y. 10021.

Several recent studies indicate that aging is associated with an increase in voltage-dependent Ca influx into hippocampal neurons (cf., Landfield, Pitler, Science, 1984; Soc. Neurosci. 1987; Pitler, Landfield, under review; Campbell, et al, this meeting). In order to determine whether this effect may be associated with an increase in the concentration of calcium channels, studies were conducted on binding of a dihydropyridine (DHP) calcium channel antagonist, nimodipine, to hippocampal and other forebrain structures. DHP ligands bind with high affinity to L-like calcium channels and much is known about DHP pharmacology and binding patterns (cf. reviews in Scriabine, Structure and Physiology of the Slow Inward Calcium Channel, Liss, 1987; Janis, Silver, and Triggle, Adv. Drug Res., 1987).

Using ^{3}H-Nimodpine as the tracer, and 100-fold excess unlabeled nimodipine to define specific binding, we prepared membrane fractions from paired hippocampi and paired remaining forebrain for Scatchard plot analyses, in individual young adult (3-5 mo. old) and aged (24-27 mo. old) specific-pathogen-free Fischer rats.

Results showed a significant aging-related decrease in the B_{max} for ^{3}H-nimodipine binding in both hippocampus (134 vs. 69 fmol/mg, $p < .05$) and forebrain (129 vs. 72 fmol/mg, $p < .05$). However, the K_d exhibited no age differences in either region. These data were unexpected in light of the increased Ca currents that have been found in aging rat hippocampal neurons (Campbell, et. al., this meeting). Thus, they may reflect some form of Ca-mediated down-regulation of Ca channels, or they may reflect the fact that DHPs bind to Ca channels most effectively in the inactivated state, and, with aging, some channels may resist inactivation even during membrane preparation. Alternatively, since the data are expressed per mg of protein, they may reflect the loss of neurons and replacement by glia containing fewer channels. Further studies will be needed to understand the relations of DHP binding to Ca currents in aged brain, but the present data indicate that a complex interaction may be present (supported in part by AG 04542 and Miles, Inc.).

37.7

SPATIAL MEMORY RELATED NEURON ACTIVITY IN MONKEY HIPPOCAMPUS. T.Ono, K.Nakamura*, R.Tamura* and M.Fukuda. Dept. Physiol., Fac. Med., Toyama Med.& Pharmaceu. Univ., Sugitani, Toyama 930-01, JAPAN

Single neuron activity in the monkey hippocampus was recorded during bar press to manipulate a mobile device. Monkey sat in a half-mirrored cabin that could move linearly and rotate. The monkey could change light, sound and temperature in the cabin by change of position (internal environment). The laboratory light was switched on or off (external environment). One of five cue tones predicted a trial. After a short delay the monkey could see food or an object. By pressing the appropriate levers, it could move some distance to get the food or a drop of juice.

Of 104 neurons analyzed, 72 responded in some tasks. Of these, 24 were place recognition related. Their activity increased maximally when the monkey moved to a restricted position related to the external and internal environment. Movement with the laboratory light off, or the internal environment fixed, suppressed the neuronal activity increase about 50%. Tewenty one direction selective neurons responded maximally when the experimenter or the monkey moved to a specific position. Responses to cabin rotation were allocentric, egocentric or reduced. The result suggest hippocampal involvement in spatial memory defined by each coordinate of self location, and external and internal environment.

37.8

TONE-EVOKED POTENTIALS INDICATE DIFFERENTIAL PROCESSING OF SENSORY INFORMATION IN THE HIPPOCAMPUS AND NEOCORTEX OF THE RAT. R.E. Hampson, H.H. Willis, III, C.R. Breese*, and S.A. Deadwyler. Dept. of Physiology & Pharmacology, Bowman Gray Sch. of Med., Wake Forest Univ., Winston-Salem, NC, 27103.

Auditory evoked potentials (AEPs) from the hippocampus exhibit characteristics of sensory information processing which differ from cortical AEPs recorded in a two-tone discrimination task (Hampson, Breese & Deadwyler, *Soc. Neurosci. Abstr.*, **14**:1015, 1988). Two main components were present in the cortical AEP with peak latencies that differed from those in the hippocampus: a negativity at a mean latency of 40 ms (N_{40}) and a positivity at 140 ms mean latency (P_{140}).

During a two-tone discrimination task, the cortical N_{40} was present on rewarded and unrewarded trials. In contrast, the cortical P_{140} was evoked by the tone only on rewarded trials. P_{140} amplitude in the rat was altered by the sequence of prior tones in a manner similar to previous observations of sequence-dependent changes in the human P_{300}.

Implementation of a single-tone signal detection task (Heyser, Breese, Hampson & Deadwyler, *Soc. Neurosci. Abstr.*, **14**:1030), revealed that N_{40} was present in the cortical potential on all trials in which the tone stimulus was detected, but also on trials when tones were not detected. In contrast, the P_{140} component was present only on trials when the tone was detected behaviorally. Hippocampal AEPs in this task showed only a late (70 ms) component of the AEP (previously described as N_2) associated with detected tones.

These two studies suggest that information regarding the current tone and preceding sequence of tones appears to be available in the hippocampus prior to the occurrence of the task-sensitive cortical components in the two-tone discrimination task. However, in the tone detection task, the same information appears to be present in the neocortex first, and hippocampus later. This suggests that separate neural processes may be used to transmit sensory information between neocortex and hippocampus as a function of the behavioral requirements of the task.

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37.9

RECEPTIVE FIELD PLASTICITY DURING CARDIAC CONDITIONING IN THE DORSAL MEDIAL GENICULATE NUCLEUS OF THE GUINEA PIG. Jean-Marc Edeline* and Norman M. Weinberger, Cntr. Neurobiol. Learn. Memory and Dept. Psychobiol., Univ. Calif., Irvine, Ca. 92717.

Associatively-induced, frequency-specific receptive field plasticity has been found in auditory cortical fields (Br. Res., 1986, 372, 357). We report here data on receptive field plasticity in the dorsal medial geniculate nucleus.

Single unit (n=4) and cluster (n=8) discharges were recorded in 10 adult guinea pigs before, during and after Pavlovian training (CS = tone, 6.0 sec.; UCS = 250 ms footshock at CS offset). Cardiac decelerative conditioned responses developed in all subjects during pairing but not during a prior sensitization period. Frequency receptive fields (FRF) were determined immediately before and following training by repeatedly presenting a range of tones (50 ms) at different intensities (40-80 db). All acoustic stimuli were delivered with controlled intensity to the contralateral ear. FRF plasticity was observed both in single or cluster recordings in 7 cases, of which 5 were specific to the frequency used as the conditioned stimulus.

These findings, the first demonstration of receptive field plasticity in the auditory thalamus, indicate that such plasticity is not restricted to the auditory cortex and suggest that interactions between subdivisions of the medial geniculate nucleus and the auditory cortex be studied to understand modifications of information processing during learning.

Supported by Lavosier fellowship (JME) and ONR (NMW).

37.10

EYE BLINK CONDITIONING IN THE FREELY MOVING MIDDLE-AGED RAT. C. Weiss, T.J. Shors, L.B. Tran, and R.F. Thompson. Dept. of Psych., U. Southern Cal., L.A., Ca. 90089-1061.

Both rabbits and humans exhibit the same basic properties of eyeblink conditioning in a closely similar manner, including severe association deficits as a result of aging. Because of the lack of age verifiable rabbits, we have implemented this paradigm in the freely moving Fischer-344 male rat.

We implanted chronic wires to record activity from the external eyelid muscle and to evoke an unconditioned blink (Skelton,1989). In addition, we have implanted some rats with a 19 gauge cannula to permit the use of an airpuff unconditioned stimulus (US).

Animals maintained electrical contact via a tether and miniature swivel. Shock intensity (100 msec, 60 Hz, AC) was adjusted to elicit a blink (<0.5mA). After habituation/threshold testing the rats were trained by pairing a tone (350 msec, 2.8 kHz, 85 dB) with the shock or airpuff US. Ten blocks of 10 trials/day (1 CS alone, 8 paired, 1 US alone) were given with an average intertrial interval of 30 sec. After training, hippocampal slices were prepared for long-term potentiation (LTP).

Our results to date suggest that middle aged (1 yr old, 400-500 gm) rats exhibit LTP (recording from CA1), and that eyeshock is not inducing stress related impairments.

BNS-8718300 to R.F.T.; NIA Training Grant AG00093.

37.11

INTRACELLULAR RECORDING FROM CORTICAL NEURONS OF CONSCIOUS RATS. M.P. Kristensen* and C.D. Woody (SPON:B. Swartz). UCLA Med. Ctr., Los Angeles, CA 90024.

In order to study subcellular mechanisms underlying changes in mammalian behavior and their modulation by pharmacological agents, the availability of intracellular recording methods allowing direct measurement of membrane properties of central neurons in the absence of trauma and anesthesia is useful. Intracellular recording in conscious animals has previously been done in cats (Woody and Black-Cleworth, J. Neurophysiol. 1973; Woody and Gruen, Brain Res. 1987) and primates (Matsumura, Brain Res. 1979; Aou et al Brain Res. 1988). The purpose of the present study was to design a preparation enabling intracellular recording from conscious rats in a manner consistent with current guidelines and comfort of the animal. Adapting previous methods used for studying primates (Jasper et al, EEG clin. Neurophysiol. 1960; Evarts, J. Neurophysiol. 1968), a fitting was attached to the skull to prevent cranial movement. It allowed recording more than 2 months after implantation. During fixation of the head-fitting (with the body free to move within in a plastic cylinder), stable intracellular recordings of cortical neuronal activity were obtained for more than 15 minutes. Using rectangular currents (60 ms duration), input resistances of 10-15 Mohm and spontaneous as well as evoked spike amplitudes of 70 mV were seen. It is the objective using this method to study cellular and subcellular changes during operant conditioning of licking behavior.

37.12

THE LEARNING-SPECIFIC REDUCTION IN CA1 NEURON AHP IS VOLTAGE-DEPENDENT. M. deJonge*, J. Black, R.A. Deyo* and J.F. Disterhoft, Dept. of Cell Biology and Anatomy, Northwestern Univ. Med. Sch., Chicago, IL 60611.

Classical eye-blink conditioning in rabbits leads to a learning-specific decrease in amplitude of the slow afterhyperpolarization (AHP) in hippocampal CA1 cells. In the present study the effect of resting membrane potential (RMP) on the conditioning-reduced AHP was measured while current clamping the resting membrane potential (RMP) to different levels.

Subjects were young adult albino rabbits: Naive (N=10), Trace conditioned (N=8) or Pseudo conditioned (N=9). Following behavioral training, and with the experimenter blind to training condition, hippocampal slices were prepared and microelectrodes filled with 2M KCH_3SO_4 were used to record CA1 cells which exhibited stable membrane potentials, action potential amplitudes larger than 70 mV and accommodation to a depolarizing pulse. Slow AHP's were measured following bursts of 4 action potentials elicited by a 100 msec intracellular current pulse. A significant learning-specific reduction in AHP amplitude was observed but only when the RMP was clamped at -70 to -80 mV. For example, the AHP amplitudes (mV) were 4.15 ± 0.50 (N=20 cells), 4.58 ± 0.39 (N=23) and 4.43 ± 0.34 (N=19) for Naive, Pseudo and Trace cells, respectively, when the RMP was held between -60 to -65 mV; and 2.66 ± 0.29 (N=15), 2.81 ± 0.26 (N=15) and 1.87 ± 0.21 (N=16) ($p<.05$), when RMP was between -70 to -75 mV.

These results suggest that the current(s) affected by conditioning was (were) inactivated at RMPs more positive than -70 mV. Recently, Numann et al. (*J.Physiol.*1987,*393*:331-353) described two $K_{(Ca)}$ currents in isolated CA1 cells. $K_{2(Ca)}$ was completely inactivated at holding potentials of -55 mV, had a low threshold, and developed and inactivated slowly. This current may be altered during conditioning.

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37.13

HIPPOCAMPAL ACTIVITY AND LATENT INHIBITION IN THE RABBIT. A. G. ROMANO. Med. Coll. of PA at EPPI, Philadelphia, PA 19129.

CA1 hippocampal activity was monitored in 2 groups of rabbits during the latent inhibition (LI) paradigm. Animals experienced either 0 or 300 preexposures of a tone CS followed by classical conditioning of the nictitating membrane response.

The 300 CS preexposures produced a decline in CS-evoked hippocampal activity ($p < .0005$) which was predictive of the subsequent rate of learning. Preexposed animals learned significantly slower than nonpreexposed animals ($p < .01$). Moreover, preexposed animals maintained baseline levels of CA1 activity throughout acquisition whereas nonpreexposed animals showed reliable increases in CS-evoked activity ($p < .05$).

The results suggest that CA1 activity may reflect two different aspects of the LI paradigm. During preexposures, activity may be reflecting the decline in the attentional value of the CS. However, during acquisition, the persistent depression in CA1 activity may be indicative of a potent memory component underlying the LI effect.

37.14

HIPPOCAMPAL RESPONSES TO TONE DURING JAW MOVEMENT DISCRIMINATION IN RABBITS. L.J.Dreshfield*, M.P.Galupo* and S.D. Berry. Dept. of Psychology, Miami Univ.,Oxford,OH 45056.

To assess the generality of conditioned hippocampal responses observed during nictitating membrane conditioning bilateral recordings were made from CA1 during go/no go jaw movement discrimination training and reversal in 5 New Zealand White rabbits. Electrodes were implanted under general anesthesia (Ketamine, 50 mg/kg; Rompun 10 mg/kg IM) In the task, one tone (CS+) signalled water 500 msec later; another tone (CS-) signalled no water. 1 KHz and 8 KHz tones were counterbalanced as CS+ and CS-. Non-parametric analysis indicated significant neural responses to the tones in all subjects. Surprisingly, individual subjects displayed a tone preference (e.g., 1 KHz or 8 KHz, regardless of whether it was the CS+ or CS-). Conditioned responses occurred to that tone only, and the effect of discrimination reversal was to reduce, but not eliminate, this response to the preferred tone even after behavioral reversal. Animals given unpaired control tones do not display hippocampal neural responses. These results suggest that hippocampal neurons may be involved in coding the behavioral significance of either the CS+ or CS- during successful behavioral responding to the CS+ only.

37.15

POSSIBLE ROLE OF KINDLING-INDUCED NEURONAL ALTERATIONS IN DISCRIMINATION-REVERSAL TRAINING OF THE RABBIT NICTITATING MEMBRANE (NM) RESPONSE. C.R. Sears* and G.B. Robinson (SPON: F.V. Szeligo). Univ. of New Brunswick, Fredericton, N.B. Canada, E3B 6E4.

We recently demonstrated that kindled rabbits acquired a discriminative response significantly faster than controls but were significantly impaired in reversal learning (Robinson et al., Behav. Brain Res., 31, 1988). Kindling of the perforant path results in significant LTP of the perforant path-granule cell (PP-GC) population spike and a long-lasting increase in the magnitude of paired-pulse inhibition. The possible contribution of these neural alterations to the effect kindled seizures have on discrimination-reversal learning of the rabbit NM response was investigated in the present experiment.

The results showed that kindling resulted in significant LTP, but it's duration was less than the maximum period allowed for reversal learning (28 days). In contrast, the increased magnitude of paired-pulse inhibition persisted throughout both discrimination and reversal training, with little recovery toward control levels. These results raise the possibility that altering the nonlinear transformational characteristics of PP-GC synapses, and thus transmission through the hippocampal formation, disrupts learning tasks dependent on an intact, neurophysiologically normal hippocampus. Supported by MRC.

37.16

LATERALITY OF THE CLASSICALLY CONDITIONED NICTITATING MEMBRANE RESPONSE IN THE RABBIT. S.L. Stewart*, V. Bracha* and J.R. Bloedel (SPON: A.S. Schwartz). Division of Neurobiology, Barrow Neurological Inst., Phoenix, AZ 85013.

Although the classically conditioned nictitating membrane response (NMR) is considered to be largely confined to the eye exposed to the unconditioned stimulus (US), differences in degree of NMR laterality in individual animals have been reported in earlier studies. Because the extent of NMR bilaterality is important to issues pertaining to the neural substrates required for conditioning, experiments were performed to examine this question.

Awake rabbits were conditioned in a standard delay paradigm: 100 ms right corneal airpuff (US); 450 ms, 1 kHz, 85 dB tone (conditioned stimulus); interstimulus interval - 350 ms; intertrial interval - 17-23 sec; 100 paired trials/day. Movements of the nictitating membrane were recorded in both eyes. Most subjects demonstrated few or no responses in the nonstimulated eye during the first day of conditioning, but the incidence of responses in this eye gradually increased in subsequent training days. By the fifth day of training conditioned responses occurred in 85% of the trials in the stimulated eye and in 40% of trials in the unstimulated eye. In the unstimulated eye the increased number of conditioned responses was accompanied by an increase in the incidence of unconditioned responses, which reached 35% on day five.

The observed increase of both conditioned and unconditioned responses in the nonstimulated eye over the course of conditioning indicates that the bilateral responding in this paradigm is complex and may not relate only to changes in pathways exclusively mediating the conditioned response. (NIH Grant NS21958)

37.17

COGNITIVE DISRUPTION FOLLOWING AMYGDALA KINDLING: EFFECTS OF HISTORY, STIMULATION PARAMETERS, AND FOOTSHOCK. D.B. Peele, M.E. Gilbert, C.M. Mack*, S.K. Acheson*, and S.D. Allison*. NSI Environmental Sciences, RTP, NC 27709.

The effects of amygdala kindled seizures on two tests of cognitive function were examined using long-delay flavor-aversion (FA) and passive avoidance (PA) conditioning paradigms in rats. The purpose of the study was to compare the cognitive effects of unilateral and bilateral kindled seizures with those due to a kindling history. FA conditioning was unaffected by a history of either unilateral or bilateral kindling. However, seizures induced during the period separating saccharin (CS) and lithium (US) disrupted FA conditioning in a delay-independent manner. These effects could not be mimicked by administering trains of potentially interfering footshock during the CS-US interval (0.5 mAmp, 0.5sec every 6 seconds for either 30 or 90 sec). In contrast to the seizure-dependent disruption of FA conditioning, PA conditioning was disrupted by a history of bilateral but not unilateral kindling. This disruption was not dependent on seizures induced during training. The data from the FA experiments suggest that kindled seizure activity produces deficits that are more a function of a failure of associative learning than of an accelerated memory loss, since the deficits lacked a temporal dependency. The findings support previous research showing an important role of the amygdala in maintaining aversive conditioning, yet suggest that the cognitive impact of kindling and kindled seizure induction may depend critically on the task.

37.18

UNIT-ACTIVITY IN THE AMYGDALOID BASOLATERAL NUCLEUS DURING ACQUISITION AND OVERTRAINING OF DISCRIMINATIVE AVOIDANCE BEHAVIOR IN RABBITS. S. Maren*, A. Cox, and M. Gabriel. Department of Psychology, University of Illinois, Champaign, IL, 61820.

Multiple-unit and integrated-unit activity were recorded in the amygdaloid basolateral (BL) nucleus during differential conditioning of avoidance (wheel-running) behavior in rabbits. The conditioned stimuli (CS+ and CS-) were pure tones of different auditory frequency, and the unconditioned stimulus (US) was a constant-current footshock delivered through the grid floor of the wheel. Both the multiple-unit and the integrated-unit activity manifested significant (onset latency = 60-90 ms) discriminative neuronal discharges (i.e., greater discharges to the CS+ than to the CS-) during acquisition and overtraining of the conditioned avoidance behavior. Significant discriminative activity developed during the first session of conditioning, increased in magnitude as training continued, and reached maximal magnitude as the rabbits reached the asymptote of behavioral discrimination. Also, a significant increase in overall (non-discriminative) discharge magnitude occurred in the first conditioning session relative to the preceding pretraining session in which the CSs and US were presented non-contingently. These overall and discriminative neuronal discharges were substantially attenuated during overtraining (i.e., training beyond the first attainment of behavioral asymptote). Rapidly developing neuronal plasticity also occurs in structures (anterior cingulate cortex; mediodorsal thalamic nucleus) directly interconnected with the BL nucleus. In contrast, plasticity in the posterior cingulate cortex and anteroventral thalamic nucleus develops more slowly and is more persistent. Thus, neurons of the BL nucleus are the first to exhibit plasticity among neurons comprising a system that generally exhibits rapid changes. The rapid changes are in agreement with other data indicating that BL amygdaloid neurons and the system in which they participate subserve the earliest stages of learning (supported by NIH grant 26736 and AFOSR grant 89-0046 to MG).

37.19

Neuronal Activity in the Rabbit's Medial Prefrontal Cortex (PFCm) is Influenced by Aversive and Appetitive Pavlovian Contingencies. C.M. Gibbs, K.L. Watson* & A.W. Gibbs*, VA Med. Center & Univ. of South Carolina, Columbia, SC 29201.

Numerous findings have indicated that the PFCm participates in the development of aversively conditioned bradycardia in rabbits (e.g. Buchanan SL, Powell DA: JCPP 96:755; Gibbs CM, Powell DA: Brain Res 442:86; Gibbs CM: Soc Neurosci Abstr 14:862). The present studies were undertaken to evaluate single-unit activity in the PFCm during either differential aversive Pavlovian conditioning (one tone (CS+) paired with eye-shock, another (CS-) presented alone) or differential appetitive conditioning (CS+ paired with an intraoral pulse of water, CS- presented alone).

Our results to date indicate that: (1) Most (63/96; 66%) PFCm cells show quantitatively distinct activity patterns to the tone CSs during aversive training, with 97% of these showing greater responsivity to the CS+; further, the tone-evoked activity of the majority of these cells is significantly correlated with concomitant changes in heart rate (HR). (2) Similarly, most (33/48; 69%) PFCm cells show discriminative activity to appetitive CSs; however, in contrast to the aversive data above, a larger percentage of these cells (30% vs. 3%) show greater responsivity to the CS- ($\chi^2 = 14.57$, $p < .001$), and the relationship(s) between unit activity and concomitant HR changes has yet to be uncovered. Thus, the PFCm may be differentially involved in aversive and appetitive Pavlovian conditioning processes.

(Supported by VA Institutional Research Funds)

37.20

Multiple Unit Activity in the Mediodorsal Nucleus of the Thalamus Evoked by Pavlovian Conditioning. D.A. Powell and K. Watson*. Neuroscience Laboratory, VA Medical Center and University of South Carolina, Columbia, SC 29201.

Chronic multiple unit recording electrodes were implanted in the mediodorsal nucleus of the thalamus (MD) in rabbits. Two weeks later the animals were exposed to orienting, Pavlovian conditioning and extinction, in which tones served as conditioned stimuli (CSs) and paraorbital electric shock was the unconditioned stimulus (US). Tone-evoked multiple unit activity (MUA) from MD and heart rate (HR) changes were recorded. The initial HR response to the tone, as well as the conditioned HR response, consisted of decelerations from pre-tone baseline. A training-induced short latency increase in MUA was also observed, which began during the first 100 msec interval following tone onset and reached its peak amplitude during the third 100 msec interval. Although this response occurred during initial tone presentations, when the CS was not yet reinforced with the US, it habituated greatly over ten unreinforced habituation trials. Presentation of the CS paired with the US augmented this response two-to-three fold, and it remained at this level until the end of paired CS/US training. The presentation of the CS without the US during extinction again resulted in a quick decline of MUA to pretraining levels.

Supported by VA Institutional Research Funds

37.21

LONG-LASTING ENHANCEMENT OF ANTERIOR THALAMIC (AT) SYNAPTIC RESPONSIVENESS BY STIMULATION OF A BRAINSTEM CHOLINERGIC (Ch) GROUP. D. Paré, M. Steriade, M. Deschênes and S. Datta. Lab. Neurophysiol., Sch. of Med., Univ. Laval, Québec, Canada.

To study how the Ch laterodorsal tegmental (LDT) nucleus influences AT neurons, a series of intra- and extracellular recordings were performed in urethane-anesthetized, reserpine-treated cats. LDT stimulation evoked a short-latency (5 msec), short-duration (≈20 msec) depolarization and enhnaced the responsiveness of AT cells to cingular and mammillary body (MB) stimuli. This effect varied as a function of the number of trials. The gradual increase in responsiveness was paralleled by a progressive increase in input resistance. In some neurons, LDT trains also increased the probability of fast prepotentials being triggered by sub-threshold orthodromic volleys. In order to distinguish the cumulative effects of repeated LDT trains from the possibly slow time-course of LDT influences, we studied the effects of a unique 1-sec LDT train (at 30 Hz) on the synaptic responsiveness of AT cells. Such LDT trains induced a 3 fold increase in synaptic responsiveness, reaching its peak 40-50 sec after the LDT train and lasting up to 4 min. Iontophoretic application of scopolamine abolished these effects, thus indicating that the long-lasting AT potentiation is a muscarinic effect.

Supported by MRC.

37.22

LATERALIZED BEHAVIORAL AND NEURONAL RESPONSES OF THE CINGULATE CORTEX DURING DISCRIMINATION CONDITIONING IN THE CAT. M. Penttonen*, T. Korhonen* and K. Hugdahl* (spon: P. Helén). Univ. of Jyväskylä, Finland and Univ. of Bergen, Norway.

The purpose of this experiment was to study the effects of symmetrical conditioned stimuli (CS) and asymmetrical unconditioned stimuli (US) on the lateralization of conditioned responses. The tone CS was delivered through earphones attached in front of both ears, and the US was the electrical stimulation of either the left or right lateral hypothalamus. The cats learned discrimination by showing greater head turning response amplitudes to the CS+ than to the CS-. In almost every cat the conditioned response was a head turn to the left. This response was accompanied with a negative evoked response (128-328 ms from the CS onset) to both CS- and CS+. The evoked response was greater in the right than in the left cingulate cortex. The magnitude of the difference was greater for the CS+ than the CS- with the advantage always in the right cingulate cortex. No differences were found in the behavior or the neural activity due to left versus right stimulation. Regardless of the hemisphere which received the US, the UR and CR headmovements were to the left, and contralateral to the hemisphere exhibiting the greater neural response. The greater activity of the right cingulate cortex might reflect some basic differences between hemispheres in this paradigm.

37.23

AUDITORY TRANSFER EFFECTS ON RECOVERY OF FUNCTION AFTER VISUAL SYSTEM LESIONS IN RATS. E.R. Delay. Dept. of Psychology, Regis College, Denver, CO 80221.

Previous studies (Delay, 1988) found that postoperative auditory discrimination (cross-modality transfer) training enhanced behavioral recovery of a brightness discrimination after occipital cortex (OC) ablations in rats. This study extended that research to examine the effects of cross-modality transfer on recovery of a brightness discrimination after lesions of the pretectal area (PA) and superior colliculus (SC). Each lesion occurred either separately or simultaneously with ablations of the OC. All rats were trained with a brightness cue to avoid shock. Twenty-four hours later surgery was performed under sodium pentobarbital anesthesia. Six days later, six rats in each surgery and control condition received either: 1) 20 trials with the original visual avoidance task, 2) 20 trials of avoidance training but with auditory intensity cues, or 3) no training. The next day, all rats were retrained on the original visual task. SC lesions produced a small behavioral deficit which was reduced by visual and auditory training. Rats with lesions of the SC plus OC showed large relearning deficits which were unaffected by auditory training. Recovery of deficits from PA, OC, and PA plus OC lesions was enhanced by auditory training. Auditory transfer training appears to aid recovery of a visual discrimination but only if either the SC or the OC are intact.

38.1

POSTSYNAPTIC LONG-TERM-ENHANCEMENT (LTE) BY DOPAMINE IS MEDIATED BY Ca^{2+} AND CALMODULIN. B.Libet and S.Mochida*, Dept.Physiology, Univ.California, San Francisco, CA. 94143.

LTE (lasting>3hr) of the muscarinic slow depolarization, or the equivalent slow (s-)EPSP, is induced either by a brief exposure to dopamine (DA) (Libet&Tosaka, P.N.A.S. 67: 667, 1970) or by preganglionic neural input which releases DA intraganglionically (Libet&Mochida, Brain Res. 473:271, 1988), in rabbit superior cervical ganglia. DA induction is effected through D_1 receptors and a rise in cyclic AMP.

We now tested the effects of reduced extracellular Ca^{2+}_o and of a specific calmodulin antagonist (calmidazolium) on DA induction of LTE, as seen in postsynaptic responses of ganglia to methacholine (MCh), in a perfusion sucrose-gap chamber. Lowering Ca^{2+}_o to either 1.0 or 0.2 mM completely abolished DA-induction of LTE, with no significant effects on the MCh depolarizing response per se. The Ca^{2+}_o action does not depend on a voltage-sensitive influx of Ca^{2+}. Calmidazolium (5μM) blocked LTE if present before treatment with DA, but did not when added after DA. Although hippocampal long-term-potentiation (LTP) exhibits similar sensitivities to Ca^{2+}_o and calmidazolium (Reymann et al., Brain Res. 461:388, 1988), LTE and LTP are quite different processes, on other grounds.

These results suggest that DA-induction of the enduring change in muscarinic responses may involve a cyclic AMP-dependent protein kinase that is activated by Ca-activated calmodulin. (Supported by U.S.P.H.S.grant NS-00884.)

38.2

IS LONG-TERM POTENTIATION (LTP) IN SYMPATHETIC GANGLIA INDUCED BY AN ENDOGENOUS MODULATOR? R. Wu*, F.G. Shon*,and D.A.McAfee. Dept. Pharmacology, University of California, Irvine, CA 92717.

Previous studies (Briggs, et al.,J. Physiol.,359:503,1985) have demonstrated that a few seconds of repetitive stimulation of the preganglionic nerve enhances ganglionic synaptic transmission for hours. Our previous observations are consistent with the hypothesis that in addition to ACh, preganglionic tetany induces secretion of a non-cholinergic, non-adrenergic modulator. Accordingly, this modulator, acting through a cyclase-coupled presynaptic receptor, produces a prolonged facilitation of the stimulus-evoked release of ACh, resulting in enhanced synaptic transmission. We now test the idea that this modulator, possibly a peptide, is susceptible to depletion by sustained preganglionic activity (Bachoo, et al.,Brain Res.400:377,1987).

Rat superior cervical ganglia were isolated, maintained in vitro as described above. Suction electrodes were used to deliver preganglionic stimuli and record postganglionic discharge. The timing of the stimuli and acquisition of the responses was controlled and quantified by computer using custom software developed in our laboratory.

Brief tetanic preganglionic stimulation (20hz/20sec) reproducibly enhanced responses to single test stimuli (1/min) about two fold. This potentiation decays exponentially (over 2 hours) to less than 20%. However, prolonged preganglionic stimulation (40hz/2hrs) inhibited these responses to brief periods of tetany. Quantitative analysis revealed that the prolonged stimulation did not inhibit generation of post-tetanic potentiation (PTP), but greatly reduced the magnitude and time course of LTP even when generated more than two hours later. The effects of prolonged stimulation were blocked if carried out in a low Ca-high Mg media which blocks release of neurotransmitters. These observations are consistent with the idea that an as yet unidentified modulator is released in a Ca-dependent manner during preganglionic activity from a pool which is readily depleted and not replenished. (This work was supported by PHS grant NS-22470.)

38.3

INVOLVEMENT OF PROTEIN KINASES IN TWO PHASES OF LONG-TERM POTENTIATION IN RAT HIPPOCAMPAL SLICES. G.L.Collingridge*, K.G.Reymann*, and S.N.Davies*. (SPON: Brain Research Association) Department of Pharmacology, University of Bristol, Bristol BS8 1TD, U.K.

We have reported that long term-potentiation (LTP) in the Schaffer collateral-commissural pathway is associated with a delayed increase in sensitivity of CA1 neurones to ionophoretically administered α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA). We suggested that this represents a slowly developing postsynaptic phase of LTP and that the initial phase may be presynaptic. We have now studied the effects of the protein kinase C inhibitor K-252b on these two phase of LTP.

In control slices tetanic stimulation usually resulted in non-decremental LTP (>180 min) and a slowly developing increase in sensitivity to AMPA. Perfusion of K-252b (50 nM, 40-90 min) starting immediately after the tetanus resulted in only an early phase of LTP (lasting 45-90 min) and no change in sensitivity to AMPA. A similar decremental LTP was seen if K-252b was applied from 15-120 min before to 40 min following the tetanus. However, if K-252b was applied for 30-60 min starting 1-3 h after the tetanus it had no effect on either the potentiated synaptic or AMPA-induced responses.

These results suggest that there is a transient K-252b sensitive process activated following the tetanus that leads to a postsynaptic change associated with LTP.

38.4

IS PROTEIN KINASE C INVOLVED IN THE MAINTENANCE OF LONG TERM POTENTIATION ? A. Arai*, J. Larson*, M. Kessler, M. Oliver* and G. Lynch. Center for the Neurobiology of Learning and Memory, University of California, Irvine, CA 92717.

Recent studies using protein kinase C (PKC) inhibitors and activators have suggested a prominent role for PKC in long term potentiation (LTP). In particular, the inhibitor H-7, which acts directly on the catalytic subunit of the kinase, has been reported to reverse LTP for the duration of its infusion, even if applied 1 hour after the establishment of LTP. We have further examined the effects of H-7 and other PKC inhibitors on the physiology and the maintenance of LTP in field CA1 of the hippocampal slice. H-7 (75-450 μM) was perfused 10-20 min after establishing LTP in one of two input pathways. Low concentrations of H-7 (75 or 150 μM) had no influence on the slope of either control or potentiated EPSP's. Above 150 μM single stimulation pulses evoked repetitive neuronal discharge, and seizures were observed at concentrations above 300 μM. An incision between CA1 and CA3 could supress the seizures and the delayed part of the spiking activity and was, therefore, routinely applied. Intracellular analysis suggested that spiking resulted from a blockade of inhibitory synaptic potentials; this could at least partially be due to an interaction with GABA receptors, since H-7 at 400 μM was found to inhibit [3H]muscimol binding by more than 50%.

At concentrations of 300 and 450 μM, H-7 reduced both control and potentiated responses but did not show a preferential effect on the latter. These results do not support the hypothesis that sustained activation of PKC is required for the maintenance of LTP. (Supported by AFOSR 86-0099 and NS 21860)

38.5

REGULATION OF CALCIUM/CALMODULIN-DEPENDENT PROTEIN KINASE II IN CEREBELLAR GRANULE CELLS. K. Fukunaga*, D.P. Rich*, and T.R. Soderling. (SPON: T. Inagami), Dept. of Mol. Physiol., Vanderbilt Med. Sch., Nashville, TN 37232-0615.

CaM-kinase II is highly enriched at synapses where it may regulate Ca^{2+}-dependent functions including long-term potentiation. In vitro studies have identified a unique consequence of autophos. of Thr-286/287 to produce a Ca^{2+}-independent form of the kinase. We have investigated generation of this Ca^{2+}-indep. form of CaM-kinase II in 8-10 day cultured rat cerebellar granule cells. Under basal conditions about 4-5% of total CaM kinase II activity (assayed with Ca^{2+}/CaM) was the Ca^{2+}-indep. form (assayed with EGTA). Depolarization with 56 mM K^+ produced a rapid increase to 10% Ca^{2+}-indep. activity within 15-30 sec followed by a decline to 5-6% at 10 min. Ionomycin produced 10% Ca^{2+}-indep. activity which remained elevated. Inclusion of 5 μM okadaic acid, a protein phosphatase inhibitor, in the incubation medium potentiated the increases in Ca^{2+}-indep. activity. Removal of Ca^{2+} from the Krebs-Ringer medium reduced Ca^{2+}-indep. activity to 1-2% and eliminated the elevations in response to high K^+. Phosphopeptide mapping of the ^{32}P-labeled 58-60 kDa subunit of the CaM-kinase II revealed a correlation between generation of Ca^{2+}-indep. activity and ^{32}P-incorporation into peptide CB1 which contains Thr-286/287. These results indicate that formation of the Ca^{2+}-indep. CaM-kinase II is regulated in cerebellar granule cells by Ca^{2+} influx and by endogenous protein phosphatase activity and correlates with phosphorylation of Thr-286/287.

38.6

DIRECT EVIDENCE THAT ACTIVATORS OF PROTEIN KINASE C INCREASE THE PHOSPHORYLATION OF SYNAPSIN I AND PROTEIN III IN RAT HIPPOCAMPAL SLICES. E. Barnes* and M.D.Browning. Department of Pharmacology, University of Colorado H.S.C., Denver, CO 80262.

Previous studies have shown that activators of protein kinase C (PKC) produce LTP-like potentiation of synaptic transmission in the hippocampus. We have recently shown in chromaffin cells that phorbol dibutyrate (PdBu, a PKC activator) increased the phosphorylation of protein III (two synaptic vesicle-associated phosphoproteins protein IIIa M_r 74,000 and protein IIIb, M_r 55,000 that are collectively referred to as protein III) in chromaffin cells. Protein III is a very poor substrate for PKC but an excellent substrate for cAMP-dependent and Ca^{2+}/calmodulin dependent protein kinases. Thus we felt that activators of PKC like PdBu might leading to activation of a kinase cascade in chromaffin cells. In this report we test the hypothesis that similar effects on protein III and synapsin I (a protein very homologous with protein III) might be obtained in hippocampal slices.

Hippocampal slices were incubated for 90 min in $^{32}PO_4$ in PO_4-free buffer followed by ten minute incubation in various treatments. After 10 min the slices were sonicated in 1% SDS and synapsin I and protein III were immunoprecipitated and run on SDS-PAGE. PdBu produced dose-dependent increases in the phosphorylation of synapsin I and protein III with half-maximal effects at 100 nM. Maximal increases at 1.0 μM PdBu were 82%, 55%, and 56% for synapsin I, protein IIIa and protein IIIb respectively. We also performed phospho-site analysis of synapsin I using limited proteolysis. These studies indicated that PdBu increased the phosphorylation of multiple sites on synapsin I. These sites have previously been shown to be phosphorylated by both cAMP and Ca^{2+}/calmodulin dependent protein kinases. Previous studies have provided direct evidence that synapsin I regulates neurotransmitter release at the squid giant synapse. Accordingly, we hypothesize that the phosphorylation of synapsin I and the related protein, protein III may play an important role in mediating the increased transmitter release elicited by activators of PKC. (Supported by PHS grants DK40483 and NS26377 to M.D.B.)

38.7

DIRECT EVIDENCE THAT NOREPINEPHRINE INCREASES THE PHOSPHORYLATION OF SYNAPSIN I AND PROTEIN III IN DENTATE SLICES OF YOUNG BUT NOT AGED F344 RATS. K. Parfitt*, B.J. Hoffer, M.D. Browning. Dept. Pharmacology, U. Colorado HSC, Denver, CO 80262.

Previous investigations have shown deficiencies in noradrenergic neurotransmission in the hippocampus of aged rats. Mobley and Greengard (PNAS 82,945, 1985) have shown that norepinephrine (NE) stimulates the phosphorylation of synapsin I in rat frontal cortex. The purpose of this study was to determine whether NE was capable of enhancing phosphorylation of synapsin I and protein III, two homologous phosphoproteins thought to be involved in transmitter release, in the dentate of young (3 mos) F344 rats and aged (26 mos) F344 rats. We focussed on the dentate because of reports that NE produces LTP in this region of the hippocampus. LTP, thought to be an electrophysiological correlate of memory, in part involves an augmentation of transmitter release. Dentate slices were incubated for 90 min in $^{32}PO_4$ in PO_4-free buffer followed by 10 min incubation in various treatments. The slices were then sonicated in 1% SDS, and synapsin I and protein III were immunoprecipitated and run on SDS-PAGE. Viability of slices was assessed by electrophysiological criteria, including evoked responses as well as LTP. NE (10 uM) and isoproterenol (ISO, 250 nM) produced an increase in the phosphorylation of synapsin I and protein III in dentate slices of young rats but failed to alter the phosphorylation of these proteins in aged rats. Phospho-site analysis of synapsin I, performed using limited proteolysis, suggested that NE and ISO increased the phosphorylation of sites modified by cAMP-and Ca^{2+}/calmodulin-dependent protein kinases. Neither of these sites exhibited NE- or ISO-enhanced phosphorylation in the aged rats. This β-adrenergic agonist-stimulated phosphorylation of synapsin I and protein III is hypothesized to play a role in the potentiation produced by NE in the dentate. Thus, the failure of the aged rats to show such phosphorylation may underlie, in part, the failure of aged rats to exhibit LTP. (Supported by PHS grants GM07635 to K.P., AG04418 to B.H. and NS26377 & DK40483 to M.D.B.)

38.8

ISOPROTERENOL AND BACLOFEN SYNERGISTICALLY PRODUCE A LONG-LASTING POTENTIATION OF EVOKED RESPONSES IN RAT DENTATE GYRUS. J. M. Sarvey and E. C. Burgard. Dept. of Pharmacology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814.

Bath application of ß-adrenergic agonists in the rat hippocamal slice preparation produces a long-lasting potentiation of evoked responses recorded in the dentate gyrus. The $GABA_B$ receptor agonist baclofen has been shown to augment the intracellular second messenger response to ß-adrenergic agonists in the hippocampus. Here we demonstrate that baclofen can also potentiate the electrophysiological effects of isoproterenol in the rat dentate gyrus.

Hippocampal slices were maintained in an interface recording chamber. Stimulation of the medial perforant path evoked a population spike (PS) recorded extracellularly in the granule cell layer of the dentate gyrus. A 20 min bath application of isoproterenol produced a dose-dependent potentiation of the evoked population spike amplitude which persisted throughout a 30min wash. ISO-induced potentiation, measured at 30 min wash was reliably induced at a concentration of 1µM (mean 191% of control, n=5). A concentration of 0.1 µM ISO had no effect on the PS (mean 96% of control, n=6). A 15 min bath application of (-) baclofen produced a dose-dependent enhancement of PS amplitude. This effect was maximal at a concentration of 5µM and was associated with a decrease in paired-pulse inhibition (interpulse intervals of 20-30ms). At a concentration of 0.1µM, baclofen did not significantly affect PS amplitude (mean 106%, n=8). However, administration of baclofen (0.1µM) together with ISO (0.1µM) produced a significant long-lasting potentiation of the PS amplitude measured at 30 min wash (mean 163%, n=6). These results demonstrate that ISO and baclofen, when administered together in doses below threshold for measurable effects, can produce a synergistic long-lasting potentiation of evoked responses in rat dentate gyrus. (Supported by NIH grant NS23865)

38.9

INDUCTION OF LONG-TERM POTENTIATION IN RAT CA1 REGION IS NOT ALTERED BY CHOLINERGIC STIMULATION. R.D. Blitzer, O. Gil*, and E.M. Landau. Dept. of Psychiatry, Bronx VA Medical Center, Bronx, NY 10468

The activation of NMDA receptors is essential to LTP in certain components of the rat hippocampal circuitry, including the Schaffer collateral - CA1 pathway. The CA1 region also receives a cholinergic innervation, and the effects of cholinergic stimulation on LTP in CA1 may give insight into the cholinergic role in memory. We have investigated the effect of carbachol (CCh) on the LTP induced in the CA1 region in rat slices. Extracellular recording and stimulating electrodes were placed in stratum radiatum. Tetanus consisted of two 100 Hz trains. CCh was present for 10-20 min before tetanus, and removed immediately after tetanus. The stimulus intensity used for tetanus was adjusted in CCh to control for EPSP depression, and returned to baseline after tetanus.

1 µM CCh depressed the EPSP by 34% before tetanus. Field potentials recorded during tetanizing trains were similar in CCh and control slices. Following tetanus, when time was allowed for CCh washout, LTP (measured either as % change EPSP amplitude or slope) was found to be unaffected by CCh administration. 30 min post-tetanus, EPSP amplitude was increased in control and CCh-treated slices to 147±11 and 146±7% of baseline, respectively; EPSP slope was increased to 162±17 and 180±14%.

5 µM CCh depressed the EPSP further (by 47%), but also failed to affect LTP. Similar results were obtained in the presence and absence of picrotoxin. The data show that a cholinergic agonist fails to reduce LTP in CA1, in contrast to the depression of LTP observed in CA3 (Williams & Johnston Science, 242: 84, 1988). Supported by NIH Grant 5-P50-AG05138-02.

38.10

INFLUENCE OF CHOLINERGIC NEUROTRANSMISSION ON HIPPOCAMPAL SYNAPTIC PLASTICITY. D. A. Engstrom and G. M. Rose. Department of Pharmacology, UCHSC & Medical Research, VAMC, Denver CO 80262

The purpose of this study is to determine what effect increasing synaptic levels of endogenously released acetylcholine has on primed burst potentiation. Primed burst potentiation, (PB), is a model of hippocampal synaptic plasticity, in which a patterned stimulation paradigm is used to evoke long term changes in synaptic efficacy.

Male Sprague-Dawley rats (275-350 g) are anesthetized with either urethane (1.5 g/kg, i.p.), secobarbital (50 mg/kg, i.p.) or pentobarbital (60 mg/kg, i.p.). Animals given barbiturates are maintained with a supplemental infusion to maintain adequate anesthesia. The animals are then prepared for acute in-vivo recordings. A glass multi-barrel recording electrode is lowered into the CA1 region of the hippocampus, and a teflon insulated wire stimulating electrode is lowered into the contralateral hippocampal commissure. Recordings and measurements are made of cell firing rates and attributes of the evoked field potentials, namely the population spike and slope of the e.p.s.p. Physostigmine, an inhibitor of acetylcholinesterase, is administered either i.p., i.c.v., or by local application through a barrel of the recording electrode.

Results to date suggest that within a narrow dose window, physostigmine can lower the threshold for eliciting PB or increase its magnitude, while doses outside this window can have opposite effects. The data suggest a complex, "inverted-U" type dose response similar to dose response relationships found for the drug in behavioral pharmacological paradigms. Differences in the quality and efficacy of the different routes of administration of physostigmine will be discussed.

38.11

LONG TERM POTENTIATION IN THE HIPPOCAMPUS INDUCED BY THE MAST CELL DEGRANULATING PEPTIDE : RELEASE OF AMINOACIDS AND PROTEINS.

M.P. ROISIN*, C. CHARRIAUT-MARLANGUE*, L. ANIKSZTEIN* and Y. BEN ARI. (SPON. by I. Hunter). INSERM U29, 123 Bld de Port-Royal, 75014 Paris.

The Mast Cell Degranulating peptide (MCD) is a bee venom peptide which produces seizures and convulsions in the rat following intracerebroventricular application. MCD, which blocks a specific K^+ channel produces in the CA_1 hippocampal region a long lasting enhancement of synaptic transmission similar to the long term potentiation (LTP) produced by a high frequency train of electrical stimulation. Using a push pull device, we have analyzed in vivo, in the CA_1 region of the hippocampus, the release of endogenous excitatory amino acids and proteins induced by MCD in relation to long term potentiation.

Application of MCD (20 µM for 10 min) produced a long lasting potentiation of the field E.P.S.P (4 hours). This potentiation was associated only with a short transient increase (10 min) in the release of glutamate (5 fold) and aspartate (6 fold), and with a delayed (1-2 hours) enhanced release of pre-existing (64, 54, 48, 45 kDa) proteins and the appearance of newly secreted proteins (33, 19, 16 kDa). These experiments confirm our earlier observations that LTP produced by a train of high frequency stimulation is not due to a sustained enhanced release of glutamate and aspartate. Our results cannot be readily reconciled with a presynaptic type of mechanism and suggest that postsynaptic mechanisms may play a crucial role in LTP.

38.12

BLOCKADE OF MAINTENANCE OF LONG-TERM POTENTIATION BY DENTATE, BUT NOT ENTORHINAL, INHIBITION OF PROTEIN SYNTHESIS. W.C.Abraham and S. Otani*. Dept.of Psychology and the Neurosci. Res. Center, Univ. of Otago, Dunedin, New Zealand.

We investigated whether the new protein synthesis, previously shown to be important for perforant path LTP maintenance, occurs in the dentate gyrus or the entorhinal cortex. Anisomycin, but not boiled anisomycin or the saline vehicle, injected into the dentate gyrus of urethane-anesthetized rats led to nearly complete decay of LTP within 3 hours. Intra-entorhinal anisomycin had no effect on LTP maintenance even over 6 hours. Intra-dentate injection of actinomycin-D (a mRNA synthesis inhibitor) also did not affect LTP maintenance over 6 hours. These results suggest that the proteins necessary for LTP maintenance over 6 hours are synthesized in the dentate gyrus from already existing mRNA, without involving protein synthesis in the cell bodies of the afferent fibers.

38.13

TETANIZATION-INDUCED LONG-LASTING DEPRESSION OF HIPPOCAMPAL CELL EXCITABILITY IS MEDIATED BY VOLTAGE-DEPENDENT CALCIUM CHANNELS. J. Wickens* and W.C.Abraham. SPON: (J.G.Anson). Depts. of Psychology and Anatomy and the Neurosci.Res. Centre, Univ. of Otago, Dunedin, New Zealand.

The induction of long-lasting depression of the orthodromic population spike in area CA1 was investigated using extracellular recordings in hippocampal slices from which area CA3 had been removed. Antidromic tetanization (6 trains of 50 pulses; 200, 50, or 15 Hz) induced transient reductions in the stratum radiatum evoked population spike and field EPSP that lasted 5-10 min. When the slices were bathed with 20 uM picrotoxin, the depression of the spike, but not of the EPSP, persisted for at least 30 min. Orthodromic tetanization (200 Hz) of the stratum oriens gave greater long-lasting depression than antidromic stimulation. An antibody to L-type calcium channels and nimodipine (20 uM) both prevented the induction of the long-lasting spike depression. These data suggest that a lasting, generalized depression of pyramidal cell excitability, mediated by voltage-dependent calcium channels, can result from large depolarizations.

38.14

BRAIN DNA SYNTHESIS DURING LONG-TERM POTENTIATION OF THE PERFORANT PATH-GRANULE CELL SYNAPSE.

A. Giuditta*, A. Neugebauer*², F. Morelli*², U.A. Gironi Carnevale*, A.G. Sadile³, G. Buzsaki⁴. (SPON: A. Cerbone). Dept. Gen. Environm. Physiol., Univ. Naples; ²Int. Inst. Genet. Biophys., Naples; ³Inst.Hum.Physiol.& Med.Biophys., Univ. Naples, Italy; ⁴Inst. Physiol., Univ. Pècs, Hungary.

Long-term potentiation (LTP) of perforant path-granule cell synapse was adopted as plasticity model to investigate its effect on DNA synthesis in hippocampal and cortical areas of rat brain. Three groups of adult Long-Evans rats were given 50μCi of ³H-thymidine intraventricularly under urethane and received a train of high (HFS: 400Hz) or low frequency (LFS: 0.1Hz) in the perforant bundles, or were not stimulated (CON). Field EPSP and population spike were monitored from dentate gyrus before and 5, 10, 15, 60 min afterwards. DNA synthesis was measured post-hoc by the incorporation of the radioactive precursor into DNA in hippocampal and cortical areas of either side. Discriminant function analyses separated the groups along 2 main axes, explaining 83% (LTP) and 17% (DNA synthesis) of between-group variance. Correlative analyses showed that LFS covaried positively and HFS negatively with field EPSP in the stimulated dentate area, and with population spike in dentate area and frontal cortex of both sides, and in CA3 and CA1 of the stimulated side. The results suggest a modulatory role of LTP on DNA synthesis in hippocampal and functionally related cortical areas.

38.15

INCREASED POSTSYNAPTIC RELEASE AND PRESYNAPTIC ACTIONS OF ARACHIDONIC ACID SUGGEST A RETROGRADE MESSENGER ROLE IN LONG-TERM POTENTIATION.

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The aims of the present ex vivo study were two-fold: to identify the source of the increased release of arachidonic acid (AA) into push-pull perfusates which is associated with LTP in the dentate gyrus (Lynch, M.A. et al., Neuroscience, 30, No 3, 1989), and to examine the presynaptic actions of AA and its lipoxygenase metabolites. Synaptosomes, and fractions enriched in glia and in postsynaptic densities (PSDs) were prepared from potentiated and control dentate gyrus. A significant increase in liberation of free AA was found in PSDs from potentiated tissue (LTP: 0.87 ±0.20;, control: 0.39±0.10 μmol AA/mg protein, n=6, $p<0.05$); no significant changes were found in membrane-associated fractions from synaptosomes or glia. The increased release of AA is therefore probably postsynaptic in origin. Enzymatic studies suggest that the increase is initiated by enhanced phospholipase A_2 activity and sustained by increased phospholipase C activity. Presynaptically, AA and its 12-lipoxygenase metabolites 12-HETE and 12-HPETE stimulated glutamate release and phosphoinositide turnover in synaptosomes.

38.16

INFLUENCE OF GROWTH FACTORS ON SYNAPTIC PLASTICITY: DIFFERENTIAL EFFECTS OF EGF AND FGF ON LTP IN THE HIPPOCAMPAL SLICE. *H. Terlau* and W. Seifert*
Max-Planck-Institut für biophys. Chem. Department of Neurobiology, Göttingen (F.R.G)

Rat hippocampal slices were perfused either with epidermal growth factor (EGF) at a concentration of 10^{-8}M or fibroblast growth factor (FGF) at a concentration of 10^{-9}M. During extra- and intracellular recordings in the pyramidal cell body layer of the CA_1-region no influence of EGF on the evoked potentials was seen if single or paired pulse stimulation was used. However the presence of EGF in the bath medium lead to an increase in the magnitude of potentiation after tetanic stimulation. This increase was maximal directly after tetanisation but still present 30 min after the tetanus was given (Terlau and Seifert, Brain Research, in press).

Also in the presence of FGF no changes in the population spike amplitude and field EPSP during low frequency stimulation were seen. However there was a little increase in the magnitude of potentiation, which becomes bigger with the time after tetanisation. The increase in the field EPSP slope 2 min after the tetanus was: Control: 155.3±8.2%, FGF: 162.0±8.6%; mean±S.E.M.; not significant. After 30 min the increase was: Control: 128.1±4.3%, FGF: 144.0±5.3%; n=20; $P<0.01$.

These observations show that growth factors with neurotrophic potential like EGF and FGF can modulate synaptic plasticity, but there are clear differences in this modulatory action.

38.17

EFFECTS OF INTERLEUKIN-2 ON LONG-TERM POTENTIATION IN RAT HIPPOCAMPUS.

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The T lymphocyte response to mitogen or antigen results in the secretion of the polipeptide growth factor interleukin (IL-2). Since in the cancer therapy IL-2 subministration causes some neurological side effects, such as memory inpairment, experiments were performed to investigate whether IL-2 may affect the long-term potentiation (LTP) of synaptic transmission that is widely regarded as a candidate substrate for aspects of memory in the CNS. We give here evidence that IL-2 at threshold dose of 1000 U/ml strongly inhibits in a dose-dependent manner, LTP in rat hippocampus. This is possibly related to a post-synaptic IL-2 action since IL-2 did not influence voltage-dependent Na+, K+, and Ca2+ currents in patch-clamped neocortical cultured neurones of the rat, while reduced mean open time of single acetylcholine channels in mouse cultured myotubes. This work is supported by a grant from Fidia.

38.18

CHANGES IN THE INPUT/OUTPUT (I/O) PROFILE WHICH ACCOMPANY HIPPOCAMPAL LONG-TERM POTENTIATION (LTP). A. AU* AND L.S. LEUNG (SPON: W.T. BLUME). DEPTS. PHYSIOLOGY AND CLIN. NEUROL. SCI., UNIV. WESTERN ONTARIO, LONDON, CANADA, N6A 5A5.

Population spikes were recorded in the CA1 cell layer following test pulses of varying intensities delivered to the stratum radiatum in the slice preparation. I/O profiles were recorded for 30 minutes prior to and 60 minutes after LTP induced by a patterned burst stimuli. Responses were normalized by the maximal population spike amplitude before LTP.

Low-amplitude baseline responses showed post-tetanic potentiation (PTP at 10 sec) and to a lesser degree LTP (>10 min) while high-amplitude responses showed no PTP although they demonstrated LTP.

	Baseline	10 sec	10 min	30 min	60 min
Percent	0	30 ± 8#	8 ± 3#	7 ± 4	1 ±.1
of	11 ± 1	72 ± 9#	54 ± 8#	38 ±11#	26 ±11
Maximal	45 ± 2	87 ± 6#	78 ± 4#	69 ± 6#	64 ±9#
Baseline	84 ± 1	104± 6#	108± 5#	106± 7#	102±4#
Response	100 ±.5	106± 5	110± 3#	109± 5	112±3#

(# $p<.05$)

The magnitude and time course of both PTP and LTP appear to depend upon the size of the population spike. Supported by MRC and NSERC.

39.1

HORMONAL INFLUENCES ON INSECT MOTONEURONS DEVELOPING IN CELL CULTURE. L.Griffin* and R.B. Levine. Arizona Research Labs. Division of Neurobiology, University of Arizona, Tucson, AZ 85721.

In the hawkmoth, Manduca sexta, many larval motoneurons (MNs) persist through metamorphosis to innervate new muscles in the adult. During this process identified thoracic and abdominal MNs undergo substantial changes in their dendritic branching patterns that are known to be under hormonal control. To facilitate the analysis of hormonal influences on MN morphological and biophysical properties, we have developed techniques for following the postembryonic development of identified motoneurons in primary cell culture. The culture conditions were adapted from those devised by Dr. Jon Hayashi (Neuro. Abs.,1989). Neurons were grown in a modified L-15 medium, on glass cover slips that had been coated with laminin and con-A. Thoracic leg MNs were labeled as a population by injecting di-I into several leg muscles early in the fifth larval instar. At various times thereafter the thoracic ganglia were desheathed and dissociated enzymatically. Although the cultures contained many neuronal types, leg MNs were identified after plating by the presence of di-I in their somata, and have been followed for up to four weeks in vitro. Intracellular soma recordings revealed that the MNs had normal resting potentials and were able to generate action potentials upon depolarization. In vivo, thoracic leg MNs undergo dendritic regression at pupation, followed by the growth of new dendrites during the first two weeks of adult development. MN somata isolated from early pupal (P0-P2) ganglia assumed a unipolar, or occasionally bipolar morphology during the first two weeks in culture. In the absence of the steroid 20-hydroxyecdysone (20-HE), the MNs grew one or two long, broad processes. Process outgrowth, especially the extent of fine branching, was enhanced by the addition of 20-HE to the medium in levels similar to those experienced in vivo (1 μg/ml).

39.2

AN ECDYSTERONE-REGULATED GENETIC LOCUS IS REQUIRED FOR CNS METAMORPHOSIS IN *DROSOPHILA*. L.L. Restifo and K. White*, Department of Biology, Brandeis University, Waltham, MA 02254.

The *Broad Complex* (*BR-C*) is an ecdysterone-regulated locus which is essential for metamorphosis in *Drosophila*. It consists of at least two distinct functional domains required for development of the adult epidermis (Kiss *et al.*, *Genetics* **188**:247, 1988). We have previously demonstrated that *BR-C* transcripts are expressed in the larval and adult CNS, and that some *BR-C* mutants exhibit abnormalities of CNS reorganization during metamorphosis (Restifo and White, *Soc. Neurosci. Abstr.* **14**:733, 1988). We have now extended this analysis to include mutants representing the several functions encoded at this complex locus. The CNS phenotypes observed in *BR-C* mutants include abnormalities of i) formation of the cervical connective between the brain/subesophageal ganglion and the thoracic ganglion; ii) movement of the subesophageal ganglion into the developing head capsule; iii) rotation of the optic lobes; and iv) expansion of the synaptic neuropil of the dorsal brain. Some *BR-C* mutations are also associated with decrease or absence of the dorsal-ventral muscle class of indirect flight muscles. These morphological abnormalities will be discussed with respect to the genetic organization of the locus. In addition, a molecular analysis of *BR-C* transcription in the CNS will be presented. (Funded by NIH grant NS01259 to LLR.)

39.3

REDUCED FERTILITY IN MALE GENETICALLY OBESE ZUCKER RATS IS NOT ASSOCIATED WITH DECREASED NUMBER OF MOTONEURONS IN THE SPINAL NUCLEUS OF THE BULBOCAVERNOSUS. J. A. Finkelstein, L. Jasenak*, W. Lee* and P. C. Doherty. Dept. of Anatomy, Northeastern Ohio Univ. Col. Med., Rootstown, OH 44272.

Genetically obese Zucker rats are known to have impaired reproductive function. This impairment has been studied in obese and lean male Zucker rats by comparing 1) ability to impregnate female rats, 2) sexual organ weights and 3) number of motoneurons in the sexually dimorphic spinal nucleus of the bulbocavernosus (SNB). Nineteen obese (fa/fa) and 9 age-matched lean (Fa/-) male rats were each placed with two lean female rats. Sperm was seen in vaginal smears from all females, regardless of whether or not pregnancy occurred. Females were removed when pregnant or at the end of forty days. While all lean males induced pregnancy, only nine of the nineteen obese males were able to sire litters. Testes weights did not differ between obese and lean rats, but weights of both the seminal vesicles and the bulbocavernosus muscle complex were lower in the group of obese rats as a whole. On these measures, however, obese males whose mating resulted in successful pregnancy did not differ from those who were not successful. Number of motoneurons in the SNB (uncorrected counts) was not associated with increased fertility. These data indicate that no single morphological parameter correlates with the ability of an obese male rat to induce pregnancy. Supported by Biomedical Research Support Grant No. S07RR05806-06 and Research Challenge Funds from the Ohio Board of Regents.

39.4

ANDROGEN METABOLITES MASCULINIZE MOTONEURON NUMBER IN A SEXUALLY DIMORPHIC RAT SPINAL NUCLEUS. L.A. Goldstein and D.R. Sengelaub. Program in Neural Science, Dept. of Psychology, Indiana University, Bloomington, IN 47405.

The development of masculine motoneuron number in the sexually dimorphic spinal nucleus of the bulbocavernosus (SNB) in rats is determined by an androgen-regulated cell loss. Females treated with testosterone propionate perinatally have a masculine SNB, but treatment with the metabolite dihydrotestosterone is ineffective. To determine if masculinization of the SNB involves the conversion of testosterone into both dihydrotestosterone and estrogen, we compared motoneuron numbers in females treated with estrogen alone or in combination with dihydrotestosterone.

Timed-pregnant female rats were injected with estradiol benzoate (EB, 100 μg) or EB and dihydrotestosterone propionate (DHTP, 2 mg) on embryonic days (E) 16-22. Pups were cross-fostered and injected on the day of birth [postnatal day (P) 1], P3 and P5. At E18, E20, E22, P4 and P10 counts of SNB motoneurons were made in hormone-treated females and compared with counts from normal animals.

At E18, counts of SNB motoneurons were low in all groups and increased through E22. At E22, SNB motoneuron numbers in both EB and EB+DHTP females were similar to that of normal females, but significantly lower than that of normal males. Between E22 and P10, cell numbers declined in all groups. This decline was greatest in EB and normal females (52-67%) who did not differ at P10 (79 *vs.* 72 motoneurons). Motoneuron loss in normal males was much less (31%), resulting in significantly more SNB motoneurons at P10 (215). Cell loss after E22 in EB+DHTP females was only 14%; by P10 the number of SNB motoneurons was not different from that of normal males (199). These data suggest that both metabolites can act synergistically to masculinize SNB development. (Supported by NIH NS24877)

39.5

TESTOSTERONE MASCULINIZES THE DEVELOPMENT OF THE SUBSTANCE P INNERVATION OF THE MEDIAL NUCLEUS OF THE AMYGDALA AND BED NUCLEUS OF THE STRIA TERMINALIS. C.W. Malsbury & S.A. Brown*. Dept. Psychology and Division of Basic Medical Sciences, Memorial U. of Newfoundland, St. John's, NF, Canada, A1B 3X9.

Our laboratory has previously described marked sex differences in the substance P (SP) innervation of the most posterior divisions of both the medial nuc. of the amygdala (MePD) and medial bed nuc. (BSTMP) in the adult rat brain. We have now examined the effects of early postnatal testosterone exposure. Four groups (n=4/group) were compared. Males were either sham-operated (SM) or gonadectomized (GM) on day 1, while females were injected sc with either vehicle (SF) or 500 ug testosterone propionate (TF) on days 2 and 5. All animals were killed at day 26. Alternate sections were either stained with cresyl violet or for SP using the PAP method. The areas of dense SP fiber staining and of relevant cell groups (cresyl) were measured. Marked sex differences were seen in the MePD and BSTMP before puberty (day 26). Neonatal testosterone injections masculinized SP staining in females, while castration of newborn males prevented the development of the masculine pattern of staining, eg. means of the total areas (mm^2) of SP staining in MePD were: SM, 4.90 > TF, 4.22 > GM, 3.04 = SF, 2.95.

39.6

ONTOGENY OF ENKEPHALIN IMMUNOREACTIVITY IN THE SEXUALLY DIMORPHIC MEDIAL PREOPTIC NUCLEUS. R.P. Hammer, Jr., D. Yoshishige* and S.A. Tobet (SPON: H.L. Gillary). Dept. Anatomy & Reprod. Biol., Univ. Hawaii Sch. Med., Honolulu, HI 96822 and Dept. Biochem. and Developmental Neurobiol., E.K. Shriver Ctr., Waltham, MA 02254.

In the adult rat medial preoptic nucleus (MPN), sex differences in the density and distribution of enkephalin-immunoreactive (ENK-IR) fibers have been reported (Simerly, et al., *J. Comp. Neurol.*, 276: 442). In the present study we examined the ontogeny of ENK-IR fibers during the early postnatal period to determine when, in relation to the formation of cytoarchitectonic sexual dimorphisms, opioid peptide immunoreactivity becomes sexually dimorphic in the MPN.

Rats of different postnatal ages through P12 were perfused with neutral buffered 4% paraformaldehyde, brains were postfixed in 5% acrolein and sectioned by Vibratome prior to processing with leu-enkephalin antibody (Immunonuclear Corp.) using the ABC technique with 3, 3'-diaminobenzidine as substrate. The most striking ontogenetic change in ENK-IR expression was the rapid increase in MPN fiber density, which achieved the adult pattern of higher density in lateral MPN than medial MPN earlier in the male. In contrast, lateral septum ENK-IR fiber density transforms from dense and unpatterned at P4 to the characteristic pericellular adult pattern in both sexes by P12. At the earliest ages, ENK-IR fiber density in the lateral preoptic area is greater than in MPN, but this changes with age in both sexes. Sexual dimorphism of MPN morphology develops in the first postnatal week (Hammer and Jacobson, *Int. J. Devel. Neurosci.*, 2: 77), before a sex difference in MPN ENK-IR is present.

We conclude that sex differences in MPN cytoarchitectonics precede sexual differentiation of MPN ENK-IR. Therefore, factors within the MPN may influence the ontogeny of MPN enkephalinergic fiber distribution, rather than the converse. (Supported by USPHS Awards DA04081 and NS01161 to RPH and HD20327 to SAT.)

39.7

CORPUS CALLOSUM: OVARIAN HORMONES AND FEMINIZATION IN THE RAT.
R. H. Fitch*, P. E. Cowell*, L. M. Schrott* and V. H. Denenberg. (SPON: E. Thoman). Biobehavioral Sciences Graduate Program , U-154, Univ. of CT, Storrs, CT 06269-4154

The rat's corpus callosum (CC) is sexually dimorphic with the male's being larger (Berrebi et al., Br. Res., 1988, 438:216-224). This sex difference appears to depend in part on the neonatal presence of testosterone in the male, and estrogen in the female (Fitch et al., ms. in preparation). To further investigate the possibility that neonatal hormones act in the development of the female's CC, rats were ovariectomized (Ovx) on postnatal days 8, 12, and 16. Female littermates received 1 mg of testosterone propionate (TP) on corresponding days, while others received sham surgery. Male littermates were also retained for comparison. All animals were handled daily from birth until weaning. Animals were sacrificed at 110 days and a mid-sagittal section of the callosum was obtained. For controls, the male CC was larger. The time of Ovx (Day 8, 12 or 16) was not significant, and thus Ovx data were pooled across days. Ovx acted to enlarge the CC of females compared to controls. Ovx female values were equal to or greater than male values for most CC measures. Brain weight was significantly larger for Ovx females, although still significantly less than for control males. TP had no effect on any CC variable when administered on Days 8, 12 or 16.

We conclude that ovarian hormones - presumably estrogen - act to feminize the callosa of females, and that the removal of estrogen from females results in defeminization. Furthermore, the fact that ovariectomy was effective as late as Day 16 while TP treatment on Day 8 or later had no effect suggests that masculinization and feminization of this structure may constitute separate processes with distinct critical periods.

39.8

CORPUS CALLOSUM: INTERACTIVE EFFECTS OF INFANTILE HANDLING AND TESTOSTERONE IN THE FEMALE RAT. V. H. Denenberg, R. H. Fitch*, P. E. Cowell*, L. M. Schrott* and N. S. Waters*. Biobehavioral Sciences Graduate Program, U-154, Univ. CT., Storrs, CT 06269-4154.

In the rat the male's corpus callosum (CC) is larger than the female's. Further, neonatal handling enhances this sex difference (Berrebi et al., Br. Res., 1988, 438:216-224). More recently, 1 mg testosterone propionate (TP) given on Day 4 of life increased the female's CC to the size of the male. These animals had been handled in infancy to maximize the base-line sex difference (Fitch et al., ms. in preparation). In the current set of experiments the effects of handling (H) vs nonhandling (NH) were studied. In Experiment 1 the Fitch et al. study was replicated using NH animals. Within a litter two males were castrated on Day 1 and two received sham surgery; two females received 1 mg TP on Day 4 and two received an oil injection. At 110 days a mid-sagittal section of the CC was obtained. In controls the CC was larger in males. Neither castration nor TP treatment had any effect on any CC parameter. Experiment 2 included both H and NH rats. Surgery and hormone regimens were the same as in Experiment 1. H pups were placed for 3 min into cans containing shavings on Days 1-20. NH pups were undisturbed until weaned at 21 days. A mid-sagittal section of the CC was obtained at 110 days. Within the H treatment, control males and TP females had a larger CC than control females while castration of males had no effect. In the NH group, the CCs of control males were marginally larger than those of females; neither TP treatment to females nor castration of males affected CC size.

In a series of three experiments TP on Day 4 was effective in masculinizing the CC of handled , but not nonhandled, female rats when measured in adulthood. This probably involves an interaction of adrenal and gonadal hormones.

39.9

REGIONAL SPECIFICITY OF SEX DIFFERENCES IN THE RAT NEOCORTEX. Silvia N. M. Reid & Janice M. Juraska. Dept. of Psych. and Neural & Behavioral Biology Program, Univ. of Ill., Champaign, IL 61820.

The cerebral cortex has both a regional and laminar organization. While several studies have indicated that the male rat cortex is thicker than that of the female, it is not known whether every cortical region or layer is dimorphic.

Coronal frozen sections (30-50 microns) were obtained from brains of paraformaldehyde perfused 90 day old Long-Evans hooded rats (10 littermate pairs; socially housed) and stained with methylene blue-Azure II. Differences in the cortical thickness (male>female) were found in all four major regions (16 of 25 measures). Sex differences in layer thickness (male>female) showed regional specificity. Sex differences were found in layers 2-3 (6.9%) and 5 (9.1%) of the primary motor area, in layer 6 (8.9%) of the forelimb part of the sensory-motor area, and in layers 2-3 (8.0%) and 6 (6.6%) of the binocular portion of the primary visual area. No sex difference was found in the monocular area of the primary visual cortex, and no sex-related left-right asymmetry was detected in any measurement. We will be examining whether there are sex differences at the cellular level that underlie these laminar dimorphisms.

39.10

THE DISTRIBUTION OF THE CALLOSAL PROJECTION NEURONS IN THE VISUAL CORTEX OF MALE AND FEMALE RATS. P. Seymoure*, J. R. Kopcik, S. K. Schneider*, J. H. Y. Kim, and J. M. Juraska. Dept. of Psychology and Neural and Behavioral Biology Program, University of Illinois, Champaign, IL 61820.

In our previous research female Long-Evans rats had more axons than male rats in the splenium of the corpus callosum (Juraska & Kopcik, Br.Res., 450, 1, 1988). In the present study we hoped to determine if the sex differences in axon number might be reflected in different patterns of callosal projection neurons. Following extensive injections of horseradish peroxidase (HRP) in the posterior cortex of rats at 60 days of age, we examined the tangential pattern of labeled projection neurons in the visual cortex. The area 17/18a border was computer reconstructed from serial-sections of five male and female pairs matched for equivalent HRP labeling. While variability in projection neurons at this border was found between animals, qualitative comparisons failed to reveal any differences between the sexes. Quantitative measures of length and width did not demonstrate any significant differences. Although no sex differences were found in the distribution of the projection neurons at the area 17/18a border, this study does not preclude the possibility of differences in the density of projection neurons.
Supported by the Univ. of Illinois Research Board.

39.11

ESTROGEN BIOSYNTHESIS IN THE DEVELOPING RAT BRAIN: REGIONAL AND TEMPORAL ASPECTS Neil J. MacLusky and C.D. Toran-Allerand Div. Reprod. Science, University of Toronto Medical School, Ontario, Canada, and Center for Reproductive Sciences, College of Physicians and Surgeons, Columbia University, New York, NY.10032

Previous studies in mice (MacLusky *et al* Soc. Neurosci 1986 p.1217) and rhesus monkeys (Clark *et al*, Endo 123:932, 1988) have suggested that the cerebral cortex is a target for developmental effects of locally-synthesized estrogens. The present studies were performed to re-evaluate the regional distribution and ontogeny of aromatase in the brain of the rat, which has been reported to lack cortical aromatase activity. METHODS: **[I]** Explant cultures from the brains of newborn rats were incubated for 72h with [1,2,6,7-^{3}H] androstenedione (A). Tritium-labelled [^{3}H] A metabolites were separated by TLC. **[II]** Aromatase activity was measured by tritium release from [1β-^{3}H] A (Roselli & Resko, Endo. 114:192, 1984) in tissues from rats killed at intervals over the first 25 days of postnatal life. RESULTS: **[I]** Estrogen formation was detectable in cultures from the preoptic area (POA), septum (SEPT), hippocampus (HIPP), mid-brain central grey (MB) and anterior cingulate cortex (ACCTX) but not in posterior cingulate cortex, olfactory bulb or cerebellum. Activity in the ACCTX was much lower than in the other aromatase positive structures (<1/10 that in HIPP; <1/50 that in POA). **[II]** At birth, aromatase activity was detectable in all brain regions studied (POA, mediobasal hypothalamus, amygdala, HIPP, MB and ACCTX). Activity/mg protein declined rapidly during postnatal development, except for HIPP, where it remained stable until postnatal day 10, then declined. These results demonstrate that aromatase activity is widely distributed in the neonatal rat brain, including regions of the cerebral cortex. However, the relatively low concentrations of cortical aromatase in the rat, as compared to previous findings in other species, suggest that there may be significant species differences in the role played by local estrogen biosynthesis in cerebral cortical development (Supported by NIH grant HD08364).

39.12

EFFECT OF SEX, INTRAUTERINE POSITION, AND ANDROGEN MANIPULATION ON THE DEVELOPMENT OF BRAIN AROMATASE ACTIVITY IN FETAL FERRETS. R. W. Krohmer and M. J. Baum. Boston University, Department of Biology, 2 Cummington St., Boston, MA 02215.

Experiments were conducted to explore the possible relationship between testicular androgen secretion and the development of brain aromatase activity in fetal ferrets. Aromatase activity in the preoptic + medial basal hypothalamus and temporal lobe was similar in fetuses of both sexes between embryonic days 26 and 36 even though whole body androgen content was invariably higher in males than females. Whole body androgen content was significantly higher in females located caudally (downstream) from two or more as opposed to 0 or 1 males in the same uterine horn; nevertheless their brain aromatase activity was similar. Finally, maternal treatment with either the androgen receptor antagonist Flutamide or 5α-dihydrotestosterone propionate beginning on gestational day 24 did not affect brain aromatase activity in fetal offspring of either sex, delivered on embryonic day 34. Previous studies suggest that the biosynthesis of estrogen in the fetal ferret brain is normally greater in males than females. The present results suggest that this sex difference results primarily from increased androgenic substrate being available to non-saturated aromatizing enzymes and not from an androgen-dependent activation of aromatase. (Supported by U.S. Public Health Service grant HD21094 and Research Scientist Development Award MH00392 to M.J. Baum.)

39.13

AROMATASE ACTIVITY (AA) IN THE HYPOTHALAMIC/PREOPTIC AREA OF NEONATAL MALE AND FEMALE RATS: TESTOSTERONE (T) AND ETHANOL (EtOH) EXPOSURE. P.K. Rudeen and S. Paredez*. Dept of Anatomy, University of Missouri School of Medicine, Columbia, MO 65212

Neuronal AA is critical to masculinization of the rodent central nervous system. The effects of EtOH and T on AA in neonatal male and female rats were determined by the measurement of stereospecific [^{3}H]-water formation from [^{3}H]-1ß-androstenedione. Neonatal male rats exposed to EtOH showed only a modest increase in AA ($p<0.05$), but were not affected by the presence of T either acutely (24h) or chronically (6 days). AA was lower on postnatal day one in female rats (235 fmol/mg prot/h) compared to AA observed in male rats (387 fmol/mg prot/h). EtOH exposure had no observable effect on the basal level of AA in female rats (235 vs 220 fmol/mg prot/h). Furthermore, acute exposure to T failed to induce AA in the neonatal female rats (235 vs 245 fmol/mg prot/hr). The data indicate that AA is greater in male than in female neonatal rats, but that neither sex significantly responds to either T or EtOH (Supported by grants AA05893 and AA00107).

39.14

TRANSIENT ELEVATION OF ESTROGEN RECEPTORS IN THE NEONATAL RAT HIPPOCAMPUS. J.A. O'Keefe* & R.J. Handa*(SPON: A.J. Castro) Dept. of Anatomy, Loyola Univ., Maywood IL 60153

The presence of sex differences in hippocampal morphology and function suggests that this brain region may be sensitive to the organizational actions of gonadal steroids. Therefore, we examined the postnatal development of estrogen receptors (ER) in the rat hippocampus. ER was measured by the in vitro cytosolic binding of ^{3}H-estradiol. Radioinert R2858 was used to determine nonspecific binding. Hippocampal ER concentrations increased from birth through postnatal day (PND) 4 when levels peaked (10.05 ± 1.2 fmol/mg protein); these were maintained through PND-7 (9.45 ± 1.4) and declined thereafter to low levels characteristic of the adult (2.05 ± .35). This ontogenic profile is similar to that found in other cortical regions but is distinct from that observed in the hypothalamus, where ER levels remain high in the adult. Saturation analysis of PND-7 hippocampal cytosols demonstrated a single, high affinity binding site (Kd: $0.46 \pm 0.13 \times 10^{-9}$ M). ^{3}H-E binding was specific in that it was displaced by R2858, DES and E but not by non-estrogenic steroids. Significantly greater ER levels were found in hippocampal nuclear extracts from DES-treated PND-7 animals compared to controls (73.40 ± 15.62 vs. 3.14 ± 2.16 fmol/mg DNA; $p < .01$). The presence of elevated hippocampal ER levels during the perinatal critical period and evidence of functional transformation to the DNA binding state after DES treatment suggests that the hippocampus is a potential substrate for estrogen-mediated organizational events.

SPROUTING AND SPROUTING MECHANISMS I

40.1

CLIMBING FIBRE COLLATERAL SPROUTING AND FORMATION OF NEW SYNAPSES IN THE CEREBELLUM OF THE ADULT RAT. F. Rossi[1]*, L. Wiklund[2]*, J.J.L. van der Want[3]* and P. Strata[1]. [1]Dept. of Human Anat. and Physiol., Univ. of Turin, I-10125 Turin, Italy; [2]Lab. Physiol. Nerv., C.N.R.S., F-91190 Gif-sur-Yvette, France; [3]The Netherlands Ophth. Res. Inst., NL-1100 AC Amsterdam, The Netherlands.

The present study was aimed at demonstrating that climbing fibres (CF), survived to a subtotal lesion of the inferior olive (IO), are able to emit collateral sprouts and reinnervate Purkinje cells (PC). IO was lesioned by means of 3-acetylpyridine (62-70 mg/kg i.p.) in adult Wistar rats (b.w. 120-250 g). The animals were killed 3 days to 6 months after the lesion. Surviving CF were visualized by means of the anterograde tracer Phaseolus vulgaris leucoagglutinin (PHA-L). At 7 days after the lesion short sprouts, emitted by CF terminal arbours, end in small terminal plexuses made of branchlets and varicosities. From 1 month on, thick branches, up to some hundreds µm long, give rise to several new terminal plexuses, surrounding PC dendrites. At longer survival times, single CF form clusters of new terminal plexuses, extended for several hundreds µm in both the longitudinal and the sagittal plane of the folium. The ultrastructural study showed new synapses formed by labeled CF on spines projecting from major PC dendrites. These results show that CF are capable of remarkable plastic changes leading to the reinnervation of PC.

40.2

A MOLECULAR MECHANISM FOR THE "TRACING CIRCUIT" MODEL FOR HUMAN MEMORY: ACTIVITY-DEPENDENT RELEASE OF ATP, SUBSTRATE OF ECTO-PROTEIN KINASE, CAN STABILIZE THE IN-CIRCUIT SYNAPSES BY SPROUTING. Y. Kuroda, Dept.of Neurochem., Tokyo Metropolitan Institute for Neurosciences, Fuchu-shi, Tokyo 183, Japan.

A "tracing circuit" model has been proposed for the human memory process (Kuroda,Y.:Neurochem. Intern. 14, 309, 1989). In the model, a "tracing circuit" of neurons corresponding to human memory is established by a mechanism in which impulses originating from sensory organ(s) make closed circuits, then repeatedly trace the connecting circuit or pathways. At every synapse in the "tracing circuit", LTP should occur. With a slower time course, dynamic changes of synaptic contacts follow: activity-dependent elimination of ex-circuit synapses and resulting sprouting of in-circuit axonal terminals, preserving the circuit pattern for the order of years. To explore molecular mechanism of sprouting, we have developed an experimental system by which we can quantitatively observe in vitro synapse formation of cerebral cortical neurons by multi-site Ca^{2+} fluorometry(Muramoto, K.et al.:Proc.Japan Acad.,64B, 319, 1988). Rat cerebral cortical neurons make glutamatergic synapses on each other after 7 days in culture. During the culture, continuous application of a protein kinase inhibitor(K-252b), which does not permeate the cell membrane, appeared to block the synapse formation in a dose-dependent manner. This result suggests that a specific phosphorylation of surface proteins on synaptic membrane by an ecto-protein kinase is involved in formation and/or stabilization of immature synapses, and can be triggered by a supply of substrate ATP released from tetanically stimulated axon terminals in the "tracing circuits", but not from terminals out of the circuit.

40.3

IPSILATERAL INTERPOSITORUBRAL PROJECTION IN THE RAT AND ITS MEANING TO PLASTICITY. W.-J. Song*, Y. Nagisa* and F. Murakami, Dept. of Biophysical Eng., Fac. of Eng. Sci., Osaka University, Toyonaka 560, JAPAN

Lesion-induced aberrant projections observed in the CNS may be interpreted as proliferations of already present sparse projections. In the interpositorubral (IN-RN) system, hemicerebellectomy induces ipsilateral IN-RN projection only in neonatal animals. Following the interpretation, this age-at-lesion effect would mean disappearance of a sparse aberrant ipsilateral IN-RN projection which exists in neonatal animals. However, evidence suggesting the existence of the projection in adult rats has been provided by a degeneration study (Castro, 1978). Since degeneration methods are not suitable for demonstrating sparse projections, we attempted to verify the projection in adult rats with the PHA-L method. Injection of PHA-L into the nucleus interpositus resulted in labeling of terminals in ipsilateral red nucleus, indicating the existence of ipsilateral IN-RN projection. The present study suggests factors other than disappearance of neonatal aberrant projections are involved in age-at-lesion effect on post-lesion plasticity.

40.4

INCREASED DENSITY AND ALTERED MORPHOLOGY OF HISTAMINE-CONTAINING NERVE FIBERS IN KAINIC ACID-INDUCED BRAIN LESIONS. P. Panula, M.S. Airaksinen, L. Kivipelto and A. Willman*.

To elucidate the role of histamine in the CNS, small injections of kainic acid (KA) were made in different parts of the rat brain selected on the basis of their histamine-immunoreactive (HA-ir) innervation pattern. In some cases, similar injection of saline was made on the contralateral side. After two or six weeks, the rats were perfused with 4% carbodiimide and processed for histamine-immunocytochemistry.

A clear increase in the density of HA-ir nerve fibers was observed in the cerebral cortex, neostriatum, amygdala and ventral pallidum (= all KA injection sites) in comparison to the control side. The lesion-induced fibers formed dense networks and contained numerous varicosities of various size. The increase was obvious after two weeks, and a further increase in the number of fibers was evident after six weeks. The results suggest that KA lesions are associated with accumulation of histamine in pre-existing fibers in the lesions, or that sprouting of histaminergic fibers occurs even in those areas of the brain where these fibers are not numerous in normal brain. HA-ir nerve fibers are also found in the brain during fetal development (Auvinen and Panula, J. Comp. Neurol. 276:289,1988). Histaminergic mechanisms may, therefore, be involved in the development of the brain. It is possible that these mechanisms are activated during regenerative processes.

40.5

MORPHOLOGY OF INDIVIDUAL VISUAL CALLOSAL AXONS IN NORMAL AND EYELESS HAMSTERS AS DEMONSTRATED BY ANTEROGRADE TRACING WITH PHASEOLUS VULGARIS LEUCO-AGGLUTININ. S.E. Fish, R.D. Mooney, C. Bennett-Clarke and R.W. Rhoades. Depts. of Anatomy, Medical College of Ohio, Toledo, OH 43699 and Marshall University School of Medicine, Huntington, WV 25704.

Phaseolus vulgaris leuco-agglutininwas used to label visual callosal axons in normal, bilaterally enucleated, and congenitally anophthalmic hamsters. Callosal axons in the area 17-18a border region of normal hamsters left the white matter and followed a trajectory that was usually orthogonal to the cortical surface. They gave off short branches and had swellings indicative of boutons en passant in layers V and VI. A smaller number of fibers did collateralize extensively in these laminae. Most callosal axons extended through lamina IV with little or no branching, but they did give off en passant swellings in this layer. Callosal fibers collateralized fairly extensively and gave off both en passant and terminal swellings in layers II and III. In layer I, callosal axons paralleled the pial surface, branched only occasionally, and gave off both en passant and terminal boutons. Some callosal fibers extended into lamina VI and lowermost layer V of medial area 17. They left the white matter near the 17-18a border, angled medially, branched very rarely, and had swellings indicative of boutons en passant. In both binocularly enucleated and congenitally anophthalmic hamsters, callosal fibers were visible in the supragranular layers of medial area 17. There was no qualitative difference between the morphological characteristics of these axons and callosal fibers in normal hamsters. It thus appears that expanded visual callosal projection in experimentally manipulated and anophthalmic hamsters is the result of axons with normal morphological characteristics occupying cortical regions that they do not in normal animals.

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40.6

INDUCTION OF CHOLINERGIC FUNCTION IN RAT SYMPATHETIC NEURONS CO-CULTURED WITH HIPPOCAMPAL CELLS. MD Johnson* and DD Potter Dept. Neurobiology, Harvard Medical School, Boston, MA 02115

Perivascular sympathetic axons from the superior cervical ganglion sprout into the parenchyma of the rat hippocampal formation after fimbria lesions; this sprouting may result from loss of the cholinergic input to the hippocampal formation from the basal forebrain (review: Crutcher, 1987, Brain Res. Rev. 12). Given evidence that denervated cardiac cells in culture produce a "conditioned medium factor" that can induce cholinergic status in initially noradrenergic postnatal sympathetic neurons in culture (e.g. Patterson, 1978, Ann. Rev. Neurosci. 1), it was of interest to see if cholinergically-denervated cells of the hippocampal formation induce not only sprouting but also cholinergic function in sympathetic neurons. Induction of cholinergic function can be tested in culture. Cells from the hippocampal formation and superior cervical ganglion, dissociated from Long Evans rats 0-6 days old, were co-microcultured for 6-44 days in medium containing NGF. In 37 co-microcultures there were neurons whose appearance and electrical behavior were characteristic of cultured sympathetic neurons; approximately 70% (60/86) of these exerted an excitatory synaptic effect on themselves or another neuron that appeared sympathetic. In all 44 trials in which the ganglionic blocker hexamethonium was used ($\leq 10^{-3}$M), the excitatory effect was strongly reduced or eliminated. It will be of interest to determine which cells are responsible for this induction of cholinergic function in the sympathetic neurons. Supported by NSO2253.

40.7

ROLE OF CYCLIC AMP IN INSULIN AND INSULIN-LIKE GROWTH FACTOR I DIRECTED NEURITE OUTGROWTH IN SH-SY5Y CELLS. C. Wang*, B. Wible*, K. Angelides* and D.N. Ishii (SPON: J. Masken) Physiology Dept., Colorado State Univ., Ft. Collins, CO 80523; Physiology and Molecular Biophysics Dept., Baylor College of Medicine, Houston, TX 77030.

We previously showed that insulin and insulin-like growth factors (IGFs) selectively increase the relative abundance of 68 kD and 170 kD neurofilament (NF) mRNAs which precedes neurite formation in cultured human neuroblastoma SH-SY5Y cells. Others have shown that cAMP can increase neurite growth. In the present study, the possibility that cAMP might regulate neurite growth through effects on NF gene expression was examined on Northern blots. Dibutyryl cAMP increased both 68 and 170 kD NF mRNA, but not histone 3.3 mRNA. Together with insulin, dibutyryl cAMP was additive, and theophylline and caffeine were either additive or synergistic. Dibutyryl cAMP and theophylline seemed additive with IGF-I. These studies suggest cAMP may mediate the capacity of insulin and IGFs to increase 68 and 170 kD NF mRNAs. (Supported by NIH grant RO1 NS24327).

40.8

INSULIN-LIKE GROWTH FACTORS INDUCE NERVE SPROUTING IN ADULT MOUSE MUSCLE. Caroni, P. and Grandes, P.* Brain Research Institute, University of Zurich, August-Forel-Str. 1, CH-8029 Zurich/Switzerland

Partial denervation or paralysis of mammalian skeletal muscle results in massive sprouting from intact neighboring motoneurons, a reaction which might initiate reinnervation. Such intramuscular sprouting has been shown to be due to a diffusible activity released by the inactive muscle fibers. IGF-2 has been shown to promote sprouting of sensory neurons in vitro. In addition, IGF-2 mRNA in muscle correlates with neurite growth phases. A possible direct involvement of IGFs in motoneuron sprouting was therefore investigated. The following finding are interpreted as being consistent with a direct physiological role of IGFs in intramuscular sprouting: 1.) rapid and massive stimulation of neurite growth in vitro from purified motoneurons by IGFs; half-maximal effect by 0.2 nM IGF-2; 2.) direct high-affinity and specific binding of IGFs by the fine processes of motoneurons in vitro (identified by retrograde labeling); 3.) induction of intramuscular sprouting in intact adult mouse gluteus muscle by non-lesioning subcutaneous injections of low doses of IGFs. Additional reactions in IGF-injected adult muscle included: 1.) enhanced GAP 43 levels in intramuscular nerves; 2.) stimulation of peanut agglutinin binding sites; 3.) no changes in N-CAM nor J1 immunoreactivity; 4.) moderate capillary growth.

40.9

RECOVERY OF HIPPOCAMPAL SEROTONIN CONTENT AFTER MEDIAN RAPHE LESION. G.L. Marshall* and J.H. Haring. (SPON: J. Arends). Dept. of Anatomy & Neurobiology, St. Louis Univ. Sch. of Med., St. Louis, MO 63104.

Six wks after lesions of the median raphe (MRN), an apparent proliferation of serotonergic (5HT) axons is seen in the area dentata using immunocytochemistry. The purpose of this study was to measure changes in hippocampal 5HT levels by HPLC and 5HT terminal density by high affinity uptake to see if axon proliferation was actually induced by MRN lesion or if 5HT synthesis was merely increased without sprouting.

Adult male rats (250-300g) received a MRN injection of 5,7DHT (3µg in 250nl artificial CSF) and were studied 2 and 6 wks later. Rats injected with vehicle served as controls. Each hippocampus was cut into 300µm slices perpendicular to the septotemporal axis for the biochemical analyses. Two wks after MRN lesion, depletions of hippocampal 5HT ranging from 80-92% were observed. Histological assessment of lesion sites showed that the residual 5HT represented not only dorsal raphe projections but also spared MRN neurons. The projections of these spared neurons to the hippocampus was confirmed in similar lesion cases by retrograde tracing. Six wks after lesion, 5HT levels of 66-82% of normal values have been observed. Uptakes indicate that terminal density changes generally parallel changes in hippocampal 5HT content. These observations suggest that axon sprouting contributes to the recovery of 5HT innervation in the hippocampus after MRN lesion. Support: NS25752.

41.1

TEMPORALLY REGULATED CHANGES IN ALPHA$_1$-TUBULIN AND APOLIPOPROTEIN E mRNA's IN THE DENTATE GYRUS FOLLOWING ENTORHINAL CORTEX LESION. M. A. Hess, J. Poirier,* P. C. May, and C. E. Finch. Andrus Gerontology Center, Dept. Biological Sciences, USC, Los Angeles, CA 90089. We sought to identify differentially expressed mRNA's underlying the morphological changes that occur after deafferentation of the hippocampus by entorhinal cortex lesioning. Male Fischer 344 Rats underwent bilateral, electrolytic lesions of the entorhinal cortex. At 14 days post lesion the hippocampi were removed and poly A + RNA was isolated. A cDNA library was subsequently constructed and screened for clones of differentially expressed mRNA's. We have now identified 2 such clones by sequence analysis; alpha$_1$-tubulin, a microtubule subunit protein and apolipoprotein E, a protein involved in cholesterol transport. Northern blots of hippocampal RNA isolated 2, 6, 14, and 30 days post lesion showed a differential time course of induction between the two mRNA's. Alpha$_1$-tubulin mRNA prevalence was maximal at 2 and 6 days, returning to control levels by 30 days. Apolipoprotein E mRNA was highest at 6 days and returned to control levels by 30 days. Preliminary In situ hybridization studies localized alpha$_1$-tubulin mRNA to CA1, CA3, and CA4 neurons with intense labelling of hilar neurons adjacent to the granule layer. Increased apolipoprotein E mRNA was detected predominantly in astrocytes of the outer molecular layer of the dentate gyrus at 6 days post lesion. These results suggest differential temporal regulation of neuronal and astrocyte specific mRNA's and a possible role for astrocytes in membrane remodelling and subsequent synaptogenesis. Supported by the John D. and Catherine T. MacArthur Foundation Research Program on Successful Aging, Le Fond de la Recherche en Santé du Québec, and by a Leadership and Excellence in Alzheimer's Disease research grant (NIA AG07909) and ADRDA Grant IIRG-88-069 (PCM).

41.2

AGE-RELATED CHANGES IN NEURONAL PLASTICITY AND FUNCTIONAL RECOVERY FOLLOWING PARTIAL DENERVATION OF A TARGET G.A.Kuchel*[1,2] and R.E.Zigmond[1] (SPON:M.S.Albert) Dept. Biol. Chem. & Mol. Pharm.[1], Division on Aging[2], Harvard Medical School, Boston, MA 02115

The activity of the rat pineal gland enzyme N-acetyltransferase (NAT), exhibits a dramatic circadian rhythm. Increased NAT activity at night is dependent on stimulation of the gland by its sympathetic innervation which reaches the gland via the two internal carotid nerves (ICN). Specific neuronal ^{3}H-norepinephrine (NE) uptake was measured in homogenates of this tissue and utilized as an index of its innervation. Studies were conducted on 3,9,15 and 24 month old male F344 rats. Surgical lesion of the right ICN resulted in an approximately 50% reduction in ^{3}H-NE uptake compared to controls in all age groups when examined 15 days after surgery. Ten days after surgery, ^{3}H-NE uptake was similar to control values in both 3 and 9 month old animals. At the same time point, ^{3}H-NE uptake remained at 49% and 45% of the sham values in 15 and 24 month old aged animals respectively. We have previously shown that unilateral denervation results in impaired NAT function the first night, followed by a rapid recovery by the second night after surgery. While 3 month old animals exhibited full nighttime NAT activity 10 days after unilateral denervation (114% of control), 24 month old lesioned animals showed somewhat lower NAT activity (78% of control).

These data demonstrate a dramatic failure of collateral sprouting following partial denervation in aged animals. Further studies are required to determine the functional consequences of this age-related deficit for the recovery of function after partial denervation. (NS17512, MH00162, the MacArthur Foundation Program on Successful Aging and a fellowship from the Brookdale Foundation[2])

41.3

COLCHICINE-INDUCED CHOLINERGIC DENERVATION OF THE HIPPOCAMPUS ELICITS SYMPATHETIC INGROWTH. S.R. Ginn, G.W. Lanford and G.M. Peterson. Department of Anatomy and Cell Biology, East Carolina University School of Medicine, Greenville, NC 27858-4354.

It has been demonstrated that sympathetic neurons from the peripheral nervous system will invade the hippocampus following destruction of its septal inputs. It is thought that this is due to the loss of cholinergic innervation since the medial septum-diagonal band complex (MSDB) provides the major source of cholinergic fibers projecting to the hippocampus. However, non-cholinergic (e.g., GABAergic) neurons also arise in the MSDB and project to the hippocampus. Thus, the role of cholinergic denervation in sympatho-hippocampal sprouting cannot be directly tested by non-specific lesion techniques. Colchicine, a plant alkaloid that blocks tubulin transport, has previously been demonstrated to be specifically toxic to cholinergic neurons in the medial septum. We have injected colchicine into the lateral ventricles of female Sprague-Dawley rats (10 μg in 2.5 μl 0.9% saline). Following a survival period of one, two, or three weeks the rats were killed by transcardiac aldehyde perfusion, and four sets of 40 μm thick adjacent sections were cut through the MSDB and the hippocampus. Septal sections were stained with thionin, acetylcholinesterase (AChE), choline acetyltransferase (ChAT) and nerve growth factor receptor (using the monoclonal antibody 192-IgG). Hippocampal sections were similarly stained except that dopamine-β-hydroxlase (DβH) replaced ChAT. Colchicine toxicity was demonstrated by a decrease in the number of ChAT and 192 IgG-immunoreactive (-ir) neurons in the MS, and a decrease in AChE-positive fibers in the hippocampus. Coarse, branched DβH-ir fibers were observed in CA3 and the dentate gyrus. Morphologically similar fibers were immunoreactive for 192-IgG, suggesting that the DβH-ir fibers were of peripheral origin. These results support the hypothesis that the invasion of the hippocampal formation by sympathetic fibers results from cholinergic denervation. *(Supported by ADRDA IIRG-88-059)*

41.4

NGF-INDUCED ELIMINATION OF UNINJURED AXONAL PROJECTIONS IN THE MATURE RAT: EVIDENCE FOR TROPHOMORPHISM B.N. Saffran and K.A. Crutcher. Dept. of Neurosurgery, Univ. of Cincinnati, Cincinnati, Ohio 45267

The occurrence of transient axonal projections within the developing mammalian nervous system has been documented in some detail, but evidence for the continued remodeling of mature, uninjured axonal projections has been less well established. One example of axonal remodeling within the mature brain is the growth of sympathetic axons into the septal-denervated rat hippocampal formation. In a previous study (Saffran et al., 1989), we found that intraventricular infusion of NGF failed to elicit sympathetic sprouting into the hippocampal formation but increased the sympathetic innervation of the internal carotid artery. Furthermore, intraventricular infusions of NGF given at various times after sympathetic axons have been induced to grow into the brain by a medial septal lesion reduced the amount of sprouting and delayed its onset. Cessation of NGF infusion permitted subsequent sprouting, indicating that the hippocampal tissue retained its receptivity to sympathetic axonal growth and that sympathetic axons could still respond to the sprouting signal. To test the possibility that NGF directly inhibits sympathetic axon growth in the brain, animals received a unilateral superior cervical ganglion transplant and a medial septal lesion followed by NGF infusion. In these animals, NGF did not inhibit sympathetic ingrowth from the transplant. In fact, in addition to the growth into the denervated HF, sympathetic fibers were observed in the fimbria, corpus callosum and tissue surrounding the lateral ventricle. On the contralateral side there was no evidence of sympathetic sprouting within the hippocampal tissue in spite of extensive septal denervation. These results suggest that the loss of sprouted fibers within the hippocampal formation is not a result of direct inhibition by NGF but likely occurs secondary to the proliferation of vascular sympathetic fibers. If this intrepretation is correct, NGF, in the absence of injury, can elicit remodeling of axonal projections in the mature nervous system. (Supported by NIH grant # NS 17131)

41.5

NGF-INDUCED SPROUTING OF MATURE, UNINJURED NEURONS. L. G. Isaacson, B.N. Saffran, and K.A. Crutcher. Department of Neurosurgery, University of Cincinnati, Cincinnati, OH 45267.

Nerve growth factor (NGF) mediates trophic interactions between sympathetic neurons and their peripheral targets (Levi-Montalcini and Angeletti 1968;Thoenen and Barde 1980) and exerts local effects on growing neurites in vitro (Campenot 1977;1981;1982) and developing axons in vivo (Menesini-Chen et al. 1978). NGF has also been reported to increase the sympathetic innervation of targets in mature animals (Bjerre et al. 1975), but it is not known whether this represents an increase in the number of axons or in the norepinephrine content of individual fibers. Following the intraventricular infusion of NGF, we observed an increase in sympathetic fluorescence associated with the internal carotid artery (Saffran et al. 1989). In the present study, we investigated this response at the ultrastructural level in order to determine whether the NGF-induced increase in perivascular catecholamine histofluorescence represents an increase in the number of axons. Intraventricular infusion of NGF or vehicle (cytochrome C) was accomplished by the use of an osmotic minipump (Alzet #2002) as described by Williams et al. (1987). Infusions took place over a two-week period delivering solution at a rate of 0.5 ul/h. Segments of the intradural portion of the internal carotid were processed using routine electron microscopic techniques. The total number of axons located in the tunica adventitia of the internal carotid artery at three rostral-caudal levels was tallied. Axonal counts revealed a statistically significant (ANOVA, $p < .0001$) 3-fold increase in the number of fibers in the NGF-infused animals. The increase in perivascular axons in NGF-infused animals demonstrates that NGF infusion can elicit growth from mature uninjured axons. These results also suggest that NGF normally may play a role in the regulation of the autonomic innervation of cerebral blood vessels in the mature animal and that infusion of NGF into humans, as has been suggested for Alzheimer's disease, may elicit similar changes in autonomic vascular innervation. (Supported by NIH grant NS 17131.)

41.6

COMPENSATORY SPROUTING OF MAGNOCELLULAR NEUROSECRETORY AXONS FOLLOWING PARTIAL DENERVATION OF RAT NEURAL LOBE. J.A. Watt* and C.M. Paden. Dept. of Biology, Montana State University, Bozeman, MT 59717.

Sprouting of vasopressinergic axons has previously been demonstrated following transection of the pituitary stalk or lesion of the paraventricular nucleus in adrenalectomized animals. In a continuing investigation of the plasticity of the magnocellular neurosecretory system we have partially denervated the neural lobe (NL) using unilateral knife cuts of the hypothalamo-neurohypophysial tract. Animals were sacrificed at 5, 10, 32 and 90 days post-surgery and the NL prepared for ultrastructural analysis. A total of 15 low magnification electron micrographs were collected randomly throughout the NL for each animal (n=3 per group). Mean axon density and axon number were estimated for each group. A significant reduction in axon number was observed in the 5 and 10 day post-surgical groups (60.11% and 57.9% of control values) with a partial recovery in axon number observed in the 32 and 90 day groups (72.4% and 78.8% of control values). These data, in conjunction with results of immunohistochemical and morphometric analysis in similarly prepared subjects (Soc. Neurosci. Abst. 47.6, 1988) indicate that intact magnocellular axons arising from the contralateral hypothalamus undergo a sprouting reaction in the partially denervated rat NL. Supported by NIH grant NS23642 and RCDA NS01318 to CMP.

41.7

ANATOMICAL CHANGES IN CENTRAL TERMINALS OF INTACT VIBRISSAE AFTER NEONATAL NERVE SECTION. P.M.E. Waite and P.J. de Permentier. Sch Anat, Univ. New South Wales, Sydney 2033 Australia

Section of the infraorbital nerve (ION) in neonatal rats is followed by functional reorganization throughout the trigeminal nuclear complex (nuclei principalis, oralis, interpolaris and caudalis: NP, NO, NI and NC). Cells deafferented by the nerve section develop new receptive fields on the nose, cheek and on uninjured whiskers over the eye and in front of the ear. The present study examines the mechanism by which these changes occur by labelling the terminals of the 'ear' whisker. This whisker is innervated by the zygomaticofacial nerve, a branch of maxillary trigeminal uninjured by ION section. The infraorbital nerves were sectioned unilaterally within 12 hours of birth and regeneration prevented by repeated weekly sectioning. At 6-12 weeks of age, the 'ear' whiskers were injected bilaterally with horseradish peroxidase (0.9µl of 30% HRP in 2% DMSO over 1 hr). After 24-30 hrs survival, the animals were perfused and the brain stem sectioned and reacted for HRP by the TMB method. Serial sections throughout the whole trigeminal nuclear complex were examined. All patches of labelled terminals were drawn on both the normal and lesioned sides and the areas compared.

On the normal sides, terminal labelling for the 'ear whisker was found in discrete oval patches localized ventrolaterally in NP and NO and laterally in NI. In NC and C_1 cervical cord the labelling occurred centrally in the magnocellular region. On the lesioned side, the patches in NP, NO and NI were less localized and extended medially into the nucleus. For these nuclei, labelled terminal areas were approximately doubled after lesioning and this increase was highly significant in all animals ($p < 0.001$). In contrast, NC and C_1 showed no significant change with lesioning.

These results suggest that for NP, NO and NI, the functional changes associated with ION section may be related to anatomical alterations, such as sprouting, in the central terminals of adjacent uninjured nerves.
(Supported by NH & MRC and Ramaciotti Grants to P.M.E. Waite).

41.8

EFFECTS OF FETAL INFRAORBITAL NERVE DAMAGE UPON THE PROJECTIONS OF INDIVIDUAL TRIGEMINAL PRIMARY AFFERENT NEURONS. N.L. Chiaia, G.J. Macdonald, W.R. Bauer and R.W. Rhoades. Depts. of Anatomy, Medical College of Ohio, Toledo, OH 43699 and Robert Wood Johnson Medical School, Piscataway, NJ 08854.

Transection of the infraorbital nerve (ION) in newborn rats produces marked alterations in the peripheral projections of trigeminal (V) ganglion cells, but only subtle alterations in the central arbors of large caliber axons whose parent cells survive the lesion (Renehan, W.E. et al., in submission). It may be that the central axons of these damaged fibers have passed the stage in development during which reorganization of peripheral connectivity can alter central projections. We have tested this possibility by transecting the ION on embryonic day 16 and assessing the peripheral and central projections of surviving ganglion cells when the animals reached at least 6 wks of age. Retrograde tracing and extracellular single unit recording from the V spinal tract revealed numerous abnormalities in the peripheral connectivity of surviving ganglion cells. Retrograde tracing demonstrated that the somatotopic representation of the rows of vibrissa follicles in the V ganglion was disrupted and single fiber recording showed that 50.7% of 73 axons that projected into the regenerate ION did not have peripheral receptive fields. Furthermore, 16.7% of 60 fibers with non-ION receptive fields were also activated by stimulation of this nerve. The central arbors of HRP injected fibers that projected into the regenerate ION were reconstructed in V subnucleus interpolaris and caudalis (SpC) and they were generally much larger than those in normal rats. The central arbors of several ION fibers showed qualitatively abnormal structure-function relationships. Two of these fibers are of particular interest. These rapidly conducting, large caliber axons were activated only by vibrissa deflection, but had their major terminations in layers I and II of SpC.

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41.9

NEUROMUSCULAR JUNCTION FINE STRUCTURE FOLLOWING TENOTOMY. B.R. Pachter and N.I. Spielholz*. Dept. of Rehab. Med., New York Univ. Med. Ctr., New York, NY 10016.

Experimental tendon transection is often studied as a model of muscle disuse. The present study was designed to assess qualitatively and quantitatively the fine structure of the neuromuscular junctions in rat soleus muscle after tenotomy. Female Wistar rats (250-300g) had their Achilles tendon cut about 5-7mm proximal to their insertion into the calcaneus and the cut ends left free. After two weeks, the soleus muscles were removed whole and prepared for EM examination. The innervation zone was located and ultrathin sections were taken. Morphologically, the tenotomized soleus muscle fibers appeared atrophic and the mitochondria and fibrillar size reduced. The majority of the endplates contained two or more axonal terminals separated by Schwann cell cytoplasm within the same synaptic cleft. The junctional folds appeared highly irregular shaped and attenuated; some folds exhibited signs of degeneration. Quantitatively, the mean nerve terminal area and mean postsynaptic area of junctional folds and clefts per nerve terminal was significantly reduced as compared to those of controls. It would appear that Achilles tenotomy results in an altered muscle morphology as well as degenerative and regenerative changes at the endplate.

41.10

PLASTICITY OF SPINAL SYSTEMS AFTER UNILATERAL LUMBOSACRAL RHIZOTOMY IN NEONATAL RATS. S. D. Wang, M. E. Goldberger and M. Murray. Dept of Anat., The Med. Coll. of Pa., Phila., PA 19129.

Previous studies of spinal systems in adult rat have shown anatomical plasticity of substance P and serotonergic systems in response to complete unilateral lumbosacral rhizotomy. In the present study, we used the same paradigm to examine the plasticity of these systems in neonatal rat. Dorsal roots were cut and/or ganglia were removed from L1 to S2 in 5 day old rats. The animals survived up to 126 days postoperatively. Substance P (SP) and serotonin (5HT) immunocytochemical staining were compared on the control and deafferented sides at the L4 level. An image analysis system was used to quantify SP and 5HT immunocytochemical reaction product in the dorsal horn. After deafferentation, SP decreased to 65% of normal on the experimental side in laminae I and II by 10 days. The density of SP staining recovered to between 97% and 92% of normal values after 30 and 60 days postoperatively. Recovery of SP in the dorsal horn is thus more complete after neonatal deafferentation than adult deafferentation where recovery is only between 51% and 74%. After rhizotomy, the area fraction occupied by 5HT immunoreaction product increased more than two times normal on the experimental side in laminae I and II. Dorsal rhizotomy in neonatal rats produces 17% shrinkage of the dorsal horn by 10 days postoperative. Therefore shrinkage contributes to but does not account for the increase in density of 5HT staining. The increase in 5HT staining and the extent of shrinkage are similar in adult and neonatal deafferented rats. Thus there is evidence for more plasticity of SP in rat dorsal horn in response to neonatal deafferentation than in adult. Supported by NIH grants NS16629, NS16556 and NS24707.

41.11

DISSOCIATION OF TERMINAL SPROUTING AND ENHANCED TRANSMITTER RELEASE INDUCED BY CHRONIC DISUSE AT RAT NEUROMUSCULAR JUNCTIONS. T.Tsujimoto*, M.Umemiya* and M.Kuno* (SPON: Y. Kayama). Dept. of Physiol., Kyoto Univ. Sch. of Med., Kyoto 606, Japan.

An increase in transmitter release observed at neuromuscular junctions in chronically paralyzed muscle has been postulated to result from the formation of sprouts. We examined this assumption in the rat extensor digitorum longus muscle following chronic block of the sciatic nerve by locally applied tetrodotoxin. Transmitter release was measured by the quantal analysis, and motor nerve terminals were stained with nitro blue tetrazolium. Terminal sprouts or terminal enlargement were not expressed until 3 days after nerve block, whereas transmitter release was significantly enhanced already 24 hr after nerve block. When nerve block had been maintained for 6 days, approximately 50% of the terminals examined showed sprouting. Under this condition, terminal size was measured at junctions identified by injections of carboxy-fluorescein during electrophysiological studies. Terminal size was significantly larger in the terminals with sprouts than in those without sprouts. However, the amount of transmitter release was uniformly enhanced at all junctions, being independent of the presence or absence of sprouts. It is concluded that the formation of terminal sprouts and enhancement of transmitter release induced by prolonged elimination of nerve-muscle activity are not causally related.

41.12

SPROUTING OF CORTICOSPINAL FIBERS INTO DENERVATED CONTRALATERAL SPINAL CORD REVEALS SPECIFICITY OF NEW AXON ARBORS. R.Z. Kuang*, and K. Kalil. (SPON: A.W. Clark). Dept of Anatomy and Neuroscience Training Program. University of Wisconsin, Madison, WI 53706.

Corticospinal projections in the hamster are completely crossed at all ages and project topographically from sensorimotor cortex to all segments of the spinal cord. We recently showed an anatomical segregation of sensory cortical axons to the dorsal horn and motor cortical axons to the ventral horn, and wished to determine whether corticospinal axons sprouting into territory denervated by a contralateral lesion of the corticospinal pathway would maintain this topographic and functional specificity.

Unilateral lesions of the medullary pyramidal tract were made in hamsters ranging from 5 days to adult, and 12-15 days later the intact corticospinal pathway was labeled by iontophoretic injections of PHA-L (Phaseolus vulgaris leucoagglutinin) into sensorimotor cortex opposite the lesion. This method reveals details of the entire corticospinal axon arbor. Sprouting from the uninjured corticospinal pathway into the denervated spinal cord was robust after a 5-day lesion and almost equalled the density of the normal projection. Sprouting after a 12-day lesion was somewhat less dense, after a lesion at 19 days was relatively sparse, and after lesions in animals older than 19 days ceased to occur. At all ages, corticospinal axons sprouted into the topographically correct segments of the cord. Moreover, the arbors from sprouting axons maintained their functionally appropriate territories in the dorsal or ventral horn, depending on their origin from sensory or motor cortex. These results suggest that sprouting of corticospinal axons is maximal after early lesions, but can persist beyond the time period for corticospinal axon outgrowth. Moreover, since sprouting corticospinal axons maintain their topographic and functional specificity even when innervating the incorrect side of the spinal cord at a time later than normal, the signals within the target regions that determine this specificity must persist until relatively late in development. (Supported by NIH Grant NS14428 to K. Kalil.)

42.1

SYNTHESIS OF NA,K-ATPASE IN THE RETINA OF THE BB SPONTANEOUSLY DIABETIC RAT. S.C. Specht. Dept. Pharmacol., Inst. Neurobiology, Univ. P.R. San Juan, Puerto Rico 00936

Synthesis of Na,K-ATPase was determined in retinae of diabetic and diabetes-resistant BB/Wor rats labeled with a two-hour intravitreal pulse of 35S-methionine. Retinal Na,K-ATPase was isolated, separated by SDS-PAGE and the protein bands containing the alpha and alpha(+) subunit isoforms were dissolved and analyzed by liquid scintillation counting. Retinal incorporation was 11-16% higher than control in rats that had been diabetic for 4 weeks, although the difference was not significant. Degradation of the two bands, measured at 16, 24 and 48 hours, was significantly slower in diabetic retinae. In rats that had been diabetic for 16-18 weeks, retinal incorporation was significantly higher: labeling of the alpha(+) band was increased from 1460 to 2460 cpm/retina, alpha from 890 to 2000. In other systems, inhibition of Na pump activity stimulates its synthesis. Hence, these data are compatible with observations of decreased Na pump activity in diabetic rats. (NIH grants NS-07464 and S06RR08224).

42.2

MATURATION OF OLFACTORY RECEPTOR NEURONS IN DEVELOPING HYPO- AND HYPERTHYROID RATS. M.A. Paternostro and E. Meisami. Dept. of Physiology and Biophysics, Univ. Illinois Urbana, IL 61801.

The total number of olfactory receptor neurons (ORN) increases markedly (10x) during the rat's suckling period (Meisami, Dev. Brain Res. 46: 9-19, '89). This proliferation is reduced by 35% in hypothyroid rats as is the increase in olfactory epithelium (OE) surface area. The thickness of OE and packing density of ORNs remains unaltered, however (Paternostro & Meisami, Int. J. Dev. Neurosci. 7, '89). To examine the role of thyroid hormones on maturation of ORNs, we determined the surface density of ORN dendritic knobs, in thin (lum) sections, obtained from nasal septum of 25-day male hypothyroid rats [treated from birth with PTU (0.1% w/v in drinking water)] and hyperthyroid pups [thyroxin (3 ug/g bw, daily sc)]. The dendritic knobs are characteristic of mature ORNs (1 knob/mature ORN). The surface density of the knobs increases significantly during the postnatal period, reaching a value of about 45,000/sq mm of OE surface in normal 25-day pups. Hypothyroid pups show about 25% ($p<0.05$) reduction in this parameter, indicating a delay or retardation in maturation of ORNs. This deficit grows larger in 50-day hypothyroid animals, because increases in knob density continue in normal animals but not in the hyperthyroid ones. The results of knob counts in hyperthyroid pups will also be reported.

42.3

ONTOGENY OF GNRH NEURONS IN THE FETAL RHESUS MACAQUE. O.K. Ronnekleiv and J.A. Resko*. Dept. of Physiology, Oregon Health Sciences University, Portland and Oregon Regional Primate Research Center, Beaverton, OR.

We studied the ontogeny of GnRH neurons in the fetal rhesus macaque from days 36 to 135 of gestation. The nasal region, pituitary and the brain were dissected, fixed in 4% paraformaldehyde, sectioned on a cryostat (10μm) and mounted on slides. Immunohistochemistry and in situ hybridization were performed as described previously (Ronnekleiv et al., Mol Endocrin. 3:363, 1989). At 36 days, clusters of GnRH cells were found in the nasal region only. GnRH fibers extended into the brain and large bundles projected laterally towards the basal hypothalamus (BH). By day 38 GnRH cells were also localized in the olfactory region of the brain. With increasing fetal age a gradual caudal extension of GnRH cells occurred. These cells were first observed in the BH at 47 days. Cells containing prolactin and LH were detected in the pituitary at 47 and 50 days, respectively. Low levels of proGnRH mRNA were present in the nasal septum, the nervus terminalis and the brain by day 50 of gestation, which significantly increased by day 135. These data suggest that GnRH neurons in the primate brain originate in the nose. Furthermore, GNRH neurons exhibit low levels of synthetic activity at the early fetal stages but higher synthetic activity close to term. (Supported by PHS HD16793 and HD16022)

42.4

NEONATAL MSG ADVANCES, BUT GLUTAMATE ANTAGONISTS DELAY THE ONSET OF PUBERTY IN THE FEMALE RAT. M. Wilkinson and M.C. MacDonald*. Obst. and Gynecol. and Physiol. Biophysics, Dalhousie University, Halifax Nova Scotia, Canada B3H 4H7

We have suggested (Endocr. 113 596) that a gradual decrement in opioid feedback, coupled with the emergence of an excitatory drive, may be part of the mechanism which regulates the timing of puberty. Based on this premise, the effect of glutamate antagonists was investigated as well as the influence of neonatal monosodium glutamate (MSG) which is known to destroy POMC neurons in the arcuate nucleus.

The glutamate antagonist MK-801 (0.1 mg/kg) was injected daily from day 27 until vaginal opening (VO) was observed. MK-801 significantly delayed VO (31.6±0.3 days vs 33.5±0.6) and elevated body weight at VO ($p<0.05$). The effect of MK-801 was completely prevented by co-injection of the agonist NMDA (15 mg/kg)

In a second series of experiments we examined whether neonatal MSG treatment might advance puberty through removal of arcuate ß-endorphin neurons. Four injections of MSG (4 mg/gm; days 2,4,6 and 8) slightly advanced the time of VO (33.6±0.6 vs 34.4±0.5 $p<0.05$) and lowered body weight ($p<0.005$). However, when MSG dose was reduced (days 2 and 4; 4mg/gm) VO was significantly precocious (30.1±0.2 vs 34.8± 0.4 days) and in keeping with a smaller body weight. (80.1±1.1 gms vs 114.6±2.1). All rats ovulated at VO.

These data provide evidence for glutamate as an excitatory neurotransmitter at puberty. The neonatal MSG lesions, however, possibly remove an opiatergic, inhibitory ,pathway.
Supported by the Canadian MRC.

42.5

CHRONIC TREATMENT WITH OPIATES ONLY PARTIALLY ATTENUATES ONSET OF PUBERTY IN FEMALE RATS. M.C. MacDonald* and M. Wilkinson. (SPON: R.E.Brown). Depts. Obst. Gynol. and Physiol. Biophysics, Dalhousie University, Halifax, Canada B3H 4H7

We have suggested that the hypothalamic opioid system becomes gradually less able to suppress LH in peripubertal rats (Endocr. 113 596). However, little is known concerning the influence of exogenous opioid agonists, e.g. morphine, on puberty in female rats. We have now investigated the effects of opiate treatment which avoids daily injections. Firstly, morphine (MOR) or fentanyl citrate (FEN) was administered orally, via the drinking water. Secondly, rats were treated with FEN released from s.c. osmotic minipumps.

Beginning at age 22 days the rats were habituated to MOR by slowly increasing the concentration in the drinking water (over 2 days) to give final concentrations of 100, 400 and 800 mg/L at day 24. Controls received sucrose and water. Vaginal opening (VO) was significantly delayed at both the higher doses; CON: 33.6 days; 400 and 800 mg/L groups: 38.2 and 38.5 days. The treated rats were 15% heavier than controls ($p<0.01$) on the day of VO and produced a normal set of corpora lutea. Experiments with FEN gave similar results (concentrations: 9.6, 19.2 and 30 mg/L) with delay times of 33.7, 35.0 and 36 days (CON: 32.1). We next treated immature rats with FEN released from osmotic minipumps (implanted day 24; pump concentration: 4.5 mg/ml). VO was significantly delayed (32.4 vs 35.9 days $p<0.005$).

Our results show that the hypothalamic opioid system can be desensitized to allow VO to occur even in the face of continous agonist treatment. Supported by the Canadian MRC.

42.6

ONTOGENY OF MINERALOCORTICOID (TYPE 1) RECEPTORS IN BRAIN AND PITUITARY. P. Rosenfeld*, W. Sutanto*, S. Levine and E. R. de Kloet. Rudolf Magnus Institute for Pharmacology, University of Utrecht, Utrecht, The Netherlands.

The ontogeny of high affinity (^{3}H)corticosterone uptake and retention in brain and pituitary of 24-hour adrenalectomized rats was examined using autoradiography of in vivo labelled brain sections. Our data indicate: (1) There is specific uptake of radiolabelled steroid in both brain and pituitary already at 2 days of age, following administration of a tracer (2 µCi/gbw) dose of (^{3}H)corticosterone. This uptake is maximum around 4 to 8 days of age and decreases towards adult values around postnatal day 16. (2) High affinity uptake, at least in the brain, probably represents mostly binding to the mineralocorticoid receptor (MR) and not to the glucocorticoid receptor (GR), as it was not displaced by an excess dose of a GR antagonist, RU 38486, and its location in the hippocampus resembled that of MRs in the adult animal. The tracer amounts of (^{3}H)corticosterone circulating after injection in the rat pups resulted in steroid levels comparable to basal levels of non-adrenalectomized animals during the SHRP. Thus, MRs may be the receptors mainly responsible for mediating physiological effects of glucocorticoids in the hippocampus during early ontogeny.

42.7

REDUCTIONS IN BRAIN OPIATE RECEPTORS IN PRENATALLY-STRESSED MALE AND FEMALE RATS. C.H. Kinsley, T.R. Insel[1], P.E. Mann and R.S. Bridges. Harvard Medical School, Laboratory of Human Reproduction and Reproductive Biology, Boston, MA 02115 and [1]N.I.M.H., Laboratory of Clinical Science, Poolesville, MD 20837

Prenatal stress affects the display of numerous opioid-regulated behaviors and physiological responses in adulthood, e.g., maternal and sexual behavior and morphine-induced analgesia and prolactin release. The present work examines alterations in central opiate receptors which may underlie some of the effects observed in prenatally-stressed (P-S) animals. Forty-two day old offspring from females stressed on days 15-22 of pregnancy and their non-stressed Controls had their brains removed. Membrane homogenates from striatum, but not several other brain regions, exhibited decreased binding of the mu-opioid ligand ^{3}DAGO in P-S animals compared to Controls. Saturation studies suggest that this difference is due to significantly fewer mu-opiate receptors in the striatum of P-S offspring. Employing in vitro receptor autoradiography, the decreased binding in striatum was found mostly in the rostral striatum, extending into the nucleus accumbens, with conservation of the normal anatomical distribution of receptor-rich patches. These data indicate that the aberrant perinatal endocrine environment associated with the prenatal stress procedure may contribute to an alteration in brain-opiate dynamics.

42.8

POSTNATAL CHANGES IN ACTH RESPONSE TO ACUTE PHYSOSTIGMINE ADMINISTRATION IN THE RAT. J. T. McCracken* and R. E. Poland*, (SPON: W.O. Shekim). Dept. of Psychiat., Harbor-UCLA Med. Ctr., Torrance, CA 90509.

In order to explore possible changes in cholinergic regulation of the hypothalamo-pituitary-adrenal axis associated with pubertal development, the release of ACTH in response to an acute injection of the cholinergic agonist, physostigmine (PHYSO), was measured in four age groups of male rats (12, 28, 45 and 90 days old). Within each age group PHYSO 0.25 mg/kg or saline was injected IP followed 20 min later by rapid decapitation and collection of trunk blood for analysis. Each group also contained a non-treated group for comparison. ACTH was analyzed by double antibody radioimmunoassay. Mean ACTH concentrations for the 12, 28, 45 and 90 day old animals were 376, 555, 316, and 1189 ng/ml respectively. An analysis of variance revealed significant differences between age groups ($F_{3,26}$=4.54; P< 0.012). Post-hoc comparisons revealed significantly less ACTH release in the 12, 28, and 45 day old animals after PHYSO when compared to the adult 90 day old animals. While preliminary, these data suggest that HPA axis sensitivity to cholinergic agonists undergoes significant modification during or following pubertal development in the male rat.

42.9

EFFECT OF STRESS DURING DEVELOPMENT ON SPATIAL LEARNING IN RATS. P.J. Gutierrez* and J.S. Meyer (SPON:N.Carlson). Dept of Psychology, Univ. of Massachusetts, Amherst, MA 01003.

We previously reported that histamine stress in developing rats inhibits hippocampal cell proliferation, possibly by stimulating corticosterone secretion (Gutierrez and Meyer, Soc. Neurosci. Abstr. 14:281, 1988). In the present research, the possible behavioral effects of such treatment were investigated. Rats were either untreated or injected daily with histamine from days 7 to 19 postnatal, then tested for spatial learning in the Morris water maze. Separate groups were tested early in development (days 20 to 26) and in adulthood (days 90 to 96). Testing was carried out for 6 days (4 trials/day), the first 5 days representing task acquisition and the 6th day a reversal test in which the hidden platform was moved to the opposite quadrant of the tank. Although no group differences were found in acquisition performance, effects of stress were noted on the reversal day. During reversal testing, all control animals (both young and adult) showed a high latency on trial 1, followed by 3 trials with significantly lower latencies. In contrast, stressed animals tested early in life did not initially perseverate in the original platform location but instead showed low latencies across all reversal trials. Finally, among the stressed subjects tested in adulthood, males but not females displayed relatively high latencies across all reversal trials, suggesting a deficit in adjusting to the new escape location. (Supported by NSF BNS-8704702 and BRSG RR07048 from NIH)

42.10

PROOPIOMELANOCORTIN POST-TRANSLATIONAL EVENTS DURING THE STRESS NONRESPONSIVE PERIOD. D.M. Vazquez* and H. Akil. Mental Health Research Institute and Department of Pediatrics, University of Michigan, Ann Arbor, MI 48109-0720.

The POMC producing cells of the anterior (AL) and intermediate lobe (IL) of the pituitary are derived from a common primordium. However, these cells exhibit distinct tissue specific post-translational processing. The AL response to biosynthetic and secretory demands differs depending on the type of challenge. Acute stress activates translational or post-translational events such that the precursor is processed to the final products twice as rapidly. With repeated stress the cell resorts to pre-translational events reflected by an increase of POMC mRNA (Endocrinol. 119:1793). Unlike the adult, the developing POMC cell products are similar in both lobes and a tissue specific pattern is not achieved until day 21. POMC mRNA levels increase (Endocrinol. 124:60), but the capacity of the developing animal to respond to stress is blunted during the first two weeks of life. A pulse paradigm was used to assess cellular dynamics on rats age 3,7,14 and 21 days. AL cell suspensions were prepared by the Mulder and Smelik method and ^{3}HLeu was used for incorporation. Cell extracts obtained were purified by immunoprecipitation using a midportion β-endorphin antibody and the different POMC derived molecular forms were separated on a SDS-PAGE. Day 14 AL cells appeared to process the POMC precursor at a faster rate when compared to adult (31K:β-LPH ratio 1:0.86 vs. 1:0.06). It is conceivable that the developing corticotroph relies on efficient translational events to maintain basal levels of the circulating peptides. Unlike the adult corticotroph, the capacity to increase the rate of translation may be limited when confronted with a greater demand, thus contributing to the Stress Nonresponsive Period. This work is supported by NIMH MH09720.

42.11

HYPOTHALAMIC NEURONS IN NON-CONTACTING THREE-DIMENSIONAL CULTURE RELEASE VASOPRESSIN AND OXYTOCIN. P.A. Doris, M.S. Walker * & P.W. Coates. Dept. Cell Biol. & Anat., TTU HSC Sch. Med., Lubbock, TX 79430.

Contact with neurons and glia may provide signals that regulate release of neuropeptides from hypothalamic neurons. It is not known whether these neurons are inherently capable of this important function in the absence of cell contacts because connections normally form *in vivo* and under standard culture conditions. However, hypothalamic neurons from late gestation fetal rats grow and differentiate rapidly as single cells without cell contacts when cultured at low cell density in a three-dimensional matrix (3D gel) (Coates and Walker, Biol.Rep.38:186, 1988) and express immunohistochemically detectable vasopressin (VP) (Dudley et al., SN Abs.14:417, 1988). In order to determine whether hypothalamic neurons are intrinsically capable of release, radioimmunoassays were used to quantitatively assess VP and oxytocin (OXY) in this system. Cells and medium were harvested after two days, as were blank controls (gels with no cells and medium from gels with no cells). Quadruplicate dishes were pooled for sample analysis with data expressed in pg (mean ± SEM) per sample. Repeated experiments gave consistent results. Statistical evaluation was performed using Student's t test. VP content of a typical sample of hypothalamic neurons in non-contacting 3D culture was 1.37 ± 0.24, more than 5 times that present in blank gels lacking cells (0.25 ± 0.25). VP content of medium from cells grown in 3D gels was 3.5 ± 0.29, twice that of control medium (1.75 ± 0.75). OXY was undetectable in blank gels, but was 0.13 ± 0.1 in gels with cells. OXY content of medium from cells in 3D gels was twice that for medium from blank gels (1.26 ± 0.32 and 0.65 ± 0.65, respectively). Results demonstrate that it is feasible to assay quantitatively for VP and OXY despite low numbers of neurons in this system. The data suggest that single hypothalamic neurons can release neuropeptides independent of synaptic or other cell contact mediated mechanisms. (Supported by NS20802, HD22806 and HL36217)

42.12

CHANGES IN THERMAL RESPONSIVENESS OF RAPHE NEURONES DURING PROESTRUS LIKE PHASES. P.Hinckel,H.B.H.Dick* and C.Hiemke*. Physiologisches Institut, Universität Giessen, Aulweg 129, D-6300 Giessen 1, F.R.G. and Institut für Physiologische Chemie, Universität Essen, Hufelandstrasse 55, D-4300 Essen 1, F.R.G.

In female mammals the preovulatory phase is characterized by surges of luteinizing hormone (LH) and a shift in the basal body temperature. The stimulation of gonadotropin release is triggered primarily by estradiol via the central nervous system. The elevation of body temperature seems to be due to increased production of progesterone in the ovary. The present study examines the effects of s.c. progesterone application on thermoresponsive neurones in the nucleus raphe magnus (NRM) during proestrus like phases in ovariectomized rats.

Spike rate maxima of warm-responsive neurones decreased and peak activity of cold-responsive neurones increased 20 - 50 minutes after progesterone injection, whereas no significant change in spike rate was seen after sham or control injection. After pretreatment with estradiol the progesterone induced inhibitory effect on warm-responsive units and the excitatory effect on cold-responsive units was amplified. Serum concentrations of LH and the two steroids were measured by using specific radioimmunoassays.

It is concluded that the hormonal control of basal body temperature change is mediated by complex effects of the steroids on thermal neurones in the lower brain-stem.

42.13

DEVELOPMENTAL APPEARANCE OF ALTERED HYPOTHALAMIC DOPAMINE AND TYROSINE HYDROXYLASE LEVELS IN AMES DWARF MICE. D. L. Hurley*, S. W. Carlson*, and C. J. Phelps (SPON: J. Hansen). Dept. Neurobiology & Anatomy, Univ. Rochester Med. Ctr., Rochester, NY 14642.

The hypopituitary Ames dwarf (df/df) mouse presents a model system of hypothalamic dysfunction in the absence of normal target hormone secretion. Dwarf mice lack growth hormone and prolactin (PRL); normal growth ceases beyond 10 days of age. Because of hypothalamic arcuate nucleus (catecholaminergic area A12) dopamine (DA) involvement in PRL regulation, adult dwarf mice have been assessed with regard to the A12 regions of the hypothalamus. Median eminence DA levels (Morgan et al., Endocrinol. 109: 2069, 1981), and histofluorescence are decreased, concomitant with a decrease in visible cell bodies in the A12 region (Phelps et al., Cell Tiss. Res. 240: 19, 1985). In order to determine whether this adult condition is primary or regressive, brains of dwarfs and normal (Df/?) littermates at 7, 14, 21 and 90 days of age were prepared for catecholamine histofluorescence (formaldehyde/ glutaraldehyde perfusion fixation). At 7 days, strongly fluorescent perikarya appeared in A12, and rich fiber innervation of the median eminence was observable in normal mice (DF/?); slight reduction in the fluorescence of both areas was noted in df/df littermates. This difference continued to increase through development; continued decline in fluorescence intensity was seen in 3 month old dwarf animals relative to normals, and maximal differences were observed in dwarfs greater than 1 yr old. Immunocytochemical staining for tyrosine hydroxylase (TH), the rate-limiting enzyme in the DA pathway, was performed on alternate hypothalamic sections to compare levels of TH during development. Numbers and staining intensity of TH-positive cell bodies were reduced in dwarf A12 regions, and TH-reactive fibers were less pronounced in the median eminence of the dwarfs, each with a pattern of gradual decline through the first months after birth. In situ hybridization to TH mRNA is being performed to quantify the decrease in dwarfs during these developmental stages. The long-term decline of DA neurons in the Ames dwarf mouse is indicative of a primary effect of the mutation on PRL gene expression with secondary alterations in the hypothalamus. Supported by NIH grant NS25987 (CJP)

42.14

THYROID HORMONE EFFECTS ON MICROTUBULAR COMPOSITION IN DEVELOPING CEREBRAL CORTEX. [1]S.A. Stein, [2]G.S. Bloom*, [1,2]G.A. Mihailoff, [3]P.M. Adams and [4]D.R. Shanklin*. Depts. of [1]Neurology, [2]Cell Biol. and [3]Psychiatry, UT Southwestern Med. Ctr., Dallas, TX 75235; and [4]Depts. of Path. and Ob.-Gyn, Univ. of Tenn.-Memphis, Memphis, TN 38163.

Hypothyroidism may lead to reduced microtubule numbers (Faivre et al, Dev. Br. Res., 8: 21-30, 1983), which may contribute to the observed abnormal and delayed axonal and dendritic process development in Layers II-V cerebral cortex (CC) as early as 15 days post conception (d pc) to 10 days after birth (d ab). To illuminate the potential mechanisms behind these abnormalities we have used the hyt/hyt mouse (Stein et al. Neuroendo., 49: 509-519, 1989), which has primary inherited congenital hypothyroidism starting at 15 d pc, their euthyroid hyt/+ littermates and the progenitor strain BALB/cBY mouse, to look at the abundance and localization of isotype-specific mRNA's and polypeptides of tubulin, the principal microtubule protein. In BALB/cBY mice, the Mß5 tubulin isotype mRNA is abundant in CC starting at 15 d pc, falls at 8 d ab and is localized to Layers II-V. In comparing hyt/hyt to hyt/+ littermate CC, the Mß5 mRNA is depressed 3-5-fold on the day of birth (Stein et al., in Iodine in the Brain, Adv. Exp. Med. Biol., pp. 59-78, 1989) and remains depressed until 7 d ab. However, total ß-tubulin mRNA and protein are unchanged. These results are being evaluated in terms of *in situ* hybridization and immunofluorescence. The timing and duration of the decrease in Mß5 mRNA abundance may influence the composition of functionally important neuronal microtubules at a critical time in CC development and contribute to some of the previously reported neuroanatomical abnormalities (Escobar et al., in Congenital Hypothyroidism, pp. 85-127, 1983) in specific CC regions to which Mß5 is localized.

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42.15

COMPARISONS OF THE NUCLEAR UPTAKE OF TESTOSTERONE AND ITS METABOLITES IN THE BRAINS OF MALE AND FEMALE MACAQUE FETUSES. R.P. Michael and R.W. Bonsall*, Department of Psychiatry, Emory University School of Medicine, Atlanta, Georgia 30322 and the Georgia Mental Health Institute, Atlanta, Georgia 30306.

To investigate the mechanism by which testosterone (T) might influence the development of the primate brain during fetal life, we compared the uptake of T in cell nuclei from the brains of 7 intact female and 5 intact male fetuses (*Macaca fascicularis and M. mulatta*) at 121-124 days gestation 60 min after the injection of ^{3}H-T (250 μCi i.v. or 500 μCi s.c.). Purified nuclear pellets prepared from 8 brain regions were analyzed by HPLC to measure levels of ^{3}H-T and its metabolites. In both males and females, concentrations of radioactivity extracted from cell nuclei were significantly higher in the hypothalamus-preoptic area than in other brain regions ($P<0.001$). ^{3}H-estradiol represented 65.0 ± 5.7% of this radioactivity, and nuclear concentrations of this aromatized metabolite were 73% lower in males than in females ($P<0.001$). ^{3}H-T represented 68.9 ± 8.8% of the radioactivity in cell nuclei from pituitary gland, and concentrations were 48% lower in males than in females ($P<0.001$). Levels of ^{3}H-T in plasma and in tissue supernatants in males and females did not differ significantly, but males had significantly higher levels of endogenous T in plasma (599.8 ± 208.2 ng/100 ml) than did females (37.7 ± 28.5 ng/100 ml)($P<0.01$). Thus, the differences between males and females in steroid uptake by cell nuclei could not be explained by differences in the bioavailability of ^{3}H-T, but may have been due to the prior occupation of receptors by endogenous steroids in the male fetus. This supports the view that T produced by the male testis may act on the fetal brain, and suggests that aromatization might be involved in some of its actions.

Work supported by USPHS grant MH 40420 and by the Georgia Department of Human Resources.

42.16

IMMUNOCYTOCHEMICAL LOCALIZATION OF INTRACELLULAR ANDROGEN RECEPTORS IN SPINAL MOTOR NEURONS R. C. Yu, P. Vuong*, C. Chang*, S. Liao*, and B. G. W. Arnason. The Brain Research and Ben May Institutes, Univ. of Chicago, Chicago, Il 60637.

Somatic motor neurons(MN) are one of the major sites for androgen(A) action within the central nervous system. The effects of A on responsive cells are mediated by an intracellular receptor(R) which in turn interacts with the genome to activate specific genes. We have examined, immunohistochemically, the distribution of AR in rat spinal cord and ventral prostate (VP). Monoclonal and polyclonal antibodies specific for AR were generated by using a fusion protein containing a segment of human AR corresponding to amino acid positions 331 to 527 from the N-terminal. The sensitivity of the immunoperoxidase stains(IS) of AR on the tissue sections was greatly affected by the fixative and the duration of fixation. Tissues fixed in 4% paraformaldehyde for 2 hours displayed IS in both nucleus and cytoplasm of spinal cord MN. Some neuronal processes including the axon hillock were also stained. Only nuclear IS was observed in VP epithelia. Prolonged(>4 hrs) fixation with aldehyde-based fixative reduced IS intensity. IS was also diminished following alcohol-based fixation (OmniFix) or fresh frozen unfixed tissue. Acetone fixation obliterated IS in VP cells and reduced nuclear IS in MN while the intensity of IS in the perikaryon remained unchanged. Our results demonstrate that the distributions of nuclear and cytoplasmic intracelluar AR are different in spinal cord MN and VP.

GENETIC MODELS OF NERVOUS DISORDERS I

43.1

HPRT DEFICIENT MICE ARE BEHAVIORALLY SUPERSENSITIVE TO AMPHETAMINE. H.A. Jinnah, F.H. Gage, and T. Friedmann*. Depts. of Neurosciences and Pediatrics, UCSD, La Jolla, CA 92093.

Congenital HPRT deficiency in humans results in a severe neurological disorder know as Lesch-Nyhan syndrome. Behavioral abnormalities in this disorder are thought to result from dysfunction of dopamine systems in the basal ganglia. Recently however, mice with a large HPRT gene deletion resulting in complete HPRT deficiency were reported to exhibit normal behavior. In the present study, we tested the sensitivity of these mice to amphetamine, a drug which stimulates dopamine release and which can produce Lesch-Nyhan like self-injurious behavior in normal rodents. Mice (n=20) were scored for stereotypic behavior using a stereotypy scale. No difference between the scores of HPRT+ and HPRT- animals was observed after sc injection of saline, 2, or 4 mg/kg amphetamine. However, after a dose of 8 mg/kg amphetamine, HPRT- animals engaged in stereotypic gnawing, sniffing, and licking much more frequently than HPRT+ animals, resulting in significantly higher scores for HPRT- animals ($p<.05$). Additional mice (n=32) were tested for locomotor activity in photocell chambers. In five hour test periods, no consistent differences between HPRT+ and HPRT- animals were observed after injection of saline, 2, or 4 mg/kg amphetamine. However, after a dose of 8 mg/kg, the activity profiles of HPRT+ and HPRT- animals were strikingly different. HPRT+ animals displayed a single peak of activity which subsided after 2 hours. HPRT- animals exhibited biphasic activity profiles, indicative of the intervening phase of stereotypy observed in the previous study. This difference was also statistically significant (ANOVA for repeated measures, $p<.01$). These results document the existence of an abnormal behavioral phenotype in HPRT deficient mice, and provide further support for a relationship between HPRT and dopamine systems in the brain. We are currently testing the sensitivity of these mice to other pharmacologic agents.

43.2

TRANSGENIC MICE EXPRESSING THE HUMAN SOD-1 GENE IN PRIMARY CELL CULTURE OF NEURONS: EFFECT OF OXIDATIVE STRESS. P.H. Chan, S. Chen,* L. Chu,* E. Carlson,* C.J. Epstein*Depts. of Neurology & Pediatrics, University of California, San Francisco, CA 94143

Oxygen radicals have been implicated in cerebral ischemia and reperfusion injury. Supplementation of liposome-entrapped CuZn-superoxide dismutase (SOD) ameliorates cerebral ischemic infarction. Recently transgenic mice carrying the human CuZn-SOD (h-SOD-1) gene have been developed as animal models for the study of the effects of the increased SOD-1 activity in Down syndrome (Epstein *et al.*, PNAS 84:8044, 1987). The availability of these animals has allowed us to examine the effects of oxidative stress on neurons. Primary cultures of cerebellar neurons (14 days *in vitro*) were developed from 7-day-old transgenic mice and from the normal diploid littermates. The total SOD activity in the transgenic neurons was 2.3-fold higher than in normal diploid cells. In addition to the mouse SOD activity, the h-SOD-1 activity was identified when the transgenic neurons were subjected to native gel electrophoresis followed by staining with nitroblue tetrazolium. Since increased h-SOD-1 activity will affect the oxidative states and may provide protection for neurons from oxidative stress, we studied the levels of glutathione (GSH) and the effects of H_2O_2 on transgenic cells. The levels of GSH were 10.9 ± 1.1 and 10.8 ± 0.9 nmoles GSH/mg protein for normal diploid and transgenic neurons, respectively. Exposing neurons to H_2O_2 at various concentrations (0, 10, 50, 100 mM) at 1 hour caused dose-dependent cell injury as measured by the release of lactate dehydrogenase (LDH) into the incubation medium by the cells. However, LDH releases were significantly lower at 10 mM (4.7 vs. 11.3 U/L/h) and at 50 mM (7.1 vs. 16.2) for transgenic neurons. LDH release was similar for both normal and transgenic neurons at 100 mM (34.4 vs. 32.4). These data suggest that transgenic neurons are more resistant to low levels of H_2O_2 toxicity, and this resistance may be due to adaptation of the neurons to the H_2O_2 produced constantly by increased h-SOD-1 activity. Supported by NS-14543, NS-25372, and HD-17001.

43.3

DIFFERENTIAL MURINE SENSITIVITY TO SCOPOLAMINE IN TWO DISSOCIATED TASKS. M. Buckley and M.A. Pelleymounter. Abbott Laboratories, Abbott Park, IL 60064

Cell loss in the basal forebrain cholinergic system and memory deficits are well-documented characteristics of Alzheimer's Disease (AD). There is also evidence for a genetic component in AD. It is unclear, however, whether the cholinergic degeneration found in AD has genetic basis. We have examined a possible genetic basis for cholinergic involvement in AD-like memory impairments by observing the effects of a cholinergic antagonist on spatial memory in DBA mice. Several groups have shown that this strain has a severe spatial learning impairment. Male DBA, C57 and CD1 mice were injected with saline or scopolamine (1 mg/kg;ip) 20 min prior to training in the plus maze version of the Morris water maze. Latencies to reach a hidden, stationary escape platform were measured, along with the number of trials to reach a spatial bias criterion. DBA mice required significantly more trials to criterion and had longer overall latencies than C57 or CD1 mice. Further, DBA mice were more disrupted by scopolamine than either of the other two strains. These animals were also tested for retention of a step-through, passive avoidance response after pretraining injections of scopolamine or saline. Again, DBA mice were the most sensitive to the disruptive effects of scopolamine, suggesting that a hyper-responsive cholinergic system might be part of the genetic component for the memory impairments in these mice. We are currently assessing the effects of scopolamine (1 mg/kg) on hippocampal and cortical high affinity choline uptake in the three strains of mice.

43.4

INCREASED SENSITIVITY TO CHOLINERGIC AGONISTS: A GENETIC TRAIT M.Steiner, T.Sota*, and B.G.Orpen. St. Joseph's Hospital Research Institute, and Dept. of Psychiatry and Biomedical Sciences, McMaster University, Hamilton, Ontario, Canada.

Wistar-Albino-Glaxo (WAG) rats are supersensitive to the temperature-depressing effects of the muscarinic agonist, oxotremorine. We have cross-bred the WAG with the pigmented Dark Agouti (DA) rat to provide a pigmented, cholinergically-supersensitive rat model for the cholinergic supersensitivity demonstrated in depression. We report here on our preliminary studies of the cholinergic supersensitivity observed in the F1 offspring of WAGxDA crosses.

Cholinergic supersensitivity, as indicated by the drop in core body temperature 30 minutes after giving oxotremorine (.25 mg/kg, i.p.) in the presence of peripheral receptor blockade by methyl atropine, was studied in male and female WAGxDA and DA rats.

Body temperature dropped significantly more in WAGxDA than in DA rats (Males: 2.25 C.$\pm$ 0.41 vs. 1.84 $\pm$ 0.56, df=46, p<0.01; Females: 2.85 $\pm$ 0.49 vs. 2.03 $\pm$ 0.48, df=27, p<0.01). Also, female WAGxDA rats showed a greater temperature drop than males (2.85 $\pm$ 0.49 vs. 2.25 $\pm$ 0.41, df=53, p<0.01). Thus, the cholinergic supersensitivity appears to be a dominant, but complex, genetic trait in these animals. Preliminary results from studies of muscarinic receptor binding in "live" punches from brain slices suggest that the difference in drop in body temperature is due to increased numbers of muscarinic receptors in the hypothalamus of the WAGxDA rats.

(Supported by St. Joseph's Hospital Foundation. B.G.O. is an Ontario Mental Health Foundation fellow.)

43.5

PHYSIOLOGICAL ANALYSIS OF THE BRAIN OF THE MUTANT HAN-WISTAR RAT: XENOPUS OOCYTE ASSAY OF GLUTAMATE RECEPTORS. R.W. Cohen, R.S. Fisher, A.T. Campagnoni and C.D. Hull. MRRC, UCLA, Los Angeles, CA 90024.

A mutant strain of Han-Wistar rats carries an autosomal recessive gene that produces spastic paresis which is characterized by hyperexcitability, tremor and rigidity in the hindlimbs (Neurosci. Lett. 2:45-49). Immunohistochemical analysis of the brains of these animals revealed three major sites of degeneration: (1) in purkinje cells of the cerebellar cortex, (2) in myelinated fiber tracts to and from the cerebellum, and (3) in ascending fiber tracts to the thalamus. We utilized the Xenopus oocytes assay which involves the expression of foreign mRNA injected into oocytes to search for a possible source of this brain dysfunction. Brains of affected rats and unaffected littermate controls were bisected at the mesencephalon into rostral and caudal halves (the caudal half contained the cerebellum). Each brain half was homogenized and its mRNAs were isolated. Samples of these mRNAs were injected into Xenopus oocytes. These oocytes were voltage clamped at -60mV and exposed to 1mM glutamate which produces a strong inward current. Unaffected (31.6$\pm$7.2nA) and affected (31.0$\pm$8.5nA) rostral halves showed similar responses to glutamate; the caudal halves had statistically different responses: Unaffected=41.7$\pm$6.0; affected=95.8$\pm$7.1 ($\bar{x}\pm$S.E.). These results suggest that the Han-Wistar dysfunction involves either a large increase in glutamate receptors or a change in receptor sensitivity of the caudal brain. Presently, we are attempting to determine which type of glutamate receptor is contributing to this phenomenon.

43.6

MORPHOMETRIC ANALYSIS OF NEOCORTEX IN THREE MALES WITH THE FRAGILE X SYNDROME.
V.J. Hinton*, W.T. Brown* and R.D. Rudelli*. (SPON: J. Currie). NY State Institute for Basic Research in Developmental Disabilities, 1050 Forest Hill Rd, Staten Island, NY 10314.

Fragile X syndrome [fra (X)] is the second most prevalent chromosomal developmental disorder which clinically presents as a form of mental retardation (MR) and is associated with a position q27 marker on the X chromosome. Postmortem investigation of three males with karyotype confirmed fra (X) included cortical intra-layer neuronal cell counts. Layers II-VI were identified by neuronal characteristics in cingulate and temporal association areas, (Brodmann's 23 and 38). For each layer, cresyl violet stained neurons were counted in ten visual fields (an area of 20μm X 15μm magnified to 1400X on a video screen), and the mean computed. A 3-way ANOVA (condition by area by layer) performed on the transformed counts showed no significant differences, [$F(1,4)=.61$, $p>.05$], between fra (X) and control neocortex. Thus, fra (X) associated MR may not be related to either neuronal developmental deficits or postnatal neuron loss.

43.7

DELIVERY OF UV-INACTIVATED RADIOLABELED HERPES VIRUS ACROSS THE BLOOD-BRAIN BARRIER AFTER OSMOTIC BLOOD-BRAIN BARRIER DISRUPTION. E.A. Neuwelt*, R. Dix*, & M.A. Pagel*. (SPON: Fred O. Risinger). Departments. of Neurosurgery and Biochemistry, Oregon Health Sciences University, Portland, Oregon and University of Miami, Miami, Florida.

The present studies were undertaken to evaluate the possibility of delivering a viral particle across the blood-brain barrier (BBB). Osmotic BBB modification with intracarotid (IC) mannitol (25%) was immediately followed by bolus IC administration of UV-inactivated 35S-labeled Herpes virus (2.0×10^6 cpm). After 60 min, intravascular virus was cleared by saline perfusion and the animals were then sacrificed. A marked increase (fourfold, $p \leq .02$) in protein-precipitable radioactivity was observed when compared with controls without barrier modification. Administration of virus intravenously (IV) immediately after BBB modification displayed no difference in delivery compared with IC saline-infused (non-BBB modified) controls suggesting the importance of a first-pass phenomenon. There were no significant differences in serum concentrations among IC or IV groups. These preliminary studies would suggest the possibility of delivering viral particles across the BBB with the osmotic technique which may permit delivery of genetic material in replication-defective viral vectors for gene therapy in our feline model of Gm2-gangliosidosis. (Supported by the Veterans Administration).

43.8

PATHOLOGIC CHANGES IN SKELETAL MUSCLE IN AN INHERITED CANINE HYPOKALEMIC PERIODIC PARALYSIS. G.A. Hegreberg. Dept. Vet. Pathol., WSU, Pullman, WA. 99164-7040

An autosomal recessive neuromuscular disorder in the dog resembles human hypokalemic periodic paralysis and is clinically accompanied by skeletal muscle weakness which is especially expressed as intolerance to exercise, cold and excitement stresses. Muscle atrophy is generalized and slowly progressive. Studies were performed to characterize the skeletal muscle morphologic changes. Biopsies were surgically removed from the biceps femoris of 5 affected and 5 nonaffected adult dogs. Frozen and 4F-1G fixed specimens were examined. A consistent and pronounced change in the skeletal muscle from affected dogs was the variation in fiber diameter size with increased atrophy and hypertrophy factors. There was a marked apparent decrease in the number of type II fibers. Some sarcoplasmic masses were present and consisted of peripherally located myofibril-poor sarcoplasm. Some nuclei were internally placed and in rows. Perinuclear vacuoles were associated with the centrally located nuclei and some perinuclear regions contained coarse PAS positive granules. Large vacuoles, present in the sarcoplasm of muscle fibers from older affected dogs, contained a floccular material which was PAS positive and diastase susceptible. Supported in part by NIH 00515 and Washington State University.

44.1

EYE-OPENING IN MUTANT ALBINO VS. PIGMENTED RAT LITTERMATES, CONGENIC HYBRIDS OF F344 AND WLE INBRED STRAINS. I.S.Westenberg.Psychol.Dep't.,Glendale Com.Col.,Glendale,AZ 85302.

Precocious eye-opening in albinos has been seen in albino-vs.-pigmented comparisons of rats in which a difference at the albino (c) gene locus was confounded with other genetic differences. This problem is solved by comparing congenic rats that differ only at the c locus, e.g., albino (c/c) vs. pigmented (c/+) F_1 hybrids of the Fischer (F344) inbred and Westenberg-Long-Evans (WLE) segregating inbred strains. I bred c/c F344 females with c/+ WLE males to get 12 litters of F3WLF1 hybrid rats. All littermate F3WLF1 pups were c/c or c/+ but otherwise almost identical genetically, matched for maternal and paternal effects, prenatal and post-natal environment, etc. In each litter, checked daily, 3 outcomes were possible; the first-detected eye-opening could have been in c/c, c/+, or c/c & c/+ (tie). There was 1 tie; in all other litters (11/11) the first-detected eye-opening was in a c/+ pup. Often the eyes of all c/+ in a litter opened before eye-opening in any c/c. Statistical analysis conservatively included only litters where the number of c/c was $\geq$ the number of c/+; in each of 8 such litters the first-detected eye-opening was in c/+. This statistically significant finding ($p < 0.01$, sign test) reinforces warnings about the use of albinos as "normal" subjects and supports the albino mutation's value as a research tool to be used with proper genetic controls.

44.2

A NEW LINE OF CONVULSION-PRONE MICE. R.E. Wimer and C.C. Wimer*. Division of Neurosciences, Beckman Research Institute of the City of Hope, Duarte, CA 91010

Mice that exhibit convulsions were an unanticipated result of a breeding experiment designed to produce recombinant inbred strains differing in the number of pyramidal cells in hippocampal regio superior (CA1). Animals were selectively bred for convulsion-proneness, and simultaneously inbred, resulting in a new strain in which nearly all of the mice have convulsions during weekly cage changing, from the age of 10-12 weeks. Severity ranges from immobility, rapid breathing, body tremor, and exopthalmia to vocalization, back arching, falling over, occasionally followed by tonic extension of the limbs frequently terminating in death. Otherwise the strain appears to be behaviorally and neurologically normal, with an average life span and normal reproductive behavior. Preliminary tests suggest that products (probably chemo-olfactory) of other mice may be effective in eliciting the convulsions. Abnormalities in cell lamination in the hippocampus have been observed, although hippocampal involvement remains to be demonstrated.

44.3

INCREASED NUMBER OF SOMATOSTATIN-IMMUNOREACTIVE NEURONS IN PRIMARY CULTURES OF TRISOMY-16 MOUSE CORTEX. G. Capone, P. Corsi, M. Caserta, M.L. Oster-Granite, R. Reeves* and J.T. Coyle. Depts. of Neuroscience, Psychiatry and Physiology, The Johns Hopkins School of Medicine, Baltimore, Maryland 21205.

Since the gene encoding pre pro-somatostatin (ppSS) is located on mouse chromosome 16 (MMU 16), we have examined the effects of trisomy (Ts) of MMU 16 on SS immunoreactivity (IR) and ppSS mRNA expression by *in situ* hybridization in primary cultures of papain dissociated neocortex from 15 day gestational Ts16 and euploid littermates. Cultures were grown for 1-3 weeks on polylysine coated 35 mm plastic plates in MMEM containing 10% heat inactivated horse serum. Neurons were identified by neuron specific enolase IR. Immunocytochemical analysis indicated an increase in the relative density of SS-IR neurons, ranging from 1-3-fold above euploids in Ts16 cultures at 1 and 2 weeks *in vitro* although differences become attenuated at 3 weeks. SS-IR was more intense in Ts16 neurons but was expressed in the same cell types. No difference in the relative density of glutamate decarboxylase or neuropeptide Y IR neurons was observed. *In situ* hybridization for ppSS mRNA and SS-IR on the same cultures revealed the same density of positive neurons. Thus, under the culture conditions used, the presence of an extra copy of ppSS gene in Ts16 appears to result in SS expression in an increased number of cortical neurons.

44.4

ACCLERATED DEVELOPMENT OF SODIUM CHANNELS IN CULTURED ASTROCYTES FROM THE TRISOMY 16 MOUSE. P.J. Yarowsky[1], T.A. Bunting*[3], S. Baharloo*[1], M.L. Oster-Granite[3] and B.K. Krueger[2]. Depts. of Pharmacology & Experimental Therapeutics[1] and Physiology[2], Univ. of Maryland Sch. of Med., Baltimore, MD 21201, Developmental Genetics Lab[3], Johns Hopkins Univ. Sch. Med., Baltimore, Md. 21205.

Previous studies revealed that cultured neonatal rat cerebral astrocytes have two types of Na channels; channels with low STX-affinity were present throughout four weeks in culture and high STX-affinity Na channels appeared in the second week (Yarowsky and Krueger, J. Neurosci. 9:1055, 1989). This spontaneous change in STX-affinity coincided with changes in astrocyte morphology from polygonal immature cells with few processes to mature stellate cells with numerous processes. In primary cultures of cortical astrocytes from trisomy 16 mice we observed predominantly $GFAP^+$ stellate astrocytes (75%) as early as 8 days in culture. At the same time in culture, astrocytes from normal siblings were mostly polygonal (85%). Also, at 8 days in culture, trisomic cells showed a two-fold increase in high STX-affinity Na channels over normal astrocytes. This increased and premature expression of Na channels persisted throughout three weeks in culture. (NSF BNS 8711829 and NIH NS16285, HD19920, HD19932)

44.5

EFFECT OF MPTP ON STRIATAL DOPAMINE LEVELS IN THE WEAVER MUTANT MOUSE. E.H. Stotz*, J.R. Simon, B. Ghetti and J.A. Richter (SPON: A.N. Siakotos). Indiana Univ. Sch. of Med., Indpls., IN 46202.

Compared to normal (+/+) mice, weaver mutant mice (wv/wv) have a substantial decrease in the number of dopaminergic neurons in the nigrostriatal pathway; heterozygous (wv/+) mice do not undergo such a cell loss (Triarhou et al., 1988). In one effort to compare the neurochemical characteristics of the surviving nigrostriatal dopamine (DA) neurons in wv/wv with those in the wv/+ and +/+ mice, we have tested if the effects of the neurotoxin MPTP on striatal DA levels are the same in the three genotypes.

A total of four injections of 6 mg/kg of MPTP-HCl or saline were given (s.c. every 2 hr) to all three genotypes. The mice were decapitated one week later, and the striata were assayed for DA levels by HPLC-EC. In the saline-treated groups, the DA levels in +/+ and wv/+ were the same whereas the levels in wv/wv were much decreased. MPTP treatment decreased the striatal DA levels in both +/+ and wv/+ mice but seemed not to affect the striatal DA levels of the wv/wv mice.

MPTP action requires the uptake of its metabolite MPP^+ into DA neurons. A depressed DA uptake system in the remaining nigrostriatal DA neurons in these adult wv/wv mice could explain the lesser MPTP effect. Currently we are testing this idea by measuring several DA markers in the three genotypes. (Supported by NS 14426).

44.6

STRIATAL AND MESENCEPHALIC SUBSTANCE P CONTENT IN THE WEAVER MUTANT MOUSE AND THE EFFECT OF MPTP. J.R. Simon, J.A. Richter, B. Ghetti and M.R. Vasko. Indiana University School of Medicine, Indianapolis, IN 46202.

The weaver mutation induces a decrease in the number of dopaminergic neurons in the nigrostriatal pathway of the weaver (wv/wv) relative to homozygous (+/+) and heterozygous mice (wv/+). One consequence of this cell loss is a substantial decrease in dopamine (DA) content in the striatum (STR) of the wv/wv. In view of the evidence suggesting interactions between DA- and substance P (SP)-containing neurons, we determined the striatal and mesencephalic(MES) SP content in wv/wv, wv/+ and +/+ mice. We also investigated the effect of the DA neurotoxin, MPTP, on SP content in STR and MES of the three genotypes.

A total of four injections of 6 mg/kg MPTP-HCl or saline were given (s.c. q 2h) to adult mice of the three genotypes. Mice were sacrificed one week later and the STR and MES assayed for SP by RIA. In the saline-treated groups, SP content was greater in the MES than in the STR with no apparent differences in content among the three genotypes. Following MPTP treatment, SP content tended to decrease in the STR and MES of +/+ and wv/+ mice, but did not appear to be affected in either region of the wv/wv. Thus, there appear to be differences in the manner in which SP-containing neurons respond to acute (MPTP) and chronic (wv/wv) depletion of DA. (Supported by NS 21697 and NS 14426)

44.7

MONOAMINERGIC ALTERATIONS AND MATERNAL EFFECTS IN ATAXIC HOTFOOT MICE. L.J. Draski* and G.A. Gerhardt[1] (SPON: J.M. Masserano). Depts. of Pharmacology and Psychiatry[1], Univ. of Colorado Health Sciences Center, Denver, CO 80262.

The single gene mouse mutation, hotfoot, is characterized by a progressive ataxia and body tremor which first can be observed after approximately the second postnatal week. In an attempt to investigate neurochemical correlates of the pathogenesis of the ataxia, tissue levels of monoaminergic neurotransmitters and their metabolites were determined in cerebellum and striatum of 30 homozygous recessive male mice (ho/ho) and 30 phenotypically normal (+/-) littermates by HPLC-EC methods. In addition, animals were identified as being raised by either a phenotypically normal or hotfoot mother. Normal males born to hotfoot dams and hotfoot males, regardless of maternal genotype, exhibited significantly greater amounts of norepinephrine (NE), as well as lower MHPG/NE and 5-HIAA/serotonin (5-HT) ratios, in cerebellum, relative to phenotypically normal males raised by normal dams. However, in striatum, significantly greater levels of 5-HT were observed only in hotfoot males with no significant effects of maternal genotype noted. These results suggest that monoamine neurotransmitter systems are involved in the expression of the hotfoot ataxia, as well as illustrate the susceptability of the normally developing central nervous system to the effects of early environment. Supported by USPHS AG06434, AG00441.

44.8

QUANTITATIVE ANALYSIS OF VARIATION OF FUR PATTERN AS A MARKER FOR NEUROCHEMICAL ABNORMALITY IN FEMALE BRINDLED MICE. P.M. Martin, M. Irino*, Q.D. Walker*, Kinuko Suzuki, M. Lewis and R.B. Mailman. Toxicol. Curric., BSRC, and Dept. of Pathology, Univ. of North Carolina, Chapel Hill, NC 27599.

We have developed a method of quantifying the fur pattern of mice with varied coat color (e.g., chimeric mice), and have used it to study mice heterozygous for the brindled gene. Brindled (MO^{br}) is an X–linked neurological mutation in mouse, arising spontaneously in the C57BL inbred strain. The hemizygous males (MO^{br}/y) have low copper concentrations in brain, an almost total lack of fur pigmentation, and neuronal degeneration of the cerebrum. Thirty day old female mice heterozygous for the brindled gene have similar characteristics, including large patches of lightly colored fur, the extent of which appears to be in proportion to the number of abnormal Purkinje cells in the cerebellum. Eleven female mice (5–11 mo of age) were tested in locomotor chambers, then photographed from a standardized height and position. An imaging system measured absorbance, yielding a mean density (± S.D.) for each mouse. After euthanasia, concentrations of dopamine, norepinephrine, serotonin, and their metabolites in various microdissected brain regions were measured by HPLC–EC. NE from the hypothalamus, but not other brain regions, was higher in the more lightly colored females ($r=-0.516$). Since copper is a cofactor for dopamine–β–hydroxylase, the extent of lightly colored fur may serve as a marker for neurochemical abnormalities due to abnormal copper distribution in the brain. This method of quantifying fur patterns may also be useful in measuring the extent of introduction of new genetic material into chimeric mice. (Supported by Grants ES70126, NS24453 and ES01104).

44.9

BRAIN CATECHOLAMINE CONCENTRATIONS IN THE BRINDLED MOTTLED MOUSE RESEMBLE THOSE SEEN AFTER INHIBITION OF DOPAMINE–β–HYDROXYLASE. Q.D. Walker*, M.H. Lewis, K. Suzuki, P.M. Martin, S.W. May, and R.B. Mailman. Univ.of North Carolina, Chapel Hill NC 27599 & Georgia Institute of Technology, Atlanta, GA.

The brindled mottled mouse has an X–linked mutation resulting in abnormal copper homeostasis, compromised function of copper–dependent enzymes, and neurodegeneration. The neuropathology in this murine model of the human neurodegenerative disease, Menkes' kinky hair syndrome, is prominent in a few neural loci, the fronto–parietal cerebral cortex and thalamic nuclei. As the susceptible nuclei are terminal fields for norepinephrine (NE), their degeneration may be associated with a decrease in dopamine–β–hydroxylase (DBH) activity and subsequently, NE depletion. To test this hypothesis, catecholamine concentrations in selected brain regions of both brindled mottled mice and mice treated with the DBH inhibitor, disulfiram, were compared. Administration of disulfiram (200 mg/kg) to normal mice produced > 70% depletions of NE in all cerebral regions assayed. Concomitantly, dopamine concentrations increased at least 2–3 fold in all but the dopamine–rich midbrain. NE concentrations in all brain regions of brindled mottled mice were lower than the detection limits of the assay. Dopamine levels in the mutants were higher than those of control mice but similar to the concentrations in disulfiram–treated mice. These data with disulfiram were compared with those using novel inhibitors of DBH. Together, this work provides evidence for the hypothesis that a profound lack of noradrenergic innervation during a critical period of development may interfere with synapse formation and lead to neuronal degeneration (ES01104, HL28167, NS24453 & ES07126).

RESPIRATORY REGULATION I

45.1

TOPOGRAPHIC ORGANIZATION OF RETICULOSPINAL PROJECTIONS TO RESPIRATORY MOTONEURON POOLS IN THE RAT. S. Manaker, T.L. Bigler*, A.R. Morrison and A.I. Pack*. Depts. of Medicine and Animal Biology, University of Pennsylvania, Philadelphia, PA 19104

During rapid eye movement sleep (REMS), the somatic musculature is inhibited by descending pathways from the reticular formation. However, during REMS the activity of the respiratory musculature continues. This different behavior in REMS may reflect differential organization of projections from pontomedullary inhibitory areas to the different motoneuron pools. We therefore examined in rats the origin of reticulospinal projections to three motoneuron pools: the lumbar spinal cord, the cervical spinal cord, and the hypoglossal nucleus as representing somatic, respiratory and upper airway motoneuron pools, respectively.

Ten nl of Fast Blue (3% solution) were injected into one of the motoneuron pools, and the animal allowed to survive up to five weeks. Rats with extension of tracer out of the ventral cord or hypoglossal nucleus were excluded from analysis. Every third tissue section (32 μm) through the medulla and pons was examined under epifluorescence, and retrogradely labeled fluorescent neurons plotted on camera lucida drawings.

Greatest numbers of fluorescent neurons were observed after hypoglossal injections, and fewest after lumbar injections. Reticular formation neurons were topographically organized: neurons projecting to the hypoglossal nucleus were most dorsal, neurons projecting to cervical spinal cord were intermediate, and neurons projecting to lumbar spinal cord were most ventral. Relatively little overlap among these three groups of neurons was observed. These data indicate a distinct topography to the organization of reticulospinal projections that may mediate motor inhibition in REMS. (Supported by SCOR grant HL-42236)

45.2

EFFERENT PROJECTIONS FROM NEURONS IN THE NUCLEUS TRACTUS SOLITARIUS WHICH MAY PLAY A ROLE IN THE BREUER-HERING REFLEX. A. C. Bonham, D. R. McCrimmon, & S. K. Coles*, Northwestern Univ. Med. School, Chicago, IL 60611.

We previously identified an area in the nucleus tractus solitarius (NTS) just medial to the tractus and centered about 300μm caudal to the obex in which: 1) chemical excitation mimicked the Breuer-Hering (BH) reflex (produced apnea), 2) interruption of synaptic transmission impaired the BH reflex and, 3) slowly adapting pulmonary stretch receptor interneurons (pump cells) were recorded. (Soc. Neurosci 114:189.2, '88). To identify potential efferent projections from the cells in this region, we injected *Phaseolus vulgaris* leucoagglutinin (PHA-L). The site was physiologically identified by injecting the excitatory amino acid DL-homocysteic acid (DLH) through one barrel of a double barrel pipette. Once the center of the region was identified in which DLH (3nl of 5 or 20mM) produced an apnea (cessation of diaphragm EMG), PHA-L was injected through the adjacent barrel. From the injection site a band of axons swept caudally (up to 700μm) and medially (up to midline) within the NTS. In separate preliminary experiments, DLH injections in this area produced apnea; in contrast to the rostral region, the apnea elicited from this caudal area was accompanied by increases or decreases in arterial pressure. Following PHA-L injections, which were larger but still restricted to the medial NTS, labelled axons and varicosities were located in the rostral NTS, para ambigual region, locus coeruleus, and Kölliker-Fuse.

These data suggest that some neurons with a presumptive role in the BH reflex synapse caudally within the NTS. Supported by R01 HL 40336 and HL 07717.

45.3

WITHDRAWN

45.4

HISTOLOGICAL STUDIES ON BRAINSTEM RESPIRATORY CHEMOSENSITIVE AREA IN RATS. Y. Pan*, F. Bada *, D.G. Bernard*, J.A. Holloway, C.O. Trouth. Dept. of Physiol. & Biophysics, Coll. of Med., Howard Univ. Washington, D.C. 20059.

The caudal respiratory chemosensitive area on the ventral medullary surface (VMS) of choralose-urethane anesthetized rats was explored electrophysiologically for neurons which increased their firing rate in response to increased inspired CO2 (PICO2) but not to other modalities of sensation. Recording sites of CO2 sensitivity were found between and lateral to the hypoglossal rootlets at the exit from the brainstem. The recordings sites were marked electrolytically and controlled histologically. Light and electronmicroscopic examination showed neuronal elements with a rich array of synapses scattered among astrocytic processes within the marginal glia, similar to those described in the cat. Neurons rich in synapses were also found within the marginal glia along blood vessels as they pierce the VMS making intimate contact with vascular walls. These latter are reminiscent of neurovascular elements and may be involved in 1)neurosecretion, 2)chemoreception, or 3) vasomotor function. (Supported by the NIH Grant-MBRS 2-S06-RR-08016 AND PH 55-T 32 GM-07800.)

45.5

ONTOGENY OF OPIOID RECEPTORS IN THE RAT BRAINSTEM. Y.Xia * and G.G. Haddad (SPON: J. Stitt). Dept. of Pediatrics (Div. Respir. Medicine),Yale Univ., New Haven, CT 06510.

Opioids have been shown to play a role in modulating cardiorespiratory function during hypoxia. Differences in hypoxic responsiveness between young and adult can be attributed in part to differences in the maturation of the opioid system. Little is known, however, about the ontogeny of opioid receptors in the brainstem. In this work, we use autoradiographic techniques to study opioid receptor distribution in the pons and medulla of rats at ages of 1, 5, 10, 21 and 120 days. Ten μM sections from 4 standard planes were incubated for 45 min. at 25°C with 4 nM of either 3H-DAGO for labelling mu-receptors or 3H-DADLE with cold DAGO for labelling delta-receptors. Results show that the density of delta-receptors is much less than that of mu-receptors in most regions. High concentration of mu-receptors is found in nuclei such as Nucleus Tractus Solitarius (NTS), Dorsal Vagal Nucleus (DMNX), Nucleus Ambiguus, Parabrachial and Kölliker-Fuse Nuclei. Fewer receptors are present in the Hypoglossal and Raphe nuclei. Although different concentrations of mu-receptors are found in various areas at birth, mu-receptors increase with age in all regions, especially in NTS and DMNX. We conclude that: 1) brainstem regions containing the highest mu-receptors are cardiorespiratory-related; 2) marked differences in mu-receptor density exist in the respiratory-related areas and 3) the developmental time schedule of mu-receptors varies among brainstem nuclei.

45.6

RESPIRATORY PATTERNING FOLLOWING ACUTE COCAINE ADMINISTRATION. R.R. Terreberry, R.M. Harper, R.K. Harper*, R.C. Frysinger and C.A. Richard. Dept. of Anatomy & Cell Biology and Brain Research Institute, UCLA, Los Angeles, CA 90024.

We examined patterning of diaphragmatic activity in intact, freely moving cats following intravenous or intraventricular administration of 3 dose levels of cocaine (5, 7.5, and 10 mg/kg intravenously; .625, 1.25, and 2.5 μg intraventricularly). EEG, EMG, EOG, diaphragmatic EMG, hippocampal slow wave, and core temperature recordings were continuously gathered during sleep and waking states following which cocaine was administered intravenously or intraventricularly. Low dose cocaine administration was followed by tachypnea; high dose cocaine was followed by convulsive seizures in which respiratory patterning continued independently of the phasic motor activity associated with seizures. Extreme elevation of core temperature associated with high levels of cocaine induced panting with greatly enhanced genioglossal and facial motor upper airway muscle activity. Both intravenous and intraventricular administration resulted in tachypnea. Cocaine greatly altered respiratory patterning, with pronounced reduction of respiratory period and recruitment of upper airway muscles; some of these patterning changes may be secondary to cocaine-induced hyperthermia. Supported by DA04913.

45.7

ROLE OF ENDOGENOUS OPIATES AT BRAINSTEM RESPIRATORY CHEMOSENSITIVE AREAS. D.G. Bernard*, F. Bada*, Y. Pan*, P. Archer*, C.O. Trouth. Dept. of Physiol. & Biophysics, Coll. of Med., Howard University, Washington, D.C. 20059.

Sensing elements on the ventrolateral medullary surface (VMS) are involved in the central chemical regulation of breathing. In spontaneously breathing cats anesthetized with chloralose-urethane, the ventilatory responses to morphine (MOR), Beta-endorphin (END) and naloxone (NAL) applied topically to the caudal chemosensitive area were investigated. MOR and END depressed minute ventilation $\dot{V}E$, whereas NAL increased $\dot{V}E$. Increased $\dot{V}E$ induced by increased inspired CO2 or topically applied acetylcholine was augmented by NAL. These findings suggest a modulatory role for endogenous opiates at VMS chemosensitive sites. Immunocytochemical studies by light microscopy revealed positive reaction product in cell bodies and axons of superficial neurons using peroxidase-antiperoxidase technique. Electron microscopy identified reaction products in mitochondria and secretory granules. No reaction products were found in control sections without antibody. (Supported by the NIH Grant-MBRS 2-S06-RR-08016 AND PH 55-T 32 GM-07800.)

45.8

MODULATION BY μ OPIOID ANTAGONISM OF RESPIRATORY PATTERN IN RELATION TO SLEEP/WAKE STATE IN BEHAVING PIGLETS. S.C. Scott, J.G. Inman and I.R. Moss*. Depts. Pediatrics, Physiology/Biophysics, UT Southwestern, Dallas, TX 75235

Young (8-13 days; y) and older (28-34 days; o) piglets were instrumented aseptically for chronic recording of sleep/wake states (biparietal electroencephalogram, horizontal and vertical electrooculogram, nuchal electromyogram (EMG)), posterior cricoarytenoid and diaphragmatic EMG (EMG_{pca}, EMG_{di}), arterial pressure and heart rate, and measurement of arterial pH and gas tensions. Following recovery from surgery, 1-2 ∿1.5 h daily sessions of control recordings were conducted with the piglet in a sling. Then, on each of three successive days, recording sessions were divided into control and post μ antagonism (CTP, a somatostatin analog, 10-40 mg/kg i.v.). In quiet sleep, that state which best reflects the uninfluenced respiratory oscillator, respiratory frequency increased following CTP, inspiratory time decreased in the o and y, peak amplitude of EMG_{di} increased in the y and the rate of rise of EMG_{di} increased in both but to a greater extent in y. The effect of CTP on EMG_{pca} was inconsistent. Similar results were seen during transitional sleep and wakefulness but not during active sleep. We conclude that μ opioid antagonism enhances basic diaphragmatic respiratory drive in neonatal piglets, young more so than older. (HL36939)

45.9

A GLUTAMATERGIC SYSTEM IN THE VENTROLATERAL MEDULLA IS REQUIRED FOR RESPIRATION. T.P. Abrahams, P.J. Hornby, D.P. Walton, A. Taviera DaSilva* and R.A. Gillis*. Depts. of Pharmacology and Medicine, Georgetown University, Washington, D.C. 20007.

Recently, we have described a site in the ventrolateral medulla of cats where blockade of tonic GABAergic tone by microinjection of bicuculline produced dose-dependent decreases in mean arterial pressure (MAP; Neurosci. Abstr. 12:536, 1986). This area is approximately 2.5mm rostral to obex, 4mm lateral to the midline and 1mm below the ventral surface. The purpose of the present study was to determine whether blockade of tonic glutamatergic tone at this site by microinjection of kynurenic acid would alter cardiorespiratory activity. Kynurenic acid (50nl of 250mM solution) was microinjected bilaterally into this region in chloralose-anesthetized cats (N=6), while monitoring MAP, heart rate (HR), tidal volume (V_t) and respiratory rate (f). Kynurenic acid produced a decrease in V_t (-18.5±2.6ml, $P<0.05$) and an increase in f (+17±5 breathes/min, $P<0.05$). These changes progressed to apnea in each animal tested. No significant changes in MAP and HR were observed. Unilateral microinjection of kynurenic acid was ineffective in producing pronounced respiratory depression and apnea. The inactive analogue of kynurenic acid, xanthurenic acid, microinjected bilaterally into this site did not result in significant alterations of cardiorespiratory activity. These data suggest that a glutamatergic system in the ventrolateral medulla is required for breathing to occur in chloralose-anesthetized cats. Supported by USPHS grant MH 42322.

45.10

MICROINJECTION OF AN EXCITATORY AMINO ACID ANTAGONIST INTO THE VENTROLATERAL MEDULLA INCREASES RESPIRATORY ACTIVITY AND POTENTIATES THE RESPONSE TO HYPOXIA. G.H. Dillon*, D.E. Welsh* and T.G. Waldrop (SPON: G.Iwamoto). Dept. of Physiology, Univ. of Illinois, Urbana, Il. 61801.

Several studies suggest that an excitatory amino acid (EAA) mechanism in the ventrolateral medulla (VLM) may be involved in modulation of cardiorespiratory reflexes. The purpose of this study was to determine if an EAA mechanism in the VLM modulates the respiratory response to hypoxia or hypercapnia in anesthetized rats. Respiratory responses (diaphragmatic EMG activity) to hypoxia and hypercapnia were determined before and after bilateral microinjections of the EAA antagonist kynurenic acid (KYN) into the VLM. Bilateral microinjections of KYN into the VLM produced significant increases in respiratory activity. Hypoxia elicited a significantly larger increase in respiratory activity after KYN injection. The response to hypercapnia tended to be larger also, but was not significantly different from control levels. Accentuated responses returned to pre-injection levels after a 45-60 min. recovery. Furthermore, xanthurenic acid, an inactive analog of KYN, had no significant effects on resting levels or on the reflex responses. We conclude that an excitatory amino acid mechanism in the VLM exerts a tonic effect on respiration and modulates the respiratory response to hypoxia. (NIH HL 38726 and AHA)

45.11

BETHANECHOL MICROINJECTED INTO THE MEDIAL PONTINE RETICULAR FORMATION (mPRF) CAUSES STATE-DEPENDENT CHANGES IN RESPIRATION.
H.A. Baghdoyan, R. Lydic, and K.A. Gilbert.* Medicine and Anesthesia, Pennsylvania State University, College of Medicine, Hershey,Pa 17033.

Microinjection of cholinergic agonists into the mPRF of intact, unanesthetized cats produces a state which is similar to naturally occurring rapid eye movement (REM) sleep. Following our recent demonstration that the mixed cholinergic agonist carbachol causes state-dependent changes in respiration (Neurosci. Letts. 102:[In Press], 1989) the present study is examining the hypothesis that these respiratory effects are muscarinically mediated. To date we have performed 12 microinjection trials in 2 cats using the muscarinic cholinergic agonist bethanechol (4.3ug/0.25 ul). Respiratory frequency (f), tidal volume in ml (VT), and minute ventilation in ml/min (Vdot) were analyzed for 30 one min bins during waking (W) and for 30 one min bins during the REM sleep like state evoked by bethanechol (D-BETH). The means (±s.d.) during W were: f=30.7(±5.7), VT =22.4(±4), and Vdot=675(±105). During D-BETH the means(±s.d.) were: f=19.8(±3.2), VT=27.1(±6.5), and Vdot=527.4(±105). In D-BETH the respiratory measures were significantly different from respiration during W, and mPRF injections of atropine sulfate (15ug/0.25ul) blocked D-BETH. These findings demonstrate that muscarinic cholinergic mechanisms in the mPRF can cause state-dependent changes in respiration.

45.12

THE CHOLINOCEPTIVE MEDIAL PONTINE RETICULAR FORMATION (mPRF) MEDIATES STATE-DEPENDENT CHANGES IN THE VENTILATORY RESPONSE TO HYPERCAPNIA (HCVR) AND HYPOXIA (HVR). R. Lydic, H.A. Baghdoyan, and R. Wertz. Medicine and Anesthesia, Pennsylvania State University, College of Medicine, Hershey,Pa 17033.

In most mammals, including humans, the ability to generate a compensatory ventilatory response to hypoxic or hypercapnic stimuli is highly dependent on states of consciousness. The central mechanisms underlying this state-dependence is unknown. Since the mPRF is known to play a role in sleep cycle control, this study is testing the hypothesis that the cholinoceptive mPRF can also cause state-dependent alterations in the HVR and HCVR. Using 2 cats, we have performed 24 HCVR and 24 HVR drives during W and during the REM sleep-like state (D-BETH) evoked by mPRF injections of the muscarinic cholinergic agonist bethanechol (4.3ug/0.25ul). Regression analyses of min ventilation (y) and end-tidal CO_2 (x) described the HCVR in W as Y=52.2(x)-897 and during D-BETH as Y=33.4(x)-452. HVR regressions describing y as a function of x (O_2 saturation) were Y=-35.4(x)+4118 during W and Y=-27.3(x)+3551 during D-BETH. For both the HCVR drives (n=302 x,y pairs) and the HVR drives (n=375 x,y pairs) the slopes of the ventilatory response were significantly less during D-BETH than during W. Thus, muscarinic cholinergic mechanisms in the mPRF can cause state-dependent alterations in the ventilatory responses to hypoxia and to hypercapnia.

45.13

EFFECTS OF INTRAVENOUS BICUCULLINE AND STRYCHNINE ON TRANSIENT INSPIRATORY INHIBITORY RESPONSES IN THE CAT. D.F. Speck, D. Karius* and L. Ling*. Dept. of Physiology, Univ. of Kentucky Med. Ctr., Lexington, KY 40536-0084.

Single shock stimulation of the superior laryngeal nerve (SLN), the intercostal nerves (ICN), the phrenic nerve (PN) or within the dorsal respiratory group (DRG) has been shown to produce a transient, short-latency attenuation of inspiratory motor activity. This study determined if i.v. injection of either strychnine or bicuculline could affect these transient inhibitory responses. Experiments were conducted in decerebrate cats initially anesthetized with 50 mg/kg pentothal (i.p.). A microelectrode was positioned within the right DRG and the right PN, SLN and left ICN were mounted on bipolar electrodes for stimulation. After recording individual control responses of left PN activity to threshold stimulation of the three nerves and the DRG, bicuculline (1 mg/kg) or strychnine (50 ug/kg) was injected. Five minutes after injection the responses to stimulation were again recorded. With strychnine administration, the procedure was reiterated until the cumulative dose elicited marked convulsions. Systemic bicuculline had no effect on transient inspiratory inhibitory responses. However strychnine prolonged the onset latency of all four inhibitory responses examined. Since the duration of inhibition was not shortened, the onset prolongation is unlikely to result solely from increased motoneuronal excitability. (Supported by P01 HL40369).

45.14

HYPOTHALAMIC GABAERGIC MODULATION OF THE RESPIRATORY RESPONSE TO HYPERCAPNIA. C.A. Peano* and T. G. Waldrop. Dept. of Physiology, Univ. of Illinois, Urbana, IL 61801.

Previous reports from this laboratory have shown that a GABAergic mechanism in the posterior hypothalamus (PH) regulates cardiorespiratory activity. The purpose of the present study was to determine if a similar mechanism modulates the respiratory responses to hypercapnia and hypoxia in the anesthetized rat. Cardiovascular and respiratory responses to hypercapnia (5% CO_2) and to hypoxia (10% O_2) were recorded before and after microinjection of an inhibitor of GABA synthesis (3-mercaptopropionic acid, 3-MP) into the PH. Respiratory output was derived from diaphragmatic EMG activity. Microinjection of 3-MP evoked increases in arterial pressure, heart rate and respiratory frequency which occurred after a latency of $\sim$ 20 minutes. Moreover, the fall in heart rate as well as the increase in respiratory activity evoked by hypercapnia were accentuated after microinjection of 3-MP. In contrast, the arterial pressure response to hypercapnia and the responses to hypoxia were not altered by the microinjections. These results indicate that a GABAergic mechanism exerts a tonic inhibitory action upon posterior hypothalamic neurons. Lifting this inhibition provides a specific excitatory effect upon resting cardiorespiratory activity and responses to hypercapnia. (Supported by NIH HL 38726 and the AHA).

46.1

A POSITION-SPECIFIC ANTIGEN IN THE EARLY QUAIL EMBRYO
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Transplantation studies in avian embryos have suggested the existence of positional cues that can inform donor neural tube and neural crest of their location along the rostrocaudal axis. This rostrocaudal, positional information has been postulated to exist on cells or in the extracellular matrix. We report here on the generation of monoclonal antibodies to position-specific antigens using the cyclophosphamide immunosuppression technique. One antibody, atipo-1[< Jap *atama* = head + *shipo* = tail] recognizes an antigen located on the surface of ectodermal cells, in the subectodermal space, and around the neural tube and notochord. Antibody binding is present only in the most caudal region of stage 13 quail embryos, just before neural crest migration. However, in the cranial region when crest migration occurs(stage 7), atipo-1 does not bind. This caudal-specific distribution of the atipo-1 binding differs strikingly from that of known molecules thought to be involved in embryonic morphogenesis. Thus, the atipo-1 antigen is a candidate for providing position-specific information in the early quail embryo.

46.2

SPECIFIC POSITIONAL CUES USED BY AFFERENT PROJECTIONS ARE PRESENT IN ECTOPIC NEUROPILS IN THE LEECH.
M.B. Passani*, A. Peinado, C.A. Baptista* and E.R. Macagno. (SPON: D. Gorlick) Dept. of Biological Sciences, Columbia University, New York, N.Y. 10027.

Afferent projections segregate into four distinct bilateral longitudinal tracts in the neuropils of most segmental ganglia in the leech *Hirudo medicinalis*, as revealed by staining with the monoclonal antibody Lan 3-2 which is specific for afferent axons in *H. medicinalis*. We found that an additional tract exists in the neuropils of the fifth and sixth body ganglia (SG5 and SG6, also known as sex ganglia). These fibers appear to originate in the reproductive tissues, since they are anterogradely labelled by rhodamine isothyiocianate (RITC) applied to the gonopores. Also, animals which had the sexual organs removed at early embryonic stages did not have the additional tract. To assess (a) whether segment-specific differences exist in the pattern of cues available to incoming fibers and (b) the degree of specificity with which the incoming fibers recognize appropriate pathways, we transplanted the primordia of the male reproductive tissue to body segments that normally do not receive afferents from the male organ (SG7-SG15). Transplants were carried out in 9-10 day old animals. Transverse sections of the SG adjacent to the ectopic male reproductive tissue in animals 30-60 days old were stained with the mAb Lan 3-2. In 6 cases out of 22, the afferent fibers appeared to be sorted out in five fascicles, as seen in SG5 and SG6. In contrast, when homotopic patches of skin were transplanted, the afferent fibers organized themselves in four bundles as in non sex ganglia. We propose that the ganglionic neuropils in all segments express the positional cues required by afferent axons to select a particular tract, regardless of whether such cues are normally required in any particular location.

46.3

RHOMBOMERE DEVELOPMENT IN THE CHICKEN EMBRYO.
P.G. Layer, R. Alber* and S. Kaulich*. Max-Planck-Institut für Entwicklungsbiologie, D-7400 Tübingen, FRG.

In order to correlate patterns of neuronal proliferation, migration and differentiation during the development of rhombomeres (called R1 - R7 according to their rostrocaudal position) in the chick hindbrain between stages HH 9 - HH 22, we have combined cholinesterase- (AChE, BChE), peanut lectin- (PNA) and immunohistochemistry (BrdU-, HNK-1-, G4-antibodies). From the onset of rhombomere formation at HH 9-10, four preotic (R2-R5) and one postotic (R6/7) rhombomeres are discernible. Pairs of R4+R5 and R2+R3 corresponding to the acusticofacial and the trigeminal complexes respectively, differentiate almost concomitantly with a delay in R5 and R3. A segregation of R1 from the 2nd rhombomere is indicated after HH 13/14 by a weak PNA-border. Then the postotic rhombomere (Glossopharyngeal-Vagus-Complex) is further subdivided into R6 and R7; R6 is delayed. We failed to locate the caudal limit of an 8th rhombomere. We conclude, that in addition to a segmental pair rule (see Lumsden, A. & Keynes,R., Nature 337, 424-428, 1989), superimposed mechanisms at both ends of the hindbrain (R1, R7/8) and at the otic placode have to be postulated.

46.4

CUES USED BY MUSCLES FOR THEIR POSITIONING IN CAENORHABDITIS ELEGANS.
C. Li[1,2] and M. Chalfie[2]. [1]Dept. of Biology, Boston Univ., Boston, MA; [2]Dept. of Biol. Sci., Columbia Univ., New York, NY.

The positioning of the vulval muscles in the egg-laying system of the nematode Caenorhabditis elegans appears to be dependent on cues from the somatic gonad and vulva. Both the position of the vulval muscles and their orientation around the vulva can be visualized with an anti-myosin antibody (kindly provided by Dave Miller).

Ablation of the somatic gonad precursors (Z1 and Z4) in wild-type animals, resulting in the loss of the somatic gonad and vulva, leads to the appearance of vulval-like muscles that are randomly distributed and oriented along the length of the animal. When only the vulva is absent, as in the Vulvaless mutants *n300*, *lin-2*, and *lin-10*, vulval-like muscles develop in their correct positions, but the muscles are randomly oriented. Thus, the muscles can be positioned with cues from the somatic gonad alone, but they are oriented incorrectly.

The presence of extra vulval cells in the Multivulva mutant *lin-15* has little or no effect on either the position or orientation of the vulval muscles. In *lin-15* animals in which Z1 and Z4 are ablated, however, vulval-like muscles in their correct orientation are visible at the vulva and extra vulval cells. No vulval-like muscles are detected elsewhere. These results suggest that the vulval cells also provide cues for positioning and orienting the vulval muscles.

The VC and HSN neurons innervate the vulval muscles. In lin-*15* mutants in which Z1 and Z4 are ablated, vulval-like muscles appear in positions independent of VC and HSN processes. The neurons, therefore, do not appear to provide cues to the muscles for positioning.

46.5

MAINTENANCE OF STRIATAL COMPARTMENTS IN THE REELER MOUSE SUGGESTS PATTERN FORMATION INDEPENDENT OF RADIAL GLIAL GUIDANCE.
J.G. Johnston, L.A. Krushel and D. van der Kooy, Neurobiology Research Group, Department of Anatomy, University of Toronto, Toronto, Ontario, Canada, M5S 1A8.

Radial glial fibers appear to be critical in the guidance of young postmitotic neurons to their final destinations in the cortex. However, it is not clear whether radial glial cells play a similar role in the development of the other major structure of the telencephalon, the striatum. The striatum is a compartmentalized structure which can be subdivided into the patches and surrounding matrix, on the bases of distributions of neurotransmitters, receptors and connections. Several studies have suggested that similar developmental mechanisms (commitment to birthdate, adhesion) underly pattern formation in the striatum and cortex. To test if radial glial cells are necessary for the compartmentalization of the striatum, we have studied the development of the patch compartment in the mutant reeler mouse(*rel+/rel+*). The reeler's deficit appears to be expressed primarily in radial glial cells. Migrating neurons, which normally detach from radial glial fibers, fail to do so, resulting in the reversal of cortical lamina. We have shown that opiate receptor patches in the reeler appear normal in number, size and distribution in comparison to unaffected littermates (*rel-/rel-*; *rel+/rel-*). These results suggest that in the absence of a glial guidance mechanism, that is critical to cortical lamina organization, other developmental mechanisms (such as selective adhesion of early born patch cells or the afferent connections of patch cells) are sufficient to constrain the formation of striatal compartments.

46.6

THE LEECH HOMEOBOX GENE LOX2 IS EXPRESSED EMBRYONICALLY IN POSTERIOR SEGMENTAL GANGLIA OF *HIRUDO MEDICINALIS*.
J. W. Wysocka-Diller* and E. R. Macagno. Dep. of Biological Sciences, Columbia University New York, NY 10027, USA

Homeobox genes, first characterized in *Drosophila*, have been shown to be expressed in the developing nervous systems of many species. We have isolated several homeobox genes of the leech *Hirudo medicinalis* on the basis of sequence homology to fly *Antp*-type homeobox genes and determined that at least two of them are expressed in the developing leech CNS in temporally and spatialy restricted domains. *In situ* hybridization studies of wholemounted, 5-14 day embryos with probes generated from one of these, Lox2, first show detectable expression of this gene in 7 day embryos, in the ganglionic primordia of body segments 7 to 12. By the 8th day of development, the signal has extended posteriorly to the 15th body ganglion and become detectable on two small bilateral clusters of cells near the posterior margin of the 6th body ganglion. By the 11th day, all posterior ganglia, including the fused tail ganglion, show expression of Lox2. At later stages there is a decrease in the level of the signal seen over the ganglia in segments posterior to the 14th. These observations indicate that the Lox2 domain of expression has an invariable anterior boundary and a dynamic posterior boundary during the developmental stages examined. Moreover, expression appears to precede neuronal differentiation. Since homologues of many if not most of the neurons in segmental ganglia that show expression of Lox2 are also present in ganglia which do not, our observations suggest that neurons with the same function may have position-dependent levels of expression of Lox2. Thus, it would seem that Lox2 by itself cannot specify cell type, but it might have such a function in conjunction with other genes that are expressed in a spatially distinct manner.

46.7

EXPRESSION OF COMPARTMENTATION ANTIGENS IN RAT CEREBELLAR TRANSPLANTS

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Zebrin I (the mabQ113 antigen) is expressed in rat by a subset of Purkinje cells that form 14 parasagittal bands that correspond to divisions in the olivocerebellar projection. The OCP compartments are present prior to the expression of the mature zebrin I phenotype, suggesting that afferent input may regulate the zebrin I phenotype of the target. Lesion studies revealed no role for afferent inputs in the regulation of zebrin I expression postnatally, but a prenatal role remained open. To explore this possibility, cerebellar anlagen were dissected from rat embryos (E14) and transplanted ectopically into adult hosts, either into the anterior chamber of the eye or into cavities prepared in the neocortex. Grafts were allowed to mature and then immunostained. Zebrin I was expressed by grafted Purkinje cells *in cortico* and *in oculo*. Double-labelling experiments using anti-zebrin and the anti-calbidin confirm that both the zebrin I^+ and the zebrin I^- phenotypes are present. It therefore seems probable that afferent input does not determine the PC zebrin I phenotype.

46.8

COMPARATIVE MORPHOLOGY OF +/+ PURKINJE CELL DENDRITES IN STAGGERER, LURCHER AND WILD TYPE CHIMERIC MICE.

J. M. Soha and K. Herrup. E. K. Shriver Center, Waltham, MA 02254.

Previous studies from this lab described aberrant and frequently atrophic dendritic morphologies among the wild type Purkinje cells (PCs) remaining in the cerebella of lurcher↔wild type chimeric mice after the loss of all lurcher PCs. A working hypothesis to account for these morphological irregularities and the enhanced survival of granule cells in these mice is that wild type PCs are deafferented, and consequently provide additional trophic support to granule cells during the postnatal episode of cell death. The hypothesis predicts normal morphology for wild type PCs in staggerer↔wild type chimeras.

To test this prediction by comparison of a statistically significant number of PCs, a subjective scoring scheme was employed. Golgi processed PCs were assessed in relation to a series of 10 morphological criteria (e.g. size, complexity, orientation). PCs were visually assigned scores of 1 (aberrant) to 5 (ideal) for each property. As reported previously, wild type PCs in lurcher chimeras differed significantly from PCs in the wild type chimera for several of the scored properties, consistent with their atrophic, deafferented appearance. In contrast, wild type PCs in a staggerer chimera scored similarly to PCs in the normal chimera, while differing significantly from lurcher chimera PCs for this set of properties. PCs in staggerer and wild type chimeras differed significantly for a second set of properties, apparently due to a more erratic orientation in staggerer chimeras. Golgi impregnated PCs displaying the staggerer phenotype were also identified in the staggerer chimera. Supported by NIH grants NS20591 and HD07251.

46.9

CEREBELLAR PHENOTYPE OF TWO ALLELES OF THE REELER MUTATION ON SIMILAR BACKGROUNDS.

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Reeler mutant mice exist as two alleles (Ed and Orl) on different backgrounds (C57 and BALB/c). Orl *reeler* mice on BALB/c are less affected and survive better than Ed mutants on C57 background. Their cerebellum has better developed folia (such as a well-defined fissura secunda) and a thicker molecular layer. To assess whether these differences are allelic or due to background, congenic strains have been produced by successive backcross, allowing the comparison of the two alleles on similar backgrounds.

The phenotype of mutant animals was progressively modified during backcrosses, in parallel to changing background. From F10, mice on similar backgrounds are indistinguishable. That is, Ed and Orl *reeler* mice survive well (approx. 50%) on BALB/c, but not on C57 background. The cerebellar phenotype also changes with modification of the background: in C57 *reeler* mice (both Ed and Orl), the cerebellum is almost smooth and the molecular layer thin, while the BALB/c *reeler* cerebellum has a well-defined fissura secunda and a thicker molecular layer.

These observations show that phenotypic differences between the Ed and Orl *reeler* mutants are due to differences in genetic background.

46.10

PATTERNS OF CEREBELLAR FOLIATION IN RECOMBINANT INBRED MICE.

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Foliation of the cerebellum was quantified by measuring depth of fissures at the mid-sagittal plane for the inbred strains BALB/cByJ(B), C57BL/6ByJ(C), and their recombinant inbred strains (D, E, G, H, I, J, K). Six extra fissures were seen in various mice: intracentral(1), intraculmen A and B(2A, 2B), intradeclive(3), intrauvular A and B (4A, 4B). In a sample of 186 mice, the strains with a larger cerebellum showed more fissures and proportionately greater depth of the extra fissures ($r = 0.63$), but size alone could not entirely account for the qualitatively different patterns of fissures for different strains. Strain B had frequent 3 and deep 4B; C had shallow 4B and few others; D usually had no extra fissures; E showed no 1 but always had 4B; G had deep 1; H had deep 2A; I resembled B; J had deep 2B; and K had deep 4A. Because only two of the nine strains resembled each other in foliation pattern (B and I) and each of the other seven was unique, the genetic difference between B and C must involve several loci. Within a strain there were noteworthy qualitative differences among individual mice.

Supported in part by grant 4878 from the Natural Sciences and Engineering Research Council of Canada.

SOMATIC AND VISCERAL AFFERENTS I

47.1

TOPOGRAPHY OF IDENTIFIED CAT DRG AFFERENTS.

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Topographical organization (TO) within the cat DRG was studied using orthograde tracers, somal reconstruction, intracellular recording, and physiological identification of peripheral receptors supplied. Some statistically significant TO was found. For example, in S1, somata supplying sural nerve are widely distributed, yet concentrated in the rostral pole. Further, there is clustered organization of somata supplying at least some muscle nerves. Finally, anecdotal observations of likelihood to next record intracellularly from a physiologically similar cell were examined statistically. In summary, while there exists some degree of topographical organization within the DRG, it is substantially less apparent and demonstrable than elsewhere. My interpretation is that extant topography is likely the consequence of system development. Particularly, large afferent populations supplying single peripheral targets are likely derived from similarly located neural crest cells; and, further, since proliferation continues after migration is complete (Honig 1982 JP) and afferents supplying similar receptors have similar somal membrane properties (Rose 1986 diss.), post-migratory clones of afferents might supply similar peripheral receptors within a given peripheral target.

Supported by NIMH MH08323 and the Hunkele Foundation (RDR) and NIH NS14899 & NS 16996 (to L. Mendell).

47.2

SELECTIVE DECREASE OF SMALL SENSORY NEURONS LABELED WITH HRP AFTER LASER IRRADIATION OF THE RAT TIBIAL NERVE.

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Recent electrophysiological evidence indicates that Q-switched Nd:YAG laser irradiation might have selective effects on neural impulse propagation in small slow conducting sensory nerve fibers as compared to large diameter afferents (Wesselmann et al., Soc. Neurosci. Abstr. 14:698, 1988). In an attempt to clarify the ultimate fate of sensory neurons after laser application to their peripheral axons, we have used HRP to retrogradely label dorsal root ganglion (DRG) cells. Q-switched Nd:YAG laser light was applied to the tibial nerve at pulse energies of 70 or 80 mJ/pulse for 5 min in seven experimental rats. Seven days later HRP was applied to the left (laser-treated) and right (untreated) tibial nerve 5 mm proximal to the site of laser application, as previously described for injured axons (Wesselmann et al., Neurosci. 13:1299, 1984). In control animals (n=3) the numbers of labeled DRG cells were not significantly different between sides (left: 3699±897, right: 3683±892)*. In contrast, after previous laser irradiation, labeling was always less on the laser-treated side as compared to the untreated side (left: 2183±513, right: 3937±225)*. The mean decrease (-47%±12 of contralateral labeling)* was significantly different from the differences in labeling between sides in the control group ($P<0.03$). Analysis of the dimensions of labeled cells suggested, that the reduction of labeled cells on the laser-treated side was mainly due to a deficit in small sensory neurons. The reduced labeling of small sensory cells after laser irradiation might be due to 1) impaired uptake, 2) altered axonal transport, or 3) increased rate of HRP breakdown. Since the conduction velocity of afferent fibers in rats is related to the size of their somata (Harper et al., J. Physiol., 359:31, 1985), our histological data imply that laser light selectively affects slow conducting fibers. This is in agreement with our previous electrophysiological observation. The functional characteristics of these slow conducting sensory fibers impaired by laser irradiation remain to be examined electrophysiologically. (Supported by the Medical FEL Program ONR/SDIO N00014-86-K-0188, and VA Merit Review to W. Z. R.) *: mean±SEM

47.3

ANALYSIS OF MU, DELTA, AND KAPPA OPIOID BINDING SITES IN RAT SPINAL CORD IN AN EXPERIMENTAL MODEL OF PERIPHERAL NEUROPATHY. C.W. Stevens, K. C. Kajander, G. J. Bennett and V. S. Seybold Department of Cell Biology and Neuroanatomy, University of Minnesota, Minneapolis, MN 55455 and NAB, NIDR, NIH, Bethesda, MD 20892.

An experimental model for peripheral neuropathy induced by loose ligation of the sciatic nerve has been developed in rats (Bennett and Xie, *Pain 33*, 1988). These rats exhibit hyperalgesia in response to chemical and thermal stimuli, and other behaviors consistent with neuropathy in the affected hindlimb. Thus, this model in rats may be useful to investigate neuropathic pain disorders in humans. The present study examined changes in mu, delta, and kappa opioid receptor binding in discrete areas of the rat spinal cord associated with primary afferent neurons putatively involved in this peripheral neuropathy.

Spinal segment L4 was obtained from control rats (N=4) and 2, 5 and 10 days after nerve ligation (N=8 rats/group). Autoradiographic studies of opioid binding sites were performed on spinal cord sections using tritiated sufentanil (0.3 nM), DPDPE (5 nM), and U-69593 (3 nM) to label mu, delta, and kappa sites, respectively. Data were analysed by computerized densitometry. Specific binding was defined as total binding minus the non-specific binding obtained in the presence of cold ligands. A significant *decrease* in the density of mu sites occurred in lamina X on the ligated side compared to the nonligated side at 10 days postsurgery. Delta sites *increased* in laminae I-II on the ligated side on day 2, however significantly *decreased* in laminae I-II on day 10. Changes in kappa opioid binding were difficult to assess due to overall low binding. These differences may represent alterations of opioid receptors in conjunction with changes previously shown for endogenous opioid peptides in this model. Supported by USPHS grants DA05309 and NS17702.

47.4

ANALYSIS OF ^{125}I-SUBSTANCE P BINDING SITES IN RAT SPINAL CORD IN AN EXPERIMENTAL MODEL OF PERIPHERAL NEUROPATHY L. M. Aanonsen, K. C. Kajander, G. J. Bennett and V. S. Seybold
Department of Cell Biology & Neuroanatomy, University of Minnesota, Minneapolis, MN 55455; NAB, NIDR, NIH, Bethesda, MD 20892

An experimental model for a peripheral neuropathy induced by tying loose ligatures around the sciatic nerve has been characterized in rats (Bennett and Xie,1988). These rats exhibit hyperalgesia in response to chemical and thermal stimuli. Thus, this peripheral neuropathy in rats may represent a model of neuropathic pain disorders in humans. In addition to behavioral changes, immunohistochemical studies showed a significant depletion of substance P (SP) in laminae I/II and V on the nerve ligated side of the spinal cord of rats, 20 days after ligation (Bennett et al., 1988). The present study examined changes in SP receptor binding in laminae I/II, V and X, regions of termination of primary afferent neurons putatively involved in this peripheral neuropathy.

Spinal segment L4 was obtained from rats 2, 5 and 10 days after nerve ligation (n=8 rats/group) and from control rats (n=4). Autoradiographic studies were performed on spinal cord sections using 50nM ^{125}I-SP. Nonspecific binding was<5% in laminae I/II and X. Significant *increases* in grain densities were observed on the side ipsilateral to the ligation compared to the nonligated side at 5 and 10 days postsurgery. In a related study, ^{125}I-SP grain densities in laminae I/II were also increased eight days after dorsal rhizotomy in rats. The increase in ^{125}I-SP grain densities after dorsal rhizotomy may be indicative of an upregulation of SP receptors due to the absence of SP release from primary afferent terminals. Thus, the increase in ^{125}I-SP grain densities in laminae I/II in this model of peripheral neuropathy may also be indicative of an upregulation of SP receptors. An upregulation of SP receptors is consistent with the decrease in SP immunoreactivity in laminae I/II previously reported in this model. Supported by USPHS grants DA05309 and NS17702.

47.5

ANALYSIS OF ^{125}I-CGRP BINDING SITES IN RAT SPINAL CORD IN AN EXPERIMENTAL MODEL OF PERIPHERAL NEUROPATHY. M.G. Garry, K.C. Kajander, G.J. Bennett, and V.S. Seybold. Dept. of Cell Biol. and Neuroanatomy, Graduate Program in Neuroscience, Univ. of Minnesota, Minneapolis, MN 55455; NAB, NIDR, NIH, Bethesda, MD 20892.

In an experimental model of peripheral neuropathy, calcitonin gene-related peptide immunoreactivity (CGRP-IR) is significantly depleted in the nerve-damaged side of the dorsal horn of the spinal cord (Bennett et al., 89). Following injury, decreases were observed at 10 and 20 days by RIA, and in laminae I-V at 20 days by immunohistochemical techniques (Bennett et al., '89). The goal of our study was to examine ^{125}I-CGRP binding sites in the dorsal horn of this model to determine whether CGRP binding sites were modified in conjunction with changes in CGRP-IR. In each animal (male rats), loosely constrictive ligatures were placed around the left sciatic nerve. The right hindlimb underwent sham surgery. Control animals received left hindlimb sham surgery only (n=4). L4 spinal cord was examined at 2, 5, and 10 days following ligation (n=8/group). Autoradiography was performed using 0.1nM ^{125}I-CGRP; nonspecific binding (NSB) was determined on adjacent sections by the addition of 100nM unlabeled CGRP with the labeled ligand. The densities of autoradiographic grains over laminae I/II, V, and X were examined using a computerized densitometry system. Specific binding (SB) was determined by subtracting the NSB value from the total binding value within each region of interest in each spinal cord. No changes in SB were observed in laminae I/II, V, or X at any time point examined. These data suggest that changes in CGRP binding sites do not occur in parallel with changes in CGRP-IR as determined by RIA. Changes in binding sites at a later time point (20 day post-ligation), however, remain to be addressed. This work was supported by USPHS grants NS 17702 and T32 DA 07234.

47.6

U-TURN AFFERENT FIBERS IN THE RAT VENTRAL ROOT. K.J. Kim, E.J. Baik-Han* and J.M. Chung. Marine Biomed. Inst. and Depts. of Anat. & Neurosci. and of Physiol. & Biophys., Univ. Texas Med. Br., Galveston, TX 77550.

Neurophysiological evidence for the existence of looping afferent fibers was obtained in the rat ventral root.

Experiments were conducted on 26 adult Sprague-Dawley rats anesthetized with an intraperitoneal injection of sodium pentobarbital. A laminectomy was performed at the lumbosacral level and ventral roots (L4-L6) were cut close to the spinal cord. The distal stump of the cut ventral root was placed on an electrode assembly which consisted of pairs of proximal and distal bipoloar recording electrodes. Action potentials from spontaneously active units were recorded simultaneously from these two pairs of electrodes.

In 6 ventral roots, single unit activity appeared as pairs of spikes with opposite polarity in both the distal and proximal recording electrodes. The timinig and polarity of the spikes can only be logically explained by supposing that they are simultaneous recordings from two pairs of electrodes of two action potentials traveling opposite directions in a single fiber. This explanation was reinforced by recording the activity after sectioning various parts of the ventral root.

These data suggest that there are spontaneously active ventral root fibers that enter the root and then loop back out toward the dorsal root ganglion. (Supported by NIH NS 21266, NS 11255 and RCDA NS 00995)

47.7

SPROUTING VENTRAL ROOT AFFERENT FIBERS PRODUCE SPONTANEOUS ACTIVITY. E.J. Baik-Han*, K.J. Kim and J.M. Chung. Marine Biomed. Inst. and Depts. Anat. & Neurosci. and Physiol. & Biophys., Univ. Texas Med. Br., Galveston, TX 77550.

Regenerating afferent nerve fibers following injury may generate spontaneous activity. We examined the possibility of producing spontaneous activity from the sprouting ventral root afferent fibers, using a paradigm which is known to make ventral root afferents sprout; neonatal peripheral nerve injury.

Experiments were done on 24 anesthetized adult Sprague-Dawley rats which were subjected to a sciatic nerve section at the neonatal stage. Ventral roots (L4-L6) were cut close to the spinal cord and the distal stump was placed on two pairs of proximal and distal recording electrodes. Action potentials from spontaneously active units were recorded simultaneously from these two pairs of electrodes to detect the direction of their propagation.

The activity which propagates proximally is commonly seen and likely originates from the periphery. However, there were other activities which propagate in the distal direction. We interpreted this activity is generated from a site in the ventral root proximal to the recording electrodes. Since the root has been cut near the cord, it is likely that the impulse generating site is the sprouting ending of afferent fibers in the ventral root. This possibility was reinforced by results obtained when the root was sectioned at different levels. (Supported by NIH NS21266, NS11255 and RCDA NS00995)

47.8

EXPRESSION OF GABA-IMMUNOREACTIVITY BY SUBPOPULATIONS OF SENSORY NEURONS IN THE CHICK DORSAL ROOT GANGLIA. G. ROY* and E. PHILIPPE* (Spon: M. Filion), Dept. of Anatomy, Laval University and Center of Neurobiology, 2075 Vitré, Quebec - Canada.

In order to determine the central and peripheral projections of sensory neurons in the chick dorsal root ganglia (DRG), characterization of phenotypes expressed by these neurons was defined by a combination of immunocytochemical and ultrastructural criteria.

Immunocytochemistry was performed with polyclonal antibodies raised against gamma-aminobutyric acid (GABA) according to the peroxydase anti peroxydase procedure (PAP).

Among the whole population of sensory neurons located in the lumbosacral DRG, a few large A and occasionally small B neuronal cell bodies exhibited a GABA-immunoreactivity in their cytoplasm. These immunostained cell bodies could be related to the A2 and B2 subpopulations of chick sensory neurons according to the ultrastructural classification of Philippe and Droz (Neuroscience, 26, 215-224, 1988).

Since defined subclasses of DRG neurons innervate defined tissues, it is assumed that the phenotypic expression reflects in part influences exerted by the central and peripheral target tissues. (Supported by grant of C.R.M.).

47.9

HISTOCHEMICAL AND IMMUNOHISTOCHEMICAL ANALYSIS OF THE RELATIONSHIPS BETWEEN CYTOCHROME OXIDASE (CO), CARBONIC ANHYDRASE (CA), PARVALBUMIN (PV), CALBINDIN D28K (CaBP), CALCITONIN GENE-RELATED PEPTIDE (CGRP) AND FLUORIDE-RESISTANT ACID PHOSPHATASE (FRAP) IN RAT DORSAL ROOT GANGLIA (DRG). P.A. Carr[1], T. Yamamoto[1], K.G. Baimbridge[2] and J.I. Nagy[1] [1]Dept. of Physiol., Univ. of Manitoba, Winnipeg, Man., Canada R3E 0W3 and [2]Dept. of Physiol., Univ. of British Columbia, Vancouver, B.C., Canada V6T 1W5

Quantitative histochemistry for CO activity in neurons of rat lumbar DRG showed a full range of CO staining densities in each size class with many more large type A cells intensely stained than small type B cells. Immunohistochemistry for CO gave similar staining patterns. Histochemically, all neurons with dense CO staining were CA positive. The majority (85%) of cells containing dense CO were also immunoreactive (IR) for PV and CaBP. Dense CO product was found in 90% of PV- and CaBP-IR cells. Staining for CA was displayed by 87% of PV-IR and 76% of CaBP-IR cells. Among all lumbar DRG cells 22% were CaBP-IR while 14% were PV-IR. Greater than 99% of PV-IR cells also contained CaBP. Less than 1% of PV-IR cells and 9% of CaBP-IR cells were immunopositive for CGRP. Thirty percent of all CGRP-IR cells (about 44% of type B CGRP-IR cells) displayed FRAP staining while 50% of FRAP-positive cells were CGRP-IR. These observations suggest that a subpopulation of primary afferent neurons contains high levels of CO, CA, PV and CaBP and that CGRP coexists with FRAP.

47.10

CHOLECYSTOKININ MESSENGER RNA IS PRESENT IN GUINEA PIG BUT NOT RAT DORSAL ROOT GANGLIA (DRG). K.B. Seroogy, N.K. Mohapatra*, M. Rethélyi, D.S. McGehee, P.K. Lund* and E.R. Perl. Dept. of Physiology, University of North Carolina, Chapel Hill, NC 27599 and Second Department of Anatomy, Semmelweis University Medical School, Budapest, Hungary.

The controversy about the immunochemical detection of the neuropeptide cholecystokinin (CCK) in rat primary sensory neurons led us to examine the expression of CCK messenger RNA (mRNA) in rat and guinea pig DRG using *in situ* hybridization and Northern blot techniques. Two different synthetic oligodeoxyribonucleotides (oligomers) complementary to different regions of rat CCK mRNA were 5' end-labeled with ^{32}P and used as probes on fresh tissue sections. After overnight hybridization and washing, the sections were processed for emulsion autoradiography. In guinea pig, CCK mRNA was detected in small and medium sized neurons comprising 10-15% of the total DRG cell population. In contrast, in neurons of rat DRG, CCK mRNA was not detectable. Northern blot analyses revealed a single CCK mRNA species of expected size (0.8kb) in guinea pig DRG but not in rat DRG. A 0.8kb CCK mRNA was detected in spinal cord and cortex of both rat and guinea pig. These results suggest that CCK is normally not synthesized in DRG of rat and that there are species differences in CCK gene expression in mammalian sensory ganglia. (Supported by grants NS 23804 and NS 08525 from NINCDS)

47.11

LOCALIZATION OF SUBSTANCE P AND SOMATOSTATIN MESSENGER RNAs IN DORSAL ROOT GANGLIA (DRG) OF SIX MAMMALIAN SPECIES. N.K. Mohapatra*, K.B. Seroogy and P.K. Lund*. (SPON: S.P. Schneider). Dept. of Physiology, University of North Carolina, Chapel Hill, NC 27599.

The cellular localization of messenger RNAs (mRNAs) encoding the neuropeptides substance P and somatostatin (SOM) was examined in DRG of mouse, rat, hamster, guinea pig, rabbit and cat using *in situ* hybridization. Synthetic oligodeoxyribonucleotides labeled at the 5' end with ^{32}P were used as probes. Sequences were complementary to regions of substance P and SOM mRNAs that are best conserved across mammalian species. Probe specificity was verified based on hybridization with rat and guinea pig substance P and SOM mRNAs on Northern blots. *In situ* hybridizations were performed overnight on fresh tissue sections followed by autoradiographic processing. In each of the six species analysed, substance P mRNA was detected primarily in small neurons comprising 10-20% of the total DRG cell population. These finding indicate a similar pattern of substance P mRNA expression across DRG of different mammalian species. SOM mRNA was detected in similar numbers of DRG neurons in mouse, rat, hamster and rabbit. In cat and guinea pig, SOM mRNA was found in distinctly fewer numbers of DRG perikarya. These findings suggest that, in contrast to substance P, SOM mRNA expression in DRG neurons is more variable and species-specific. (Supported by grants NS 23804 and NS 08525)

47.12

IDENTIFICATION OF DYNORPHIN-CONTAINING SACRAL DORSAL ROOT GANGLION CELLS IN THE CAT USING *IN SITU* HYBRIDIZATION. S. Erdman*, J. Keast and W.C. de Groat. (SPON: B. Boston). Depts. of Pharmacology and Behav. Neuroscience, Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA, 15261.

Previous immunohistochemical (IHC) studies have provided evidence that some afferent projections to the sacral spinal cord of the cat contain dynorphin (DYN), however, dynorphin cell bodies in the dorsal root ganglia (DRG) could not be identified using IHC methods. In the present studies in situ hybridization techniques were used in an attempt to identify mRNA coding for prodynorphin in these DRG's. Additional sections were processed for localization of somatostatin (SOM) mRNA, a substance which is known to be present in some primary afferent neurons. Cryostat sections (14-28 µm) of snap-frozen ganglia were fixed in paraformaldehyde solution, washed and incubated with hybridization buffer containing ^{35}S-labelled synthetic oligonucleotide probes for SOM or proDYN mRNA (Dupont NEN). After washing off unbound probe on the following day, sections were processed for emulsion autoradiography, developed 9-30 days later and counterstained. Neurons with SOM or proDYN mRNA were identified in all sections, the former being more common. This suggests that there are primary afferent neurons that synthesize DYN, but store it in amounts too small to be detected by immunohistochemical methods. (J. Keast is supported by an NH & MRC C.J. Martin Fellowship).

47.13

IDENTIFICATION OF NEUROPEPTIDES IN UTERINE AFFERENT NEURONS IN LUMBOSACRAL DORSAL ROOT GANGLIA OF THE CAT. M. Kawatani and W.C. de Groat. Dept. Physiol., Showa Univ., Tokyo, and Depts. Pharmacol. and Behav. Neurosci., Univ. Pittsburgh, Pittsburgh, PA 15261.

Vasoactive intestinal polypeptide (VIP), leucine enkephalin (LENK), substance P (SP), calcitonin gene-related peptide (CGRP) and cholecystokinin (CCK) have been identified in lumbosacral dorsal root ganglia (DRG). We showed previously that VIP is present in a high percentage of pelvic nerve (PLN) (42%) and hypogastric nerve (HGN) (45%) afferent neurons (AN) but in a lower percentage of pudendal nerve (PUDN) afferents (10%). Approximately equal proportions of these afferents contained LENK (30%) and SP (24%). In the present experiments, afferent pathways from the uterine cervix which are contained in PLN, HGN and PUDN were studied with axonal tracing and immunocytochemical techniques to examine their content of neuropeptides. The uterine cervix afferent neurons (UCAN) were small to medium size (<40 µm) and were located primarily (>90%) in the S1-S3 DRG. Most significantly, 60-82% of these neurons contained VIP. CGRP was present in 35-53% of the cells. SP, CCK and LENK were present in 20-32% of UCAN which is similar to their distribution in PLN afferents. Somatostatin was present in the DRG but not in UCAN. In summary, several types of neuropeptides are present in the UCAN. VIP and CGRP are most prominent and may play a role in afferent transmission from this region of the reproductive tract.

47.14

HORMONAL VARIATION IN SENSITIVITY OF AFFERENT FIBERS SUPPLYING REPRODUCTIVE ORGANS IN THE FEMALE RAT. A. Robbins[1,2], Y. Sato[1], and K.J. Berkley[1,3]. Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan[1]; Rockefeller University, NY, NY 10021[2]; Florida State Univ., Tallahassee, FL 32306[3].

The hypogastric and pelvic nerves contain sensory fibers that innervate the uterus and vagina. Recording simultaneously from both nerves, this study examined the responses of these afferent fibers to mechanical stimulation (distension of the uterus and vagina) across days of the estrous cycle. As determined by response thresholds, hypogastric nerve fibers were most sensitive to uterine distension on the day of estrus, and least sensitive on diestrus. Pelvic nerve fibers were most sensitive to vaginal distension on the day of proestrus and least sensitive on diestrus. These hormonal variations in sensitivity indicate that both uterine and vaginal afferent fibers are more responsive to distension during the days of the cycle associated with ovulation and mating.

Supported by grants from the Japanese government and grant RO1 NS11892 from NIH.

47.15

COEXISTENCE OF PUTATIVE NEUROTRANSMITTERS IN VISCERAL AFFERENT NEURONS OF THE NODOSE AND PETROSAL GANGLIA. A.J. Niederer* and C.J. Helke. Dept. of Pharmacol., Uniformed Services Univ., Bethesda, MD 20814.

Visceral afferent neurons of the nodose (NG) and petrosal (PG) ganglia are immunoreactive (ir) for many neurotransmitters [e.g. substance P (SP), neurokinin A (NKA; substance K), calcitonin gene-related peptide (CGRP), and dopamine (tyrosine hydroxylase-ir; TH)]. Coexistence of SP-ir with CGRP-, NKA- or TH-ir was studied in individual neurons of the rat using fluorescence immunocytochemistry. SP- and CGRP-ir were found to be similarly distributed in scattered cells concentrated mostly in the rostral pole of the NG and in numerous neurons throughout the PG. SP-ir completely coexisted with CGRP-ir. However, there was at least twice the number of CGRP-ir neurons as SP-ir neurons. SP- and NKA-ir were present in equal numbers of cells and were consistently colocalized. In contrast, SP- and TH-ir had different distributions in both the NG and the PG. SP-ir was located in the more rostal regions of both the NG and the PG whereas TH-ir was detected throughout the entire NG and only in the most caudal region of the PG. There was no colocalization of SP- and TH-ir.

The differential localization and coexistence of peptides in visceral sensory neurons in the NG and PG provide evidence for a "chemotopic" organization of these ganglia. (NIH NS20991)

47.16

CONDUCTION VELOCITY AND SEGMENTAL DISTRIBUTION OF BLADDER AND RECTAL AFFERENT FIBERS IN THE FEMALE RAT. P.L. Vera and I. Nadelhaft. VA Med. Ctr. and Depts. of Pharmacology and Neurosurgery and Center for Neuroscience, Univ. of Pittsburgh, Pittsburgh, PA.

The conduction velocity of individual afferent fibers innervating the bladder or the rectum was examined electrophysiologically by recording from post-ganglionic bladder nerves or rectal nerves arising out of the major pelvic ganglion, while stimulating the distal cut end of the ipsilateral L6 and S1 dorsal roots. The bladder nerves responded to distension of the bladder whereas the rectal nerves responded to distension of the colon. The distributions of conduction velocities were similar for bladder (0.5-21 m/s; n=243) and rectal (0.5-23.5 m/s; n=135) afferents. 64% of the bladder afferents and 58% of the rectal afferents had conduction velocities < 2 m/s and were considered to be unmyelinated. The segmental distributions, however, were quite different. 84% of the bladder afferents traveled in the L6 dorsal root and 16% in the S1 dorsal root, compared to only 4% in L6 and 96% in S1 for the rectal afferents.

47.17

AFFERENT FIBERS IN THE HYPOGASTRIC NERVE OF THE FEMALE RAT: CONDUCTION VELOCITY AND SEGMENTAL DISTRIBUTION. I. Nadelhaft and P.L. Vera. VA Med. Ctr. and Depts. of Pharmacology and Neurosurgery and Center for Neuroscience, Univ. of Pittsburgh, Pittsburgh, PA.

Rat hypogastric nerve afferents innervate the pelvic viscera; their cell bodies are located in T13-L3 dorsal root ganglia [Nadelhaft and McKenna, JCN 256:308 (1987)]. We have measured the conduction velocities of individual fibers by stimulating dorsal root fascicles and recording from the hypogastric nerve. The nerve responded to pinching of visceral structures; e.g., cervix, ureters, bladder. Fibers were activated mainly from the L1 and L2 dorsal roots bilaterally but more units were activated from the ipsilateral roots. Of a total of 167 units, 52% were from L1, 47% were from L2 and 1% were from T13. 64% were ipsilateral and 36% were contralateral. Conduction velocities ranged from 0.15 to 4.5 meters/sec and 87% of the units were below 1.5 meters/sec suggesting that the overwhelming majority of these afferents are unmyelinated.

47.18

SPINAL CORD ACTIVITY IN ANESTHETIZED FEMALE DURING VAGINAL-CERVIX STIMULATION. M. Martínez-Gómez, R. Chirino-Vargas*, C. Beyer*, B.R. Komisaruk and P. Pacheco. Univ. Aut. de Tlaxcala, Méx.; CIRA, CINVESTAV-Tlax. Méx.; IAB, Rutgers Univ., Newark, N.J., USA; and IIB-UNAM. Méx., D.F.

In our previous studies vaginal cervix stimulation (CS) by a blunt glass probe produces analgesic effects, reflex contractions of psoas major-iliacus muscles and the blockage of the ilio-pubococcygeus reflex contractions evoked by genital stimulation. Two pathways could account for these effects, one producing indirect excitatory effects upon motoneurons and other provoking inhibitory effects. For the analysis of those probable pathways, the first step was to determine the spinal dorsal (DR) and ventral (VR) roots involved during CS. After exposition of segments S_1 to L_6 in female anesthetized rats (Urethane), the left VRs were sectioned and recorded. The proximal segment of the dorsal roots sectioned near the spinal cord, was recorded. On DRs from segments S_1 to L_3 CS evoked afferent activity without adaptation, 1"off"3responses and no afterdischarges. The quantity of this response was related to the spinal segment and stimulus intensity. CS produced afterdischarges on the sectioned dorsal root. The VRs from segments S_1 to L_4 were reflexively activated during CS. Afterdischarges and syncronous activity characterized the VR response. Thus CS, through "on" receptors, evokes on several spinal cord segments dorsal horn interneuronal activity which outlasted stimulation provoking motoneuronal afterdischarge. Primary afferent depolarization and/or postsynaptic inhibition by CS still need to be determined.

SEP and CONACyT grants (PP); and RUTGERS UNIVERSITY-CINVESTAV Exchange Program (BRK and CB).

47.19

RELATIONSHIPS OF THE PELVIC NERVE BRANCHES WITH THE COPULATORY PHENOMENAE. J. Manzo-Denes*, R.A. Lucio*, M. Martínez-Gómez, B.R. Komisaruk and P. Pacheco. Univ. Veracruzana, Méx.; CIRA Univ. Aut. Tlaxcala, Méx.; IAB Rutgers Univ., Newark, N.J., USA; and IIB-UNAM, Méx.,D.F.

The differential participation on mating behavior of the viscero-cutaneous (VB) and somato-motor (MB) pelvic nerve branches was studied. Copulatory parameters, seminal plugs, pelvic movements and muscular reflexes were analyzed in sexually experienced male rats before and after surgery from trials compound of two ejeculatory series. Bilateral transection of VB in both series increased the latency of mount (ML), intromission (IL) and ejaculation (EL) as well as the number of mounts (NM), while decreasing the number of intromission (NI). MB transection increased IL, EL and NM. The copulatory efficiency (NI/NI + NM) was significantly reduced in the VB transectioned rats. Weight of the seminal plugs was reduced after the surgeries, but more with VB transection. Also acelerometric mount recordings had less duration after nerve sectioning. In urethane anesthetized animals EMG from the ilio-pubococcygeus muscles, innervated by MB, was evoked by perineal, scrotal, preputial and groin skin pressure. Also vesical and urethral pressure produced reflex contractions. It has been assumed that pelvic nerve transection does not affect copulatory behavior, however, VB sectioning alters seminal plug and copulatory parameters which could reflect delayed erection, and MB sectioning alters the seminal plug and the quality of copulatory movement.

SEP and CONACyT grants (PP); and RUTGERS UNIVERSITY-CINVESTAV Exchange Program (BRK).

47.20

COMPARISON OF SPINAL AND BRAIN STEM PROJECTIONS OF RAT MEDIAN, ULNAR, SCIATIC AND SAPHENOUS NERVES USING EITHER INJECTIONS OF WGA-HRP OR BHRP. C.C. LaMotte, S.E. Kapadia, C.M. Kocol*., Sect. of Neurosurgery, Yale Univ. of Med., New Haven, CT. 06510.

With light and electron microscopy we verified that wheat germ agglutinin HRP (WGA-HRP labelled only unmyelinated (C) and small diameter myelinated (Aδ) afferents, and that B subunit cholera toxin HRP (BHRP) labelled all sizes of myelinated afferents, but not unmyelinated afferents. For example, in the sciatic, WGA-HRP labelled lamina I and the substantia gelatinosa, but light label also was found in the N. of Clarke,and moderate label in the N.gracilis. Using BHRP, dense label was present in laminae I, III-V, the ventral horn, N. of Clarke, and N.gracilis; no label was found in lamina II. A similar segregation occurred for the other nerves; saphenous projections were minor except to the dorsal horn. Extent and location of labelled areas are summarized for the dorsal horn (DH) and for motoneuron pools (MN):

Median	DH	C3-T2	max at C6-7, entire width; C3-6 & C8-T2, medial
	MN	C3-C8	dorsolaterally at C3-8; ventrolaterally at C6-7
Ulnar	DH	C3-T2	max at C6, from medial border to root entry
	MN	C6-C8	max dorsolaterally at C6-8; ventrolaterally at C7
Sciatic	DH	L1-S2	max at L4-5 entire width
	MN	L3 -L6	dorso & ventrolaterally at L3-6; medial at L5-6
Saph.	DH	L2-L4	max at L3-4; centromedial. (Note: No MN in saph.)

The N. of Clarke received both median and ulnar afferemts from C8 to T8 with a maximum at C8-T4. Sciatic projections to N. of Clarke were from T7 to L1, and saphenous from T12 to L1, but minor. In the dorsal column nuclei, separate but overlapping territories could be differentiated between the median and ulnar, and the sciatic and saphenous. (NS10174 & NS13335)

48.1

DOPAMINE TRANSPORTER mRNA and cDNA: *XENOPUS* OOCYTE EXPRESSION. S. Shimada, B.F. O'Hara*, T. Nishimori*, J.M. DiGiorgianni* and G.R. Uhl. NIDA, Addiction Research Center, Baltimore, MD 21224 and Depts. of Neurol. & Neurosci., Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

The *Xenopus* oocyte can express biological transporting activity after injection of mRNAs encoding several carrier molecules. We have used mRNA from NGF-pretreated PC12 cells and rat midbrain as well as transcripts from a rat midbrain cDNA library to confer pharmacologically-specific dopamine uptake activity on previously-nonexpressing Xenopus oocytes.

mRNA-injected oocytes incubated with 3H-dopamine contain 3H-dopamine and metabolites, while oocytes coincubated with cocaine or uninjected oocytes contain only radiolabeled metabolites. 3H-dopamine uptake into mRNA-injected oocytes is time, sodium, and temperature dependent, as also reported by Blakely *et al.* It is blocked by cocaine or mazindol, but not by other agents including haloperidol. It is not found after injection of mRNA from other brain regions.

A size-selected rat midbrain library was constructed in the plasmid vector, pCDM8, that contains T7 promoter sequences facilitating *in vitro* transcription of mRNA corresponding to the cDNAs (see O'Hara *et al.*, this meeting). mRNA transcribed from this library yields cocaine-blockable 3H-dopamine uptake into injected oocytes. These findings suggest that cDNA(s) encoding the dopamine uptake site are present in the library, and provide a means to moniter their purification.

48.2

XENOPUS LAEVIS OOCYTE EXPRESSION OF THE RAT AND HUMAN DOPAMINE TRANSPORTERS. M.J. Bannon, C.-H. Xue*, L.J. Dragovic* and G. Kapatos. Cent. for Cell Biology, Sinai Hospital of Detroit, the Cellular and Clinical Neurobiology Program, Wayne State University and the Wayne County Medical Examiner's Office, Detroit, MI 48235.

The actions of dopamine (DA) are terminated by DA reuptake through a specific Na+/DA cotransporter. Psychostimulants such as cocaine and amphetamine enhance synaptic DA by interfering with this transporter. Thus it is important to understand the cellular/molecular biology of the DA transporter. As an alternative to purification of this low abundance membrane protein, poly A mRNA isolated from human or rat substantia nigra was injected into isolated oocytes and several days later 3H-DA accumulation was monitored as an index of DA transporter expression. After a 60 min incubation, 3H-DA accumulation in oocytes injected with nigral mRNA was several times that seen in uninjected oocytes. 3H-DA uptake into injected oocytes was completely prevented by coincubation with 1uM unlabelled DA or by removal of Na+. The DA reuptake inhibitors cocaine (3uM) and GBR 12909 (100nM) also completely blocked specific 3H-DA accumulation. Injection of poly A mRNA isolated from human globus pallidus (an area devoid of DA cells) did not result in 3H-DA accumulation above the levels in uninjected oocytes. Further pharmacological and kinetic analyses of the expressed DA transporters are currently underway.

48.3

EFFECT OF A PHYSIOLOGICAL DOSE OF 17β-ESTRADIOL OR PROGESTERONE ON OVARIECTOMIZED RAT STRIATAL DOPAMINE UPTAKE SITES. M. Morissette* and T. Di Paolo. Dept. of Molecular Endocrinology, Laval University Medical Centre, Québec G1V 4G2 and School of Pharmacy, Laval University, Québec G1K 7P4, Canada

Striatal dopamine (DA) uptake sites labelled with [^{3}H] GBR-12935 binding were investigated in ovariectomized (OVX) rats acutely treated with 17β-estradiol (17β-E_2) or progesterone (P). One injection of P (110 µg/0.2 ml, sc) to OVX rats increases these steroid plasma levels from 15 to 120 min (peak of 11.8±1.5 ng/ml plasma, $p<0.01$ vs control) while plasma prolactin (PRL) levels remained unchanged. [^{3}H]GBR-12935 binding density and affinity remained unchanged (0.43± 0.02 nM; 4177±276 fmol/mg protein) up to 120 min after the P injection. By contrast, one injection of 17β-E_2 (100 ng/0.2 ml, sc) to OVX rats increases plasma levels of this steroid after 15 min (78.8±18.7 pg/ml plasma, $p<0.01$ vs control) while plasma PRL levels remained unchanged. The 17β-E_2 injection leaves striatal [^{3}H]GBR-12935 binding affinity unchanged (0.49±0.03 nM) while the maximum density increases at 15 and 30 min after injection (+24% after 15 min, $p<0.01$ vs control; +18% after 30 min, $p<0.05$ vs control; 3541±222 fmol/mg protein for control). Thus, acutely, 17β-E_2, but not P, modulates striatal DA uptake sites in OVX rats. The effect of 17β-E_2 appears in coincidence with the peak of this steroid plasma concentration. This increase is rapid and is probably non-genomic and suggests a causal-effect relationship although the primary site of action of 17β-E_2 is yet to be determined. Supported by the MRC of Canada.

48.4

CHARACTERIZATION OF NOREPINEPHRINE AND GABA UPTAKE MECHANISMS IN THE PC12, SH-SY5Y AND TE671 CLONAL CELL LINES. Ann Marie Anderssohn[1,2,*], Allan L. Bieber[1,*] and Ronald J. Lukas[1,2] (SPON. R.F. Spetzler). Dept. Chem., Arizona St. Univ., Tempe AZ 85287[1] and Div. Neurobiol., Barrow Neurological Institute, Phoenix AZ 85013[2].

Specific uptake of tritium-labeled norepinephrine (NE) or tritium-labeled gamma-aminobutyric acid (GABA) was examined in the PC12 rat pheochromocytoma, the SH-SY5Y human neuroblastoma, and the TE671 human medulloblastoma clonal cell lines. Reserpine-sensitive uptake of NE was observed to reach maximal values within a 10 minute period of exposure to radiolabeled transmitter for both PC12 and SH-SY5Y cells, but did not occur in TE671 cells. Total NE uptake (i.e., reserpine-sensitive and -insensitive) was linear over a 2 hour time course for SH-SY5Y cells, but was nonlinear for PC12 cells, reaching a maximal value after 30 minutes of NE exposure. Nipecotic acid (NpA)-sensitive GABA uptake was observed in all three cell lines. This specific uptake was linear over a 30 minute time course in SH-SY5Y cells (IC_{50} for NpA of 10 uM at 20°C), but reached maximal values within 10 and 30 minutes, respectively, of GABA exposure to PC12 cells (IC_{50} for NpA of 100 uM at 37°C) and TE671 cells (IC_{50} of 300 uM at 20°C). These results demonstrate the usefulness of neuron-like clonal lines for studies toward a characterization of mechanisms of neurotransmitter uptake and release and co-localization.

48.5

CONFORMATIONAL AND BIOCHEMICAL ANALYSIS OF DRUG BINDING TO THE VESICULAR AMINE TRANSPORTER

Patrick M. Kelley[S] and Edwin R. Hortelano*', [S]Dept. of Biol. Sci. and 'Dept. of Chem., Wayne State Univ., Detroit MI 48202

Biogenic amines (catechol- and indoleamines) are transported into secretory vesicles, such as the chromaffin vesicles of the adrenal medulla, by a specific transporter. Since the flexible amine molecules can exist in numerous conformers, it is not surprising that the amine binding site of the transporter differs from the amine binding sites of various biogenic amine receptors. To elucidate the transporters binding site, the rigid inhibitors, reserpine and tetrabenazine, were studied using molecular mechanics. Of particular interest is the orientation of the 5-nitrogen lone pair in reserpine and tetrabenazine. Our calculations indicate that only one significant isomer of each compound exists. We propose these isomers fits in the amine binding site of the biogenic amine transporter. This proposal is supported by the following: (a) modification of the 5-nitrogen in reserpine, by oxidation to an iminium ion or by alkylation to a quaternary ammonium, causes a total loss in physiologic activity; (b) similar molecules (such as isoreserpine) have little or no activity; and (c) our data on the inhibition of biogenic amine uptake correlates well with the minimized geometry of this nitrogen. Funded by NIH grants GM 33849 and GM 30500.

48.6

IN VIVO CHARACTERIZATION OF DOPAMINE UPTAKE INHIBITORS BY BRAIN MICRODIALYSIS. G.G. Nomikos, G. Damsma, D. Wenkstern*, H.C. Fibiger. Division of Neurological Sciences, Univ. British Columbia, Vancouver, Canada.

This study employed *in vivo* microdialysis to profile the effects of dopamine (DA) uptake inhibitors on extracellular concentrations of DA in the striatum of awake, freely moving rats. d-Amphetamine, nomifensine, methylphenidate, cocaine, benztropine, bupropion and GBR 12909 were administered via the perfusion fluid in increasing concentrations, from 1 to 1000 µM. All drugs increased extracellular DA in a dose dependent manner. Although no absolute discrimination between uptake and releasing properties of the drugs could be derived from the present study, the data suggested uptake-inhibition effects for cocaine, GBR 12909, nomifensine, benztropine, methylphenidate and bupropion, and release plus uptake-inhibition for amphetamine. At the highest concentration nomifensine, benztropine, methylphenidate and bupropion also appeared to have DA-releasing effects. The rank order of potency, as determined by the relative extracellular DA increase induced by 10 µM of the drugs after correction for *in vitro* recoveries of the drugs applied, was: GBR 12909>benztropine>amphetamine>methylphenidate>nomifensine>cocaine>bupropion. Addition of tetrodotoxin (0.3 µM) to the perfusion fluid showed that the effects of all drugs, except amphetamine, were prevented suggesting that only action of amphetamine was not dependent on action potentials. The *in vivo* characterization of changes in extracellular dopamine following local administration of DA uptake inhibitors further documents the effects of these drugs on DA transmission.

48.7

DOPAMINE TRANSPORTERS/COCAINE RECEPTORS SELECTIVELY LABELED BY A NOVEL PHOTOAFFINITY PROBE: [^{125}I]DEEP. A.A. Wilson*, D.E. Grigoriadis, R. Lew and M.J. Kuhar. (SPON: A. Goldberg). Neuroscience Branch, Addiction Research Center, and the Johns Hopkins Medical Institutions, Baltimore, MD 21224.

The dopamine transporter has been studied directly in binding experiments using a variety of radiolabeled ligands. The cocaine binding site on the transporter has been related to drug self-adminstration in monkeys suggesting that it is a cocaine receptor related to drug dependence. Several photosensitive compounds related to GBR 12909, a selective inhibitor of dopamine transport, were designed. One of these compounds, [^{125}I]-[2-(diphenylmethoxy)ethyl]-4-[2-(4-acido-3-iodo-phenyl)ethyl] piperazine ([^{125}I]DEEP) had a high affinity for the transporter and was covalently attached to its binding protein component following exposure to UV light. In rat striatal homogenates, ^{125}I-DEEP bound to a protein with an apparent molecular weight of 58,000 Da. The pharmacological properties and regional distribution of the binding was that which would be expected for binding to the dopamine transporter. The ^{125}I-DEEP binding protein appears to exists in a glycosylated form since wheat germ-agglutinin lectin adsorbed the DEEP labeled protein. Thus, as a photoaffinity ligand, ^{125}I-DEEP will be a useful tool in the characterization and purification of the dopamine transporter.

48.8

DOPAMINE TRANSPORTER IN RAT CAUDATE-PUTAMEN IS GLYCOSYLATED. R. Lew*, D.E. Grigoriadis, A.A. Wilson*, and M.J. Kuhar. Neuroscience Branch, NIDA Addiction Research Center, Baltimore, MD 21224 and Johns Hopkins Medical Institutions, Baltimore, MD 21205.

Recently, we have demonstrated in rat caudate-putamen that the photoaffinity probe [^{125}I]1-[2-(diphenylmethoxy) ethyl]-4-[2-azido-3-iodophenyl)-ethyl]piperazine ([^{125}I]DEEP) binds to a 58 kDa protein with the pharmacological properties of the dopamine transporter (D.E. Grigoriadis et al. 1989, J. Neurochem., in press). Using [^{125}I]DEEP, the present study further investigates the biochemical nature of the dopamine transporter. Membrane preparations of rat caudate were prepared and photolabelled with [^{125}I]DEEP. In lectin affinity chromatography studies, photolabelled membranes were solubilized and loaded onto WGA-sepharose columns. In enzyme studies, photolabelled membranes were treated with neuraminidase (2 U/ml) in 100 mM sodium acetate (pH 5.0) containing 100 µM PMSF for 60 min at 37°C and electrophoresed on SDS-PAGE. In WGA affinity chromatography studies, labelled proteins bound to WGA Sepharose and could be specifically eluted with 200 mM ß-N-acetyl-glucosamine. SDS-PAGE studies revealed a shift of the labelled protein by 7 kDa following neuraminidase treatment. In conclusion, the present data suggest that the dopamine transporter is a glycoprotein containing terminal sialic acid residues.

48.9

EVIDENCE FOR A NUCLEOPHILIC AMINO ACID RESIDUE AT THE STIMULANT BINDING SITE OF THE DOPAMINE TRANSPORTER. M.M. Schweri* (SPON: A.Black). Div. of Basic Medical Sciences, Mercer Univ. Sch. of Med., Macon, GA 31201-1554.

N-ethylmaleimide (NEM) was used to covalently modify stimulant binding sites contained in the P_2 fraction of striatal tissue from Sprague-Dawley rats. This compound reacts rapidly with sulfhydryl groups, but can alkylate amino groups under certain conditions. NEM was used in these studies to investigate the presence of nucleophilic amino acid residues at the site on the nigrostriatal nerve terminal where psychomotor stimulants bind to block dopamine uptake. Stimulant binding was studied using the [^{3}H]methylphenidate ([^{3}H]MP) radioreceptor assay [Schweri et al, J. Neurochem., 48:102, 1987]. Reaction with 0.1 mM rapidly inactivated the site ($t_{½}$ = 7min.). The IC_{50} was 0.33 mM under the conditions of the study. Inactivation of binding was irreversible, remaining constant after the tissue was washed three times. Reaction with 0.5 mM NEM reduced the B_{max} by 61% (5.0 vs 12.7 pmols/mg protein for controls), while slightly increasing the affinity of the remaining sites for [^{3}H]MP (K_D = 62 vs 82 nM for controls). Pretreatment of the tissue with saturating concentrations of unlabeled MP (5 µM) completely protected [^{3}H]MP binding from inactivation by NEM. These results suggest that the stimulant binding site on the dopamine transport complex contains one or more nucleophilic amino acid residues which react readily with NEM.

48.10

PERIPHERAL AND CEREBRAL METABOLISM OF 6-[^{18}F]-FLUORODOPA IN MONKEYS. W.P. Melega, J.M. Hoffman*, J.S. Schneider, C.H.K. Nissenson*, M.M. Perlmutter*, M.E. Phelps, and J.R. Barrio*. UCLA School of Medicine, Los Angeles, CA 90024.

The peripheral and cerebral metabolism of 6-[^{18}F]-fluorodopa (FDOPA) was analyzed to determine its metabolic pathways and thus validate its use as a biochemical probe of presynaptic dopaminergic function with positron emission tomography (PET). Macaca nemestrina monkeys (n=5) were pretreated with a peripheral aromatic amino acid decarboxylase inhibitor (carbidopa, 5 mg/kg, i.v.) 60 min before injection of FDOPA (5-8 mCi, i.v.). Arterial plasma samples were obtained at 5, 10, 30, 60, and 120 min and analyzed by HPLC (C-18 reverse phase column, 80% 0.1 M Na_2HP0_4, 2.6 mM OSA, 0.1 mM EDTA; 20% MeOH, pH 3.5). As FDOPA plasma levels decreased (30 min:42%, 60 min:28%, 120 min: 10%), the major peripheral metabolite remained 6-[^{18}F]fluoro-3-0-methyl-L-DOPA (3-OMFD), (30 min:45%, 60 min:62%, 120 min:70% of total radioactivity) indicative of the rapid in vivo methylation of FDOPA by peripheral catechol-0-methyl transferase in primates. The cerebral metabolism (n=3) at 60 min was determined for the putamen, caudate, cortex and cerebellum. The total radioactivity ratio for the putamen/cerebellum was 1.9. The major metabolites in the putamen were 3-OMFD (36%) and 6-[^{18}F]fluorodopamine (FDA) (35%), 3,4,-dihydroxy 6-[^{18}F]fluorophenyl acetic acid (FDOPAC) (8%), and 6-[^{18}F]fluorohomovanillic acid (FHVA) (12%); for the caudate, 3-OMFD (39%) and FDA (32%), FDOPAC (10%) and FHVA (14%). Cerebellum radioactivity was restricted to 3-OMFD (80%) and FDOPA (20%), while in the cortex 3-OMFD (84%) and FHVA (5%) were detected. The peripheral origin of 3-OMFD was indicated by its distribution (counts/g) ratios: putamen/cerebellum, 0.8; caudate/cerebellum, 0.8; and cortex/cerebellum, 0.9; the (plasma/mL)/(cerebellum counts/g) ratio was 0.8. These biochemical data when correlated with tomographic data in primates indicate that a quantitative relationship between peripheral FDOPA metabolism and cerebral FDA metabolism can be established.

48.11

EFFECT OF REPEATED AMPHETAMINE (AMPH) OR L-DOPA INFUSIONS UPON DOPAMINE (DA) RELEASE FROM SUPERFUSED CORPUS STRIATAL (CS) TISSUE FRAGMENTS. W. Lin, J. McDermott*, D. Dluzen & V. Ramirez. Department of Physiology and Biophysics and Medicine-Carle Foundation Hospital, University of Illinois Urbana, Illinois 61801.

In an attempt to understand some mechanisms by which L-DOPA alters nigrostriatal dopaminergic function we compared DA release rates of superfused CS fragments in response to either repeated AMPH (10 uM) or L-DOPA (5 uM) infusions. Male rat CS were superfused using a modified KRP medium with samples collected at 10 min intervals and assayed for DA using HPLC-EC. During intervals 3,7 and 11, KRP medium containing AMPH (N=5) or L-DOPA (N=4) was directly infused into superfusion chambers. Both AMPH and L-DOPA evoked increases in DA release following this initial infusion. Assigning this first response a value of 100% we calculated the changes in response to the second and third infusions. In response to the second and third AMPH infusions, DA release decreased to 82.6±16.9 and 41.1±15.9%, respectively, while subsequent responses to L-DOPA appeared to be potentiated with DA release following the second and third L-DOPA infusions increasing to 175.5±43.4 and 266±65.1%, respectively. These results indicate that repeated AMPH infusions apparently result in a depletion of a releaseable pool of DA, while L-DOPA stimulated DA release appears to potentiate processes involved in the synthesis and/or release of DA from CS tissue fragments following repeated L-DOPA stimulation.

48.12

FURTHER CHARACTERIZATION OF THE NOREPINEPHRINE-RELEASING PROPERTIES OF 9-AMINO-1,2,3,4-TETRAHYDROACRIDINE (TACRINE, THA): COMPARISON WITH VARIOUS RELEASING AGENTS. C.P. Smith, W. Petko* and F.P. Huger Dept. of Biological Research, Hoechst-Roussel Pharmaceuticals, Inc., Somerville, NJ, 08876-1258.

There are an increasing number of reports which demonstrate that tacrine (THA) has direct effects on membrane ion channels in addition to inhibiting cholinesterases. Although the therapeutic relevance is controversial, direct ion channel effects may cause activation of other neurotransmitter systems in the brain. We have previously shown that THA increases spontaneous NE release from cortical slices in a calcium-independent manner, increases electrically-stimulated NE release and increases the turnover of phosphatidylinositol (PI) in pre-labeled cortical slices. In this report, we describe additional experiments to compare the necessary conditions for release by THA and other classes of releasing agents and also to examine the relationship between effects on PI turnover and release.

The effects of THA on spontaneous NE release differed from those of tyramine, veratridine, 4-AP and ouabain based on Ca^{2+}-dependency and sensitivity to tetrodotoxin, nomifensine and reserpine.

THA-induced PI turnover was blocked by neomycin and a Ca^{2+}-free buffer but the THA effect on spontaneous NE release was unaffected by these conditions. Furthermore, a Na^+-free medium and amiloride prevented the THA stimulation of PI turnover, but did not block the enhancement of spontaneous release, suggesting that there is not a cause-effect relationship between PI turnover and spontaneous release of NE.

48.13

MECHANISM OF TETRAHYDROBIOPTERIN (BH_4) UPTAKE INTO RAT BRAIN SYNAPTOSOMES: EFFECTS ON SEROTONIN METABOLISM. P.Z. Anastasiadis*, W.A. Wolf*, D.M. Kuhn, and R.A. Levine. Lab. of Molecular Neurobiology, Lafayette Clinic and Cellular and Clinical Neuroscience Pgm., Dept. Psychiatry, Wayne State Univ., Detroit, MI.

BH_4 is the naturally occurring cofactor for phenylalanine, tyrosine and tryptophan hydroxylases, the latter two enzymes being rate-limiting for catecholamines and serotonin synthesis. Deficiencies in $BH_{4\ synthesis}$ occur in newborns (atypical PKU) and may be involved in Parkinson's and Alzheimer's disease and familial dystonia. Enhancing dopamine and/or serotonin synthesis in brain using BH_4 therapy has been only partially successful; this may be due to not enough exogenous BH_4 entering brain or an inability of BH_4 to enhance hydroxylation. Our studies examined the mechanism and extent of BH_4 uptake into rat brain synaptosomes and its effects on 5-TH metabolism. Synaptosomes were incubated with BH_4 (.2 - 2000 uM) with and without 5 uM tryptophan; 5-HT and metabolites were measured by HPLC after 30 min and BH_4 uptake was measured by HPLC-ED at various times up to 60 min. BH_4 entrance into synaptosomes was higher at 37°C than at 0oC and linear at high concentrations, with an apparent rapid accumulation at lower levels. Omission of glucose had no effect on the uptake process at low BH_4 levels after 10 min. Regarding 5-HT metabolism, exogenous BH_4 had no effect on total synaptosomal indole content, whereas 5 uM tryptophan increased total indoleamines 3-fold; this effect was not enhanced by 200 uM BH_4. Further studies are testing whether BH_4 is actively taken up and whether BH4 can stimulate indoleamine synthesis in brain slices.

48.14

IN VIVO MEASUREMENT OF LONG-TERM EFFECTS OF MDMA ON 5-HT UPTAKE SITES AND 5-HT_2 RECEPTORS. U. Scheffel*, J.R. Lever* and G.A. Ricaurte. Departments of Radiology, Environmental Health and Neurology, The Johns Hopkins Medical Institutions, Baltimore, MD. 21205. (Sponsor: Z. Annau).

With a view towards developing a positron emitting ligand for documenting subclinical MDMA-induced 5-HT neural damage in the living human brain, the present study investigated the feasibility of detecting MDMA neurotoxicity through either in vivo measurement of ^{3}H-paroxetine binding to 5-HT uptake sites, or ^{125}I-N-methyl-iodo-LSD (^{125}I-MIL) binding to 5-HT_2 receptors.

One week after treatment with MDMA (8 x 20 mg/kg, s.c.), rats were injected intravenously with either ^{3}H-paroxetine or ^{125}I-MIL. Specific binding of these ligands was then quantified in various brain regions. In all CNS areas examined, MDMA produced a significant decrease in the specific binding of ^{3}H-paroxetine binding, suggesting loss of 5-HT uptake sites. The same MDMA treatment produced no long-term effects on 5-HT_2 receptors as measured by the in vivo binding of ^{125}I-MIL. There were no changes in ^{125}I-MIL binding at either one or two weeks after MDMA treatment.

These results indicate that in vivo binding of ^{3}H-paroxetine can be used to detect MDMA-induced 5-HT neuronal damage in living animals, and suggest that a PET ligand for the presynaptic element of 5-HT neurons would be useful for detecting MDMA neurotoxicity in the living human brain. Such a ligand should also be of value for further investigating the role of 5-HT in the human brain during both health and disease.

48.15

REAPPEARANCE OF MAO TYPE A AND TYPE B IN THE RAT STRIATUM AFTER IRREVERSIBLE INHIBITION. G.S. Carter and A.J. Azzaro. Departments of Neurology, Pharmacology/Toxicology and Psychiatry, West Virginia University Health Science Center, Morgantown, WV 26506.

The reappearance of monoamine oxidase (MAO) subtypes A and B was examined in the striatum of adult, male Sprague-Dawley rats after complete irreversible inhibition of MAO activity with the nonselective MAO inhibitor nialamide. A dose of 125mg/kg body weight of nialamide given intraperitoneally produced greater than 90% inhibition of MAO activity. Using the specific substrates serotonin (5-HT) and phenylethylamine (PEA) at their Km concentrations, the return of MAO activity was assessed. The activities of the MAO subtypes were compared with their activities in uninhibited animals of the same approximate weight and age. The deamination of PEA (MAO B activity) showed a rapid return to the uninhibitied value with a calculated half-life of 10.4 days. The deamination of 5-HT(MAO A activity) showed a slower return to uninhibited levels with a calculated half-life of 17.5 days.

Most MAO B in the rat striatum is located in astrocytes. Only a small fraction is found in serotoninergic neurons. A significant fraction of MAO A, however, is found in nigrostriatal dopaminergic neurons. As axoplasmic transport is required to replace a significant portion of MAO A, its reappearance is delayed compared to MAO B. Our findings support the concept that the primary neuronal form of MAO is type A.

48.16

NEUROTENSIN AND DOPAMINE RELEASE IN THE RAT PREFRONTAL CORTEX: IN VIVO PHARMACOLOGICAL AND PHYSIOLOGICAL STUDIES. A.J. Bean, M.J. During, R.H. Roth, Department of Pharmacology and Psychiatry, Yale Univ. School of Medicine, New Haven, CT 06510.

Dopamine (DA) and neurotensin (NT) are colocalized in a subset of mesencephalic neurons. Some of the mixed DA/NT neurons project to the prefrontal cortex (PFC), a brain region which does not contain intrinsic DA or NT cells. Stimulation of the axons of mesocortical neurons may be expected to result in the release of both DA and NT. However, studies on the stimulation-evoked release of colocalized molecules in the peripheral nervous system (PNS) by Lundberg, Bartfai and coworkers have suggested that there may be frequency and/or pattern dependent differential release. Additional studies in the PNS have suggested that autoreceptors may control the release of more than one colocalized molecule. We have studied the effects of electical stimulation and autoreceptor stimulation of mesocortical axons on PFC DA and NT release using microdialysis coupled to HPLC-EC and RIA methods. DA and NT were measured within the same dialysis sample and their release was found to be calcium dependent. Stimulation of mesocortical axons produced frequency dependent release of both DA and NT. Burst stimulation increased both DA and NT release more than tonic stimulation for the same number of spikes. DA autoreceptor stimulation decreased DA release and increased NT release. These data suggest that mixed DA/NT mesocortical neurons can release both DA and NT and that differential release can occur.

Suppored by MH 14092 and NIGMS-07324.

48.17

METAPHIT ANTAGONIZES THE EFFECT OF PCP AND NOMIFENSINE, NOT AMPHETAMINE, ON ENDOGENOUS DOPAMINE RELEASE IN RAT STRIATAL SLICES. S.T. Buxton*, G.A. Gerhardt, N.R. Zahniser, A.E. Jacobson*, R.A. Lessor* and L.P. Dwoskin (SPON: N. Bhat). Univ. Kentucy Col. Pharmacy, Lexington, KY 40536, Dept. Pharmacol., Univ. Co. Hlth. Sci. Ctr., Denver, CO 80262, and NIADDK, Bethesda, MD 20892.

Metaphit is a phencyclidine (PCP) analog which acylates PCP binding sites in vitro (Rafferty et al, 1985). The ability of Metaphit to antagonize the effect of 10 uM PCP, nomifensine (NOMI) or amphetamine (AMPH) on electrically (1 Hz, 60 pulses) evoked release of endogenous dopamine (DA) and dihydroxyphenylacetic acid (DOPAC) from superfused striatal slices was examined. Prior incubation (30 min) with Metaphit resulted in a dose-dependent decrease (EC50 10 uM) in the stimulation-evoked overflow of DOPAC. Metaphit (10 uM) antagonized both the increase in DOPAC and DA induced by PCP exposure. Metaphit antagonized the increase in DA, and further decreased DOPAC as a result of NOMI exposure. Metaphit did not alter the increase in DA and decrease in DOPAC induced by AMPH exposure. The results suggest that Metaphit interacts with a PCP receptor closely associated with the DA uptake site. Metaphit selectively antagonizes DA release induced by PCP, and not that induced by AMPH. Therefore, the results do not support the suggestion that DA release induced by PCP and AMPH is via a similar mechanism. Supported by USPHS NS09199, AG06434 and Univ. KY Med. Ctr. Research Fund.

48.18

EFFECT OF AN ACUTE COMBINED INJECTION OF HALOPERIDOL AND ESTRADIOL ON RAT BRAIN DOPAMINE METABOLISM. T. Di Paolo, M.A. Gagné* & M. Daigle* (SPON: A. Dupont), School of Pharmacy, Laval University, Qc G1K 7P4, and Dept of Mol. Endocrinol., Laval Univ. Med. Centre, Qc G1V 4G2 Canada.

We have shown that estradiol (E_2) and progesterone (P) at physiological doses, acutely increase dopamine (DA) and its metabolite levels in the rat brain. It has also been recently reported that the effect of haloperidol on rat brain DA metabolism fluctuates during the estrous cycle (Neuropharmacol. 27:73 (1988)). This report investigates the acute combined E_2 and haloperidol effect on brain DA metabolism of ovariectomized rats. DA and its metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were measured by HPLC with electrochemical detection in the frontal cortex and striatum. An intraperitoneal injection of haloperidol (0.1 mg/kg) increased DA turnover as measured by the ratio DOPAC/DA and HVA/DA in the striatum and frontal cortex 30-180 min after the injection ($p < 0.05$). An E_2 injection (100 ng/0.2 cc) 15 or 30 min before sacrifice of the haloperidol injected rats potentiated the DA turnover increase caused by the neuroleptic in the frontal cortex from 30 to 180 min ($p < 0.05$); striatal changes were smaller and more variable. Our results suggest that physiological levels of E_2 can potentiate the DA turnover increase induced by haloperidol and could explain the variations of DA turnover induced by this neuroleptic during the estrous cycle. Supported by the MRC.

48.19

Separation of vesamicol-binding and NE-containing compartments in fractionated PC12 cells. D. Blumberg and E.S. Schweitzer. Neuroscience Training Program, University of Wisconsin, Madison, WI 53706.

We examined whether sorting of integral membrane proteins occurs within the regulated secretory pathway by following the binding of vesamicol (AH5183, a putative ligand for the ACh transporter) to fractionated PC12 cell membranes, and comparing this with [^{3}H]norepinephrine (NE) (as a marker for organelles possessing the catecholamine transporter) as well as two other vesicle-specific integral membrane proteins, synaptophysin (P38) and SV2.

We first characterized binding properties of [^{3}H]vesamicol in PC12 cells. PC12 cells exhibit saturable binding for vesamicol, binding 130.8 $\pm$2.91 pmol/mg protein, with a K_d of 1.91 $\pm$0.37 μM, association rate constant of 1.65 x 10^6 $min^{-1}M^{-1}$, and dissociation rate constant of 0.056 $\pm$0.004 min^{-1}.

PC12 cell homogenates were fractionated on linear Ficoll gradients. Results showed a peak of vesamicol binding at ~10% Ficoll, which was distinct from [^{3}H]NE, which peaked at ~22% Ficoll. These data suggest that ACh and NE are packaged into separate subcellular compartments. The majority of synaptophysin immunoreactivity co-migrated with [^{3}H]vesamicol binding, whereas SV2 immunoreactivity appeared to co-migrate with both [^{3}H]NE-containing and [^{3}H]vesamicol-binding structures.

48.20

MEASUREMENT OF SUBSECOND COMPONENTS OF ENDOGENOUS DOPAMINE RELEASE AND REUPTAKE RATES IN RAT STRIATAL TISSUE. J.S. McElvain and J.O. Schenk. Dept. of Chemistry and Program in Biochemistry, Washington State University, Pullman, WA 99164-4630.

We have developed a semi-micro electroanalytical technique (500 uL volume) for making rapid measurements of endogenous dopamine (DA) release from and reuptake into homogenates of striatal tissue using a rotating disk electrode (RDE). This method makes it possible to monitor both endogenous DA release and uptake rates on msec time scales in the same tissue preparation. Using glassy carbon rotating disk electrodes, the detection limit is ca. 5-10 nM DA in physiological buffer. The magnitudes and rates of striatal DA release were shown to be K^+, Ca^{2+}, and temperature dependent. At 37°C the K^+ depolarization release rate of DA from rat striatal tissue was found to be 300 pmoles/gm wet wt/second. DA uptake rates were also shown to be temperature dependent and inhibited by the uptake blocker cocaine. At 37°C the uptake rate of DA was found to be 49.3 pmoles/gm wet wt/sec. These rates are ca. 10 x greater than previously reported. This RDE technique can also be used to determine the effects that certain drugs of abuse and autoreceptor feedback control mechanisms have on endogenous DA uptake and release rates. (Supported in part by the Washington Alcohol and Drug Abuse Program (Initiation 171) and MH42759 (J.S.))

48.21

INTERACTION OF [^{3}H]-DIHYDROTETRABENAZINE BINDING ACTIVITY FROM BOVINE STRIATUM WITH IMMOBILIZED LECTINS. Michael S. Vincent* and Joseph A. Near. Medical Sciences Program, Indiana University School of Medicine, Myers Hall, Bloomington, IN 47405.

Synaptic vesicles and other secretory granules accumulate catecholamines and serotonin by means of an uptake system that is coupled to a proton-translocating ATPase by a transmembrane proton electrochemical gradient. Two classes of catecholamine depleting agents, represented by reserpine and dihydrotetrabenazine (TBZOH), interact with the transporter but exhibit different binding properties. We have recently been engaged in the solubilization and characterization of tritiated TBZOH binding activity from bovine *corpus striatum*. A crude synaptosomal fraction (Azarian, *et al.*, J. Neurochem. 50:824, 1988) was prepared, and TBZOH binding activity solubilized with sodium cholate (1% w/v) as described by Scherman and Henry (Biochem 22:2805, 1983). While the synaptosomal fraction contains two classes of binding sites for TBZOH, only the higher affinity class of sites was observed in pellet or supernatant after cholate treatment. Because the lower affinity sites are not found in purified synaptic vesicles, but are present in liver membranes, they are probably unrelated to catecholamine storage. More than 50% of the cholate soluble binding activity was retained by wheat germ lectin sepharose and concanavalin A sepharose after incubation for 60min at 4°C. The interactions could be blocked by inclusion of 200mM N-acetyl-glucosamine or alpha-methyl-mannoside respectively, but not by glucosamine or cellobiose.

These findings suggest that a TBZOH binding subunit of the transporter, or a membrane component closely associated with this subunit, is glycosylated.

Supported by grant RO1-NS20784 from NIH.

48.22

THE ROLE OF THE HIGH AFFINITY 5-HYDROXYTRYPTAMINE CARRIER IN THE RELEASE OF 5-HYDROXYTRYPTAMINE IN THE ABSENCE OF EXTRACELLULAR CALCIUM. S.M. Evans*, K.J. Collard * and L.S. Wilkinson* (SPON: E.M. Meyer). Department of Physiology, University of Wales in Cardiff, Cathays Park, Cardiff, Wales.

The dependence on extracellular calcium of 5-hydroxytryptamine (5-HT) release from rat brain synaptosomes was examined under various conditions using a rapid superfusion system. Release was evoked from synaptosomes preloaded with tritiated 5-HT in the presence or absence of an inhabitor of monoamine oxidase (MAO) by perfusion with medium containing 50 mM potassium. The release was found to be only partially dependent on calcium, and the requirement for calcium decreased significantly when an inhibitor of MAO was present. However, the release occurring in the absence of calcium when MAO was inhibited was abolished by specific 5-HT uptake inhibitors, suggesting that this release may involve the 5-HT carrier. The release evoked by p-chloroamphetamine (p-CA), a drug whose releasing action is thought to involve the 5-HT carrier, was significantly increased when MAO was inhibited although the dependence on extracellular calcium was not significantly altered. P-CA was found to inhibit the uptake of tritiated 5-HT into synaptosomes, suggesting that p-CA may be a substrate for the 5-HT carrier. These data support the suggestion that 5-HT can be released by reversal of the carrier when tissue is depolarised or treated with p-CA, and that the magnitude of release via this route can be increased by inhibition of MAO, presumably due to the increased levels of 5-HT available to the carrier intraterminally.

48.23

THE EFFECT OF THALLIUM ON THE RELEASE OF ENDOGENOUS MONOAMINES FROM RAT BRAIN. M.G. Kolta and S.F. Ali (SPON: N. Uretsky). Division of Reproductive & Developmental Toxicology, NCTR, Jefferson, AR 72079.

Thallium (TL), a rodenticide and pesticide, has been shown to be neurotoxic to humans and animals. Recent results from our laboratory showed that acute or sub-acute doses of TL to rats produced alterations in CNS monoaminergic systems. The present study examined the effect of TL on the release of dopamine (DA), serotonin (5-HT) and their metabolites from the caudate nucleus (CN). Adult male CD rats were sacrificed and CN were dissected. CN slices were incubated with either 0, 10, 100 uM of TL or 10 uM of amphetamine (A) as a positive control. A induced a significant increase in DA levels in the medium, tissue slices and total content (medium + tissue slices) when compared to untreated control slices. TL at 10 or 100 uM also caused an increase in total dopamine content. While total DA content was equally enhanced in TL and A-treated slices, DA released into the medium from slices exposed to TL was significantly reduced and that retained in the slices was significantly higher when compared to A or control. No significant effect of TL was observed on DOPAC levels in the medium, tissue slices or total content as compared to A, nor on HVA as compared to control. These in vitro data suggest that TL-induced neurotoxicity may be due to its blocking effect on DA release.

48.24

Botulinum Neurotoxin Light Chain Inhibits 3H-Norepinephrine Release from Permeabilized PC-12 Cells. Rich Lomneth*, T. F. J. Martin*, and B. R. DasGupta* (SPON: J. Adler) Dept. of Food Microbiology and Toxicology, and Dept. of Zoology, Univ. of Wisconsin, Madison, Wisconsin 53706.

The botulinum neurotoxins (NT) are a class of neurotoxins which are among the most potent known. The well established action of NT is the inhibition of acetylcholine release from neurons. The NTs are comprised of a heavy chain (~100 kD) which is involved in the binding of the NT to the cell, and a light chain (~50 kD) which is believed to cause the intracellular lesion. To understand the NT structure-function relationships, we studied the effects of NT serotype B on the pheochromocytoma cell line PC-12. The 150 kD NT has no effect on the K^+-stimulated 3H-norepinephrine release from intact cells. However, after mechanically permeabilizing the cells so that the Ca^{+2}-stimulated secretory pathway remains functional (Martin, T. F. J., Methods Enzymol., 168;451, 1989), the 150 kD NT inhibits almost all of the Ca^{+2}-dependent 3H-norepinephrine release. The isolated light chain is at least as effective as the 150 kD NT while the isolated heavy chain has no effect. Therefore, we have developed a model system to study NT structure-function relationships and the intracellular mechanism of action (funded by NS24545 and DK40428).

49.1

LOCALIZATION OF CAT-301 IMMUNOREACTIVITY ON NEURONS IN THE GERBIL BRAINSTEM AUDITORY NUCLEI. I.R. Schwartz*# and S. Hockfield# Sections of Otolaryngology* and Neuroanatomy# Yale Univ. Sch. of Med., New Haven, CT 06510.

Cat-301, a monoclonal antibody generated against adult cat CNS, recognizes a proteoglycan on the extrasynaptic surface of a subset of mammalian CNS neurons. In the visual system of primates and cats Cat-301 appears to recognize a functionally distinct subset of neurons, those involved in processing the motion, or low contrast, component of a visual stimulus.

The distribution and identity of Cat-301 immunoreactive auditory neurons was determined in serial sections of adult gerbil brains. A characteristic, selective pattern of labeling marked distinct classes of auditory neurons. The labeled neurons were among the larger cell types present in each nucleus. Staining covered the soma and major dendritic trunks and varied in intensity depending on the specific neuronal class. Cells labeled include: giant or radiate neurons of the deep dorsal cochlear nucleus; octopus, multipolar and large elongate neurons of the posterior ventral cochlear nucleus; spherical and multipolar neurons of the anterior ventral cochlear nucleus; most neurons of the medial superior olive and of the medial, lateral and ventral nuclei of the trapezoid body; principal neurons of the lateral superior olive, primarily in the lateral limb; large oval neurons of the ventral nucleus of the lateral lemniscus; large oval and multipolar neurons of the dorsal nucleus of the lateral lemniscus; and very large multipolar neurons of the lateral (external) nucleus of the inferior colliculus. The results appear consistent with the hypothesis that Cat-301 labels a subset of auditory neurons responding to some dynamic property of sound.

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49.2

INTRINSIC CONNECTIONS OF CAT PRIMARY AUDITORY CORTEX SHOWN WITH THE ANTEROGRADELY TRANSPORTED LECTIN PHASEOLUS VULGARIS LEUCOAGGLUTININ (PHA-L). M.N. Wallace, L.M. Kitzes and E.G. Jones. Dept. of Anatomy and Neurobiology, Univ. of California, Irvine, California 92717.

Iontophoretic injections of PHA-L were made bilaterally into the primary auditory cortex (AI). Four days later the animals were reanesthetised and the best frequency of cells at the injection site and in the surrounding cortex was determined by recording single and multiple unit responses to contralateral stimulation. Electrode tracks were marked by electrolytic lesions, the brain was perfused with 4% paraformaldehyde and the cortex was flattened and sectioned tangential to the pial surface at 40μm on a sliding microtome. One series of sections was stained immunohistochemically for PHA-L and the second with the monoclonal antibody, CAT-301. CAT-301 staining, as well as staining for acetylcholinesterase and cytochrome oxidase, were useful for defining the location of AI as they all showed elevated levels within layer IV. Patches of anterogradely labeled fibers occurred in the surrounding auditory fields. In addition, between three and eight patches of terminals occurred within AI. Despite being arranged in a variety of patterns around the injection site, these patches usually occurred in parts of AI that had a similar best frequency to the injection site.

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49.3

AUDITORY KONIOCORTEX IN THE RAT: ANTEROGRADE TRANSPORT OF PHA-L TO NEOCORTEX FROM THE VENTRAL DIVISION OF THE MEDIAL GENICULATE BODY. *L.M. Romanski and J.E. LeDoux*, Div. Neurobiology, Cornell Univ. Med. Coll., NY, NY 10021

The primary auditory receptive region of cortex, the auditory koniocortex, can be defined anatomically as the cortical projection field of the ventral division of the medial geniculate body (MGV). While the cortical projections of MGV have been examined in a number of mammalian species, these remain poorly characterized in the rat. To identify auditory koniocortex in this species, PHA-L was iontophoretically injected into MGV in male Sprague-Dawley rats. Labeled fibers entered temporal neocortex from the external capsule and extended towards the pial surface. Anterograde transport was heavily concentrated in the middle laminae (III and IV). With small injections, labeling appeared to occur in discrete, column-like ramifications. The labeled areas overlapped parts of Krieg's areas 39, 40 and 41 and Zilles' area TE3. PHA-L was also injected into auditory koniocortex. Anterograde transport was present in MGV, in a region ventral to auditory koniocortex and dorsal to perirhinal cortex that may represent a secondary auditory field, and in the posteromedial striatum. These findings thus anatomically define the primary auditory projection area in the neocortex of the rat and will facilitate behavioral studies of the function of rat auditory cortex. Supported by MH 38774.

49.4

GABA IMMUNOREACTIVE NEURONS AND PUNCTA IN LAYERS IV AND V OF CAT PRIMARY AUDITORY CORTEX (AI).
B.A. Peterson and J.A. Winer. Department of Molecular and Cell Biology, University of California, Berkeley, California 94720-2097.

The organization of layer IV in primary auditory cortex is significantly different than that of layer V. Layer IV is the main thalamic recipient zone, and it has a variety of non-pyramidal cells, many with axons intrinsic to AI. In contrast, layer V receives little thalamic input, and contains pyramidal and non-pyramidal cells, many of which project in the commissural and corticofugal systems. It would be interesting to compare the patterns of GABAergic cells and puncta in these layers since each has different functional roles. Tissue from adult cats was sectioned, then immunostained with either GAD (1:2000) or GABA (1:5000) and the avidin-biotin immunoperoxidase technique (S.M. Hsu et al., *J. Histochem. Cytochem.* 29:1349-1353 [1981]).

A substantial fraction of the population of AI neurons is GABAergic; however, there are significant laminar differences in their arrangement. The main results within layer IV are that (i) members of several classes of neurons are GABAergic; (ii) many GABAergic neurons have relatively small non-pyramidal perikarya that, in Golgi studies, were common in the upper half of layer IV (J.A. Winer, *J. Comp. Neurol.* 224:535-567 [1984]); (iii) at least one class of larger neuron is also GABAergic; (iv) small and medium-sized GABAergic terminals dominate the neuropil; and (v) immunonegative cells are often ringed by puncta, less so in layer V. The findings for layer V are that (i) members of each class of non-pyramidal neuron are GABAergic; (ii) certain inverted pyramidal cells are also GABAergic, including some accumulating [^{3}H]GABA; and (iii) immunopositive axon terminals are coarser and sparser than those in layer IV.

The high density of GABAergic terminals in layer IV is consistent with results in the primary visual cortex, while the layer V GABAergic inverted pyramidal neurons represent either a unique class of pyramidal neuron or may be more properly construed as non-pyramidal.

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49.5

AUDITORY CORTEX NEURONS SENSITIVE TO CORRELATES OF AUDITORY MOTION IN THREE-DIMENSIONAL SPACE. E. Stumpf*, J.A. Manley-Toronchuk* and M.S. Cynader. (SPON: R.M. Douglas) Depts. of Psychology and Ophthalmology, University of British Columbia, Vancouver, B.C., V6T 1Y7, Canada.

Amplitude modulation at the receiver's ears is a characteristic of moving sound sources. When a sound source moves from side to side, stimulus intensity decreases in one ear and increases in the other. When a sound source moves toward or away from the organism, the two ears receive correlated increases or decreases in sound level.

We recorded from single units in the auditory cortex (AI) of anesthetized cats while presenting amplitude modulated pure tones via a sealed system. The stimuli consisted of AM ramps presented to the two ears and simulated movement in four canonical directions: toward vs. away, and toward ipsilateral vs. toward contralateral hemifield. Most cells encountered responded selectively to these correlates of moving sound, generally showing preferred directions emphasizing motion toward, away, or into the contralateral hemifield. In addition, all movement sensitive units responded selectively to the rate of rise of the AM ramps, a feature analogous to stimulus velocity. These findings suggest a role for auditory cortex units in three-dimensional localization of moving sound.

49.6

CORTICAL NEURONS SENSITIVE TO AUDITORY MOTION: UNDERLYING MECHANISMS. J.A. Manley-Toronchuk*, E. Stumpf* and M.S. Cynader. Depts. of Psychology and Ophthalmology, University of British Columbia, Vancouver, B.C., V6T 1Y7, Canada.

The traditional response properties of auditory cortical neurons, such as monotonicity vs. non-monotonicity, phasic vs. tonic, on vs. off, and E/E vs. E/I have usually been studied in isolation from one another. However, these response properties can be shown to interact synergistically, providing an underlying mechanism for selectivity to auditory motion. This poster will provide specific examples of the types of interaction found in cortical area AI in the cat. Several general rules for mechanisms underlying auditory motion selectivity have emerged from our studies. For example: 1) Neurons preferring a simple increase in sound intensity respond to the binaural correlates of sounds moving toward the head. Neurons reponding to stimulus offset prefer movement away. 2) E/E cells respond best to simulated movement toward the head, while E/I cells respond, best on average, to movement toward the contralateral hemifield. 3)Non-monotonic cells tend to show a preference for lower rates of sound source movement than do monotonic cells.

The rules described above are only first approximations, however, and the interactions among these response properties can be subtle and intricate. Illustrative examples will be presented.

49.7

LEVEL-DEPENDENCE OF FREQUENCY AND LATENCY TOPOGRAPHY IN CAT PRIMARY AUDITORY CORTEX. C.E. Schreiner Coleman Laboratory, University of California, San Francisco, CA 94143-0526.

With near threshold stimuli, a strict cochleotopic organization can be demonstrated for mammalian primary auditory cortex (AI). This study defined the topographic representation of a pure tone in AI as a function of stimulus level. Frequency-response areas (FRAs) were measured for multiple-unit responses in AI of barbiturate-anesthetized cats. From these FRAs, a measure of cortical activity strength (spike count) in response to a pure tone of fixed frequency (8 kHz) was extracted for each recording site, and normalized to the maximum activity observed at that location. A cortical area was mapped corresponding to CFs spanning approximately 1.5 octaves. For stimulus levels of 10 to 40 dB SPL, the spatial distribution of the tone response evoked a band of increasing activity. This band was oriented in the dorso-ventral dimension of the iso-frequency domain, as determined with near threshold stimuli. By contrast, for stimulus levels above 40 dB SPL, no clear frequency-specific pattern of activity strength was observed. Instead, three distinct bands of activity were obtained that were oriented along the rostro-caudal extent of AI, i.e. orthogonal to the iso-frequency domain. The dorsal and ventral bands were associated with a high level of activity, whereas the central band showed only a relatively small amount of excitatory activity. On the other hand, a level-dependent spatial distribution of minimum latencies exhibited a clear frequency-specific pattern up to the highest levels tested (70 dB SPL), foremost in the center of AI. It is concluded that at low levels, spectral information is represented by a rate distribution. At high stimulus levels, spectral specificity in AI is preserved in the spatial pattern of response timing. Supported by NIH NS-10414, HRI, and the Coleman Fund.

49.8

CONNECTIONS OF AUDITORY CORTEX IN OWL MONKEYS. A.E. Morel, L.A. Krubitzer and J.H. Kaas. Dept. of Psychology, Vanderbilt University, Nashville, TN 37240.

Cortical and thalamic connections of AI and surrounding cortex were investigated in owl monkeys (Aotus trivirgatus) with injections of WGA-HRP and fluorescent dyes (FB and NY). Fields AI and R were identified by patterns of tonotopic organization in microelectrode mapping experiments and were related to areas of dense myelination and cytochrome oxidase reactivity in brain sections cut parallel to the surface of flattened cortex.

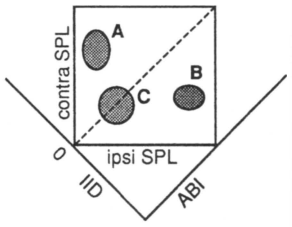
Injections in AI demonstrated major connections with field R, the cortex adjoining AI caudally and medially, and the principal division of the medial geniculate body (MGB). Injections including cortex immediately lateral to AI and R labeled AI, R and cortex medial, lateral and rostral to AI and R. Three of these injections also labeled the frontal cortex. Thalamic connections to cortex lateral to AI and R were largely with the medial and dorsal divisions of the MGB, the suprageniculate nucleus and the medial pulvinar.

These results support previous conclusions that auditory cortex in owl monkeys includes both a core (AI and R) and a surrounding fringe of cortical fields. The fringe is distinguished from the core by having more widespread thalamic and cortical connections, including, for some part of the fringe, connections with the frontal cortex. (NS-16446).

49.9

TWIN (TWO-WAY INTENSITY NETWORK) NEURONS IN THE CAT PRIMARY AUDITORY CORTEX. M. N. Semple and L. M. Kitzes. Dept. of Anatomy and Neurobiology, Univ. of California Irvine, Irvine, CA 92717.

We have previously described a response type in gerbil inferior colliculus (IC) and cat primary auditory cortex (AI) for which the optimal tonal stimulus is a two-way intensity network (TWIN), constituting a particular sound pressure level (SPL) at one ear and a particular SPL at the other (Semple and Kitzes, *Soc. for Neurosci.*, 1987, 13: 1467). TWINs in IC are always contra-excited with a mixed ipsi influence of low-level facilitation and higher-level suppression. Within the ipsi by contra SPL matrix, the excitatory focus always favors the contra ear (A). Here we report more extensively on TWIN tuning of single neurons in cat AI.



TWINs are abundant in AI. Some are similar to those in IC (A), whereas others have an ipsi excitatory focus (B). Predominantly-binaural neurons respond best when the SPL is similar at both ears (C). These are functionally like other TWINs, except that the optimal SPL at each ear is similar.

Excitatory foci for different TWINs span the SPL matrix and their best frequency distribution is broad. Thus, TWINs would be selectively activated according to the spectral content of a sound and the amplitudes of the component frequencies at each ear.

Supported by grant NS-25674.

49.10

CROSSCORRELATION AND SYNAPTIC STRENGTH IN A BIDIRECTIONAL ASSOCIATIVE MEMORY (BAM) MODEL. I.Espinosa, R.Lara* and F.Heredia*. Cybernetics Lab., Mexico National Univ., Mexico, D.F. 04510.

We are trying to interpret data from cat's auditory cortex AI (Espinosa I. and Gerstein G., Soc. Neurosci. Abstr. 11:249,1985) within the context of models which perform collective computation using two layers of neurons.

We simulated BAMs (Kosko,B., Byte, Sept. 1987,p.137) using a simulator which takes into account neurophysiological findings (MacGregor,R. Neural and Brain Modeling, A.P. 1987). Several sets of associations were stored in a 35x20 neurons BAM and activity of selected neuron pairs was analyzed with crosscorrelation.

These BAMs show a dominant state which results in a poor recovery of information. From one set to another synaptic strength changes massively. However, crosscorrelation barely detects those changes because of neurons' firing synchronicity.

It is not known if functional changes in AI assemblies are function of synaptic strength. In any case, crosscorrelation detects them (Espinosa I. and Gerstein G., Brain Research 450:39,1988). These experiments and simulations indicate that in the brain collective computation circuits with more layers, sparser connectivity and less synchronization are more likely.

49.11

THE INFLUENCE OF AUDITORY CORTEX ON THE ACOUSTICALLY EVOKED RESPONSES OF NEURONS IN THE INFERIOR COLLICULUS AND CEREBELLUM OF ECHOLOCATING BATS. D.X. Sun*, X.D. Sun* and P.H.-S. Jen. Department of Biology, East China Normal University, Shanghai PRC and Division of Biological Sciences, University of Missouri, Columbia, Missouri USA.

The influences of the auditory cortex on the responses of inferior collicular (IC) and cerebellar (CBL) neurons to sound stimulation in echolocating bats were studied by electrical stimulation of the auditory cortex. Among 471 IC neurons isolated, about 26% of them were affected by cortical stimulation. Responses of 103 (22%) IC neurons were inhibited and 17 (3.6%) were facilitated. The degree of inhibition is dependent upon the amplitude of both auditory and electrical stimuli. For a given acoustic stimulus, inhibition increased with increasing amplitude of electrical stimulus. Conversely, for a given electrical stimulus, inhibition decreased with increasing amplitude of acoustic stimulus. The point of cortical stimulation is crucial in influencing the responses of IC neurons. Among 186 CBL auditory neurons examined, responses of 139 (74.7%) neurons were facilitated upon electrical stimulation of the contralateral auditory cortex. Inhibitory effect was not observed. For a given electrical stimulus, the degree of facilitation varied not only among individual neurons but also among different sound amplitudes within each neuron. The facilitatory pathways to different parts of the cerebellum were studied by examining the difference in the facilitatory effect on each part of the cerebellum before and after a topical application of a local anesthetic, procaine, onto the point of electrical stimulation in the auditory cortex. The results showed that the facilitatory pathways to vermal and hemispheric neurons differ from the pathway to paraflocculer neurons. The modulation of responses of IC and CBL neurons by the auditory cortex is believed to be a part of a neural mechanism to sharpen the acoustic processing in the IC and to enhance the role of the CBL in acoustic orientation. (Supported by NIH grant)

49.12

SPECTRAL PROCESSING OF FM PULSES IN THE AUDITORY CORTEX OF AN FM BAT. S.L. Shannon* and D. Wong, Anatomy Dept, Indiana U. Sch. Med., Indpls., IN.

The echolocating bat, *Myotis lucifugus*, must extract multiple acoustic cues for target feature analysis (e.g., size, shape and texture) from the spectral content of its emitted FM signals. This species emits ultrasonic FM pulses that sweep downward approximately one octave within 4 msec. Virtually all neurons in *Myotis* auditory cortex are sensitive to specific FM sweeps. The FM signal which elicited maximal neuronal response was presented in discrete quadrants to an awake bat to determine which sweep segment presented alone was sufficent to evoke neural response, and how other sweep segments affected the response magnitude. Most FM units respond weakly when only the third quadrant of the sweep is presented and exhibit little or no response when the first half of the sweep is presented alone. However, presentation of the second or last quadrant along with the third quadrant greatly facilitates the response with little change in response latency. Resolving how different components of an FM signal shape the neuronal response may elucidate how spectral cues convey target information for an FM bat (Supported by NIH grant R01 NS27182).

49.13

EFFECT OF CALLOSAL STIMULATION ON SINGLE UNIT RESPONSES IN PRIMARY AUDITORY CORTEX OF THE FERRET. D.W. Doherty and L.M. Kitzes. Dept. of Anatomy and Neurobiology, University of California Irvine, Irvine, CA 92717.

The functional properties of the extensive callosal pathways between auditory cortices were examined. Single unit activity was recorded in primary auditory cortex (A1) of ferrets anesthetized with Nembutal. The callosal system was stimulated electrically via a pair of rostrocaudally oriented, monopolar electrodes placed in the middle layers of contralateral A1. Single electric shocks of 100 to 200 μs duration and between 100 and 600 μa in magnitude were delivered in a systematic temporal relationship to monaural or binaural tonal stimuli delivered through sealed and acoustically calibrated ear assemblies.

Callosal activity, triggered by a shock to contralateral A1, can facilitate or suppress acoustically evoked responses of EE, EO, EI, and PB units.. Callosal stimulation can evoke a burst of activity in EE units. In PB units, callosal stimulation can also evoke a burst of activity that may be accompanied by a suppression of acoustically evoked discharges. In units that exhibit suppression, the suppression of acoustically evoked responses varies parametrically with shock amplitude. Differential effects upon single unit responses to acoustic stimuli were observed, e.g., no effect upon contralaterally evoked responses with marked suppression of ipsilaterally evoked responses. These data indicate that callosal activity influences units of several response types in A1. Additionally, this influence can have complex facilitatory or suppressive effects on acoustically evoked responses.

Supported by NS-25674.

49.14

CONTRIBUTION OF THE CORPUS CALLOSUM (CC) TO AUDITORY LOCALIZATION IN THE CAT. P. Poirier*, L. Richer*, F. Lepore, M. Ptito, and J.-P. Guillemot. Univ. de Montréal and Univ. de Québec, Montréal, QC, H3C 3J7.

Anatomical and electrophysiological studies with cats suggest that the primary auditory cortex (AI) is involved in sound localization and in the treatment of interaural time (ITD) and intensity differences (IID). Behavioral studies have shown that CC is not involved in these functions. Electrophysiological and tracing studies of AI, however, suggest its implication in the fusion of auditory hemispace and its contribution to the temporal aspects of auditory localization. We report here evidence that CC fibres do contribute to sound localization. We recorded CC fibres and tested spatial tuning by varying ITDs. Cats were anesthetized and paralyzed. Pure tones (PT) and broad-band noise (BBN) bursts were delivered independently to each ear through a sealed-speaker system. Fibres exhibited binaural properties which were similar in most respects to those found in AI. ITD-sensitivity functions showed three different types of patterns: some showed a selectivity to a precise ITD; some responded maximally to a range of delays; others did not respond to ITDs. Fibres were narrowly tuned to PTs. They responded preferentially BBNs. Some fibers adapted rapidly while others slowly. Some were bimodal (auditory-somatosensory, auditory-visual). Testing auditory space representation in free-field stimulation, which we are presently carrying out, should reveal whether spatial sensitivity to static position is independent of its dynamic position.*(supported by FCAR and CRSNG).*

49.15

SPEECH PROCESSING IN PRIMATE PRIMARY AUDITORY CORTEX (A1). M. Steinschneider*, J. Arezzo and H. G. Vaughan, Jr. Albert Einstein College of Medicine, Bronx, NY 10461

Concurrent laminar recordings of current source density (CSD) and multiple unit activity (MUA) were performed in A1 of an awake monkey to /da/, /ba/ and /ta/, their formants and to tones.

Differential MUA patterns that reflected the spectral characteristics of formant frequencies and consonant place of articulation occurred at sites excited by tones within the syllables' spectral range and were determined by the sites' tonotopic specificity. In contrast, tonotopic specificity was not required to encode the temporal characteristics of fundamental frequency and voice onset time.

MUA evoked by the syllables was often a nonlinear sum of the isolated formant responses. Interactions among the formants modulated the activity to the syllables and sharpened response differences that reflected place of articulation.

CSD and MUA depict complementary aspects of laminar processing, namely synaptic events and axonal firing patterns. Laminar profiles of the CSD and MUA revealed specific response transformations between the thalamocortical input and the cortical cells. Encoding of formant transitions and steady-state vowels appeared to occur differentially within auditory cortex.

49.16

EFFECT OF UNILATERAL AUDITORY CORTEX ABLATION ON THE ABILITY OF MACAQUES TO PERCEIVE DICHOTICALLY PRESENTED VOCALIZATIONS H. E. Heffner and S. E. Mooney*. Lab. of Comparative Hearing, Dept. of Psychology., Univ. of Toledo, Toledo, OH 43606.

Sounds presented to one ear are processed mainly by the contralateral hemisphere. Which hemisphere processes sounds that are presented to both ears but lateralized (using time and intensity differences) to one ear?

Four Japanese macaques (Macaca fuscata) were tested on their ability to discriminate two types of their "coo" vocalizations following unilateral auditory cortex ablation. The coos were presented via headphones 1) to one ear at a time (monaural condition) or 2) to both ears with a binaural time delay and/or an intensity difference sufficient to produce a lateralized signal (binaural condition).

Unilateral ablation resulted in a deficit in the monaural condition for sounds presented to the ear contralateral to the lesion. However, no deficit was observed in the binaural condition even when the sound should have been lateralized to the contralateral ear.

These results demonstrate that information regarding sound arriving at one ear is sent primarily to the contralateral hemisphere. Information regarding sound arriving at both ears is sent to both hemispheres even when it is lateralized to one ear. (Supported by NIH grant NS 12992)

49.17

MINIATURE ELECTRODE ARRAYS REVEAL TONOTOPIC AUDITORY EVOKED POTENTIALS IN PIG CORTEX. D. L. Woods, T. C. Chimento#, R. J. Andrews*, R. Kirby*, Clinical Electrophysiology Laboratory, Depts of Neurology/Neurosurgery, U.C. Davis, VA Medical Center, Martinez, CA, 94553, and #Coleman Laboratory, UCSF, San Francisco, CA.

Although auditory evoked potentials (AEPs) provided important historical insights into the localization and organization of auditory cortex, contemporary studies rely almost exclusively on single and multi-unit neuronal recording. A miniature electrode array was constructed (21 electrodes in 1.6 cm^2) to determine whether the topography of AEPs would reveal a tonotopic organization of auditory cortical areas of domestic swine.

Domestic swine were sedated, intubated and maintained under inhalation anesthesia (isoflurane 1.5-2.5%). After craniectomy, the array was placed epidurally over the temporal lobe. To evaluate tonotopic organization, tonebursts (0.25-8.0 kHz, 10 ms duration, 2 ms rise/fall times) were presented binaurally at interstimulus intervals of 1.0 sec. Peak amplitudes of the P25 component, presumed to arise in primary auditory cortex, shifted systematically with frequency. A tonotopic map was generated in about 10 minutes: low frequencies were represented rostromedially and high frequencies caudolaterally. Mini-array recording of AEPs can rapidly characterize functional cortical topography (Supported by the VA Research Service and the NINCDS).

49.18

CORTICAL AUDITORY EVOKED RESPONSE IN THE ALBINO RAT: EFFECTS OF INTERAURAL TIME DELAY. S.L. SALLY*, J.B. KELLY AND G.L. KAVANAGH* (SPON: E. Peterson). Laboratory of Sensory Neuroscience, Carleton University, Ottawa, Canada, K1S 5B6.

The effects of interaural time delays (ITD's) on cortical evoked potentials have been investigated in the cat. However, similar studies have not been reported for the rat. In the present study cortical evoked potentials were used to determine the range over which manipulation of ITD's produces variation in response amplitude. Recordings were made on the surface of auditory cortex. Clicks were delivered to each ear independently through sealed loudspeakers inserted into the external meatus. ITD's from -600 usec (left stimulus leading right) to +600 usec (right leading left) were examined. The function for the left hemisphere response was relatively flat from -600 usec to -250 usec and then rose sharply to reach a maximum amplitude at about +250 usec. At this point the function reached a plateau. The function for the right hemisphere began to decline at about -250 usec and reached a minimum amplitude at about +250 usec. Beyond these values the function was relatively flat. As in the cat, the highest amplitude response appeared in the hemisphere contralateral to the ear which was first stimulated. However, in the rat variation in response amplitude occurred over a smaller range of interaural time differences than the cat.

49.19

HOMOLOGY BETWEEN HUMAN AND CAT AUDITORY BRAINSTEM POTENTIALS (ABR). M. Zaaroor*, A. Starr. Neurology, U. Calif. Irvine, CA 92717. In humans and animals a sequence of short latency (<10msec) components to clicks can be recorded from scalp electrodes. In human the vertex positive components are labelled serially with Roman numerals. In cat, the vertex components are labelled by their polarity and latency in msec. We have studied the generator sites for the cat ABR using intracranial recordings from round window, VIII nerve, cochlear nucleus and superior olive while comparing their latency with the latency of the scalp ABR. These results were then related to intracranial recordings in man reported by Moller to define the homologies between the ABR components in the two species as follows:

ABR COMPONENTS (IN MSEC) (VERTEX NON-CEPHALIC REFERENCE) TO 60 dB nHL CLICKS.

Wave	Human	Cat	Generator Site
I	1.5	1.0	ipsi VIII N, distal
II	2.8	1.4	ipsi VIII N, proximal
III	3.7	2.0	ipsi cochlear nucleus
IV	4.9	3.0	contra superior olive
V	5.6	4.0	both superior olive
VI	7.1	5.0	?

49.20

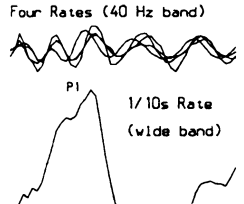
COMPARISON OF THE BRAINSTEM AUDITORY EVOKED RESPONSES (BAER) IN TAURINE-SUPPLEMENTED AND TAURINE-DEFICIENT CATS. M-H. Vallecalle*, G. Heaney, E. Sersen, J.A. Sturman*.

Cats fed taurine-deficient diets develop pronounced reductions in retinal functions, such as electroretinogram responses and visual acuity, and degeneration of the retina and tapetum lucidum. Despite the reports of many-fold reductions of taurine concentrations throughout the central nervous system in taurine-deficient cats there have been no reports of other functional or behavioral deficits. Brain-stem auditory evoked response (BAER) has proved to be a useful instrument for electrophysiological measure of brainstem dysfunction and sensorineural hearing loss. We have recorded the BAER in adult cats fed a taurine-deficient or taurine-supplemented diet for an extended period (> 2 years). The BAER were elicited by rarefaction clicks at different intensities. The responses of the cats were measured as functions of peak latency, peak amplitude and threshold. Our preliminary results are as follows: The BAER of all cats contain five peaks (I to V). A detailed analysis of peak IV, chosen because of its similarities to peak V in humans, shows no difference in threshold between taurine-deficient and taurine-supplemented cats. Analysis of all five peaks suggests that taurine-deficient cats show shorter latencies than taurine-supplemented cats. These results suggest that the reduction of the inhibitory action of taurine may lead to faster conduction of nervous impulses.

49.21

THE AUDITORY 40 HZ-BAND EVOKED RESPONSE LASTS 150 MS AND INCREASES IN SIZE AT SLOW RATES. S. Makeig* and R. Galambos Naval Health Research Center, San Diego, CA.

The auditory middle latency response (MLR) extracted from the EEG when tones or clicks are delivered at a rate near 10/s resembles two or more cycles of a 40 Hz sinusoid. Our experiments demonstrate that brief auditory stimuli actually evoke a burst of waves near 40 Hz in frequency lasting at least 150 msec. Moreover, at very slow presentation rates (<< 1/1s), the amplitude of this 40 Hz-band response increases to up to ten times its size at faster rates (> 1/s). The accompanying figure superimposes the vertex 40 Hz-band click responses (means of three subjects) for four presentation rates (1/10s-3/s).



In the Figure response amplitudes have been normalized. Below these 40 Hz-band responses, the mean wide-band 1/10s response is plotted to illustrate the presence of the 40 Hz-band oscillations in the larger and slower evoked response activity. We conclude that a major consequence of each acoustic stimulus is to evoke a rhythmic response consisting of several near-40 Hz oscillations, and that both the conventional 10/s MLR and the 40/s steady-state response (SSR) represent partial superimpositions of this 40 Hz-band evoked rhythmicity.

49.22

FORTY HERZ (40Hz) AND SENSORY MOTOR RHYTHMS (SMR) AS EEG STATE VARIABLES AND RELATED EVOKED POTENTIALS. C. C. Turbes and G. T. Schneider*. Creighton University, Department of Anatomy, Omaha, NE 68178.

There is evidence that certain brain rhythms may signal the occurrence of different brain states. Support for this hypothesis may be provided by evidence that processing of information is changed during different brain states.

The prestimulus period proceeding the auditory evoked potential (AEP) is converted to a power spectral estimate. These power spectra are used to sort for the AEP when certain power values for the (36-42Hz) and the (11-16Hz) are reached in the prestimulus period of the sensory motor cortex, nucleus accumbens and amygdala.

Attempts are made to measure the performance of the 40Hz and SMR estimators. These studies are made in the cerebral cortical and subcortical regions in cats. In these studies D and L isomers of amphetamine are used to induce a chemical brain state changes. The AEP are selected in the amphetamine altered states based on the prestimulus auto spectra.

49.23

DIFFERENCES BETWEEN HUMAN AND AUTOMATIC WORD RECOGNITION. Henry J. deHaan. U.S.Army Research Institute. Alexandria, VA. 22333.

In order to determine some of the ways in which automatic word recognition differs from human word recognition, humans spoke a list of minimally-different words into a microphone connected to a word recognizer. The overall accuracy was lower for automatic word recognition than for human word recognition. Confusion matrices were prepared and a phonetic analysis was undertaken to determine errors associated with particular phonemes and classes of phonemes. Further analyses were performed on some of the more important distinctive features of speech, such as voicing and place and manner of articulation and some tentative conclusions were drawn about particular features and phonetic classes. The differences between human and automatic word recognition indicate that at the present time there is neither a cognitive model nor brain model adequate to account for human speech perception and language processing.

49.24

DISTORTIONS IN AUDITORY GENERALIZATION OF SUPPRESSION FROM SALICYLATE INDUCED TINNITUS IN RATS. J.F. Brennan and P.J. Jastreboff. Dept. of Psychology, Univ. of Massachusetts at Boston, MA 02125.

Tonal generalization was examined in water deprived rats exposed to continuous low level, wide band noise. 3 days of lick training were followed by acclimation to 5 presentations of the to-be-conditioned stimulus (CS), consisting of 1 min offset of the noise. 2 subsequent sessions introduced Pavlovian conditioned suppression of licking through 5 CSi each terminating with a 0.5 s mild footshock. In 5 extinction sessions, CSi were accompanied by a tonal frequency: 7-, 8-, 9-, 10-, or 11-kHz. 8 rats began daily salicylate injections, 350 mg/kg, 2 hrs prior to the first Pavlovian session, while a second group started injections before the first extinction session. The remaining 8 rats served as saline injected controls. Systematic gradients revealed greater suppression at higher frequencies in the group starting injections before training, while just the opposite occurred in the group starting salicylate after training. These data are explained by the presence of subjective auditory perception from salicylate injections which interferes with the generalization at higher frequency tones. (Supported by DRF, 1988).

50.1

MEASUREMENT OF ACOUSTICAL CROSS-TALK THROUGH THE BARN OWL'S EAR CANAL. T. Haresign and A. Moiseff. Department of Physiology and Neurobiology, University of Connecticut, Storrs, CT 06269.

The Barn Owl's interaural canal transmits low frequency sound between the ears. Computer modeling predicted that a small frequency difference between the ears should produce monaural intensity modulations dependent on the instantaneous phase relationship of the sound between the ears and the length of the interaural canal.

Single unit recordings in the monaural nucleus magnocellularis confirmed that the interaural canal acts as a low pass filter, effectively isolating the ears at frequencies above 4 khz. In cases where acoustical cross- talk was present tonal stimuli differing by 3 Hz. were delivered to the two ears. Intensity sensitive units showed variations in their firing rate reflecting beat frequencies that arise at the ear. Analysis of the response revealed the canal attenuation at the test frequency in addition to the effective acoustical delay through the conductive pathway. These results were used to refine the mathematical model.

Supported by NIH Grant NS21480 to AM.

50.2

SENSITIVITY OF BARN OWL AUDITORY NEURONS TO CONSTANTLY VARYING INTERAURAL TIME DIFFERENCES. A. Moiseff and T. Haresign. Dept. of Physiology and Neurobiology, Univ. of Conn., Storrs, CT 06269.

We have adapted the binaural beat technique of Kuwada *et al* (*Science* 206:586, 1979) to determine the ITD sensitivity of owl auditory neurons.

Tonal stimuli differing by 3 Hz were delivered to each ear. At frequencies where the owl's ears are acoustically isolated such a stimulus subjected binaural cells to a continuous, smoothly varying interaural phase difference but a constant interaural intensity difference. Each action potential was treated as a vector on a unit circle at an angle equivalent to the instantaneous interaural phase difference. From these data mean vector angle was calculated, converted to an equivalent ITD (and termed dynamic ITD), and then compared to the statically obtained ITDs. Static and dynamic ITD measurements were correlated significantly ($r^2 = 0.96$).

The simplicity and rapidity of the dynamic technique allowed us to extend our understanding of ITD sensitivity in the owl with respect to the effects of frequency and interaural intensity differences.

Supported by NIH Grant NS21480 to AM.

50.3

BINAURAL CUES FOR DIRECTIONAL HEARING IN ACOUSTICALLY NON-SPECIALIZED BIRDS. O.N. Larsen, R. Tweedale* and M.B. Calford. V.T.H.R.C., Dept. of Physiol., Univ. of Queensland, 4067 Australia.

We wanted to determine the cues for directional hearing available to birds with symmetrical ears and a functional interaural canal.

Bilateral recording of cochlear microphonics on urethane anaesthetized birds (boobook owls, noisy miners, chicks and quail) was used to measure variations in interaural time differences (ITD) and intensity differences (IID) as a tonal free-field stimulus was moved to different positions in the bird's frontal field.

ITDs varied smoothly with stimulus azimuth for all frequencies and elevations tested and were close to those expected from the pathlength round the head at high frequencies, but up to two times larger at low frequencies. For any frequency and azimuth the largest ITDs were produced in the horizontal plane, successively decreasing with increasing elevations (roughly proportional to cos(elevation angle)). IIDs increased smoothly with azimuth from 0^0 (frontal) to at least $+/-40^0$ for the frequencies and elevations tested. Beyond this range more complex variations occurred. IIDs generally decreased with elevation. Thus binaural cues in "normal" birds are mainly for azimuth localization.

50.4

DEVELOPMENT OF THE DELAY LINES IN NUCLEUS LAMINARIS IN THE BARN OWL. C. E. Carr and R. E. Boudreau*. Dept. of Neurobiology and Anatomy, University of Rochester, Rochester, NY 14642.

In the barn owl, sensitivity to interaural time differences arises in the brainstem nucleus laminaris (NL). Maps of interaural phase difference are formed in the dorso-ventral dimension of NL by interdigitating axons from the isp- and contralateral magnocellular cochlear nuclei (NM). The normal development of these axonal delay lines has been studied during the first month of life when the distance between the ears almost doubles in size, subjecting the bird to constantly changing interaural time differences.

The first NM afferents find their targets in NL a few days before hatching. In the first week posthatch, NM axons continue to arborize and form synapses on NL neurons. Around hatching, large numbers of oligodendrocytes line the borders of NL, and a wave of myelination of the NM axons in NL begins towards the end of the first week posthatch. Myelination is significant for development of delay lines, as the amount of delay mapped in NL depends on their conduction velocity. The dorso-ventral dimensions of NL are also important, as the depth traversed by the NM axons in NL determines the interaural phase difference mapped. Depth of NL increases from about 200μm at hatching to about 600μm at 1 month posthatch. The increase in size is mainly due to continuing myelination of delay lines, although fiber diameter also increases from 1.5 μm at hatching to 2 μm at 1 month posthatch. At this time the circuit in NL is almost adult in appearance. The major anatomical difference between the 1 month old and the adult is the depth of NL. In the second month posthatch, the axonal delay lines grow to an average diameter of 3μm, and NL reaches its adult depth of about 700μm.

50.5

TRANSITION FROM SINGLE TO MULTIPLE FREQUENCY CHANNELS IN THE PROCESSING OF BINAURAL DISPARITY CUES IN THE OWL'S MIDBRAIN. I. Fujita[1,2] and M. Konishi[1]. [1]Div. of Biology, Caltech, Pasadena, CA 91125 and [2]Frontier Research Program, RIKEN, Wako 351-01, Japan.

Interaural level (ILD) and time (ITD) differences are the primary cues for the barn owl to localize the vertical and horizontal positions of sound, respectively. Distinct anatomical pathways in the brainstem process ILD and ITD separately and reach the external nucleus of the inferior colliculus (ICX). We analysed neuronal selectivity to ILD and ITD in the ICX and its input nucleus, the lateral shell of the central nucleus of the inferior colliculus (ICL).

ICL neurons responded to monoaural stimulation of one or both ears. ILD functions were either bell-shaped or monotonic. ICL neurons could be divided into two groups according to their noise ITD curves: one with multiple peaks of identical height (type I, "phase ambiguous") and the other with a main peak and smaller secondary peaks (type II). All neurons with monotonic ILD function belonged to type I. The peak width of bell-shaped ILD functions was narrower in type II than in type I. In type II cells, the peak was sharper with noise stimuli than with tone bursts. As reported earlier, ICX neurons were broadly tuned to frequency and responded maximally to noise at a unique ITD. They responded exclusively to binaural stimuli with a range of ILD. Monoaural stimulation evoked no response or inhibited the cells. ILD curves of ICX neurons were thus sharply peaked.

These results suggest that ILD information is first combined with ITD information in type I ICL cells and then multiple frequency inputs begin to converge in type II ICL cells and eliminate phase ambiguity. The results also suggest that convergence of different frequency channels contributes to neuronal selectivity to ILD and explain the behavioral observation that in the vertical direction, barn owls locate noise sounds much better than tones. (Supported by the Uehara Memorial Foundation and NIH).

50.6

MODEL OF THE COMPUTATION OF AUDITORY STIMULUS DIRECTION AND TUNING TO INTERAURAL LEVEL DIFFERENCE IN THE INFERIOR COLLICULUS OF THE BARN OWL. J.C. Pearson, C.D. Spence*. SRI-David Sarnoff Research Center, CN5300, Princeton, NJ 08543.

The direction of auditory stimuli are represented in a map-like form in the external nucleus of the inferior colliculus (ICx) of the barn owl. The spatial receptive field of each neuron derives from its sensitivity to a particular combination of interaural time delay (ITD) and interaural level difference (ILD). The ICx map is produced by merging the map of ITD in the IC with the map of ILD in nucleus ventralis lemnisci lateralis pars posterior (VLVp). Our two-step model of this process (an early version was presented in *Advances in Neural Information Processing Systems, I*) predicts that the lateral shell of the IC contains a three-dimensional map of cells tuned to ITD, ILD and frequency. The tuning (bell-shaped response curve) of these neurons to ITD and frequency is inherited from their inputs, whereas the tuning to ILD must be derived as the cells in the VLVp are only sensitive to ILD (sigmoidal response curve). ILD is represented by bands of activated cells oriented along the dorsal-ventral axis in the VLVp. Our model produces ILD tuning by effectively computing the spatial derivative of these bands through asymmetric lateral inhibition in the projection to the lateral shell. (Supported by the AFOSR)

50.7

SINGLE UNIT RESPONSES TO MULTIPLE SOUND SOURCES IN THE INFERIOR COLLICULUS OF THE OWL. J.A.MAZER* (SPON: J.ALLMAN). Division of Biology 216-76, Caltech, Pasadena, CA 91125.

Neurons in the external nucleus (ICx) of the inferior colliculus (IC) are tuned to interaural time differences (ITD); tonal stimuli presented at varying ITD's generate maximal responses at a neuron's characteristic delay (CD) and its phase equivalents. A plot of ITD versus response, an ITD curve, shows a characteristic pattern of peaks and troughs in ICx. By mixing two synthetic sounds, each with different delays and different spectral characteristics, I have been able to examine the effects of multiple sound sources on ITD-channels in ICx.

A conventional ITD curve was generated for ICx neurons using a single sound source; a second ITD curve was generated for the same neuron in the presence of a second sound source with a constant ITD at or near the neuron's CD. The presence of this second source appeared to cause a general suppression of activity when compared to the single source responses. This suppression occurred even though the absolute combined intensity of the multiple source stimuli may have been equal to or greater than the intensity the single source stimuli. This suppression was not uniform across ITD-space. The second source appeared to maximally suppress sidepeaks near the CD, while not dramatically affecting the troughs immediately adjacent to the CD. Finally, preliminary observations have shown shifts in peak positions on ITD curves generated in the presence of a second source source.

Previous experiments using a free-field paradigm have shown the existence of auditory center-on surround-off receptive fields in ICx (Knudsen and Konishi,*Science*, 202:778-780, 1978). The cross ITD-channel interactions seen with multiple dichotic sources may correlate with the center-surround fields seen in free-field recordings.

50.8

RESOLUTION OF AUDITORY LOCALIZATION AMBIGUITIES BY SPACE-SPECIFIC NEURONS IN THE OWL'S OPTIC TECTUM. M. S. Brainard*, S. D. Esterly and E. I. Knudsen. Dept. Neurobiology, Stanford Univ., Stanford, CA 94305.

Frequency-specific interaural differences in timing (ITD) and intensity (IID) provide important cues for sound localization. However, for a single frequency, ITD and IID cues are ambiguous in that sounds from many different locations give rise to identical cue values. To localize a sound source, the auditory system must resolve these ambiguities. Space-specific neurons in the optic tectum of the barn owl exhibit restricted receptive fields when stimulated with broadband sounds. Thus, they somehow resolve the ambiguities that are inherent to individual cue values. We studied the response properties of these neurons to determine how this was accomplished.

We used probe tube microphones placed in the ear canals to measure the values of IID and ITD produced by sounds at 178 frontal locations. We then recorded neuronal responses to free-field stimuli, and compared the spatial pattern of responses with the spatial distributions of cue values. Tectal neurons responded to tonal stimuli from regions of space outside the broadband receptive field. These regions of ambiguity occurred only where the values of both ITD and IID cues closely matched their values within the broadband field. Thus, within a narrow frequency band, tectal neurons resolve some of the ambiguity inherent to either ITD or IID alone by requiring the conjunction of specific ITD and IID values. The remaining ambiguities were reduced by the addition of a second tone to the stimulus. At a location from which one tone was excitatory, the addition of a second tone suppressed the response to the first if either the ITD or the IID of the second differed substantially from its value at the center of the broadband field. Thus, by combining information both within and across frequency, localization ambiguities are resolved and neuronal responses become space-specific.

Supported by the NIH: R01 NS16099-09 and NSF: RCD 8758111.

50.9

FREQUENCY-DEPENDENT TUNING FOR INTERAURAL DIFFERENCE CUES IN SPACE-SPECIFIC NEURONS IN THE OWL'S OPTIC TECTUM. S.D. Esterly and E.I. Knudsen. Dept. Neurobiol., Stanford Univ., Stanford,CA 94305

The binaural cues for sound localization, interaural intensity differences (IID) and interaural time differences (ITD), are frequency-dependent. Space-specific neurons in the optic tectum of the barn owl are sensitive both to IID and to ITD, and they respond to free-field broadband sounds only when the source is within a restricted receptive field. To determine whether the tuning of space-specific units to IID and ITD is frequency-dependent, we tested these units with computer-generated narrowband and broadband dichotic stimuli. Units tuned to locations where IID increases monotonically as a function of frequency were found to be tuned to progressively larger IID values as the frequency of a dichotic stimulus was increased. Conversely, non-monotonic tuning to IID was observed for units tuned to locations where IID values vary non-monotonically with frequency. Thus, the pattern of a unit's tuning to IID, as a function of stimulus frequency, agrees well with the frequency-dependent pattern of IID values produced by a free-field sound located in the unit's receptive field. The frequency-dependence of ITD is small at most free-field locations, and most units showed little frequency-dependence in their ITD tuning. Some units, however, did show substantial shifts in ITD tuning with frequency, and these units were tuned to locations where ITD is strongly frequency-dependent. Broadband dichotic stimuli containing identical cue values at all frequencies drove most space-specific units well. However, some units could not be driven by such stimuli. In these cases we found that broadband dichotic stimuli containing appropriate frequency-dependent cue values were effective in driving the units.

These results indicate that the auditory system interprets interaural difference cues in a frequency-specific manner when deriving the location of a sound source.

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RETINA I

51.1

PHOTORECEPTOR RESCUE IN THE RCS RAT WITHOUT PIGMENT EPITHELIAL TRANSPLANTATION. M.S. Silverman* and S.E. Hughes* (SPON: D.H. Eldredge). Central Institute for the Deaf and Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

Transplantation of normal retinal pigment epithelium (RPE) to the subretinal space has been reported to rescue photoreceptors in the RCS rat (Li and Turner, Exp Eye Res, 47:911, 1988; Lopez et al, IOVS, 30:586, 1989). Moreover, the rescue effect was surprisingly large considering the relatively small number of RPE cells transplanted. The reason for this widespread rescue of photoreceptors is not known, nor is the mechanism for outer segment phagocytosis in photoreceptors not apposed to the transplanted RPE cells. This suggests that the rescue effect may not be solely mediated by the transplanted cells. We therefore wished to test whether the transplantation surgery itself might contribute to the rescue of RCS photoreceptors.

Control surgery was performed on 26- to 28-day-old RCS rats. At this age, the RCS rat is just beginning to show degenerative changes and still has almost a full complement of photoreceptors. For these control experiments, we performed the surgery described by Li and Turner for the transplantation of RPE but instead of injecting RPE, we injected saline. We sacrificed the RCS control operates two months following surgery. In the area of the surgery (superior retinal quadrant) the outer nuclear layer (ONL) was up to 8-10 photoreceptor cells thick, while at the extreme inferior margin of the retina the ONL was almost eliminated. The degree of rescue of photoreceptors that we obtained with saline injections (6 cases) was comparable to that obtained with the transplantation of intact RPE by our transcorneal procedure (Silverman and Hughes, IOVS, Suppl 28:288, 1987) or by the dissociated RPE cell injection techniques of Li and Turner or Lopez et al. It therefore appears that the rescue of photoreceptors in this animal does not require transplantation of normal RPE cells, but only the surgical manipulation for such transplantation.

51.2

PHOTOPIC AND SCOTOPIC ERG MODIFICATIONS AS WELL AS NEUROCHEMICAL CHANGES IN STREPTOZOTOCIN-INDUCED DIABETES IN THE RAT. P. Olivier, A. Drumheller and F.B. Jolicoeur. Depts of Ophthalmology and Psychiatry, Univ. of Sherbrooke, Fac. of Med. Sherbrooke, Qc, Canada. J1H 5N4.

Concommitant ERG modifications and retinal neurochemical changes in light and dark adapted rats rendered hyperglycemic (above 300 mg/dl) by the I.V. administration of 65 mg/kg streptozotocin (STZ). ERG parameters were both scotopic and photopic B-wave amplitudes and implicit times generated by various intensities of stimulation. Retinal concentrations of dopamine (DA), its main metabolites DOPAC and HVA, as well as 5-HT, 5-HIAA, and NE were measured using HPLC-ECD. ERG recordings and neurochemical determinations were performed in control and treated animals prior to and at 1,4 and 8 weeks following STZ treatment. To verify the specificity of possible neurochemical alterations in the retina, the same neurochemical measurements in striatum were performed at 8 weeks after injections of STZ. Results indicate that B-wave amplitudes, but not implicit times were differentially affected by these two conditions of light adaptation: amplitudes were significantly enhanced but markedly decreased under photopic and scotopic conditions respectively. Retinal levels of NE, 5-HT and 5-HIAA were not altered. However, significant increases in the ratios of each metabolite DOPAC and HVA to DA retinal concentrations were found. These increases became more pronounced with time. Striatal levels of all amines and metabolites were not affected. Together, these results indicate that STZ induced diabetes in rats produces light adaptation dependent changes in B-wave amplitudes. Using ratios as indices of DA utilization, our result suggest that experimental diabetes produces a specific enhancement of retinal DA turnover which could underlie the observed electrophysiological modifications.

51.3

THE LEAD-INDUCED SELECTIVE ROD DEGENERATION: ROLE OF LIPID PEROXIDATION, VITAMIN E, ZINC AND CALCIUM. D.A. Fox, R.D. Weigand* and R.E. Anderson*. College of Optometry, University of Houston, Houston, TX 77204 and Cullen Eye Institute, Baylor College of Medicine, Houston, TX 77030.

The mechanism by which developmental Pb exposure produces a selective rod degeneration[1] is unknown. Direct evidence for involvement of lipid peroxidation, or a vitamin E or Zn deficiency in photoreceptor degeneration has come from several laboratories. Alternatively, the influx and accumulation of [Ca] in the rod could result from the elevated retinal cGMP[1] and lead to rod cell death. To examine the role of each these factors, we determined the composition of the major fatty acids (22:6 and 20:4) of rod outer segments (ROS), production of lipid hydroperoxides (lipid conjugated diene content) of ROS, Vitamin E content of ROS, and the neural retina [Zn] and [Ca] at end of Pb exposure and in adult hooded rats exposed to Pb (0.2% PbAc to dam) on postnatal days 0-21. There were no indications of lipid peroxidation or of a change in vitamin E. In contrast, retinal [Zn] was decreased 9-14% and [Ca] was increased 18-31%. The decrease in [Zn] and increase in [Ca] correlate with our findings of a decreased rhodopsin content[2] and impaired retinal mitochondrial energy metabolism[3], respectively, in Pb-exposed rats. These divalent cation alterations may mediate the selective rod degeneration. Supported by ES 03183 and EY 04149. 1. EER 46: 597-611 and 613-625, 1988; 2. EER 48: 237-249, 1989; 3. Toxicologist 9: 98, 1989

51.4

RETINAL MÜLLER CELLS IN PIGEONS EXPRESS GLIAL FIBRILLARY ACIDIC PROTEIN FOLLOWING CENTRAL LESIONS THAT DISRUPT NEURAL REGULATION OF CHOROIDAL BLOOD FLOW. B.A. Vana*, M.E.C. Fitzgerald and A. Reiner. Dept. of Anat. & Neurobiol., UT-Memphis, Memphis, TN.

Retinal Müller cells have been shown to express glial fibrillary acidic protein (GFAP) following various types of retinal pathologies but not in normal healthy retina. We have found that choroidal blood flow in birds is regulated by medial Edinger-Westphal (mEW) (Reiner & Fitzgerald, 1989), and EW lesions that interrupt such regulation result in pathological modifications of photoreceptors of the ipsilateral eye (Fitzgerald & Reiner, 1989). To further explore the apparently deleterious effects of mEW lesions on the health of the eye, we have examined the expression of GFAP in Müller cells, using anti-GFAP, following unilateral electrolytic mEW lesions.

One week postlesion, the GFAP label of Müller cells was confined to the nerve fiber layer while at 3 weeks postlesion, labeling spanned from the nerve fiber layer through the inner plexiform layer. In all eyes ipsilateral to mEW lesions, Müller cells and their processes throughout the retina expressed GFAP. No GFAP expression by Müller cells was observed in control eyes.

These results indicate that EW lesions that disrupt regulation of choroidal blood flow lead to the expression of GFAP by Müller cells, a phenomenon typically seen with retinal injury or disease. Thus, disruption of the role of EW in control of choroidal blood flow adversely affects the health of the retina. The expression of GFAP by Müller cells may play a role in the adaptation of the retina to this stressed state. Supported by EY-05298.

51.5

FOVEAL GLARE EFFECT ON RHESUS SPATIAL VISION FOR TRANSIENT AND PROLONGED VIEWING. D.O. Robbins, H.Zwick* and R.C. Long*. Ohio Wesleyan University, Delaware, OH 43015 and Letterman Army Institute of Research, San Francisco, CA 94129.

The transient and more persistent deficits in spatial acuity and contrast sensitivity were examined in nonhuman primates following exposure to small spot foveal laser irradiations. Retinal exposure durations varied from being relatively brief to prolonged for both on- and off-axis exposures. In these experiments, levels as low as 1000 times below the Maximum Permissible Exposure (MPE) were utilized for spot sizes of less than 50 microns on the retina.

In previous studies single 15 nsec pulses both below and above the MPE produced prolonged deficits suggesting involvement of nonpigmental factors. In comparison, the current study employed extended duration, low-energy exposures that produced deficits suggestive of a combination of retinal edema and photochemical/neural saturation of retinal receptor mechanisms. Both acuity and contrast sensitivity were immediately affected by the onset of exposure and resulted in periods of significantly depressed visual functioning. Full recovery typically occurred upon termination of relatively low level exposures but this trend is not expected as energy levels increase. These results may be compared to visual problems associated with extended source viewing of lasers in a variety of present day laser applications.

51.6

LOCALIZATION OF RETINAL HEAT SHOCK (STRESS) PROTEIN AND mRNA INDUCED BY HYPERTHERMIA. M. Tytell[1], M.F. Barbe[2], D.J. Gower[3], and I.R. Brown[4], [1]Anatomy, Bowman Gray Sch. Med., Wake Forest U., Winston-Salem, NC; [2]Anatomy, Med. College Penn., Philadelphia, PA; [3]Neurosurgery, Univ. Oklahoma Health Sci. Ctr., Oklahoma City, OK; [4]Zoology, Univ. Toronto, Scarborough Campus, Toronto, Ontario, Canada.

A 4-5° C rise in body temperature for 15 min makes the retina of the albino rat resistant to light damage, while stimulating the accumulation of the 70,000 Dalton heat shock (stress) protein (HSP70; Barbe et al., Science 241:1817, 1988). The relationship between that resistance and HSP70 was examined further in retinal sections from control and hyperthermia-treated rats by in situ hybridization for the HSP70 mRNA and immunohistochemical detection of the protein itself. Autoradiographic localization of the HSP70 mRNA with a labeled riboprobe showed that it was increased primarily in the photoreceptor nuclear layer, especially between 4 and 18 hrs after hyperthermia. An anti-HSP70 monoclonal antibody (W.J. Welch, Univ. Calif., San Francisco) showed that the protein also was increased at those times, particularly in the layers including the cytoplasm of the photoreceptors; however, the retinal ganglion cells (RGCs) and inner plexiform layer (IPL) contained elevated HSP70 as well. These results support the possibility that the induction and accumulation of HSP70 in the photorecetors is responsible for their resistance to light damage after hyperthermia. Furthermore, the increase in HSP70 in the RGCs and IPL without a similar rise in the mRNA suggests that some of the photoreceptor HSP70 may be transferred to those layers. Supported by grants from the NEI (EY07616; MT) and MRC (Canada; IRB).

51.7

CYTOSKELETAL ALTERATIONS OF THE BASAL SURFACE OF THE RETINAL PIGMENT EPITHELIUM OF PEARL MUTANT MICE. M. A. Williams*, Dept. Biol. Sci., Purdue Univ. and D. Drenckhahn*, Dept. Cell Biol., Univ. of Marburg, F.R.G. (Spon: G. Das)

Lack of infoldings over 20% of the basal surface of the retinal pigment epithelium (RPE) of pearl mice could affect transport of macromolecules and ions. Electron microscopic immunolocalization and morphometric analyses were used to analyze the distribution of components of the cytoskeleton associated with the basal surface of the RPE. Substantial amounts of bound antibodies to actin, α-actinin and vinculin were localized over the basal infoldings of pearl and wild-type RPE. Surfaces of the pearl RPE which lack infoldings exhibited virtually no bound antibodies. The normalized density of coated vesicles (CVs) over the basal surface was four times greater for pearl compared to wild-type due to the high density of CVs over the flattened surface of the pearl RPE. Cytosolic pH (pHi) < 6.8 has been shown to prevent CV disassembly and endocytosis. If localized acidic pHi contributes to the high density of CVs of the pearl RPE, alkalinization should reduce the density. The RPE of pearl mice, perfused with PBS containing 20 mM NH_4Cl, showed a 55% reduction of CV density at the flattened surface compared to controls perfused with PBS. Therefore, CVs may be "stabilized" at the flattened surface due to localized pHi reduction, consequently excluding actin filaments from associating with the surface.

51.8

ULTRASTRUCTURAL LOCALIZATION OF BLOOD-RETINAL BARRIER (BRB) BREAKDOWN IN EXPERIMENTAL MODELS OF DIABETES. S.A. Vinores, R. McGehee*, A. Lee*, C. Gadegbeku* and P.A. Campochiaro*. Dept. Ophthalmol., Univ. of Virginia Sch.Med., Charlottesville, VA 22908

Breakdown of the BRB is associated with macular edema, the major cause of visual morbidity in diabetics. Immunohistochemical localization of extravascular albumin demonstrated that disruption of the inner BRB, composed of the retinal capillary endothelium (RCE), predominates in human diabetic retinopathy, but outer BRB disruption (RPE) also occurs (Am J Path 134:231, 1989). To explore the nature of the BRB disruption, we performed electron immunocytochemical staining for albumin in the retinas of diabetic and galactosemic rats. In these models, inner BRB breakdown also predominates with occasional disruption of the outer BRB. In the inner retina, albumin was demonstrated on the abluminal side of the RCE in intercellular spaces, and within pericytes, astrocytes, and some neural cells. In the outer retina, albumin was detected in the subretinal space and within photoreceptors. Albumin did not appear to cross RCE or RPE cell junctions, but was demonstrated (although infrequently) in RCE and RPE cytoplasm of diabetic, galactosemic, and aged rats. These findings suggest that specific sites of BRB compromise are infrequent, but once albumin has penetrated the RCE or RPE, it freely fills intercellular spaces and permeates the membranes of cells not directly involved in maintenance of the BRB.

51.9

CHOROIDAL VASOACTIVE INTESTINAL PEPTIDE IMMUNOREACTIVITY IS INCREASED IN DYSTROPHIC RATS. M.E.C. Fitzgerald, R.B. Caldwell, and A. Reiner, Dept. of Anatomy and Neurobiol. UT-Memphis, TN 38163 and Dept. of Anatomy, Medical College of GA., Augusta, GA 30912.

In the dystrophic Royal College of Surgeons (RCS) rat, vascularization of the retinal pigment epithelium (RPE) is associated with retinal neovascularization (Weber *et al.*, '89) and increased retinal vascular density (Caldwell *et al.*, '89), suggesting that RPE alterations stimulate retinal vascular proliferation. Since RPE alterations could also affect choriocapillaris and choroidal vessels, we have now studied these vessels in RCS and control rats during the period that retinal vascular changes are observed (7 mos and later).

Light microscope morphometry of numerous 1.25 mm^2 areas showed no significant differences in number or size of choriocapillaris or coroidal vessels (choriocapillaris: RCS=14.6 vessels, 15 μm^2, control=12.2 vessels, 16 μm^2; choroid: RCS=8.0 vessels, 35 μm^2; control=6.0 vessels, 46 μm^2, p>.2, t-test). Electron microscopic study showed similar vascular morphology in both groups, but indicated that vascular innervation was increased in RCS rats. Nerve fibers with varicosities containing densely packed round vesicles and a few large dense core vesicles were more abundant in the RCS choroid. Such fibers were less common in control rats. We next studied the distribution of choroidal neurotransmitters using immunofluorescent techniques. In both groups antibodies for substance P (SP), dopamine-ß-hydoxylase (DBH) and vasoactive intestinal peptide (VIP) labeled thin fibers with varicosities. Immunoreactive fibers were located on the vessel walls and in the extracellular matrix between vessels. VIP positive fibers were more abundant in RCS rats than the controls in the same area where electron microscopy showed increased fibers and varicosities. Numbers of fibers immunoreactive for SP and DBH were similar in both groups.

Because released VIP is likely to dilate choroidal vessels, this change may allow for increased blood flow in the absence of vascular density changes. This could reflect a long term adaptation to RPE alterations in the RCS retina. Supported by NIH-EY-04618 (RBC), Juvenile Diabetes Foundation (RBC) and NIH-EY-05298 (AR).

51.10

VARIABILITY IN THE PATTERN ELECTRORETINOGRAM DUE TO EYE SQUINTING. J. Mishra, E. Reszel* and T.C. Prager*. Dept. Of Ophthal., Univ. of Texas Med. Sch., Houston, TX 77030.

Reliability of pattern electroretinogram (PERG) technique depends upon identification and careful study and control of the variables affecting the PERG amplitude. In this study we focused on the effect of eye squinting on PERG.

A total of 14 normal control subjects, between 18-56 yrs, with 20/20 visual acuity, were tested. The pattern ERG was recorded monocularly with a DTL thread electrode. The visual stimuli was 30' black and white checks with 98% contrast reversing at 8.3 hz, stimulating the central $16^0 x 19^0$ retina with 200 presentations. The waveforms were replicated and combined into a single evoked potential. Subjects were instructed to 1) keep both eyes open during recording 2) to squint the contralateral occluded eye.

Results show that squinting produced variability in the PERG waveforms with a significant reduction in the amplitude (normal amp=3.13uv, 0.64 SD squinting amp=1.80uv, 1.03 SD). Changes in the shape and latency of the PERG waveform were also noted. Analysis of Variance demonstrated a significant value for amplitude change (P=.0004) but not latency (P=.0925). Since squinting masks the inner retinal layers response it is important to instruct the patients to keep both eyes open to minimize the effect of eye muscle tremor on the PERG.

51.11

CHEMICAL SENSITIVITIES OF THE SCOTOPIC THRESHOLD RESPONSE OF THE CAT ERG. F. Naarendorp and P. A. Sieving. Kellogg Eye Center, University of Michigan, Ann Arbor, MI 48105

Previous work from several laboratories has demonstrated a proximal retinal origin for the Scotopic Threshold Response (STR) of the Electroretinogram (ERG) of cat (Frishman and Steinberg, J. Neurophys., 1989; Sieving, et al. J. Neurophys., 1986) and monkey (Wakabayashi et al. Invest. Ophthal., 1988). In this communication we present our most recent studies done in cat on the chemical sensitivity of the STR to neuropharmacological agents that are known to have active sites on third order neurons. Specifically, we evaluated effects of Glycine, Gaba, Dopamine, Acetylcholine, Serotonin and their antagonists given by intravitreal injection. Our results with Gaba and Glycine showed selective, but reversible suppression of the STR; other neurotransmitter analogues showed considerably less selective influence on the STR. As reported last year, Strychnine enhanced the STR and completely blocked Glycine-induced effects. Gaba-effects on the STR, however, were only partially blocked by Gaba-a receptor antagonists. We now report that Bicuculline, when combined with a dopamine antagonist, blocks Gaba-effects on the STR and PII, suggesting that bicuculline induces dopamine release in the cat retina. Dopamine in very low concentrations has noticeable suppressive effects on STR and PII. Preliminary results suggest that at scotopic levels of illumination, dopamine may modulate the potency of selected neuroactive substances in the inner retina of the cat. NEI R01-06094

51.12

Q-WAVESHAPE ANALYSIS OF SINGLE TRIAL FLASH EVOKED HUMAN RETINAL POTENTIALS. D.S. Weiss* and S. Reinis. (SPON: J.P. Landolt). Department of Psychology, University of Waterloo, Waterloo, ONT., Canada.

Sets of forty single trial events were recorded from human volunteers at the School of Optometry of the University of Waterloo. The retinal potentials were recorded using a nylon fiber electrode. Twenty potentials were recorded with the subjects reclining in a supine position; twenty potentials were recorded with the subjects tilted 40 degs head downward. In the latter condition, changes in intra-occular blood perfusion pressure are known to alter the waveform morphology of *averaged* retinal potentials. The purpose of this study was to determine whether or not such alterations would be evident in single trial potentials as well.

The data were evaluated using a method of pattern recognition called Q-Waveshape Analysis, a system for cross-validating complete linkage Euclidean distance cluster analysis with rotated factor analysis of waveforms. The matrix of *factor loadings* in association with the *cluster dendrogram* provides the separation of single trial events into clusters. The *factor scores* may be plotted as a function of time to provide a standardized representation of idealized or *cluster specific* waveforms. Taken together, these procedures may confirm the cluster solution, establish limits on the number of acceptable clusters, help visualize important waveform characteristics which separate the clusters, and provide a measure of variability for the solution.

In all cases, Q-Waveshape Analysis divided the single trial data into two meta-clusters which corresponded exactly to the two categories of body orientation. Each analysis was replicated with analogous results. It is suggested that Q-Waveshape Analysis may be an accurate and efficient method of evaluating patterns of variation and change in large sets of single trial data without the concomitant loss of waveform information associated with ensemble averaging and other univariate approachs to biological signal analysis. Furthermore this method of analysis may be helpful in assisting the researcher or clinician divide seemingly homogenous data into more uniform subgroups prior to ensemble averaging, thus insuring a consistent preservation of relevent biological signal.

VISUAL CORTEX I

52.1

COMPARISON OF VISUALLY EVOKED POTENTIALS GENERATED WITH LIGHT FLASH AND OPTIC NERVE SHOCK IN RAT. I. Siegel and D.F. Sisson. School of Life and Health Sciences, University of Delaware, Newark, DE 19716 and J.H. Lucas. U.S. Army Engineering Lab., Aberdeen, MD 21005.

VEPs from OC1, generated by light flash (fVEPs) of optic nerve shock (sVEPs), were compared in acute chloral hydrate anesthetized rats. Shapes of both VEP types were similar when corrected for latency differences. The first components of both fVEPs and sVEPs were positive potentials (P_1) with 27 and 4 msec latencies, respectively; the second were negative (N_1) with 33 and 12 msec latencies; the third were positive (P_2) with 55 and 28 msec latencies. Later components of the fVEPs did not coincide with sVEP components.

Current source density analysis was used to compare the early components of fVEPs and sVEPs. Current sinks with latencies coinciding with P_1 were distributed in layer IV and superficial layer V; their sources were found in layer I, superficial layer II and layer VI. Sinks with latencies coinciding with N_1 appeared in layer II/III and along the layer V-VI border; sources were in layer IV. Sink with latencies coinciding with P_2 were located in layers I and VI; sources were found in layers II and V. This distribution of sinks and sources indicates that the initial components of fVEPs and sVEPs are generated by the same cortical circuitry.

This work was supported in part by ARO Contract DAAL 0388K0043.

52.2

HUMAN AND RAT VISUAL EVOKED POTENTIALS IN RESPONSE TO STIMULUS CONTRAST. W.K.Boyes and H.K. Hudnell. Neurotoxicology Div. USEPA, RTP, NC 27711.

The extent to which visual evoked potentials (VEPs) recorded from humans and rats reflect similar visual processes is unknown. Previously, we compared steady state pattern on/off VEPs in the two species by varying stimulus spatial and temporal frequency (Boyes and Hudnell, SFN abstract 1988). The spectral amplitude at the stimulus rate (1F) was bandpass in spatial frequency (SF) profile, whereas that at twice the stimulus rate (2F) was low pass for both species.

The current studies examined the response to contrast. VEPs were recorded from rats and humans using vertical sinusoidal gratings at several SFs (0.05-0.8 cpd(rat); 0.5-16.0 cpd(man)). Stimuli were temporally modulated at 3 Hz. The range of contrast values was adjusted for each SF.

The data were similar for both species in that: (1) the 2F response reached higher amplitude than 1F; (2) at low contrast, the 1F and 2F responses predominated at high and low SFs, respectively; and (3) neither species showed response amplitude saturation for either 1F or 2F as contrast increased. These results suggest that, although the spatial resolution limit is lower in rats than humans, qualitatively similar processes operate in both species.

52.3

HUMAN AND RAT VISUAL EVOKED POTENTIALS IN RESPONSE TO DIAZEPAM. H.K. Hudnell and W.K.Boyes. Neurotoxicology Div. USEPA, RTP, NC 27711.

The extent to which visual evoked potentials (VEPs) recorded from humans and rats reflect similar visual processes is unknown. One avenue of comparison is to examine the response to drug treatments.
VEPs were recorded before and after diazepam treatment from both rats and humans. Stimuli were 30% contrast, vertical, sinusoidal gratings at several spatial frequencies (SFs) (0.05-0.8 cpd(rat); 0.5-8.0 cpd(man)). Gratings were temporally modulated in an on/off fashion at 5 Hz. Dosage was 0-0.5 mg/kg for rats and 0 or 10 mg for humans.
The data for both species showed a response depression of spectral amplitude at twice the stimulus rate (2F). The rat 1F response was generally unaffected, whereas the human 1F was slightly depressed. In addition, the human study, which used a battery of VEP tests, found effects of diazepam on transient flash and pattern VEPs. Similar flash VEP changes have been reported previously for rats (Dyer, SFN Abstract, 1987). These results suggest that diazepam, thought to potentiate GABA mediated inhibition in sensory cortex, similarly affected the two species' visual function.

52.4

INTERACTIONS OF AMBIENT LIGHT AND FLASH INTENSITY ON N_{160} PEAK AMPLITUDE OF FLASH-EVOKED POTENTIALS (FEPs). D.W. Herr*[1], V.T. Griffin*[2], and R.S. Dyer. Neurotoxicol. Div., US E.P.A., and [2]NSI, RTP, NC 27711.

The N_{160} peak of FEPs may reflect a sensitization and/or habituation process (Dyer, R.S., Physiol. & Behav., 45:355-362, 1989). The influence of the "contrast" between ambient lighting and flash intensity in modulating the N_{160} peak was examined. Long-Evans hooded rats were implanted with epidural electrodes over the visual cortex and allowed to recover. Overhead lighting (0, 115, 250 lux) and flash intensity (409, 81, 24, 5 lux-sec) were between-groups factors. On each of 13 consecutive test days 64 flashes/day (0.3 Hz) were presented. The N_{160} peak amplitude (from baseline) increased over several days, reaching a plateau by day 13. Overhead lighting and flash intensity interacted in influencing N_{160} development. Low flash intensities produced larger N_{160} amplitudes when rats were tested in the dark than in a lighted chamber. Conversely, bright flash intensities promoted larger N_{160} amplitudes in lighted chambers than in the dark. Data suggest that optimizing "contrast" between ambient lighting and flash intensity will maximize development and amplitude of the N_{160} peak, and that processes in addition to sensitization may be involved. [1]DWH
Supported by a NRC Research Associateship.

52.5

DOES BILATERAL CORTICAL REPRESENTATION OF TEMPORAL VISUAL FIELDS EXIST IN MAN? C Iadecola, M M Conte and J D Victor. Dept of Neurology Cornell Univ Med Coll, New York NY 10021. Ganglion cells of the temporal retina project to the ipsilateral area 17 while those of the nasal retina project to the contralateral area 17. However, in primates there are cells in the nasal half of the fovea which project to the ipsilateral area 17 (Leventhal et al., Science 240:66, 1988). If this double representation is physiologically significant, macular viewing of homonymous hemifields (HFs) would produce unequal patterns of cortical activation a portion of area 17 ipsilateral to the stimulus would be activated through the ispsilateral, but not the contralateral, eye. We studied visual evoked responses (VERs) elicited by HF stimulation in man to determine whether such differences between homonymous HFs could be detected. Stimuli (4 min checks, 40% contrast, 8.4Hz reversal) were delivered monocularly in 30 sec episodes to each HF. VERs were recorded from 9 electrodes placed at 2.5 cm intervals along a horizontal line centered at OZ. Scalp topography of VERs was characterized by the spatial weights corresponding to the first principal component extracted from the 8 bipolar pairs. In 3 of 4 subjects there was a statistically significant ($p<0.02$) eye dependence of the VER topography elicited from homonymous HFs. Such eye dependence is consistent with models of retino-calcarine projections. We conclude that the bilateral cortical representation of temporal visual fields found in primates is likely to be present also in man. Thus, postchiasmatic lesions may cause an incongruous loss of macular vision resulting in varying degrees of macular sparing.

52.6

A Low Dose Barbiturate Effect on the Cat Visual Evoked Response. A.W. Kirby and A.T. Townsend*. USAARL, P.O. Box 577, Ft. Rucker, AL 36362.

We previously have reported a preferential loss to low spatial frequency stimulation in the cat visual evoked response (VER) following cholinesterase inhibition, while others report uniform reduction under similar conditions. The only apparent difference was that others added supplemental surital (thiamylal sodium, 1.0 mg/kg/hr) to the maintenance anesthesia. We report here results which show that the nature of VER reduction changes, based upon the presence of barbiturate, even at this low dose.

Anesthesia was induced in 4 adult cats with i.v. surital, and they then were prepared for VER recording. Each cat received a continuous infusion of surital (1 mg/kg/hr), and was maintained on nitrous oxide. Following capture of baseline VERs, physostigmine was given (0.4 mg/kg). The VERs showed a uniform reduction. Surital then was removed from the infusion mixture and the animal allowed to recover for 3 hours. The physostigmine dose then was repeated and the VER showed a preferential loss of low spatial frequencies. We believe this demonstrates the effect of even small amounts of barbiturate on central neural processing.

52.7

RECEPTIVE FIELDS OF NEURONS AT THE CONFLUENCE OF AREAS 17, 18 AND 20 IN THE CAT. Bertram Payne and Donald Siwek*. Department of Anatomy, Boston University Medical School, Boston, MA 02118.

In the course of electrophysiological studies on the boundary between areas 17 and 18 on the posterolateral gyrus of the cat we recorded the activity of neurons with very unusual receptive field properties. The most notable feature of the receptive fields was their extraordinary large size which could range in size from 40 or more degrees per side to half or even most of the visual field of the contralateral eye. At least part of each receptive field could be activated by both eyes, and small (2°) or large, flashing or moving rectangles of light were potent stimuli, and the neurons exhibited spatial summation but not internal inhibition when the stimuli were increased in size within the receptive field. In addition, some neurons had receptive fields with distinct "hot spots". Subsequent anatomical and histological identification of the recording sites revealed that the neurons studied were located at the ventral end of the posterolateral gyrus where it blends with the posterior suprasylvian gyrus. In this region portions of areas 17, 18, 20a and 20b lie adjacent to each other in a rather complex arrangement with each area bounded at least partially by at least two of the other areas. In reconstructing the region in more detail it became clear to us that the neurons we had studied lay in a common transition zone separating all four of these areas. While the morphological basis for these large receptive fields is not known, we propose that the large receptive fields may result from disturbances in the individual areal mapping functions as the individual representations merge in this region. (Supported by EY06080 and EY06404)

52.8

A SINGLE CONTINUOUS VISUAL AREA EXTENDS ALONG THE ANTERIOR ECTOSYLVIAN SULCUS (AES) IN CAT. Gy. Benedek*, Y. Katoh*, G. Sáry*, G. Kovács*, and M. Norita. Dept. Physiol., Albert Szent-Györgyi Med. Univ., Szeged, Hungary and Dept. Anat., Fujita-Gakuen Med. Univ, Toyoake, Japan.

Two distinct visual areas with similar physiological characteristics have been described recently: the anterior ectosylvian visual area (AEV) and the insular visual area.

Extracellular microelectrode recording was performed along the AES in pentobarbital anesthetized cats. We were successful in finding visually sensitive cells along the whole extent of the sulcus when we approached the area from an oblique angle, which eliminated the technical limitations encountered in the earlier experiments. Two main types of visually responsive cells were recorded: "tonic" cells discharging steadily while a light stimulus was moving in the fairly large and uniform receptive fields of the cells, and "phasic" ones that respond to a moving light stimulus only with a short burst of discharge. The two types of cells exhibited showed different velocity and length sensitivity. The cells had a rather homogeneous distribution pattern, although a predominance of "phasic" cells could be seen in the caudal one-third of the sulcus and in the deep sulcal regions. Cells with a sensitivity to squares increasing or decreasing in size were often found.

We conclude that there is only one visual area that plays a role in movement-detection in this part of the feline brain.

52.9

VISUAL PROCESSING IN AWAKE MACAQUES UNDER ACTIVE AND PASSIVE CONDITIONS. C.E.Schroeder, S.J.Givre, C.E.Tenke, J.C.Arezzo and H.G. Vaughan Jr. Neurosci. and Neurol. Depts., Albert Einstein Coll. Med., Bronx, NY.

Profiles of visual evoked potentials (VEP), current source density (CSD) and multiunit activity (MUA), elicited by bar gratings subtending 4^{o} radial to a fixation point were obtained from V1 and V2 using multicontact electrodes. Pattern responses were recorded during a "passive" fixation task, and during an "active" task requiring a match between a feature of the bar grating and a cue in the fixation stimulus. Initial activation in V1 occurs in thalamorecipient laminae, followed by responses in supra- and infragranular laminae. Initial activation of V2 is reflected in two groups of responses in the middle laminae, and lags the earliest activity in V1 by only a few ms. Activity in both V1 and V2 extends well beyond 200 ms poststimulus. The most prominent active/passive effect in V1 is increased amplitude and duration of activity in supragranular laminae. Enhancement of the later response in upper 4C was noted occasionally. Similar enhancement was noted in V2, but any gradient of effect is not yet clear. Preliminary results from LGN do not show active/passive effects in the time frame of the initial retinal volley; such effects in LGN occur at longer latencies than those in V1. The time frame of activity across LGN, V1 and V2 is essentially concurrent. Motivational/attentional variables may affect visual processing at its ear-liest cortical stage.

52.10

REACTIVATION RESPONSES IN STRIATE AND EXTRASTRIATE VISUAL CORTEX DURING A PERIPHERAL ATTENTION TASK. B.C. Motter* (SPON: S. Mitchell) V.A. Med. Ctr. & Depts. Physiology & Neurosurgery, SUNY-HSC @ Syracuse, Syracuse, NY 13210.

A reactivation of neural activity occurs to a stimulus already present within the visual receptive field of V1 & V4 neurons when the stimulus becomes a potential target for a saccadic eye movement (Fischer & Boch, Brain Res. 345(1985)111-123; Boch, Exp. Brain Res. 64(1986)610-614). A similar reactivation response, reported here for those same areas and for areas V2 & V3, occurs in a paradigm requiring maintained fixation of a central target and peripheral attention to a cued spatial location. Reactivation activity was observed to a small, non-optimal cue used to direct attention to one of several locations in the visual field at which the macaque monkey was required to make a bar orientation discrimination. The cue was present in the receptive field for 500-1200 msec before it became 'selected' by the disappearance of several other false cues. The onset latencies of the reactivation response were found to be nearly the same in each cortical area, about 150 msec or 2-3 times the response latency to cue onset. The dissociation of the reactivation response from saccades and from the explicit release of visual fixation suggests it reflects a more general attentional phenomena.

Support: VA Med. Res. Svc. and EY07059.

52.11

FACTORS AFFECTING PURSUIT RECOVERY FOLLOWING LESIONS VISUAL AREA MT. Dwayne S. G. Yamasaki and Robert H. Wurtz. Lab. of Sensorimotor Research, National Eye Institute, Bethesda, MD 20892.

Punctate chemical lesions in the middle temporal area (MT) of the monkey produce deficits in pursuit eye movements that recover within about a week. We further investigated the effects on the rate of recovery of: 1) type of lesion, 2) visual motion experience after the lesion, and 3) size of the lesion.

Monkeys (*Macaca mulatta*) were trained to pursue a spot of light moving at 16^{o}/sec in a step-ramp paradigm. Small chemical lesions were produced by injecting 2-6 µl of ibotenic acid (IBO, 4 hemispheres) or AMPA (2 hemispheres), and one large AMPA lesion (51.8 µl) was made in the superior temporal sulcus to remove all of MT and much of MST. An electrolytic lesion (500 µA/15 min, 5 sites) was also made in one monkey.

1. Pursuit recovery was only slightly longer for AMPA (3-11 days) than for the IBO (2-5 days) lesions. Recovery still occurred after the electrolytic lesion (9 days), suggesting that the recovery seen after previous chemical lesions is not due to an artifact of the chemical lesion method. 2. To study the effect of visual experience on recovery, we made a lesion (2 µl, IBO) in MT and placed the monkey in a light-tight room illuminated by a 4 Hz strobe light. When compared to the recovery time of previous IBO lesions, this recovery took longer: 10 days for eye speed, and 21 days for eye position error. This effect indicates that experience is a factor that facilitates the recovery process. 3. Recovery was dramatically delayed following a large lesion. At post lesion day 175, the monkey was not yet fully recovered. This suggests that previous recoveries were facilitated by active processes occurring in areas removed by the large lesion. Furthermore, immediately after the lesion eye speed was not reduced to zero (0.1 ± 0.3 to 4.4 ± 0.8^{o}/sec), suggesting that other areas normally contribute only a small amount of information on target velocity to the pursuit system.

52.12

EFFECT OF THE PERIPHERAL VISUAL FIELD ON DISPARITY SENSITIVE CELLS IN CORTICAL VISUAL AREA MST. J. P. Roy and R. H. Wurtz. Lab. of Sensorimotor Research, National Eye Institute, Bethesda, MD 20892.

We have previously shown that neurons in the medial superior temporal area (MST) of extrastriate visual cortex are sensitive to visual motion at different disparities in the central region of their visual receptive fields. In the present experiments we determined the effect of disparity and other factors in peripheral regions of the visual field on the response of these cells to stimuli falling in the central visual field. While monkeys (Macaca mulatta) fixated on a spot on a tangent screen and the position of both eyes was measured, a random dot pattern moved within the central 80 degrees of the visual field. This pattern was presented at different disparities by using a red-green color anaglyph viewed by the monkey through a red or green filter over each eye. We identified the central region of the receptive field and moved the pattern in this region in the preferred direction, speed, and disparity (corresponding usually to near or far) for the particular MST cell under study. We then added outside of this central region a second random dot pattern moving in the same or the opposite direction as the center, with different speeds, and at different disparities (near, far, or zero). The effect of these variables in the peripheral regions influenced the response of different neurons to varying degrees, but the factors identified as more effective were: 1) Motion in the peripheral area in the direction opposite to the preferred direction of the central area increased the discharge rate of the cells. 2) With such antiphase motion, motion of the periphery at a depth that was the same or nearer than that of the center was the most frequently effective. 3) Motion of the periphery at a speed higher than that in the central region was usually more effective than motion at the same speed in the center and periphery. These characteristics are all appropriate for cells that contribute to the differentiation of figure from ground.

52.13

RADIAL AND AXIAL DIRECTIONALITY IN CORTICAL VISUAL AREA MST. C. J. Duffy and R. H. Wurtz. Lab. of Sensorimotor Research, National Eye Institute, Bethesda, MD 20892.

A subset of neurons in monkey medial superior temporal area (MST) selectively respond to the radial motion of expanding or contracting patterns (Saito, et al., *J. Neurosci.*, 1986). We have studied the receptive fields of these neurons in the awake monkey (Macaca mulatta) to understand the mechanisms of their response selectivity and their possible role in conveying information on optic flow fields. The visual receptive fields studied were 50 to 100 degrees wide, and centered up to 50 degrees from the fixation point. They were mainly contralateral but extended as far as 50 degrees into the ipsilateral visual field. Responses to wide field motion were more robust than responses to spot motion. We have confirmed that many of these neurons have radial selectivity for inward or outward motion covering the receptive field. We also found that when the stimulus was limited to a 10 or 30 degree square in different areas within the receptive field, the radial selectivity was frequently maintained. Therefore neither a change in the position of the center of radial motion nor changes in the local directions and speeds of motion eliminate the radial selectivity. Radially selective neurons also frequently showed preference for motion along a single axis during full field stimulation. When small parts of the fields were stimulated, different axial directions frequently were preferred in different parts of the receptive field. There was a tendency for this to vary in systematic ways throughout the visual field. For example, inward axial motion tended to be preferred in fields that preferred inward radial motion; outward axial motion in fields that preferred outward radial motion. We conclude that radial and axial selectivity are nearly coextensive but their relative strength varies across the receptive field. This may reflect the overlap of superimposed fields of selectivity or one of these properties may be more fundamental, creating others by the interactions of parts of the field.

52.14

ATTENTIONAL INFLUENCES ON NEURONS IN PARIETAL CORTEX OF THE MACAQUE. Caroline Kertzman and David Lee Robinson. Laboratory of Sensorimotor Research, National Eye Institute, Bethesda MD 20892.

Clinical and physiological studies have linked parietal cortex with attentional functions. We studied the activity of neurons in this area while a monkey performed a variety of attentional tasks. In one paradigm the monkey fixated a spot of light and contacted a bar when target lights appeared. Reaction times for responding to the onset of visual targets were modified by antecedent visual cues. Faster reaction times occurred when the cue correctly predicted the subsequent target location. The cue is hypothesized to control the direction of attention. We found that parietal cells responded differently to the identical target depending on cue validity. The intensity of the modulation varied as a function of how close in time the target followed the cue. For some cells we found enhanced responses in a saccade task: neurons responded better to a light when it was the target for an eye movement than when the same light was presented during continuous fixation. Cells modulated in the cuing paradigm were not always enhanced in the saccade task. These data show that neurons in parietal cortex are influenced by attentional processes. Since damage to this region of cortex dramatically alters performance in the cuing paradigm, these neurons probably participate in attentional shifts.

52.15

COLOR SELECTIVITY OF NEURONS IN THE INFEROTEMPORAL CORTEX OF THE MONKEY. H. Komatsu, S. Kaji*, S. Yamane*, K. Kawano and T. Ideura*. Neuroscience Section, Electrotechnical Lab., Tsukuba, Ibaraki, 305, Japan.

In an attempt to study the role of the inferotemporal cortex (IT) on color recognition, we recorded single unit activities from IT of an awake macaque monkey and examined their color selectivity. Spots or rectangles with various hue, saturation and luminance were used as visual stimuli. The monkey fixated a stationary spot to detect its dimming. Visual stimuli were presented on a monitor display while the fixation spot was blinked off for a short period of time. In the anterior part of IT, we found a cluster of cells with high selectivity to the color of the stimulus. These cells were activated by stimuli with certain range of hue and saturation. In some cells, such a range was constant regardless of the luminance of the stimuli, but in others, the preferred range shifted with the change of the luminance of the stimuli. Preferred hue varied from cell to cell. A small number of cells in this area were activated by non-colored stimuli (white, gray and black) but responded little to colored stimuli. Activities of cells in this area were only weakly influenced by the size or shape of the stimuli. The receptive fields of these neurons always included the fovea, and most of them extended 10 degree or more from the fovea in any direction. We concluded that these cells are mainly involved in the processing of the color information.

52.16

RESPONSES OF INFERIOR TEMPORAL NEURONS IN A SHORT-TERM MEMORY TASK. Lin Li* and Robert Desimone, (SPON: J. Bachevalier) Lab. Neuropsychology, NIMH, Bethesda, MD 20892.

Previous studies have found that inferior temporal (IT) neurons show maintained, stimulus-specific activity during the delay interval in delayed matching-to-sample tasks (Fuster and Jervey, '82, Miyashita and Chang, '88), suggesting that the maintained activity "holds" the representation of the sample, thereby mediating short-term or recency memory. To test this possibility, we recorded from 104 neurons in the ventral aspect of middle and anterior IT cortex in 1 rhesus monkey. A sample stimulus (not trial-unique) was followed by a series of up to 5 different test stimuli, presented successively, and the monkey was required to respond when one of the test stimuli matched the sample. All stimuli were separated by 500 msec delay intervals, and fixation was required throughout the trial. Although many cells showed maintained, stimulus-specific activity in the delay interval immediately following the sample, the activity during subsequent test stimuli and delays did not appear to vary with respect to the sample stimulus. That is, the activity averaged across all test stimuli and delays was invariant across different samples. Furthermore, we did not find consistent differences in response to matching versus non-matching test stimuli. The major task-related activity found was that over 90% of the cells responded best to a given stimulus when it was a sample than when it was a matching or non-matching test stimulus, which we interpret as enhancement due to increased attention to the sample. Our results support an attentional, rather than mnemonic, explanation for task-related IT responses in recency memory tasks (also see Baylis and Rolls, '87), at least for the portion of IT cortex we studied.

52.17

THE RESPONSES OF NEURONS IN THE INFERIOR TEMPORAL CORTEX OF THE MACAQUE TO PATTERNS DEFINED BY TEXTURE ELEMENTS. P.M.Mueller*, B.O.Moore, P.Alvarez-Royo, H.Pashler* and G.C.Baylis Depts. of Neuroscience and Psychology, University of California, San Diego, La Jolla, CA 92093.

Human subjects are readily able to perceive objects when their boundaries are defined by different types of texture elements. It is clear that neurons in the visual system must be able to represent objects as equivalent whether defined by gray-scale, color or texture.

We have recorded the activity of single neurons in the inferior temporal cortex and superior temporal sulcus in two awake behaving macaque monkeys as they viewed patterns defined by gray-scale and the same patterns defined only by equiluminant texture fields. The patterns chosen were 2D Walsh functions, since this set allows inferences to be made about the degree of selectivity between patterns. These were presented on fixation for 1.0 sec. It was found that most neurons responded selectively to patterns defined either by gray-scale or texture elements. In many cases the response was the same to a pattern and its contrast-reversed pair. However, in many cases, there was poor correlation between the response to a pattern defined by gray-scale and the response to the same pattern defined by texture elements. These results suggest either that the patterns were not perceived as equivalent by the monkey or that equivalence has not been achieved by the time information reaches the inferior temporal cortex. We are currently investigating these two possibilities.

This work was supported by O.N.R. contract N00014-88-K-0281.

52.18

TRANSLATION INVARIANCE IN THE RESPONSES OF NEURONS IN THE INFERIOR TEMPORAL VISUAL CORTEX OF THE MACAQUE. P.Azzopardi* and E.T.Rolls. Dept. of Exptl. Psychology, University of Oxford, England.

To investigate whether neurons in the inferior temporal visual cortex and cortex in the anterior part of the superior temporal sulcus operate with translation invariance when these cortical regions are operating normally in the awake behaving primate, their responses were measured during a visual fixation (blink) task in which stimuli could be placed in different parts of the receptive field. Neurons with responses selective for faces (Rolls 1984 Hum. Neurobiol.3: 209-222) were investigated. It was found that in many cases the neurons responded (with a greater than half-maximal response) even when the monkey fixated 2-5 degrees beyond the edge of a face which subtended 3-20 degrees at the retina. Moreover, the stimulus selectivity between faces was maintained this far eccentric within the receptive field. These results held even across the visual midline. It is concluded that some neurons in the temporal lobe visual areas do have considerable translation invariance so that this is a computation which must be performed in the visual system, and that their selectivity is maintained in different parts of their receptive fields.

52.19

FACE-RESPONSIVE COMPONENTS IN THE TEMPORAL LOBE VISUAL EVOKED POTENTIALS (EPs) OF JAVA MONKEYS. O.-J. Grüsser and L. Fuhry*. Dept. of Physiol. Freie Universität Berlin, Arnimallee 22, 1000 Berlin 33 (West) Germany.

EPs were recorded with nine epidural electrodes implanted above the right hemisphere of a Java monkey trained to distinguish monkey or human faces (F-stimuli) from complex non-face stimuli (NF-stimuli, b/w slides 5.1° diameter). Eye movements were monitored (EOG). Either F-stimuli or NF-stimuli were rewarded. EP averaging began 200 ms before stimulus change and lasted 1 s. Stimuli were presented within a randomized time slot of 2.9-4.1 s. In one set 53 F- and 59 NF-stimuli were applied three times. Fig. 1 illustrates the responses to monkey F- and NF-stimuli (electrodes -9/4 and 1/30 mm ant./lat.). A face-responsive component began about 110 ms after stimulus change, having a positive peak at about 150 ms. This component did not change when NF-stimuli were rewarded, and also appeared with human F-stimuli (fig. 2). The distribution of the face-responsive component recorded over the temporal lobe showed marginal regional differences for monkey and human F-stimuli. (DFG-grant Gr 161).

Fig. 1: monkey faces

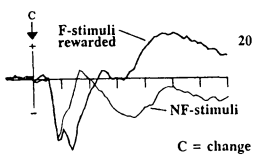
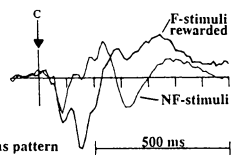
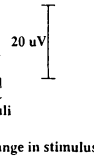


Fig. 2: human faces





C = change in stimulus pattern

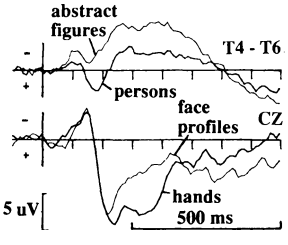
52.20

COMPONENTS OF VISUAL EVOKED POTENTIALS RESPONSIVE TO SILHOUETTES OF PERSONS OR HANDS. Margitta Seeck*, Elke Heusser* and O.-J. Grüsser. (SPON: EBBS). Dept. of Physiol., Freie Universität Berlin, Arnimallee 22, 1000 Berlin 33, Germany.

Visual evoked potentials (EP) were recorded when silhouettes of persons (P-stimuli) in various positions (neutral standing, boxing, greeting, depressed body posture) were presented. As controls "non-persons" (flowers, tools, abstract figures) were applied in random sequence (EEG-recordings from T3, T5, T4, T6, CZ and OZ, reference electrode linked mastoids, 9 male and 9 female subjects). Category-specific differences between EPs evoked by P- and non-P-stimuli were small, but most pronounced in the temporo-occipital electrodes, i.e. the "bipolar" recordings (T4-T6, T3-T5). Differences between EPs evoked by the various P-stimuli were absent.

In a second study (5 males, 3 females) we investigated the EPs of silhouettes of hands, face profiles and abstract figures. Again the greatest category-specific differences were found in CZ, T5, T6, starting about 200 ms after the stimulus change. In CZ the "hand-responsive" EP-component consists of a broader, more positive amplitude, whereas in the temporo-occipital electrodes it differs from non-hand stimulus EPs in having a "W"-form.

(DFG grants Gr 161, S 520).



53.1

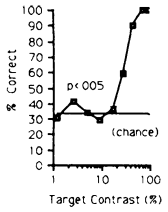
SPARING OF FUNCTIONS IN CORTICAL BLINDNESS. S. AGLIOTI*, M. CORBETTA*, M. BENASSI* (Spon. European Neuroscience Association), Institute of human physiology, University of Verona, I-37134.

Evidence of visual information processing into separated pathways has been obtained in a 33 years old brain damaged patient, with a complex cortical blindness-like syndrome consequent to an electrocution accident that provoked heart failure and a two-week lasting coma. Electrophysiological measurements (EEG and Evoked potentials) and imaging techniques (CT scan, NMR and SPECT) documented a diffuse cortical damage more pronounced in the posterior areas. The intelligence of the patient is preserved to a large extent. Despite poor visual acuity and very bad form perception, color vision results highly preserved and the use of color-related cues improves acuity discrimination tasks. Furthemore, perception both of real and apparent movement is mildly impaired for light displacement across visual hemifields along the horizontal meridian but is preserved if the light is moved along the vertical meridian. Finally, preliminary data indicate an improvement of form recognition when the stimulus is moved across the visual field. Our patient's visual behavior is in agreement with the idea of modularly organized, but strictly cooperant, visual pathways.

53.2

NON-CONSCIOUS 'VISION' IN NORMAL HUMANS: EVIDENCE FOR TECTAL MEDIATION. R. Lahav* and H.A. Buchtel. Departments of Psychiatry and Psychology, University of Michigan and Psychology Service, VA Medical Center, Ann Arbor, MI 48109-2399.

We found that under scotopic conditions, shape-discrimination of sub-threshold visual targets accompanied by a higher-contrast stimulus displays a non-monotonic function. This is manifested as a significantly better-than-chance discrimination ability in a narrow band of target figure-to-background contrasts (around 1%-5%), which is lower than, and not adjacent to, the traditional range of shape-discrimination contrast values. Discrimination was found to be dependent on the presence of a second stimulus of about 70% contrast, and appeared in 10 out of 12 human subjects tested.



Several findings implicate the retino-tecto-cortical pathway as responsible for the effect: discrimination was accompanied by the subjects' absence of (reported) visual experience; acuity was found to be low (about 3°); discrimination was found to quickly habituate upon repeated presentation of the same target; and it was found to dishabituate upon changes in target-contrast. The latter two effects have been seen in the superior colliculus of the monkey. In addition, a subject whose striate cortex was unilaterally removed showed a similar discrimination capacity in her blind field.

Despite some similarities, the effect seems to be different from previously reported phenomena, such as 'blindsight' (which is monotonic: Pöppel, Held & Frost, Nature, 1973), or the pedestal effect, which, among other things, occurs at much lower contrasts, and does not show habituation (e.g. Barlow et al., J. Opt. Soc. Am., 1987).

Supported by the VA Department of Medicine and Surgery (HAB).

53.3

MODELLING OF VISUAL RECOGNITION STRATEGIES IN THE TWO CEREBRAL HEMISPHERES. M.B.Pavlovskaya*, I.A.Vol* and V.M.Bondarko* Pavlov Institute of Physiology, Academy of Sci. of the USSR, Leningrad, 199034, USSR.

In terms of computer simulation model we have analyzed whether there is a difference in visual recognition strategies in the left and the right hemispheres. The stimuli (four meaningless line drawings) were randomly presented in the left or the right visual hemifield (LVF,RVF) for 10-100 ms. Object confusion matrices for different exposure durations were obtained for the LVF and the RVF separately. The statistical model of visual processing based on the spatial-frequency approach (Fourier transforms) was used to generate predicted confusions among stimulus pairs. The probability of confusion of two objects was suggested to be determined both by the similarity of image spectra and the probability of a priory preference for the given stimulus. Good fits of theoretical to experimental similarity matrices were obtained only for the RVF (Pearson's correlation coefficient reached 0.93), whereas in the LVF this value didn't exceed 0.34. Then the suggested recognition strategy is realized in the RVF only.

53.4

REACTIVITY OF NEUROMAGNETIC ALPHA FREQUENCY ACTIVITY IN HUMAN SUBJECTS. C. Gallen*, S. Hampson*, W. Young, F. Bloom, Div. of Preclinical Neuroscience, Research Institute of Scripps Clinic, La Jolla, CA 92037.

Reactivity (event-related desynchronization) of intrinsic cortical rhythmical activity has been proposed as a potentially sensitive measure of cortical function. Determination of the optimal conditions for generation of stimulus-related reactivity is necessary before such a measure can be applied to the magnetoencephalographic study of human cortical function.

The time course and extent of reactivity of the human alpha rhythm was studied in three subjects using a 14-channel Neuromagnetometer. Serial recordings during 20 epochs of cycling lights-off/lights-on stimulus conditions were made from 100+ sites distributed across the cranium. The total alpha power in the five one-second periods preceding and following the onset of visual stimulus was measured and used to calculate an index of alpha reactivity. Occipital alpha power was markedly increased in darkness compared to the light stimulated condition (5-20-fold) while frontal and temporal regions showed much less alteration (0-2-fold). The field patterns for magnetic alpha power and reactivity were tightly and reproducibly localized in the posterior superior cranial region suggesting an occiptial lobe source. Reduction of alpha power with light stimulus occured rapidly (less than 1 sec), persisted for 1-3 seconds, then rebounded even with continuing light. Reactivity varied markedly from epoch to epoch and was maximal when the stimulus occured in the middle of an alpha spindle. These findings indicate that recordings of magnetic alpha reactivity offer a vehicle for the study of the neuronal generator sources underlying the cortical response to visual field stimulation.

53.5

SUPPRESSION OF CHROMATIC AND ACHROMATIC LETTER PERCEPTION BY MAGNETIC COIL STIMULATION OF HUMAN VISUAL CORTEX. P.J. Maccabee*, V.E. Amassian, R.Q. Cracco, V. Zemon*, A. Rudell* and L. Eberle*, (SPON: J.B. Ranck, Jr.), Depts. Physiol. and Neurol., SUNY, Hlth. Sci. Ctr. Bklyn. and Lab. of Biophys., Rockefeller Univ., N.Y.

Studies of primate cellular physiology and human perception reveal separate pathways for chromatic and achromatic stimuli. We now extend the perceptual studies by using a magnetic coil (MC) over visual cortex to suppress recognition of briefly presented alphabetic letters (J. Physiol., 1988, 398, 40P). Subjects with normal color vision were briefly presented stimuli consisting of: 1) against a grey background, randomly presented letters at positive contrast; 2) against a red background, isoluminant green letters. Following letter presentation, the MC was energized at various randomized latencies. In the youngest subject, 45-60 msec intervals resulted in progressive loss of perception; at 60-100 msec, perception was absent and at 100-140 msec perception recovered to normal. Curves in older subjects had similar shapes but with longer onset latancies. In all subjects, isoluminant stimuli revealed sharper curves which were significantly prolonged by e.g. 20-40 msec. Isoluminant (chromatic contrast) stimuli are apparently processed by the parvocellular - blob pathway whereas achromatic (luminance contrast) stimuli are processed by magnocellular and remaining parvocellular pathways. The increased latency differences may reflect different dynamics in these subsystems.

53.6

VISUAL PERCEPTION UNMASKED BY MAGNETIC COIL STIMULATION OF HUMAN CEREBRAL CORTEX. V.E. Amassian, P.J. Maccabee*, R.Q. Cracco, A. Rudell* and L. Eberle*. Depts. of Physiology and Neurology, SUNY Health Sci. Ctr. at Brooklyn, New York 11203.

We used a Cadwell magnetic coil (MC) over visual cortex (VC) to suppress perception of 3 random letters, briefly presented 80-100 ms before the MC pulse (J. Physiol. (1988) 398, 40P). However, single words could not be suppressed with the MC over Wernicke's area, supramarginal and angular gyri. To reduce the possibility of VC 'refreshing' subsequent processing areas, a 2nd random word was presented 50-83 ms after the 1st; an MC pulse given 90-150 ms after the 1st word significantly reduced its retrieval. Next, a paradigm was sought yielding a positive response with the MC. Two word or 3 letter arrays or arbitrary linear patterns, with the second stimulus (S_2) 4X brighter than S_1 masked the response to S_1 at intervals of, e.g. 100 ms, the masking then occurring after VC. The response to S_1 was reliably retrieved (unmasked) when the MC pulse was delivered to VC 80-100 ms after S_2. An MC pulse delivered to tempero-parieto-occipital cortex 100-150 ms after S_2, also unmasked letter responses to S_1, but less than with VC stimulation. Beyond visual cortex, suppression is weaker possibly because multiple pathways conveying the information must be affected.

53.7

RESONANT CORTICAL EEG PATTERNS EVOKED IN RHESUS MONKEYS BY A CHOICE TASK. D. Krieger, R. Coppola, R. J. Sclabassi, R. K. Nakamura. NIMH, Bethesda, MD 20892 and Lab. Comp. Neurophys., U. Pitt., Pittsburgh, PA 15213.

This work was directed to the question: Are behaviorally relevant resonant EEG patterns present in the cortex of the rhesus monkey during performance of a GO/NOGO visual discrimination task? The results support an affirmative answer to the experimental question. EEG recordings were obtained simultanously from 16 bipolar placements distributed across the cortical convexity of chronically implanted rhesus monkeys. A machine algorithm was used to identify resonant activity electrode by electrode and trial by trial. Resonances were then analyzed to identify temporal and spatial relationships to task relevant parameters. Strong temporal relationships were found between resonance latency and both stimulus latency and reaction time. The pattern of intensity across the cortex at the resonant frequency was a consistently good classifier for which of four stimuli was presented (up to 77% accuracy; chance = 25%), stimulus orientation, i.e. slanted left vs right (up to 90% accuracy; chance = 50%), stimulus type, i.e. diagonal vs diamond (up to 67% accuracy; chance = 50%), and trial type, i.e. GO vs NOGO (up to 84% accuracy; chance = 50%).

53.8

DEVELOPMENT OF VISUAL TEXTURE SEGMENTATION IN KITTENS. F. Wilkinson & R. Ohayon* Department of Psychology, McGill University, Montreal, Canada, H3A 1B1.

In this study, we have used preferential looking and two-alternative forced choice discrimination on the jumping stand to investigate the development of texture segmentation in very young kittens. Beginning at 3 weeks of age, detection thresholds for texture arrays were assessed by preferential looking. In this paradigm, textures were paired with homogeneous grey fields of matched mean luminance. Kittens as young as three weeks showed clear preferences for textures over homogeneous fields provided the texture elements were above a critical size. Pairs of such textures (e.g., with vertically and horizontally oriented elements) were then presented to the kittens on the jumping stand in a texture segmentation task (Wilkinson, **Behav. Brain Res.**, 1986, 19, 71-82). Twelve kittens have been tested in this sequential paradigm on textures with oriented elements. In spite of clear evidence of detection of the textures before 6 weeks of age, no animal was successful on the texture segmentation task before 80-120 days of age. The generality of this effect is currently being examined using other texture patterns. The results will be discussed in relation to the development of striate and extrastriate visual mechanisms.

This work was supported by NSERC grant. OGG0007551

53.9

VERNIER ACUITY IN STEREOBLIND CATS.

B. Timney, L.A. Symons* and K. Keil* . Dept. Psychology, Univ. Western Ontario, London, Canada, N6A 5C2.

The similarities between vernier acuity and stereoacuity have led to the suggestion that they may share a common mechanism. However, the evidence in support of such a suggestion is mixed. One way to compare two abilities is to eliminate one and look for an equivalent deficit in the other. We measured vernier acuity in stereoblind kittens (induced by monocular deprivation between 40 and 50 d) and compared it with that of control animals. Vernier and stereoacuity were measured in these animals using a jumping stand procedure. In control animals vernier thresholds were typically less than 5 min arc and binocular depth thresholds less than 10 min arc. The MD animals showed little difference between monocular and binocular depth thresholds, indicating a loss of stereopsis. These animals found the vernier task very difficult to learn and final thresholds were much worse than those of the controls, ranging from 30 to 50 min arc. The deficit was not attributable to a reduction in resolution acuity. We conclude that the mechanisms for vernier and stereoacuity must at some level share a common pathway.

53.10

APB ALTERS VISUAL CONTRAST SENSITIVITY IN GOLDFISH. J. Bilotta*, P. DeMarco* and M. Powers. Dept. of Psych., Vanderbilt University, Nashville TN 37240.

APB (DL-2-amino-4-phosphonobutyric acid) is believed to suppress activity in the ON-pathway of the visual system (Slaughter, M.M. & Miller, R.F. Science, 211:182, 1981). However, we have shown that APB reduces the sensitivity of ON and OFF responses from the retina (Powers M.K. et al, IOVS sup., 29:104, 1988). We now report the behavioral consequences of this sensitivity reduction. Contrast sensitivity functions (CSFs) were measured from goldfish before and after intraocular injections of APB. Fish were classically conditioned to suppress respiration to a sinusoidal grating drifting at 1 Hz. CSFs were derived from an experimenter-based two alternative forced choice procedure (Bilotta, J. & Powers, M. IOVS sup., 30:505, 1989). Baseline CSFs were first obtained; in subsequent sessions injections of APB (100 µM or 1000 µM) or fish Ringer's were made prior to testing. Results showed that (1) CSFs obtained from fish injected with Ringer's were identical to baseline CSFs. (2) APB-treated fish had CSFs shifted to lower spatial frequencies and had lower contrast sensitivity. (3) 1000 µM APB decreased contrast sensitivity more than 100 µM APB. We conclude that APB not only reduces the absolute sensitivity of the goldfish retina, but that it also reduces spatial contrast sensitivity and resolution.

53.11

THE ROLE OF CORTICAL DIRECTIONAL SELECTIVITY IN PERCEPTION OF COHERENT MOTION. Daniel Harvitt*, Joanne Albano*, and Tatiana Pasternak, (SPON: R. Sekuler). *Dept of Neurobiology and Anatomy and Center for Visual Science, Univ. of Rochester, Rochester, NY 14627.*

We examined the perception of coherent motion in cats with severely reduced cortical directional selectivity. Such animals, are unable to discriminate the direction of moving gratings at low contrasts (eg. Pasternak, et al, 1985). We used high contrast dynamic random-dot displays (Williams and Sekuler, 1984), in which the direction of motion for each dot could be chosen at random from a specified distribution. When the bandwidth of the distribution is broad (360 deg), the display consists only of local random motion of individual dots and no coherent motion is reported. However, when the bandwidth is limited, the dots appear to flow coherently in a single direction. We measured the bandwidth needed to reliably discriminate between opposite directions of motion and manipulated the strength of motion signal by varying the proportion of dots in the display with bandwidth of 360 deg (noise). When no noise was present in the display, cats with reduced directionality were able to discriminate direction as well as the normal cats (threshold bandwidth: 270-300 deg). As the strength of motion signal was reduced, a narrower bandwidth was required to perform the discrimination. With a very narrow bandwidth (1 deg) normal cats were able to discriminate direction when only 10% of the dots contributed to the motion signal. However, cats with a reduced number of motion detectors required a much greater signal strength (25-30%) to perform the same task. Thus, in the absence of noise, even an abnormally small number of directionally selective cells can perform spatial pooling of local directional signals and support normal perception of coherent motion. However, a full complement of directional detectors is needed when the motion signal is weak (low contrast) or is masked by noise. (Supported by EY06175 and EY01319)

54.1

RECOVERY IN HEMIPARKINSONIAN RATS FOLLOWING INTRASTRIATAL IMPLANTATION OF ACTIVATED LEUKOCYTES. R.J. Weber*, S.E. Ewing*, A. Zauner*, and R.J. Punkett*. (Spons. D.M. Jacobowitz). Laboratory of Medicinal Chemistry, NIDDK, and Surgical Neurology Branch, NINCDS, NIH, Bethesda, MD 20892.

A hemiparkinsonian syndrome resembling idiopathic Parkinson's disease in humans was induced in Sprague-Dawley rats that had undergone prior superior cervical ganglionectomies, using a modification of a recently reported technique for selectively lesioning the pars compacta of the substantia nigra by unilateral stereotactic injections of 6-hydroxydopamine. Phytohemagglutinin-stimulated rat peritoneal cells, predominantly T-cells and macrophages, were stereotactically implanted in the lesioned caudate-putamen, and behavioral recovery assessed by measuring rotation induced by D-amphetamine, a dopamine releasing agent. Animals receiving implants of activated leukocytes (n=13), showed a 47% decrease in amphetamine-induced turning 8 weeks after implantation (p=0.05), which was not seen in control (n=10) or sham-operated (n=7) animals. The recovery in leukocyte-implanted animals was correlated with an increases in striatal dopamine content and the presence of increased tyrosine hydoxylase reactivity on the lesioned side. We conclude that implantation of leukocytes promotes recovery in hemiparkinsonian rats, possibly through restoration of striatal dopaminergic activity.

54.2

CRYOPRESERVED HUMAN FETAL NEURAL TISSUE REMAINS VIABLE 4 MONTHS AFTER TRANSPLANTATION INTO HUMAN CAUDATE NUCLEUS. D.E.Redmond, Jr., D. Spencer, F. Naftolin, C. Leranth, R.J. Robbins, T.L. Vollmer, R.H. Roth, B.S. Bunney, J.H. Kim, Depts. Psychiat., Neurosurg., Ob.Gyn., Neurol., Med., Pharmacol., & Neuropath. (Yale Neural Transplant Program),Yale Univ. Sch. Med., New Haven, CT 06510.

Cryopreserved human fetal mesencephalic tissue survived transplantation into monkeys in a previous study (*Science*, 242:768,1988), but viability of neural tissue grafted into the brain of a human subject has not been demonstrated.

We retrieved mesencephalic tissue from an 11 week aborted human fetus, using the method previously reported, and transplanted it into two areas of one caudate nucleus in a 69 year old patient who was deteriorating from end-stage parkinsonism. The study was approved by the Yale Human Investigation Committee. During the first 8 weeks, the patient received cyclosporin (blood levels 100-150 ng/ml). After 4 months the patient died from complications of parkinsonism. Examination of the brain revealed no complications from the implantation of tissue, and a diagnosis of Parkinson's disease was confirmed by usual pathological findings, but without Lewy bodies. Both grafts found in the caudate nucleus appeared to have been viable at the time of death. They contained erythrocyte-packed capillaries and pyramidal-shaped neurons with synapses, reactive glia, and some macrophages, based on light and electron microscopy. No inflammatory process indicating tissue rejection was apparent. Tyrosine hydroxylase (TH) immunohistochemistry did not label cells in the grafts, but labelled a few in surrounding caudate tissue.

These data demonstrate that cryopreserved human fetal mesencephalic neural tissue survives four months after transplantation into the mature human caudate nucleus. The absence of TH-labelling of identified neurons in the grafts is unexplained and unexpected since similar tissue which was cultured or grafted into monkeys contained TH-positive neurons (*Science, ibid.*).*(Supported by private funds).*

54.3

HUMAN PARKINSON'S DISEASE TREATED BY FETAL DOPAMINE CELL TRANSPLANT. CR Freed, RE Breeze*, NL Rosenberg*, JN Barrett, D Eidelberg* and DA Rottenberg, U. Colorado Sch. of Med., Denver, CO, U. Miami Sch. of Med., Miami, FL, and Memorial Hosp., NY, NY.

We have implanted fetal dopamine cells from a 7 week human embryo into caudate and putamen of a 52 year old man with a 20 year history of Parkinson's disease, Hoehn and Yahr stage 4. Fetal tissue was obtained from elective abortion with informed consent obtained after the procedure. Maternal blood was negative for HIV and hepatitis B. Five months prior to transplant the patient began evaluations by a neurologist and by a home based computer system for daily measurement of walking and hand movements. Preop fluorodopa PET scan showed severe bilateral dopamine depletion. Tissue was implanted stereotaxically in caudate and putamen on the right side via 10 needle tracks. Fetal tissue and the patient were ABO matched and the patient was not immunosuppressed. Data 26 weeks after surgery show a 50% increase in walking speed on a full day basis. There was a 30% increase in left finger speed and a smaller increase in right finger speed. Left sided performance became equal to right sided. Peak dose dyskinesias were seen on the left. Reaction time was not affected. Drug doses have been reduced. These data indicate that transplants of human fetal dopamine cells may be of benefit in patients with Parkinson's disease.

54.4

HISTOLOGICAL ANALYSIS OF THE BRAIN AND THE ADRENAL GLAND OF A PARKINSONIAN PATIENT TREATED BY UNILATERAL TRANSPLANT OF ADRENAL MEDULLA. E.C.HIRSCH, C. DUYCKAERTS*, F. AGID*, J.-J. HAUW*, Y. AGID*. INSERM U289 and Laboratoire R. Escourolle, Hôpital de la Salpêtrière, 75013 Paris, France.

An autologous unilateral adrenal medullary transplant to the caudate nucleus was performed on a 54 yrs old patient with idiopathic Parkinson's disease (PD) as described by Madrazo. After surgery no clinical benefit was observed and he died 120 days later, suffering from meningitis. Autopsy findings showed an important astrocytic gliosis and an inflammatory infiltrate surrounding the graft. No cell bodies containing catecholaminergic markers (tyrosine hydroxylase TH, dopamine-beta-hydroxylase, chromogranin A and mRNA coding for TH) were detected in the graft, in contrast such cells were numerous in the non grafted adrenal medulla. A few TH-positive DBH-negative fibers were visualized in the graft. In the host striatum, a hyperdensity of TH-positive sprouting fibers was observed in periphery of the graft. However, no TH-positive fibers were evidenced throughout the remaining part of the grafted striatum nor in the non-grafted striatum. The fiber density of other striatal fiber systems (methionine-enkephalin, neuropeptide Y or choline acetyl-transferase) was similar in both striata. The data suggest that in PD the adrenal transplants may have a selective stimulating effect on the host.

54.5

ADRENAL AUTOTRANSPLANT IN HEMIPARKINSON MONKEYS. B. Brooks[1], D.C.German[2], and C. McGregor*[1]. [1]Univ. of TX Hlth Sci. Ctr. (Physiol.), San Antonio, TX. [2]UT SW Med. Ctr. (Psychiat.), Dallas TX.

A monkey was trained to bar-press rapidly with either hand as a measure of motor function. After press rates were stabilized, MPTP (.5 mg/kg) was injected via one internal carotid artery. Bradykinesia, rigidity, and severely reduced bar pressing occurred contralateral to injection; the ipsilateral limb remained normal. SINEMET (oral L-dopa/carbidopa) transiently restored the disabled limb. The deficits persisted for 7½ weeks until an adrenal medulla-to-caudate transplant was performed on the injected side. Within 76 hours, a stepwise improvement in bar pressing occurred, which gradually increased over 4½ months to about 90% recovery. SINEMET had no effect on pressing in the recovering limb.

Sections of perfused brain received either tyrosine hydroxylase (TH) antibody or Nissle stains. TH immunoreactivity throughout the rostral-caudal extent of the caudate and putamen nuclei on the treated side was markedly depleted, compared to the contralateral hemisphere, which showed intense staining typical of normal animals. An ipsilateral reduction in midbrain DA containing cells affected areas A8, A9, and A10. Evidence of a viable graft was found in the walls of the lateral ventricle; a gradient of enhanced TH-IR extended from the ventricular surface of the caudate inward. Sponsored by NIH Grant #EY5R01NS22187-03 and DAPS.

54.6

SPECT IMAGING OF DOPAMINE D_2 RECEPTORS IN MPTP-TREATED MONKEYS FOLLOWING BRAIN TRANSPLANTATION. R.H. Roth, P.B. Hoffer, D.E. Redmond, Jr., J.D. Elsworth, J.R. Taylor, J.R. Sladek Jr.†, T.J. Collier†, H.F. Kung††*, A. Alavi††*, and R.B. Innis. Yale Univ. Sch. Med., New Haven, CT 06510; †Dept. Neurobiol. and Anat., Univ. Rochester Sch. Med. & Dentistry, Rochester, N.Y. 14642 and ††Section of Nuclear Med., Univ. Penn., Philadelphia, Pa.

Pilot studies in non-human primates indicate the feasibility of using brain imaging with Single Photon Emission Computed Tomography (SPECT) as a noninvasive technique to assess D_2 receptor localization *in vivo* (see abstract by Innis et al). In this study we examine the feasibility of monitoring possible dopamine receptor changes associated with transplanted neural tissue in parkinsonian primates. Two MPTP-treated vervet monkeys received either fetal substantia nigra or cerebellum transplanted unilaterally into striatum. They were examined with SPECT brain imaging 21 months after grafting. Animals were injected with 4-5 mCi ^{123}I-labelled IBZM (iodobenzamide, a specific dopamine D_2 receptor probe). They were scanned for 2 hr. in a Strichman 810X Brain Imager with repeated 2 min scans through the striata. Small lateralized differences in radiolabel uptake were seen in the nigral-transplanted monkey. The uptake of radiolabel was markedly different in the two monkeys: at 120 min post injection, striatal to extrastriatal ratio of label was 1.8 in nigral-transplanted animal but only 1.3 in the cerebellar-transplanted animal. Animals were sacrificed immediately after the scan. Direct measures of ^{123}I in tissue punches removed from brain slices were consistent with the SPECT results. These results, added to morphological and chemical analyses, suggest that SPECT neuroreceptor imaging may be useful in assessing PD and monitoring its treatment with brain transplantation.

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54.7

MRI OF FETAL NEURAL TISSUE TRANSPLANTS IN MONKEYS: STRIATAL GRAFTS SHOW NO ENHANCEMENT AFTER Gd-DPTA.

E.L.Kier*, D.E.Redmond, Jr., J.R.Sladek, Jr.†, J.R.Taylor, J.D.Elsworth, T.J.Collier†, and R.H.Roth (SPON: P.D.Shepherd). Dept. Diagnostic Radiology*, Psychiat. & Pharmacol., Yale Univ. Sch. of Med., New Haven, CT. 06510 and †Dept. of Neurobiology and Anatomy, Univ. of Roch. Sch. of Med., Rochester, N.Y. 14642.

Magnetic resonance (MR) imaging may allow *in vivo* assessments of the accuracy of tissue placement, surgical complications, breaches in the blood-brain-barrier, and the development of neural tissue grafted into the brain. Six St. Kitts green monkeys *(Cercopithecus aethiops sabaeus)* were imaged approximately 21 months after unilateral grafts of fetal neural tissue from fetal substantia nigra or two types of controls.

Several sequences of spin echo and gradient echo images were performed in a 1.5 Tesla *GE Signa* MR Scanner in sagittal, axial, and coronal projections. Pre- and post-gadolinium-DPTA scans were obtained. Several imaging parameters were used to establish the best resolution. Coronal sections were made at sacrifice and correlations were made between the MR coronal images and the actual locations and conditions of the grafts using histological techniques. MR scans in the coronal projection were found to be the most informative. Both tracts and graft areas with hemosiderin deposits were visualized. Gadolinium enhanced a prominent vascularity in the lateral ventricle of one monkey, but no enhancement was seen in any graft located in the striatum.

The high quality resolution and anatomic detail provided by a 1.5 Tesla magnet may make MR scanning useful for the evaluation of neural grafts and also provide an anatomic foundation for localizing receptor images derived from Single Photon Emitting and Positron Emitting tomographic techniques *in vivo*. The lack of gadolinium enhancement in identified grafts within the striatum suggests the absence of a significant break in the blood-brain-barrier at 21 months after transplantation.

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54.8

BIOCHEMICAL STUDIES ON THE REVERSAL OF MPTP-INDUCED PARKINSONISM IN PRIMATES FOLLOWING TRANSPLANTATION OF FETAL SUBSTANTIA NIGRA TO STRIATUM

J.D.Elsworth, J.R.Taylor, D.E.Redmond,Jr., †T.J. Collier, †J.R. Sladek,Jr. and R.H.Roth. Depts. Pharmacol. & Psychiat., Yale Univ. Sch. Med., New Haven, CT 06510; †Dept. Neurobiol. & Anat., Univ. Rochester Sch. Med., NY 14642.

Previous studies have shown that transplantation of fetal substantia nigra to the caudate nucleus of MPTP-treated monkeys reverses the toxin-induced parkinsonism. This study (and abstract by Roth et al.) provides biochemical data that address the mechanism of recovery.

MPTP-treated vervet monkeys received fetal substantia nigra grafted bilaterally or unilaterally to caudate nucleus or putamen; other MPTP-treated subjects received non-dopaminergic fetal tissue transplanted similarly or underwent "sham" surgery. CSF homovanillic acid (HVA) concentrations were measured over time and parkinsonian behavior was regularly rated. After 18 months brains were examined by a method that allowed dopamine (DA) and HVA measurements and tyrosine hydroxylase immunohistochemistry to be performed in the same slice of tissue.

In order to distinguish DA production by transplanted cells from that generated by remaining host DA neurons, DA concentrations and HVA/DA ratios in striatum were related to cannula placements and tyrosine hydroxylase staining.

These data provide information on survival and function of transplanted cells and on the mechanism of behavioral recovery from MPTP-induced Parkinsonism.

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54.9

EFFECTS OF DOPAMINERGIC GRAFTS IN THE STRIATUM AND SUBSTANTIA NIGRA ON TURNING BEHAVIOUR IN 6-HYDROXYDOPAMINE-LESIONED RATS.

George S. Robertson[1], Harold A. Robertson[1] and Alan Fine[2] Dept. of Pharmacology[1], Dept. of Physiology and Biophysics[2], Dalhousie University, Halifax, Nova Scotia, Canada B3H 4H7.

L-Dopa administration elevates dopamine (DA) levels in the substantia nigra (SN) and striatum (ST) reduced by prior 6-OHDA-lesions of the SN. L-Dopa-induced circling in these lesioned animals may result from increased nigral DA levels (Robertson and Robertson, Neurosci. Lett. 89, 204-208). The reduction in turning to dopaminergic agonists which occurs after fetal dopaminergic grafts to the 6-OHDA-denervated ST might therefore be further augmented by a dopaminergic graft to the ipsilateral SN. To test this possibility, fetal nigral grafts were placed in the SN, ST and SN+ST ipsilateral to 6-OHDA-lesion of the SN. A fourth group of ungrafted 6-OHDA-lesioned rats served as additional controls. Circling produced by the selective D1- and D2-receptor agonists SKF 38393 and LY 171555, respectively, was evaluated 1, 2 and 3 months after transplant surgery. Animals with a graft to the SN only did not show a consistent reduction in rotation. Animals grafted to the ST or the SN+ST showed a greater reduction in D1- than D2-receptor-mediated rotation. However, a combined SN+ST graft did not produce a greater reduction in turning behaviour than a ST graft. Supported by Parkinson Foundation and MRC of Canada

54.10

COMPARISON OF SUBCUTANEOUS AND INTRAVENTRICULAR ADMINISTRATION OF DOPAMINE AGONISTS IN ANIMAL MODELS OF PARKINSON'S DISEASE,

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Chronic levodopa therapy in Parkinson's disease (PD) is limited by response fluctuations and loss of efficacy. Parenteral forms of administration may offer certain advantages. We have therefore compared the effects of apomorphine and PHNO, a D-2 dopamine agonist, administered subcutaneously (s.c.) versus intracerebroventricularly (i.c.v.; cannulae in the left lateral ventricle) in normal rats and in rats with unilateral 6-OH dopamine lesions of the substantia nigra. Apomorphine (0.1, 0.25, 0.50, and 0.75 mg/kg) and PHNO (1.0, 5.0, 50.0 and 100 ug/kg) produced dose-dependent stereotypic behavior (SB) both with s.c. and i.c.v. injections. Comparison of the two routes revealed a more immediate effect with i.c.v. and a more intense behavioral response in some animals (i.e. gnawing and licking), however, there was no statistical difference ($p < 0.05$) in the percent of animals with SB and in the duration of SB. Several animals had seizures following i.c.v. injection. Apomorphine (0.1 to 0.50 mg/kg) but not PHNO (1 to 50 ug/kg) produced dose-dependent turning behavior measured for ten minutes following injection in 6-OH dopamine lesioned animals. There was no difference in turning behavior between the two routes of administration. It is concluded dopamine agonists can be given by the s.c. and i.c.v. routes and i.c.v. confers no major advantage over s.c. administration.

54.11

L-DOPA PRODUCES HIGHER LEVELS OF EXTRACELLULAR DOPAMINE IN DOPAMINE DEPLETED VS. INTACT STRIATA.

A.E.Bonatz*, H.J. Morris*, M.J. Zigmond, and E.D. Abercrombie (SPON: O.M.Reinmuth) *Dept. of Behavioral Neuroscience and Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260.*

The effect of L-DOPA administration upon dopamine (DA) in extracellular fluid was examined both in the intact striatum and in the DA-depleted striatum of rat using in vivo microdialysis. The basal amount of DA in 20µl of dialysate (corrected for recovery of the probe) in DA-depleted animals was significantly less than in controls when striatal tissue DA was reduced by more than 86%. Peripheral dopa decarboxylase (DDC) inhibition (RO4-4602, 50 mg/kg i.p.) followed by L-DOPA treatment (100 mg/kg i.p.) elevated extracellular DA in controls from 37 ± 5 pg to 84 ± 13 pg (n=7). In animals with bilateral striatal DA depletions (mean tissue DA depletion 82%; n=6), L-DOPA increased extracellular DA from 17 ± 9 pg to 244 ± 53 pg. In unilaterally DA-depleted animals (mean tissue DA depletion 98%; n=6). The increase in extracellular DA after L-DOPA was greater on the lesion side (from 7 ± 4 pg to 245 ± 67 pg) than on the intact side (from 28 ± 11 pg to 61 ± 8 pg). Unilaterally lesioned animals showed contralateral circling behavior after L-DOPA suggesting that DA formed from L-DOPA functionally stimulates postsynaptic receptors. It is proposed that the greater absolute effect of L-DOPA on extracellular DA concentration in the DA-depleted striatum is due to: (1) a substantial reduction in high affinity DA reuptake as a consequence of DA nerve cell degeneration and (2) DDC activity in non-dopaminergic striatal elements. Thus, the failure of Parkinsonism patient to continue to respond to L-DOPA treatment may be due in part to the overstimulation of DA receptors leading to desensitization. (Supported by Grant NS19608 and the National Alliance for Research on Schizophrenia and Depression.)

54.12

DOPA-INDUCED INHIBITION OF STRIATAL ACH RELEASE: INCREASED EFFECTIVENESS AFTER 6-HYDROXY-DOPAMINE.

D. Jackson, and M. J. Zigmond, *Dept. of Behavioral Neuroscience and Center for Neuroscience, University of Pittsburgh, Pittsburgh, Pa. 15260*

Although the motor activity of normal rats is relatively unaffected by large doses of DOPA, increased locomotion is observed after administration of DOPA to rats lesioned with 6-hydroxydopamine (6-HDA). In the present study, we looked at the effects of DOPA on the release of striatal dopamine (DA) and acetylcholine (ACh), a transmitter normally inhibited by DA. Striatal slices (350 um) were prepared from control and lesioned rats (250 ug 6-HDA,ivt), preincubated with [^{3}H]choline (.07 uM), and superfused (100 ul/min) with Krebs bicarbonate buffer containing hemicholinium (10 uM). Slices then were exposed to two periods of electrical field stimulation. Tritium, an index of ACh efflux, and endogenous DA were measured. DOPA (10 uM), added after S_1, elevated the basal efflux and overflow of DA from intact and lesioned slices, although these effects were greatly reduced by the lesion. Nonetheless, 6-HDA pretreatment greatly enhanced the inhibitory influence of DOPA on ACh overflow. Thus, while DOPA had no detectable effect on ACh overflow from intact slices (-4±4%), it caused a considerable inhibition of ACh overflow from lesioned slices (-37±4%). A comparable potentiation of the inhibitory influence of DOPA could be produced by the addition of nomifensine (1 uM) to intact slices. Collectively, these data suggest that the ability of DOPA to restore striatal function results from an DOPA-induced increase in DA release coupled with the relative lack of high affinity DA uptake sites. (Supported in part by USPHS grants NS-19608 and MH-00058 and the American Psychological Association)

54.13

PRESYNAPTIC AND POSTSYNAPTIC INDICIES OF STRIATAL DOPAMINERGIC FUNCTION IN THE 10 DAY RAT PUP. R.A. Wallace and M.J. Zigmond, *Department of Behavioral Neuroscience and the Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 152-60.*

The striatum of 10-day old rat pups contains 40% less dopamine (DA) than that of the adult, yet neurological function appears relatively mature. To examine the capacity of endogenous DA to control striatal function, tissue slices (350 um) were preincubated with ^{3}H-choline and exposed to electrical field stimulation (10 Hz, 9 sec). Tritium, a measure of acetylcholine (ACh), and endogenous DA overflow then were determined. Sulpiride (1 uM) increased ACh overflow approximately 2-fold from slices prepared both from adult and neonatal rats. No evidence of postsynaptic supersensitivity was observed in neonatal slices, and DA overflow was significantly less than from adult slices. However, in the presence of nomifensine (10 uM), DA overflow from neonatal slices increased 12-fold to a level comparable (3.14 ± 0.51 ng/mg protein) to that seen from adult slices examined under the same conditions (2.46 ± 0.10 ng/mg protein). These results suggest that the amount of DA released from individual DA terminals in neonates may be much greater than from adults but that it is rapidly inactivated by reuptake. (Supported in part by NS-19608, MH-18273, and MH-00058.)

54.14

L-DOPA FACILITATES GABA RELEASE ACTIVATING D-1 DOPAMINE RECEPTORS IN THE BASAL GANGLIA OF THE RAT BRAIN. J. Aceves* and B. Florán* (SPON: J. Alanís). Dept. of Physiology. CINVESTAV-IPN. 07000 México, D.F.

L-Dopa increases GABA levels in the CSF of parkinsonian patients (Manyam, B., Arch. Neurol. 39: 391, 1982). This could be due to L-dopa-induced facilitation of GABA release. Here we have tested this possibility. The experiments were done on slices of substantia nigra pars reticulata, entopeduncular nucleus, globus pallidus and caudate-putamen of 6-OHDA lesioned rats. The lesion was produced by applying 6-OHDA into nigra compacta. [3-H]GABA release was induced by high (15 mM) K^+. L-Dopa markedly increased GABA release in all these nuclei (EC-50 = 2 µM). The increase was competitively antagonized by the selective D-1 DA antagonist SCH-23390. The effect was stereo-specific, since D-Dopa also stimulated the release but at higher concentrations (EC-50 ≃ 300 µM). The effect was not due to L-Dopa acting as DA precursor because the experiments were done having Dopa-decarboxylase inhibited by NSD (500 µM). Dopamine also stimulated GABA release in all of the nuclei; the stimulation was blocked by SCH 23390. These results suggest that in the parkinsonian patient L-Dopa could activate striatal output acting as both DA precursor and D1 DA agonist facilitating GABA neurotransmission at the output relay nuclei of the striatum.

54.15

PARKINSONIAN TREMOR AND PETIT MAL EPILEPSY: COMMON MECHANISMS. G. Buzsaki, A. Smith*, A. L. Roskies*, L. J.Fisher and F. H. Gage. Dept. of Neurosciences, UCSD, La Jolla, CA 92093

Neocortical activity, face muscle EMG and body tremor were recorded in young (3-10 mo) and aged (22-30 mo) Fischer 344 and Sprague-Dawley rats. During immobility high voltage spindles (HVS) appeared in the neocortex, the rhythmicity of which was phase-locked to the vibrissal and body tremor. The HVS had spike and wave components (< 5 mV) in F344 rats but only wave components in SD rats. The incidence of both HVS and tremor was 5-8 times higher in aged rats than in young adults. Peripheral administration of ethosuximide, valproic acid, scopolamine and apomorphine reduced the incidence of HVS and tremor. Chlorpromazine, acepromazine and haloperidol increased both parameters when injected peripherally or into the head of the caudate nucleus (CP; 1-10 nM) but not when administered into the thalamus. 6-OH lesion of the nigrostriatal dopamine system similarly inreased the incidence of HVS and tremor. Neuorons in the neocortex, thalamus, and CP, fired in a phase-locked manner with the HVS, and single pulse stimulation of the CP triggerred HVS during immobility. We suggest the thalamopetal GABAergic inputs (pars reticulata of substantia nigra, entopeduncular nucleus, pallidum) are burst- and oscillation-promoting systems, whose output is controlled by the striatum. Experimental or disease-related decrease of the striatal dopamine levels increase the efficacy of the GABAergic burst-promoting systems resulting in rhythmic network oscillation of thalamocortical neurons during rest.

SPECIFICITY OF SYNAPTIC CONNECTIONS

55.1

OLIVOCEREBELLAR FIBER MATURATION IN NORMAL AND LURCHER MUTANT MICE: DEFECTIVE DEVELOPMENT IN LURCHER. J. A. Heckroth, D. Goldowitz, and L. M. Eisenman. Department of Anatomy, Jefferson Medical College, Philadelphia, PA 19107

Olivocerebellar fiber maturation was examined in normal and lurcher mutant mice between postnatal day 5 (P5) and P15, using the anterograde transport of WGA-HRP from the inferior olive. Immunocytochemistry for the Purkinje cell specific peptide PEP-19 (antiserum provided by J.I.Morgan) was used to demonstrate Purkinje cell development in the same material. In mutant and normal animals a regional developmental variation is observed, such that, when compared at a given age, cortex lining the vermal fissures appears developmentally advanced to cortex in the cerebellar hemispheres. In the primary fissure of the normal animals the first recognizable Purkinje cell dendrites appear on P6, and the olivocerebellar fibers first enter the climbing stage of their development on P9. In lurcher animals Purkinje cell development proceeds on this schedule, although their somata and dendrites are abnormal in appearance. In contrast to normal, lurcher olivocerebellar fibers are never observed to enter the molecular layer, and instead maintain dense perisomatic nests around Purkinje cells, even at P13-15. Examination of WGA-HRP labeling in P14 lurchers by transmission electron microscopy indicates that the olivocerebellar fibers form synapses on Purkinje cell somatic spines, and that the basket cell axons fail to form their typical perisomal nests around Purkinje cells. In addition, parallel fibers are observed to have synapses on dendritic spines of the Purkinje cell primary dendrites. We interpret these results as indicating a specific recognition defect between olivocerebellar fibers and Purkinje cell dendrites. An analysis of this defect in lurcher may reveal how the developmental transformation of olivocerebellar fibers, from "perisomatic nests", to the "climbing" phase is achieved in the normal animal. Supported by NINCDS grant #NS22093.

55.2

DENDRITES OF PURKINJE CELLS IN REELER MUTANT CEREBELLUM INVADE THE MOLECULAR LAYER OF THE DORSAL COCHLEAR NUCLEUS. A. S. Berrebi and E. Mugnaini. Laboratory of Neuromorphology, U-154, Univ. of Connecticut, Storrs, CT. 06269-4154.

The murine mutation reeler affects neuronal migration and orientation. In the cerebellum, the defect results in grossly underdeveloped folia, disruption of lamination, substantial loss of Purkinje cells and secondarily, of granule cells. Lamination is also altered in the adjacent dorsal cochlear nucleus (DCoN), and the granule cells of the cochlear nuclei are reduced in number. We have examined the cerebellum and DCoN of reeler mice using Purkinje cell specific immunocytochemical markers. One of these, L7 (gift of Dr. J. I. Morgan, Roche Research Center), reveals all surviving Purkinje neurons, including those clustered in the deep region of the cerebellum. Some large clusters of Purkinje cells are adjacent to the DCoN, and their spine-laden dendrites enter and ramify within the DCoN molecular layer. In this layer, cerebellar-like parallel fibers, the axons of cochlear granule cells, presumably provide synaptic input not only to DCoN neurons, but also to the ectopic Purkinje cell dendrites. This finding suggests that growth of Purkinje cell dendrites can be chemotropically directed by vicinal axons. Moreover, such ectopic cells may be inappropriately activated via connections normally involved in acoustic processing.

This work was supported by a NINCDS NRSA Postdoctoral Fellowship (A.S.B.) and PHS grant NS-09904 (E.M.).

55.3

SPATIAL COOPERATIVITY BETWEEN NEARBY IMMATURE PURKINJE CELLS MEDIATED BY THEIR INHIBITORY RECURRENT COLLATERALS.

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In the 7 days potsnatal rat the cerebellar cortex is composed of immature Purkinje cells (PC), forming a monoplanar layer, coupled to each other by recurrent collaterals of their Gabaergic inhibitory axons. It is devoid of interneurons and PCs receive only few excitatory inputs. It is thus an interesting model to study how inhibitory coupling modifies ongoing activity and the spatiotemporal extent of cooperativity in a simple neuronal network. Extracellular recordings of pairs of PCs were made with methyl blue filled micropipettes (in 3M Nacl) in the cerebellar vermis of 6-8 days pups under control conditions and after blocking all inhibitory actions by superfusion of a Gaba antagonist. Relative positions of electrode tips was deduced from 3D morphologic reconstruction of the recorded folium. Treatment of data was done off-line and included classical methods as well as a method to calculate the informational entropy (Axelrad & al: 1st INNS Conf., 4:59,1987). Analysis of 8 pairs of PCs studied in both the coupled and uncoupled mode clearly indicate that: 1)recurrent collaterals exert a powerful inhibitory action leading to a 30% reduced rate of activity ; 2) in this case the shape of the interspike interval histogram (usually of Poisson or Gaussian types) is broader with greater values; 3) as judged from crosscorrelograms the cell seem to have symetrical connections if they are separated by less than 80µm, asymetrical ones from 80 to 300µm and none if they are further apart; 4)a minute fall in entropy takes place in the control mode (i.e the network has more informational capabilities). These results indicate that due to the action of the PC's inhibitory recurrent collaterals a spatially oriented cooperativity takes place between cells in the immature cerebellar network.

55.4

GRAFTED MESENCEPHALIC DOPAMINERGIC NEURONS, BUT NOT OTHER EMBRYONIC DA CELLS, DEVELOP IN ACETALDEHYDE/MPTP LESIONED, BUT NOT IN NORMAL, MOUSE ADULT STRIATA.

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We have shown that acetaldehyde/MPTP treated mice represent a good model to study in rodents the development of grafted embryonic DA neurons. Lesioned C57BL mice, grafted with dissociated mouse ventral E13 mesencephalic cells, develop an amphetamine induced turning behavior which well correlates to the number of TH^+ neurons that survive two to four months after the implant. On average, the number of TH^+ neurons corresponded to 0.1-0.2% of the implanted cells. They develop a rich network of fibers selectively and extensively innervating the lesioned host striatum. In implanted untreated controls, substantially fewer TH^+ neurons were found, a few localized in areas of the striatal parenchyma destroyed during the injection; most located in the corpus callosum or cortex and their fibers did not enter the normally innervated striatum. When hypothalamic E17 cells were used for the graft, implanted lesioned mice showed little or no rotation after amphetamine. In these animal the number of TH^+ neurons was much lower than that found when mesencephalic cells were implanted, and their network of fibers was very poor. Taken together, these data indicate that striatal cells deprived of the normal DA innervation, but not the normally innervated striata, can very effectively sustain the survival and development of DA neurons from the embryonic mesencephalon, but not that of other DA cells. It is postulated that trophic and/or tropic factor(s), specific for mesencephalic DA neurons, are produced by striatal cells when nigral DA cells are lost. It is also possible that such putative factors may be equally produced by the normal striatum but are not available for exogenous DA neurons when the endogenous DA fibers are intact.

55.5

AGE DEPENDENT CHANGES IN DISTRIBUTION OF SUBSTANCE P-LIKE IMMUNOREACTIVITY IN COHO SALMON.

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In an effort to understand the neural plasticity during smolt transformation (ST) in salmon, a number of neurotransmitters and neuromodulators are examined systematically. We report here our findings on substance P (SP). Brains and retinas of presmolt (n=6), postsmolt (n=3) and adult (n=1) coho salmon were fixed by perfusion with 4% paraformaldehyde and 0.25% picric acid dissolved in 0.1M Sorensens phosphate buffer (pH 7.2) and post fixed in the same fixative overnight. After several rinses in Tyrode buffer to which successively 25% sucrose was added, serial cryostat sections were prepared (33 µm). The tissue sections were incubated with anti-substance P (SP) (immunonuclear) diluted 1:1600-1:2000 overnight and the immunoreactive sites were visualized by standard PAP techniques. Method specificity was checked by using SP that had been preabsorbed with synthetic substance P (100 µg/ml antisera) or with omission of the primary antibody. Controls abolished all immunoreactivity.

The distribution of SPLI fibers and cells is similar to those reported in adult goldfish (Sharma, S.C. *et al., J. Comp. Neurol.*:279:104; 1989) with some exceptions. The dorsomedial area (DM) of the salmon telencephalon e.g., contains a large number of SPLI fibers, the DM increasing in size significantly with ST. The SPLI fibers in the tectum and habenula are also numerous, changing their distribution with aging. The quantity and distribution of SPLI change dramatically throughout life suggesting that SP systems are important in neural plasticity in salmon.

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55.6

THE ELIMINATION OF LOCUS COERULEUS AXONS DURING DEVELOPMENT CAN BE PREVENTED BY EARLY TRANSECTION OF THE NORMALLY MAINTAINED COLLATERALS.

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In adult rats, coeruleospinal (c⇒s) neurons are found only at mid-rostrocaudal levels of the nucleus, where they are essentially confined to its ventral, wedge-shaped half. However, during early postnatal development, c⇒s neurons are found throughout the nucleus. This developmental restriction of the distribution of c⇒s neurons is due to axonal elimination rather than to cell death, since neurons retrogradely labeled through their spinal axons perinatally are still present in the dorsal portion of the locus coeruleus at survival times beyond the age at which these cells lose their spinal projection (Chen & Stanfield, *Brain Res.* 410[1987]154). In the present study unilateral transections of the dorsal adrenergic bundle were made in newborn rats (n=12). Over one month later diamidino yellow was injected into the cortex of four of these rats and fast blue was injected into the upper spinal cord of all twelve. Similar injections were made in age-matched control rats (n=15).

In control animals which received the cortical diamidino yellow injection, many labeled cells are seen in the locus coeruleus. But none is found in the locus coeruleus of lesioned rats, though some are seen in other regions known to project to the cortex, including the midline raphé. The distribution of fast blue labeled c⇒s neurons is consistently more widespread in the lesioned animals than in controls. While still concentrated ventrally, many c⇒s cells are present in the dorsal half of the nucleus; some are even at its dorsalmost tip. In addition, c⇒s cells are found within the posterior pole of the nucleus. These results indicate that the absence of the normally maintained collateral of a locus coeruleus neuron is sufficient to prevent the elimination of a c⇒s collateral which would otherwise be lost, and also imply that during normal postnatal development, the presence of the maintained collateral may somehow contribute to the elimination of the transient c⇒s collateral.

55.7

THE FORMATION OF SPECIFIC MONOSYNAPTIC SENSORIMOTOR CONNECTIONS IN CHICK EMBRYOS IS NOT DEPENDENT ON PATTERNED NEURONAL ACTIVITY OR MOTONEURONAL CELL DEATH.

B. Mendelson and E. Frank. Dept. of Neurobiology, Anatomy and Cell Science, University of Pittsburgh, School of Medicine, Pittsburgh, PA 15261.

In vertebrate animals the sensorimotor connections involved in the stretch reflex exhibit a stereotypical pattern. Muscle spindle afferents monosynaptically excite motoneurons that innervate the same and synergistic muscles while they polysynaptically inhibit motoneurons that project to antagonistic muscles. The importance of patterned neuronal activity and motoneuronal cell death in the formation of these connections was studied by injecting d-tubocurarine (dtc) into eggs during the period when sensorimotor connections are made (St 28-40). Dtc blocked limb movements, thereby interrupting stretch evoked activity in spindle afferents. Short latency, fatigue-resistant synaptic potentials were recorded intracellularly from identified brachial and lumbosacral motoneurons upon stimulation of identified populations of spindle afferents. In both normal and curare-treated animals large excitatory potentials were evoked in homonymous motoneurons (those that innervate the same muscle) and were often observed in synergistic motoneurons as well. In contrast, these potentials were uncommon in antagonistic motoneurons. We saw no difference in the pattern of sensorimotor connections in normal versus curare-treated embryos. These observations suggest that neither normal patterns of neuronal activity nor motoneuronal cell death play a large role in the development of specific connections between sensory and motor neurons.

(Supported by NSF)

55.8

NETWORK ACTIVITY, AFFERENT ACTIVITY AND THE ROLE OF NMDA CHANNELS IN SYNAPTIC PLASTICITY IN VITRO.

R.D. Fields, C. Yu* and P.G. Nelson. Lab. of Developmental Neurobiology, NICHD, NIH, Bethesda, MD 20892.

Three factors which may be involved in the mechanism of activity-dependent synaptic plasticity: (1) electrical activity of afferents converging on a postsynaptic neuron, (2) activity in the neuronal network impinging on the postsynaptic neuron, and (3) NMDA channels, were studied in a multicompartment tissue culture chamber containing neurons dissociated from the mouse spinal cord (Nelson et al., Science, 1989). A three-way analysis of variance showed that synapses from stimulated afferents developed significantly stronger EPSPs than unstimulated convergent afferents ($p < 0.03$). Suppression of the network activity also significantly increased the amplitude of EPSPs from both active and inactive convergent afferents ($p < 0.01$), but the effect of blocking the NMDA channel depended on network activity ($p < 0.0001$; interaction term). If network activity was not suppressed, the NMDA channel antagonist APV (100 µM) inhibited the competition between active and inactive convergent afferents; but when network activity was suppressed with medium containing 3 mM Ca^{++}/3 mM Mg^{++}/5 nM TTX, the activity-dependent competition between convergent afferents was not inhibited by the NMDA channel antagonist. These changes in amplitude of synaptic potentials were paralleled by similar changes in the number of synaptic connections. As the medium used to decrease network activity contained elevated calcium, the influence of high calcium under normal network activity needs to be examined (Nelson, P.G. et al., these proceedings).

55.9

NMDA RECEPTOR AND CALCIUM INVOLVEMENT IN SYNAPSE ELIMINATION. P.G. Nelson, C.Yu*, R.D. Fields and D. Brenneman. Lab. of Dev Neurobiology, NICHD, NIH, Bethesda, MA 20892.

In medium containing 1.8 mM calcium and 100 μM amino-phosphonovaleric acid (APV), a specific blocker of the N-methyl-d-aspartate (NMDA) receptor, the activity-dependent plasticity occurring in APV-free medium in a spinal cord (SC)-dorsal root ganglion culture system (Fields, R.D., et al., these proceedings) is blocked. In media containing elevated calcium (3 mM) plus 100 μM APV, plasticity does occur despite the NMDA receptor blockade. The number of unstimulated afferents synaptically connected to each VH neuron was significantly reduced in medium containing 3 mM calcium + APV compared to unstimulated afferents in medium containing APV but normal (1.8 mM) calcium. The stimulated afferents connected to SC neurons in the two conditions were not significantly different. Thus, the elimination of connections from unstimulated afferents occurred in high calcium medium and was not dependent on NMDA receptor activation. Previous experiments (Brenneman, D., et al., J. Cell Biol., 104:1603, 1987) have shown that neural activity is accompanied by release of VIP which stimulates release of neurotrophic material from glia cells. We propose a dual effect of neural activity (1) a synapse (and perhaps neuronal) elimination process dependent on calcium ingress into neurons through NMDA and non-NMDA channels; (2) a synapse and neuronal stabilizing effect mediated by neurotrophic material released from glia in response to specific stimulation by neurally released glial secretogogues such as VIP. These processes and their appropriate spatial and temporal organization could subserve activity-dependent neural circuit formation in the developing brain.

55.10

MUSCLES DENERVATED AT BIRTH ARE REINNERVATED BY A NEW POPULATION OF MOTONEURONS. S. Presley*, and L. Ziskind-Conhaim. Dept. of Physiology and Center for Neuroscience, University of Wisconsin, Madison WI 53706

We have examined whether denervated muscles of newborn rats are reinnervated by the original or by a new motoneuron population. Fluoro-Gold (FG), a long-lasting fluorescent dye, was injected into one intercostal muscle (T_8) to retrogradely label its motoneurons. Two days later intercostal nerves T_7-T_9 were cut. Intercostal muscle T_8 was reinnervated within two to three weeks. Three to four weeks after axotomy tetramethylrhodamine isothiocyanate (TRITC) was injected into T_8 to label the population of motoneurons that reinnervated it. The Table summarizes the percentage of labeled motoneurons stained with FG, TRITC or double-labeled with FG and TRITC.

	2-3 days after birth		2-3 weeks after birth	
	axotomy	no axotomy	axotomy	no axotomy
FG	14*	38	36	29
TRITC	83	58	51	36
FG-TRITC	3	4	13	35
	(n=7)	(n=3)	(n=5)	(n=3)

*FG was absent in sections of 3 spinal cords.

Our results suggest that, in newborn rats, axotomy results in motoneuron death and muscle reinnervation by a new population of motoneurons. Supported by Research Career Development Award and NIH grant NS 23808.

55.11

DEVELOPMENT OF A NEURON-SPECIFIC TYPE III INTERMEDIATE FILAMENT PROTEIN AT THE RAT NEUROMUSCULAR JUNCTION. A.W. English, J.G. Wood, and G. Schwartz*. Dept. Anatomy & Cell Biology, Emory Univ., Atlanta, GA 30322

A type III intermediate filament protein has been described in adult motoneurons (57k IF, peripherin, clone 63) (e.g.Parysek and Goldman, J. Neurosci. 8:555, 1988). To examine its role in the development and stabilization of neuromuscular synapses, we examined its developmental appearance and localization in rat hindlimb muscles. In adults, 57k IF immunoreactivity (IR) was found in pre-terminal axons and throughout neuromuscular synaptic terminals. In embryos as young as E17, 57k IF was expressed in axons, but was not seen in terminals until P8. During early postnatal life, synaptic terminals associated with more than one 57k IF IR pre-terminal axon were seen uncommonly. Labelling of multiple synaptic terminals was found at these ages on muscle cells stained either for neurofilament proteins or for the microtubule asscociated protein Tau, but not with anti-57k IF. This selective presence of 57k IF at some synaptic sites but not others may be related to their ultimate survival of the process of synapse elimination. Supported by NS 20545.

55.12

JUVENILE ANDROGEN TREATMENT PERMANENTLY PREVENTS SYNAPSE ELIMINATION IN THE LEVATOR ANI MUSCLE . J.L. Lubischer, C.L. Jordan and A.P. Arnold, UCLA Dept. of Psychology, Los Angeles, CA 90024-1563.

In rats, the levator ani (LA) is one of the target muscles of motoneurons of the spinal nucleus of the bulbocavernosus (SNB). SNB cell size and number and the presence of the LA in adults are sexually dimorphic and can be masculinized by neonatal androgen treatment.

The pattern of innervation of the LA is also sensitive to the effects of androgen. Synapse elimination normally occurs between two and four weeks after birth. However, multiple innervation is maintained in the LA in juvenile castrates given daily androgen injections from postnatal day 7 (P7). This effect persists in the young adult rat (P63 and P90), even after androgen treatment has ended (at P34). The current study was undertaken to determine if this androgen-induced maintenance of multiple innervation is a permanent effect or if synapse elimination eventually occurs during adulthood in these animals.

Male rat pups (N = 5 per group) were castrated on P7 and given four weeks of daily subcutaneous injections of testosterone propionate (TP; 100 μg / 50 g body weight) or the oil vehicle. At 13 months of age, the LA was stained with tetranitroblue tetrazolium to visualize motor nerve terminals. Muscles were analyzed without knowledge of the hormone treatment received, and the percentage of fibers multiply innervated was determined for each muscle. Animals who received TP as juveniles showed elevated levels of multiple innervation in the LA (63%) compared to oil-treated animals (23%), despite the absence of androgens for one year. The effect of androgen on synapse elimination in the LA thus appears to be permanent.

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55.13

EXPRESSION AND LOCALIZATION OF DIFFERENT GAP JUNCTION PROTEINS IN RETINA AND OTHER REGIONS OF THE CNS. EA Finch* and DL Paul* (Spon: SM Shankland) Prog. in Neuroscience and Dept. of Anatomy and Cellular Biology, Harvard Medical School.

The expression of different gap junction proteins in the CNS was studied using antibodies and cDNA's specific for either rat connexin32 (Cx32) or rat connexin43 (Cx43). The retina was examined by immunofluorescence because its distribution of gap junctions is well characterized. Staining patterns consistent with known gap junction size and distribution were observed in horizontal cells using anti-Cx32 antibodies in mouse, rabbit and monkey retina. Rabbit retinal ganglion cells and cells in monkey inner nuclear layer were also labelled with anti-Cx32 antibodies. Cx43 immunoreactivity was detected in mouse outer plexiform layer and outer nuclear layer, mouse pigment epithelium and the stria medullaris of rabbit retina. Anti-Cx43 antibodies also strongly labelled cell borders in mouse optic nerve. Cultured astrocytes, studied using Northern blot analysis and immunocytochemistry, exhibited high levels of Cx43 expression. Cx32 was not detected. The levels of Cx43 and Cx32 mRNA in different brain regions were assayed by Northern blot analysis. Highest levels of Cx32 mRNA were detected in spinal cord and brainstem, moderate levels in midbrain, caudate and thalamus, and lower levels in cortex, hippocampus, hypothalamus, and cerebellum. Cx43 mRNA was present at relatively uniform levels in all brain regions examined except cortex. These results suggest that Cx32, a junction protein associated with epithelial cell types, may be expressed in some neurons. Cx43, which is expressed in many mesenchymal cell types, is clearly expressed in some glia, although we do not exclude neuronal expression.

55.14

SUPPRESSION OF NEURONAL ACTIVITY DOES NOT AFFECT SPECIFICITY, STABILITY OR COUPLING RESISTANCE OF NEW ELECTRICAL SYNAPSES. R.C. Berdan and A.G.M. Bulloch. Department of Medical Physiology, University of Calgary, Calgary, Alberta, Canada T6G 2E9

Our aim was to determine whether Na+-dependent regenerative activity plays a role in synaptogenesis of electrical synapses between identified neurons following axotomy. Buccal neurons 4 and 5 from the mollusc, Helisoma trivolvis, were induced to extend neurites and form new electrical synapses within cultured buccal ganglia by axotomy. Electrical coupling occurs between heterologous and homologous neurons within five days after axotomy (J. Neurophysiol. 48:569, 1982). We compared the incidence of coupling and coupling resistance between neurons B4-B5 and the contralateral pair of neurons B5 after axotomy in the presence and absence of tetrodotoxin (TTX). TTX (2 X 10-5M) effectively and reversibly blocked activity of buccal neurons B19, 5 and 4. Neurite outgrowth was unaffected by its presence, as determined by filling neurons with Lucifer Yellow. No differences in the incidence of electrical coupling or the coupling resistance were detected in the presence of TTX within 5 days after axotomy. We conclude that Na+-dependent neural activity does not play a role in the formation, maintenance or specificity of new electrical synapses formed following axotomy. Supported by AHFMR and MRC of Canada.

55.15

DEVELOPMENT OF SYNAPTIC CONNECTIONS IN A FILIFORM HAIR INTERNEURON SYSTEM IN LOCUSTS. H.J. Pflüger. Institute of Neurobiology, Free Univ. Berlin, D-1000 Berlin 33, FRG

An identified locust interneuron (A4I1) extends from the brain to the first unfused (fourth) abdominal ganglion (A4). The cell body is in A4 and the main input zone in the prothoracic ganglion (PRO). Its receptive field includes filiform hairs on the prosternum, similar hairs on the pronotum and a few hairs of windsensitive head hair field 1, all projecting to the PRO. Hairs on the ipsilateral prosternum, pronotum and head connect monosynaptically with the ipsilateral interneuron. Those of the ventral prosternum connect with the contralateral interneuron. Epsps in A4I1 generated by receptor spikes differ enormously in size. A few connections are powerful enough to generate spikes within A4I1. It appears that the most powerful connections are made by hairs already present in a first instar. In following instars these hairs increase in length and become those most sensitive to air displacements. Developmental age may determine the number of synapses made with A4I1. Supporting this conclusion, smaller filiform hairs, which appear later in postembryonic development, have much weaker connections.

55.16

PATTERNS OF SYNAPTIC INTERACTIONS WITHIN A PERIPHERAL NERVE IN *DROSOPHILA*: IN WILDTYPE STRAINS AND IN *PASSOVER*. M.D. Egger, R.S. Nowakowski, B. Peng* and R.J. Wyman†. Dept. of Anatomy, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854; †Dept. of Biology, Yale University, New Haven, CT 06511.

The jump-flight escape response in the fruitfly, *Drosophila melanogaster*, is mediated in part by a peripherally synapsing interneuron (PSI) that synapses with axons which innervate the dorsal longitudinal flight muscles (DLM) (King & Wyman, (1980) *J. Neurocytol.* 9, 753-770). In a search for possible neuroanatomical defects in *Passover* (*Pas/Pas*), a mutant in which the jump-flight escape response is defective, we examined electron microscopically, at intervals of 0.7 μm, the patterns of synapses formed between the PSI and the DLM motoneuron axons in *Passover*, and in two strains of wildtype flies, Canton-S and Oregon-R. Because of the stereotypy of the anatomy of the posterior dorsal mesothoracic nerve (PDMN) in which the peripheral process of the PSI runs, each of the five DLM motoneuron axons can be recognized individually. The PSI processes and the DLM axons within the PDMNs displayed no obvious ultrastructural abnormalities in *Passover*. Synapses were located in the PDMN from near the thoracic ganglion to about 30 - 50 μm distal to it. The total numbers of synaptic contacts within the PDMN were similar for wildtype (mean: 46.0 for n = 8 nerves) and *Passover* (mean: 50.3 for n = 6 nerves). The cumulative distributions of synaptic contacts in both wildtype and *Passover* are fit closely by a function of the form $f(x) = A(1 - e^{-Bx})$, where x = the distance from the thoracic ganglion, and A & B are constants. With respect to the individual axons, the distributions of synaptic contacts between the PSI and the DLM motoneuron axons (percent total contacts on each axon) were as follows:

Axon:	#1	#2	#3	#4	#5
Wildtype:	4.4	21.5	16.9	25.3	32.1
Passover:	0.0	18.2	35.4	17.6	28.8

The *Passover* pattern differed significantly from that of the wildtype (χ^2 = 84.6, $p < 0.001$), with most of the difference accounted for by the contacts with axon #3.

55.17

CELL CONTACT REDUCES NON-SYNAPTIC CHANNEL ACTIVATION BY TRANSMITTER (5-HT) DURING SYNAPSE FORMATION. P. Drapeau, McGill Univ. Ctr. Res. Neurosci. & Montreal Gen. Hosp., 1650 Cedar Ave., Montreal, Quebec, Canada H3G 1A4.

When serotonergic Retzius neurons of the leech contact pressure sensitive (P) neurons in culture, they selectively reduce a cationic response to 5-HT and reform the inhibitory, Cl-dependent synapse seen in vivo. Single cationic channel recordings (cell-attached patches) were performed in order to determine whether the number or activity of the channels is reduced by cell contact.

The largest i observed at V_{rest} (~-50 mV) had a γ = 60 +/- 2 pS (n=11), V_{rev} = +62 +/- 1 mV (i.e. E_{rev}~+12 mV), P_{open}= 10^{-4}-10^{-3} and τ_{open} ~1 ms. This cationic channel was observed in 52% (26/50) of the patches recorded from single P cells. In the presence of 10 μM 5-HT, 69% (18/26) of these channels showed a 2-10 fold increase in the number (but not the duration) of openings. No other channels were activated by 5-HT at V_{rest} (near E_{Cl}).

Recordings from P cells paired with Retzius cells showed a similar incidence (56% - 24/43 patches) of this channel. However, only 21% (5/24) of these channels were activated >2 fold by 5-HT. These results suggest that activation (and not the number) of cationic channels is reduced upon cell contact. The early clearing of the non-synaptic (excitatory) response to 5-HT appears to be a prelude to (inhibitory) synapse formation.

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TRAUMA I

56.1

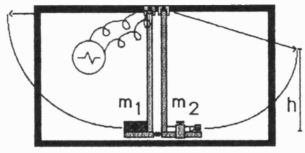
TIME COURSE OF LOCAL CEREBRAL GLUCOSE UTILIZATION (LCGU) ALTERATIONS AFTER MOTOR CORTEX ABLATION IN THE RAT. R.L.Sutton, D.A.Hovda, and H.T.Chugani. Brain Res. Lab., Rutgers Univ., Newark, NJ 07102 and UCLA School of Med., Los Angeles, CA 90024.

The quantitative 2-[14C]deoxyglucose autoradiographic method of Sokoloff et al. (1977) was used to compare LCGU in normal rats to that of rats sacrificed at 1, 3, 9 or 15 d. after right (R) sensorimotor cortex (SMC) ablation. Injury reduced LCGU in R and left (L) cortex (parietal, prefrontal, temporal, occipital), with largest decreases at d. 3 (L: -4.3 to -12.1%; R: -15.1 to -18.6%). LCGU in L SMC decreased on d. 1 & 3 (-3.1 to -2.9%) but increased 13.9% by d. 15. Greatest subcortical reductions in LCGU occurred at d. 1 or 3 in the n. accumbens, caudate, medial dorsal, anterior, & ventrobasal thalamic n. (L: -3.8 to -11.8%; R: -9 to -17.6%), with smaller reductions in the red n., nigra, locus coeruleus, cerebellar n. & globus pallidus (L:-1.3 to -7.3%; R: -2.6 to -8.1%). Reduced LCGU in cerebellar cortex (Crus I) at d. 1 & 3 was greater ipsilateral to injury (L: -7.7 to -10.1%; R: -8.3 to -10.6%). Gradual recovery towards normal LCGU values is evident by d. 9 to 15 in all regions, with some showing hypermetabolism. Data on LCGU alterations at 6, 12, 20 & 30 d. postinjury will also be presented. Support: USPHS Grant HD-07032.

56.2

AN IN VITRO MODEL OF RAPID ACCELERATION CNS INJURY (RAI) J.H. Lucas and A. Wolf*, Dept. of Biological Sciences and Center for Network Neuroscience, The University of North Texas, P.O. Box 5218, Denton TX 76203

We have developed an in vitro model of RAI which utilizes a ballistic pendulum impact apparatus. Impacts are delivered to flasks containing monolayer cultures of mouse embryo brain or spinal cord tissue. Impacts can be either tangential or normal to the plane of growth. The magnitude of an impact is expressed as Impulse (J) which equals the maximum momentum transferred to m_2. Thus, $J = m_2 [2gh]^{1/2}$. By measuring the impact duration (T), we can obtain force ($F = J/T$) and acceleration ($A = F/m_2$, also monitored by a g-meter). Initial studies have examined the effects of multiple tangential RAI on nerve cell survival. Six small neuronal circuits (5-10 cells each) were selected in separate regions of each flask. Flasks were given 5 or 10 tangential impacts (J_{cum} = 17.5 or 35 N·s). Time between impacts was approx. 3 s. The culture medium was removed just before RAI and replaced immediately after the last impact. Viability was determined by erythrosine B at 15 min, 2 h, 24 h, and 48 h after RAI. Mean cell death after 5 impacts was 37%, 41%, 40% and 34%. Mean cell death after 10 impacts was 40%, 44%, 43% and 51%. In the control group (medium removal, no RAI) mean cell death was 4%, 7%, 7% and 8%. Each mean represents 3-5 separate observations (flasks). Preliminary data indicate that tangential RAI are more damaging than normal RAI of the same J_{cum}. Cell death starts at cumulative tangential accelerations above 350g.



Planned studies will compare the effect on survival of: 1) multiple and single RAI of the same J_{cum} and 2) multiple RAI of the same J_{cum} but different intervals between impacts. We will also examine subcellular reactions to normal and tangential RAI including: 1) damage due to organelle shifts, 2) changes in DNA, RNA and protein synthesis, and 3) stress responses. Supported by the Hillcrest Fndtn. of Dallas, TX.

56.3

FOCAL STIMULATION OF STRESS PROTEIN SYNTHESIS DJ Gower, RS Glasser Section on Neurosurgery and Department of Anatomy, University of Oklahoma, Oklahoma City, OK 73103

Stress protein (SP, heat shock protein) synthesis is a primary cellular response to trauma. Barbe et al (1988) demonstrated in vivo protection of the retina from light damage was possible if the SP synthesis was initiated, by elevation of core body temperature, 18 hours prior to light exposure. This project develops a model with SP synthesis in a single retina without stressing the entire animal.

Anesthetized rats were administered 2ul intraocular injection of a 1mM solution of ten test substances known to induce the SP response in culture. The opposite (control) eye was injected with carrier vehicle only. Eighteen hours after injection the retinas were harvested and evaluated using 2-D gel electrophoresis and western blots probed with antibodies against SP-70 (H11, H7, N27 kind gift of Dr. William Welch). Silver nitrate, cadmium and arsenite are effective stimulants of retinal SP synthesis.

These data suggest that SP synthesis can be stimulated in a small subset of cells in vivo and this model will be useful to study the role of SP's in the protection of CNS from injury.

56.4

ANATOMICAL BASIS OF SENSORY MOTOR DYSFUNCTION FOLLOWING TRAUMATIC BRAIN INJURY. A.S.Merians, A.W.Deckel University of Medicine and Dentistry of New Jersey, Newark, N.J. 07107.

Defining morphological changes occurring post TBI in relationship to changes in behavior provides an understanding of the anatomical basis of sensory motor dysfunction. Previous work demonstrated that this impact model manifests persistent behavioral deficits resembling the hemiplegia, balance and tactile deficits evident clinically. Lesioned animals demonstrated dysfunction in vertical locomotor activity, sensation, and balance and increased ipsilateral rotation at 1, 3 and 6 weeks. There were consistent changes in peripheral physiology and metabolic brain activity.

Image analysis was used to quantify the morphological effects of TBI on 23 rats (10 control and 13 lesion). Analysis indicates a decrease in the depth of the neocortex and the area of the striatum, and an increase in the area of the ventricles ipsilateral to the lesion. Stepwise regression analysis using these neuroanatomical measures to "predict" behavioral outcome measures indicates that the left anterior striatum and neocortex are strongly associated with the regulation of horizontal movement in the controls, while in animals with left TBI this relationship shifts. The ipsilateral or contralateral neocortex and the contralateral striatum become dominantly associated with locomotor activity.

56.5

ALTERATION OF CUTANEOUS SENSATION FOLLOWING BURN INJURY. R.S. Ward,* J.R. Saffle* and R.P. Tuckett. Dept. Physiol., Univ. Utah School of Medicine, Salt Lake City, UT 84108.

Though alterations in cutaneous sensation following severe burn injury have been frequently reported, there have been few quantitative psychophysical studies of such changes. Sensory testing was performed on burn patients (N=60) ranging in age from 18-65 years (mean [M] = 34) with burn size ranging from 1-60% of total body surface area (M = 27%). All patients had undergone skin grafting. Fifty-eight patients (97%) exhibited significantly diminished or absent responses to sharp/dull, hot/cold and light-touch over their grafted areas. Deep pressure sensation remained intact (N = 60). In all but one case the difference between measures in normal skin and skin in donor areas or in areas of healed (ungrafted) partial thickness burns was not significant. Grafted areas in which some dermis remained intact (i.e., tangentially excised, deep partial thickness burns) showed significantly greater recovery than full thickness or deeply excised areas of injury. In addition, patients described alterations in sensation including chronic pruritus (25%) and pain (25%). There was no significant correlation between sensory loss, age, burn size or type of burn. In conclusion, alterations in sensory profile following thermal injury can be highly significant both in terms of sensory deficit and ongoing sensations of pruritus and pain. Supported by USPH grant NS15102.

56.6

A PHOTOCHEMICALLY-INDUCED SPINAL CORD INJURY MODEL. V.R. Holets, A. Salvatierra, R. Prado, M.B. Bunge, R.P. Bunge and B.D. Watson[1]. Depts. of Neurological Surgery and [1]Neurology, Univ. of Miami, Miami, FL 33136.

The goal of the present study was the development of a spinal cord injury model with characteristics similar to the human lesion. Using a modification of the photochemical method of spinal cord injury in non-laminectomized rats (Prado et al, J Neurosurg 67:745, 1987), a model has been developed in which there is a central cyst, lesion of the entire central grey at the epicenter, and demyelination of some white matter tracts.

Young adult rats received a photochemically-induced spinal cord lesion using a focused argon dye laser beam and the photosensitizer Rose Bengal. Two to four weeks later, the animals received an injection of lysolecithin into the cyst at the epicenter of the lesion (T7). Immediately after surgery the animals had flaccid paralysis of the hindlimbs, but recovered some movement of the hindlimbs after 3-5 days. Histological examination using one micron thick plastic sections showed demyelination of the some white matter tracts in the ventral and ventrolateral funiculi. Future studies will demonstrate the physiological and functional consequences of this lesion. The potential use of this model for transplantation of cells into the repository provided by the cyst will also be examined. Funded by The Miami Project and The Daniel Heumann Fund for Spinal Cord Research.

56.7

Delayed loss of function, and long term recovery after spinal cord compression in the rat. J.A. Gruner, K. Sakatani, H. Iizuka*. Dept. of Neurosurgery, NYU Medical Ctr., 550 First Ave., New York, NY 10016.

Following spinal cord injury (SCI) a period of delayed or "secondary" cell damage or death is presumed to occur, which constitutes the primary rationale for efforts to develop clinical SCI treatments. Still, there is little evidence to support this assumption, and no model of the phenomenon. Here we report on the course of recovery of somatosensory evoked potentials (SEP) and auditory evoked "startle" responses (ASR) after spinal compression in the rat.

Rats were anesthetized with sodium pentobarbital. Using sterile surgical procedures, the skull was exposed and 3 metal screws implanted for recording SEP. Metal staples were placed over the vastus laterals (VL) and tibialis anterior (TA) muscles of the hindlimb for recording ASR. A laminectomy was performed to expose the cord at T8 with the dura left intact. A 40 gram vascular clip was used to compress the sides of the cord for 5-20 sec. SEP were recorded before and after laminectomy, and 10 min after compression. At 24 and 48 hrs and 1, 2, 4, and 8 wks after injury, SEP and ASR were recorded. ASR were evoked in the awake animal by a 10 ms tone, after which SEP were evoked by sciatic stimulation under light ketamine anesthesia.

The predominant response pattern following compression was a partial to complete loss of SEP at 10'. Several animals showed strong SEP at 10' and complete loss at 24 hours; all animals showed some deficit at 24 hours. SEP and ASR amplitudes recovered in parallel between 48 hours and 8 weeks, reaching maximum after 4 weeks. ASR and SEP during this period appeared correlated with motor function. Thus animals which could support and move their limbs at 24 hours showed ASR and SEP, while completely paralyzed animals did not. As animals progressively became able to move their hindlimbs, then achieve full standing posture, and finally locomote (generally by 4 weeks), ASR and SEP also progressively improved. In conclusion, a) rat spinal cord compression may represent a useful model of delayed SCI, and b) ASR appear to provide a reliable motor function test for awake rats.

56.8

DYNAMICS OF EXTRACELLULAR LACTATE, PYRUVATE, ASCORBATE, AND PURINES FOLLOWING BRAIN CONTUSION IN THE RAT. L.Hillered (1,2), P.Nilsson*(2), U.Ungerstedt*(3), and U.Pontén*(2).
Depts. of Clinical Chemistry(1) and Neurosurgery(2), University Hospital, S-751 85 Uppsala, and Dept. of Pharmacology(3), Karolinska Institute, Stockholm, Sweden.

The aim of this study was to measure changes in extracellular energy metabolite levels following a compression contusion trauma. A craniotomy (6x9 mm) was made over the right parietal cortex in artificially ventilated rats. A microdialysis probe (CMA/10, Carnegie Medicin, Stockholm) was inserted stereotaxically into the cortex at the medial edge of the craniotomy. The probe was perfused with Ringer solution (2 ul/min) using a CMA/100 microinjection pump (Carnegie Medicin) and samples were collected in 10 min fractions for 2 hours after implantation. At that time the probe was removed and the contusion device put in position. After the trauma, the probe was reinserted into the same position within 45 s, using the stereotaxic instrument. Microdialysis samples were collected for another 2 hours following this procedure, and analyzed by HPLC. The trauma induced a dramatic increase in the dialysate concentration of lactate, ascorbate, adenosine, inosine, and hypoxanthine while pyruvate increased slightly. The changes virtually normalized within 2 hours. In control animals (subjected to removal and reinsertion of the probe without the trauma) no concentration changes occurred.

56.9

XANTHINE OXIDASE IN EXPERIMENTAL SPINAL CORD INJURY (ESCI) C.Y. Hsu, J. Xu*, J.S. Beckman*, P.L. Perot Jr.* and E.L. Hogan (SPON: N.L. Banik) Depts. of Neurology and Neurosurgery, Medical University of South Carolina, 171 Ashley Avenue, Charleston, SC 29425, USA; and Dept. of Anesthesiology, University of Alabama, Birmingham, AL 35233, USA.

Conversion of xanthine dehydrogenase (XDH) to xanthine oxidase (XD) may produce oxygen free radicals which are mediators of inflammation, and have been implicated in the pathogenesis of brain edema. Proteolytic formation of XO may be mediated by calpains which are activated in ESCI. We studied XDH and XO activity in ESCI using a sensitive fluorometric assay. Trauma caused an increase in both XDH and XO activity (Sham: XDH, 27±2 μunits mg protein, XO, 10±1 [N=11]; Trauma XDH, 51±7, XO, 21±3 [N=9]). Allopurinol (AL) treatment (100mg/kg, IP x 1 hr prior to and 6 hr after trauma) resulted in no detectable XDH or XO activity in traumatized spinal cord. AL treatment did not affect post-traumatic edema formation (H_2O content, control 76.54±0.30 %; AL 76.67±0.32, N=10) or polymorphonuclear cell (PMN) infiltration (myeloperoxidase activity, control 1.29±0.17 units/g wet weight, AL 1.14±0.15, N=15). The results suggest that if oxygen free radicals contribute to post-traumatic edema and PMN infiltration in ESCI, XO is not likely to be the main source of free radicals.

56.10

LACK OF EFFICACY OF A 21 - AMINOSTEROID IN THE RAT VENTRAL SCI MODEL E.Wilcoxson*, E.C.Benzel, T.Woods*, M.Fowler*, J.Lancon*, L.Kesterson*, T.Hadden*

A ventral rat SCI model was used to test the efficacy of a 21-aminosteroid, U74500A. 39 animals were studied in 2 groups: (1) treatment group (1 mg/kg at 45 min and 0.5 mg/kg U74500A I.P. at 2 1/2 hrs following lesioning), and (2) control group. Subsequent to pre and postinjury neurologic assessment, histologic evaluation at the level of injury and 1 cm above the injury was also performed. No difference between treatment and control groups was noted with respect to neurologic outcome or histologic changes. The lack of efficacy of U74500A is in contrast to previously published data. Perhaps the use of a different 21-aminosteroid, the use of the same agent at a higher or lower dose or its administration at a different time following lesioning may offer superior results. The model utilized may also yield results which are at variance with other techniques. It should be kept in mind, however, that the model used here closely mimics the human SCI pathophysiological situation and that it may be a more appropriate model for the assessment of potentially useful therapeutic regimens.

56.11

OPIOID VS. NON-OPIOID MECHANISMS OF DYNORPHIN A-INDUCED PARALYSIS: INVOLVEMENT OF κ-OPIATE AND N-METHYL-D-ASPARTATE (NMDA) RECEPTORS. R. Bakshi* and A.I. Faden.(SPON: B.Lester) Center for Neural Injury, University of California, San Francisco, CA 94121.

Endogenous dynorphin A (Dyn A)-related peptides may contribute to the pathogenesis of spinal cord injury. Intrathecal administration of such peptides causes paralysis; these include Dyn A(1-17) and Dyn A(1-13), which bind to opiate receptors, as well as Dyn A(2-17) and Dyn A(2-13), which are inactive at opiate receptors. It has been suggested that either opiate receptor antagonists or N-methyl-D-aspartate (NMDA) antagonists may modify effects of intrathecal (i.t.) Dyn A. In the present studies, the κ–active opiate receptor antagonist nalmefene, the κ-selective antagonist nor-Binaltorphimine (nor-BNI), the competitive NMDA antagonist CPP, and non-competitive NMDA antagonist dextrorphan were tested for their ability to modify rat hindlimb paralysis produced by Dyn A(1-17) and Dyn A(2-17). Intrathecal pretreatment with each of the drugs significantly limited the acute motor dysfunction ($p<.005$) and mortality ($p<.05$) associated with Dyn A(1-17). Effects of nor-BNI and CPP were dose related ($p<.01$). However, while CPP and dextrorphan also significantly attenuated the paralytic effects produced by Dyn A(2-17) ($p<.005$), nor-BNI and nalmefene did not. From these data we suggest that Dyn A-induced paralysis includes an opioid component involving κ-opiate receptors and a non-opioid component involving NMDA receptors.

56.12

SEPARATE ROLES FOR SODIUM AND CALCIUM IN NEURONAL INJURY. **D. G. Emery, J. H. Lucas and G. W. Gross. Zoology Dept., Iowa State University, Ames IA 50011, and Biology Dept., University of North Texas, Denton, TX 76203.**

After laser dendrotomy, neurons from dissociated mouse spinal cord cultures were examined by TEM. After injury in medium with normal Na and Ca, mitochondria (MIT) were dilated, many vacuoles formed in the cytoplasm, and the cytoskeleton (CS) was disrupted. Vacuoles appeared in the somata before vacuolization in the dendrite had spread to the perikaryon, indicating a two-phased pathology. If all extracellular ions were replaced with sucrose, dendrotomy had little effect on ultrastructure. After injury in low-Ca, MIT did not dilate but became electron dense, and many vacuoles formed in dendrites and somata. In low-Na few vacuoles formed in somata after injury. The sodium ionophore, monensin, caused dilation of the Golgi in uninjured neurons. Dilation of the Golgi also occured with monensin if isothionate replaced the extracellular Cl.

Therefore, we hypothesize the following roles for ions in physical injury: rows of vacuoles in injured dendrites (dilated smooth endoplasmic reticulum) are due to Na and Ca influx through the lesion. Dilation of Golgi is due to general influx of Na, perhaps due to neuronal depolarization after injury. MIT dilation and CS disruption are due to influx of Ca through the lesion. There is no clear role for anions (chloride) in physical trauma.

56.13

ALTERATION IN EXTRACELLULAR AMINO ACIDS AFTER TRAUMATIC SPINAL CORD INJURY S. Scott Panter, Sabrina W. Yum*, Paul Demediuk, and Alan I. Faden., Department of Neurology (127), University of California San Francisco and Veterans Administration Medical Center, San Francisco, CA 94121.

Tissue damage following spinal cord trauma is diminished by the posttraumatic administration of N-methyl-D-aspartate antagonists, suggesting that excitatory amino acids (EAA's) play a significant role in the secondary injury response. Using microdialysis, we have studied the concentrations of extracellular amino acids in rabbit spinal cord after the administration of impact trauma. Nine different excitatory, inhibitory, and non-transmitter amino acids were evaluated; the extracellular concentration of each was elevated following trauma. Of note were the prolonged elevations of glutamate, aspartate and glycine. Following moderate (40 gm-cm) trauma, the concentrations of glutamate, aspartate, and glycine increased 357, 261, and 330%, respectively. Peak levels were detected 10 minutes posttrauma but returned to control values by 20 minutes. Following severe (150 gm-cm) trauma, a more significant elevation of glutamate, aspartate and glycine was observed, attaining increases of 410, 542, and 454%, respectively. Peak levels were measured at 20 minutes posttrauma, and a significant elevation was maintained for 50-60 minutes. Peak concentrations of glutamate, aspartate, and glycine were elevated beyond levels that might arise from the leakage of plasma free amino acids into the injury area. These results are consistent with the hypothesis that EAA's may contribute to delayed tissue injury after CNS trauma.

56.14

EFFECTS OF ANTAGONISTS OF EXCITATORY AMINO ACIDS ON FUNCTIONAL DEFICITS AFTER CONTUSIVE SPINAL CORD INJURY. M. Reynolds*, C. McCauley*, B. Sullivan* and J. Wrathall (SPON: H. Bernstein-Goral). Dept. of Anat. & Cell Biol., Georgetown Univ., Washington, D.C. 20007.

Recent studies indicate a potential role of excitatory amino acids (EAA) in traumatic injury of the brain and spinal cord. To investigate this hypothesis we examined the effects of EAA antagonists present at the time of injury on functional deficits resulting from a standardized contusive spinal cord injury. Rats were anesthetized with chloral hydrate, cannulated for intravenous drug infusion and a laminectomy performed at the T8 vertebral level. They were randomly assigned to groups (n = 12) receiving the EAA antagonists MK-801 (1 mg/kg), dextromethorphan (10 mg/kg), kynurenate (KYN, 300 mg/kg), or controls receiving an equal volume of saline. One half of the total dose was given 5 min prior to, and the remainder 15 min after a moderate contusive injury. Functional deficits were assessed at 1, 7, 14, 21 and 28 days after contusion using a battery of behavioral tests. All groups showed maximal deficit at 1 day with recovery of some function over time. However, the KYN group showed greater and especially more rapid recovery of several hindlimb reflexes as well as weight-bearing and use of the hindlimbs in locomotion. There was a significantly better combined behavioral score (CBS) by day 14. The results suggest that EAA-mediated processes may be involved in some aspects of the functional deficits resulting from contusive injury.

56.15

NMDA-RECEPTORS MEDIATE ACTIVATION OF THE c-fos PROTO-ONCOGENE IN A MODEL OF BRAIN INJURY. D.G. Herrera and H.A. Robertson. Department of Pharmacology, Faculty of Medicine, Dalhousie University, Halifax, Nova Scotia, Canada, B3H 4H7

The proto-oncogene c-fos is rapid and transiently induced in central nervous system by a variety of stimuli. Brain injury, disruption of pia-arachnoid in a limited area, is one of the situations that leads to a dramatical increase in c-fos immunoreactivity. This increase is limited to the lesioned hemisphere Injection of atropine (25 mg/kg i.p.), naltrexone (5 mg/kg i.p.), nifedipine (5 mg/kg i.p.), and W7 (20 mg/kg i.p.), prior to the injury, did not affect the activation of c-fos as assesed by immunohistochemistry in adult Sprague-Dawley rats perfused two hours after the lesion. The non-competitive NMDA antagonists ketamine (100 mg/kg i.p.) and MK-801 (3 mg/kg i.p.) markedly reduced c-fos activation. PCP (10 mg/kg i.p.) produced a slight reduction in damage-induced c-fos activation. This study suggests that c-fos activation in this particular model is N-methyl-D-aspartate receptor-mediated and supports the idea that the c-fos proto-oncogene might play a role in plasticity and/or neurotoxicity.
(Supported by the MRC of Canada)

56.16

HYPOTHERMIA PROTECTS SPINAL CORD NEURONS AFTER INJURY
G. Wang*, J. H. Lucas, and G. W. Gross, (SPON: J.B.Kirkpatrick) Center for Network Neuroscience, University of North Texas, P.O. Box 5218, Denton, TX 76203

There have been many investigations of the protective effects of low temperature following spinal cord injury (SCI). Despite strong interest, the therapeutic efficacy of hypothermia is still controversial. The cascade of responses to SCI in the intact animal makes it difficult to determine the specific action of a given intervention. In the present study we used an in vitro model of injury to evaluate the direct effects of low temperature on neuron survival after dendrite amputation.

A laser microbeam was used to transect primary dendrites from mouse spinal cord neurons growing in monolayer cultures. In each experiment, 10 neurons were operated and 10 others served as unoperated controls. Lesion distance was 100 μm from the soma of each operated cell. Experiments were performed as pairs with one culture at a reduced temperature and one at 37°C. After 2 h at the selected temperatures (T), erythrosine B was used to determine cell viability.

Studies to date have shown that the probability of nerve cell survival 2 h after lesioning is a function of 1/T. At 27°C survival was 70% ± 9 (SD) compared to 60% ± 9 survival at 37°C. At 17°C survival was 86% ± 13 compared to 68% ± 8 at 37°C. At 7°C survival was 100% compared to 56% ± 6 survival at 37°C. There were 5 matched pair experiments in each study. Unoperated control survival was 100% in all studies.

Below 17°C both lesioned and control neurons tended to swell, and, upon rewarming to 37°C, some cells died. One day after lesioning, viability of nerve cells maintained initially at 17°C for 2 h and then restored to 37°C was 64% ± 11; viability of operated normothermic neurons was 50% ± 12 (N=5 pairs of experiments). These data suggest that low temperature not only slows cell deterioration, but may actually increase the numbers of neurons which survive a physical trauma. Experiments are in progress to determine whether longer intervals at 17°C will cause a further increase in cell survival. We will also examine how soon after injury hypothermia must be applied in order to be effective.

Supported by PHS grant NS23686-03 and by a grant from the Hillcrest Foundation of Dallas, TX.

56.17

EXOGENOUS AND ENDOGENOUS OPIATES AFTER SEVERE HEAD INJURY R.S. Zimmerman,*[1] R.L. Hayes[1], D.L. Morris,*[2], and W.L. Dewey[2] Divisions of Neurosurgery[1] and Neuropharmacology[2] Medical College of Virginia, Richmond, VA 23298

It has been suggested that release or production of endogenous opiates after brain trauma worsens the prognosis for neurological recovery. Thus, the present study was undertaken to examine if an association existed between acute levels of endogenous opiates in ventricular CSF (vCSF) and functional outcome. All severely head injured patients at the Medical College of Virginia were entered into a uniform treatment protocol which included the placement of a ventricular catheter. Samples of vCSF were obtained <24 hrs after injury in 15 patients. Immunoreactive beta endorphin (ir-βE) was then quantified with radioimmuno assay. Ir-βE had *no* significant correlation with either 6 month or 1 year outcome based on the Glascow Outcome Score, even when stratified by age, sex, or initial degree of injury. However, levels of ir-βE *were* significantly lower if the patients had received exogenous opiates for sedation (85.9 pg/ml vs. 47.4 pg/ml with morphine sulfate, $p<0.05$). These results suggest that 1) there is no correlation between acute vCSF ir-βE levels and long-term outcome after severe head injury, and 2) systemic administration of exogenous opiates (morphine) reduces the level of ir-βE in vCSF.

Supported in part by NIH grant NS-12587

TRAUMA II

57.1

HISTOCHEMICAL AND BEHAVIORAL CHANGES FOLLOWING EXPERIMENTAL BRAIN INJURY IN RAT. D. Becker, B. Shook, L. Gorman, and Y. Katayama. BRI, Depts. Neurosurgery and Anatomy & Cell Biology, UCLA Med. School, Los Angeles, CA 90024.

Cytochrome oxidase (CYO) histochemistry and an operantly conditioned bar press (bp) were used to study the effects of fluid percussion (animal model of concussion). The effects were compared to normals and sham operates.

Fluid percussed rats exhibited a selective absence of oxidative metabolism in the medial septum and hippocampal cell layers. Sham operates showed a mild decrease in CYO activity in the same regions. These effects cannot be attributed to a contusion, since in all animals the intervening striatum showed no deficit in metabolic activity.

Relative to pre-operative bp rates (mean = 13.39 bp/min), post-injury rates covaried with the extent of CYO activity; percussed mean = 2.07 and sham mean = 5.94 bp/min.

This is the first report of a localized functional deficit which correlates with a concussive behavioral disorder.

57.2

MEASUREMENT OF DEPRESSION LEVELS IN MINOR HEAD INJURED ADOLESCENTS USING EEG SLEEP AND PSYCHOMETRIC TESTS. L. C. Parsons, and T. Britt*, Sleep Lab., College of Nursing, Univ. of Ariz., Tucson, AZ 85721.

One purpose of this study was to correlate the relationship between electroencephalographic (EEG) sleep variables indicative of depression with psychometric measurements of depression in persons following Minor Head Injury (MHI) within 72 hours, 6 weeks, and 12 days of injury. Sleep variables which identified depression were latency to the first rapid eye movement (REM) period, expanded time in the first REM period, decreased deep (delta) sleep, early morning awakening, and increased sleep stage cycling. The psychometric instruments used to measure depression were the Beck Depression Inventory (BDI) and the Center for Epidemiologic Studies-Depression (CES-D). The sample consisted of 20 subjects, 10 adolescent MHI patients and 10 gender and age matched control subjects. The variables of gender and age were treated as covariates during statistical analysis. Randomization was achieved by matching MHI patients with control subjects. ANOVA was used to determine significant changes within groups. Independent t-tests were used to assess between group differences. Findings indicate that depression levels when measured using the BDI and CES-D were significantly higher ($p < .05$) in the MHI group when compared to the control subjects at the measured time intervals. Alterations within the sleep patterns of the MHI patients indicative of depression were also identified. Interpretation of these data raise two questions, 1) were the MHI patients depressed before the injury and if so could this have contributed in some way to their accident, or 2) could the MHI have contributed to the depression levels? Identified depression indicators within the sleep patterns appeared to support the hypothesis of a preexisting depression. Factors indicative of depression in both the EEG sleep pattern and psychometric tests may provide a profile related to the time course of depression following MHI.
Supported by NINDS NS24169

57.3

REPAIR OF DAMAGED SQUID GIANT AXON. P.E. Gallant, K. Hammar* and T.S. Reese. Laboratory of Neurobiology, NINDS, NIH, & Marine Biological Laboratory, Woods Hole, MA 02543

Repair of mechanically injured squid giant axons was studied with four microscopic techniques, dark-field and DIC microscopy of living axons and light and electron microscopy of fixed axons, at various intervals after injury. After transection constriction of the axon began within mins near its severed ends and progressed for approximately 1 hr. A condensed plate of axoplasm formed at the neck of this constriction 50-100 μm from the cut end. This condensed axoplasm marked the densest part of the white area formed at the cut ends of living axons and it shrank radially as the axon progressively constricted. The axolemma and cytoskeletal elements closest to the transection were severely disrupted but became progressively more normal until they finally appeared completely normal at 500-600 μm from the transection; they remained normal for up to 10 hrs after transection. In living axons punctured with a micropipette a small plate of disrupted axoplasm developed and soon surrounded the puncture site. The disruption of axoplasm, axoplasmic whitening and constriction were inhibited when the extracellular calcium, magnesium and chloride were replaced with sodium aspartate. What powers axonal constriction is unclear, but an ion-dependent contraction of the axoplasm might be responsible since the axoplasm in the constricted area occupied progressively less space without folding, while the axolemma, Schwann cells, and connective tissue became highly folded around the shrunken axoplasm.

57.4

TASKS TRADITIONALLY USED TO ASSESS CALLOSAL FUNCTION REVEAL PERFORMANCE DEFICITS ASSOCIATED WITH MILD CLOSED HEAD INJURY. B.D.Fantie† and B.Kolb, Psychology Dept, University of Lethbridge, Lethbridge, Alberta, Canada T1K 3M4.

Neuropathological studies have consistently indicated that there is disproportionately severe damage to the corpus callosum in cases of closed head injury (CHI) and that this can occur in cases when the trauma seems relatively minor. Our aim was to determine if we could assess hemispheric disconnection or callosal dysfunction in the processing of various stimuli presented preferentially to one hemisphere or the other. Visual stimuli (words, alphabetic trigrams, and numerals) were tachistoscopically presented (150 ms) to the right or left of a fixation point and the subjects were required to identify verbally what they had just seen. A haptic information transfer test required subjects to match small patterns of pinheads using only their sense of touch. On the visual task, the CHI group (n=18, mean age=24 years, mean WAIS-R IQ=114) made more errors identifying words, $F(1,43)=4.50$, $p=.040$, and alphabetic trigrams, $F(1,43)=4.60$, $p=.038$. Males did more poorly than females overall ($F(1,23)=7.66$, $p=.011$) and were more affected by injury. The CHI group also made more errors on the haptic transfer task, $F(1,99)=14.22$, $p<0.001$. Therefore, we have evidence that CHI causes deficits in the performance of tasks traditionally used to assess callosal functioning. Although likely the consequence of some degree of callosal dysfunction, the pattern of results is not compatible with a simple hemispheric disconnection explanation. [Supported by an AHFMR grant to BDF.](†present address: Neuropsychology Laboratory, The American University, Washington, D.C. & LPP-NIMH, Bethesda, MD.)

57.5

HEAT SHOCK PROTEIN EXPRESSION IN RAT BRAIN AFTER EXPERIMENTAL BRAIN INJURY. T. McIntosh, H. Soares, M. Gonzalez and F. Sharp, Dept. of Surgery, Univ. of Conn. Health Ctr., Farmington, CT 06032 and Dept. Neurology, Univ. California San Francisco, CA 94121.

Heat shock proteins (HSPs) of 70-80 kDa molecular weight are synthesized in the mammalian brain in response to a variety of stressors including hyperthermic shock, ischemia and various pharmacological agents. Recently, Gonzalez et al (1989) suggested that the 72 kDa HSP (HSP72) may be used as a marker of cellular injury in the mammalian brain. Little is known concerning the response of HSPs to traumatic brain injury. We examined whether HSP72 expression was correlated with the severity of injury following experimental fluid-percussion (FP) traumatic brain injury. Male Sprague-Dawley rats (350-400 g, n=12) were anesthetized with sodium pentobarbital (50 mg/kg i.p.) and subjected to mild (1.9-2.0 atm), moderate (2.3-2.4 atm) or severe (2.7-2.9 atm) parasaggital FP brain injury centered over the left parietal cortex. All rats were sacrificed at 48 h postinjury, 50-100um coronal sections were cut and reacted immunohistochemically using a monoclonal antibody to HSP72 and the avidin-biotin-peroxidase (ABC) method. Moderate FP brain injury but not mild or severe injury induced dark, specific HSP72 immunostaining in the injury site (left parietal cortex), left hippocampus and right (contralateral) hippocampus. This pattern of immunostaining coincided with previous histological data on neuronal damage produced by FP brain injury. Although it is unclear whether cells exhibiting HSP72 immunoreactivity survive or die, these results suggest that HSP72 immunocytochemistry may be used to identify injured cells in neural tissue after traumatic brain injury.
Supported by VA Merit Review 74R and NIH RO1-NS26818

57.6

ALTERATIONS IN BRAIN NEUROPEPTIDE Y AFTER EXPERIMENTAL BRAIN INJURY. V. Head*, H. Soares, D. Ferriero and T. McIntosh (SPON: S. Wentzel). Dept. Surgery, Univ. of Conn. Health Ctr., Farmington, CT 06032 and Dept. Neurology, Univ. of California, San Francisco, 94143.

Immunocytochemical studies have demonstrated that cerebral blood vessels are supplied by perivascular nerve fibers containing Neuropeptide Y (NYP)-like immunoreactivity. NPY has also been implicated in cerebral vasospasm. Although vasospasm may be involved in the pathophysiological sequelae of traumatic brain injury, nothing is known of the response of central NPY to brain trauma. Male Sprague-Dawley rats (n=40) were anesthetized with pentobarbital (50 mg/kg i.p.) and subjected to fluid-percussion (FP) brain injury centered over the left parietal cortex. Animals were sacrificed prior to injury (baseline) and at 1 min, 15 min, 1 h and 24 h following brain injury. Regional brain homogenates were analyzed for NPY concentration using radioimmunoassay. A second group of animals (n=4) were sacrificed at 15 min postinjury, brains sectioned and subjected to NPY immunocytochemistry. No significant changes in NPY were observed in the contralateral (uninjured) cortex, brainstem or hypothalamus. NPY concentrations increased significantly at the injury site within the first minute postinjury ($p < 0.05$), reached a peak at 15 min ($p < 0.05$) and remained significantly elevated up to 1 h postinjury ($p < 0.05$). At 15 min, NPY immunoreactive neurons were present at the injury site and around the areas of hemorrhage. By 24 h postinjury, NPY concentrations had returned to baseline values. These data demonstrate that NPY concentrations are elevated acutely in the injury site after brain injury and suggest that NPY may be involved in the pathophysiology of posttraumatic cerebral vasospasm or ischemia.
Supported in part by VA Merit Review 74R and NIH RO1-NS26818

57.7

RESERPINE PRETREATMENT BLOCKS AMPHETAMINE FACILITATED LOCOMOTOR RECOVERY FOLLOWING SENSORIMOTOR CORTEX ABLATION. K.A. Krobert, S.A. Queen*, & D.M. Feeney. Depts. of Phys. and Psych., UNM, Albuquerque, NM. 87131.

Unilateral injury to sensorimotor cortex (SMCX) produces a transient contralateral hemiplegia observed on a beam walking (BW) task. A single administration of amphetamine (AMPH) given 24 hrs after SMCX injury, combined with BW experience, produces an enduring facilitation of BW recovery. Prior work implicated norepinephrine in this promotion of functional recovery (Crit. Rev. Neurobiol., 3(2):135-197.). To further assess differential roles of catecholamines, this study examined the effect of reserpine given prior to AMPH. Reserpine enhances AMPH evoked striatal dopamine (DA) release and behavioral stereotypies (TIPS, Oct, 1983., p.429-432). If the AMPH facilitation of recovery from hemiplegia involves striatal DA, reserpine pretreatment should enhance this effect. Rats received a right SMCX ablation or sham surgery and at 20 hrs post surgery received a BW test, showing normal BW in sham controls and hemiplegia in SMCX rats. Reserpine (5 mg/kg;i.p.) abolished BW performance. At 4 hrs post-reserpine (24 hrs post-surgery), AMPH (2 mg/kg;i.p.) or saline was administered. During the first hr after AMPH, both SMCX and sham animals displayed a marked increase in activity and motor stereotypies. For 2 hrs after AMPH BW performance returned to normal in sham controls. However, for reserpinized SMCX rats given AMPH, BW performance only returned to pre-reserpine baseline disability levels. These results indicate reserpine blocks the enduring AMPH facilitation of BW recovery after SMCX lesion but not AMPH evoked "striatal DA" activity and stereotypies. Supported by DHHS RO1 NS20220-03.

57.8

d-AMPHETAMINE ATTENUATES LEARNING AND MOTOR DEFICITS FOLLOWING CORTICAL INJURY IN RATS. G.L. Dunbar, G.A. Smith*, S.K. Look*, and R.J. Whalen*. Department of Psychology, Central Michigan University, Mt. Pleasant, MI 48859.

In this experiment, we examined whether daily treatments of d-amphetamine counteract lesion-induced learning and motor deficits when rats were not under amphetamine "intoxication" (i.e., when injections were given 22 hrs prior to testing). Rats were given unilateral aspiration lesions of the frontal cortex or sham surgery, followed by IP injections of saline or d-amphetamine. Injections started immediately (seconds) after surgery and were given at 24 hr intervals for 15 days. Postoperative testing on a balance beam and a linear maze began the day after surgery and continued daily for 15 days. d-Amphetamine-treated rats with lesions (AL) showed less impairment on a balance beam task than saline-treated rats with lesions (SL). However, d-amphetamine treatments caused initial disruptions on this task for the sham-operated rats, compared to saline-treated sham-operated rats (SC). Maze acquisition was facilitated by d-amphetamine treatments for both rats with lesions and those with sham operations, compared to their respective saline-treated counterparts. In addition, AL rats made significantly fewer errors than SL rats, and performed at the same level as SC rats. These results indicate that (1) d-amphetamine treatments can attenuate lesion-induced learning and motor deficits, and (2) these effects can occur when specific training is dissociated from acute amphetamine "intoxication."

57.9

EFFECTS OF ALCOHOL INTOXICATION ON RECOVERY OF SENSORY CONDUCTION AFTER SPINAL CORD TRAUMA. **S. Katz, H.F. Martin, Dept. of Physiology and W.O. Boggan, Dept. of Psychiatry. Medical University of South Carolina, Charleston, South Carolina, 29425.**

The objective of this study is to clarify the role of alcohol on spinal cord transmission following trauma to the spinal cord. The results of this study should enable us to develop a model to better predict prognosis of spinal cord injured patients who were intoxicated at the time of injury.

Previous experimental studies found that acute intoxication may potentiate the outcome of spinal cord injury resulting in increased spinal cord necrosis and impaired functional recovery. There is however, a paucity of information relating blood to tissue alcohol concentration at the time of trauma and to the degree of recovery from injury. The initial studies in this investigation measured the blood and tissue ethanol concentration produced at various times after a 15 min. infusion of 5, 10, or 15% solution of ethanol in saline. The 15% solution produces a blood concentration of 173.8 mg%, and spinal cord and brain concentration of 7.81 and 9.42±1.51 mg/gm tissue, respectively. The effect of alcohol intoxication on the recovery of sensory conduction was studied by obtaining somatosensory evoked potentials (SEP's), before and after impact trauma to rat spinal cord. Recovery of SEP's are compared between ethanol treated rats and control animals given a caloric equivalent sucrose solution.

57.10

CHANGES IN PHOSPHOLIPID PHOSPHORUS CORRELATE WITH INCREASED BRAIN PHOSPHOLIPASE C ACTIVITIES FOLLOWING TRAUMATIC BRAIN INJURY IN THE RAT. B. Vishwanath*, G. Clifton*, R. Franson*, B. Lyeth, R. Hamm, L. Jenkins and R. Hayes. Department of Biochemistry & Molecular Biophysics and Division of Neurosurgery, Medical College of Virginia, Richmond, Va 23298.

Previous studies of traumatic brain injury (TBI) have demonstrated disturbances in arachidonic acid metabolism in both the cat and rodent (J Neurochemistry 37:892, 1981; J Neurotrauma 5:331, 1988) and changes in total phospholipase C (PLC) activity in the cat (J Neurosurgery 56:695, 1982). However, the activity of specific brain PLCs following rodent or cat fluid percussion injury have not been measured. In the present study, both acid and neutral pH sensitive PLC activities were evaluated 5 minutes following moderate fluid percussion injury (2.2 ATM) and compared to sham animals. Phospholipid phosphorus alterations in whole brain homogenate were correlated with in vitro measurements of phospholipase C activity in homogenate and supernatant fractions using [N-methyl-^{14}C] sphingomyelin (SM) and ^{3}H-phosphatidylinositol (PI) as substrates. Five min post-injury, total phospholipid phosphorus (umols/mg) was decreased 9.1±1.2% (mean ± SEM). At the same time metal-ion independent PLCs, most active at pH 4.5-5.0, hydrolyzing SM and PI, were increased 25±8.3% and 35±6.8%, respectively. Mg^{2+}-dependent PLC mediated hydrolysis of SM at neutral pH was increased 106±21%. These results demonstrate that loss of phospholipid phosphorus immediately following TBI in the rat is associated with activation of both acid and neutral active PLCs.

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57.11

MOVEMENT RELATED CORTICAL POTENTIALS (MRCP) IN POST ACUTE TRAUMATIC BRAIN INJURED (TBI) SUBJECTS. A. Nativ,* R. Rosas-Ramos* and R. Balliet* (SPON: P. Bach-y-Rita). Neuromuscular Retraining Clinic, Dept. of Rehab. Med., Univ. of Wisconsin, Madison, Wisconsin, 53705.

Slow cortical potentials, putatively associated with the planning and initiation of simple goal directed forearm and finger movements, were recorded in post-acute, hemiplegic, TBI and in normal control subjects to operationally define motor related brain dysfunction. Subjects activated a mechanical trigger with either flexion of the index finger, or by flexion or extention of the elbow. Two seconds of movement related EEG activity were recorded from frontal and parietal electrodes placed on the scalp according to the 10/20 system and averaged on line by a Nicolet Pathfinder 1 computer.

Post-acute TBI subjects showed reduced MRCPs compared to the control group for hemiplegic arm movements, and also, to a lesser degree, for movements of their relatively 'non-affected' arm. TBI subjects also demonstrated unusual waveforms (e.g., opposite deflections in homologous recordings) and uncharacteristic cross-cortical MRCP correlations associated with specific electrode configurations, as well as with specific movement conditions. These preliminary data suggest that, aside from its general sensitivity to motor related brain dysfunction in clinical populations (e.g., Shibasaki et al., 1987; Barrett et al., 1987), the study of MRCPs may also offer a non-invasive diagnostic tool to investigate specific cerebral disorders associated with motor dysfunction.

57.12

DURA EXERTS LONGITUDINAL TENSION ON SPINAL CORD, B.R. Fink, Anesthesiology, Univ. of Washington, Seattle, WA 98195.

The tendency of spinal dura mater to retract upon incision was investigated. Direction of connective tissue fibers was evaluated in an orthogonal morphometric survey on human posterior L3-4 dura from three autopsies; in x5000 micrographs the dural collagen fiber bundles pursued wavy, irregular courses whereas the elastic fibers took rectilinear orthogonal paths, either longitudinal or transverse in approximately equal quantity. In Papio, Macaca, and sheep laminectomized 1-2 h after death longitudinal or circumferential incision of dura led to spontaneous separation of cut edges by up to one third of the cord diameter, depending on the length of the cut. Partial transverse dural incision followed by complete transverse section of cord produced a wedge-shaped gap which lengthened as the dural incision was extended circumferentially and suddenly became cylindrical and surged to 3% of the cord length when the last shred of the dural circumference was divided. Successive transections at T9, L3, and T3 were each followed by a similar train of events, indicating that in the non-humans, including fetal lambs, probably the entire length of the cord was under longitudinal tensile stress originating in the dura. The human functional significance of dural elasticity is unclear and raises important questions regarding repair of cord injury.

57.13

EVALUATION OF THE EFFICACY VERSUS SAFETY OF SPINAL CORD STIMULATION FOR THE GENERATION OF MOTOR EVOKED RESPONSES. S. Sabato*, C.A. Agresta* and S.K. Salzman. Alfred I. duPont Institute, Research Dept., Wilmington, Delaware 19899.

Direct and indirect spinal cord stimulation paradigms were evaluated for their ability to produce viable motor evoked potentials (MEPs) versus their effect on spinal function. Male Sprague-Dawley rats (N=50) were anesthetized with pentobarbital (60 mg/kg, i.p.) and positioned in a spinal stereotaxic unit. A laminectomy was performed at T6, and bipolar silver ball stimulation electrodes were positioned 0.5-1.0 mm above the intact dura and covered with saline. In other animals, stimulation was obtained from screw electrodes threaded into the intact spinous process. Varying intensities of both constant voltage and constant current stimulation were employed. MEPs were recorded as compound muscle action potentials (cMAPs) using bipolar platinum needles inserted into the quadriceps muscle. Spinal cord function was evaluated using three methods: somatosensory-evoked potential (SEP) morphology during the experiment, post-operative neurologic testing, and post-mortem spinal serotonin content and metabolism.

Stimulation intensities sufficient to produce a reproducible N-P or N-P-N complex (latency of 7, 8 and 10 msec, respectively) in the recorded cMAP consistently resulted in neurologic deficits, SEP abnormalities and depletion of spinal serotonin when stimulation was applied directly to the cord. In contrast, with intraspinous stimulation, lower intensities were required to obtain reproducible cMAPs and spinal function was always preserved. Implications for intraoperative spinal cord monitoring of motor function are discussed.

57.14

VISUAL REHABILITATION TRAINING IN BRAIN-DAMAGED PATIENTS. H.J. Markowitsch^{+} and K. Pommerenke^{+} (SPON: ENA), Fac. of Psychology, Univ. Bochum, D-4630 Bochum, Fed. Rep. Germany

In patients with damage of the occipital lobe and some surrounding brain tissue it was investigated whether it is possible to use computer programs as screening tests to diagnose visual field defects. Second, visual exploration was trained using an electronic reading and exploration training apparatus (ELEX). With ELEX it was hoped to increase the visual field or search field - measured by standard perimetric conditions - even a considerable time after brain damage. Thirdly, ELEX was used to improve reading performance with respect to speed, number of errors, comprehension.

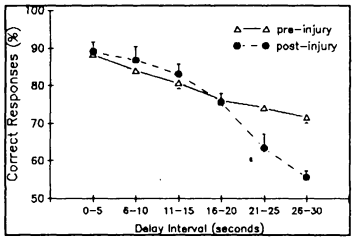
While a number of quite specific relations were found between various variables in individual patients, the overall results indicated that specific training may improve reading abilities even after a considerable post traumatic period, and that a computer monitored perimetry is an effective screening device. On the other hand, a training dependent expansion of the visual field could not be established unequivocally.

57.15

EFFECTS OF EXPERIMENTAL TRAUMATIC BRAIN INJURY ON SHORT-TERM MEMORY. C.H. Napper*, D.S. Prough*, D.S. DeWitt* (SPON: J. Turner) Dept. of Anesthesia, Bowman Gray Sch. of Med., Winston-Salem, NC 27103

Impairment of short-term memory is the most common cognitive disorder reported by patients following traumatic brain injury (TBI). Short-term memory deficits assessed using the 8-arm radial maze occur in rats following TBI (Lyeth, et al, Soc Neurosci Abstr 13:1253, 1987). Our study examines the effects of fluid percussion injury (FPI) on memory using a delayed matching-to-sample (DMTS) task (Dunnett, SB: Psychopharm 87:357-363, 1985) in rats.

Male Sprague-Dawley rats (n=5) were water restricted to 85% of their free-feeding weight and trained to a stable performance level on the DMTS task prior to moderate FPI (2.3 atm.). After injury, animals were assessed daily for short-term memory deficits.



Pre-injury, rats achieved 88.4±0.5% correct responses at shortest delays (0-5 sec.) and 71.7±2.5% correct responses at longest delays (26-30 sec). These values are comparable to values previously reported in rats (see Dunnett). After injury, memory performance at shortest delays remained at pre-injury levels (89.7±1.2%). At longest delays, performance dropped to 55.7±1.7%. These data indicate that TBI produces deficits in short-term memory which persist for several days. (Supported, in part, by NS 19355)

57.16

ATTENUATION OF SYNAPTIC TRANSMISSION IN HIPPOCAMPAL SLICES FOLLOWING WHOLE ANIMAL EXPOSURE TO IONIZING RADIATION. G.E. Hollinden and T.C. Pellmar. Physiology Dept., AFRRI, Bethesda, MD 20814

Ionizing radiation directly alters neuronal excitability in vitro (Tolliver & Pellmar 1987, Rad. Res. 112, 555). However, in vivo many other factors (e.g. blood flow, neuromodulators) are likely to influence CNS function. To examine the contribution of these factors to neuronal deficits, we irradiated male guinea pigs in vivo (20 and 50 Gy, 20 Gy/min, 60-Co radiation) and prepared slices of hippocampus from animals euthanized 30 min, 1 day, 3 days or 5 days post-radiation. Hippocampal slices were incubated 1-2 hrs, transferred to a submersion chamber and perfused with aCSF (30°C). A stimulating electrode was placed in s. radiatum. Recording microelectrodes were placed in s. radiatum to record the afferent volley and population postsynaptic potential and in s. pyramidale to record the population spike. Following a 30 min equilibration period, an input-output curve was produced by varying stimulus intensity from 0 to 0.5 mA.

Both 20 and 50 Gy γ-radiation decreased synaptic transmission and spike generation at 3 days post-radiation. The combination of longer survival and in vivo exposure revealed deficits at 20 Gy, a previously ineffective dose.

57.17

IONIZING RADIATION ALTERS ELECTROPHYSIOLOGY OF HIPPOCAMPUS IN VITRO. T.C. Pellmar, D.A. Schauer*, and G.H. Zeman*. Physiology and MRA Dept., AFRRI, Bethesda, MD 20814-5145

A new x-ray source has made it possible to directly examine radiation damage to isolated neuronal tissue. With the x-ray machine inside a lead Faraday cage, electrophysiological properties can be recorded before, during and immediately after exposure.

Hippocampal slices were prepared from euthanized guinea pigs and placed in a submersion chamber perfused with aCSF (1-2 ml/min; 30°C). The afferent volley and population synaptic response (pPSP) were recorded in s. radiatum of CA1. The population spike was recorded in s. pyramidale of CA1. Afferents to CA1 in the s. radiatum were stimulated (0.2 Hz) to monitor slice excitability. By varying stimulus intensity (0.0 to 0.5 mA, 300 μs), input-output (I/O) curves were obtained before, 5, 30, and at some doses, 60 min. after radiation exposure (17 keV x-rays; 1.5 Gy/min, 5 to 65 Gy). I/O curves in control slices were obtained at similar time points.

Following exposure to doses as low as 40 Gy the pPSP was enhanced, reaching a steady level soon after exposure. The ability of the pPSP to generate a spike was reduced and damage progressed after termination of the radiation. No unusual activity was observed during the exposure. These results demonstrate that an isolated neuronal network can show complex changes in electrophysiological properties following moderate doses of ionizing radiation.

57.18

EXTRACELLULAR ELECTRIC FIELD EFFECTS IN A MODEL OF MAMMALIAN MYELINATED MOTOR NERVE EXCITABILITY. J.D. Sweeney[1,3], K. Deng[2*], E. Warman[3] and J.T. Mortimer[3]. (1) Dept. Chem., Bio., and Materials Engin., Arizona State Univ., Tempe, AZ 85287; (2) Dept. Elec. Engin. and Applied Physics, and (3) The Applied Neural Control Lab., Dept. Biomed. Engin., Case Western Reserve Univ., Cleveland, OH 44106.

Neural Prostheses elicit changes in the excitability of the nervous system through the introduction of electric fields. By solving for the extracellular electric field created by a neural prosthesis, and by modelling the excitability changes elicited by the field, we can potentially improve the design of neural prosthetic electrode systems and predict their performance. Towards this goal, our present objective has been to determine the effects of changes in extracellular electrode type and position (e.g. monopolar or linear; aligned or offset with respect to nodes) and extracellular medium conductivities (e.g. isotropic, anisotropic) using an improved version of a previously developed computer model of mammalian myelinated motor nerve [see Soc. Neurosci. Abstr. 12:1306, 1986]. In this compartmental cable model (implemented in FORTRAN, and run on an IBM AT with INMOS transputer) the nodal and internodal parameters are based on known or estimated mammalian peripheral nerve values [from e.g. J. Physiol., 292:149, 1979; J. Physiol., 298:171, 1980]. For a given motor axon diameter, electrode type and configuration, and extracellular medium representation, strength-duration curves have been generated (and fit to Lapicque's and Weiss's equations) for rectangular current stimuli with pulse widths of 10 to 500 usec. In general, representation of the extracellular space as a highly anisotropic medium increased both spatial selectivity and selectivity relative to axon size (although, for very small monopolar electrode-node separations axon size selectivity was minimal). In all instances, increased electrode-node separation or offsetting an electrode over the internodal region resulted in larger chronaxies.

This work is supported by NIH-NINCDS Grant No. NS26476.

HYPOTHALAMIC-PITUITARY-ADRENAL REGULATION: CRH

58.1

THE EFFECTS OF CRH IMMUNONEUTRALIZATION ON ULTRADIAN RHYTHMS OF PLASMA ACTH IN THE RAT. M. Carnes, S.J. Lent*, S. Erisman*, J. Saydoff, C. Mueller*. VA Medical Center and University of Wisconsin, Madison, WI 53705.

Time series of plasma ACTH concentrations reveal circadian and ultradian rhythms. The diurnal surge of ACTH has been shown to require endogenous CRH. To determine whether ultradian fluctuations in plasma ACTH also require CRH, 6 rats were immunoneutralized with 0.5 ml i.v. of an antiserum raised against rCRH (anti-CRH) and 6 rats were given 0.5 ml normal rabbit serum as a control (NRS). All rats had jugular and femoral venous cannulae inserted at least 48 hr prior to blood sampling. Blood was withdrawn by peristaltic pump at 35 ul/min from the jugular cannula with simultaneous infusion of warmed, extracted rat plasma into the femoral cannula. Two-minute samples were collected for 4 hr. Plasma was assayed for irACTH. Resultant time series were analyzed by PULSAR (P) and Cluster Analysis (C). Comparison of pulse parameters identified in anti-CRH and NRS groups revealed a significant decrease in amplitude (P,C), and area (C) in anti-CRH rats but no significant difference in frequency (P,C), interpeak interval (P,C), or peak width (P,C). These results suggest that some ultradian rhythms of ACTH secretion occur independently of CRH stimulation and support in vitro findings of an endogenous rhythm of ACTH release by the corticotroph.

Supported by the Veterans Administration and NIH grant 1R01-DK40759-01.

58.2

SHORT-LOOP FEEDBACK EFFECTS OF ADRENOCORTROPHIC HORMONE ON CORTICOTROPIN-RELEASING FACTOR AND VASOPRESSIN-IMMUNOREACTIVITY IN THE PARAVENTRICULAR NUCLEUS. P.E. Sawchenko, The Salk Institute, La Jolla, CA 92037.

The observation that hypophysectomy (HYPOX) results in a more pronounced enhancement of corticotropin-releasing factor-(CRF) and arginine vasopressin- (AVP) immunoreactivity (IR) in the parvocellular division of the paraventricular nucleus (PVH) than does adrenalectomy (ADX) suggests a role for adrenocorticotrophic hormone (ACTH) in modifying peptide dynamics in this system. To test this notion, ACTH or vehicle was administered systemically via osmotic minipump for seven days to rats submitted to both HYPOX and ADX surgeries. Lower replacement doses (0.5 ug/day) of ACTH reduced the number of detectable AVP-IR cells in the parvocellular PVH to 53% of that seen in vehicle-treated HYPOX/ADX controls; the number of CRF-IR cells was not significantly affected. Higher doses of ACTH (2.0 ug/day) resulted in counts of AVP- and CRF-IR neurons that were 32% and 70%, respectively, of control values. Qualitatively, staining patterns for the two peptides in the external lamina of the median eminence generally followed the cell count data. The results suggest that ACTH can in fact serve as an inhibitor of corticotropin-releasing peptide expression in the PVH, and that AVP-IR appears to be preferentially targeted. The sites and mechanisms by which short-loop feedback effects may be exerted remain to be specified.

58.3

BED NUCLEUS OF STRIA TERMINALIS PROJECTIONS TO HYPOTHALAMIC PARAVENTRICULAR CRF, OXYTOCIN AND VASOPRESSIN CELLS IN THE RAT. D.J. Magnuson and T.S. Gray, Department of Anatomy, Loyola University Stritch School of Medicine, Maywood, IL. 60153

The present study was designed to determine whether the bed nucleus of stria terminalis (BST) projects to oxytocin, vasopressin and/or corticotropin - releasing factor (CRF) cells in the hypothalamic paraventricular nucleus (PVN). Phaseolus vulgaris leucoagluttinin (PHA-L) fibers and terminals were visualized with horseradish peroxidase-brown diaminobenzidine and then processed with standard immunocytochemistry for oxytocin, vasopressin and CRF cells with a glucose oxidase-nitro-blue tetrazolium reaction product. The densest projections to the paraventricular nucleus are from ventral portions of BST. Ventral BST projects to all parts of the PVN, with labeling densest in medial and lateral parvocellular nuclei and slightly less dense in anterior parvocellular nucleus. Lighter labeling was present in posterior magnocellular paraventricular nucleus and the least amount of labeling was in anterior magnocellular paraventricular nuleus. Ventral BST projections to the paraventricular nucleus are much heavier in contrast to central nucleus of amygdala projections to the same nuclei (Gray et al, Neuroendocrinology, in press, 1989). Brown PHA-L labeled fibers and terminals were found in close proximity to all three types of cell bodies throughout the PVN. Vasopressin and CRF cells were more likely to have BST PHA-L terminals in close proximity than were the oxytocin cells due to heavier projections to the caudal magnocellular and parvocellular areas of the PVN. The parvocellular regions of the PVN known to have projections to the autonomic spinal cord received heavy projections from ventral BST. The BST receives afferents from a variety of limbic areas including hippocampus, septum, amygdala, and insular and prefrontal cortex. Via BST projections to the PVN including oxytocin, vasopressin and CRF cells the limbic system can strongly modulate autonomic and hypothalamo-hypophyseal function. (Supported by ONR N000-14-88-k-0010)

58.4

INVOLVEMENT OF CALCIUM IN RELEASE OF CORTICOTROPIN-RELEASING FACTOR (CRF) BY THE BOVINE MEDIAN EMINENCE. T.H. Welsh,* M.R. Sutton,* P.L. Chen,* P.G. Harms* and N.H. McArthur. Depts. of Animal Science and Veterinary Anatomy, Texas A&M University, College Station, TX 77843.

The involvement of calcium in the release of CRF by the bovine median eminence (ME) was studied in vitro. Individual ME halves from steers were perifused for 300 min with Krebs-Ringer bicarbonate medium (500-µl chambers; 5.5 mM glucose; 95% O_2:5% CO_2; 37°C; 1 ml/10 min fractions for CRF radioimmunoassay). ME halves were challenged for 30-min periods at 90 and 200 min of perifusion with depolarizing agents [50 µM veratridine (V) or 60 mM potassium (K^+)] in the presence or absence of calcium-free medium containing 1.25 mM EGTA. Depolarization with veratridine (a sodium channel activator; n=7) at 90 min of perifusion increased CRF release (from 130 ± 48 pg/ml pre-V to 378 ± 73 pg/ml at 30 min post-V). At 200 min of perifusion, veratridine treatment (n=3) also increased CRF release (from 14.6 pg/ml pre-V to 156 pg/ml at 30 min post-V). However, over a similar time period EGTA treatment (n=3) blocked V-induced CRF release (44.5 pg/ml pre-V vs 43.2 pg/ml at 30 min post-V). EGTA treatment also inhibited K^+-induced CRF release at 90 and 200 min of perifusion. The inhibitory effect of EGTA on veratridine- and potassium- induced CRF release supports the concept that release of this neurohormone by the bovine median eminence is calcium-dependent.

58.5

CHRONIC BEHAVIORAL STRESS INCREASES CRF IMMUNOREACTIVITY AND CRF/VP CO-LOCALIZATION. J.A.Barrett*, A.J.Silverman, A. Hou-Yu and D.D.Kelly, Depts. of Psychiatry and Anatomy & Cell Biol., Columbia Univ., & NYS Psychiatric Inst, NY,NY 10032.

We previously found that chronic stress increased the size, staining intensity and detectable number of CRF cells in the paraventricular nucleus. To evaluate whether stress-sensitive CRF cells might also contain vasopressin (VP), a synergist of CRF in the release of ACTH, we employed a double label protocol with antibodies against CRF and the glycopeptide portion of VP neurophysin (courtesy of A. Robinson). Five sets of male Fischer 344 rats were housed in groups of four. One from each group was removed and exposed to 240 hrs of stress (alternating 30-min sessions of white noise and of conditioned emotional response training), another to 72 hrs of stress, and a third served as a no-stress control. In the hypothalamus of intact control subjects, few cells were CRF positive, and only a very small percentage of these contained VP. Exposure to stress increased both the number of CRF and CRF/VP cells; the more prolonged the stress, the more numerous the cells. Because a pilot study suggested that isolation housing might also alter stress-sensitive CRF cells, the remaining subject from each group was isolated for 4 weeks. Relative to the group-housed controls, isolation stress markedly enhanced both the number of detectable CRF cells and the proportion of these containing VP. These results illustrate the sensitivity of CRF cells to the behavioral history of the individual, in particular, the prior exposure to stress. NS 23858

58.6

HIPPOCAMPAL-HYPOTHALAMIC CIRCUITS MEDIATING TONIC INHIBITION OF THE HYPOTHALAMO-PITUITARY-ADRENOCORTICAL (HPA) AXIS. I.P. Herman, E.A. Young, A. Savina* and S.I. Watson. Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109-0720.

Removal of the hippocampus results in up-regulation of the ACTH secretagogues corticotropin-releasing hormone (CRH) and vasopressin (VP) mRNA in the parvocellular paraventricular nucleus (PVN) and in increased release of corticosterone and β-endorphin into plasma (Herman et al., J. Neurosci., in press). These data are consistent with the hypothesis that hippocampus exerts a tonic inhibitory influence on the HPA axis. In an effort to define presumably multi-synaptic hippocampal-hypothalamic circuitry mediating this inhibition, electrolytic lesions were made to the fimbria-fornix or medial corticohypothalamic tract (MCHT) of Sprague-Dawley rats. Expression of CRF mRNA in the PVN was analyzed by semi-quantitative *in situ* hybridization histochemistry, and ACTH in plasma was determined by RIA. Fimbria-fornix lesions, which disrupt the majority of hippocampal outflow to subcortical structures connecting with PVN, results in a two-fold increase in CRF mRNA in PVN and a significant rise in plasma ACTH. MCHT lesions, which specifically interrupt connections between the ventral subiculum and hypothalamus, had no effect on either CRF mRNA or plasma ACTH. Results indicate that inhibitory pathways between hippocampus and hypothalamus are carried in the fimbria-fornix system exclusive of the MCHT. Primary structures receiving afferents from the fornix and projections to the PVN or proximity include the lateral septum and bed nucleus of the stria terminalis; studies are presently being conducted to determine the role of these nuclei in HPA regulation. Supported by MH422251, NS08267.

58.7

EFFECT OF LOCUS COERULEUS LESIONS ON mRNA EXPRESSION AND CSF LEVELS OF CORTICOTROPIN RELEASING HORMONE IN RAT. Evagelia Mamalaki*, Linda S. Brady, Sam Listwak*, and Miles Herkenham (SPON: M. A. Smith) Unit on Functional Neuroanatomy, Clinical Neuroendocrinology Branch, NIMH, Bethesda, MD 20892.

Although norepinephrine stimulates *in vitro* release of corticotropin releasing hormone (CRH), previous studies have provided conflicting results regarding its effects on the CRH system *in vivo*. Levels of CRH in the cerebrospinal fluid (CSF) and CRH mRNA in the hypothalamic paraventricular nucleus (PVN) were assessed in male Sprague-Dawley rats with electrolytic destruction of the locus coeruleus (LC) or no lesion CSF (100-250µl) was drawn from the cisterna magna 4 days before and again 10 days after the operation and assayed for CRH by radioimmunoassay. Cryostat-cut sections through the PVN and LC were hybridized with ^{35}S-labeled oligonucleotide probes for CRH mRNA and tyrosine hydroxylase mRNA, respectively, and exposed to film for quantitative autoradiography. CRH levels in CSF were unaffected by the lesion (p>.05). CRH mRNA in the PVN was increased by unilateral and bilateral lesion of the LC (p<.03):

Condition	CSF CRH levels (pg/ml)	CRH mRNA (µCi/g tissue)
Control (N=4)	24.2 ± 6.1	3.2 ± 0.2
Bilateral LC lesion (N=4)	31.4 ± 5.6	3.9 ± 0.7
Unilateral LC lesion (N=4)	31.0 ± 9.8	3.5 ± 0.3

The data suggest that the LC has a minor influence on hypothalamic CRH production but not on CSF levels of CRH in non-stress conditions. Further studies should be done to investigate the relationship of the central noradrenergic and CRH systems during stress.

58.8

NPY-INDUCED RELEASE OF HYPOTHALAMIC CRF IS POTENTIATED BY REDUCTION OF NORADRENERGIC/ ADRENERGIC NEUROTRANSMISSION. D.A.Haas and S.R.George. Depts.of Medicine and Pharmacology, Univ. of Toronto, Toronto, ONT, M5S 1A8, Canada

We have reported that neuropeptide Y (NPY) administration increases hypothalamic corticotropin-releasing factor (CRF) concentration and release (Life Sci. 47:2725, 1987), and that there exists a tonic alpha-1 adrenergic inhibition of CRF release (Can. J. Physiol. Pharmacol., 66:754, 1988). Since adrenergic neurons appear to regulate CRF, and are closely associated with NPY neurons anatomically, their role in the NPY-induced changes of CRF was investigated.

All studies conformed with the guidelines for experimental procedures and involved adult male rats. Noradrenaline and adrenaline were selectively depleted by i.c. 6-OHDA prior to administration of NPY. This resulted in decreased median eminence CRF ($p < .025$) and increased plasma adrenocorticotropin (ACTH) (26-fold compared to 6-OHDA treatment alone, $p < .0005$). NPY induced a 2-fold increase in ACTH without 6-OHDA treatment ($p < .05$). Administration of the alpha-2 agonist clonidine resulted in a significant decrease in median eminence CRF ($p < .005$) and a 24-fold increase in plasma ACTH ($p < .005$). All of these results are consistent with the release of hypothalamic CRF. Concurrent administration of clonidine with the alpha-2 antagonist yohimbine prevented the clonidine-induced changes in plasma ACTH and hypothalamic CRF, consistent with mediation of effect through alpha-2 receptors. These data imply that NPY stimulation of CRF release is inhibited by normal adrenergic tone. Depletion of these neurotransmitters allowed NPY to profoundly stimulate CRF release, a result common to alpha-2 stimulation.

58.9

EFFECT OF NEUROPEPTIDE Y (NPY) ON HYPOTHALAMIC-PITUITARY-ADRENAL FUNCTION IN THE RAT. T.C. Kamilaris*, A.E. Calogero*, E.O. Johnson*, S.J. Listwak*, M.T. Gomez,* P.W. Gold and G.P. Chrousos (SPON: G. Guroff). CNB/NIMH & DEB/NICHD, Bethesda, MD, 20892.

Neuropeptide Y (NPY), a 36 amino acid peptide, co-exists and is co-secreted with catecholamines at the terminals of catecholaminergic neurons. These include the ascending noradrenergic bundle neurons, originating from the central arousal/sympathetic system in the brainstem and terminating at the paraventricular nucleus of the hypothalamus, and the catecholaminergic cells of the adrenal medulla. Recent data indicate that catecholamines are excitatory to the corticotropin releasing hormone (CRH)-secreting neurons of the hypothalamus and to ACTH secretion by the rat pituitary corticotrophs. The purpose of this study was to evaluate the possible effects of NPY on the function of the hypothalamic-pituitary-adrenal (HPA) axis. Intravenous administration of NPY to chronically cannulated, freely moving male rats resulted in dose-dependent elevation of plasma ACTH and corticosterone concentrations. Previous sectioning of the pituitary stalk, and pretreatment with rCRH antisera or with dexamethasone significantly decreased or eliminated the plasma ACTH response to NPY, suggesting that NPY exerts direct effects on the hypothalamus stimulating secretion of CRH. Interestingly, however, in all of the above three situations there was an unexpectedly adequate plasma corticosterone response to NPY, consistent with direct effects of this peptide on the adrenal cortex. We hypothesize that NPY represents another link between the arousal/sympathetic system and the HPA axis on the levels of both the hypothalamus and the adrenal cortex.

58.10

POLYCLONAL ANTIBODIES AGAINST RECOMBINANT HUMAN PRE-PROCORTICOTROPIN RELEASING HORMONE (h-PRE-PROCRH). M.G. Castro*, R. Dils*, P. Saphier*, E. Linton*, D. Savva* and P.J. Lowry*.(SPON: Brain Research Assn., U.K.) Department of Biochemistry and Physiology, School of Animal and Microbial Sciences, University of Reading, Reading RG6 2AJ, United Kingdom.

Corticotropin releasing hormone (CRH) is cleaved from a larger precursor by the action of endopeptidases at pairs of basic amino acids. In this study we have expressed h-pre-proCRH fused to β-galactosidase in E. coli (TG2) and used the recombinant protein as a ready made hapten-carrier molecule for raising antibodies against h-pre-proCRH. A human pre-proCRH genomic clone (kindly provided by S. Numa) was digested with HinfI, the 584 base pair restriction fragment was blunt ended, ligated to BamHI linkers and cloned into the BamHI cloning site of the pUR292 expression vector. The recombinant plasmid was transformed into competent TG2 cells. The identity of the chimeric β-galactosidase-pre-proCRH was confirmed by western blotting using a rabbit anti h-proCRH fragment (a.a. 125-151) antibody, DomI, and a two site immunoradiometric assay using a radio-labelled sheep anti h-CRH (a.a. 36-41) antibody and the DomI antibody. The chimeric protein was purified by SDS-PAGE followed by electroelution and was injected into rabbits. The presence of specific antibodies was determined by binding studies using 125I-h-CRH (a.a. 1-41) and 125I-h-pro-CRH fragment (a.a. 125-151), and by western blotting using E. coli (JM83) expressing h-pre-proCRH as an unfused protein. The antibodies raised using this approach recognise the full length precursor as well as h-CRH-(1-41), hence, they should provide useful tools for studying post-translational processing as well as tissue distribution and different circulating forms of the CRH precursor.

58.11

ALTERED RATIO OF VASOPRESSIN (VP) TO CRF IN HYPOPHYSIOTROPHIC CRF NEURONS FOLLOWING ADRENALECTOMY. L.T. Bertini* and J.Z. Kiss*. Dpt. of Morphology, Univ. of Geneva, Sch. of Med., Geneva, Switzerland. (SPON: J.-J. Dreifuss)

Paraventricular CRF neurons represent the final common hypothalamic pathway that regulates pituitary ACTH secretion. CRF neurons seem to regulate ACTH secretion by releasing a neuromediator mixture including CRF and VP. Physiological evidence that: 1) VP potentiates the action of CRF on ACTH secretion, and 2) this potentiation is resistent to the inhibitory effect of dexamethasone, suggest that the ratio of VP to CRF is important in regulating ACTH release. Quantitative electron microscopic study was conducted to estimate VP and CRF immunoreactive sites over neurosecretory vesicles of CRF neurons in control and adrenalectomized rats. Immunoreactive sites were visualized on consecutive thin sections by the postembedding protein A-gold technique. Adrenalectomy significantly increases VP immunoreactive sites over neurosecretory vesicles while CRF immunostaining remains unchanged. In addition, the size of CRF/VP containing neurosecretory vesicle increased about 25% after ADX. These results suggest, that the quantitative proportion of VP to CRF is variable, and can be modified by endocrine manipulation such as adrenalectomy.

58.12

ANGIOTENSIN RECEPTORS IN THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS REGULATE CRF RELEASE. E. Castrén and J.M. Saavedra. Dept. of Anatomy, University of Helsinki, 00170 Helsinki, Finland, and LCS, NIMH, Bethesda, MD 20892.

We have studied the role of hypothalamic angiotensin (Ang) receptors in the regulation of pituitary hormone release by measuring Ang receptor densities in the hypothalamic nuclei of hypophysectomized, adrenalectomized or repeatedly stressed rats.

Ang receptors in the parvocellular paraventricular nucleus (pPVN) were significantly decreased after both hypophysectomy and adrenalectomy. Replacement therapy with either gluco- or mineralocorticoid after adrenalectomy restored the receptor densities. After repeated stress, Ang receptors in the pPVN were significantly increased. Magnocellular PVN had a low number of Ang receptors, and these were regulated similarly to those in pPVN. Lack of vasopressin in Brattleboro rats did not influence Ang receptors in the PVN. Ang receptors were not found in the supraoptic nucleus.

These results suggest that Ang receptors in the pPVN regulate CRF release and might have a physiological role in the adaptation to stress.

58.13

CHRONIC ADMINISTRATION OF CORTICOTROPIN-RELEASING HORMONE (CRH) ATTENUATES PLASMA ACTH RESPONSE TO ETHER STRESS. Y. Tizabi and G. Aguilera* Dept. of Pharmacology, College of Medicine, Howard Univ., Washington, D.C. 20059 and Endocrinology and Reproduction Research Branch, NICHD, NIH, Bethesda, MD 20892.

The responses of the hypothalamic pituitary-adrenal axis during chronic stress are characterized by normal or slightly elevated plasma ACTH, increased hypothalamic CRH secretion, decreased pituitary CRH receptors and hypersensitivity of the ACTH and glucocorticoid responses to a superimposed stress. To determine the role of CRH in the pituitary hyperresponsiveness to a novel stress, pituitary CRH receptors and plasma ACTH responses were measured in rats receiving a minipump infusion of CRH, 3 μg/hr for 50 hr. Rats were killed by decapitation with or without prior exposure to ether vapors for 5 min. Blood was collected for ACTH RIA. Pituitary CRH receptor concentration, measured by binding of ^{125}I-Tyr oCRH was reduced by 45% in CRH infused rats, with no change in receptor affinity. Ether exposure had no effect on pituitary CRH receptors. Adrenal weight was significantly increased in CRH infused rats ($p<0.001$ n=21). Plasma ACTH levels were markedly increased after ether exposure in both groups ($p<0.001$). However, in contrast with the responses during chronic stress, the increases in ACTH were 40% lower in CRH infused rats ($p<0.05$, n=10). The data indicate that CRH alone does not account for the enhanced ACTH response to a novel stimulus superimposed on chronic stress and emphasize the multifactorial nature of the regulation of the corticotroph function.

58.14

CHRONIC ETHANOL TREATMENT LEADS TO REDUCED MEDIAN EMINENCE CRH AND PITUITARY POMC mRNA IN THE PRESENCE OF NORMAL PLASMA ACTH AND CORTICOSTERONE LEVELS. A. Thiagarajan, M. Grino*, M. Palkovits*, E. Mezey* and R. Eskay*. Laboratory of Clinical Studies, NIAAA and Laboratory of Cell Biology, NIMH, National Institutes of Health, Bethesda, MD 20892.

In order to explore the effects of ethanol on the hypothalamic-pituitary-adrenal axis from the acute-activation phase through the less-responsive, chronic-exposure period, male rats received a standard liquid diet containing ethanol via a gastric cannula to continuously maintain blood ethanol levels between 150-300 mg% for 0.5, 1, 3 or 7 days. Asynchrony between plasma ACTH and corticosterone (Cs) was observed with elevated Cs on 0.5, 1 and 3 days only of ethanol treatment. Plasma ACTH levels did not differ from controls at any time interval. Median eminence levels of CRH were reduced on days 3 and 7 without a change in hypothalamic CRH mRNA levels. Median eminence AVP and hypothalamic AVP mRNA remain unchanged throughout. Anterior and intermediate lobe POMC mRNA levels were reduced by 75 and 50%, respectively, only on day 7 of sacrifice. The lack of change in plasma ACTH levels during continuous ethanol exposure in spite of significant reductions in pituitary POMC mRNA and median eminence CRF suggests the involvement of other compensatory stress-axis regulators.

58.15

CORTICOTROPIN RELEASING FACTOR DECREASES SLOW WAVE (SW) SLEEP WHILE IT INCREASES RAPID EYE MOVEMENT (REM) SLEEP IN REM DEPRIVED RATS. F.Marrosu*, M.Giagheddu*, G.Mereu* and W.Fratta*. (SPON: S.L.Pocotte).Inst. of Neurology and Dpt. of Pharmacology, University of Cagliari, ITALY.

The intracerebro-ventricular injection of CRF causes behavioral modification known as "stress-like behavior" characterized by prolonged period of arousal, and complex hormonal manifestations (release of ACTH and endorphins etc.). We report here a novel action of CRF on the sleep patterns of REM deprived rats. The rats were REM deprived by keeping them for 72 hrs in a platform surrounded by water, then injected with 1 mg of rCRF. The animals showed an important delay in the first sleep onset, a strong decrease of SW while a prolonged REM sleep which lasted about 50% of the total sleep recorded in the first 100 min. Since CRF increases the firing rate of noradrenergic (NA) cells we might hypothesize that this action prolongs the "on-phase" in the NA cells (responsible for the long-lasting arousal) and that, soon after, the necessity of a prolonged restoration (or up-regulation) in NA system causes an increase in REM which is considered crucial in order to up regulate NA receptors.

58.16

CORTICOTROPIN SECRETAGOGUES HAVE DIFFERENT ELECTROPHYSIOLOGICAL ACTIONS IN AtT-20 PITUITARY CELLS. S. J. Kom, J. A. Connor and R. Horn. Neurosciences Dept., Roche Institute of Molecular Biology, Nutley, NJ 07110.

Stimulation of corticotropin (ACTH) secretion by corticotropin releasing factor (CRF) and β-adrenergic agonists is accompanied by a rise in intracellular [Ca^{++}] and cAMP. We are using the perforated patch recording method and fluorescent Ca^{++} indicators to examine the mechanisms by which these secretagogues act. With the calculated Cl^- equilibrium potential (E_{Cl}) set between -53 mV and -22 mV, AtT-20 cells had a resting potential of approximately -50 to -60 mV and spontaneously fired Ca^{++} action potentials (APs). CRF (30 nM) decreased the duration of, or abolished APs, and decreased the amplitude of Ca^{++}-dependent Cl^- currents (I_{Cl}). In contrast, isoproterenol (ISO; 1 μM) prolonged APs and prolonged I_{Cl} (E_{Cl}= -22 mV). Resting $[Ca^{++}]_i$ in AtT-20s, measured with fura-2, was 46.9 ± 23.8 nM (n=24). CRF (20-50 nM) and ISO (10 μM) stimulated a rise in $[Ca^{++}]_i$ to 446.9 ± 154.1 nM (n=9) and 285 ± 51.9 nM (n=8) respectively, but with different latencies. The CRF-induced [Ca^{++}] rise was delayed by ~25 sec (measured with 5 sec resolution) following the start of CRF application, and reversed very slowly. The ISO-induced [Ca^{++}] rise had a more rapid onset (~8-9 sec), and reversed rapidly after ISO application was terminated. These data suggest that CRF and isoproterenol may elevate intracellular Ca^{++} and stimulate secretion *via* different mechanisms.

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NEUROTOXICITY: DOPAMINE

59.1

TOXIC EFFECTS OF POTENTIAL ENVIRONMENTAL TOXINS RELATED TO MPP+ ON RAT DOPAMINERGIC NEURONS IN CULTURE. F. Hefti, P.P. Michel, B.K. Dandapani*, B. Knusel*, J. Sanchez-Ramos*, and S. Efange.. Andrus Gerontology Center, U.S.C., Los Angeles, CA 90089.

Exposure of cultures of dissociated embryonic rat mesencephalic cells to 0.1-30uM MPP+ results in selective degeneration of dopaminergic neurons. At 10uM MPP+ reduced TH activity by 89%, the number of TH-positive cells by 85%, and [3H]dopamine uptake by 96%, whereas protein content, GABA uptake and the number of neurons visible in phase contrast microscopy remained unchanged. MPP+ also failed to affect cholinergic neurons in septal cultures.

A total of 48 compounds with structural similarity to MPP+ were tested for their ability to produce a selective dopaminergic degeneration. Only MPP+ and its close derivatives, 2'-methyl MPP+, p-amino-MPP+, 1-methyl-4-cyclohexyl-, 1-methyl-4-(4'-acetamido)phenyl- (MACPP+), and 1-ethyl-4-phenyl pyridium exhibited selective dopaminergic toxicity. After MPP+, MACPP+ was the most potent selective toxin. It is concluded that the structural requirements for a selective dopaminergic neurotoxin are rather strict and that our studies so far failed to support the environmental hypothesis of Parkinson's disease.

59.2

SELECTIVE DECREASE IN EXTRACELLULAR DOPAC CONCENTRATIONS IN RAT STRIATUM FOLLOWING *IN VIVO* DIALYSIS WITH LOW CONCENTRATIONS OF MPP^+

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Doses of MPTP which do not result in nigral cell death or striatal dopamine loss in the dog do cause a long term but reversible loss of the dopamine metabolite DOPAC without affecting total MAO activity. Since MPP^+, the toxic metabolite of MPTP, is retained in monkey and dog dopamine terminals for long periods of time and since MPP^+ has been shown to be an effective inhibitor of MAO-A, low concentrations of MPP^+ may cause selective inhibition of the small pool of MAO-A within dopaminergic terminals. While the rat does not retain MPP^+ for long periods of time and is thus an inappropriate model for studying the effects of systemically administered MPTP, using *in vivo* dialysis the concentration of extracellular MPP^+ can be regionally maintained for extended periods while the effects on amine metabolism are simultaneously determined.

Dialysis probes (concentric, 3mm) were inserted into the striatum of chloral hydrate anesthetized rats (3-4/group) and operated at 1.5μL/min using an artificial CSF. Following a 3 hr baseline period, effects of the following compounds were tested: MPP^+ at 1 or 10 μM, GBR-12909 at 1 μM, or a combination of MPP^+ and GBR-12909 at 1 μM each. Twenty minute fractions were collected onto dry ice and analyzed for DA, DOPAC, HVA, 5-HT and 5-HIAA by HPLC-EC.

Unlike millimolar concentrations of MPP^+, micromolar concentrations administered through the probe did not produce a detectable increase in extracellular DA concentrations. There were observed decreases in extracellular DOPAC concentrations of 50% and 80% in response to 1 hr of dialysis with 1 and 10 μM MPP^+ respectively. These changes were selective, since concentrations of HVA and 5-HIAA were uneffected. The addition of 1 μM GBR-12909, a selective dopamine uptake blocker, prevented the DOPAC decrease induced by 1 μM MPP^+, indicating that uptake of MPP^+ into dopaminergic terminals mediates this response. GBR alone had no effect on DOPAC efflux. These results suggest that low micromolar concentrations of MPP^+ can be taken up and concentrated within dopamine terminals to produce a selective inhibition of presynaptic MAO-A without affecting surrounding neuronal (e.g. serotonergic) or glial metabolism.

59.3

COMPARISON OF THE EFFECTS OF INTRACEREBRALLY ADMINISTERED MPP^+ IN THE MOUSE, RAT AND MONKEY: MICRODIALYSIS OF DOPAMINE AND METABOLITES.

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Division of Toxicology, School of Pharmacy and Department of Anatomy, School of Medicine, University of California, San Francisco, CA 94143.
Department of Neurology, Jefferson Medical College, Thomas Jefferson University, Philadelphia, PA 19107.

Intracerebral microdialysis in three awake species allowed the measurement of the basal output of dopamine (DA), dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) from rat and mouse striatum and monkey caudate in vivo. The DOPAC/HVA ratios in dialysates from mouse and rat striatum were about 1 and 2 respectively, but only 0.06 in monkey caudate dialysates. The estimated extracellular levels of the metabolites correlated well with reported tissue levels; extracellular DA levels were 3 orders of magnitude lower than tissue concentrations. The effects of intracerebrally administered MPP^+ were essentially similar in the three species. In all cases an immediate, massive release of DA was accompanied by a pronounced, persistent decrease in the output of the metabolites. Basal DA release was no longer detectable 5-12 hours after MPP^+ administration and a second MPP^+ perfusion failed to induce another increase in the release of DA. Also systemically administered MPTP had comparable acute effects on the output of DA and metabolites from rat striatum and monkey caudate.

59.4

EVALUATION OF THE NEUROTOXICITY OF MPP^+ ANALOGS BY INTRACEREBRAL MICRODIALYSIS. N. A. Naiman,* H. Rollema*# and N. Castagnoli, Jr.* (SPON: R. Young). Dept. of Chemistry, Virginia Tech , Blacksburg, VA 24061 and # Dept. of Medicinal Chemistry, State University Groningen, The Netherlands

The neurotoxicity of the Parkinsonian inducing agent MPTP is mediated by its MAO-B generated 1-methyl-4-phenyl-pyridinium metabolite MPP^+ which appears to be formed extraneuronally and then concentrated within striatal dopaminergic terminals. Cell death ultimately results from the the MPP^+ mediated inhibition of mitochondrial respiration and the concomitant depletion of ATP. With the aid of an intracerebral microdialysis assay we have examined the neurotoxic potential of a variety of N- and pyridine ring-substituted MPP^+ analogs. Unlike the strict structural requirements observed for compounds which display MAO substrate properties, a variety of pyridinium species appear to be neurotoxic as measured by their ability to deplete striatal dopamine irreversibly following intracerebral perfusion. Additionally, some non-toxic analogs may potentiate the dopamine depleting effects of MPP^+. Since few tetrahydropyridine derivatives are likely to be MAO substrates, these results point to the importance of identifying alternative pathways that may lead to the formation of potentially neurotoxic pyridinium and presumably related (isoquinolinium, β-carbolinium) metabolites.

59.5

TETRAPHENYLBORON POTENTIATES THE INHIBITORY EFFECTS OF MPP^+ ON MITOCHONDRIAL RESPIRATION. S. Ofori*, R.E. Heikkila, J. Hwang* and W.J. Nicklas. Dept. of Neurology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854.

The inhibition of mitochondrial respiration by MPP^+ has been proposed to be an important mechanism in MPTP-induced neurotoxicity. We have confirmed recent findings that tetraphenylboron anion (TPB) potentiates both the accumulation of MPP^+ by mitochondrial preparations and the inhibition of Complex I respiration. However, present data suggest that the potentiation by TPB of the inhibitory effects of MPP^+ on mitochondrial respiration is only partly due to the facilitation by TPB of MPP^+ accumulation. For example, when mitochondria were incubated with MPP^+ at levels which produce a 50% inhibition (IC_{50}) of Complex I respiration (200 μM MPP^+ alone or 3 μM MPP^+ plus 10 μM TPB), the accumulation of MPP^+ was 8.46 nmoles/mg protein and 0.14 nmoles/mg protein respectively. When mitochondrial preparations were incubated with 3 μM MPP^+ without TPB, 0.06 nmoles of MPP^+/mg protein were accumulated. A second potential mechanism to explain some of the effects of TPB might be that it enhances the accumulation of the pyridiniums in the membrane close to the inhibitory site on NADH dehydrogenase of Complex I.

59.6

CARDIAC INOTROPIC EFFECTS AS A MODEL TO STUDY THE MECHANISM OF MPTP'S TOXICITY. D.T. Shearer,* J.S. Wilson, and R.G. Carpentier* (SPON: D.E. Matsumoto). Dept. Anat., Dept. Phys., Howard Univ., Wash., D.C. 20059.

Because of structural similarites to Ca++ channel agonists (dihydropyridines), it has been suggested that MPTP or its oxidative metabolite, MPP+, may induce Ca++ loading of neurons resulting in their death. In support of this hypothesis, we found that MPTP's effects on neostriatal brain slices were blocked by superfusing with high Mg++, low Ca++ (Neuro Sci Abst 86 12:1309). We have also found that MPTP caused high amplitude swelling of mitochondria similar to that produced by kainic acid and seizure induced Ca++ loading of neurons. To analyze the mechanism of action of MPP+, we studied its effects on the contraction of rat heart atrial muscle, in vitro. MPP+ caused dose-dependent increases in peak tension developed and velocity of development of tension. The positive inotropic effect at 10 uM was completely blocked by propranolol and in the reserpinized rat; and therefore, can be explained by the indirect activation of beta-adrenergic receptors and not by the direct activation of dihydropyridine receptors. MPTP also caused a dose-dependent positive inotropic effect which was smaller than that of MPP+. Depletion of norepinephrine by reserpine blocked MPTP's positive inotropic effect and uncovered a negative inotropic effect. Muscle contraction did not recover to control values following washout. Instead, contraction continued to deterioriate progressively. In pilot experiments, MPTP's negative inotropic effect was blocked by both MAO inhibitors and SOD/catalase. These preliminary data suggest that the oxidation of MPTP may produce radicals responsible for the negative intropic effect. Supported by NIH-MBRS 2S06RR 08016 to JSW.

59.7

THE UPTAKE AND SUBCELLULAR LOCALIZATION OF MPTP IN CULTURED CEREBELLAR ASTROCYTES. A. Marini*, J.P. Schwartz, R. Lipsky*+ and I.J. Kopin (SPON: J. Stevens) Clinical Neuroscience Branch, NINDS, NIH Bethesda, MD 20892 and +American Red Cross, Rockville, MD 20894.

MPTP is a potent neurotoxin that destroys nigrostriatal dopaminergic neurons. The conversion of MPTP to MPP+ is thought to occur in astrocytes, the principle site of MAO B. Cerebellar astrocytes rapidly take up MPTP (2.5 nM) with equilibrium reached within 15 min: no conversion to MPP+ was detected at this time but by one hour MPP+ was being formed and released into the medium. Temperature had no effect on uptake, indicating that it occurs by passive diffusion. Uptake of MPTP was linear over a wide concentration range (up to 1 mM) for 15 min. Radiotracer studies showed that MPTP is concentrated about 30-fold. Since lysosomotropic agents like chloroquine, ammonium chloride and monensin (a Na+/H+ antiporter) abolished MPTP accumulation, lysosomes are a possible MPTP storage site. High micromolar concentrations of MPTP (up to 100 uM) had no effect on cell number or GFAP as analyzed by immunoassay.

59.8

EFFECTS OF NICOTINE AND HYDRAZINE ON MPTP-INDUCED NEUROTOXICITY. L.A. Carr. Department of Pharmacology, University of Louisville, Louisville, KY 40292.

We have previously reported that chronic exposure to cigarette smoke significantly attenuates the decrease in striatal dopamine and metabolite levels caused by MPTP, 10 mg/kg, in the C57Bl/6 mouse and that this effect is probably not due to decreased oxidation of MPTP to MPP^+ in cerebral tissues. The purpose of this study was to determine whether various individual components of cigarette smoke were responsible for this protective effect. Mice were chronically infused with nicotine (3.3 mg/kg/day) or hydrazine (10 mg/kg/day) for 14 days via osmotic minipumps. On day 7, mice were treated with MPTP, 10 mg/kg subcutaneously. The striata were assayed for content of dopamine, dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA). Treatment with nicotine alone significantly increased striatal HVA levels. In mice treated with MPTP, nicotine increased levels of DOPAC and HVA. Treatment with hydrazine significantly increased DOPAC and HVA levels when administered alone and in combination with MPTP. These results suggest that both nicotine and hydrazine may contribute to the attenuation of MPTP toxicity caused by smoke exposure. There is some indication that these components may exert direct effects on nigrostriatal neurons unaffected by MPTP to increase turnover and/or release of dopamine and thus compensate for the loss of function caused by MPTP.

59.9

EFFECTS OF GBR 12909, A SPECIFIC DOPAMINE UPTAKE BLOCKER, ON MPTP'S TOXICITY IN DOG. J.S. Wilson. Dept. Anatomy, Howard Univ., Washington, D.C. 20059.

The selective toxicity of MPTP is thought to be mediated by its oxidative metabolite MPP+. Although MPP+ is toxic to most cells, it has high affinity for dopamine uptake sites and thus is thought to be concentrated to toxic levels in catecholaminergic neurons. The selective toxicity of MPTP can be explained by the fact that the nigrostriatal system has the highest dopamine uptake levels in the brain. In support of this hypothesis, it has been shown that inhibitors of dopamine uptake block MPTP's toxicity in mice; however, the data in monkey are equivocal. Therefore, we decided to test this hypothesis in dog. Two dogs received injections (2.5 mg/kg i.v.; 2.5 mg/kg i.p.) of GBR 12909 (Res. Biochem.) 30 minutes before MPTP (3.0 mg/kg; i.v.) and were sacrificed one month later. GBR completely blocked the acute effects of MPTP such as dilation of eyes; however, within 24 hrs, dogs began to show chronic hypokinesia. After sacrifice, histological examination of the brains revealed an exasperation of damaged which in many respects was equivalent to dogs receiving twice (6.0 mg/kg) the dose of MPTP, only. Thus there was an absence of TH-immunostaining in not only the caudate N. and putamen but also in the N. accumbens. In addition, cell loss was conspicuous in areas A8, A9 and A10. Although GBR is thought to have a half life of approximately 16 hrs in dog, it may not be inhibiting dopamine uptake for a long enough period to block the concentration by nigral cells of MPP+ still remaining in the brain. To resolve this issue, GBR will have to be administered for a prolonged period to determine if inhibition of dopamine uptake blocks or exasperates MPTP's toxicity in dog. Supported by NIH-MBRS 2S06RR-08016 to JSW.

59.10

EFFECTS OF MPTP ON PROENKEPHALIN mRNA AND MET-ENKEPHALIN CONTENT IN THE MOUSE STRIATUM. C.Cosi, H.Shinoda, J.D.Harvey-White*, A.Zuddas and J.P.Schwartz
Clinical Neuroscience Branch, NINDS, NIH, Bethesda, MD 20892

MPTP causes a selective destruction of the nigrostriatal dopaminergic cells in mice. It is known that dopamine (DA) exerts a tonic inhibition on striatal enkephalinergic turnover. We thus investigated the effect of a sub-lesioning dose of MPTP (30 mg/kg, i.p., free base) on brain proenkephalin (PE) mRNA gene expression and Met-enkephalin (ME) peptide level in C57/Bl mice, in vivo. DA, PE mRNA and ME contents were measured in the striata of the same animal. One week after the drug treatment DA content was depleted to 40% and PE mRNA decreased to 65% of control whereas ME increased to 140% of control. PE mRNA did not change in the olfactory tubercle or in the prefrontal cortex of the same animals.

These data suggest that a partial depletion of DA differentially affects PE mRNA gene expression and ME levels in the striatum and that this effect is region-specific.

59.11

ULTRASTRUCTURAL CHANGES INDUCED BY 1-METHYL-4-PHENYLPYRIDINE (MPP+) IN NIGRAL AND STRIATAL COMPONENTS OF AN *IN VITRO* MODEL OF THE CANINE NIGROSTRIATAL SYSTEM. B. Christie-Pope and W.O. Whetsell, Jr. Neuropathology, Vanderbilt Sch. of Med., Nashville, TN 37232-2561.

Incubation of organotypic cultures of canine substantia nigra (SN) with micromolar concentrations of MPP^+ results in a generalized destruction of neuronal and glial cells within 72 hours (Christie-Pope et al, Exp. Neurol., 104:235-240, 1989). The present study was designed to examine whether candate nucleus (CA) in co-culture with substantia nigra (SN) would also exhibit degenerative changes in response to MPP^+. Portions of CA and SN were cultured in an SN-CA-SN configuration with a separate CA explant placed in the same culture but 2-4 mm away. Ultrastructural examination of SN components of such co-cultures after 72 hours of exposure to 0.1 µM MPP^+ showed severe degeneration of glia and neurons; severe degeneration was also observed in both the separated CA and the CA in SN-CA-SN. In cultures of CA alone (without SN present), no ultrastructural changes were observed after incubation in 0.1 µM MPP^+ for 72 hours. The medium of SN-CA cultures developed greater acidity by 24 hours of MPP^+ exposure than the medium of cultures of CA alone. Results suggest that acidosis, possibly resulting from MPP^+ toxicity in SN neurons of SN-CA-SN cultures may induce or contribute to a non-specific degeneration in CA co-cultured with SN. [Supported by a grant from the United Parkinson Foundation and a Pharmaceutical Manufacturers Foundation Fellowship Award (BCP)].

59.12

MPP+ INHIBITS MITOCHONDRIAL ENERGY PRODUCTION AND DEPLETES ATP IN SYNAPTOSOMES. K.P. Scotcher*, D.A. Di Monte*, I. Irwin*, L.E. DeLanney and J.W. Langston. Institute for Medical Research and California Parkinson's Foundation, San Jose, CA 95128.

The neurotoxic effects of MPTP are thought to be mediated via its conversion to the fully oxidized metabolite, MPP+. Neuronal damage by MPP+ may result from its ability to block NADH-linked substrate oxidation in mitochondria. We investigated the effects of MPP+ on the energy metabolism of neuronal terminals using 95% pure mouse forebrain synaptosomes (non-synaptosomal mitochondria were separated by centrifugation through discontinuous Ficoll gradient). Addition of MPP+ to synaptosomes caused a dose-dependent depletion of ATP; about 40% less ATP was detected after incubation for 60 min with 100 µM MPP+. Thus, MPP+ was effective at concentrations in the same range as those reached in the brain after administration of MPTP. In order to identify the site of inhibition of ATP production, we compared the toxic effects of MPP+ to those of known inhibitors of mitochondrial electron transport (rotenone and antimycin A) and glycolytic production of ATP (iodoacetate). As expected in view of the glucose-dependent metabolism of our synaptosomal preparations, iodoacetate was the most effective of the three compounds tested in depleting ATP. ATP depletion caused by antimycin A, which completely blocks mitochondrial electron flow, was more pronounced than that produced by rotenone, an inhibitor of mitochondrial NADH oxidation. The extent of ATP depletion induced by rotenone was the same as that induced by MPP+; MPP+ and rotenone together had no additive effect. Synaptosomal preparations are a useful model for studying the effects of neurotoxicants on neuronal terminals. Inhibition of mitochondrial NADH-linked substrate oxidation by MPP+ may be critically involved in the neurotoxic action of its parent compound MPTP.

59.13

REGIONAL EFFECTS OF 6-HYDROXYDOPAMINE(6OHDA) AND NEUROLEPTIC ON FREE RADICAL SCAVENGING SYSTEM IN RAT BRAIN. A.S.Perumal*,T.B.Cooper*,S.Fahn andJ.L Cadet. (SPON:S.P.Bagchi). Dept. of Psychiatry and Neurology, Columbia Uni. and NYSPI, NY, NY 10032

It has been hypothesised that free radicals may play an important role in the pathogenesis of neurodegenerative diseases. The free radical scavenging system such as superoxide dismutase(SOD), catalase(CAT), glutathione peroxidase(GSH-Px), glutathione reductase (GR) and total glutathione (GSH) were analysed in caudate-putamen(CP), brain stem(BS), hippocambus(HIP) and frontal cortex(FC) of control and rats treated with either intracerebroventricular infusion of 6OHDA or Prolixin(IP for a month. Injection of 6OHDA resulted in significant decreases in the activity of SOD in CP (20%) and BS(30%) but not in HIP. CAT was reduced only in BS(25%). There were no changes in GSH-Px in any of the brain regions studied. GSH levels dropped in CP(23%) and BS(38%) without affecting HIP. In Prolixin treated animals, there were significant decreases in SOD(12%), CAT(34%) and GR (17%) in HIP. CAT activity was increased (62%) in FC. Prolixin treatment did not show any significant effect on other regions. These changes may be secondary to the production of free radicals due to the drug treatment in rat brain.

59.14

ENZYMES OF OXY-RADICAL METABOLISM AFTER HALOPERIDOL TREATMENT OF RAT. J. Murthy*, H. Laev, S. Karpiak, S. Mahadik, Div Neurosci NYS Psychiat Inst, Depts Psychiat, and Biochem & Mol Biophys, P&S, Columbia U, NY, NY 10032.

Molecular mechanism(s) underlying the deleterious effects associated with potent neuroleptic treatment are poorly understood. Haloperidol is a potent neuroleptic commonly used for the treatment of psychiatric disorders. Acute haloperidol treatment increases the oxidative metabolism of catecholamines resulting in the production of oxy-radicals and other toxic intermediates. If these are not eliminated by specific enzymes (superoxide dismutase, SOD; glutathione peroxidase, GSHPOD; catalase, CAT) neuropathological changes can occur in target tissues. These enzyme levels were measured in different rat brain regions after haloperidol treatment. Six rats (male, Sprague-Dawley) were injected with haloperidol (5mg/kg/day, i.m.) or saline. Enzymes were analyzed at 1,7,14,21,28,42 days of treatment. All three enzyme levels differed significantly among the brain regions. When comparing control and haloperidol treated rats, enzyme levels were unaltered in hippocampus, thalamus & striatum. While there was no change in GSHPOD levels in cortex, the levels of SOD and CAT were 30% higher in the haloperidol treated rats. This variable marginal response against oxy-radical toxicity following haloperidol treatment probably reflects the variable deleterious effects and the vulnerability of CNS to toxicity.

59.15

TRIETHYL LEAD SELECTIVELY ENHANCES THE MOTOR STIMULANT EFFECTS OF DOPAMINE AGONISTS. L.A. Taylor and T.J. Walsh, Rutgers University, Department of Psychology, New Brunswick, NJ 08903.

Triethyl lead (TEL) is a neurotoxic organometal that has been shown to produce functional supersensitivty of dopamine (DA) receptors which manifests itself as an enhanced behavioral response to both indirect (amphetamine) and direct acting (apomorphine) DA agonists. Neurochemical evidence also supports an enhanced sensitivitity of DA-stimulated adenylate cyclase activity following exposure to TEL (Walsh et al. Brain Res 363, 1986; DeHaven-Hudkins et al. Neurotoxicol. Teratol. 10, 1988.)

The present experiment examined whether TEL-treated rats were differentially sensitive to other drugs that enhance motor behavior via non-DA mechanisms of action. Male Sprague-Dawley rats were injected with 7.88 mg/kg TEL chloride or vehicle. Seven days later, the motor stimulant effects of the indirect acting DA-agonist, d-amphetamine sulfate (2mg/kg), the serotonin agonist, 5-methoxy-N,N-dimethyltryptamine (5-MeDMT) (0, 2.5, & 5.0 mg/kg), and the adenosine antagonist/phosphodiesterase inhibitor, caffeine (0, 6.25, 16.5, & 40 mg/kg) were examined. Our results demonstrated that TEL significantly enhanced amphetamine (2mg/kg) induced locomotor activity but it did not enhance the motor stimulant effects of caffeine, or the profile or degree of the motor symptoms associated with the 'serotonin syndrome' induced by 5-MeDMT.

These results support the hypothesis that TEL produces its behavioral supersensitivity through brain DA systems, and not through other neurotransmitter or modulatory systems involved in the regulation of motor activity.

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59.16

AUTORADIOGRAPHIC ANALYSIS OF DOPAMINE (DA) AND GABA/BENZODIAZEPINE RECEPTORS FOLLOWING CHRONIC NEUROLEPTIC TREATMENT IN RATS. R.E.See and G.D. Ellison. Dept. of Psychology, UCLA, Los Angeles, CA 90024.

Repeated administration of neuroleptics has previously been found to alter dopamine and GABA receptor binding in extrapyramidal brain regions. In the present study, female, Sprague-Dawley rats were administered the typical neuroleptic haloperidol, one of the two atypical neuroleptics clozapine or raclopride, or no drug for 8 months and then withdrawn from treatment for 21 days. Coronal brain sections at various AP levels were sectioned and prepared for autoradiography using the following ligands: [^{3}H]spiperone and [^{3}H]raclopride for DA D-2 receptor binding, [^{3}H]SCH23390 for DA D-1 receptors, [^{3}H]muscimol for GABA receptors, and [^{3}H]RO15-1788 for benzodiazepine receptors. Haloperidol, but not the two atypical neuroleptics, increased D-2 receptor binding in striatal regions and GABA receptor binding in the substantia nigra reticulata. No significant differences were found between groups for [^{3}H]SCH23390 or [^{3}H]RO15-1788 in any regions examined. These results support previous findings of altered DA/GABA function following prolonged HAL, but not atypical neuroleptic treatment. Implications for neuroleptic-induced dyskinesias will be discussed.

59.17

FORMATION OF 6-HYDROXYDOPAMINE (6-OHDA) AND 5,6-DIHYDROXYTRYPTAMINE (5,6-DHT) ARE FOUND AFTER A LARGE SINGLE DOSE OF METHAMPHETAMINE (MA) IN GUINEA PIGS. Lewis S. Seiden and Georgetta Vosmer* Dept. Pharm/Phys. Sciences, Univ. of Chicago, Chicago, IL 60637.

Male Hartley guinea pigs (650 grams) were injected with MA (100 mg/kg, s.c.) or saline and killed 15, 30, or 60 mins after injection. 6-OHDA was detected in the caudate of 2/7 (.003 ng/mg tissue) at 30 min and 3/7 (.005 ng/mg tissue) at 60 min. Lower levels of 5,6-DHT were detected in hippocampi in 2/4 (.001 ng/mg tissue) at 15 min, 3/7 (.001 ng/mg tissue) at 30 min and 6/7 (.0025 ng/mg tissue) at 60 min. DA levels in the caudate were 75% of control and 5-HT levels in the hippocampus were 46% of control 1 hr after injection. These results are consistent with results found in rats showing formation of either 6-OHDA or 5,6-DHT in approximately one half the rats after any given experiment. Reasons for the variability in findings will be discussed. This is consistent with the notion that neurotoxicity of MA is related to formation of neurotoxins from the transmitters DA and 5-HT. This research supported by NIDA #00250 Project VI, RSA MH-10562 (L. Seiden).

59.18

MODIFICATION OF METHAMPHETAMINE-INDUCED NEUROTOXICITY IN MICE. C. Konradi*, L. Manzino, P.K. Sonsalla and R.E. Heikkila. Dept. of Neurology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854.

The present study was an attempt to determine the effects of pretreatment with various drugs on methamphetamine induced neurotoxicity to neostriatal dopaminergic neurons. On four consecutive days, male Swiss-Webster mice received vehicle or increasing doses of either methamphetamine, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) or cocaine, three times a day at 3 hour intervals. On day five, the animals were challenged with three injections of methamphetamine, 16mg/kg per injection at three hour intervals. Five days later the mice were killed and the neostriatal content of dopamine was determined. In mice pretreated with methamphetamine prior to the methamphetamine challenge, there was a significantly smaller reduction of dopamine content than in mice pretreated with vehicle. In MPTP or cocaine pretreated mice there was no such tolerance to the dopaminergic neurotoxicity of methamphetamine. In fact, of the four experimental groups, the cocaine pretreated animals had the largest decrease in dopamine content after the methamphetamine challenge. Possible explanations for these interactions will be presented.

59.19

TETRAHYDROPYRIDINE ANALOG OF HALOPERIDOL CAUSES PROFOUND DEPLETION OF STRIATAL DOPAMINE. N. Castagnoli*, B. Subramanyam*, and G.A. Ricaurte (SPON: M. Bleecker). Department of Chemistry, Virginia Polytechnic Inst., Blacksburg, VA. 24061 and Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, MD. 21224.

The haloperidol derived compound 4-(4-chlorophenyl)-1-[4-(4-fluorophenyl)]-4-oxobutyl-1,2,3,6-tetrahydropyridine (PCOTP) bears close structural resemblance to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a parkinsonogenic compound with known dopaminergic neurotoxic activity. One of the major side-effects of haloperidol therapy is an extrapyramidal syndrome with prominent parkinsonian features. This study examined the dopaminergic neurotoxic potential of PCOTP, and compared it to that of MPTP.

Male C57 black mice weighing 25-30 grams were injected with equivalent doses of PCOTP and MPTP (20 mg/kg/hour X 4; i.p.). Each drug was tested in a group of 6 animals. Control animals received an equivalent volume of saline. Four days later, all animals were sacrificed and striatal dopamine content was determined. PCOTP-treated mice showed a large (70-80%) depletion of striatal dopamine. The dopamine-depleting effects of PCOTP were comparable to those of MPTP.

These results demonstrate that the haloperidol analog PCOTP produces a severe depletion of striatal dopamine in mice. The duration and basis of the dopamine depletion are currently under investigation. The relevance of these findings to haloperidol-induced parkinsonism in humans remains to be determined.

59.20

AMPLIFICATION OF THE ACTION OF 6-HYDROXYDOPAMINE BY ETHANOL F.F. Ahmad*, D.L. Cowan*' and A.Y. Sun (SPON: Vincent V. St. Omer). Sinclair Comparative Medicine Research Farm and Departments of Biochemistry and Physics*, University of Missouri, Columbia, MO 65203

6-hydroxydopamine (6-OHDA) has been used extensively to induce brain lesion in dopaminergic neurons for the study of dopamine (DA) deficient state such as Parkinsonism. Production of free radicals by 6-OHDA has been suggested as the cytotoxic mechanism for neuronal degeneration. However, direct evidence for free radical formation is lacking. By using electron spin resonance (ESR) and spin trapping technique (Ahmad, Cowan and Sun, Life Sci. 43:1169-1176, 1988), we demonstrated the direct participation of 6-OHDA in the free radical reaction. In the presence of ethanol, the free radicals formed were converted to the longer lived hydroxyethyl free radicals (·OHEt) which may cause more damage than the short lived species. The formation of ·OHEt increased in proportion with increasing ethanol concentration (0-.05%). The results suggest that ethanol ingestion may amplify the action of neurotoxin, such as 6-OHDA in causing the brain lesion and Parkinsonism. (Supported in part by Grant AA02054 from DHHS.)

NEUROTOXICITY I

60.1

TAURINE DEFICIENCY EXACERBATES CYSTINE NEUROTOXICITY IN CAT RETINA: A MORPHOLOGICAL STUDY. H. Imaki and John Sturman*, N.Y.S. Inst. for Basic Res. in Devel. Disabil., Staten Island, New York 10314.

Dietary taurine deprivation in cats causes severe visual dysfunction associated with photoreceptor degeneration. To examine if such effects of taurine deficiency may be offset by excess dietary precursor, we fed mature cats a synthetic diet containing 5% cystine and either 0 or 0.05% taurine. All the cats fed cystine without taurine exhibited clear neurological symptoms and died or were killed when they appeared terminal, while some cats fed taurine in addition to cystine showed no adverse effects for up to a year, the rest dying after showing minimal symptoms. The most common and conspicuous changes noted in these retinas were reductions in the number of cells in INL and GCL, and swelling of many cells and their processes in INL and IPL. Many of such cells exhibited irregularly shaped nuclei containing dense clumps of chromatin, and greatly distended mitochondria and vacuolar profiles in unusually pale cytoplasm, most frequently among amacrine cells of cats fed cystine and no taurine, but also among some horizontal, bipolar and ganglion cells of most cats fed cystine. Muller cells appeared normal as did astrocytes, microglial and endothelial cells. Photoreceptor cells remained relatively intact, but subtle ultrastructural changes, such as dilated disc membranes in outer segments and swollen mitochondria in inner segments, were more prevalent in the retinas of cats fed taurine-free than -supplemented diet with cystine. These results suggest that taurine plays a protective role not only in photoreceptor cells but in other retinal neurons against the neurotoxic effects of cystine.

60.2

FACILITATION AND INHIBITION OF NEURONAL EXCITABILITY BY LEAD IN THE ISOLATED TURTLE CORTEX. D. M. Wilkison. Dept. Pharmacology and Toxicology, Med. Col. Wisconsin, Milwaukee, WI 53226

The actions of lead on central nervous system synaptic function were investigated by studying the effects of bath-applied lead acetate (Pb-Ac) on the dynamic firing response to pairs of stimuli in the isolated cortex. Turtles (Pseudemys scripta) were cooled in ice water prior to decapitation. The anterior dorsal (visual) cortex was removed to a tissue chamber. Tissue was perfused with a balance buffer at room temperature. Extracellular recordings were made by standard glass-microelectrode techniques. Cells identified by application of glutamate and GABA were activated by electrical stimulation of afferent, thalamocortical, or intracortical fibers. Paired-stimulations (10-200 msec) delivered at 0.3 Hz produced potentiation of the evoked discharges. This potentiation was inhibited by Pb-Ac (1-30uM). In contrast, single shock stimulation was facilitated by Pb-Ac over the same exposure range. Interactions between Pb+2 and Ca+2 and GABA function are under investigation. The data are somewhat consistent with data from synaptosomes (TAP 84:400-411, 1986) in which Pb increased spontaneous release and decreased evoked release. Studies are supported by NIEHS, ES04184.

60.3

THE EFFECTS OF CADMIUM CHLORIDE ON SPONTANEOUS LOCOMOTOR ACTIVITY. M.A. Blackshear, K. Phillips*, R.A. Sawrie* and E.J. Olson*. Dept. of Biological Sciences, Tennessee State University, Nashville, TN and Dept. of Pharmacology, Meharry Medical College, Nashville, TN 37208.

This study investigates the acute and chronic effects of cadmium chloride ($CdCl_2$) administration on spontaneous locomotor activity (SLA) and examines the possible involvement of brain dopamine receptors. Male mice (Swiss ICR, 20-26g) received either a single injection of 2 mg/kg $CdCl_2$ or 14 daily injections of 0.14 mg/kg $CdCl_2$. Control animals received 0.01 ml/mg physiological saline. Locomotor activity was measured for 20 minutes post $CdCl_2$ or saline injections in the acute studies. To determine the effects of chronic $CdCl_2$ administration, locomotor activity was monitored on days 1, 3, 7 and 14 for 20 minutes post Cd treatment and compared to that of saline controls.

Acute administration of $CdCl_2$ caused a marked decrease in SLA, 46.3 ± 2.5 and 9.5 ± 0.7, respectively, for control and Cd treated mice. With chronic Cd treatment, SLA was decreased on day 1 (40 ± 3.9 vs 59 ± 2.9 for controls) and on day 3 (30.4 ± 3.9 vs 49.4 ± 3.0 for controls). [^{3}H]-spiroperidol binding to striatal membranes was increased after acute Cd treatment (1.80 ± 0.17 fmol/mg protein vs 1.06 ± 0.5 fmol/mg protein for controls), but was unaltered after chronic treatment. Although Cd was not detected in the brain, it was present in the liver (0.144 ± 0.5 mg/100 dry wt.) of acutely treated mice. Cd was not detected in the brains or livers of control animals.

These findings indicate that both acute and chronic administration of $CdCl_2$ decrease SLA, and suggest that central dopamine receptors are probably not involved in this behavior.
(Supported by NSF grant BNS-8711253).

60.4

EFFECTS OF IN VITRO LEAD EXPOSURE ON VOLTAGE-SENSITIVE CALCIUM CHANNELS DIFFER AMONG NEURONAL TYPES IN LYMNAEA STAGNALIS. T. Audesirk and G. Audesirk. Biology Dept., U. Colorado-Denver, 1200 Larimer Street, Denver, CO 80204.

The effects of acute in vitro lead exposure on barium currents through voltage-sensitive calcium channels were studied under voltage clamp. Three physiologically distinct cell types were used: the pedal giant neuron (RPeD1) and two subsets of the B cell cluster (B_{pos} and B_{neg}). Free Pb^{2+} concentrations were buffered by using a Leibovitz-L15-based saline, and were measured with a Pb^{2+}-selective electrode. In B_{pos} neurons, 5 nM free Pb^{2+} irreversibly inhibits current flow through calcium channels by 38 ± 10%. In B_{neg} neurons, 5 nM free Pb^{2+} slightly inhibits inward currents (12 ± 6%) and may shift their voltage dependence to more depolarized voltages. The inhibition and voltage shift are irreversible. In RPeD1 neurons, Pb^{2+} inhibits inward current by 18 ± 19% (5 nM free Pb^{2+}) to 31 ± 23% (30 nM free Pb^{2+}). The effects of Pb^{2+} are fully reversible. These data indicate that (1) voltage-sensitive calcium channels in Lymnaea neurons are inhibited by nanomolar concentrations of free Pb^{2+}; (2) different neuronal types in Lymnaea possess different types of calcium channels; and (3) the effects of in vitro lead exposure differ qualitatively among channel types.

60.5

ULTRASTRUCTURAL FINDINGS IN FROGS WITH CIS-PLATIN INDUCED NEUROTOXICITY. Karen S. Blisard PhD MD and Deborah A. Harrington. Research Service (151), Veterans' Administration Medical Center, and Department of Pathology, University of New Mexico Medical School, Albuquerque, NM 87108.

Cis-diamminedichloroplatinum II (cis-platin) is a cancer chemotherapeutic agent whose usefulness is often limited by side effects, including peripheral sensory neuropathy or central nervous system (CNS) toxicity with seizures. We have developed a model system for CNS toxicity using the frog. The animals had tonic-clonic seizures 3 to 5 weeks after a single dose of 10 mg/kg cis-platin. The principle pathology was found in the spinal cord, where there was vacuolization in the anterior gray horns. Ultrastructurally, the vacuoles consisted of swelling of astrocytic processes in the neuropil and around neurons. Generalized edema with swelling of perivascular astrocyte foot processes was not seen. There was increased nuclear irregularity in motor neurons from treated animals. Cup-shaped or ring-shaped mitochondria occasionally were seen in processes and mitochondrial pleomorphism could be observed in motor neurons. Further study of this model may help elucidate the mechanisms of cis-platin's neurotoxicity in humans.

60.6

MANGANESE TOXICITY: FREE AMINO ACIDS AND TRACE ELEMENTS IN THE MOUSE BRAIN E. Bonilla, A. Arrieta*, J.O. Dávila* and I. Quiroz*. Instituto de Investigaciones Clínicas, Universidad del Zulia and INBIOMED-FUNDACITE, Aptdo. 376, Maracaibo-Venezuela.

We studied the effect of manganese administration on the levels of 20 free amino acids and 4 trace elements in the striatum and olfactory bulb.

Male albino mice weighing 20-25 g were injected intraperitoneally with manganese chloride (5mg Mn/Kg b.w.) in 0.9% NaCl solution. Both control and Mn-treated mice received one daily injection five days per week. After nine weeks, 11 animals of each group were sacrificed by decapitation and the brain extracted immediately. The analysis of the amino acids was performed by HPLC. Brain manganese, copper, zinc and iron concentrations were determined by flameless atomic absorption spectrophotometry.

The growth rate and water intake of the Mn-intoxicated mice were normal. The means ± S.E. of the manganese content (ug/g dry weight) at the ninth week were: a) striatum: 4.05 ± 0.34 in controls and 11.44 ± 0.61 in Mn-loaded mice; b) olfactory bulb: 5.22 ± 1.16 and 13.78 ± 2.62. The iron concentrations were as follows: a) striatum: 46.79 ± 1.85 in controls and 70.39 ± 4.30 in Mn-treated mice; b) olfactory bulb: 56.18 ± 9.55 and 91.02 ± 8.65 . The striatal and olfactory bulb copper and zinc levels did not change with the manganese administration.

No alterations were observed in the levels of free amino acids in the striatum of Mn-treated mice. However, in the olfactory bulb the contents of alanine, alpha-amino-n-butyrate, arginine, asparagine, aspartate, citrulline, GABA, glutamine, glutamate, glycine, isoleucine, leucine, methionine, phenylalanine, serine, threonine, tyrosine, and valine were significantly diminished. The changes produced in the olfactory bulb must be thoroughly evaluated to determine its importance on the pathophysiology of manganese poisoning.

60.7

***IN VIVO* EFFECTS OF DIFLUROMETHYLORNITHINE ON INDUCED BRAIN ODC-mRNA & ACTIVITY.** N. Zawia*, C. Huntington* and S. Bondy* (SPON: C. GORENSTEIN). Dept. of Pharm., Dept. of C.&E. Med. & the S.O.H.C., U. of California, Irvine, CA 92717.

Ornithine decarboxylase (ODC) is the rate limiting enzyme in the polyamine pathway, which is known to be involved in the adaptive mechanisms of the CNS. We previously found that the induction of enzyme activity in the neocortex in response to electroconvulsive shock (ECS) was accompanied by an increase in ODC-mRNA which persists longer than the transient change in activity, while the elevation of enzyme activity in the hippocampus was transcription-independent. This prompted us to study the effect of difluromethylornithine (DFMO), a specific ODC inhibitor, on the regional regulation of ODC activity and mRNA. DFMO was administered to rats in 2 doses (5 hr. interval) of 500 mg/Kg I.P.. ECS was applied 15 min. after the second injection and the animals were sacrificed 5 hrs. later and their brain regions were dissected. ODC-mRNA was studied by Northern blot analysis using a mouse cDNA as a probe. Enzyme activity was determined by counting evolved $^{14}CO_2$ from ^{14}C-ornithine. Our results show that the basal ODC activities in the neocortex and the cerebellum are resistant to *in vivo* DFMO inhibition, while the hippocampal ODC activity which is higher than other regions is very susceptible to DFMO blockade. Upon ECS treatment, the hippocampus exhibits greater susceptibility to *in vivo* DFMO than other brain regions. The ODC-mRNA in all cases was not significantly altered by DFMO applications. The susceptibility of hippocampal ODC implies that here the enzyme may have a distinctively longer half-life than that in other regions.

60.8

A RODENT MODEL OF METHANOL-INDUCED VISUAL TOXICITY. J.T. Eells. Dept. of Pharmacology and Toxicology; Medical College of Wisconsin, Milwaukee, WI 53226.

Methanol is an important environmental neurotoxin with selective actions on the primate visual system. Methanol poisoning in primates is characterized by metabolic acidosis and ocular toxicity accompanied by the accumulation of formate in body fluids and tissues. Rodents do not exhibit these signs of toxicity and do not accumuate formate following methanol administration. The objective of this investigation is to establish a rodent model in which to study the pathogenesis of methanol-induced ocular toxicity. To accomplish this objective, we studied the development of formic acidemia and visual dysfunction following methanol administration in rats made folate-deficient by treatment with N_2O. Rats were exposed to N_2O/O_2 (50/50) for 4 hr prior to the administration of methanol (4 g/kg) and throughout the course of the experiment. Accumulation of blood formate (peak values 13.5 ± 1.0 mM) was observed in these animals. Visual dysfunction was assessed by measurements of the electroretinogram (ERG) and the flash-evoked cortical potential (FEP). A reduction in the amplitude of the ERG (b wave; 56 ± 12 % of control) and the FEP (P20-N30 component; 50 ± 9 % of control) occurred coincident with the accumulation of blood formate indicative of a temporal relationship between methanol-induced metabolic and visual disturbances. The highest concentrations of formate were measured in the vitreous humor and retina suggesting that the target organ toxicity of methanol may result from the differential distribution of this toxic metabolite.
(Supported by Fight for Sight, Inc., New York City. GA 88-048).

60.9

ETHANOL-INDUCED AXONAL TRANSPORT CHANGES IN SENSORY NERVES DETECTED BY COMPUTER-ENHANCED VIDEO MICROSCOPY. J.A. McLane[1], M.B. Atkinson[*2] and A.C. Breuer[2]. [1]Neuroscience Research, VA Hospital, Hines, IL 60141, [1]Dept. [2]of Biochem., Loyola University, Maywood, IL 60153, and [2]Dept. of Neurology, Cleveland Clinic Fndt., Cleveland, OH 44195.

We hypothesize that ethanol-induced alterations in axonal transport lead to the peripheral neuropathy often observed in chronic alcoholism. In rats, we have shown that chronic ethanol exposure through a liquid diet leads to reduced accumulation of anterogradely transported proteins at a nerve ligation (Alcohol 4:385, 1987), but no change in transport rate (Trans Am Soc Neurochem 20:234, 1989). Also, the amount of material delivered to the dorsal root ganglia by retrograde transport was reduced (Abstr Soc Neurosci 13:124, 1987). We have used computer-enhanced video-microscopy to obtain preliminary transport rate data in sural nerves from chronic (5 month) ethanol-fed rats. This data confirms that the rate of fast anterograde axonal transport was unchanged (ethanol = control = 1.50±0.06 um/sec). However, this treatment produced a significant increase in the rate of organelle transport in the retrograde direction (ethanol = 1.78±0.02 um/sec, control = 1.60±0.03 um/sec, $p<0.001$). These results suggest that a compensatory mechanism in the retrograde transport system attempts to offset the reduced delivery of materials to the cell body from the sensory nerve ending by increasing the transport rate.

60.10

AXONAL AND TERMINAL DEGENERATION IN THE CNS OF THE FERRET FOLLOWING EXPOSURE TO BIS (1-METHYLETHYL) PHOSPHOROFLUORIDATE (DFP). D. Tanaka, Jr., S.J. Bursian*, and E. Lehning*. Depts. of Anatomy and Animal Science, Michigan State Univ., East Lansing, MI 48824.

Although it has been well established in mammals that exposure to the organophosphorus delayed neurotoxin DFP results in delayed degeneration in spinal tracts and peripheral nerves, it is not clear to what extent other areas of the CNS may be affected. Ferrets were injected subcutaneously with 4mg DFP/kg body weight and killed 7, 14, 21, and 28 days after exposure. Brains and spinal cords were processed using a modified Fink-Heimer method. At 7 days, minimal degeneration was noted only in the nucleus gracilis. From 14 to 28 days, increasing amounts of degeneration were noted in the fasciculus and nucleus gracilis, medial and dorsal accessory olivary nuclei, inferior vestibular nucleus, lateral reticular formation, dorsal and ventral spinocerebellar tracts, and cerebellar folia I-IV. Degenerating fibers were also noted in laminae VI-VII of the lumbar spinal cord. Hindlimb ataxia and paralysis were also noted at 21 and 28 days post-DFP. These results suggest that exposure to DFP primarily results in degeneration of selected ascending tracts associated with exteroceptive and proprioceptive pathways.

Supported by funds from the Michigan Agricultural Experiment Station and BRSG funds awarded to the College of Veterinary Medicine.

60.11

PROTECTIVE ENZYMES ARE NOT INVOLVED IN NGF-INDUCED RESISTANCE OF PC12 CELLS TO OXIDATIVE INJURY. Margaretann Halleck* and Frederick Kauffman. Lab. for Cellular and Biochemical Toxicology, Rutgers University, Piscataway, NJ 08854

NGF enhances the resistance of PC12 cells to H_2O_2 toxicity as indexed by trypan blue uptake (Perez-Polo, *et. al.*, Clin. Neuropharm, **9**, 98, 1986) or maintenance of high energy phosphate (O'Brien & Kauffman, Pharmacologist, **30**, A47, 1988). Accordingly, we examined enzymes involved in detoxification of reactive O_2 species in PC12 cells after 2, 5 and 14 days of treatment with NGF. Enzymes studied were catalase, glutathione reductase (GR), glutathione peroxidase (GPO) and superoxide dismutase (SOD). H_2O_2 (250 µM) caused rapid and profound (>80%) decreases in ATP and P-creatine at 15 min in both groups of cells; however, recovery occurred only in NGF-treated cells. Although this pattern of NGF-induced protection was seen at each day studied, enzyme activities were the same in both groups (Table).

Enzyme	*Control*	*5 day NGF-Treated*
Catalase (µmole/min/mg protein)	1.4 ± 0.1	1.4 ± 0.1
GPO (nmole/min/mg protein)	30.8 ± 1.0	31.6 ± 0.8
GR (nmole/min/mg protein)	34.0 ± 2.4	33.3 ± 1.2
SOD (units/mg protein)	69.7	64.8

Rates of removal of H_2O_2 by intact cells were the same in control and NGF-treated PC12. Thus, protection conferred by NGF is not due to enhanced degradation of H_2O_2. Experiments employing flow cytometry and ion sensitive dyes to monitor intermediates involved in cell injury and protection are being applied to elucidate the novel mechanism of resistance to oxidative injury in NGF-treated PC12 cells. (Supported by NIH Grant HD-16596)

60.12

CHICK EMBRYO EXPOSURE TO CARBAMATES ALTERS NEUROCHEMICAL PARAMETERS AND BEHAVIOR. M. Farage-Elawar* and W. D. Blaker (SPON:B.S. Jortner). VA/MD Regional College of Veterinary Medicine, Department of Biomedical Sciences, Blacksburg, VA 24061.

Recent evidence has shown that exposure to pesticides can lead to long-term neurophysiological and functional deficits. We have demonstrated that behavior and locomotion in chicks exposed to some organophosphates and carbamates could be persistently altered without concomitant central or peripheral esterase inhibition. Furthermore, histopathology on the ataxic chickens showed no lesions in either the central or peripheral system. In this study, we examined the hypothesis that locomotion alterations seen in chicks exposed to carbaryl and aldicarb are accompanied with perturbations in particular central neurotransmitter systems. Carbaryl and aldicarb were injected in ovo on day 15 of incubation at 6, 16 and 65 mg/kg and at 0.2, 0.4 and 3.5 mg/kg respectively. Neurotransmitter levels (assayed by HPLC-ED) and locomotion were measured at various times (1-50d) after dosing. At the lower doses of both carbaryl and aldicarb a trend towards prolonged decreases in cerebral dopamine and HVA was seen. The high dose of carbaryl significantly reduced dopamine and the high dose of both compounds significantly decreased HVA and 5-HIAA. Persistent behavioral alterations were observed only at the higher doses of both carbaryl and aldicarb.

60.13

GENE PROBE FOR PO MESSAGE USED TO INDEX ACRYLAMIDE TOXIC NEUROPATHY IN RATS. B.Veronesi[1]*, K.Jones [1]* S.Gupta, J. Pringle* and C.Mezei (SPON: D.L.Brown) United States Environmental Protection Agency,[1] HERL, RTP, NC, 27711, and Dalhousie University, Department of Biochemistry, Halifax, Nova Scotia, B3H 4H7, Canada

Cumulative exposure to acrylamide produces axonal damage to the distal lengths of both central (CNS) and peripheral (PNS) nerve fibers and subsequent hind-limb paralysis. Gene probes for the major PNS myelin glycoprotein PO (PO-mRNA) were used to monitor this toxic neuropathy in Sprague Dawley rats prior to, concurrent with and subsequent to ultrastructural pathology. Rats were dosed every other day with acrylamide (50 mg/kg, IP) and sacrificed intermittently throughout a 4 wk exposure. Slot Blot and Northern gel analysis of the sciatic nerve was used to determine the quantity and quality of PO-mRNA. PO-mRNA was depressed 12% after 3X exposures in the distal sciatic nerve in the absence of discernable ultrastructural neuropathy. Levels of PO-mRNA continued to decline 20% and 40% after 6X and 12X treatment. PO-mRNA from rats exposed 12X and allowed to recover hind-limb function for 30-40d, was depressed by 28%. These results suggest that gene probes are highly sensitive to neurotoxic damage, that they can be used to monitor the progression of pathology and may be used to index toxic neuropathy at low levels and early time-points when PNS fiber damage is reversible.

60.14

THE EFFECTS OF ARACHIDONIC ACID METABOLISM INHIBITORS ON METHYL IODIDE-INDUCED NEUROTOXICITY IN VITRO. C.J. Davenport and K.T. Morgan*. CIIT, RTP, NC 27709.

The monohalogenated methanes - methyl chloride (MeCl), methyl bromide (MeBr) and methyl iodide (MeI) - are established neurotoxins. Whereas, following inhalation exposure of rodents to monohalomethanes most organs show degenerative rather than inflammatory changes, the dual lipoxygenase-cyclooxygenase antagonist 3-amino-1-[m-(trifluoromethyl)phenyl]-2-pyrazoline (BW755C) inhibited MeCl and MeBr toxicity in vivo and MeI neurotoxicity in vitro (EC50 100µM). The present experiments, using mature dissociated murine (CD-1 strain) neocortical cultures, assessed the effects of 1 hr pretreatment with the lipoxygenase inhibitor nordihydroguaiaretic acid (NDGA) or the cyclooxygenase inhibitors salicylic acid, lysine acetyl salicylate and indomethacin on MeI (10mM, 5min) neurotoxicity. Cytotoxicity was attenuated in a concentration-dependent fashion by 1-10µM NDGA (EC50 3µM), with almost complete protection at 10 µM NDGA. Lysine acetyl salicylate (27mM), salicylic acid (10mM) and indomethacin (100µM) failed to inhibit cytotoxicity. In addition to correlating well with existing in vivo and in vitro data on the inhibition of monohalomethane toxicity by BW755C, and the lack of protection by indomethacin, these results indicate that prostaglandins do not play an essential role in MeI-induced neurotoxicity.

60.15

AIDS VIRUS ENVELOPE PROTEIN GP120 KILLS RODENT RETINAL GANGLION CELLS IN VITRO. Peter K. Kaiser, Jeffrey T. Offermann*, Rafael Campo, Jorge Arroyo, Edward Lamperti, and Stuart A. Lipton. Department of Neurology, Children's Hospital and Harvard Medical School, Boston, MA 02115.

The envelope protein gp120 of HIV-1 kills rat hippocampal neurons in culture (Brenneman et al., *Nature* 335:639, 1988). Here we report that another mammalian central neuron, the rat retinal ganglion cell, is also susceptible to gp120 and that this lethal effect can be prevented by anti-gp120 sera. Postnatal ganglion cells were identified with fluorescent markers, dissociated from the retina and cultured as previously described (Leifer, Lipton et al., *Science* 224:303, 1984). As little as 0.1 pM-1.0 nM purified gp120 from two native isolates as well as an extremely pure recombinant preparation of gp120 killed retinal ganglion cells in vitro in a dose-dependent manner. Neurotoxicity could be abated by specific anti-gp120 sera but not by control preimmune sera, suggesting that the lethal effects of the purified preparations of the envelope protein were due to gp120 and not to a contaminant. Taken together with the previously reported neurotoxic effects of gp120 on hippocampal cells, these findings in retinal ganglion cells may account at least in part for the dementia and blindness that develop in some patients with AIDS.

60.16

CALCIUM CHANNEL ANTAGONISTS PARTIALLY PREVENT NEURONAL CELL KILLING BY HIV-1 COAT PROTEIN GP120 IN VITRO. Rafael Campo, Jeffrey T. Offermann,* Jorge G. Arroyo, Peter K. Kaiser, Huei-sheng V. Chen and Stuart A. Lipton. Depts. of Neurology and Biological Chemistry & Molecular Pharmacology, Children's Hospital & Harvard Medical School, Boston, MA 02115.

Picomolar concentrations of the viral envelope protein gp120 increase a prolonged component of Ca current and kill rat retinal ganglion cell and hippocampal neurons in culture (S.A. Lipton et al.; P.K. Kaiser et al., these abstracts). An increase in $[Ca^{2+}]_i$ is associated with and apparently responsible for several forms of neurotoxicity, including that mediated by excitatory amino acids binding at the NMDA receptor. Thus, the increase in Ca current engendered by gp120 could conceivably be related to its toxic action. Since the dihydropyridine antagonists nifedipine and nimodipine were found to offset the increase in Ca current produced by gp120 (Lipton et al., these abstracts), these agents were used in an attempt to block the neurotoxicity of gp120 on rat retinal ganglion cells in vitro. The addition of 1-10 μM nifedipine or nimodipine partially prevented the death of 1 week postnatal retinal ganglion cells cultured in the presence of gp120 (20 of 22 experimental trials, $P < 0.001$). However, the higher concentrations of nifedipine and nimodipine were toxic by themselves, although to a far lesser extent than gp120.

OPIATES, ENDORPHINS AND ENKEPHALINS: TOLERANCE AND DEPENDENCE

61.1

EFFECTS OF OPIATE WITHDRAWAL ON RAT LOCUS COERULEUS NEURONS: BEHAVIORAL, BIOCHEMICAL, AND PHYSIOLOGICAL CORRELATES. D.B. Beitner, K. Rasmussen, J.H. Krystal, G.K. Aghajanian, and E.J. Nestler. Laboratory of Molecular Psychiatry, Depts. of Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06508.

We have compared the onset and duration of the behavioral manifestations of opiate withdrawal to the *in vivo* activity of locus coeruleus (LC) neurons and to increases in the levels of G-proteins, adenylate cyclase, and cAMP-dependent protein kinase that occur in the LC during chronic morphine treatment.

Rats were given morphine by daily implantation of subcutaneous morphine pellets (75 mg) for 5 days. On the sixth day, morphine withdrawal was induced by administration of naltrexone (100 mg/kg, s.c.), with additional doses given 6 and 24 hours later.

We found a striking parallel between the time courses of the behavioral symptoms and the increased activity of LC neurons during withdrawal. Both were most pronounced within 30 min, recovered rapidly (>50%) within 6 hr, but did not recover completely until after 72 hr of withdrawal. Adenylate cyclase and cAMP-dependent protein kinase activities in isolated LC membranes, both elevated in tolerant animals, recovered to control levels in 6 hr, in parallel with the rapid phase of withdrawal. Levels of Gi/Go, also elevated in tolerant animals, returned to normal by 24 hr. Taken together, these data suggest that increased neuronal activity in the LC is associated with the behavioral morphine withdrawal syndrome and that increased levels of G-proteins and an up-regulated cAMP system may contribute to the withdrawal activation of these neurons.

61.2

MORPHINE REGULATION OF C-FOS EXPRESSION IN THE LOCUS COERULEUS AND OTHER REGIONS OF RAT BRAIN. M.D. Hayward, R.S. Duman, E.J. Nestler (SPON: S. Stine). Laboratory of Molecular Psychiatry, Depts. of Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06508.

The proto-oncogene c-fos has been shown to be induced rapidly in neurons in response to seizures and increased neuronal activity. In this study, we investigated morphine regulation of c-fos induction in the locus coeruleus (LC), since firing rates of LC neurons are known to increase 4-fold during opiate withdrawal (Beitner et al, this volume).

In morphine tolerant rats, levels of Fos immunoreactivity increased several-fold 2 hr after the initiation of opiate withdrawal by naltrexone. Levels of Fos immunoreactivity returned to normal after 6 hours of withdrawal even though LC neuronal activity remains elevated 2-fold at that time. Induction of c-fos was also seen at the mRNA level, analyzed by northern blot and in situ hybridization, after 1 hr of withdrawal. In contrast, c-fos is not induced in the LC in response to naltrexone administration to control animals, in response to acute morphine, or in non-withdrawing, morphine tolerant animals. We also found similar regulation of c-fos induction in frontal cortex and amygdala, but not in a number of other brain regions, including pons cross-sections (from which LC had been excised), hippocampus, dorsal raphe, and paragigantocellularis.

Region-specific regulation of c-fos during morphine withdrawal indicates that c-fos can be used as a marker to map neuronal pathways and neuronal cell types activated in response to acute and chronic opiate administration and during opiate withdrawal, as well as in response to other psychotropic drug treatments.

61.3

OPIOID-INDUCED REWARD AND AVERSION: INVOLVEMENT OF THE MESOLIMBIC DOPAMINE SYSTEM. T.Shippenberg* R.Spanagel*, R.Bals-Kubik*, C.Stein and A.Herz* Dept. Neuropharmacology, Max-Planck Institute for Psychiatry, D-8033 Martinsried, FRG.

Opioids produce rewarding or aversive effects depending on the receptor types with which they interact. The neurochemical substrates mediating such motivational (MOT) effects of opioids is unknown. This issue was addressed using place preference conditioning and in-vivo microdialysis. μ-(DAGO, morphine) or δ-(DPDPE) agonists functioned as reinforcers, producing marked place preferences in rats. In contrast, the k-agonists U69593 and E2078; a dynorphin analog produced aversions. Mapping studies revealed the mesolimbic dopamine (DA) system as a critical site for these effects. Injections of μ-agonists into the ventral tegmentum (VT) were reinforcing whereas injections of κ-agonists into either the VT or n. accumbens (NAC) were aversive. Both MOT effects were abolished after 6-OHDA lesions of the NAC or NAC D-1 but not D-2 receptor blockade. Microdialysis studies showed that μ- and δ-agonists increased NAC DA release whereas k-agonists decreased DA release. These results demonstrate a role of the mesolimbic DA system in opioid reward and aversion and suggest that both result from an alteration in the tonic activation of D-1 receptors in the NAC.

61.4

NOREPINEPHRINE TURNOVER IN CORTICAL BRAIN AREAS OF MICE WITHDRAWN FROM REPEATED MORPHINE TREATMENT AND IN RESPONSE TO A MORPHINE CHALLENGE. M. Attila*, E. Etemadzadeh* and L. Ahtee. Div. of Pharmacology and Toxicology, Dept. of Pharmacy, Univ. of Helsinki, SF-00170 Finland.

In contrast to its effects in rats acute morphine administration accelerates the cortical norepinephrine (NE) turnover in mice (Attila, L.M.J. et al., Pharmacol. Toxicol., 61:26, 1987). The α-methyl-p-tyrosine (αMT)-induced NE depletion is enhanced in morphine-withdrawn rats and a morphine challenge during withdrawal counteracts such an enhancement in cortical hemispheres (Attila, L.M.J. & Ahtee, L., Naunyn-Schmiedeberg's Arch. Pharmacol., 327:193, 1984). To study the effects of morphine withdrawal and morphine challenge in mice we gave male NMRI mice 3 daily morphine injections (100-200 mg/kg, s.c.) for 5 days followed by a morphine challenge (10 or 30 mg/kg) after various withdrawal periods. Withdrawal did not alter the rate of αMT-induced NE depletion in the brain area consisting mainly of cerebral cortex. In control mice morphine challenge clearly enhanced the αMT-induced NE depletion as well as increased the MHPG levels but a clear tolerance to these effects was found in morphine-withdrawn mice. Thus, in contrast to rats cortical NE turnover is not accelerated in mice spontaneously withdrawn from morphine. Furthermore, during withdrawal mice are tolerant to the NE turnover accelerating effect of morphine challenge.

61.5

SPINAL MET-ENKEPHALIN CONTENT INCREASES FOLLOWING PRECIPITATED WITHDRAWAL IN THE MORPHINE-DEPENDENT RAT. R. Cridland*, M. Sutak* and K. Jhamandas (SPON: J. Milligan), Department of Pharmacology and Toxicology, Queen's University, Kingston, Ontario, K7L 3N6.

The present study examined changes in spinal met-enkephalin (ME) levels following a naloxone precipitated withdrawal in morphine tolerant/dependent rats. Morphine (5 µg/µl/hr) or saline was given by continuous intrathecal infusion for 6 days via osmotic minipumps (ALZET). Development of tolerance was assessed by monitoring daily reaction time in the tail flick and paw pressure tests. Acute intrathecal injection of naloxone (5 µg) on day 6 precipitated classical withdrawal symptoms in morphine tolerant rats. On day 7, cords were removed, rapidly frozen and analyzed for ME content by radioimmunoassay. In saline-treated rats, ME levels were 2.6 ± 0.4 and 1.4 ± 0.2 ng/mg protein (mean ± S.E.M.; n=4) in the sacral and lumbar regions, respectively. In morphine-treated rats (n=5), ME levels increased to 3.4 ± 0.5 and 3.5 ± 0.9 ng/mg, respectively. These results suggest that spinal morphine withdrawal is associated with augmented levels of met-enkephalin.

(Supported by the Medical Research Council of Canada)

61.6

SUFENTANYL AND MORPHINE DEPENDENCE: BEHAVIOR AND FUNCTIONAL ANATOMY DURING WITHDRAWAL. **R.E. Adams and G.F. Wooten, Department of Neurology, Univ. of Virginia, Charlottesville, VA. 22908**

Regional cerebral glucose utilization (RCGU) and behavior during precipitated withdrawal were studied in rats made dependent on morphine, or the selective mu agonist, sufentanyl. Sufentanyl citrate was infused into the right jugular vein in graduated doses over 7 days (2 µg/.5 µL/hr - 16 µg/.5 µL/hr) using Alzet model 2002 osmotic minipumps. Subcutaneous morphine base pellets were implanted for a total of 5 days (1 pellet/45 g for 3 days followed by 1 pellet/70 g for 2 days). After completion of the dependence protocols rats were given an IV bolus of naloxone, 5 mg/kg followed by ^{14}C-2-deoxy-D-glucose, 10 µCi/100 g. Withdrawal behaviors were observed for the next 50 min after which time rats were killed and brains prepared for autoradiography. RCGU in both withdrawing groups was increased compared to controls in the following structures: medial and lateral preoptic areas, lateral septum, central nucleus of the amygdala, periventricular nucleus, lateral hypothalamus, lateral habenula, and interpeduncular nucleus. Withdrawal behaviors, including autonomic signs of withdrawal, withdrawal jumping, and weight loss were comparable between the two groups.

The similarity of the RCGU pattern and behaviors during withdrawal in rats dependent on sufentanyl, a selective mu agonist and morphine, an opiate agonist of arguable selectivity, indicates that dependence and withdrawal are phenomena mediated by the mu opiate receptors.

61.7

OPIOID MODULATING OCTAPEPTIDE PRECIPITATES MORPHINE ABSTINENCE SYNDROME IN RAT. D.H. Malin, D.E. Fowler*, J.R. Lake*, J. Sims*, S. Brown*, M.V. Hammond* and J. Miller*. Univ. of Houston - Clear Lake, Houston, TX 77058.

Yang et al. (PNAS 82:7757, 1985) have isolated an endogenous octapeptide ("opioid-modulating" or "morphine modulating" or "FMRF-like peptide") with strong anti-opiate actions. At these meetings last year, our laboratory reported that infusion of 15 µg of this octapeptide i.c.v. induced a quasi-abstinence syndrome in opiate-naive rats. In the present study, i.c.v. infusion of 2 µg octapeptide induced a vigorous abstinence syndrome in morphine-dependent but not in non-dependent rats.

Twelve rats were infused with morphine via Alzet osmotic minipump (0.3 mg/kg/hr s.c. for 7 days), while 12 rats were infused with saline vehicle alone. Half of each group was then injected in the 3rd ventricle with octapeptide (2 µg in 20 µl saline with 0.003 ug/µl bestatin), while the other half was injected with saline and bestatin alone. Each rat was observed under blind conditions for standard abstinence signs over 20 mins. with the following results:

	OVERALL ABSTINENCE SIGNS M ± SEM	
	OCTAPEPTIDE ICV	VEHICLE ICV
MORPHINE INFUSED	49.3 ± 7.0 **	5.3 ± 1.9
SALINE INFUSED	7.5 ± 2.4	3.8 ± 0.9

** $p<.01$ vs all other groups (Dunnett's Test)

ANOVA revealed significant, $p<.01$, morphine effect, octapeptide effect and interaction effect.

61.8

RIA OF FMRF-NH_2-LIKE OCTAPEPTIDE: INCREASED IMMUNOREACTIVITY IN CSF OF MORPHINE-DEPENDENT RATS. J.R. Lake*, D.E. Fowler*, T. Dougherty*, J. Leyva*, J.B. Sloan*, D.H. Malin and H-Y. T. Yang (SPON: R.E. Marc). Univ. of Houston - Clear Lake, Houston, TX 77058 and Lab. of Biochemical Genetics, NIMH, Washington, D.C. 20032.

The FMRF-like octapeptide (F-8-F-NH_2) discovered by Yang et al. (PNAS 82:7757, 1985) appears to have some anti-opiate properties such as precipitating opiate abstinence syndrome (Malin et al., this vol.). Therefore, it was of interest to determine whether CSF levels of F-8-F-NH_2 are increased in morphine-dependent rats. Thirteen rats were rendered morphine-dependent by 7 days continuous s.c. infusion of 1.5 mg/kg/hr morphine sulfate via 2ML1 Alzet osmotic minipump. Thirteen rats were infused with saline alone. Bestatin(.06µg) was injected into the cisterna magna of each rat and approximately 180µl CSF was withdrawn. HCL was added to a final concentration of 0.1N and the CSF was heated (100°C,10 min.). Following centrifugation (20,800 g, 20 min.), 50µl aliquots of supernatant were taken for F-8-F-NH_2 analysis as previously described (Majane et al., Peptides 9:1137, 1988)except that a 1ml reaction mixture was used. CSF from morphine dependent rats had F-8-F-NH_2 LIR of 0.082 ± 0.029 pmol/ml (M±SEM), while saline-infused rats had 0.025 ± 0.016 pmol/ml, a significant difference, $p<.05$. In another experiment, acute exposure to morphine failed to affect F-8-F-NH_2 LIR. F-8-F-NH_2 LIR appears to reflect actual F-8-F-NH_2, since it co-chromatographs with F-8-F-NH_2 in reverse-phase HPLC of pooled CSF.

61.9

OPIOID MEDIATION OF COCAINE-INDUCED HYPERACTIVITY AND REINFORCEMENT. A.A. Houdi, M.T. Bardo and G.R. Van Loon. Departments of Medicine and Psychology, University of Kentucky and VAMC, Lexington, KY 40536.

The mechanisms by which cocaine produces hyperactivity and reinforcement remain poorly understood. Since reinforcement is also a property of other drugs of abuse including opiates, we examined the possible mediation of these cocaine-induced behaviors by endogenous opioid peptides. Adult Male Sprague-Dawley rats (275-375gm) were pretreated with either naloxone HCl(0.5mg/kg s.c.) or saline, followed immediately by an injection of cocaine (20 mg/kg i.p.) or saline, and locomotor activity was then assessed. For the conditioned place preference study, rats were pretreated with naloxone HCl or saline and conditioned with cocaine (5 mg/kg i.p.) paired with white compartment or saline paired with black compartment. Our results confirmed previous reports that cocaine increases locomotor activity and conditioned place preference in rats. We have also demonstrated that opioid receptor blockade, using a mu-selective dose of naloxone, antagonizes completely the locomotor-activating effect of cocaine and attenuates the strength of the place preference conditioning produced by cocaine. These data support the thesis that endogenous opioids are involved in mediation of cocaine-induced behavior.

61.10

BILATERAL LESIONS OF THE MEDULLA OBLONGATA ALTER MU-OPIOID MODULATION OF AUTONOMIC OUTFLOW. M. Palkovits*, L. Marson, F. Bobbitt*, A. Houdi and G.R. Van Loon. (SPON: D.Frazier). Dept. Medicine, U. Kentucky and VAMC, Lexington, KY 40536 and Dept. Anatomy, Semmelweis Univ., Budapest.

The role of medulla oblongata and more rostral areas of brain in mu-opioid receptor modulation of autonomic outflow was examined by studying the effects of icv DAGO on plasma catecholamines (CA), blood pressure (BP) and heart rate (HR) in conscious rats with bilateral medullary lesions. Knife cuts were made at the rostral extent of the medulla (RM), the caudal extent of the motor facial nucleus (MM), or at the caudal extent of the medulla (CM), 0.7-2.5mm lateral to the midline. Three days later, rats received DAGO, 5nmoles. Cuts at the MM and CM levels blocked the DAGO-induced increases in BP and plasma CA, but did not affect the DAGO-induced bradycardia. Cuts at the RM level blunted the sustained aspect of the DAGO-induced increase in BP and produced a delayed potentiated fall in HR, without significantly altering the plasma CA responses to DAGO. The data are consistent with mu-opioid activation of rostral medullary bulbospinal pathways to augment sympathetic outflow and of vagal nuclei to activate parasympathetic outflow. Higher centers appear to play a modulatory role.

61.11

NICOTINE-INDUCED RELEASE OF ENDOGENOUS OPIOIDS IN BRAIN. K. Davenport*, A. Houdi and G.R. Van Loon (SPON: J.Dougherty). Dept. of Medicine, Univ. of Kentucky and VA Med. Center, Lexington, KY 40536.

In this study, we tested the hypothesis that nicotine-induced release of endogenous opioids in brain mediates some of the effects of nicotine. Specifically, we hypothesized that nicotine-induced release of endogenous opioids would protect against the antagonistic effects of subsequently administered β-funaltrexamine (BFNA) on morphine-induced antinociception. Morphine antinociception was studied in adult male rats using a tail-flick method in which trials of repeated exposure to radiant heat were carried out every 3 min after sc injection of morphine. Animals received icv injections of either BFNA or saline vehicle 24 hrs before testing for morphine antinociception. Animals also received either nicotine 0.1 mg/kg sc or saline vehicle -40 and -10 min before icv injections. BFNA antagonized greatly the antinociceptive effect of morphine. Nicotine pretreatment partially protected against this antagonistic effect of BFNA. These data suggest that nicotine enhanced the release of endogenous opioids which protected opioid receptors from subsequent alkylation by BFNA. Thus, some effects of nicotine are mediated by neuronal release of endogenous opioids.

61.12

NICOTINE-INDUCED INCREASES IN ENKEPHALIN RELEASE IN DISCRETE BRAIN NUCLEI. G.R. Van Loon, A.A. Houdi, L. Marson and M. Palkovits*, Department of Medicine, University of Kentucky and VA Medical Center, Lexington, KY 40536 and Department of Anatomy, Semmelweis University, Budapest.

Some effects of nicotine may be mediated by endogenous opioids, and more specifically by proenkephalin A-related peptides. Thus, it would be of interest to assess nicotine-induced changes in activity of brain enkephalin neurons by demonstrating changes in brain enkephalin levels. However, acute administration of nicotine may alter enkephalin release without affecting brain enkephalin levels. Tyr-Gly-Gly (YGG) concentrations in brain provide an index of enkephalin release *in vivo*. Thus, we examined the thesis that nicotine alters brain neuronal enkephalin release by measuring YGG levels in specific brain nuclei punched 30 minutes after acute administration of nicotine 0.3 mg/kg s.c. in adult male rats. Acute nicotine increased YGG in several brain nuclei including nucleus accumbens, dorsal raphe, pontine reticular formation, locus ceruleus, sensory trigeminal nucleus and caudal ventrolateral medulla. This single exposure to nicotine did not alter YGG in caudate nucleus, hypothalamic paraventricular nucleus, median eminence, central amygdala, rostral ventrolateral medulla and dorsal horn of spinal cord. These brain areas in which we found nicotine-induced increases in YGG are known to be involved in mediation of analgesia, autonomic function and reinforcement. Thus, effects of nicotine on these parameters may be mediated by nicotine-induced release of enkephalins at these brain sites.

61.13

EVIDENCE FOR AGONIST AND ANTAGONIST ACTIVITY OF (+)-NICOTINE AT BRAIN SITES REGULATING AUTONOMIC OUTFLOW. L. Dong*, A. Houdi and G.R. Van Loon (SPON: R.Miller). Depts. Medicine and Pharmacol., Univ. of Kentucky and VAMC, Lexington, KY 40536.

We hypothesized that (+)-nicotine might act as a nicotinic receptor antagonist. We examined the responses of plasma catecholamines (CA), blood pressure (BP) and heart rate (HR) to icv (-)-nicotine after initial icv exposure of equimolar (-)-nicotine or (+)-nicotine or saline vehicle. (+)-Nicotine was less potent than (-)-nicotine in decreasing HR and increasing BP, but equipotent in increasing plasma CA. On the other hand, (+)-nicotine was more effective than (-)-nicotine in inhibiting HR and BP responses to subsequent (-)-nicotine, but less effective in inhibiting plasma CA responses. The data are consistent with the thesis that (+)-nicotine acts as partial agonist at nicotinic receptors on neurons regulating parasympathetic outflow and on neurons regulating outflow to sympathetic nerves, but as a full agonist on neurons regulating outflow to adrenal medulla. Using the site classification of Martin et al., the data are also consistent with the thesis that Sites 2 or 4 are the agonist site and Site 3 the antagonist site, and the brain areas regulating different aspects of autonomic outflow have differential populations of these sites.

61.14

EFFECT OF CHRONIC ETHANOL TREATMENT ON THE BIOSYNTHESIS AND POST-TRANSLATIONAL PROCESSING OF PRO-OPIOMELANOCORTIN BY THE RAT HYPOTHALAMUS. C. Gianoulakis, Douglas Hospital Research Centre and Department of Psychiatry, McGill University, Montreal, Quebec.

Previous studies have shown that chronic ethanol treatment (using a liquid diet containing 6.5% ethanol V/V), increases the biosynthesis of β-endorphin like peptides (β-EPLPs) by the anterior and neurointermediate lobe of the pituitary gland. To study the effect of chronic ethanol treatment on the biosynthesis of hypothalamic β-EPLPs animals were fed with a liquid diet for 15 days. As controls were used rats pair-fed to the ethanol group with an isocaloric sucrose diet or rats fed ad libitum with regular laboratory chow diet (basic control group). Immediately following sacrifice of the animals the hypothalamus was dissected and incubated in the presence of radioactive amino acid (^{3}H-phenylalanine or ^{35}S-methionine). The biosynthesized pro-opiomelanocortin, β-lipotropin and β-endorphin were purified by immunoprecipitation with an antiserum to β-endorphin and analyzed by polyacrylamide disc gel electrophoresis with sodium dodecyl sulfate. Results indicated that ethanol induces an increase in the rate of incorporation of radioactive amino acids into pro-opiomelanocortin β-lipotropin and β-endorphin. Thus chronic ethanol treatment alters the activity of the hypothalamic β-endorphin system. Supported by the MRCC grant MA-6923

61.15

DIFFERENTIAL RESPONSES IN PITUITARY AND HYPOTHALAMIC DYNORPHIN GENE EXPRESSION AFTER ESTROGEN AND ANTIESTROGEN IN THE RAT. S.Spampinato, T.Bachetti*, M.Canossa* and S.Ferri*. Inst.Pharmacol., Univ. of Bologna, 40126 Bologna, Italy.

In this study we have evaluated the pituitary and hypothalamic content of ir-dyn A and B (by RIA) and prodyn mRNA (by an extremely sensitive solution hybridization assay using a 400 bp RNA probe) in the adult female rat, with the aim to ascertain if estradiol benzoate (EB) and the antiestrogen tamoxifen (TAM) influence gene expression of these peptides or act at posttranslational level only. EB (administered for 14 days by sc implants) significantly reduced ir-dyn A and B as well as prodyn mRNA in the anterior pituitary (0.85±0.15 vs 1.47±0.20 pg/ug total RNA, $p<0.01$); TAM (50 ug/rat/day, sc), concomitantly administered, prevented these effects and injected alone for 14 days induced a marked increase of ir-dyn A and B and mRNA (2.94±0.46 vs 1.47±0.20 pg/ug total RNA, $p<0.01$). As regards the neurointermediate lobe, ir-dyn A and B were not changed in rats exposed to EB whereas TAM greatly reduced both peptides. However, no significant changes in mRNA were observed in the supraotic and paraventricular nuclei of the hypothalamus from which posterior pituitary dyn originates.

61.16

IDENTIFICATION OF OPIOID PEPTIDES ASSOCIATED WITH CELL PROLIFERATION IN THE DEVELOPING BRAIN. K.L. Cook*, P.J. McLaughlin and I.S. Zagon (SPON: T. Pritchard). Dept. Anatomy, Penn. State Univ. Coll. Med., Hershey, PA 17033.

Endogenous opioid systems are known to modulate the growth of developing brain; cell proliferative events are a target of opioid action. To ascertain which opioid peptide(s) is(are) related to growth, a series of studies using the cerebellar cortex (lobule VIII) of 6-day old rats subjected to opioid peptides and monitored with ^{3}H-thymidine and autoradiography were conducted. Previous findings indicated that [Met^{5}]-enkephalin (ME) is a potent opioid involved with growth. In the first experiments, the effects of ME were measured 1-24 hr following injection. Rats receiving ME (100 μg/kg) had significant reductions in labeling index (LI) from 2-24 hr (mean decrease = 15%) relative to controls. Dosages of ME ranging from 0.1 to 1000 μg/kg were tested and the LI determined 4 hr after drug exposure. ME dosages of 0.1-50 μg/kg did not alter the LI, but 100 to 1000 μg/kg markedly reduced the LI (mean decrease = 17%) relative to controls. Using these parameters to establish an appropriate paradigm, testing of a wide variety of opioid compounds revealed that ME was the most potent inhibitor of cell replication in developing cerebellum.

Supported by NIH Grant NS-20500.

61.17

ENHANCED GROWTH OF CELLS IN CULTURE BY NALTREXONE, AN OPIOID ANTAGONIST. P.J. McLaughlin and J.S. Zagon. Dept. Anatomy, Penn. State Univ. College of Medicine, Hershey, PA 17033.

Endogenous opioids and opioid receptors regulate the growth of murine neuroblastoma (-NB), as well as other neural and non-neural cells of human and animal origin; opioid peptides exert an inhibitory influence on cell replication. Paradigms using opioid antagonists to perturb interaction of opioids and receptors *in vivo* have demonstrated that complete blockade of this equilibrium enhances tumorigenic events. To further explore the role of endogenous opioid systems *in vitro*, S20Y NB cells were seeded and, 24 hr later, various concentrations (10^{-5} to 10^{-13}M) of naltrexone (-NTX) or sterile water (-CO) were added to the media; media and drugs were changed daily. Cells were counted by trypan blue exclusion 48 hr later. Cultures exposed to 10^{-6} to 10^{-8}M NTX had 38-43% more cells than CO levels. The mitotic and labeling indexes of NB cells grown in 10^{-6}M NTX for 48 hr was increased 13% and 60%, respectively, over CO values. NTX (10^{-6}M) also increased the growth of mouse N115 NB, human SK-N-MC NB, and human fibrosarcoma HT-1080 by 33-53% from CO values. These results indicate that human and animal cells in culture are tonically regulated by endogenous opioid systems.

Supported by NIH Grants NS-20623 and NS-20500.

61.18

CHARACTERIZATION OF LUNG ORNITHINE DECARBOXYLASE (ODC) RESPONSIVITY TO INSULIN AND ITS MODULATION BY CNS BETA-ENDORPHIN (BE) IN RAT PUPS N.L. Greer, S.M. Schanberg*, and J.V. Bartolome. Dept. of Pharmacology, Duke Univ. Med.Ctr., Durham, N.C. 27710

Opposite regulatory roles for insulin and opioids, particularly BE, on lung development have been reported. The importance of ODC (a key developmental enzyme) in lung maturation has also been documented. This study examined whether the opposite effects of insulin and BE on lung development could be explained by opposite actions on lung ODC activity. Both 2- and 6-day-old rat lungs were analyzed representing two distinct stages in lung maturation.

Central (but not peripheral) BE markedly reduced ODC activity while insulin increased activity in both ages. BE pretreatment altered ODC responsivity to insulin in an age-dependent manner. Changes in Vmax rather than Km accounted for these changes in ODC activity. Dialyzing or mixing supernatants from treatment groups indicated a minor role for an endogenous activator of ODC activity. Actinomycin pretreatment completely prevented the increased ODC activity evoked by insulin. While de novo ODC synthesis by trophic agents appears to be mediated through cAMP in some tissues, neither diBut-cAMP, 8-BR-cAMP, PGE-1, nor cGMP increased basal lung ODC activity. These findings indicate that changes in lung ODC activity induced by insulin or BE might explain, at least in part, the opposite effects of these endogenous compounds on lung development. (Sup. by NIH Grants RO1-NS25738 and RO1-MH13688)

61.19

NICOTINE ALTERS OPIOID PEPTIDE LEVELS IN STRIATUM AND HYPOTHALAMUS OF MOUSE BRAIN K.P. Gudehithlu*, J.P. Hubble*, M. Hadjiconstantinou and G.A. Tejwani. Dept. of Pharmacology, Col. of Med., Ohio State Univ. Columbus, OH 43210

In vitro studies have shown that nicotine(N) stimulates dopamine(DA) release from striatal(STR) slices. DA has been shown to alter the level of opioid peptides(OP). The purpose of this study was to investigate whether cholinergic and/or DA receptors are involved in the effect of N on OP. Nicotine(1mg/kg, s.c.,n=27) was injected with or without mecamylamine(M)(1mg/kg, i.p.,n=7) or haloperidol(H)(1mg/kg, i.p.,n=8) and animals were sacrificed after 60 min. Nicotine elevated striatal DA(30%) and DOPAC(58%) content and M prevented this effect. Nicotine decreased β-endorphin(β-E) level in hypothalamus(HYP,31%) and STR(44%). Met-enkephalin (ME) level increased in HYP(81%) while decreased in STR(58%). Mecamylamine prevented the effect of N on β-E only in STR while, H prevented the effect in HYP and STR. Mecamylamine and H both prevented the effect of N on ME in HYP and STR. The changes observed in opioid system with nicotine treatment may be due to the binding of nicotine to cholinergic receptors and its effect on dopamine content. (Supported in part by State of Ohio, Dept. Aging)

OPIATES, ENDORPHINS AND ENKEPHALINS: PHYSIOLOGICAL EFFECTS I

62.1

OPIOIDS INHIBIT GABA-MEDIATED SYNAPTIC POTENTIALS IN NUCLEUS RAPHE MAGNUS IN VITRO. Z.Z. Pan* and J.T. Williams. Vollum Institute, OHSU, Portland, OR 97201

Neurons in raphe magnus were recorded intracellularly in slice preparations. Focal electrical stimulation was used to evoke synaptic potentials (SP) which consisted of two components, excitatory amino acid (EAA) and $GABA_A$. Combination of excitatory amino acid and $GABA_A$ antagonists completely blocked the SP. The action of opioids was studied on the membrane properties and synaptic potentials. Opioids had no effect on the membrane properties of most raphe magnus neurons, but both [Met^5]-enkephalin (ME) and DAGO depressed the amplitude of the GABA mediated synaptic potential. This action was dependent on the concentration applied and the maximum depression was to 47% of control. The EC_{50} for DAGO was 74 nM in control and 5 μM in the presence of naloxone (100 nM) giving an estimated naloxone K_d of 1.5 nM. ME (30 μM) had no effect on the EAA-SP. The depolarization induced by GABA applied exogenously was not changed by ME or DAGO suggesting that the action of opioids on the GABA-SP was presynaptic. The GABA-SP was not changed by the δ-opioid agonist, D-Pen,D-Pen-enkephalin (300 nM). These results suggest that opioids increase the activity of raphe magnus neurons by inhibition of GABA-mediated inhibitory input through μ-opioid receptors. This action may be one mechanism by which opioids modulate descending inhibition from raphe magnus in the endogenous pain-modulating system. Supported by USDHHS DA 04523.

62.2

NEURONAL ACTIVITY OF DORSAL PERIAQUEDUCTAL GRAY NEURONS OF FEMALE RATS: RESPONSIVENESS TO GABA AND ENKEPHALIN. Sonoko Ogawa, L.-M. Kow and D.W. Pfaff. The Rockefeller University, New York, NY 10021.

To test responsiveness to GABA as well as enkephalin, we recorded extracellular single-unit activity of dorsal periaqueductal gray (dPAG) neurons in tissue slices prepared from either estrogen-treated or nontreated ovariectomized female rats. GABA inhibited 86% (109/126) of dPAG neurons; and both GABA-A (THIP) and GABA-B (baclofen) agonists were effective. Some inhibited neurons were sensitized to repeated GABA (28%), THIP or baclofen applications. Bicuculline methiodide (GABA-A antagonist) excited 60% (55/92) of dPAG neurons suggesting that many dPAG neurons are tonically inhibited by intrinsic GABA through GABA-A receptors. 21 out of 55 excited neurons fired in bursts after bicuculline application. Inhibition was the predominant response to met-enkephalin. Out of 98 tested dPAG neurons, 37 showed inhibition, 8 showed excitation and 4 showed biphasic response. Most dPAG neurons fired regularly (78%) and the mean resting firing rate was 5.8±0.5 spikes/sec. Electrophysiological properties, either resting activity or responsiveness to GABA and enkephalin, were not influenced by in vivo estrogen treatment. A small percentage of units were excited by enkephalin and inhibited by GABA or excited by bicuculline as might be related to lordosis (Mobbs et al., 1988).

62.3

DELTA-SELECTIVE OPIOID AGONISTS PRODUCE SUSTAINED INCREASES IN MOSSY FIBER-CA3 RESPONSES: OPIOID PEPTIDE-INDUCED LONG-LASTING POTENTIATION ?

B.E. Derrick & J.L. Martinez Jr., Dept. of Psychology, University of California, Berkeley 94720

Previously, we reported that naloxone blocks induction, but not maintenance, of mossy fiber LTP (Soc. Neurosci. Abstr., 13:767, 1988). Since NMDA produces only a short-term potentiation at other hippocampal synapses, we investigated whether selective opioid agonists produce similar transient effects.

The delta-selective agonist D-Pen^2-[L-Pen^5]-enkephalin (DPDPE, 10 nmols) initially produced increases in CA3 responses evoked by both mossy fiber (MF) and commissural (COMM) afferents when measured < 30 min following application. After 1.5 hr, COMM-evoked responses had return to baseline, but MF-evoked increases were greater than the increase measured < 30 min. DPDPE in quantities (1 nmol) that did not produce initial excitatory effects in either MF- or COMM-evoked responses still produced increases in only MF-evoked responses when measured at 1.5 hr. These effects were not observed with mu- or kappa agonists at equivalent concentrations. Thus, a delta agonist appears to produce an LTP-like potentiation of MF responses similar to that observed following tetanization.

Supported by NIDA #DA 04195 and the Rennie Fund.

62.4

OPIOID MODULATION OF THE IN VIVO RELEASE OF CCK-8 FROM THE DORSAL HORN OF THE RAT .

R.E.Rodriguez and M.P.Sacristán. Dept of Biochemistry Univ. of Salamanca 37007 Salamanca, Spain. (SPON: M. Lafarga)

CCK-8 immunoreactivity is observed in the superficial layers of the dorsal horn of the rat. Most of the CCK-8 containing fibers as seen by immunohistochemistry, represent primary sensory neurons, suggesting that this peptide may be involved in pain control and transmission.

We show that CCK-8 like immunoreactivity, as determined by RIA, is released from the spinal cord of the rat in vivo (1) following potassium stimulation (2) by direct activation of high threshold peripheral afferents. The addition of potassium to the media resulted in a 160% increase in the levels of CCK-8 above resting levels. Peripheral stimulation resulted in a 283% increase. This release was completely abo abolished by analgesic doses of the mu selective agoniat Dagol, and partially affected by the delta selective agonist DPDPE in a naloxone (0.1mg/Kg and 1mg/Kg respectively) reversible fashion. Perfusion with the Kappa agonist U69593 did not affect the in vivo release of CCK-8. These results suggest a mu/delta opioid modulation of the in vivo release of CCK-8

62.5

CHOLERA TOXIN (CTX) BLOCKS OPIOID-INDUCED PROLONGATION OF THE ACTION POTENTIAL DURATION (APD) OF MOUSE DORSAL ROOT GANGLION (DRG) NEURONS IN CULTURE WHEREAS PERTUSSIS TOXIN (PTX) BLOCKS OPIOID-INDUCED SHORTENING.

K.-F. Shen* and S.M. Crain (SPON: D.P. Purpura). Dept. of Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461.

APD shortening elicited by 1-10μM DADLE is mediated by opioid receptors coupled to PTX-sensitive inhibitory Gi/Go proteins (Shen & Crain, Br.Res.'89), whereas APD prolongation by 1-10nM DADLE is mediated by excitatory opioid receptor subtypes whose effects are blocked by injection of an inhibitor of cyclic AMP-dependent protein kinase (Chen et al, Br.Res.'88). The A fraction of CTX, which ADP-ribosylates Gs and attenuates ligand-activation of associated receptors, was used to determine if excitatory opioid receptors on DRG neurons are coupled via Gs to the adenylate cyclase (AC)/cAMP system. After treatment of DRG-cord explants with CTX-A (0.1-1ng/ml; >15min) or with whole toxin (at even lower concentrations), APD prolongation elicited by 1-10nM μ, δ, or k opioids was blocked (28/31 DRG cells tested), whereas APD shortening by opioids was unaffected. Higher levels of CTX (0.1-1μg/ml) also blocked opioid excitatory effects when tested after initial APD prolongation elicited by CTX alone. Our results indicate that excitatory opioid receptors on DRG neurons are coupled via Gs to the AC/cAMP system (resembling β-NA receptors) in contrast to inhibitory opioid receptor linkage to Gi/Go (resembling α_2-NA receptors). (Supported by NIDA grants DA-02031 & DA-05203 to SMC.)

62.6

DYNORPHIN PROLONGS THE ACTION POTENTIAL DURATION (APD) OF MOUSE DORSAL ROOT GANGLION (DRG) NEURONS BY DECREASING A K^+ CONDUCTANCE WHEREAS THE SPECIFIC KAPPA OPIOID, U-50,488H DOES SO BY INCREASING A Ca^{2+} CONDUCTANCE.

S.M. Crain and K.-F. Shen. Dept. of Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461.

High (μM) concentrations of kappa opioids shorten the APD of DRG neurons by decreasing a voltage-sensitive Ca^{2+} conductance (Werz & Macdonald, JPET'85). In addition to these inhibitory effects, low (nM) levels of dynorphin and U-50,488H prolonged the APD in >80% of DRG neurons in DRG-spinal cord explants (tested in 5mM Ba^{2+}/BSS). However, when opioid responsivity tests were carried out in the presence of multiple K^+ channel blockers (Ba^{2+},Cs^+,TEA), 1nM dynorphin prolonged the APD in only 10% of the neurons (n=21), whereas 1nM U-50,488H still elicited APD prolongation in 60% of the cells (n=37). During K^+ channel blockade, 1nM dynorphin shortened the APD in 33% of the DRG cells (vs 7% in control media), presumably by unmasking inhibitory kappa receptors that attenuate Ca^{2+} channel activity. These data suggest that dynorphin prolongs the APD of DRG neurons by activating a kappa receptor subtype that decreases a voltage-sensitive K^+ conductance(see Fan, Shen & Crain, this vol.), whereas U-50,488H prolongs the APD by activating another kappa receptor subtype that increases a voltage-sensitive Ca^{2+} conductance. Thus U-50,488H-induced APD prolongation appears to be mediated by an excitatory subtype of kappa opioid receptors that produces the opposite effect on Ca^{2+} channels as occurs during APD shortening mediated by inhibitory kappa receptors. (Supported by NIDA grants DA-02031 & DA-05203 to SMC.)

62.7

DYNORPHIN-INDUCED SUPPRESSION OF THE ACTIVITY OF A SPECIFIC SUBTYPE OF VOLTAGE-SENSITIVE K^+ CHANNELS IN MOUSE DORSAL-ROOT GANGLION (DRG) NEURONS MAY UNDERLIE PROLONGATION OF THE ACTION POTENTIAL DURATION(APD).

S.F.Fan[1], K.-F.Shen[2] and S.M.Crain[2] (SPON:S.Yazulla). Dept.of Anat. Sci.[1],HSC,SUNY at Stony Brook,NY 11794 and Dept.of Neuroscience[2],Albert Einstein Coll. of Med.,Bronx,NY 10461.

Tight-seal whole-cell voltage-clamp recordings were made on DRG neurons in dissociated cell cultures. Outward K^+ current during depolarizing pulses was markedly decreased by 1nM dynorphin (in 5mM Ba^{2+}/BSS). These data confirm our current-clamp results suggesting that APD prolongation elicited by 1nM dynorphin in >80% of DRG neurons in DRG-cord explants is mediated by a kappa receptor subtype that decreases a voltage-sensitive K^+ conductance (Crain & Shen, this vol.). Cell-attached patch-clamp recordings from dissociated DRG neurons revealed two subtypes of voltage-sensitive K^+ channels with different conductances and activities. Bath application of 1nM dynorphin decreased the probability of opening of K^+ channels with smaller(~40pS) conductance, whereas it increased opening of K^+ channels with larger (~100pS) conductance. Attenuation of ~40pS K^+ channel activity was so prominent, that it might account for the observed opioid suppression of the whole-cell K^+ conductance and resulting APD prolongation. In contrast, the enhanced openings of ~100pS K^+ channels may account, in part , for the 1nM dynorphin-induced APD shortening in some DRG cells. Since dynorphin added to the bath solution altered the activities of both types of K^+ channels in the patch sealed off by the pipette tip, our results provide direct evidence that some modes of excitatory and inhibitory modulation of the APD of DRG neurons are mediated by diffusible second messengers (regulated by G proteins: Shen & Crain, this vol.).(Support: NIDA grants DA-02031 & DA-05203 to SMC and HL-31299 to P.R. Brink, SUNY.)

62.8

OPIOID REGULATION OF ADENYLATE CYCLASE AND PRO-ENKEPHALIN mRNA IN NEONATAL RAT PRIMARY NEURONAL CULTURES.

D.R. Marckel and S.R. Childers. Dept. of Pharmacology, Univ. of Florida Coll. Med., Gainesville, FL 32610.

Opioids regulate adenylate cyclase activity in mammalian brain and several cell lines. Proenkephalin mRNA levels are in part regulated by cAMP levels through protein kinase A. Neonatal rat primary neuronal cultures were chosen to study the relationship between opioid regulation of adenylate cyclase and proenkephalin mRNA levels, both acutely and chronically.

Primary neuronal cultures were made from 1 day old rats in which the forebrain was removed, cleaned of pia, triturated, treated with trypsin and DNAse I, and plated. After 2 days, cultures were treated with cytosine arabinoside for 3 days to decrease the glial population and obtain cultures of 80-85% neurons. After 10 days of culture, cAMP levels were measured in cells incubated for 5 min at 37° with isobutylmethylxanthine (IBMX) and various agents. Both isoproterenol and forskolin stimulated cAMP levels in intact cells 5-10 fold. D-Ala enkephalinamide decreased stimulated cAMP levels by 20-30%. These effects were also observed when adenylate cyclase activity was measured in membrane preparations by an HPLC method.

Proenkephalin mRNA levels in primary neuronal cultures, measured by Northern blot analysis, were significantly increased by 18 hour treatment with isoproterenol or forskolin . This effect was diminished by including opioid compounds with the stimulatory agent. These results suggest that opioid agonists may regulate the level of endogenous enkephalin, possibly through the inhibition of adenylate cyclase

Supported by PHS grant DA-04534 from the National Institute on Drug Abuse.

62.9

CHOLINERGIC TONE IS REQUIRED FOR OPIOID ENHANCEMENT BUT NOT OPIOID INHIBITION OF STIMULATED ENKEPHALIN RELEASE. H.Xu*and A.R. Gintzler, Department of Biochemistry, SUNY Health Science Center at Brooklyn, Brooklyn, NY 11203.

This laboratory has previously demonstrated that opioids can enhance and inhibit the evoked release of methionine enkephalin from the myenteric plexus. Low doses (nanomolar) enhance release whereas higher concentrations (10-100 nM) inhibit release. Following pretreatment with forskolin, the opioid inhibition of stimulated met-enkephalin release is no longer observed. Instead, a previously inhibitory concentration of opioid now produces an enhancement of release. Excitatory responses to low concentrations of opioids are not affected by an identical pretreatment with forskolin. The muscarinic receptor antagonist atropine selectively blocks opioid-mediated enhancement of evoked enkephalin release but has no effect on the opioid inhibition of release. Furthermore, in the presence of atropine, forskolin still completely attenuates opioid inhibition but the opioid enhancement of release is no longer unmasked. These results indicate the divergence of the biochemical pathway that mediates opioid enhancement or inhibition of stimulated enkephalin release. It is suggested that the intracellular concentration of a muscarinic receptor coupled second messenger(s) might be crucial for the manifestation of opioid enhancement of stimulated met-enkephalin release.

62.10

ELECTRICALLY-INDUCED RELEASE OF PRO-ENKEPHALIN-DERIVED PEPTIDES FROM THE GUINEA-PIG MYENTERIC PLEXUS PREPARATION. A.D.Corbett*, M.G.C.Gillan* and H.W.Kosterlitz. U.R.A.D., University of Aberdeen, Aberdeen AB9 1AS, Scotland.

Two preparations were set up in organ baths and perifused with Krebs solution containing cycloheximide, tetraethylammonium and peptidase inhibitors. One preparation was a non-stimulated control while the other was stimulated at 2 Hz, 50mA for 15 min. The tissue contents of opioid peptides and the amounts released into the Krebs solution were determined by bioassay after HPLC fractionation. Stimulation caused 45% decrease in tissue contents of [Met^5]enkephalin and [Leu^5]enkephalin. The amounts of [Met^5]enkephalin and [Leu^5]enkephalin in the perifusing Krebs solution were 58 and 10 pmol/g, being 40-50% of this decrease in tissue contents. In contrast, the tissue contents of [Met^5]enkephalyl-Arg-Gly-Leu and [Met^5]enkephalyl-Arg-Phe were decreased by 20-30%, the amounts in the perifusing Krebs solution being only 25% of this decrease in tissue contents. Thus, pro-enkephalin-derived opioid peptides are released differentially by electrical stimulation of the guinea-pig myenteric plexus. (Supported by NIDA grant DA00662).

62.11

THE ENKEPHALINASE INHIBITOR SCH 32615 INCREASES DOPAMINE (DA) TURNOVER IN THE NUCLEUS ACCUMBENS (NACC) OF THE RAT BRAIN. G. Biggio, O. Giorgi, E. Ongini, M. Trampus*. Dept. of Exp. Biology, Univ. of Cagliari and Res. Labs., Schering-Plough, Milan, Italy (SPON: W. Fratta)

Previous studies have shown that the enkephalinase (ENKase) inhibitor acetorphan stimulates locomotion in rats and mice. This effect depends on the integrity of the mesolimbic DAergic system, since it is prevented by the infusion of 6OH-DA into the NACC (J.P.E.T., 243: 1062, 1987). Therefore, we considered of interest to investigate the effects of the novel ENKase inhibitor SCH 32615 (J.P.E.T., 245: 829, 1988) on the activity of dopaminergic neurons as reflected by the alterations in the content of the major DA metabolite dihydroxyphenyl acetic acid (DOPAC). SCH 32615 (10-30-100 mg/kg, s.c., 60 min before sacrifice) elicited a dose-dependent increase in the concentration of DOPAC in the NACC without affecting the DA content. The maximal increase in DOPAC content was 48% above the control value at the dose of 100 mg/kg. No changes in DA turnover were detected in the s. nigra, striatum and prefrontal cortex. The acceleration of DA metabolism in the NACC induced by SCH 32615 (50 mg/kg, s.c.) was maximal by 60 min and DOPAC content returned to basal values by 2 h after drug injection. Our results support the view that endogenous enkephalins may modulate the activity of mesolimbic DAergic neurons.
(Spon. : W. Fratta)

62.12

ALTERATIONS IN METABOLIC ACTIVITY OF RAT BRAIN STRUCTURES FOLLOWING INJECTIONS OF MORPHINE INTO THE SUBSTANTIA NIGRA H.D. Everist and A. Pert (SPON: D. Lozovsky). BPB, NIMH, Bethesda MD 20892 and OEP, ADAMHA, Rockville MD

Microinjections of opiate agonists into the substantia nigra produce profound behavioral effects. We have employed 2-DG metabolic mapping procedures to assess the functional activity in rat brain following bilateral intranigral injections of morphine (10 nmoles) or saline (1 µl). Immediately following intranigral injections, all rats were injected with 150 µCi/kg of ^{14}C-2DG (i.p.). Forty-five minutes later the animals were sacrificed, their brains removed and prepared for autoradiographic analysis. Intranigral injections of morphine had little effect on metabolic activity in the striatum or n. accumbens. Structures receiving nigrothalamic input, such as the mediodorsal, laterodorsal and parafascicular nuclei of the thalamus had decreased activity. Modest increases in metabolic activity were seen in the superior colliculus which also receives afferents from the substantia nigra. Increases in metabolic activity were also found in structures that receive nigrotegmental input such as the pedunculopontine tegmental nucleus, central gray matter, as well as other pontine reticular nuclei. It appears that the behavioral effects seen following nigral injections of morphine are determined through nigrotegmental or nigrotectal pathways and not through nigrostriatal activation.

62.13

OPIATES INHIBIT RELEASE OF IMMUNOREACTIVE BRADYKININ (iBK) FROM INFLAMED TISSUE, AS EVALUATED BY PERIPHERAL MICRODIALYSIS PROBES. Laureen Wells*, Anna Solodkin* and Kenneth Hargreaves*, (Spon: R. Traub), Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20892.

We have reported that systemic opiates suppress edema and plasma extravasation during carrageenan (carra)-induced inflammation (Pain Res. Clin. Mngmt., 3:55;1988, Elsevier). This study determined if systemic opiates inhibit iBK release. A 2 cm cellulose dialysis probe (Travenol 15-11) was inserted s.c. into the plantar hindpaws of anesthetized rats (n=8-17/group) who were then injected with 8 mg carra. iBK in the diasylate was measured by RIA. Rats were given either i.p. levorphanol (L; 1.5 mg/kg), dextrorphan (D; 1.5 mg/kg), or saline (S) every hour starting 1 hour before anesthesia. Data were analyzed by ANOVA followed by Duncan's test. In vitro studies indicated a 8.9% recovery of iBK at 8 µ l/min flow with the amount of iBK collected linearly related to its external concentration (r=0.96). During an initial study, iBK levels from carra-treated paws (260 ± 45 fm/ml) were significantly greater than levels in saline-injected paws (45.2 ± 9 fm/ml; $p<0.005$). In carra-injected rats treated with L, iBK levels (140± 32 fm/ml) were significantly lower than iBK in rats given either D (212± 67fm/ml; $p<0.05$) or S (239± 52fm/ml; $p<0.05$). L ($p<0.05$), but not D, significantly reduced carra-induced edema by approximately 20% as compared to saline-treated rats. The results indicate that the suppression of carra-induced inflammation by systemic opiates is accompanied by a reduction in the levels of peripheral iBK, one of the chemical mediators involved with plasma extravasation and edema.

62.14

THE ZINC CHELATOR, DITHIZONE, LOWERS HIPPOCAMPAL LEVELS OF OPIOID PEPTIDES. C.L. Mitchell, M.I. Barnes*, J.S. Hong and J.F. McGinty. LMIN, NIEHS/NIH, Research Triangle Park, NC 27709 and Dept. of Anatomy and Cell Biology, School of Medicine, East Carolina University, Greenville, NC 27858.

Zinc is present in particularly high concentrations in the giant boutons of dentate granule cell mossy fibers (Haug, Histochemie 8:355, 1967). One postulate for its function is an involvement in storage of vesicular products (Storm-Mathisen, Prog. Neurobiol. 8:119, 1977). We, therefore, examined the ability of dithizone (D) to alter the levels of hippocampal opioid peptides. D was administered subcutaneously in doses of 25 and 50 mg/kg. Both doses lowered the level of dynorphin A(1-8) (DYN). The 25 mg/kg dose lowered DYN by 13% and the 50 mg/kg dose lowered it by 21%. The higher dose lowered the level of methionine enkephalin (ME) by 31%. The effect on DYN was transitory, with DYN returning to control levels within one hour. The effect on ME was more sustained (>2.5 hrs). These doses of D reduced TIMM staining, thus indicating a reduction in mossy fiber zinc. The data are consistent with the notion that this zinc may play a role in the storage and/or release of these peptides in the mossy fibers.

62.15

REPEATED STRESS OF DEFEAT INCREASES LOCOMOTOR RESPONSE TO MORPHINE. M.L. Thompson* and C. A. Paronis* (SPON. L. Shuster), Dept of Pharmacology, Tufts Medical School, Boston, MA 02111.

It has previously been demonstrated that acute stress induces an activation of the mesocorticolimbic dopaminergic system (Herman et al 1982). Furthermore, Antelman & Chiodo (1983) demonstrated that repeated exposure of rats to stress increases the stereotypy response to amphetamine, similar in extent to that observed following repeated injections of amphetamine. Herman et al (1984) extended these results to amphetamine induced locomotor activity as well. Repeated exposure to amphetamine leads to "reverse tolerance" or enhanced locomotor activity, rather than a decrease. "Reverse tolerance" has also been demonstrated following repeated morphine exposure (Shuster, 1975). In this experiment, we report that mice injected with morphine following repeated exposure to a biologically relevant form of stress, social conflict, show "reverse tolerance" to morphine similar in extent to that seen in mice injected repeatedly with morphine. Male B6AF1 mice, 3-4 months old, were either injected with morphine, 5 mg/kg ip every other day for 10 days or subjected to a bout of social conflict (70 bites duration, about 3-4 min.) as intruders in a resident-intruder paradigm. 5 days after the last injection or bout of conflict, mice were housed individually overnight in a standard mouse cage. The next afternoon mice were injected with 5 mg/kg ip morphine and locomotor activity was assessed for 30 min. in an Opto- Varimex (Columbus Instruments) set to record ambulatory activity. The resulting scores were expressed as percent of a control group of mice receiving an injection of saline on test day. A fourth group of mice had no prior experience but was injected with morphine on the test day. Morphine significantly suppressed locomotor activity in this latter group, to a level of 50% of controls. Repeated morphine treated mice showed significant "reverse tolerance", showing activity levels 160% of controls. Mice which were exposed to repeated defeat also showed significantly increased ambulatory scores compared to controls or the group receiving a single injection of morphine. Activity levels in the defeated group were almost identical to those of the repeated morphine mice. Thus in addition to its marked effects on the endogenous opioid system, defeat also appears to mimic the effects of morphine on dopaminergic systems of the brain as well.

62.16

CARDIAC LEVELS OF DYNORPHIN A AND LEU-ENKEPHALIN DURING THE POSTNATAL DEVELOPMENT OF SPONTANEOUSLY HYPERTENSIVE RATS. M. Dumont, L. Sabourin* and S. Lemaire. Department of Pharmacology, University of Ottawa, Ottawa, K1H 8M5, CANADA.

The levels of immunoreactive dynorphin A (ir-Dyn A) and ir-Leu-enkephalin (ir-Leu-Enk) were measured in acid extracts of hearts of male Wistar (WR), Wistar Kyoto (WKY) and spontaneously hypertensive (SHR) rats at different ages (4-16 weeks). Heart contents in ir-Leu-Enk were much higher than those of ir-Dyn A in all strains studied. The hearts of 8 week-old WR, WKY and SHR rats contained comparable levels of ir-Leu-Enk (7.4, 6.4 and 4.8 pmol/g, respectively). On the other hand, the cardiac levels of ir-Dyn A were significantly higher in SHR rats (731 fmol/g) than in WR (84 fmol/g) and WKY (250 fmol/g) rats at 8 weeks of age. Ir-Dyn A and ir-Leu-Enk were also measured during the development of hypertension. In addition to the increased levels of ir-Dyn A in 8 week-old SHR rats, significant changes were also found in the levels of both opioid peptides at 16 weeks. At this age, where hypertension is well established, we observed a 2 fold decrease in the levels of ir-Dyn A and a concomitant 1.8 fold increase in the levels of ir-Leu-Enk in SHR compared to WKY rats. These results suggest that cardiac Dyn A and Leu-Enk may play a role in the development and/or maintenance of hypertension. Supported by the HSFO.

62.17

ROLE OF β- ENDORPHIN AND cAMP IN MANIA. A.J. Giannini, R.Q. Quinones, D.M. Martin. Ohio State University, P.O. Box 2169, Youngstown, Ohio 44505.

Forty male subjects had assays of serum β-endorphin and serum cAMP. Twenty subjects were actively manic while 20 were age matched, race-matched controls. Assays were performed by double antibody radioimmunoassay.

Ratios of cAMP (cyclic adenosine monophosphate) β-endorphin were compared. Manics showed a significant lower ($p > .02$) ratio than did the controls. There were no significant differences in manic/control cAMP levels or manic/control β-endorphin levels. The characteristic rise of cAMP and fall in β-endorphin bipolar (manic-depressive) disease may have contributed to the significant differences in the ratios. Since the significance occurred only in the ratio rather than in individual comparison, it is posited that a physiological relationship exists between β -endorphin and cAMP.

62.18

GLUCOSE UTILIZATION DURING ALFENTANIL INDUCED SEIZURES IN RATS. W.A. Kofke, R.A. Hawkins. Pennsylvania State University, Hershey, PA 17033

Seizure activation of subcortical foci has been reported in rats with a variety of narcotics. Our aim was to determine, using ($6\text{-}^{14}C$) glc autoradiography, if a similar phenomenon occurs with alfentanil (A). Methods. Rats were anesthetized with halothane, intubated, paralyzed, ventilated, and catheterized. After surgery all rats were ventilated with N_2O/O_2 for one hour, then they entered 1 of 3 grps: (1) N_2O/O_2, (2) low dose A (150 ug/kg IV then 15 ug/kg/min), (3) high dose A (1000 ug/kg IV then 100 ug/kg/min). After another hr each rat was injected with ($6\text{-}^{14}C$) glc and glc consumption was measured over 5 min by autoradiography (Am J Physiol 245:C428, 1983). Results. High dose alfentanil produced seizure on EEG with thalamic depression and activation of ventral hippocampus and lat. septal N ($p<.001$). Conclusions. Seizures occur with high dose alfentanil with activation apparently arising from the hippocampus and lat. septal N. These data from us and others (Young;Tommasino) suggests existence in rats of a proconvulsant opiatergic system which manifests with hippocampal activation and thalamic depression.

PAIN MODULATION: CNS PATHWAYS

63.1

THE RELATIVE EFFICACY OF MONOPOLAR VS. BIPOLAR ELECTRODES IN STIMULATION PRODUCED ANALGESIA. K.Jones* and B.Thorn. Dept. of Psychology, The Univ. of Al., Tuscaloosa, Al. 35487

This study explored the differences in the analgesic properties elicited between bipolar electrodes delivering a biphasic current and monopolar electrodes delivering a biphasic or monophasic current in the ventral area of the periaqueductal gray (PAG). Analgesia was evaluated by relative increases in tail-flick latencies upon focal brain stimulation (FBS). Ability of naloxone to reverse the stimulation-produced analgesia was also evaluated.

Results indicate that electrode configuration is crucial to whether analgesia is likely to be produced by FBS of the ventral PAG. Also, while naloxone reliably attenuated the analgesia produced by either stimulation parameter with monopolar electrodes, it failed to alter the analgesia elicited by bipolar/biphasic stimulation. A potential confound is that monopolar electrodes were located in the dorsal part of the ventral PAG, whereas bipolar electrodes were located in the ventral aspect. It is possible that the analgesia elicited from the dorsal and ventral areas of the ventral PAG may differ in underlying mechanisms.

63.2

INVOLVEMENT OF PERIAQUEDUCTAL GRAY NMDA RECEPTORS IN STRESS-INDUCED ANALGESIA IN MICE. B.Siegfried* (1) and R.L. Nunes de Souza*(2) (SPON: European Brain and Behaviour Society). (1) Inst. Pharmacol. Univ. Zurich, CH-8006 Zurich, Switzerland. (2) Lab. Psychobiol., FFCLRP, Campus Univ. Sao Paulo, 14049 Ribeirao Preto, Brazil.

Recently, a functional role for PAG NMDA receptors in pain inhibition has been suggested (Jacquet, Eur. J. Pharmacol. 154: 271, 1988). The present study assessed the effect of PAG NMDA receptor blockade on stress-induced analgesia, using the model of social conflict (Siegfried et al., Behav. Neural Biol. 42: 91, 1984). Saline injected, attacked mice exhibited a marked analgesia (tail-flick latency, TFL 1 min postexposure, median and interquartiles; 3.30(2.62-4.87)s) which was prevented by prior injection into the PAG of AP-7 (2.0 nmol; TFL 1.33(1.23-1.43)s, $p<0.005$, Wilcoxon test) or naloxone (6.0 nmol; TFL 1.33(1.23-1.43)s), as well as by i.p. injection of the non-competitive NMDA antagonist MK 801 (33 nmol; TFL 1.55(1.50-1.70)s). The results indicate an involvement of NMDA receptors in endogenous analgesic mechanisms activated by stress, and show that social conflict analgesia can be antagonized by intervention at either opioid or nonopioid receptor sites within the PAG.

63.3

ELECTRICAL BRAINSTEM STIMULATION IN THE C5 - T2 DORSAL ROOT GANGLIONECTOMIZED RAT J. Ovelmen-Levitt, L. Fox*, J. Bazoukis*, J. Aruda* and B.S. Nashold, Jr. Div. of Neurosurgery, Duke U. Med. Ctr., Durham, N.C. 27710

Central pain states in humans have been reported to be relieved in some cases by electrical stimulation in certain brainstem locations. An animal model of central or deafferentation pain has been developed in the rat with C5 - T2 ganglionectomies. The efficacy of intermittent brainstem stimulation is being studied in this model to alter or ameliorate the behavioral manifestations (biting and/or scratching of the deafferented limb). Fifty nine (59) rats with bipolar implants in VPL, Internal Capsule (IC), PAG-PVG, and Lateral Hypothalamus (LH) and/or C5-T2 ganglionectomies have been studied. No rats with implants only (IO) exhibited biting. Animals with implants in VPL, IC, and PAG followed by ganglionectomies, but without stimulation, had mean onset days of biting which were similar to those with ganglionectomies only (GO); while ganglionectomized animals with LH implants started biting significantly earlier than other controls. So far, animals with stimulation in the IC showed a reduced tendency to bite, while those with PAG-PVG stimulation a greater tendency to bite when compared with (GO) controls. The results indicate that intermittent brainstem stimulation in certain regions can alter the progress of a deafferentation syndrome which follows C5-T2 ganglionectomies in rats.

63.4

TIME COURSE FOR THE DISSIPATION OF TOLERANCE TO THE ANTINOCICEPTIVE EFFECT OF CONTINUOUS PAG STIMULATION IN RATS. M. M. Morgan and J. C. Liebeskind. Dept. of Psychology, UCLA, Los Angeles, CA 90024-1563.

Tolerance to the antinociceptive effect of electrical stimulation of the periaqueductal gray (PAG) has been shown to occur with prolonged stimulation. The objective of this study was to determine the duration of this tolerance. Two groups of male Sprague-Dawley rats were chronically implanted with a bipolar electrode in the PAG. Nociception was assessed using the tail-flick test to noxious heat. Brain stimulation consisted of 50 Hz monophasic pulses (0.4 ms) preceding by 10 s and remaining on throughout the test. The first group was used to assess the stability of stimulation-produced antinociception (SPA) by determining the threshold for SPA twice at an interval of 4 to 8 days. The threshold for SPA was found to be unchanged at 6 of 17 stimulation sites. A slight change (10-20 μA) occurred at 7 other sites. Aversive reactions occurred at the 4 remaining sites. The second group of rats was made tolerant to SPA by administering 6 min of continuous stimulation at the SPA threshold level. Six of 15 sites supported antinociception throughout the stimulation period. Tolerance to SPA occurred at the remaining 9 sites. One day after tolerance was induced, 4 of these 9 sites supported SPA at the stimulation intensity used to induce tolerance. SPA threshold had returned to pre tolerance levels at 8 of 9 sites 1 week after induction of tolerance. These findings demonstrate that SPA threshold is relatively stable across test sessions, and that tolerance to SPA induced with continuous stimulation dissipates with time. (NIH grant NS07628).

63.5

COMPARISON OF DESCENDING INHIBITION OF NOCICEPTIVE HINDPAW- AND TAIL-FLICK REFLEXES FROM STIMULATION OF THE PERIAQUEDUCTAL GRAY MATTER (PAG) IN THE RAT. R. Levine*, M. M. Morgan, J. T. Cannon, and J. C. Liebeskind. Departments of Psychology, UCLA, Los Angeles, CA 90024-1563 and University of Scranton, Scranton, PA 18510.

Electrical stimulation of the PAG is known to inhibit nociception. The goal of this study was to compare the strength of this descending inhibition for the tail and the ipsilateral and contralateral hindpaws. Male Sprague-Dawley rats were anesthetized with pentobarbital, implanted with a bipolar electrode in the right or left caudal PAG, and the thresholds for stimulation-produced inhibition of the tail-flick and paw-flick reflexes to noxious heat were determined. PAG stimulation consisted of 50 Hz monophasic pulses (0.4 ms) for 10 s preceding and throughout the test. The mean current required to inhibit nociception in the hindpaw ipsilateral to the electrode was significantly lower than that needed in the contralateral hindpaw (119 vs 164 μA). Mean baseline paw-flick latencies did not differ from each other, but were significantly shorter than the mean baseline tail-flick latency (3.0 vs 3.4 s). In addition, the threshold for inhibition of the tail-flick reflex (66 μA) was significantly lower than that of the hindpaws. In a follow-up study, the heat source was adjusted so that the baseline paw-flick latencies approximated that of the tail-flick latency (3.8 to 3.6 s). This change resulted in a reduction in the thresholds for inhibition of the paw-flick. The threshold for inhibition of the ipsilateral paw-flick was not significantly different than that of the tail-flick (57 vs 48 μA). However, a difference in threshold between ipsilateral and contralateral paws was still evident (57 vs 97 μA). (NIH grant NS07628).

63.6

NALOXONE-SENSITIVE INHIBITION OF LIMB-WITHDRAWAL REFLEXES PRODUCED BY ELECTRICAL STIMULATION OF THE ARCUATE NUCLEUS IN ANESTHETIZED MICE. J. T. Cannon, R. Yirmiya and J. C. Liebeskind. Departments of Psychology, University of Scranton, Scranton, PA 18510 and University of California, Los Angeles, CA 90024.

Electrical stimulation of the periaqueductal gray (PAG) can inhibit limb-withdrawal reflexes in several strains of mice. A striking characteristic of this inhibition is the marked degree to which it can be reduced by opiate antagonists. Such sensitivity stands in stark contrast to work with rats in which the effects of opiate antagonists either prove elusive to demonstrate or, when observed, are typically only small to moderate in magnitude. As projections from the arcuate n. are a major source of opioids in the PAG, we determined whether electrical stimulation of the arcuate also produces naloxone-sensitive inhibition of limb-withdrawal. Male Swiss Webster mice were anesthetized with Nembutal and maintained at a level of anesthesia sufficient to block spontaneous movements throughout testing. Baseline limb-withdrawal latencies to a noxious heat source were determined for both hind limbs. Brain stimulation consisted of 0.4 msec cathodal pulses delivered at 50 pulses per sec for 10 sec preceding and throughout a withdrawal trial. The lowest stimulation threshold for inhibiting the withdrawal of either limb was determined before and after an injection of either naloxone (5 mg/kg) or an equal volume of saline. Stimulation of the arcuate n. consistently inhibited limb-withdrawal. Paralleling PAG stimulation in the mouse, naloxone elevated stimulation thresholds for this inhibition by over 200%. Supported by NIH grant NSO7628.

63.7

AN EXAMINATION OF THE TIME COURSE OF ANALGESIA PRODUCED BY INJECTION OF LIDOCAINE INTO THE ANTERIOR CINGULUM. A.L. Vaccarino and R. Melzack*, Department of Psychology, McGill University, Montreal, Quebec, Canada, H3A 1B1.

In a previous study (Soc. Neurosci. Abstr. 14:855, 1988) we reported that injection of lidocaine, a local anesthetic, into the anterior cingulum bundle of the rat produced a significant reduction in formalin pain scores but had no effect on foot-flick latencies. The analgesia in the formalin test persisted for the entire 40 minute observation period.

In the present study we examined the time course of the functional block produced by lidocaine. A cannula was stereotaxically implanted into the anterior cingulum on one side in anesthetized rats. Seven to ten days after surgery the rats were injected with 1 ul of 2% lidocaine or saline into the cingulum at various times before and after injecting one hind paw with 50 ul of 2.5% formalin. Rats injected with lidocaine 60 minutes prior to formalin injection showed no analgesia, suggesting that no permanent damage was produced by the lidocaine. Rats injected at other times showed various analgesic profiles relating to time of injection and laterality. These results are discussed. Supported by NSERC grant A7891.

63.8

EFFECTS OF VENTROLATERAL ORBITAL CORTEX STIMULATION ON THE JAW-OPENING REFLEX (JOR) AND SPINAL CORD DORSAL HORN NEURONS. C.Y. Chiang*, N. El-Yassir*, E.M. Moustafa*, J.O. Dostrovsky and B.J. Sessle. (SPON: L. Spero) Dept. of Physiology, and Fac. of Dentistry, Univ. of Toronto, Ontario, Canada.

Anatomical studies have reported that the ventrolateral orbital (VLO) region of prefrontal cortex (PFC) sends a descending projection to the periaqueductal gray (PAG). It is also known that the VLO receives a dense projection from nucleus submedius, a thalamic nucleus which receives direct spinal and trigeminal ascending projections, and which has been implicated in nociception. These findings suggest that the VLO may be involved in descending modulation and thus we have investigated the effects of prefrontal cortex stimulation on the tooth pulp-evoked JOR and on spinal cord dorsal horn neurons. All the experiments were conducted on rats anesthetized with chloralose-urethane. Dorsal horn neurons were activated by percutaneous electrical stimulation and mechanical stimuli. Conditioning stimuli consisted of trains of stimuli (100Hz, 100ms) delivered, 150ms prior to the test stimulus, to the VLO region of PFC; in some mapping experiments, the effects of stimulation at many sites within PFC were examined. Stimulation sites were reconstructed from histological sections. Modulatory effects were assessed by comparing 16 averages of the JOR before and immediately following the conditioning stimulation, and for dorsal horn neurons on the basis of rate-meter and post-stimulus histogram plots. Stimulation at some sites in PFC, including medial VLO, were found to reduce the magnitude of the JOR. However PFC stimulation did not result in marked alterations in the responses of nociceptive or non nociceptive neurons in the spinal dorsal horn. In summary, PFC stimulation depresses the JOR but appears to have little effect on dorsal horn neurons.
Supported by the Canadian MRC.

63.9

ELECTRICAL STIMULATION OF SENSORY CORTEX INCREASES THE NOXIOUS THRESHOLD OF SPINOTHALAMIC TRACT CELLS, C. Owens, D. Zhang and W.D. Willis, Marine Biomedical Institute and Department of Anatomy & Neurosciences, University of Texas Medical Branch, Galveston, TX 77550

Stimulation of the cortex caudal to the central sulcus affected responses of spinothalamic tract (STT) cells to natural cutaneous stimulation. In 9 chloralose/pentobarbital anesthetized, Flaxedil paralyzed Macaca fascicularis, unit recordings were made from 14 STT cells. The responses of each cell to four cutaneous stimuli ranging from innocuous to frankly noxious were quantified. The stimuli were repeated during cortical stimulation (1mA, 333Hz, 0.5mS duration) near the location of the maximum evoked potential from sural nerve. The predominant (n=8) effect on the transmission of sensory information by the STT cells was an increase in threshold for noxious stimulation and a reduction in response to innocuous stimulation. Similar results were obtained from microstimulation (100μA) of the cerebral peduncles. Ablation of these modulatory centers might cause decreased thresholds for noxious stimulation and hyperexcitability to innocuous stimuli, as is characteristic of central pain. (Supported by NIH grants NS 11255 and NS 09743 and Bristol-Myers Co.)

63.10

THE RESPONSES OF SPINOTHALAMIC TRACT (STT) NEURONS TO ELECTRICAL STIMULATION OF CORTEX AND CEREBRAL PEDUNCLE (CP). D. Zhang, C.M. Owens and W.D. Willis. Dept. of Anatomy & Neurosciences and Marine Biomedical Institute, Univ. of Texas Medical Branch, Galveston, TX 77550.

The activity of STT neurons can be modulated by electrical stimulation of cortex. The present study employs extracellular and intracellular recording to investigate the spinal mechanism involved in such modulation. Fifteen anesthetized adult monkeys (M. fascicularis) were used. Extracellular recording from 25 STT neurons showed that stimulation of cortex or CP could excite or inhibit STT neurons. The responses to sural nerve Aδ-fiber volleys were preferentially inhibited. Intracellular recording showed that the stimulation of CP or cortex evoked either depolarization or hyperpolarization in 14 STT neurons. The depolarization proved to be an EPSP, since there was an increased membrane conductance and an increased amplitude of the depolarization as the membrane potential was hyperpolarized. The hyperpolarization was, in some neurons, due to an IPSP proved by an increased membrane conductance and a decreased amplitude when the membrane potential was hyperpolarized. However, in some neurons the amplitude of hyperpolarization was increased by hyperpolarizing the membrane potential and the membrane conductance was slightly decreased during hyperpolarization, suggesting a mechanism of disfacilitation. (Supported by grants from NIH (NS 09743, NS 11255) and the Bristol-Myers Co.)

63.11

ORGANIZATION OF CORTICAL INPUTS TO RAT PERIAQUEDUCTAL GREY. M.T. Shipley[1], L.B. Fu[1]*, M. Ennis[2] and M. Behbehani[2]. Dept. Anat.[1] & [2]Dept. Physiol.[2], Univ. Cinti. Coll. Med., Cincinnati, OH 45267.

Previous studies indicate that periaqueductal grey (PAG) receives inputs from medial prefrontal and lateral cortical areas. Our recent findings reveal that innervation of PAG from these areas is much more substantial than previously reported. Here, we have examined the distribution of PAG-projecting cortical neurons, and their terminal labeling patterns in PAG.

Single discrete or large multiple injections of WGA-HRP into several rostrocaudal levels of PAG retrogradely labeled substantial populations of neurons in both medial prefrontal and lateral cortex. Neurons in medial cortex were heavily distributed in orbital, prelimbic, infralimbic and cingulate fields, forming a continuous band of neurons spanning the entire dorsoventral extent of medial cortex. Laterally, labeled neurons were centered primarily adjacent to the rhinal fissure; caudal to the genu of the anterior commisure, labeled cells were also located just dorsal to the fissure. This lateral population of labeled neurons extends the entire rostrocaudal length of the cortical mantle.

Injection of WGA-HRP into subregions of medial or lateral cortex revealed heterogeneous terminal patterns within PAG. However, one common innervation pattern was observed from both inputs: discrete bands of labeling in dorsomedial and lateral/ventrolateral PAG.

These results indicate that PAG receives heavy input from cortical fields associated with visceral function and limbic-sensory integretion. These cortical inputs may overlap within two distinct subregions of PAG. (Supported by PHS Grants NS20643 and NS24698.)

63.12

CHOLINERGIC MEDIATION OF ANTINOCICEPTION PRODUCED BY ELECTRICAL STIMULATION OF THE PEDUNCULOPONTINE TEGMENTUM. LF Fitzgerald and HK Proudfit. Dept of Pharmacology, University of Illinois at Chicago, Chicago, IL 60680.

Activation of neurons in the nucleus raphe magnus (NRM) by injection of cholinergic agonists produces antinociception. The current studies defined the origin of the cholinergic projections to the NRM, and determined if stimulation of the origins of those projections could produce antinociception.

Retrograde labeling and immunocytochemistry were used to demonstrate cholinergic neurons. These experiments revealed cholinergic projections from both the pedunculopontine tegmental nucleus (PPT) and the cuneiform nucleus to the NRM.

The area of the PPT in lightly anesthetized rats was electrically stimulated at various depths in the brain stem for 60 seconds and tail flick response latencies were measured. Atropine sulfate (5mg/kg, sc) was given to determine if the stimulation-induced antinociception was mediated by acetylcholine.

Stimulation of the site corresponding to the PPT produced antinociception lasting from 4-150 minutes. This effect was blocked by atropine. Immunocytochemistry revealed cholinergic neurons around the PPT stimulation site. (Supported by USPHS Grant 03980.)

63.13

GABA NEUROTRANSMISSION IN ROSTRAL VENTROMEDIAL MEDULLA OF THE LIGHTLY ANESTHETIZED RAT: ROLE IN NOCICEPTIVE MODULATION. M.M. Heinricher and H.J. Kaplan*. Dept. Neurology, Univ. California, San Francisco, CA 94143

Local microinjection of GABA receptor agonists and antagonists into the rostral ventromedial medulla (RVM) was used to examine the contribution of GABA to the nociceptive modulating function of the RVM in the lightly anesthetized rat.

Animals were maintained in a lightly anesthetized state using a continuous infusion of methohexital. The tail flick response (TF) was used as an index of nociceptive responsiveness. Microinjection of the $GABA_A$ receptor antagonist bicuculline methiodide (BMI, 25-200 ng in 0.5 ul, injected over 3 min) produced a dose-related inhibition of the TF (10-s cut-off) that was reversed by the GABA antagonists THIP and muscimol microinjected at the same site. BMI-induced TF inhibition was seen within one minute of completing the injection and lasted from 10 to 30 min. Microinjection of BMI outside of the RVM, in n. reticularis gigantocellularis or n. raphe pallidus, had no effect on TF latency. Microinjection of THIP (12.5-100 ng) or muscimol (1-50 ng) alone produced an immediate and consistent decrease in TF latency. Microinjection of saline did not affect the TF.

These results demonstrate that GABAergic transmission within the RVM plays a key role in the nociceptive modulating functions of the region. The observation that local application of a $GABA_A$ receptor antagonist produces TF suppression would indicate that a class of inhibitory output neurons within RVM receives a GABA-mediated inhibitory input. Moreover, the fact that microinjection of $GABA_A$ receptor agonists results in enhancement of the TF would suggest that an RVM neuron which is susceptible to GABA-mediated inhibition exerts an on-going suppressive effect on spinal nociceptive transmission.

Supported by a grant from NIDA (DA-05608).

63.14

ANTINOCICEPTIVE AND BEHAVIORAL EFFECTS OF STIMULATION IN THE DORSAL RAPHE, LATERAL HABENULA, PRETECTAL AREA, AND VENTRAL TEGMENTAL AREA. C. Preston*, P. Dougherty*, and N. Dafny. (SPON: D. Marshak). Dept. Neurobiol. & Anat., UT Medical School at Houston, TX 77225.

Focal brain stimulation is reported to produce both analgesia (SPA) and aversive effects. This study compared the analgetic and aversive effects of stimulating the dorsal raphe (DR,n=6), lateral habenula (LHb,n=4), pretectal area (PTA,n=8), and ventral tegmental area (VTA,n=4). Male Sprague Dawley rats were implanted with bipolar 120 μm stainless steel electrodes, teflon insulated except at the tips. Tail-flick latencies (TFLs), before (baseline), and during a 10 min period of stimulation with constant current (100-400 μA), 0.2ms, 20 Hz pulses were averaged. The lowest current intensity at which the TFLs exceeded baseline by at least 2 SDs was designated the threshold current for analgesia. The threshold currents for each site were similar. VTA stimulation at threshold produced less elevation of a computed analgesia index. PTA stimulation at threshold produced the longest lasting post-stimulation analgesia. Threshold stimulation induced site specific behavioral patterns. Stimulation of DR and VTA appeared to be more aversive than stimulation of LHb or PTA. Thus, the SPA of VTA may be partially stress induced, while PTA stimulation may be a clinically useful pain therapy.

63.15

DESCENDING FACILITATION (AND INHIBITION) FROM THE NUCLEI RETICULARIS GIGANTOCELLULARIS (NRGC) AND GIGANTOCELLULARIS PARS ALPHA (NRGCα) IN THE RAT. M. Zhuo* and G.F. Gebhart (SPON: W. Steele). Dept. Pharmacology, The University of Iowa, Iowa City, Iowa, 52242

It is well documented that the ventral medulla is important to descending modulation of spinal nociceptive transmission. In the present work, descending inhibitory and facilitatory influences on the spinal nociceptive tail-flick (TF) reflex produced by eletrical stimulation and glutamate microinjection in the NRGC/NRGCα were examined and characterized in pentobarbital-anesthetized rats. Inhibition and facilitation of the TF reflex were produced by eletrical stimulation at identical sites in the NRGC/NRGCα; glutamate microinjection only inhibited the TF reflex. The chronaxie of stimulation was 140 μsec; the inhibitory effect of stimulation on the TF reflex did not outlast the period of stimulation. At threshold intensities of stimulation, blood pressure was not significantly affected. Inhibition of the TF reflex was produced throughout the NRGC/NRGCα; thresholds for inhibition were least (10-25 μA) in the ventral NRGC and NRGCα. Facilitation of the TF reflex was produced at many of the same sites, but always at lesser intensities (mean, 10 μA vs 45 μA for inhibition, n=21) and primarily in the NRGCα. These results reveal intensity-dependent, bi-directional modulatory descending influences from the NRGC/NRGCα.

63.16

THE SOMATODENDRITIC MORPHOLOGY OF ON- AND OFF-CELLS IN THE RAPHE MAGNUS AND ADJACENT RETIULAR FORMATION. P. Mason and H.L. Fields. Depts of Neurology, University of California, San Francisco, CA, 94143

Neurons in the nucleus raphe magnus (NRM) were recorded intracellularly during the hindlimb flexion withdrawal reflex evoked by noxious pinch or heat in the lightly anesthetized cat and ferret. Cells that either increased (on-cells) or decreased (off-cells) their discharge rate during the nociceptive reflex were labeled with horseradish peroxidase. Labeled cells were reconstructed in semi-coronal sections.

On-cells in the rostral NRM were either large multipolar or small fusiform neurons with their long axes oriented dorsoventrally. Off-cells in NRM and the adjacent ventral reticular formation were typically fusiform with a mediolateral orientation. Large multipolar off-cells were also observed.

The dendritic arbors of both on- and off-cells that had somata within NRM extended up to 1.5mm lateral to the cytoarchitechtonic boundaries of the NRM. These dendrites arborized throughout the ventral magnocellular reticular formation bilaterally. In contrast, the dorsal extent of the dendritic field was typically more restricted; few dendrites reached the dorsal pontomedullary reticular formation. Several on- and off-cells in rostral NRM had dendritic arbors that extended ventrally into the trapezoid body.

The somatodendritic morphology of on- and off-cells in the cat is further evidence for the functional connection between the NRM and the adjacent ventral reticular formation.

63.17

INTRATHECAL THEOPHYLLINE ATTENUATES ANTINOCICEPTION EVOKED BY STIMULATION IN THE VENTRAL MEDIAL MEDULLA. S. Aran, and H.K. Proudfit. Dept. of Pharmacology, Univ. of Illinois at Chicago, Chicago, IL. 60680.

We examined the capacity of intrathecal (i.t.) theophylline (THEO)* to attenuate the antinociception evoked by electrical stimulation of the ventral medial medulla (VMM).

Animals were implanted with an i.t. catheter and a bipolar stimulating electrode. One week after surgery baseline nociception was determined using the tail flick test. After determination of baseline tail flick latencies (TFL), a stimulus-response curve was generated for electrical stimulation of the VMM. Animals were then given an i.t. injection of THEO (100 μg). TFLs were redetermined 7 min after drug injection and stimulus-response curves were again constructed. VMM stimulation produced significant increases in TFL before THEO injection. However, VMM stimulation failed to increase TFL after THEO injection. These data suggest that antinociception produced by stimulation of VMM neurons is at least, in part, mediated by the release of adenosine in the spinal cord. This work was supported by USPHS Grant DA03980.

63.18

EFFECTS OF STIMULATION OF NUCLEUS RAPHE MAGNUS ON RENAL AFFERENT INPUT. M.M. Knuepfer and I.L. Holt*. Dept. of Pharmacol., St. Louis Univ, St. Louis, MO 63104.

Electrical stimulation (ES) of the raphe magnus (NRM) inhibits dorsal horn neurons responding to somatic nociceptive input. Supraspinal modulation of visceral input is less well understood. We reported that the NRM contains tonically active neurons inhibiting some renal afferent input (RAI) to the spinal gray. In this study, we sought to define the extent and nature of projections from the NRM modulating RAI. Chloralose-anesthetized rats were prepared for ES of the renal nerve, ES or chemical stimulation of the NRM, and extracellular recording of single neurons in the lower thoracic spinal gray. In 8 of 10 neurons, ES of the NRM inhibited spontaneous activity and in 6 of 9 neurons RAI was also inhibited. Glutamate (10 nmol/μl) injections into the NRM (0.5-1 μl) elicited either an increase (3), a decrease (5) or no change (5) in spontaneous activity in neurons excited by RAI. Evoked activity was increased (3), decreased (5) or unaffected (5). These neurons were unaffected by equivalent injections of saline. Neurons receiving RAI were also tested with cervical cold block. Opposite changes in spontaneous (8/12) and evoked activity (6/12) were often observed compared to glutamate. Therefore, ES of the NRM activates primarily inhibitory projections whereas selective stimulation of neuronal soma demonstrates a mixed population of facilitatory and inhibitory neurons modulating RAI. These data suggest that projections through the NRM actually mediate much of the descending inhibition observed with ES. (Supported by HL38299).

63.19

RADIATION-CONTROLLED FOCAL BRAIN PHARMACOLOGY INCREASES TAIL-FLICK LATENCY.

M.P. Remler, W.H. Marcussen*, and K.A. Sigvardt. Department of Neurology, University of California, Davis.

Radiation-controlled focal brain pharmacology is a method to concentrate a drug in a selected part of the brain by irradiating that area and thereby lowering the BBB in that area and that area only. Then, when a drug that does not cross the normal BBB is administered systemically after the radiation has lowered the BBB in that area, the drug will preferentially penetrate the selected part of the brain. Systemic carbachol, which cannot cross the BBB, has no effect on the tail-flick latency in control rats. 200 days after rats received 20 Gy to the medulla, the BBB is open in that region and systemic carbachol 0.5 mg/kg and 1.0 mg/kg IP caused a 38% increase in tail-flick latency which lasted for two hours, demonstrating that with a radiation-induced focal lesion of the blood-brain barrier in the ventral medulla systemic carbachol can act pharmacologically and the effect monitored as an increase in the latency of the rat heat-induced tail flick. Activation of the rostral ventral medulla using radiation-controlled focal brain pharmacology could provide a method for pain control by drugs that pharmacologically activate the pain modulating system and would be isolated from the pharmacologically identical non-antinociceptive CNS effects of these drugs.

63.20

REFRACTORY PERIOD ESTIMATES OF THE SUBSTRATE FOR ANALGESIA DERIVED FROM STIMULATION OF THE ROSTRAL MEDULLA. S. Schenk and R. Peltier*. TAMU, Dept. Psychol., College Station, TX. 77843

Stimulation of the rostral medulla produced an analgesic effect as indicated by an increased latency for the rat to flick it's tail out of heated water. Refractory periods of the directly stimulated substrate for this behavior were estimated using psychophysical techniques. Electrical stimulation consisted of 10 sec trains of single pulses or pairs of pulses. The intrapulse-pair interval was varied from 1.0-10.0 msec and frequency thresholds for analgesia were determined at each interval. The effectiveness of paired pulse stimulation increased steadily between pulse pair intervals of 1.5 and 6.0 msec. These data provide an estimate of the refractory period of the directly stimulated substrate. They were comparable to those obtained when dorsal and ventral PAG sites were tested. Thus, the substrates for the analgesic effects of dorsal and ventral PAG stimulation and stimulation of rostral medullary sites is comprised of similar caliber neurons. The refractory period estimates obtained suggest that these substrates consist of small, unmyelinated neurons with slow conduction speeds.

63.21

THE EFFECTS OF MELATONIN MANIPULATIONS ON PAIN THRESHOLD AND AFTERDISCHARGE THRESHOLD FROM THE AMYGDALA IN THE RAT. J.H. Peck, A. LaBanca*, F. Scholl* and L.J. Grota, Dept. of Psychology, Ithaca College, Ithaca, NY 14850.

Past research has shown that pinealectomy lowers afterdischarge threshold from the amygdala and lowers the nighttime rise in pain threshold on the hotplate. We sought to replicate these two findings by directly injecting melatonin (afterdischarge test) and doing superior cervical ganglionectomy (SCGX, pain test).

AFTERDISCHARGE THRESHOLD. Rats were implanted with an intraventricular cannula, bipolar amygdala electrodes and cortical screw electrodes. One week later, they were injected with either melatonin or saline during the light portion of their daily cycle. The amygdala was given an ascending series of constant current shocks every 5 min. and afterdischarge as well as seizure stage was noted on the record. Melatonin marginally raised the afterdischarge threshold but did protect the rats from behavioral seizures ($p<.05$).

PAIN TEST. Rats were either SCGX'd or sham operated and were tested on a hotplate for analgesic responses during the light and dark phases of the daily cycle. The sham rats showed an increase in latency to pawlick on the hotplate at night relative to the day. The SCGX rats had this rhythm greatly attenuated with day and night latencies being about the same. This produced a significant interaction between type of surgery and time of testing ($p<.05$).

PAIN MODULATION: OPIOID MECHANISMS

64.1

BIOAVAILABILITY, PHARMACOKINETICS AND AUTORADIOGRAPHIC DISTRIBUTION STUDIES WITH PD 129290, A NOVEL ANALGESIC KAPPA OPIOID RECEPTOR AGONIST. C.M. Barksdale, W.P. McNally, G.D. Nordblom*, M.J. Coon*, P.D. DeHart* D.S. Wright*, R.J. Guttendorf*, D. Turluck*, L.A. Pachla* and T. Chang*. Pharmacokinetics/Drug Metabolism Dept., Parke-Davis Pharm. Res. Div., 2800 Plymouth Road, Ann Arbor, MI 48105

Kappa opioid receptor agonists offer the possibility of producing effective analgesia via a novel mode of action when compared with existing therapies. PD 129290 (Eur. Pat. Pub. #0207773; 06/30/86) has a high *in vitro* affinity, selectivity and specificity for the k opioid receptor. We now report the *in vivo* bioavailability and pharmacokinetics of PD 129290 in rat plasma using a RIA, and the autoradiographic distribution of ^{3}H-PD 129290 in rats. In male Wistar rats given 1 mg/kg dose, half-life of the drug was 4.6 and 9.1 hours following IV and IM administration, respectively. Absolute IM bioavailability was 10%. Rats given 40 µg/kg of ^{3}H-PD 129290 by IM injection were sacrificed at intervals from 10 min to 24 hr for whole body autoradiography. Concentrations in brain were primarily in gray matter with indication of higher concentration in cortex and areas of hippocampus corresponding to regions in CA3 reported to have high k receptor density. Radioactivity was rapidly cleared from most tissues and by 6 hours postdose preliminary exposures showed residual label in liver and kidney only.

64.2

INHIBITORS OF ENDOPEPTIDASE 24.15 & 24.11: COMPARISON OF ANTINOCICEPTIVE AND OPIOID PROPERTIES IN RATS. B. Kest, M. Orlowski*, C. Molineaux, and R. J. Bodnar. Department of Psychology, Queens Col., CUNY, Flushing, N Y,11367, & Department of Pharmacology, Mt Sinai School of Medicine, New York, N Y, 10029.

Endogenous opioid antinociception is limited by succeptibility to peptidase degradation. While endopeptidase 24.11 degrades pro-enkephalin opioids, endopeptidase 24.15 degrades pro-dynorphin and alpha neo-endorphin opioids. Inhibitors of the former (EPI 24.11) elevate antinociceptive thresholds and potentiate antinociception induced by pro-enkephalin-derived opioids.The present study compared the central antinociceptive properties of EPI 24.11 and two recently developed inhibitors of endopeptidase 24.15 (EPI 24.15) using threshold measures of reactivity to thermal (tail-flick test) and aversive (jump test) stimuli. Intracerebroventricular administration of all three inhibitors produced a dose-dependent (0.05-50 nmol) antinociception on both tests, with more pronounced effects on the supraspinally-mediated jump test.The gradual (5-7 h) appearance of antinociception suggested an effect upon peptidases rather than receptor substrates. The reversal of antinociception by naloxone (1 mg/kg, sc) suggests that EPI 24.15 and EPI 24.11 antinociception is mediated in part through an opioid synapse. The two inhibitors of endopeptidase 24.15 were selective for this enzyme relative to endopeptidase 24.11 and differed in their relative potencies and inherent resistance to degradation themselves. These data indicate the putative involvement of pro-dynorphin-related peptides in supraspinal antinociception, and suggests that the relative inability of these peptides to induce antinociception may be due to their rapid enzymatic degradation.

64.3

SENSORY-MOTOR FUNCTION IN RATS FOLLOWING INTRANIGRAL MORPHINE INJECTION. A.A. Baumeister, M. Nagy*, G. Hebert*, M.F. Hawkins, and M.O. Chatellier*. Department of Psychology, Louisiana State University, Baton Rouge, LA 70803.

Intranigral (IN) injection of morphine suppresses pain-related behavior on the tail flick and hot plate tests (Brain Res., 1987, 411, 183). The following study was conducted to determine whether this effect may be due to nonspecific sensory-motor impairment. Male Sprague Dawley rats received bilateral IN injections of morphine sulfate (10 µg/0.5 µl saline) or saline (0.5 µl). Between 5 and 10 minutes after the injection animals were assessed on the following measures: 1) hot plate and formalin analgesia tests, 2) rotorod and catalepsy tests, 3) righting, placing, pinna, eye blink, and startle reflexes, and 4) wet dog shakes after brief submersion in water. Latency to rear paw lick on the hot plate test was significantly increased, whereas duration on the rotorod and number of wet dog shakes were significantly reduced in animals that received IN morphine. On all other measures, sensory-motor function appeared normal following IN morphine. Thus, simple reflexes, other than the nociceptive tail flick reflex, are unaffected by IN morphine. Impairments are demonstrable on more highly integrated tasks. However, a general sensory-motor defect does not appear to account for the analgesic-like effect of IN morphine on the hot plate test because 8 of 9 animals that received IN morphine licked, bit or sniffed the rear paw on the formalin test.

64.4

MORPHINE IN THE VTA AND VENTRAL STRIATUM PRODUCES ANALGESIA IN THE FORMALIN TEST. M.J. MORGAN and K.B.J. FRANKLIN., Dept. of Psychology, McGill University, P.Q., Canada, H3A 1B1.

Recently we found that systemic administration of the mixed DA antagonist alpha-flupentixol or 6-OHDA lesions of the ventral tegmental/substantia nigra areas abolished morphine analgesia in the formalin test (Morgan and Franklin, in press). This suggests that morphine analgesia in this test may be mediated by limbic DA systems. We examined this possibility by micro-injecting 5 nM morphine sulphate, bilaterally, in a 0.5 ul volume, into the peri-aqueductal gray (PAG), ventral tegmental area (VTA), ventral striatum (VS) and surrounding regions. This dose of morphine produced strong analgesia (>90%) in the VTA and PAG, whereas it produced only moderate analgesia in the VS (>40%) and marginal analgesia (<30%) at sites dorsal to the VTA or VS. These results suggest that mesolimbic DA neurons are involved in morphine analgesia in the formalin test and that morphine acts at receptors pre- and/or post-synaptic to DA neurons innervating the ventral striatum.

64.5

CENTRAL ALLOXAN DIFFERENTIALLY ALTERS OPIOID AND NONOPIOID ANALGESIA IN RATS. E. Lubin and R. J. Bodnar, Dept. of Psychology, Queens Coll., CUNY, Flushing, NY 11367.

While peripheral administration of alloxan destroys pancreatic beta cells and thereby produces diabetes, central administration of far lower doses of alloxan (40 and 200 µg, ICV) selectively reduces opioid-mediated 2-deoxy-D-glucose hyperphagia and analgesia, but not hyperglycemia, presumably by altering either brain glucoreceptors or a glucoprivic control mechanism. We recently found that central alloxan treatment significantly reduces morphine (2.5 and 5 mg/kg, SC) analgesia on two threshold measures of nociceptive reactivity to thermal (tail-flick test) and aversive (jump test) stimuli in rats. To evaluate possible opioid-nonopioid differences, the present study examined whether central alloxan (200 µg, ICV) alters nonopioid analgesia induced by either continuous cold-water swims (CCWS: 2°C for 3.5 min) or the muscarinic receptor agonist, pilocarpine (0.5-5 mg/kg, IP), in rats. CCWS analgesia on the jump test was initially reduced but subsequently potentiated by central alloxan. In contrast, alloxan failed to alter CCWS analgesia on the tail-flick test, nor did it alter CCWS hypothermia or pilocarpine analgesia on either nociceptive measure. The dissociations of opioid and nonopioid analgesia following alloxan occurred in the absence of changes in basal nociception.

64.6

INHIBITION OF MORPHINE (MOR) ANTINOCICEPTION BY CENTRALLY - ADMINISTERED H_2 - ANTAGONISTS. J.W. Nalwalk*, K.R. Gogas* and L.B. Hough (SPON: S. Glick). Dept. Pharmacol. & Toxicol. Albany Medical College, Albany, NY 12208.

To directly test the hypothesis that brain histamine and brain H_2 receptors play a role in opiate antinociception, the effects of several H_2 antagonists of varying structure and H_2 affinity were assessed on nociceptive (radiant heat) thresholds in the presence and absence of MOR (10 mg/kg, 30 min, ip). When given directly into the lateral ventricle of conscious rats, 5 H_2 antagonists exhibited dose - dependent inhibition of MOR antinociception 10 min after administration, with no effects on baseline nociception. As shown below, the potencies of these compounds for inhibition of MOR antinociception were highly correlated with their potencies as H_2 antagonists ($r = 0.98$, $P < 0.005$), supporting the hypothesis that activation of brain H_2 receptors is important for opiate-induced attenuation of nociception. (Supported by DA-03816).

Drug	MOR IC50 (nmol)	H_2 Affinity (nM)*
WY45,727	0.06	5.9
zolantidine	0.33	34.0
ranitidine	0.78	64.0
SKF95565	1.75	320.0
SKF95299	38.45	1900.0

*Gogas and Hough, JPET 248:262-267, 1989.

64.7

ACTIONS OF MORPHINE (MOR) ON CNS HISTAMINE (HA) DYNAMICS IN MICE. S.P. LICATA,* J.W.NALWALK* & L.B. HOUGH. Dept. Pharm & Tox, Albany Medical College, Albany, NY 12208.

The effects of MOR on the levels of HA and its metabolite tele-methylhistamine (t-MH), and on t-MH synthesis rates were examined in brains and spinal cords of mice. In DBA/2J (DBA), a strain sensitive to MOR anti-nociception, MOR (1-56 mg/kg, s.c.) suppressed t-MH levels, had no effect on HA levels and produced a dose-dependent decrease in whole brain t-MH synthesis rates, measured by the pargyline-induced accumulation of t-MH. In DBA, MOR (10 mg/kg) produced a generalized inhibition (11 to 48%) of t-MH synthesis in 8 brain regions, and *increased* t-MH synthesis in the spinal cord. In contrast, MOR had no effects on HA metabolism in either whole brain or spinal cord in C57/BL6 mice, a strain resistant to morphine antinociception. In Swiss-Webster mice, MOR exerted a slight inhibition of HA metabolism.

These results find no evidence in 3 strains of mice for a MOR-induced increase in brain HA metabolism, in contrast to results in ddY mice (Nishibori et al., J. Neurochem. 45: 719, 1985). In the strains studied presently, MOR inhibits and stimulates HA metabolism in brain and spinal cord, respectively, and the magnitude of these effects in a strain parallels that strain's sensitivity to morphine antinociception. Although further studies are needed to show this, morphine may release HA from the spinal cord as a component of its antinociceptive mechanism. (Supported by DA-03816).

64.8

GENDER AND GONADECTOMY MODULATE NONOPIOID CHOLINERGIC ANALGESIA IN RATS. J.M. Kiefel and R.J. Bodnar, Department of Psychology, Queens College, CUNY, Flushing, NY 11367.

Gender differences appear to modulate opioid-mediated and nonopioid-mediated analgesia induced by exposure to environmental stressors, such that females display a smaller magnitude of both forms of analgesia than males. Adult gonadectomy reduces the magnitude of both forms of analgesia relative to same-sex, sham-operated controls. Our laboratory found gender, and to a lesser degree gonadectomy, differences in analgesia elicited by central and systemic morphine and endogenous opioids with male rats displaying greater responses at significantly lower effective doses than females, and gonadectomy reducing the magnitude of effects. The present study evaluated whether a nonopioid form of analgesia displayed similar gender and gonadectomy differences by examining analgesia induced by the muscarinic receptor agonist pilocarpine (0.25-10.0 mg/kg, IP) using two threshold measures of nociceptive reativity to thermal (tail-flick test) and aversive (jump test) stimuli in rats. Male rats displayed significantly greater pilocarpine analgesia (4-fold shift) on both tests than estrous females across doses. In contrast, gonadectomy produced smaller reductions in pilocarpine analgesia. These data indicate a modulatory influence of gonadal steroids upon both opioid and nonopioid analgesia.

64.9

GENDER AND GONADECTOMY MODULATE CENTRAL ENDOGENOUS OPIOID ANALGESIA IN RATS. K.L. Kepler and R. J. Bodnar, Department of Psychology, Queens College, CUNY, Flushing, NY 11367.

Analgesic responses induced by systemic morphine as well as opioid and nonopioid forms of environmental stressors are sensitive to gender differences such that females display a smaller magnitude of analgesia than males. Adult gonadectomy reduces the magnitude of these forms of analgesia relative to same-sex sham-operated controls. We found profound gender differences in central morphine analgesia with male rats (5 µg, ICV) displaying peak effects at significantly lower effective doses than females (40 µg, ICV). Significant reductions in central morphine analgesia were less pronounced in gonadectomized rats. The present study evaluated gender and gonadectomy effects upon analgesia induced by the specific μ_2 opiate receptor subtype agonist, D-Ala^2, *N*-Me-Phe^4, Gly-ol-enkephalin (DAGO: 0.5-20 µg, ICV) using threshold measures of reactivity to thermal (tail-flick test) and aversive (jump test) stimuli. Male rats displayed significantly greater DAGO analgesia (2-fold shift) than estrous females across doses on both tests. Gonadectomy produced significant, though smaller, reductions in analgesia, especially in gonadectomized males. These data indicate a modulatory influence of gonadal steroids upon endogenous supraspinal opioid analgesia.

64.10

EFFECTS OF MORPHINE ON REFLEXES AND AVOIDANCE RESPONSES IN MONKEYS. D.C.Yeomans, C.J.Vierck, Jr., and B.Y.Cooper, Dept. of Neuroscience and Center for Neurobiological Research, Univ. of Florida, Gainesville, FL

Low doses (0.25-0.75 mg/kg) of systemic morphine attenuate operant responses to C fiber stimulation when given to monkeys, whereas higher doses are required to attenuate responses of monkeys to stimuli which produce predominantly A-delta associated pain in humans. An autonomic reflex (skin temperature response) is affected by doses in the same range as that which affected C fiber responses. To determine the effects of a similar range of morphine dosages on a somatic reflex, the force of shock-evoked nociceptive reflexes was measured in monkeys. In addition, the effect of morphine on components of electromyographic recordings of the reflex evoked by activation of A-beta, A-delta and C fibers respectively was determined. The lowest dose (0.5 mg/kg) facilitated the force of the response and both the A-delta and C fiber evoked components of the EMG, while the highest dose (2.0 mg/kg) attenuated both of these components. The A-beta component was unaffected by morphine in this dosage range.

Following the initial stimulus, which was used to elicit reflexes, animals could avoid further electrocutaneous stimulation by pulling up on a foot lever. The force of these avoidance responses was used to assess the affects of systemic morphine on operant motoric capacity in the absence of nociceptive stimulation. Avoidance response force was unaffected by 0.5 to 2.0 mg/kg of morphine, suggesting that neither the facilitation of reflexes seen in the same animals nor the attenuation of operant responses to nociceptive stimuli are induced by a general alteration in motor function. Supported by USPHS Grant NS07261.

64.11

CONDITIONAL HYPOALGESIA ON THE FORMALIN TEST IS BLOCKED BY NALTREXONE APPLIED TO THE PAG F.J. Helmstetter & J. Landeira - Fernandez*, Psychology Department, Dartmouth College, Hanover, N.H. 03755

The mesencephalic central gray is involved in the endogenous modulation of nociceptive reactivity and the expression of defensive behavior in rats. The present study was designed to determine if the ability of peripherally administered naltrexone (NTX) to reverse the hypoalgesia shown by rats upon presentation of shock-associated conditional stimuli is related to PAG opioids. Rats were given an s.c. injection of 15% formalin into a hind paw followed by microinjection of either 5µg/0.5µl of NTX or an equal volume of the saline vehicle into the ventral PAG before being placed in an observation chamber in which footshock (1 mA/.5 sec) occurred 24 h earlier. Shocked rats tend to suppress stereotyped reactions to the formalin injection. NTX reversed this suppression. Thus while the PAG may not be critical for the suppression of formalin behavior by systemically administered morphine, the conditional hypoalgesia measured with this test may involve opioids within the PAG.

64.12

PAIN-RELATED BEHAVIORS AND NOXIOUS STIMULUS-EVOKED EXPRESSION OF FOS PROTEIN IMMUNOREACTIVITY IN THE SPINAL CORD ARE INHIBITED BY SUPRASPINAL ADMINISTRATION OF THE MU-SELECTIVE OPIOID RECEPTOR AGONIST, DAMGO. K.R. Gogas, R.W. Presley*, J.D. Levine* and A.I. Basbaum, Departments of Anatomy, Physiology, Anesthesiology and Medicine, University of California, San Francisco, CA, 94143.

Noxious stimulation induces neuronal expression of the *Fos* protein product of the c-fos proto-oncogene in the rat spinal cord. We previously reported that systemic morphine produces a dose-related, naloxone-reversible, inhibition of noxious stimulus evoked *Fos* immunoreactivity (FI) in the spinal cord (Presley et al., 1988). To characterize the relationship between FI and pain behaviors and to examine the contribution of descending inhibitory pathways to opioid analgesia, we studied the effect of supraspinal (icv) administration of the selective mu opioid-receptor agonist, DAMGO ([D-Ala^2, NMe-Phe^4, Gly-ol^5] - enkephalin), on pain behaviors and on spinal cord FI evoked by unilateral formalin injection into the hindpaw of rats.

Formalin elicited a quantifiable behavioral syndrome and evoked spinal cord FI in neurons of laminae I and IIo, the nucleus proprius, the neck of the dorsal horn and in laminae VII, VIII and X of the ventromedial gray. The most intense staining was found in laminae I and IIo and in the neck of the dorsal horn. DAMGO (icv) produced a dose-related (0.006-0.60 µg), naloxone-reversible, inhibition of both the behavior and the spinal cord FI. Although the highest dose of DAMGO tested completely suppressed the formalin-evoked behaviors, the FI was never completely eliminated; substantial labelling persisted in laminae I and IIo. The inhibitory effects of icv DAMGO (0.6 µg) on both the pain behavior and FI were prevented by bilateral dorsolateral funiculus lesions, providing strong support for the contention that stimulation of supraspinal opioid receptors produces analgesia by increasing descending inhibition of spinal cord nociceptive neurons. Supported by: NS14627, 21445 & Training grant NS07265.

64.13

MORPHINE AND U50488H SUPPRESS NOXIOUS VISCERAL STIMULATION-EVOKED FOS PROTEIN IMMUNOREACTIVITY IN THE SPINAL CORD AND NUCLEUS OF THE SOLITARY TRACT (NTS) OF THE RAT. R.W. Presley*, D.L. Hammond, K.R. Gogas, J.D. Levine* and A.I. Basbaum, Depts. of Anesthesia, Anatomy, and Medicine, UCSF, CA 94143, and Dept. of Anesthesia and Critical Care, U. of Chicago, Ill. 60632

The *c-fos* proto-oncogene encodes a nuclear phosphoprotein, Fos, that has been proposed as a marker of neuronal activity in the CNS. We have previously shown that Fos immunoreactivity (FI) evoked in spinal cord neurons by somatic noxious stimulation is suppressed by systemic morphine (Presley *et al.*, Neurosci. Abst. 1988). Here we report on the effect of systemic morphine and U50488H, a κ opioid-receptor agonist, on FI evoked in a model of visceral nociception, intraperitoneal injection of acetic acid in awake rats. The pattern of FI was correlated with the behavior produced by the noxious stimulus.

Fifty micron frozen sections of spinal cord and medulla, from rats perfused with 4% paraformaldehyde, were immunostained with an antiserum directed against Fos. In unstimulated rats, FI was limited to very light labelling in lamina III and IV; only a few labelled cells were noted in the NTS. Two hours after injection of acetic acid, intense FI was present bilaterally in the mid and lower thoracic segments in lamina I, lateral lamina V, VIII and X, and in the intermediolateral cell column (IML). The spinal distribution of FI extended into rostral cervical and sacral segments, primarily in lamina I. Very dense labelling was recorded bilaterally in the NTS; the staining was particularly intense in the commissural nucleus and the nucleus medialis of the NTS. Both morphine (1.0, 3.0, and 10.0 mg/kg sc) and U50488H (3.0, 10.0, and 30 mg/kg sc) injected 20 minutes prior to the acetic acid, produced a dose-dependent suppression of FI in all laminae of the cord, and in the NTS. The suppression was most apparent in the deeper laminae of the cord and in the IML, and was naloxone reversible. Importantly, the opioid effects on staining paralled their effects on stretching behavior evoked by the noxious stimulus. We are presently examining the differential contribution of spinal and vagal inputs to the visceral-evoked Fos changes in the NTS. Supported by NS 14627, 21445, and G.D. Searle.

64.14

TIME COURSE OF PERIPHERAL NEUROMA-INDUCED EXPRESSION OF FOS PROTEIN IMMUNOREACTIVITY IN THE SPINAL CORD OF RATS AND EFFECTS OF LOCAL ANESTHETICS. S.-I. Chi*, J.D. Levine* and A. I. Basbaum, (SPON: D.M. MacDonald). Depts. Anatomy, Physiology and Medicine, UCSF, San Francisco, CA 94143.

It has been suggested that persistent pain produced by nerve injury results, in part, from the development of hyperactivity of regenerating sprouts in the neuroma. To test this hypothesis and to study the pattern of central changes produced by such nerve injury, we have examined the expression of the c-fos proto-oncogene product, Fos, in the spinal cord of the rat up to one month after unilateral transection and ligation of the sciatic nerve to induce a neuroma. Consistent with the topographic organization of primary afferents, acute nerve injury evoked Fos-immunoreactivity (FI) most densely in neurons of laminae I-VII of the ipsilateral L4/5 segments; labelled neurons, however, were detected at all lumbar levels and in the contralateral cord (with a different laminar distribution). After the initial, acute induction of FI by nerve injury, the staining gradually diminished, however, two days after nerve injury, many labelled cells were again detected in laminae I-VII. By 7 days, the staining had again diminished. At two weeks a third peak of FI appeared, also in laminae I-VII; this pattern persisted for the next two weeks.

To test whether the persistent FI observed after injury was dependent on hyperactivity arising in the neuroma, we maintained a proximal sciatic nerve block in the 2 (n=3) and 14 day (n=4) neuroma rats for a four hour period prior to sacrifice. Local anesthetic reduced the early (2 day) peak of Fos expression in laminae I, II and V-VII. The most profound effect was on cells in laminae V-VII. In contrast, the reduction in FI by sciatic blockade at 14 days was no different from that produced by systemic (sc) injection of the local anesthetic. These data suggest that factors in addition to hyperactivity of the neuroma contribute to the central effects of peripheral nerve injury. (Supported by NS 14627, NS 21445 & NS 21647).

64.15

THE ANTINOCICEPTIVE EFFECT OF MORPHINE IS MODULATED BY GABAERGIC NEUROTRANSMISSION. J.H. Rosland* and K. Hole (SPON: B.T. Walther). Dept. of Physiology, University of Bergen, N-5009 Bergen, Norway.

Benzodiazepines and other drugs acting on the GABA-benzodiazepine receptor complex may change the antinociceptive effect of morphine and other opioid drugs. We have shown that diazepam given intraperitoneally to mice antagonizes the antinociceptive effect of subcutaneously injected morphine using the tail flick test. This effect was totally reversed by the benzodiazepine antagonist flumazenil (10 mg/kg), and partially reversed by the $GABA_A$ antagonist bicuculline (4 mg/kg). Bicuculline, although having no effect in itself, potentiated the effect of morphine. Flumazenil had no effect on morphine analgesia. Phenobarbital, which stimulates GABAergic neurotransmission by a direct effect on the Cl^--channels, had a similar effect on morphine analgesia as diazepam. None of these effects could be explained by a change in tail skin temperature.

It was concluded that the antinociceptive effect of morphine is modulated by the GABA-benzodiazepine complex. Stimulation of the GABA-benzodiazepine complex seems to reduce the antinociceptive effect of morphine, while inhibition of the complex may increase the antinociceptive effect of morphine.

64.16

EFFECTS OF NALOXONE, TYR-MIF-1, AND MIF-1 ON MORPHINE-INDUCED ANALGESIA IN THE LIZARD. A.S.Wensel*, M.C.Liles*, M.D.Brown*, A.J.Kastin, G.A.Olson, and R.D.Olson. Dept. of Psychology, Univ. New Orleans, VAMC, New Orleans, LA 70148.

To assess the efficacy of members of the Tyr-MIF-1 family of peptides as opiate antagonists, lizards (**Anolis carolinensis**) were injected sc with 0.0, 0.2, or 2.0 mg/kg of naloxone, Tyr-MIF-1, or MIF-1 followed 10 min later by an injection of diluent or 10 mg/kg of morphine. After another 10 min wait, lizards were either handled for 15 sec or induced into tonic immobility (TI), and then tested for analgesia in a tail-flick apparatus. Half of the lizards were tested after reversal of injection order.

Results showed a significant difference among the antagonists, with the faster latencies associated with naloxone. The agonist was reliable, with morphine producing significantly longer latencies. Injection order was also significant, with the reversed order leading to much quicker times. Finally, injection order interacted with TI. No difference existed after handling, but after TI the latencies increased significantly with the original order but decreased when the order was reversed.

In summary, only naloxone functioned as an opiate antagonist in this paradigm.

64.17

DIFFERENTIAL EFFECT OF TRIGEMINAL AND SPINOTHALAMIC TRACT NOXIOUS STIMULI ON THALAMIC INTRALAMINAR NEURONS IN THE CAT. C.E. Poletti, K. Baker* and W.E. Foote. Pain Research Laboratory, Mass. General Hospital, Boston, MA 02114.

225 single neurons were studied in nucleus centralis lateralis (CL) (140 units) and parafascicularis (Pf) (85 units). The median spontaneous firing rate was 5-10 spikes/sec. The spike durations, contours and spatial distributions represented somato-dendritic neuronal recordings. The noxious stimuli applied to the spinothalamic tract (STT) were: limb joint rotation and hyperextension; and firm forceps pinch of body, limbs, and tail. The stimuli applied to the trigeminal system were: firm forceps pinch of facial skin and tongue; hyperextension of the jaw; and cornea brush.

In CL 44% of units (11/25) responded to STT noxious stimuli. In contrast, only 1.5% (3/200, 115 in CL; 85 in Pf) responded to trigeminal noxious stimuli. The 3 trigeminal responsive units showed convergent STT input.

This differential influence of trigeminal and STT mediated nociception on CL and Pf may be partially responsible for the unique features of oro-facial pain syndromes.

64.18

PAIN THRESHOLDS AND MORPHINE EFFICACY MODIFIED IN HABITUATED RATS: BULBOSPINAL SEROTONIN IMPLICATED.

R.J. Milne and G.D. Gamble*, Dept. of Physiol., Univ. of Auckland, Auckland, New Zealand.

Repeated exposure of a rat to a testing environment ('habituation') enhances its sensitivity to noxious thermal stimuli and reduces its responsiveness to a subsequent acute dose of morphine ('behavioural tolerance'). We have interpreted this and other findings in terms of reduction of tonic descending inhibition of nociceptive transmission [Gamble, G.D. & Milne, R.J., Neurosci. Lett., 96:312-317, 1989; Milne, R.J. et al, Brain Research, (in press)]. Do these effects involve serotonin which is known to be involved in stress and arousal? Wistar rats (200-250g) were housed in an animal facility until the commencement of the experiment ('novices') or habituated by extensive handling and sham nociceptive testing for at least 5 days. Both novice and habituated rats were treated with either parachlorophenylalanine (PCPA: 350 mg/kg daily for 3 days) or 5-hydroxytryptaphan (5HTP: 80 mg/kg i.p) to depress or enhance serotonin synthesis respectively, or vehicle and then randomly subjected to a variety of morphine doses (D). Data were fitted to the model: $E(D) = E_0 + ((E_{max} \times D^n)/(ED_{50}^n + D^n))$, where E_{max} is the extrapolated maximal effect of morphine from baseline (E_0). In novice animals, PCPA reduced E_0 and E_{max} on tail flick and leg flexion (49°C) tests. 5HTP increased both parameters. In habituated animals, both drugs had little effect. PCPA had no effect upon E_0 or E_{max} in acute spinal animals. 5,7-DHT [20µg ith with DMI 25mg/kg s.c) or methysergide (30µg) reduced tail flick latencies of novice animals by 37%, to that of habituated animals. Neither drug had any effect on tail flick latencies of habituated animals. Mean cutaneous tail temperatures were unchanged. These results suggest that the bulbospinal contribution to morphine analgesia as established in experimentally naive animals may represent an interaction between the stress of environmental novelty and the action of morphine.

64.19

PREVIOUSLY FOOD DEPRIVED RATS EXHIBIT A NALTREXONE REVERSIBLE INCREASE IN BOTH REACTIVITY TO SHOCK AND SHOCK-INDUCED ANALGESIA. P.A. Illich & J.W. Grau. Dept. of Psychology, Texas A&M University, College Station, TX 77843.

Prior work has shown that exposure to uncontrollable shock elicits a strong, hormonally mediated, opioid analgesia (Terman et al., Science, 226:1271, 1984). In addition, it sensitizes subjects to becoming analgesic 24 hrs later upon exposure to mild shock (Grau et al., Science, 203:1409, 1981). Here we assess the impact of another manipulation that elicits a hormonally mediated opioid analgesia, food deprivation (Hamm et al., Physiol. & Beh., 35:879, 1985). After 2 days of baseline testing, deprived subjects had their food removed. At the end of day 3, they received 9 grams of food. Food was returned on day 4. Contrary to other reports, food deprivation per se did not induce a significant analgesia. On day 5, 24 hrs after food was returned,we assessed the impact of mild shock (3, 0.75-sec, 1-mA). Interestingly the subjects who had been previously food deprived vocalized and strained significantly more to the mild shock. Oddly, this heightened reactivity was blocked by naltrexone. After the last shock, pain reactivity was assessed with the tail-flick test. Previously food deprived subjects exhibited a potentiated analgesia. This effect too was attenuated by naltrexone.

64.20

EFFECTS OF FREQUENCY, INTENSITY, AND OPIATE-RECEPTOR ANTAGONISM ON VAGAL AFFERENT-MEDIATED ANTINOCICEPTION.

D.F.B. Bossut, E.A. Whitsel, W. Maixner, Dental Research Ctr., Univ. North Carolina at Chapel Hill.

This experiment further evaluated conditioning parameters of the right thoracic vagus (RTV) which inhibit the jaw-opening reflex (JOR) in cats and the putative role of opioids in mediating these effects. An array of frequencies (1-10 Hz) and intensities (0.1-2.0 mA) were delivered for 90 sec to the central end of the RTV before and during naloxone infusion (1 or 10 mg.hr/kg, iv). JOR inhibition occurred at stimulation above 1 Hz and above 0.1 mA and appeared maximal at 5 Hz x 2 mA. The magnitude of inhibition correlated with the density of stimulation (frequency x intensity) but was independent of cardiovascular responses to vagal stimulation. Post-stimulation inhibition depended on the magnitude of inhibition observed during stimulation. Naloxone treatment failed to alter JOR inhibition at all conditioning parameters examined.

These findings indicate that antinociceptive effects induced by RTV afferent stimulation increase with frequency and intensity, are independent of cardiovascular responses, and are insensitive to opiate-receptor blockade.

Supported NIDR grant DE08013 and DE0750901 (W.M.).

CYTOSKELETON, TRANSPORT AND MEMBRANE TARGETING

65.1

A NOVEL DOMAIN FOR INTRACELLULAR TRAFFIC GUIDES GAP-43 TO PARTICULAR MEMBRANE DOMAINS. M.X. Zuber*, S.M. Strittmatter* and M.C. Fishman. Howard Hughes Medical Institute, Developmental Biology Laboratory of the Massachusetts General Hospital, Boston, MA 02114

GAP-43 is a membrane protein enriched in the growth cones of neurons. It is not known what regions of this molecule cause its hydrophobic association with the membrane. We have found that the GAP-43 protein, when expressed in neuronal or in non-neuronal cell lines, binds tightly to the membrane and can be extracted by hydrophobic reagents under conditions similar to that used to extract GAP-43 from growth cone membranes. As determined by site-directed mutagenesis, particular amino acids at the amino terminus have been found to be critical to the membrane binding. Mutants that lack large internal pieces of the molecule or differ at the carboxy terminus from GAP-43 are unaffected in membrane binding. The domain of GAP-43 defined as important for its membrane binding is sufficient when coupled to chloramphenicol acetyl transferase to cause membrane association of this normally cytosolic protein. The distribution of transfected GAP-43 and these chimeric proteins appears to be the same in NGF-treated PC12 cells, including an enrichment of these proteins on the growth cone membrane. This work suggests that the amino terminus of GAP-43 includes a novel type of sorting signal that is responsible for its distribution to particular regions of the nerve, including the growth cone. This distribution is compatible with our previous suggestion that GAP-43 may play a direct role in causing the extension of filopodia (Zuber et al., Science, 1989).

65.2

COMPARTMENTATION OF CREATINE KINASE ISOFORMS IN RAT BRAIN. David L. Friedman, Bruce R. Kenwood, Peter R. Puleo, M. Benjamin Perryman, (SPON: Michael D. Schneider). Departments of Medicine, Molecular Cardiology Unit, Baylor College of Medicine, One Baylor Plaza, Room 506C, Houston, Texas 77030.

Multiple creatine kinase (CK) isoforms serve to sustain the available energy supply within cells through the interconversion of ADP and creatine phosphate. In muscle, the enzymes are physically associated with specific cellular ATPases such as the actinomyosin ATPase, sarcoplasmic Ca^{++} ATPase, and plasma membrane Na^+ pump. Brain tissue was previously thought to contain only BB-CK and the mitochondrial isoform. We now provide evidence for the presence of MM-CK isoforms in rat brain, which have electrophoretic and chromatographic properties distinct from muscle MM-CK. Using *in situ* hybridization and immunocytochemistry we have localized CK isoforms to specific cell populations in hippocampus and cerebellum. Our results demonstrate that these CK isoforms are differentially distributed within cells. The BB-CK isoform is primarily associated with the nuclear membrane of most Pyramidal and granule cells of the hippocampus, and some Purkinje cells of the cerebellum. The MM-CK isoform, in contrast, is associated with the dendritic fields of these cells, especially in the locunosum molecular layer of CA1-CA3, and the molecular layer of the cerebellum. In contrast, the mRNA for these CK isoforms is found in the cytoplasm of the somata in cells which express these CK proteins. These results suggest a complex cell specific regulation of CK isoenzyme gene expression and compartmentation in rat brain.

65.3

SECRETION OF VGF8a: A NGF-INDUCIBLE GENE. R.Possenti*°, M.Benedetti*, P.Galeffi*, B.Paterson#, A.Levi*. °Dept. Exper. Med. Univ. Tor Vergata, Rome; #NIH, NCI, Bethesda MD; *I. Neurobiology CNR V.le Marx 15, 00156 Rome Italy. We have previousy isolated a cDNA clone induced by NGF in PC12 cell line (Levi et al.1985 Science 229,1393-1395) and the complete sequence of the protein named VGF8a, has been deduced from the cDNA sequence. Two different portions of the proteine, as fusion products with ßGal, have been expressed in E. Coli to generate antisera. By these antisera we show that VGF8a is associated with intracellular vescicles and we also prove that the secretion of this protein is under stimuli of secretagogue-agents. (Possenti et al.EMBO J. in press). Immunoblot with antisera shows two bands in SDS page and preliminary data indicate that the larger molecule is post translationally processed into the smaller one. Moreover sulfatation and phosphorilation of this protein can be evidentiated by immuno-precipitation. We are now investigating which portion of the VGF8a is involved in the regulated secretion by fusing different parts of the protein with \chain of the immunoglobuline (Clone \1S43). In this way we hope to elucidate a possible interference of the VGF8a protein over the constitutive secretory pathway of the \chain.

65.4

MEMBRANE-BOUND PHOSPHOLIPASE A2 IN BOVINE ADRENAL MEDULLARY SECRETORY GRANULES IS CALCIUM DEPENDENT. E. Hildebrandt* and J. P. Albanesi* (SPON: L. C. Ellis, Jr.). Dept. of Pharmacology, Univ. of Texas Southwestern Medical Research Center, Dallas, TX 75235.

In chromaffin cells, catecholamine secretion requires Ca and is associated with release of arachidonate (AA), a precursor for eicosanoid biosynthesis which may also act as a membrane fusogen. Release of AA from phospholipids is believed to be controlled by a Ca-dependent phospholipase A2 (PLA2), but the enzyme participating in exocytosis has not been identified. We prepared subcellular fractions of bovine adrenal medulla and assayed PLA2 activity as follows. Fractions were incubated at 37°C with sonicated vesicles of diarachidonylphosphatidylcholine (0.1 mM) containing 1-palmitoyl-2-(^{14}C-arachidonyl)-phosphatidylcholine ($7 \cdot 10^5$dpm/ml) in 0.15 M Tris pH 9.0 for 90 min. The released ^{14}C-AA was extracted, isolated by TLC, and counted. When comparable amounts of protein (0.25 mg/ml) from each fraction were assayed, PLA2 activity was greatest in the secretory granule membrane (GM) fraction. The activity was not removed by washing in 0.25 M KCl, and hence appeared tightly bound. GM PLA2 was stimulated 2.5-3 fold by 2.5 mM Ca, but 7-10 fold stimulation by Ca was achieved in the presence of 1.25 M sucrose or 50% glycerol. The Ca-dependent PLA2 had an alkaline pH optimum, and it was inhibited by addition of NaCl, KCl, or Tris-Cl. The GM PLA2 exhibits properties appropriate for a regulable enzyme involved in exocytosis.

65.5

THYROID HORMONE PROMOTES INTERNALIZATION OF MEMBRANE PROTEINS VIA AN INTERACTION WITH THE ACTIN CYTOSKELETON IN ASTROCYTES. A.P. Farwell*, R.M. Lynch*, W.C. Okulicz*, J.L. Leonard* (SPON: J. Cooke). Mol. Endo. Lab., Depts. Physiol. & Ob/Gyn., UMass. Med. Sch., Worcester, MA 01655

In cultured astrocytes, dibutyryl cAMP (bt2cAMP) induces synthesis of iodothyronine 5'deiodinase, a short-lived integral membrane protein. Enzyme turnover is modulated by a thyroxine (T4)-dependent mechanism that requires an intact actin cytoskeleton. The effects of hormones on the redistribution of this enzyme, labeled with N-bromoacetyl-T4, were examined with an anti-T4 antibody. Immune complexes were visualized by indirect immunocytochemistry and F-actin with FITC phalloidin. In serum-free bt2cAMP-stimulated cells, a distinct punctate pattern was seen at the cell periphery that was absent in unpermeabilized cells, showing an intracellular location. Laser scanning confocal microscopy localized this pattern to the plasma membrane. Cell F-actin content was markedly reduced. After 10-20 min exposure to T4, most of the membrane-associated labeling was internalized, coinciding with repolymerization of the actin cytoskeleton. Inhibition of actin polymerization blocked this rapid, T4-stimulated response. Treatment for 10-20 min with T3 or BSA failed to promote movement or to polymerize actin. These data suggest that T4 regulates polymerization of the actin cytoskeleton and promotes the rapid internalization of specific membrane proteins in cultured astrocytes.

65.6

AXONAL TRANSPORT OF UBIQUITIN IN NORMAL AND HEAT SHOCK CONDITIONS. A. Bizzi*, B. Schaetzle*, L. Autilio-Gambetti and P. Gambetti Div. of Neuropathology, Case Western Reserve University, Cleveland OH 44106.

Ubiquitin (Ub), a heat shock protein, is found in cells either free or covalently bound to various proteins. Ub is thought to play a regulatory role by either modifying the function of proteins or targetting them for degradation. Recently, Ub has been found to be associated with abnormal inclusions in several neurodegenerative diseases. In Alzheimer Disease, Ub is associated with the paired helical and straight filaments in neuronal perikarya and distal neuronal processes. Ub is also present in abnormal axons of giant axonal neuropathy and of infantile neuroaxonal dystrophy. We have used the rat visual system to investigate whether free Ub is axonally transported and if there are changes in the amount of transported Ub following heat shock. Heat shock was induced in 5 week-old rats by raising their body temperature to 41°C for 15 min in a heated box. Controls were kept in a box at room temperature for the same length of time. Two hrs later, animals were injected intraocularly with ^{35}S-methionine and sacrificed after 4 hrs, 9 days and 25 days to study the fast and slow components of axonal transport. Labeled proteins from retinas and segments of optic system were analyzed in flurograms of 1D- and 2D-PAGE. Nine days after ^{35}S administration, labeled Ub (8 kDa, pI 6.2) was present in the optic tract, along with labeled polypeptides characteristic of slow component b. Labeled Ub was not detected in either the fast or slow componet a of axonal transport. Following heat shock, the amount of transported labeled Ub, relative to the major SCb components, was not different from that of controls. Supported by NIH Grants NS14509 and AG00795.

65.7

CHANGES IN THE RATE OF SLOW TRANSPORT AFTER AXOTOMY AND DURING THE REGENERATION OF ADULT RAT RETINAL GANGLION CELL AXONS. L. McKerracher, M. Vidal-Sanz*, C. Essagian*, A.J. Aguayo. Neurosci. Unit, Montreal Gen. Hosp., and McGill Univ. Montreal, Quebec, H3G 1A4.

To investigate changes associated with injury and regrowth in the adult mammalian CNS we examined the slow transport of cytoskeletal proteins following optic nerve crush, during the regeneration of retinal ganglion cell (RGC) axons, and in controls. Axotomized RGC axons were prelabelled with ^{35}S-methionine by an intraocular injection then crushed 6 days later. To obtain labelled regenerating RGC axons, peripheral nerve (PN) grafts were attached to the ocular stump of transected optic nerves 7 days prior to injection. Five to 60 days after labelling the segments of normal, axotomized or PN-grafted RGC axons were examined by SDS PAGE. The rate of tubulin and neurofilament (NF) transport was decreased following injury compared to the unoperated controls. These changes were first detected 6 days after crush and lasted at least 60 days. In contrast, the rate of tubulin and NF transport was increased 2-fold in regenerating RGC compared to controls. In addition, there was only one slow transport rate rather than the two separate rates observed in unoperated animals. The changes in slow transport during RGC regeneration may reflect growth-associated responses which are not elicited by injury alone.

65.8

DENDRITIC TRANSPORT OF RECENTLY SYNTHESIZED RNA IN HIPPOCAMPAL NEURONS IN VIVO. R. Kleiman, G. A. Banker[1] and O. Steward. Dept. of Neuroscience, Univ. of VA, Charlottesville, VA 22908 [1]Dept. of Anatomy, Albany Medical College, Albany, N.Y., 12208

Recent studies have shown that hippocampal neurons in culture selectively transport newly synthesized RNA into dendrites (Davis et al., 1987, Nature, 330:477-479). The rate of transport is about 250-400 µm/day. The present study evaluates RNA transport in hippocampal neurons in vivo. 17 day old rat pups received stereotaxic, intracranial injections of ^{3}H-uridine (20µCi/pup) to selectively label newly synthesized RNA. Pups were perfused with 10% phosphate buffered formalin at 15 min, 30 min, 1 hour, 3 hours, 6 hours, 12 hours, and 24 hours following the uridine injection. Brains were embedded in paraffin and 10µm sections were cut and prepared for autoradiography. 15 min post-injection, the label was primarily localized to the nucleus; at 30 minutes label began to appear in the cytoplasm. One hour following injection, the label had expanded to cover the cell body, although there was a higher density of label in the nucleus relative to the cytoplasm. Three hours post injection proximal portions of the dendrites became labeled. By six hours dendritic profiles were heavily labeled, and could be seen to extend into the molecular layer for at least 50-100µm. At longer intervals there was considerable redistribution of the label making it difficult to trace individual dendritic profiles. By 12 hours the relative density of label in the nucleus compared to the cell body had decreased, and by 24 hours post-injection there was no difference in the density of labeling in the nucleus and cell body. These results demonstrate that hippocampal neurons in vivo transport newly synthesized RNA into their dendrites. The rate of transport is similar to that described in vitro. Supported by NIH NS23094 to GB and OS. RK received a predoctoral fellowship from NIH HDO7323.

65.9

QUANTITATIVE ANALYSIS OF THE VELOCITY OF DENDRITIC TRANSPORT OF RNA BY MATHEMATICAL MODELING. L. Davis, B. Burger, G.A. Banker[1], and O. Steward. Dept. of Neuroscience and the Neuroscience Program, Univ. of VA, Charlottesville, VA 22908. [1]Dept. of Anatomy, Cell Biology and Neurobiology, Albany Medical College, Albany, NY, 12208.

We previously demonstrated that recently synthesized RNA is transported into the dendrites of cultured hippocampal neurons (Davis, *et al.*, (1987) Nature 330; 477-479). We reported that the transport velocity was approximately 0.25mm/day based on measurements of the distance of label in the total population of dendrites and 0.5mm/day in the dendrites with the longest distance of label. Because our measurement of transport velocity would be compromised by the fact that the total population of dendrites included some very short dendrites, we devised a mathematical model to determine transport velocity in populations of dendrites of varying lengths. The length of the dendrites was determined following MAP2 staining. The mean distance of transport in either the total population of dendrites, or the longest dendrites, was modeled where the maximum distance of transport was equal to the length of the dendrite. The modeled time course of transport was compared with the observed time course of transport in sister cultures that were pulse-labelled with 3H-uridine and either immediately fixed or allowed to transport 3H-RNA for 1.5, 3, 6, 9, 12, or 24 hours pdor to fixation and processing for autoradiography. Use of the model suggested that the average transport velocity in the total population of dendrites was 11um/hour (0.26mm/day). However, the modeled transport velocity that best fit the time course of transport in the dendrites with the longest distance of label was 21um/hour (0.5mm/day). These results suggest that the velocity of dendritic transport may be faster in longer dendrites. Supported by NIH NS23094 to GB and OS. LD was the recipient of a predoctoral fellowship from NIH NS07199.

65.10

TRANSPORT KINETICS OF SLOW COMPONENT b PROTEINS IN OPTIC NERVE AND TRACT WINDOWS. P. Paggi[1,2], R.J. Lasek[1] and M.J. Katz[2]. Bioarchitectonics Cntr CWRU School of Medicine, Cleve. OH 44106. [2]Dip. Scienze e Tecnologie Biomediche e Biometria, Università de L'Aquila, 67100 L'Aquila, Italy.

The transport kinetics of three slow component b (SCb) proteins (clathrin, a 30 kDa protein, and actin) were studied in axons of mouse retinal ganglion cells. ^{35}S-methionine was injected into the right vitreous body of anesthetized C57Bl mice. At 1-119 days after injection, the mice were killed and 2 mm segments from the right optic nerves (N) and the contiguous left optic tracts (T) were removed; these segments represented "windows" through which we watched the movement of axonally transported proteins. Radiolabeled proteins were separated by 1- and 2-dimensional SDS-PAGE, and individual radiolabeled bands were quantified. The three studied proteins entered and cleared the optic axons between 1 and 119 days post-labeling. Each protein had a broader transport wave in the more distal T window than in the N window. This spreading of the transport waves as the proteins advance along the axon appears to be produced by a playing out of the natural heterogeneity of axonal transport rates within each population of proteins. Although these proteins can all be classified with SCb, their detailed kinetics differed: for instance, the median transit times and the rates of movement differed. The residence times of clathrin and the 30 kDa protein were similar between N and T. This is consistent with the simple hypothesis that the local environment of the optic axon remains unchanged throughout the length of the axon.

65.11

ACCELERATED TRANSPORT OF NEUROFILAMENTS IN 2,5-HEXANEDIONE NEUROPATHY IS ACCOMPANIED BY A DECREASE IN PHOSPHORYLATION-DEPENDENT IMMUNOREACTIVITY. D.F. Watson, K.P. Fittro* and J.W. Griffin. Dept. of Neurology, Johns Hopkins Hospital, Baltimore MD 21205.

Previous experiments have shown that high levels of neurofilament (NF) phosphorylation (P), as detected by immunoassay, are correlated with slow rates of NF transport along axons. We have examined the immunoreactivity of NF in rats with accelerated NF transport due to intoxication with 2,5-hexanedione (HD) 0.5% in drinking water for 8 weeks. Segments of L5 dorsal roots, ventral roots, and peripheral sensory axons were dissolved in 8M urea\ 5% 2-mercaptoethanol, and immunoreactivity was determined toward antibodies SMI31 (P-dependent) and SMI32 (P-inhibited) and BM68 (P-independent epitope on the low molecular weight NF subunit) by quantitative enzyme-linked immunosorbent assay.

In HD rats the transport of labeled NF was accelerated. The ratio SMI31/SMI32 was significantly decreased in HD rats at all sites. This result extends prior observations that lower NF P-dependent immunoreactivity is associated with higher NF transport rate.

65.12

SLOW AXONAL TRANSPORT OF TUBULIN: A QUANTITATIVE ANALYSIS. A.P. Amaratunga*, J.F. Skee* and R.C. Weisenberg. Biology Department, Temple University, Philadelphia, PA 19122

The slowest phase of Axonal transport, termed slow component a (SCa), transports tubulin in the axon. The transport state of axonal tubulin is an unresolved issue: one view is that the microtubule lattice itself moves; an alternative is that tubulin moves relative to a stationary microtubule lattice. To help resolve this issue we quantitatively analyzed the SCa (labeled) tubulin and the total (labeled and unlabeled) tubulin in the mouse optic nerve. We compared the amounts of soluble and particulate tubulin using microtubule stabilizing (3 Mm $MgCl_2$, 1Mm EGTA, 25% glycerol at 37°c) and destabilizing (5 Mm $CaCl_2$, 1M Nacl at 4°C) media. Preliminary results indicate that more than 60% of the transported tubulin in the axon is present in an insoluble state under conditions that will depolymerize both cold labile and stable microtubules. However, our results indicate that this labeled tubulin fraction represents less than 20% of the total tubulin in the axon. When microtubule stabilizing medium is used, there is only about a 5% increase in the amount of particulate labeled tubulin while the total particulate tubulin increases by more than 50%. These results demonstrate that the labeled SCa tubulin is a minor fraction of the total tubulin in the axon and that a large majority of axonal microtubules are stationary. Using negatively stained electron microscopy, we observed microtubules only in the particulate fraction prepared in microtubule stablilizing medium; no microtubules were visible in the microtubule destabilizing particulate fraction. These results support the hypothesis that SCa involves the movement of an insoluble, non-polymeric form of tubulin along a stationary microtubule lattice (Weisenberg et al. Science (1987) 238:1119, Bamburg et al. Nature (1986) 321:788).

65.13

AXONALLY TRANSPORTED PROTEINS AND ORGANELLES DIFFER IN THEIR TIME REQUIRED TO REVERSE DIRECTION AT A LESION. R.E. Snyder, X. Chen* and R.S. Smith. Depts. of Applied Sciences in Medicine, Surgery, and Anatomy and Cell Biology, University of Alberta, Edmonton, Canada T6G 2G3.

Although both proteins and organelles which undergo rapid axonal transport are known to be able to reverse their direction of transport adjacent to the proximal side of a lesion, it has not been established whether they do so as a common unit. The question of whether proteins and organelles reverse their direction independent of one another was studied by measuring the time required for each to reverse direction at a lesion. Transport and reversal of ^{35}S-methionine labelled protein was monitored using a position-sensitive detector of ionizing radiation; organelle transport and reversal was monitored in isolated myelinated axons using computer-enhanced video microscopy. Both studies were performed using amphibian sciatic nerve maintained *in vitro* at room temperature. Under similar conditions, protein was found to require ≥1.50 h to reverse its direction of transport at a lesion, whereas organelles were observed to reverse direction within ≤10 min following their arrival at a lesion. The results of this study demonstrate that proteins and organelles differ in their kinematics of reversal, and suggest that proteins must dissociate from organelles prior to reversal at a lesion. (This work was supported by the Medical Research Council of Canada.)

65.14

REVERSAL OF RAPID AXONAL PROTEIN TRANSPORT ADJACENT TO A LESION IS BLOCKED BY LEUPEPTIN, BUT REVERSAL OF ORGANELLE TRANSPORT IS UNAFFECTED. R. S. Smith and R. E. Snyder. Depts. of Surgery, Anatomy and Cell Biology, and Applied Sciences in Medicine, University of Alberta, Canada.

It has been suggested that proteolysis is a necessary event in the reversal of rapid axonal transport adjacent to a lesion. We have tested this idea by investigating in crushed sciatic axons of Xenopus laevis, 1) the effect of leupeptin on the reversal of rapid anterograde protein transport, and 2) the effect of leupeptin and low calcium on the reversal of organelle transport. In the studies of protein transport, the progression of a pulse of protein, metabolically labeled with ^{35}S-methionine, was followed in living sciatic nerves using a position-sensitve detector of ionizing radiation. Control preparations showed that 30 - 50% of anterogradely transported protein became retrogradely transported at the proximal side of a lesion. Treatment of the specimens with 0.1 mM leupeptin abolished all detectable reversal of anterograde protein transport. Organelle transport was detected in isolated myelinated axons using computer-enhanced video microscopy. In preparations bathed in a potassium glutamate based medium with added ATP, 65 % of anterogradely transported organelles and 41 % of retrogradely transported organelles reversed direction adjacent to a crush lesion. Modification of Ca^{2+} over the range 10^{-8} - 10^{-4} M with or without the addition of 1.0 mM leupeptin did not significantly change the proportion of organelles that changed transport direction. This result suggests that proteolysis is required for the reversal of protein transport, but not for the reversal of organelle transport. (Supported by MRC, Canada and by AHFMR)

65.15

TIME DEPENDENT ACCUMULATION OF RADIOLABEL IN THE DORSAL ROOT GANGLIA AFTER INTRANEURAL INJECTION OF [^{3}H]myo-INOSITOL INTO THE RAT SCIATIC NERVE. S. Padilla, C.N. Pope[1], and V.Z. Wilson[2]. (SPON: M. Gage) Neurotox. Div. (MD-74B), US E.P.A. and [2]NSI, Res. Tri. Pk, NC 27711.

Although autoradiography has demonstrated local axonal incorporation of [^{3}H]inositol after intraneural injection (Gould,1976,Br.Res.,117,169-174),retrograde axonal transport of phosphatidylinositol has only been demonstrated after injection of lipid precursor into the cell body regions (Armstrong et al,1986,J. Neurosci.5,965-969). We now report that after microinjection (1µl) of myo-[2-^{3}H]-inositol (12.8Ci/mmol;10µCi) into the rat sciatic nerve (45-50mm distal to L_4 and L_5 DRGs) time dependent accumulation of [^{3}H] occurred in the DRGs ipsilateral (ipsiDRGs) to the injection site: at 2 or 8 hours after isotope injection, the ratio of dpm present in the ipsiDRGs to that in the contralateral DRGs was not significantly different from unity, but by 24hrs this ratio rose to 10.0(±2.5, sem); at 46.3hrs=12.3(±3.2) and at 72 hrs=10.4 (±1.4). At 46.3 hrs post injection, only about 43% of the [^{3}H] in the injection site was $CHCl_3$/MeOH soluble, while >90% of the [^{3}H] in the ipsiDRGs was $CHCl_3$/MeOH soluble. The presence of this delayed, primarily lipid, radiolabeling in the ipsiDRGs is probably due to retrograde axonal transport because: (1)if the isotope is injected closer to the DRGs, the label appears sooner in the ipsiDRGs, and (2)nerve ligation prevents the radiolabel accumulation.

[1]Supported by a National Research Council associateship.

65.16

THE ESSENTIAL ROLE OF MICROTUBULES IN RESPONSES TO A HYPOTONIC ENVIRONMENT IN TISSUE CULTURED NEURITES. H.Takahashi[1]*, H.Horie[3], Y.Tanaka[2]*, and T.Takenaka[3]. Dept. of Ophthalmol., UOEH[1], Keio Univ.[2] and Dept. of Physiol., Yokohama City Univ.[3], Japan.

Mammalian neuronal cell bodies can adapt to a hypotonic environment with a cell volume regulatory mechanism (Horie, H. et al., Brain Research, 477, 233, 1989). In this study, responses of neurites growing from cultured dorsal root ganglion neurons dissected from 3-month-old mice to a hypotonic environment were analyzed by a Nikon phase contrast microscope equipped with a video-system. When a half osmolal concentration solution was applied to neurites, neurites partially swelled and then shrank to a smaller size than the initial. These partial swellings were strongly enhanced by the application of a hypotonic solution after the 1 hr treatment with 1×10^{-5}M colchicine. However, after the 5 hr treatment with 2×10^{-6}M taxol, the hypotonic solution scarecely induced morphological changes in neurites. These results indicate that swelling might occur in a specific region in neurites where microtubule density is very low when a hypotonic solution is applied.

65.17

A PREPARATION OF NEURONAL CELL BODIES FROM RAT SUPERIOR CERVICAL GANGLION PERMITTING STRUCTURAL ANALYSIS AFTER DIRECT RAPID FREEZING. L.L. Hall and T.S. Reese. Lab. of Neurobiology, NINDS, NIH, Bethesda, MD 20892.

Direct freezing yields optimal preservation of native tissue structure and chemistry, permitting subsequent analyses by a variety of structural and analytical techniques. Application of these techniques to neuronal cell bodies has previously been limited to one preparation, the ventral cochlear nucleus, due to the physical requirement for close proximity to the cooled copper freezing surface. A method for desheathing superior cervical ganglia (SCG) from adult rats *in vivo* was developed in order to expose these neuronal cell bodies to optimal rapid freezing; our goal was to use freeze-substitution to analyze the reorganization of microtubules and organelle domains during chromatolysis, which is clearly manifested in axotomized SCG neurons. Ice crystals were imperceptible in the freeze-substituted neuronal cell bodies adjacent to the freezing face of the SCG. When compared with perfusion-fixed ganglia, the hallmarks of effective cryopreservation were present: smooth membrane contours; prominent and completely stained microtubules; an abundance of smaller cytoskeletal elements, evenly distributed throughout the perikarya; and increased electron density of the mitochondrial matrix. Additionally, cisternae of the rough endoplasmic reticulum were grouped more loosely in the perikarya of freeze-substituted neurons and their wider lumens contained a sparse filamentous network. Individual Golgi cisternae typically had narrow lumens. Further experiments suggested that handling does not produce these changes; our images appear to represent the native state of the ER and Golgi membranes. Thus, the SCG preparation described here appears to be suitable for a variety of analytical and structural studies of neuronal cell bodies which depend on direct rapid freezing.

65.18

SEPARATION OF NEUROFILAMENT POLYPEPTIDES AND PROTEOLYTIC FRAGMENT BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC). J.C. Troncoso, J.L. March*, E. Barbosa*, V.W. Vogel*, M. Häner* and U. Aebi*. Neuropathology Lab., The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

To assess variations in neurofilament (NF) composition in different neuronal compartments and anatomical regions, we have begun to use HPLC to separate and quantitate NF polypeptides from bovine spinal cord. Following solubilization of NF in 6 M urea, reproducible separation of the three NF subunits (NF-H, NF-M, and NF-L) was achieved using a Mono Q anion exchange column (Pharmacia) eluted with a multistep NaCl gradient (200-500 mM). All three NF subunits reassembled into filaments following removal of urea. There was consistent separation of the 54-kD protein. Sequencing of this protein revealed three regions of complete homology with the reported protein sequence of porcine NF-L (residues 99-105, 400-409, and 438-442). We were not able to reconstitute filaments from the 54-kD polypeptide. Subsequently, we have determined that this polypeptide accumulates distal to a ligature placed around rat sciatic nerve. We think that the 54-kD polypeptide represents a major proteolytic fragment of NF-L. Studies of the axonal transport of this NF proteolytic fragment are in progress.

65.19

CHARACTERIZATION OF NEUROFILAMENT ASSOCIATED PROTEIN KINASES AND RESOLUTION OF A FRACTION THAT PREFERENTIALLY PHOSPHORYLATES THE MID SIZE NEUROFILAMENT SUBUNIT. A. Dosemeci*, C. Floyd* and H.C. Pant. Laboratory of Neurochemistry, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD 20892, USA

Protein kinases associated with a neurofilament (NF) preparation from bovine spinal cord were extracted with 0.8M KCl solution and the extract was assayed for its ability to phosphorylate endogenous and exogenous substrates. It was shown that the extract contains regulator independent kinase activity that can phosphorylate high-, middle- and low-molecular weight NF subunits (NF-H, NF-M, NF-L) and casein. In addition, assays utilizing activators, inhibitors, protein and peptide substrates specific for identified kinases revealed a significant level of Ca^{2+}-calmodulin dependent protein kinase activity and some cyclic nucleotide- and Ca^{2+}-phospholipid-dependent kinase activities. Fractionation of the salt extract by gel filtration chromatography (Ultragel AcA44) resolved a kinase activity identified by its ability to phosphorylate purified NF-M. This fraction was shown to be devoid of Ca^{2+}- and cyclic nucleotide-dependent kinases. The kinase activity in the fraction could phosphorylate all three NF-subunits, either in isolated form or in assembled form, as well as following exhaustive dephosphorylation with alkaline phosphatase. When equimolar concentrations of proteins were employed, the level of phosphate incorporation was in the following order: α-casein>NF-M>NF-H≅NF-L. The results suggest that this fraction contains a unique protein kinase which shows a preference for NF-M compared to NF-H and NF-L.

65.20

PHOSPHORYLATED NEUROFILAMENT ACCUMULATION IN NEURONAL PERIKARYA AND DENDRITES IN HUMAN BRAIN FOLLOWING STROKE OR OTHER FOCAL LESIONS. J.C. Hedreen, V.E. Koliatsos and D.L. Price. Neuropathology Lab., The Johns Hopkins Univ. Sch. Med., Baltimore, MD 21205.

The accumulation of phosphorylated neurofilaments in neuronal perikarya and dendrites has been demonstrated in rats following experimental transection of axons in the central nervous system [Koliatsos et al., *Brain Res.* 482: 205-218, 1989] and in humans in a variety of degenerative diseases. In the present study, we examined autopsy cases of stroke or other focal lesions of human brains (survival from five days to ten weeks) and control cases without neurological disease. Following formalin fixation, tissues were processed for immunocytochemistry of phosphorylated neurofilaments.

Neurons whose axons were thought to pass through the lesion sites often contained perikaryal and dendritic accumulations of phosphorylated neurofilaments. Such neurons were identified in the locus coeruleus, thalamus, and homotopic contralateral cortex following neocortical lesions, and in stratum oriens of hippocampal field CA3 following an anoxic lesion of Sommer's sector. The abnormal accumulation of phosphorylated neurofilaments in cell bodies and dendrites is presumed to be a retrograde response of the neuron to axonal injury and may prove useful for the study of neuronal connections in human brain.

65.21

cDNA CLONING OF A NEUROFILAMENT-LIKE GENE FROM THE SQUID. B.G. Szaro, J. Battey*, and H.C. Pant. Lab. Neurochem., NINDS, NIH, Bethesda, MD 20892.

Neurofilament (NF) proteins with conserved biochemical and immunological properties have been identified in all vertebrates and in several invertebrate phyla. Although cloned and sequenced in some vertebrate species, no invertebrate NF sequences have yet been described. Using a cDNA probe to a mouse NF protein at low stringency, we screened a cDNA library made from squid optic lobe. We isolated three distinct clones which hybridized with an abundant 5 kilobase RNA on Northern blots of total RNA from squid optic lobe and from stellate ganglia. Two of the three clones overlapped and contained nucleotides coding for the amino acid sequence: LeuGluLeuGluIleAla-AlaTyrArgLysLeuLeuGluGlyGluGlu. This sequence contains an intermediate filament protein consensus sequence and additional amino acids highly homologous with amino acids from the 2b portion of the rod domain of the mouse NF protein. It is also more homologous with mammalian NF proteins than with other intermediate filament proteins, e.g., vimentin, GFAP or cytokeratins. We are now determining the remaining sequence of this 5 kilobase RNA and its cellular specificity. By highlighting those portions of the NF protein that are conserved between these two animals, which diverged around 600 million years ago, the sequence of this mRNA will help elucidate functionally important domains of NF proteins.

65.22

ENHANCEMENT OF ^{3}H-ACh RELEASE BY THA IN HIPPOCAMPAL SLICES FROM RATS TREATED WITH AF64A P.E. Potter & S. Nitta*. Department of Anesthesiology, Albert Einstein Coll. Med., Montefiore Medical Center, Bronx, NY 10467

The effects of tetrahydroaminoacridine (THA) and physostigmine were studied on the release of ^{3}H-acetylcholine (ACh) evoked by electrical field stimulation (2 Hz, 2 msec, 30 V, for 2 min) from rat hippocampal slices. In slices from control animals, THA, in concentrations ranging from 10^{-8}M-10^{-5}M, significantly decreased ^{3}H-ACh release in a dose dependent manner. Physostigmine, 10^{-6}M, also decreased ACh release. In contrast, in hippocampal slices taken from rats which had received intraventricular injection of 2 nmoles of ethylcholine mustard aziridinium (AF64A), and in which hippocampal choline acetyltransferase activity was decreased by at least 50%, THA caused a marked enhancement of ACh release. In addition, ACh release from these tissues was not decreased by physostigmine. The increase in release by THA was prevented by simultaneous addition of 10^{-5}M d-tubocurarine to block nicotinic receptors, suggesting that it may have been mediated through presynaptic nicotinic receptors.

65.23

PHOSPHORYLATION OF TAU PROTEIN BY GLYCOGEN SYNTHASE KINASE-3. C. W Scott*, A.I. Salama and C.B. Caputo* (SPON: M.E. Goldberg). Dept. of Pharmacology, ICI Pharmaceuticals Group, ICI Americas Inc., Wilmington, DE 19897.

Glycogen synthase kinase-3 (GSK-3) was recently shown to associate with microtubules (MT) and phosphorylate the MT-associated protein MAP-2 (Li et al., J. Cell Biol., 107:840a, 1988). We have found that GSK-3 phosphorylates an additional set of MT-associated proteins, the microheterogeneous group of tau proteins. GSK-3 from rabbit skeletal muscle phosphorylated porcine brain tau to a stoichiometry of 0.6 mol/mol. Pretreating tau with alkaline phosphatase had no effect on its subsequent phosphorylation by GSK-3. Phosphorylation of tau by GSK-3 resulted in a change in the electrophoretic mobility of some, but not all, tau isoforms. The reactivity of tau protein with tau-1, a monoclonal antibody which recognizes a phosphate-dependent site on tau, was unaffected by phosphorylation with GSK-3, suggesting that GSK-3 phosphorylated a site(s) distinct from the tau-1 epitope. Since GSK-3 appears to require a phosphoserine for substrate recognition (Fiol et al., Arch. Biochem. Biophys. 267:797, 1988), experiments are underway to test various kinases for their ability to generate a GSK-3 phosphosubstrate sequence on tau protein, thereby increasing subsequent phosphorylation of tau by GSK-3.

65.24

CALPAIN-INDUCED PROTEOLYSIS OF TAU, MAP-2 AND TUBULIN. G.V.W. Johnson, L.I. Binder and R.S. Jope. Dept. Neurology, Cell Biology & Anatomy, Pharmacology and Neuropsychiatry Research Program, University of Alabama, Birmingham, AL 35294.

Tau, MAP-2 and tubulin are cytoskeletal proteins necessary for the maintenance of neuronal integrity and function. In this study we have examined the characteristics of calpain-induced degradation of each protein in vitro. In a microtubule preparation with a substrate to calpain ratio of 100:1, the relative rates of proteolysis of tau, MAP-2 and tubulin were determined using quantitative immunoblot analysis with monoclonal antibodies. Of these 3 proteins, MAP-2 was most rapidly hydrolyzed by calpain, with 50% of the MAP-2 being degraded within 30 sec and almost total degradation occurring within 2 min. Tau was 50% degraded in 90 sec and nearly completely proteolyzed in 10 min. In contrast to these findings, microtubule β-tubulin was found to be a relatively poor substrate for calpain. After 30 min at an increased substrate to calpain ratio of 20:1 less than 10% of the protein was degraded. These microtubule protein degradation rates are now being compared to the degradation rates of purified tau, MAP-2 and tubulin. Supported by NIH Fellowship AG05382.

65.25

CHARACTERISTIC RATES OF PROGRAMMED DEATH WITH AGING FOR DIFFERENT AMINERGIC MURINE NEURONAL POPULATIONS. W.G. Tatton, D. Holland*, M. Kwan*, N. Seniuk*, M. Verrier, C.E. Greenwood and F. Biddle* (SPON: J.Stevens). Depts.Physiology, Nutritional Science and Rehab. Medicine Univ. of Toronto,Toronto and Dept. Biochemistry,Univ. of Calgary, Calgary, Canada.

Immunocytochemistry for tyrosine hydroxylase, dopa beta hydroxylase, dopamine, serotonin and neurofilament proteins was carried out for serial brainstem sections and retinal whole mounts in order to identify, count and three dimensionally reconstruct the populations of dopaminergic (Dag) neurons in the substantia nigra compacta (SNc) and the retina (amacrines), noradrenergic (NEg) locus coeruleus (LC) neurons and serotonergic (5HT) raphe obscurus and pallidus (Rpe) neurons. Six to ten nuclei or retina were examined at ages of 8, 14, 20, 26, 35, 44, 60, 75 and 88 weeks from isogenic (approximately 200 sister-brother matings) C57BL/6Jbid mice. Each of the identified monoaminergic populations showed characteristic relationships between total neuronal number and age. All of the realtionships could best be characterized by the linear equations shown in the following table where Neuronal Number (normalized against mean number at 8 weeks) = slope x age in weeks + Y Intercept:

Population	Slope	Y Intercept	R^2
SNc DAg	-0.010	1.076	0.860
LC NEg	-0.009	1.037	0.813
Rpe 5HT	-1.67E-4	1.003	- 0.069
Amacrine DAg	-0.003	1.010	0.841

The neurons were deleted randomly within each nuclei/retina and neurofilament immunoreaction revealed cytoskeletal alterations that paralleled the neuronal loss. Cellular/molecular mechanisms underlying the characteristic relationships for the different neuronal populations will be considered. (Supported by MRC Canada)

65.26

INJURY-INDUCED VESICULATION AND MEMBRANE REDISTRIBUTION IN SQUID GIANT AXON. H.M. Fishman*, K.P. Tewari* and P.G. Stein* (SPON: D. Rassin). Dept. of Physiology and Biophysics, Univ. Texas Med. Branch, Galveston, TX 77550.

Injury to a squid (Loligo, Loliguncula or Sepioteuthis) giant axon initiates the following previously unreported processes (easily observed by phase and differential interference contrast microscopy): i) intraaxonal vesiculation extending along the axon several mm from the site of injury, followed by ii) vesicular fusions that result in the formation of large vesicles ("axosomes") and finally iii) axosome migration to and accumulation at the injury site, which eventually seals. Vesiculation does not occur after axonal injury unless divalent cations (Ca^{+2} or Mg^{+2}) are present in the external solution. Recordings of propagated action potentials in regions of profuse vesiculation, subsurface localization of the vesicles and the recognized role of the endoplasmic reticulum (ER) as a Ca^{+2}-regulating organelle that can swell after Ca^{+2} loading together suggest that these vesicles originate from ER. Patch clamp of axosomes (up to 250 μm diam.) that emerged from transected axons prior to sealing showed the presence of ion channels. Vesiculation and membrane redistribution seem to be fundamental processes in the short-term (mins to hrs) neural response to injury preceding repair and regeneration. These processes appear related to "vacuolar degeneration" observed in some mammalian neuropathies. Aided by ONR Contract, NIH grant and AHA-Wyeth-Ayerst Laboratory Grant-In-Aid (PGS).

67

SYMPOSIUM. NEW OPPORTUNITIES FOR STUDY OF MECHANISMS OF CENTRAL NERVOUS SYSTEM ISCHEMIA. J. ZIVIN, Univ. California, San Diego (Chairperson); T. WIELOCH*, Lund Univ. Hospital; D. CHOI, Stanford Univ.; R. KRAIG, Univ. Chicago; B. RANSOM, Yale Univ.

For many years, most basic investigations of stroke primarily centered on cerebral neuropathology, energy metabolism, and blood flow. Several recent advances in the study of CNS ischemia permit a wider scope of evaluations of the mechanisms of acute cell death and suggest new ways that damage may be limited or delayed. It is probable that major improvements in clinical therapy for stroke will soon be available, but further advances depend on new scientific developments. Our objective is to acquaint neuroscientists with some of these exciting possibilities. J. Zivin will discuss new *in vivo* stroke models for investigation of protein phosphorylation changes during ischemia. T. Wieloch will review receptor mediated processes in selective neuronal death following cerebral ischemia. D. Choi will comment on the use of *in vitro* methods for examination of mechanisms of cell death induced by excitatory neurotransmitters and hypoxia. R. Kraig will talk about the use of new selective microelectrodes for studies of glial pH behavior in ischemic brain injury and edema. B. Ransom will address the pathophysiology of anoxic injury in white matter of the brain by electrophysiological methods.

68

SYMPOSIUM. FROM EARLY EMBRYOGENESIS TO CIRCUITS: CELLULAR AND MOLECULAR STRATEGIES APPLIED TO THE ANALYSIS OF VERTEBRATE BRAIN DEVELOPMENT. D. Goldowitz, Jefferson Med. Coll. & P. Levitt, Med. Coll. Penn. (Chairpersons); C. Kintner*, Salk Inst. Biol. Studies; J. Rossant*, Mt. Sinai Hosp. Res. Inst.; R. McKay, MIT.

Technical advances now make it possible to integrate molecular and embryological strategies in understanding vertebrate CNS development. The goal of this symposium is to bridge these disciplines and present new concepts and approaches to investigate vertebrate neurodevelopment. Using Xenopus embryos, neural plate RNA transcripts have been identified and their activation studied during neural induction. Transgenic Xenopus embryos have been made to alter expression of specfic molecules to assay the role of these molecules during early neurogenesis (C. Kintner). Using experimental interspecies mouse chimeras, the spatial and temporal features of cell allocation in the CNS have been explored yielding novel insights into the construction of the mammalian CNS (D. Goldowitz). The use of insertional mutagenesis in transgenic mice and targeted mutations in embryonal stem cells are presented as means to study how genes are involved in neural development (J. Rossant). The use of immortalized cell lines from rat brain is presented as a means to study and reconstruct complex cellular systems both in vitro and in vivo (R. McKay). Finally, studies will be described that relate molecular specificity of functional systems to the assembly of circuits during mammalian brain development (P. Levitt). Each of the participants will highlight the use of emerging cellular and molecular techniques to investigate a particular stage of vertebrate brain development.

VISUAL CORTEX II

69.1

DISTRIBUTED HIERARCHICAL PROCESSING IN MACAQUE VISUAL CORTEX. D.J. Felleman[1] and D.C. Van Essen[2], Neurobiology and Anatomy, UT Medical School[1], Houston, TX 77225, Div. of Biology, Caltech[2], Pasadena, CA 91125.

In recent years, many new visual areas have been identified in the macaque monkey, and there has been an even more dramatic increase in the number of identified connections. We report here on: (1) a computerized database for storing and representing, in easily accessible fashion, the large amounts of information on cortical connectivity patterns, and (2) the application of these data to the analysis of hierarchical organization of the visual pathway.

Our analysis deals with 26 cortical areas that are predominantly or exclusively visual in function, plus an additional 7 areas that we regard as visual association areas by virtue of their extensive visual inputs. A total of 236 cortico-cortical connections among these areas have been reported, and there are an additional 16 connections whose existance is more questionable. Altogether, these represent 21% of the possible number of pathways if each area were connected with all others. Many pathways have yet to be adequately tested, and we estimate that the actual degree of connectivity is closer to 30-35%. The great majority of pathways involve reciprocal connections between areas, and there are only a few reported exceptions to this rule.

An analysis of the laminar patters of connections has allowed us to extend the cortical hierarchy proposed by Maunsell and Van Essen (*J. Neurosci.*3:2563,'83). However, this has entailed a significant modification of the criteria for identifying feedforward and feedback directions. In the revised scheme, a bilaminar (infragranular and supragranular) distribution of cell bodies after retrograde tracer injection can be associated with feedforward as well as feedback and lateral pathways. Consequently, the anterograde labeling pattern is, in general, more useful for establishing unequivocal hierarchical relationships. The current hierarchy includes 11 cortical levels and extends from V1 to high-level association areas such as the hippocampus and amygdala. Within this hierarchy, there are multiple, intertwined processing streams, which at low levels are related to the compartmental organization of areas V1 and V2 and at a high level are related to the distinction between processing centers in the temporal and parietal lobes. Supported by grant EY-02091.

69.2

FUNCTIONAL ARCHITECTURE OF VISUAL AREA 18 OF MACAQUE MONKEY. *D.Y. Ts'o, C.D. Gilbert, R.D. Frostig, A. Grinvald, T.N. Wiesel.* Laboratory of Neurobiology, The Rockefeller University, NY, NY 10021 and IBM Research Division, Yorktown Heights, NY 10598.

The *in vivo* optical imaging of intrinsic signals has enabled us to directly view the functional architecture and the cytochrome oxidase-rich thick and thin stripes of area 18. We have combined this optical imaging method with single unit electrophysiology in the same experiment. These studies have shown a close correspondence between the functional maps obtained with optical imaging and the receptive field properties of single cells in the imaged regions. For example, regions with poor orientation tuning indicated with optical imaging contained cells that were often unoriented, as seen electrophysiologically. These regions were localized to alternate sets of stripes. We have also found regions of rapid changes in orientation tuning or ocular dominance within a single vertical penetration and between two adjacent penetrations, while other regions contained cells with very uniform orientation tuning or binocular responses.

We have also used this combination of recording techniques to explore the receptive field properties within a single stripe. For example, among disparity-sensitive cells, the clustering of sharply tuned excitatory cells was often found to be spatially separated from the clustering of near and of far cells. Color-selective cells were also clustered within a particular stripe according to color-opponency. Color oriented cells were found in proximity to unoriented color cells of the same color selectivity. One intriguing type of oriented color cell had ON responses to red and green slits that were spatially distinct but adjacent. This type of cell responded best to a stimulus with a red-green border at a particular position and orientation. Taken together, our studies revealed a clustering of receptive field properties and suggests a substructure or a compartmentalization of function within a stripe of area 18.

69.3

ELEMENTS OF FORM PERCEPTION IN MONKEY V2 - A CORRELATION WITH THE CYTOCHROME OXIDASE PATTERN. E. Peterhans* and R. von der Heydt* (SPON: M.R. Dürsteler). Dept. of Neurology, University Hospital, CH-8091 Zürich.

We have studied the receptive fields of single cells in the second visual area (V2) of the alert rhesus monkey. From series of vertical sections we have reconstructed the recording sites with respect to the cytochrome oxidase pattern.

We found orientation selective cells in all parts of the stripe pattern, most frequently in the thick dark (85%) and pale (81%) stripes, less frequently in the thin stripes (63%) which also held the cells unresponsive to our monochromatic stimulation. Neuronal signals related to the perception of contour (real and illusory) were recorded from half of the oriented cells in the pale and thick dark stripes, but not in the thin stripes. Cells involved in the analysis of shape from coherent motion were most frequently found in the thick stripes, as were cells concerned with stereoscopic depth. By contrast, cells with end-stopped receptive fields were more evenly distributed. In conclusion, our results suggest for neurons of V2 in the alert monkey a functional segregation which correlates with the cytochrome oxidase pattern.

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69.4

MAGNOCELLULAR AND PARVOCELLULAR CONTRIBUTIONS TO AREA MT IN MACAQUE EXTRASTRIATE CORTEX.

T.A. Nealey*, D.D. DePriest*, J.H.R. Maunsell
Dept. Physiology, Univ. of Rochester, Rochester, NY 14642

Visual signals relayed by the parvocellular and magnocellular subdivisions of the lateral geniculate nucleus (LGN) remain largely segregated in striate cortex (V1). However, the degree of separation between these two classes of signals in extrastriate cortex remains an important question. We used reversible inactivation of individual LGN layers to determine directly the relative contributions of these channels to responses in the middle temporal visual area (MT). We tested 75 single or multiunit sites in MT using a small monocular stimulus while inactivating its representation in individual LGN layers with small injections (25 - 200 nl) of lidocaine (2%) or MgCl2 (40 mM).

Magnocellular blockade consistently abolished or greatly reduced responses in MT. Parvocellular blockade generally produced a less robust and more variable effect, even in anterior LGN where there is only one contralaterally-driven parvocellular layer. A few sites in MT showed a clear and repeatable reduction in response with parvocellular blockade, but many others showed no effect at all. Thus it appears that MT receives its primary excitatory drive from the magnocellular LGN layers, but also a contribution from the parvocellular layers.

Supported by NIH EY05911

69.5

MOTION ANALYSIS IN THE INFERIOR PARIETAL AREA 7A OF THE RHESUS MONKEY. R.M. Siegel. Lab. Neurobio., Rockefeller Univ., New York, NY 10021.

Human and monkey subjects use similar visual motion cues to obtain two and three-dimensional perceptions (Siegel and Andersen, '88). The present study attempts to analyze the role of area 7a in these phenomena with single unit recordings in the awake behaving monkey performing reaction time tasks.

Simple stimuli were used to map the receptive fields (RFs) of area 7a neurons while the monkey fixated a central point. In one hemisphere 200 of 298 neurons studied were found with directional selectivity to drifting squares or random dot fields, opponent vector fields (Motter and Mountcastle, '81), and/or responses to flashed, rotating, expanding and shrinking squares. These cells were scattered over area 7a.

In order to directly test the involvement of these neurons in the monkey's extraction of two and three-dimensional motion perceptions, the animal was also trained to fixate a central point and then to release a key when an unstructured 10° display became structured. The structured display consisted of 128 dots moving along the motion vectors associated with planar rotations, expansion or compression, and the three-dimensional rotation of a sphere. In the unstructured displays, the motion trajectories were randomly shuffled destroying the percept.

Of the 71 neurons tested under these conditions, 20 clearly varied their rate of firing when the stimulus changed from unstructured to structured motion. The response of the neurons was dependent on the type of structured motion; cells differed in selectivity to the three different structured motions. Of these 20 cells, 13 were restricted to a 2x2mm region within area 7a. There was no obvious correlation between the response to the structured motion and the simpler stimuli. This inability to obtain a RF underlying the structured motion response indicates either extremely non-linear RFs or attentional modulation of RFs.

These results suggest that area 7a is involved in the analysis of structure from motion. This dependency on form is interesting from the clinical perspective of the inferior parietal lobule as a visuo-spatial, not shape, processing region.

69.6

VISUAL RESPONSE PROPERTIES AND ATTENTIONAL MODULATION OF NEURONS IN THE VENTRAL INTRAPARIETAL AREA (VIP) IN THE ALERT MONKEY.

C. L. Colby, J.-R. Duhamel and M. E. Goldberg. Laboratory of Sensorimotor Research, National Eye Institute, Bethesda, MD 20892.

We have investigated the visual responsiveness of neurons in the intraparietal sulcus of the alert monkey. Area VIP has previously been defined on the basis of receiving ascending projections from area MT (Maunsell and Van Essen '83; Ungerleider and Desimone '86). We have now identified a physiologically distinct zone in the depths of the intraparietal sulcus which corresponds to area VIP. This zone includes the fundus and most ventral portions of the lateral and medial banks in the anterior portion of the sulcus. Cells in this area are strongly responsive to visual stimulation and typically exhibit the following characteristics: large receptive fields; direction selectivity; speed selectivity; responsiveness to whole field motion. A striking feature of VIP is the similarity of these response properties to those observed in area MST, a visual area which also receives strong inputs from area MT. Unlike cells in MST, VIP neurons exhibit enhanced responses to stationary visual stimuli when they are the target for a subsequent saccade. The attentional modulation commonly found in other parietal regions thus extends to the depths of intraparietal sulcus.

69.7

PARIETAL VISUAL NEURONS ACHIEVE SPATIAL ACCURACY BY COORDINATE TRANSFORMATION OF THE VISUAL MAP.

M. E. Goldberg, C. L. Colby, and J. R. Duhamel. Lab. Sensorimotor Research, National Eye Institute, Bethesda, MD 20892.

Posterior parietal cortex neurons discharge before particular saccades even when the retinal position of a target does not predict the direction of eye movement necessary to acquire that target. In order to see if such neurons require a visual stimulus, we studied them using a number of tasks in the monkey: 1) visual fixation with a peripheral visual stimulus; 2) visual saccade task in which the monkey had to make a saccade to the peripheral visual stimulus; 3) visual memory task, in which the stimulus appeared briefly during fixation, and the monkey had later to make a saccade in total darkness to where the target had been; 4) learned saccade task in which visual saccade trials were mixed with trials in which no target ever appeared and the monkey had to make the same saccade; and 5) double step task in which two stimuli, A and B, were flashed within a saccadic reaction time and the monkey had to make saccades from the fixation point to A to B. Saccades to the second stimulus from the first could not be computed from the retinal location of the target, but required knowledge of either the intervening saccade or the spatial location of the target.

A subset of parietal neurons discharged after the first and before the second saccade of the double step task. Of these neurons, the majority discharged in the visual saccade and visual memory tasks but not in the learned saccade task, establishing that for these neurons the visual presence of the target is necessary for discharge. Although some of these neurons have orbital-position dependent gain fields, none discharge to targets acquired by non-preferred saccades. The results suggest that spatial accuracy in parietal visual neurons is maintained through coordinate transformation of a retinal signal by a discharge corollary to the intervening saccade, and not by explicit coding of target position in space.

69.8

CONGRUENT VISUAL AND SOMATOSENSORY RESPONSE PROPERTIES OF NEURONS IN THE VENTRAL INTRAPARIETAL AREA (VIP) IN THE ALERT MONKEY.

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Projections from somatosensory and visual association cortices converge in the intraparietal sulcus (IPS). We have mapped the IPS physiologically and found that single cells in visual area VIP, located in the depths of the sulcus, also have robust somatosensory responses. The receptive fields are most often found on the head, face and forelimbs. These cells respond well to passive somatosensory stimulation (light touch) as well as to stimulation produced by active movement. The somatosensory (S) response properties correspond to the visual (V) response properties in two ways. First, the locations of the S and V receptive fields match: cells with S fields on brow or forehead invariably have upper field V receptive fields, while cells with S fields around the mouth or on the hands have V receptive fields in the lower field. Second, S and V receptive fields exhibit comparable direction selectivities: cells which prefer visual motion away from the fixation point for a stimulus on the screen also prefer somatosensory stimulation moving away from the center of the face with eyes closed. This correspondence between somatosensory and visual response properties suggests that such cells contribute to the elaboration of a supramodal spatial reference system.

69.9

EFFECTS OF DEACTIVATION OF LATERAL PULVINAR OR SUPERIOR COLLICULUS ON THE ABILITY TO SELECTIVELY ATTEND TO A VISUAL STIMULUS.

Robert Desimone, Mark Wessinger*, Linda Thomas*, and Walter Schneider*. Lab. Neuropsychology, NIMH, Bethesda, MD, and LRDC, Univ. of Pittsburgh, Pittsburgh, PA.

It has previously been found that extrastriate neuronal responses to ignored receptive field stimuli are suppressed. To identify possible sources of the suppression, we have studied the effects of unilateral deactivation (with the GABA agonist muscimol) of either the posterior lateral pulvinar (LP) or superior colliculus (SC) on the ability of two rhesus monkeys to attend to one extrafoveal stimulus (the target) and ignore another (a distractor), in the absence of eye movements. A brief spatial cue indicated the location of the subsequent target. The monkey's task was to indicate the color of the target (on for 100 msec), and ignore the distractor, which could have the same or different color. With no distractor, deactivation had no effect on correct performance. Reaction times, as well as the reaction time "cost" when the target was invalidly cued, were also normal. By contrast, the presence of a distractor had a devastating effect. When the distractor was located in the field ipsilateral to the affected LP or SC and the target was in the contralateral field, correct performance dropped as much as 50%. Errors were caused by the animal basing its behavior on the distractor rather than the target. When both stimuli were located in the contralateral hemifield, the effects of SC deactivation were larger than LP deactivation. The results support the notion that there is a close correspondence between attentional and oculomotor control structures and suggest that at least some attentional structures are critical for object recognition only when there is more than one stimulus in the visual field.

69.10

NEURONAL CORRELATES OF VISUAL SEARCH IN THE FRONTAL EYE FIELDS OF RHESUS MONKEY.

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Prior studies of presaccadic activity in the frontal eye fields (FEF) have utilized single visual stimuli as targets for saccades. These studies have shown that many of the cells exhibit visual responses either alone or in conjunction with a saccade related discharge. The purpose of this study was to record the activity of visually responsive cells in the FEF of a monkey confronted with multiple stimuli, among which the target had to be discriminated. Two groups of stimuli were used. The first consisted of a red spot among 3-7 green spots, and the second consisted of the letter T among 3-7 L's. The red/green search was achieved with shorter saccade latencies than the letter search. The unit recordings yielded four main results: 1) The activity of most of the visual and visuomovement cells during visual search with multiple stimuli could be predicted from their spatial tuning and temporal discharge characteristics in response to a single stimulus. 2) The response latency of a few visual cells was longer when presented with multiple stimuli. 3) The response duration of other visual cells was prolonged during search. 4) During visual search some visuomovement cells exhibited spatial tuning according to the location of the correct target relative to the receptive/movement field. These results suggest that while the reponses of many FEF neurons appear to be dictated soley by the characteristics of the visual stimulus and the resulting eye movement, some neuronal activity in FEF may be related to the process of saccade target selection during visual search.

Supported by NEI NRSA 05959.

69.11

ATTENUATION OF RESPONSES OF INFERIOR TEMPORAL NEURONS IN AWAKE MONKEYS BY ADDITION OF A SECOND STIMULUS E.K. Miller, P.M. Gochin, C.G. Gross, G.L. Gerstein. Dept. Psychol., Princeton Univ., Princeton, NJ 08544, Dept. Physiol., Univ. Penn. Sch. Med., Phila., PA 19104

We compared the responses of inferior temporal (IT) cortex neurons to individual and pairs of pattern stimuli. Monkeys were trained to fixate a small (0.5 deg) spot of light while the stimuli were presented extrafoveally. Each stimulus subtended about 1.5 deg and was projected at one of two sites 5 deg contralateral to fixation. On a given trial, stimuli were presented either at one of the sites or simultaneously at both sites.

We found that for eighty-seven percent of responsive IT neurons, addition of a second stimulus decreased the cell's response relative to the response of the more effective of the two stimuli presented alone. While many of the neurons tested responded preferentially to a specific single stimulus, the magnitude of the response attenuation was similar whether the second stimulus was the same as or different from the first stimulus. Furthermore, this effect occurred whether the second stimulus was within or outside the cell's excitatory receptive field. While previous investigations have shown that directed attention can attenuate responses of IT neurons, the effect we have observed is unlikely to be due to attention since our behavioral task only required the animal to maintain fixation of the fixation point.

69.12

VISUAL RESPONSE PROPERTIES OF INFERIOR TEMPORAL NEURONS IN ALERT INFANT MACAQUES. H.R. Rodman, J.P. Skelly and C.G. Gross. Dept. of Psychology, Princeton University, Princeton, NJ 08544.

Previously, we found that visual responsiveness is virtually absent in inferior temporal (IT) cortex before about 4 months of age in macaques under immobilization and N_2O anesthesia, although control recordings in striate cortex and area MT showed normal responsiveness and selectivity (Rodman et al., NS Abs., 14: 11). As a further check on the effects of anesthesia, we recorded in alert infant macaques implanted with a search coil and trained to fixate on a spot of light.

Over 70 single IT units were studied in 2 male and 1 female M. fascicularis ranging between 5 weeks and 3 months of age. Stimuli were typically presented for 500 ms at the fixation point and included pictures of faces, boundary curvature descriptors, lines, and other patterns. Objects were also presented by hand.

About three-quarters of all IT units studied in the alert infants were visually responsive, in contrast to our findings in anesthetized infants. Moreover, there was no tendency to find fewer responses at younger ages. IT cells showed stimulus selectivity in the earliest recording sessions, particularly for boundary curvature; a few cells selective for faces or color were also found. As in adults, responses were strongest at the fovea when compared with responses at other locations. Although responses were found in IT in each animal in the alert condition, IT cortex was not responsive when the same animals were studied under N_2O.

These findings show that IT, a critical substrate of visual recognition, has adult-like properties as early as 5 weeks. These properties are masked by anesthesia until about 4 months, indicating that developmental changes in cellular metabolism, extrinsic modulating influences or other factors persist up to or occur at this point.

SYNAPTOGENESIS I

70.1

AN HYPOTHESIS TO EXPLAIN THE TRIGGERING OF NEUROMUSCULAR SYNAPTOGENESIS. E. Rodriguez-Marin* and M.W. Cohen. Dept. of Physiology, McGill University, Montreal, Que. H3G 1Y6.

In cultures of spinal cord neurons and myotomal muscle cells derived from Xenopus embryos the neurite-muscle interactions which lead to co-localization of a synaptic vesicle antigen and acetylcholine receptors (AChRs) can occur along pre-existing, non-growth-cone portions of neurites. Following neurite-muscle contact the portion of neurite proximal, but not distal, to the contact loses its capacity to participate in these interactions.

These findings can be explained by assuming that a neural synaptogenic factor (nSF) is inserted into the surface membrane only at the growth cone and is mobile within the surface membrane. A muscle SF is also mobile within the surface membrane and is assumed to be the AChR. In regions of neurite-muscle contact the nSF and AChR cross-link with each other and become immobilized. With time membrane nSF is depleted proximal to the contact as more of it enters the contact region and becomes immobilized there. Synaptic vesicles accumulate at sites of immobilized nSF and thereby become co-localized with AChRs. Only cholinergic neurons possess the appropriate nSF for cross-linking the AChR. The cross-linking is short-lived and is replaced by indirect linkages when molecules are externalized to form the synaptic basal lamina. (Supported by MRC of Canada.)

70.2

REDISTRIBUTION OF AGRIN BINDING SITES DURING ACETYLCHOLINE RECEPTOR CLUSTERING ON CULTURED MYOTUBES. J. R. Fallon, Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545

Agrin derived from Torpedo electric organ induces the clustering of AchR when added to cultured chick myotubes. We have assessed the distribution of Torpedo agrin binding sites on the myotube surface before and during AchR clustering using immunofluorescence microscopy with species-specific antibodies directed against Torpedo agrin. Torpedo agrin is initially distributed over the entire myotube surface in a uniform, finely punctate pattern. Preferential binding to pre-existing, spontaneous AchR clusters is not observed. This distribution is distinct from the more coarsely punctate and/or fibrillar pattern that is seen for chick muscle-derived agrin (Lieth and Fallon, this volume) or for LN, COLL IV, FN, and HSP. After two hours of incubation, increasing levels of Torpedo agrin binding are observed in the vicinity of newly formed AchR microaggregates; by 24 hrs the binding sites are concentrated almost exclusively at AchR clusters. These results indicate that Torpedo agrin binding sites are uniformly distributed on the surface of untreated myotubes, and that these binding sites redistribute during agrin-induced AchR cluster formation.

Supported by NIH grant HD 23924.

70.3

AGRIN: A SYNAPTIC ORGANIZING MOLECULE CLOSELY RELATED TO NON-SYNAPTIC BASAL LAMINA PROTEINS. Earl W. Godfrey. Dept. of Anatomy & Cellular Biol., Medical College of Wisconsin, Milwaukee, WI 53226.

Agrin is a synapse-organizing protein that is found in motor neurons and is concentrated in the synaptic basal lamina at the neuromuscular junction; agrin is also associated with the basal laminae of many tissues. Agrin purified from extracellular matrix (ECM)-enriched fractions of several Torpedo tissues, including skeletal muscle, heart and intestine, is similar biochemically to agrin from the nervous system and electric organ, and aggregates acetylcholine receptors (AChRs) on cultured skeletal muscle cells regardless of tissue source [Godfrey et al., J. Cell Biol. 106: 1263-1272; 1988].

Here I report that, in the chicken, while agrin-like proteins were associated with most basement membranes, the AChR-aggregating activity of agrin preparations differed depending on the tissue of origin. Agrin enriched by immunoaffinity chromatography from the cytosol of embryonic or adult chicken CNS aggregated AChRs with a dose-response relationship similar to that seen with Torpedo agrin. However, agrin preparations from embryonic skeletal muscle and heart and adult kidney induced a small number of AChR aggregates at low doses, but had little or no biological activity at higher doses. Despite this apparent difference in AChR-aggregating activity, the major agrin-like proteins from the nervous system and other tissues were biochemically and immunochemically similar. Basement membranes of several tissues, as well as spinal cord motor neurons, were stained by antisera against agrin-like proteins from chicken kidney. These antisera also precipitated and inhibited AChR-aggregating activity from chick embryo brain and spinal cord, adult chicken brain and Torpedo electric organ. Thus, agrin-like proteins of non-neural tissues in the chicken are closely related to agrin from the nervous system. The apparent difference in biological activity between these agrin preparations may be due to inhibitory molecules that co-purify with agrin from non-neural tissues.

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70.4

AGRIN-LIKE MOLECULES ARE TRANSPORTED IN AN ANTEROGRADE DIRECTION IN MOTOR AXONS C. Magill-Solc & U.J. McMahan. Dept. of Neurobiology, Stanford University, Stanford, CA 94305

Several lines of evidence indicate that molecules in the synaptic basal lamina at the neuromuscular junction that direct the formation and maintenance of acetylcholine receptor (AChR) and acetylcholinesterase (AChE) aggregates on the muscle fibers and that the active basal lamina molecules are similar to agrin, a protein extracted from Torpedo electric organ. We reported previously that agrin-like molecules are concentrated in the cell bodies of motor neurons. This led us to suggest that motor neurons synthesize agrin-like molecules in their cell bodies, transport them anterogradely along their axons, and secrete them at their axon terminal, to become incorporated into the basal lamina at the neuromuscular junction. An alternative possibility is that agrin-like molecules are produced by muscle fibers and are taken up by axon terminals to be transported retrogradely along motor axons to their cell bodies. The experiment described here tests these hypotheses.

We ligated the sciatic nerves of adult frogs and 3 days later examined the distribution of agrin-like molecules on each side of the ligature. Others have shown that proteins synthesized in the cell bodies of neurons and transported anterogradely along their axons accumulate on the cell body side of such a ligature. In normal frog nerves axoplasm does not stain with our anti-agrin antibodies. However, in the ligated nerves the axoplasm of some of the myelinated axons on the cell body side of the ligature stained intensely. Severing the ventral roots that carry the motor axons to the sciatic nerve prior to ligation resulted in the absence of anti-agrin staining in such nerves. Extracts of the portion of ligated nerves on the cell body side of the ligature contained a high concentration of molecules that cause AChR aggregation on cultured myotubes, as does agrin. Such molecules were immunoprecipitated by anti-agrin antibodies and were not detected in normal nerves or in nerves where ventral roots had been severed prior to ligation. Altogether, these findings provide evidence that agrin-like molecules in motor neurons are transported from cell body to axon terminal.

70.5

IDENTIFICATION OF AN AGRIN cDNA. M.A. Smith, F. Rupp, P. Snow, J.W. Schilling, B.G. Wallace, R.H. Scheller and U.J. McMahan. Dept. of Anatomy and Neurobiology, California College of Medicine, University of California, Irvine, CA 92717. Dept. of Zoology, University of California, Berkley, CA 94720. Cal. Biotech. Inc. Mountain View, CA 94043. Dept. of Neurobiology and Dept. of Biology, Stanford University, Stanford, CA 94305.

Agrin proteins, isolated from a basal lamina-rich fraction of *Torpedo* electric organ, induce specializations on cultured chick myotubes that contain a high concentration of acetylcholine receptors and other postsynaptic components of the neuromuscular junction. Anti-agrin antibodies recognize four proteins Mr. 150, 135, 95 and 70kD. Two proteins, Mr 150 and 95kD representing agrin, induce the aggregation of postsynaptic components; the 135 and 70kD agrin-like proteins are inactive by this assay. As a step towards understanding the relationships between the different forms of agrin and agrin-like proteins, determining their source and assessing their role at the neuromuscular junction we have isolated an agrin cDNA clone and deduced its amino acid sequence.

A cDNA library was constructed in λgt11, from poly(A)$^+$ RNA isolated from the electric lobe of *Discopyge ommata*, an electric ray closely related to *Torpedo*, and screened with an antiserum raised against a mixture of SDS denatured, affinity purified, agrin and agrin like proteins. One of four clones initially isolated was determined by epitope selection of the antiserum, to encode a fusion protein containing antigenic determinants shared with all four *Torpedo* agrin/agrin-like proteins. This recombinant contains a 4.3kb cDNA insert that predicts an amino acid sequence containing a stretch of 11 out of14 amino acids previously identified by Edman degredation as the N-terminus of the 95/70kD *Torpedo* proteins. Two of the three mismatches represent single base changes. The deduced sequence contains several EGF-like domains and a region of sequence similarity to the active site of pancreatic secretory typsin inhibitor.

Thus a cDNA has been isolated from a *Discopyge ommata* electric lobe library, that codes for a protein that exhibits strong antigenic and structural similarity to *Torpedo* agrin.

70.6

FURTHER CHARACTERIZATION OF AN ACETYLCHOLINE RECEPTOR-INDUCING PROTEIN AND DEVELOPMENT OF AN OLIGONUCLEOTIDE PROBE FOR THIS MOLECULE. D.L. Falls, D.A. Harris, R.M. Dill-Devor*, R.L. Cole*, W.D. Walsh*, and G.D. Fischbach. Dept. of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, Missouri, 63110.

The 42kD AChR Inducing Activity (ARIA), a glycoprotein purified from chicken brain, may be a trophic factor released by motoneurons which promotes synthesis and insertion of acetylcholine receptors (AChRs) at nascent neuromuscular synapses. ARIA appears as a broad band on SDS polyacrylamide gels and as a cluster of sharp OD_{210} peaks eluted from a reverse phase HPLC column. We have found that this apparent heterogeneity is due, in large part, to variable glycosylation of the core protein. Each of the peaks eluted from the HPLC column exhibits the same N-terminal amino acid sequence. With recoveries in the 10 pmole range, we unambiguously identified 24 of the first 27 amino acids. A rabbit antiserum raised against a synthetic peptide corresponding to the N-terminal 11 amino acids labeled bands that ranged between Mr 34,000 and 42,000 on western blots of partially purified ARIA. When the same preparation was digested with N-glycanase to remove N-linked carbohydrate side chains, all of these bands migrated at Mr 31,000. Receptor inducing activity eluted from gel slices was localized to the same region of the gel as the protein labeled by the anti-peptide antibody and, after N-glycanase treatment, shifted with the labeled protein. When ARIA was exposed to reducing agents, the activity was completely destroyed. Starting with cDNA prepared from chick brain poly(A)$^+$ RNA, we have used the polymerase chain reaction and degenerate oligonucleotide primers based on the N-terminal sequence to amplify a 34-base nucleic acid sequence corresponding to the 3' base of the 7th amino acid through the 3' base of the 18th amino acid. A cDNA clone identified with the PCR product (see Harris et al., this volume) predicts a protein with four potential N-glycosylation sites and two cysteine residues. We conclude that a disulfide bond is essential for receptor inducing activity, but that N-linked carbohydrates are not. The fact that the receptor-inducing activity shifts along with the protein band following removal of the N-linked carbohydrate provides evidence that the amino acid sequence and cDNA clone are in fact ARIA.

70.7

MOLECULAR CLONING OF AN ACETYLCHOLINE RECEPTOR-INDUCING PROTEIN. D.A. Harris, D.L. Falls, W. Walsh*, and G.D. Fischbach. Dept. of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, Missouri 63110.

42-kD ARIA (AChR-inducing activity) is a glycoprotein isolated from chicken brain that stimulates synthesis of acetylcholine receptors (AChRs) by cultured chicken myotubes. This protein may play a role in the accumulation of receptors at the developing neuromuscular junction. Using the polymerase chain reaction we amplified a 34-nucleotide segment from chicken brain poly(A)$^+$ RNA that encodes 11 amino acids of the chemically determined N-terminal sequence of ARIA (see Falls et al., this volume). This 34-nucleotide sequence was then used to isolate several cDNA clones from adult and embryonic chicken brain libraries. Complete nucleotide sequencing of a 2.2 kb embryonic brain cDNA revealed an open reading frame encoding 267 amino acids, including a putative signal peptide. The sequence includes four potential N-glycosylation sites, a proline- and glycine-containing hexapeptide repeat near the N-terminus, and two hydrophobic regions: one at the C-terminus, and one near the middle of the molecule that is long enough to span the plasma membrane. Northern blots demonstrate a prominent 2.9 kb mRNA in brain and spinal cord from adult chickens as well as from embryos as young as E5. We have noted significant similarity between the amino acid sequence encoded by our cDNA and that of the prion proteins PrP 33-35^{Sc} and PrP 33-35^{C}, which are thought to play a critical role in the pathogenesis of certain degenerative neurological diseases. To confirm the identity of our cDNA clone, we are attempting to express AChR-inducing activity by transfection of mammalian cells and by translation in vitro.

70.8

ACh RECEPTOR CLUSTERING IS TRIGGERED BY LOCAL ACCUMULATION OF NON-AChR MOLECULES. J. Stollberg and S.E. Fraser. Dept. Physiology and Biophysics, UC Irvine, Irvine, CA 92717.

We have shown previously that a simple model system - cultured *Xenopus* muscle cells exposed to electric fields - preserves the components and interactions required to initiate specific ACh receptor clustering (Stollberg and Fraser *J. Cell Biol.* 107, 1988). Using digitally-analyzed fluorescence video-microscopy, we found that receptor aggregation is due to lateral migration of receptors, and is mediated by a diffusion-trap mechanism. Other experiments have ruled out the possibility that receptor aggregation is triggered by electromigrational accumulation of AChRs (*Nrsci. Abstr.* #360.5, 1988). The only remaining triggering mechanisms are *i*) the electromigrational accumulation of non-AChR molecules, or *ii*) a voltage-sensitive mechanism (at 8 V/cm there is a depolarization of about 16 mV at the cell pole where clustering occurs).

Several experiments appear to rule out voltage-sensitive mechanisms. First, receptor clustering can be induced by fields which result in a depolarization as low as 1-2 mV; this is a small effect compared to the sensitivity of voltage-gated ion channels. Furthermore neither 10 mM cobalt nor the removal of sodium affect field-induced receptor clustering, indicating that neither calcium nor sodium fluxes are involved in the process. Finally, alternating electric fields can induce bipolar receptor clusters if the period of field reversal is on the order of 40', but not if the period is shorter. Thus the time-constant of the triggering event is on the order of 10's of minutes - consistent with a mechanism in which the critical event is the electromigrational accumulation of components, but not with the much faster response characteristics of voltage-gated ion channels.

These results suggest that voltage-sensitive mechanisms do not trigger the observed AChR clustering in the model system. The only possibility consistent with all the data is that field-induced receptor clustering is triggered by the accumulation of non-AChR molecules.

70.9

S-LAMININ INHIBITS NEURITE EXTENSION PROMOTED BY LAMININ. J. Weis*, D.D. Hunter*, J.P. Merlie[1] and J.R. Sanes. (SPON: J. Ferrendelli) Depts. of Anatomy & Neurobiology, and [1]Pharmacology, Washington University School of Medicine, St. Louis, MO 63110

Motor axons preferentially reinnervate original synaptic sites in denervated skeletal muscle, a selectivity mediated at least in part by the basal lamina of the synaptic cleft. We have recently isolated a protein, s-laminin (SL), that is concentrated in synaptic basal lamina, and is adhesive for chick ciliary motoneurons. Molecular cloning revealed that SL is homologous to laminin (L) a potent promoter of neurite outgrowth (Hunter et al., Nature 338: 229, 1989). The use of recombinant fragments and synthetic peptides has allowed us to map a site in SL to which motoneurons adhere but sensory or tectal neurons (which adhere to L) do not; thus, this site is selectively recognized by motoneurons.

To assess the consequences of neuronal interaction with SL, ciliary neurons were incubated overnight on dishes coated with L, recombinant SL, or L+SL. Neurons extended neurites on L but not on SL. Furthermore, SL reduced adhesion to and neurite outgrowth on L, suggesting that SL may inhibit outgrowth rather than merely failing to support it. This hypothesis was tested by growing neurons on a dish uniformly coated with L, then overlaid with stripes of SL. Neurons adhering to L extended neurites that stopped abruptly at the L/L+SL border. This result suggests that whereas L directs axons to grow, SL directs axons to stop. In vivo, motor axons regenerating along L-rich pathways might stop growing and/or differentiate upon encountering SL at original synaptic sites. (Support: NIH, MDA, M. Kade Foundation, and Monsanto)

70.10

DEVELOPMENT OF SYNAPTIC TRANSMISSION AND TRANSMITTER SENSITIVITYOF SYMPATHETIC NEURONS INNERVATED *IN VITRO* BY APPROPRIATE VS INAPPROPRIATE PRESYNAPTIC INPUT. R. Gardette & L.W. Role, Dept of Anat. & Cell Biol., Ctr for Neurobiol. and Behav., Columbia Univ, P & S, 630 W 168th, NY, NY 10032

We have studied the development of embryonic sympathetic neurons innervated by either dorsal (dSPX) or ventral (vSPX) spinal cord explants which, in the chicken, contain the sympathetic preganglionic neurons or skeletal motoneurons, respectively. We monitored spontaneous synaptic currents and acetylcholine (ACh) induced currents in voltage clamped neurons to determine the development of innervation and transmitter sensitivity from 1 day (D1) to 4 days (D4) after addition of presynaptic input.

Innervation by either input was detected by D1, but the percentage of neurons innervated with vSPX was half that with dSPX, probably due to relatively poor adhesion and neurite extension from the vSPX. In contrast, the development of enhanced ACh sensitivity was similar regardless of the source of cholinergic input. Both dSPX and vSPX induced a steep increase in the ACh sensitivity by D1 and a smaller linear increase between D1 and D4. Non-innervated cells co-cultured with the vSPX showed a similar (though smaller) increase in ACh sensitivity, perhaps due to the influence of a soluble factor as previously suggested (Role, L. PNAS 1988 85:2825). There is little change in transmitter sensitivity of neurons maintained *in vitro* in the absence of presynaptic input or in the presence of non-cholinergic explants. Thus, although the extent of innervation of sympathetic neurons is somewhat dependent on the appropriate presynaptic input, there is apparently little specificity with respect to the source of cholinergic input in the developmental regulation of ACh sensitivity. *Supported by NS22061, Klingenstein and McKnight Foundations (LWR). CNRS. NATO. FRM. & Philippe Foundation (RG)*

70.11

SELECTIVE REINNERVATION OF MUSCLE BASEMENT MEMBRANES BY THEIR ORIGINAL MOTONEURONS. V. Boss and D.J. Wigston. Departments of Biology and Physiology, Emory University, Atlanta, GA 30322.

The reinnervation of axolotl hindlimb muscles by their original motoneurons suggests the existence of muscle-specific cues that promote selective synaptogenesis during reinnervation. We have investigated whether these cues are associated with muscle fibers or their basement membrane sheaths. Motoneurons were retrogradely labeled by injecting the long-lasting tracer diamidino yellow (DY) into either the left anterior or posterior ilio-tibialis (aILT or pILT). Myofibers in the right a- and pILT muscles were destroyed and their regeneration prevented by γ-irradiation. The left a- and pILT were removed and replaced with the right myofiberless a- and pILT, but in reversed a-p orientation. Motoneurons were able to reinnervate these myofiberless muscles. After reinnervation, we labeled motoneurons projecting to the myofiberless former pILT, now in the position of the original aILT, by intramuscular injection of rhodamine-HRP (R-HRP). We found that a higher percentage of motoneurons contained both labels when equivalent muscles were injected (DY → left pILT, R-HRP → transplanted myofiberless pILT) than when different muscles were injected (DY → left aILT, R-HRP → transplanted myofiberless pILT), despite the novel position of the transplanted muscles and their lack of myofibers. This suggests that the basement membranes of different muscles are distinguished by cues that are recognized by regenerating motoneurons.

70.12

COMPETING MOTOR NERVE TERMINALS AND THE ACETYLCHOLINE RECEPTORS UNDERLYING THEM ARE REARRANGED DURING SYNAPSE ELIMINATION. R.J. Balice-Gordon & J.W. Lichtman, Department of Anatomy & Neurobiology, Washington University School of Medicine, St. Louis, MO 63110

By repeatedly visualizing both the pre- and postsynaptic elements of the same developing neuromuscular synapses, we have studied the elimination of multiple innervation in living mouse pups. During the first days after birth, some motor nerve terminals are rapidly eliminated at multiply innervated neuromuscular junctions. At the same time, the acetylcholine receptors underlying these nerve terminals become progressively less dense and are also eliminated. To determine whether pre- or postsynaptic elimination occurs first, we have followed motor nerve terminals and acetylcholine receptors continuously over many hours. Several different fluorescent compounds, particularly dicationic voltage-sensitive dyes (e.g., RH 795; Hildesheim and Grinvald, unpublished) were found to vitally stain the membranes of axons and nerve terminals and were used with 4-Di-2-ASP, a vital mitochondrial marker, to determine how competing motor nerve terminals are rearranged during synapse elimination. Using rhodamine- and coumarin-conjugated alpha bungarotoxin, we have begun to differentially label receptors at individual multiply innervated synapses to follow their redistribution during competition. Finally, we have differentially labeled nerve terminals converging at the same synapses using anterograde diffusion of DiI (red; Honig and Hume, 1986) and a second lipophilic dye, Di10ASP (green). In this fashion we have studied the spatial segregation of motor nerve terminals and the temporal sequence of events during synaptic competition.

70.13

PROGRESSIVE LOSS OF SYNAPTIC EFFICACY DURING SYNAPSE ELIMINATION. J. Nabekura and J.W. Lichtman, Department of Anatomy & Neurobiology, Washington University School of Medicine, St. Louis, MO 63110

The amplitude of each of the synaptic inputs to multiply innervated muscle fibers was measured in the sternomastoid muscle of neonatal mice by recording intracellularly while stimulating the motor axons with multiple electrodes. At birth, all muscle fibers were innervated by more than one axon (usually 2), whereas at 2 weeks of age, all of the muscle fibers were singly innervated. At birth, the amplitude of each input to a muscle fiber was approximately the same. However, during the next 14 days, the relative amplitudes of the multiple inputs to individual fibers became progressively different. Near the end of the period of multiple innervation, there was roughly a five-fold difference in the amplitudes of the synaptic inputs to each of the few muscle fibers still maintaining multiple innervation. For several reasons, this shift in synaptic strength may be due in part to a decrease in the density of postsynaptic acetylcholine receptors under the terminal being eliminated. First, in some cases, the smaller evoked potentials were smaller than the miniature endplate potentials emanating from the other axon terminal. Furthermore, such unequal innervation occurs at developmental stages when endplates contain areas of high and low density of acetylcholine receptors (Balice-Gordon and Lichtman, Soc. Neurosci. Abs. 14: 894, 1988). Because synapse elimination also involves the structural loss of some presynaptic nerve terminals, we are presently attempting to resolve the relative roles of pre- and postsynaptic elements in the functional loss of synaptic transmission at multiply innervated junctions.

LONG-TERM POTENTIATION II

71.1

LONG-TERM DEPRESSION IN HIPPOCAMPAL PYRAMIDAL NEURONS INDUCED BY LOW-FREQUENCY PRESYNAPTIC ACTIVITY PAIRED WITH POSTSYNAPTIC HYPERPOLARIZATION. P.K. Stanton and T.J. Sejnowski (spon: W. Lytton). Albert Einstein Coll. Med., Bronx, NY 10461 and Salk Institute, La Jolla, CA 92037.

Long-term potentiation (LTP) is a persistent increase in synaptic strength following high-frequency afferent stimulation. Recently, we have shown that low-frequency inputs *in phase* with a high-frequency bursting input exhibit LTP, while *out of phase* inputs exhibit long-term depression (LTD) of synaptic strength. Out of phase inputs arrive while the postsynaptic neuron is hyperpolarized by i.p.s.p's and intrinsic conductances. This suggests that pairing postsynaptic hyperpolarization with low-frequency presynaptic activity should also yield LTD of the activated input.

We recorded intracellularly from CA1 pyramidal neurons in rat hippocampal slices (400 μm thick), stimulating Schaffer collateral or subicular inputs onto apical dendrites. The test input received low-frequency stimuli (5 Hz, 2 sec x 4 trains), and constant current was injected (starting 1-2 min before) to either depolarize (+20 mV) or hyperpolarize (-20 mV) the neuron during stimulation.

Pairing postsynaptic hyperpolarization with low-frequency synaptic stimulation produced LTD specific to the activated pathway, while pairing depolarization yielded homosynaptic LTP. Cells fired action potentials during injection of depolarizing, but not hyperpolarizing, current. Pairing hyperpolarization with stimulation decreased synaptic e.p.s.p.'s of the stimulated pathway -57.6 ± 6.8% (30 min post-stimulation), but only -13.4 ± 8.4% in the control, unstimulated pathway. Thus, hippocampal plasticity follows a two-way Hebbian rule which can either enhance or depress synaptic strength as a function of pre- and postsynaptic covariance.

71.2

INDUCTION OF ASSOCIATIVE LONG-TERM DEPRESSION (LTD) IN HIPPOCAMPAL FIELD CA3 IS NOT MEDIATED BY NMDA RECEPTORS. S. Chattarji, P.K. Stanton[1] and T.J. Sejnowski. Salk Institute, La Jolla, CA 92037 and [1]Albert Einstein Coll. of Med., Bronx, NY 10461.

Brief, high frequency activation of excitatory afferents in the hippocampus produces long-term potentiation (LTP) of synaptic strength. In field CA3, pyramidal cells receive separate inputs via commissural/associational (COM/ASSOC) and mossy fiber (MF) synapses. LTP of COM/ASSOC synapses depends on activation of glutamate receptors of the N-methyl-D-aspartate (NMDA) subtype, but MF synapses do not. CA3 COM/ASSOC synapses exhibit an associative form of LTP when low frequency (LF) stimuli are *positively* correlated in time with a train of high frequency MF bursts (HF). Recently, we have shown that when LF COM/ASSOC stimulation is *negatively* correlated in time with HF stimulation of MF, associative long-term depression (LTD) at COM/ASSOC synapses is induced. Is associative LTD of COM/ASSOC synapses also mediated by NMDA receptors?

Extracellular recordings from rat hippocampal slices (400μm thick) were made in CA3 pyramidal cell somatic and apical dendritic fields. HF stimuli to MF consisted of trains of 10 bursts of 5 pulses each at a frequency of 100 Hz, with an interburst interval of 200 msec. The LF stimuli to COM/ASSOC, a 5 Hz train of single shocks, was given either superimposed on the middle of each burst (in phase), or symmetrically between bursts (out of phase).

In control experiments, the HF stimuli alone induced homosynaptic LTP of either COM/ASSOC or MF synapses, while LF stimuli alone induced no change. In bath applied AP5 (30μM), the HF stimuli alone elicited homosynaptic LTP at MF, but not COM/ASSOC synapses. In AP5, out-of-phase LF COM/ASSOC stimuli given with HF bursts to MF still elicited associative LTD, while associative LTP normally induced by in-phase stimuli was blocked. Thus, induction of associative LTD at CA3 COM/ASSOC synapses does not require NMDA receptor activation.

71.3

DIFFERENTIAL EFFECTS OF NMDA RECEPTOR ANTAGONIST APV ON CALCIUM INDUCED AND TETANIC STIMULATION INDUCED POTENTIATION IN AREA CA1 OF IN VITRO HIPPOCAMPUS. L. Grover & T. Teyler, Neurobiology Dept., N.E. Ohio Univ. Col. Med., Rootstown, OH 44272.

The dependence of calcium induced potentiation on extracellular potassium concentration in area CA1 may reflect involvement of a postsynaptic, voltage-dependent mechanism (Grover & Teyler, Brain Res., in press). Since tetanus induced long-term potentiation is blocked by APV, we asked if calcium induced potentiation is also blocked by APV. Population spike (PS) and population EPSP (PE) were recorded in s. pyramidale and s. radiatum. Potentiation was induced by 10 min exposure to high calcium and potassium, or by high frequency stimulation (200 Hz, 0.5 s, 4X) of s. radiatum fibers. Effects of potentiating treatments were assessed after a 40 min interval. In the absence of APV, tetanic stimulation (n=8) resulted in a 159 ± 13% (mean ± SEM) increase in PS and a 50 ± 13% increase in PE. Calcium induced potentiation (n=6) of PS was 67 ± 21%, and of PE was 19 ± 7%. In the presence of 50 uM D,L-APV, tetanic stimulation (n=5) induced significantly less potentiation: PS was increased by 21 ± 13% ($p<.03$), and PE decreased by 19 ± 5% ($p<.001$). In contrast, potentiation induced by exposure to high calcium (n=7) was not significantly reduced by 50 uM APV: PS increased by 99 ± 20% ($p<.28$), and PE increased by 7 ± 3% ($p<.10$). Calcium induced and tetanus induced potentiation may therefore depend on different mechanisms for induction. (Supported by EPA and ONR.)

71.4

MODULATION OF $[Ca]_i$ BY A CAGED EGTA AFFECTS NEURONAL PLASTICITY IN THE RAT HIPPOCAMPUS. M. Segal* and A. Patchornik* (Spon: U.Z. Littauer) Center for Neuroscience, The Weizmann Institute Rehovot 76100 ISRAEL

The crucial role of intracellular calcium ($[Ca]_i$) in neuronal plasticity has been illustrated extensively in recent years (Lynch et al. Nature 305 719, 1983; Malenka et al. Science 242 81, 1988). We have used a new caged EGTA which is activated by UV irradiation. Experiments were conducted with hippocampal slices maintained in an interface chamber or with dissociated cultured hippocampal neurons voltage clamped with a patch pipette which allows a more precise control of $[Ca]_i$. Tetanic stimulation of stratum radiatum produced long term potentiation (LTP) of EPSP's as seen before. Cells recorded with EGTA (0.1M in 2M K-acetate pH 7.2) containing pipettes failed to express a large afterhyperpolarization (AHP) following a burst of action potentials evoked by a depolarizing current pulse. In addition, these cells failed to express LTP. Cells recorded with caged EGTA containing pipettes expressed normal AHP's and LTP's. However, following UV irradiation the cells ceased to express AHP's. The time course of disappearance of AHP's was used to assess the effect of the activated EGTA on $[Ca]_i$, on EPSP's and LTP. Newly activated EGTA did not affect EPSP size but did prevent generation of LTP. Once an LTP is established, activation of the EGTA could only partially reduce the potentiated EPSP. We are currently studying the critical duration of Post-tetanic elevated $[Ca]_i$ needed to maintain LTP.

71.5

INHIBITION OF POSTSYNAPTIC PROTEIN KINASE BLOCKS INDUCTION BUT NOT EXPRESSION OF LONG-TERM POTENTIATION. R. Malinow, H. Schulman and R. W. Tsien. *Depts. Mol. Cell. Physiol. & Pharmacol., Stanford Univ.*

Long-term potentiation (LTP) of synaptic transmission is a widely studied cellular example of synaptic plasticity. However, the identity, localization and interplay among the biochemical signals underlying LTP remain unclear. It is generally accepted that postsynaptic NMDA activation and calcium entry are events critical in the triggering of LTP. To learn more about the role of the postsynaptic cell in LTP, and the possible involvement of particular protein kinases, we used intracellular microelectrodes to deliver protein kinase inhibitors to postsynaptic cells. We tested four inhibitors: H-7, a general protein kinase blocker; PKC(19-31) and PKC(19-36), peptide fragments of PKC that act as potent and specific inhibitors of PKC activity; and P3, a synthetic calmodulin-binding peptide. We find that delivery of these inhibitors to postsynaptic cells prior to tetanic stimulation blocks LTP. This suggests that postsynaptic kinase activity is required to produce LTP. Previous studies have shown that established LTP can be blocked by bath-applied H-7, which presumably does not discriminate between pre- and postsynaptic protein kinases. To test if persistent *postsynaptic* kinase activity is required to express LTP, we delivered H-7 to postsynaptic cells after the establishment of LTP. We find that once established, LTP appears unresponsive to postsynaptic H-7. Furthermore, postsynaptic H-7 does not reduce the inhibition of potentiated transmission caused by bath-applied H-7. Our results suggest the involvement of postsynaptic PKC in the induction of LTP and a presynaptic protein kinase in the expression of LTP.

71.6

ESSENTIAL ROLE OF POSTSYNAPTIC CALMODULIN AND PROTEIN KINASE ACTIVITY IN LTP D.J. Perkel, R.C. Malenka, J.A. Kauer, P.T. Kelly, M.N. Waxham*, M.D. Mauk & R.A. Nicoll. Depts. Physiol., Pharmacol., & Psychiatry UCSF, San Francisco, CA 94143 & Depts. Neurol. & Neurobiol. & Anat., Univ. Texas Health Sci. Ctr. Houston, TX 77225

The mechanisms by which the influx of calcium ion through NMDA receptors contributes to the generation of LTP in the CA1 region of the rat hippocampus remain unclear. We have investigated, in the slice preparation, the possible role of postsynaptic calmodulin (CaM) and protein kinase activity by intracellular application of compounds known to interfere with such biochemical processes. Tetanic stimulation elicited a slowly decaying potentiation (approx. 30 min.) in pyramidal cells recorded with electrodes filled with calmidazolium (0.5 mM, 1% DMSO; n = 5) or with the peptides (CBP or CBP_{-3}, 190 µM; n = 11, 8) that bind CaM and prevent Ca/CaM-dependent substrate phosphorylation by Ca/CaM-dependent protein kinase type II. In all experiments included, the initial slope of the simultaneously recorded field EPSP remained greater than 120% of control values 60 min. after the tetanus. A control peptide sharing many properties with CBP but not its CaM antagonism did not block LTP (CTP_2, 190 µM; n = 8). Intracellular application of H7 (20 mM, n = 8), a non-specific inhibitor of protein kinase activity, also blocked LTP. We conclude that LTP requires activation of postsynaptic CaM and protein kinase activity.

71.7

ZIF/268 mRNA LEVELS AND INDUCTION OF LTP ARE REGULATED IN PARALLEL BY PERFORANT PATH STIMULATION. P.F. Worley, A.J. Cole, D.W. Saffen* and J.M. Baraban. Dept. of Neuroscience, Johns Hopkins University, Baltimore, MD.

Recent studies have demonstrated that neuronal stimulation elicits rapid increases in c-fos as well as several other transcription factor mRNAs in brain. To study synaptic mechanisms involved in regulating this rapid genomic response in brain, we stimulated neurons of the dentate gyrus via perforant path afferents in vivo and monitored mRNA levels of 4 genes previously shown to be rapidly induced by seizures (Saffen et al., PNAS 1988) using in situ hybridization. High-frequency stimulation of the perforant path (twelve 20 msec 500 hz trains), which induces long-term potentiation, but not low frequency stimulation (0.1 hz), markedly increases zif/268 mRNA levels in ipsilateral granule cell neurons within 15 minutes. mRNA levels of c-fos, c-jun and jun-B are less consistently increased by this stimulus. NMDA antagonists CGS-19755 and MK-801, which block neuronal NMDA responses and LTP, also block high-frequency stimulus induced increases in zif/268 mRNA. Furthermore, tetanic stimulation of CA4 neurons in the contralateral hippocampus prior to the conditioning stimulus, which blocks LTP by a feed-forward inhibitory mechanism, also blocks increases in mRNA levels. These results indicate that like LTP, zif/268 is rapidly induced by synaptic activation of NMDA receptors and may be involved in mediating long-term synaptic plasticity.

71.8

EVIDENCE SUGGESTING THAT AMBIENT GLUTAMATE LEVELS ACTIVATE NMDA RECEPTORS IN HIPPOCAMPAL CA1 NEURONS. S. Hestrin*, P. Sah*, and R.A. Nicoll (SPON: M. Block). Depts. of Physiology and Pharmacology, University of California, San Francisco, CA 94143

The high sensitivity of NMDA receptors to glutamate raises the possibility of activation by ambient levels of extracellular glutamate. To test this possibility experiments were performed in rat hippocampal slices *in vitro*. We found that in CA1 pyramidal neurons under voltage clamp (hp: -35 mV), application of 50 µM dl-APV induced an outward current (250-500 pA). To characterize this current further, tight-seal whole-cell recordings were made from thin hippocampal slices. These recording conditions revealed a standing current noise with the following properties: 1) The variance of the membrane current at -40 mV was much larger than that measured at -80 mV; 2) At -40 mV application of APV reduced the variance to that measured at -80 mV; 3) At -80 mV removal of extracellular magnesium increased the variance, which was APV-sensitive; 4) Power spectra of this membrane noise were Lorentzian and similar to those induced by application of NMDA. These observations suggest that there is a persistent activation of NMDA receptors in hippocampal CA1 neurons.

71.9

MECHANISMS UNDERLYING LTP AT AN EXCITATORY SYNAPSE IN RAT ANTERIOR CINGULATE CORTEX. P. Sah* and R.A. Nicoll. Depts. of Pharmacology and Physiology, University of California, San Francisco, CA 94143.

Although a great deal is now known about LTP in the hippocampus, the existence of similar synaptic plasticity at cortical synapses is not well established. We have studied the monosynaptic synapse between the commissural fibres and layer V pyramidal neurones in rat anterior cingulate cortex. At the resting membrane potential (-90 mV) the EPSP at this synapse was blocked by CNQX indicating that quisqualate/ kainate receptors mediate the EPSP. Depolarization of the postsynaptic cell revealed a slower APV-blockable component. High frequency stimulation of the commissural afferents (100 Hz, 1 sec) resulted in a large potentiation of the initial slope of the EPSP which lasted approximately 60 minutes. A critical stimulus strength was required to elicit potentiation, indicating that LTP at this synapse exhibited cooperativity. The potentiation also was blocked by 50 μM APV indicating that NMDA receptor activation is required for LTP. Loading the cell with the calcium chelator BAPTA also blocked the induction of the LTP. These experiments show that there are many similarities between LTP at this cortical synapse and LTP at excitatory synapses in the CA1 region of the hippocampus. However, one clear difference is the failure to elicit LTP by pairing postsynaptic depolarization with synaptic activation. Possible explanations for this difference will be discussed.

71.10

HORIZONTAL LONG-TERM POTENTIATION OF RESPONSES IN RAT SI CORTEX. S.M. Lee, M.G. Weisskopf*, and F.F. Ebner. Center for Neural Science, Brown University, Providence, RI 02912

Although the role of LTP in neocortex is not as established as in the hippocampus, there is an emerging line of evidence that in neocortex, NMDA receptors strongly influence cells' responsiveness to sensory input. Here we provide direct evidence that a conditioning stimulus (CS) presented in layer VI of a rat neocortical slice results in an APV-sensitive enhancement of responses in layer II/III horizontal to the radial recording site.

Neocortical slices 400 um thick were taken from five adult Long-Evans rats (300-350 g) and placed in an interface chamber superfused with an oxygenated medium containing (in mM) 126.0 NaCl, 3.0 KCl, 1.25 NaH_2PO_4, 2.0 $MgCl_2$, 2.0 $CaCl_2$, 26.0 $NaHCO_3$ and 10.0 dextrose. Field potentials in layer II/III to single shocks (200 us, 100 uA) in layer VI were recorded directly above ("radial") and at various distances away ("off-radial") from a concentric stimulating electrode. This intensity elicited a radial response 75-80% of maximum in layer II/III. The CS was delivered in the presence of low levels of Mg^{2+} (100-500 uM). The degree of potentiation at various sites was measured >1 hr following reinfusion of the normal medium.

The most effective CS for eliciting LTP in the superficial layers was 2 Hz for 45-80 sec. Two types of APV-sensitive long-term enhancements were seen in the superficial layers: i) 50-400% potentiation over baseline responses and ii) induction of novel responses (1000-1500 um "off-radial").

In vivo, this horizontal potentiation may determine the cortical domain of active sensory inputs and could support the reorganization of neocortical sensory maps. (Supported by NIH grant #NS-13031)

71.11

DIFFERENTIAL CONDITIONING AT CELLULAR LEVEL (CAT MOTOR CORTEX) WITHOUT ASSOCIATED POSTSYNAPTIC FIRING ACTIVITY. A. Baranyi, M.B. Szente and C.D. Woody. UCLA Med. Ctr., MRRC, BRI, Los Angeles, CA 90024

Mechanisms of long-lasting potentiation (LLP) of excitatory postsynaptic potentials and currents (EPSPs/EPSCs) were studied in intracellular microelectrode and single channel voltage clamp experiments in the motor cortex of conscious cats. Two different EPSPs/EPSCs were elicited in neurons in which the firing activities were blocked by intracellular injection of QX-314. One of the postsynaptic responses was explicitly paired with intracellular current pulses in voltage clamp mode (ISI:0-200 ms, ITI:0.1-0.5 Hz), and the other unpaired response was tested simultaneously in the same target neuron. Input-specific LLP of paired vs. unpaired EPSPs/EPSCs was induced in the absence of firing activity after 20-60 pairings. However, LLP occurred only in neurons where the paired voltage responses regularly attained a membrane potential of -35mV or the command pulses were larger than 30 mV from the holding potential of -65mV. Intracellularly injected aminopyridine, Cs^+ (vs. TEA and apamin), Co^{2+}, Cd^{2+}, EGTA and extracellularly injected dendrotoxin precluded the induction of LLP. The results suggest that input-specific LLP in neocortical neurons requires local postsynaptic depolarizations which provide sufficient Ca^{2+} levels at the postsynaptic zones of paired inputs. (Supported by HD05958.)

71.12

LONG-TERM FACILITATION OF ELECTROTONIC AND CHEMICAL COMPONENTS OF MIXED EXCITATORY SYNAPSES ON THE MAUTHNER CELL. X.-D. Yang* and D.S. Faber. Neurobiology Lab, Dept. Physiology, SUNY Buffalo, Buffalo, NY 14214.

The goldfish Mauthner (M-) cell receives a monosynaptic excitatory input from the VIIIth nerve. Impulses in single fibers produce both electrotonic coupling potentials and glutamatergic EPSPs. The EPSP is transiently facilitated when two or three presynaptic action potentials are evoked at ~500 Hz. We now report that when the nerve is stimulated repeatedly with similar brief trains once every 2 sec for 4 min, both responses exhibit prolonged enhancements.

Intracellular recordings were obtained from the lateral dendrite at the site of synaptic input. The conditioning stimulus was suprathreshold for the M-cell while the test stimulus was subthreshold. Within 10 min after training the coupling potential and EPSP were increased, on average by 23% (range 0-62%, n=6) and 85% (range 31-144%), respectively, while resting membrane potential was unchanged, and antidromic spike height was slightly reduced (5%). Electrotonic and chemical facilitations could be quantitatively dissociated, indicating they were not due to changes in stimulating electrode properties. They persisted throughout the recording period, up to 80 min after training.

Long-term facilitation of the coupling potential might be due to an increased junctional conductance, which would be consistent with the reduction in antidromic spike height. It would be interesting to determine if a common intracellular modification triggers both enhancements. Supported in part by NIH grant #NS 15335.

71.13

N-METHYL-D-ASPARTATE ANTAGONISTS INFUSED INTO THE AMYGDALA BLOCK ACQUISITION OF FEAR-POTENTIATED STARTLE. M.J.D. Miserendino*, K.R. Melia*, C.B. Sananes & M. Davis. (Spon: R.J. Storella). Psychiatry Dept., Yale Univ. Med. Sch., 34 Park St., New Haven, CT. 06508.

The amygdala plays a critical role in a variety of aversive conditioned responses. N-methyl-D-aspartate (NMDA) receptors, which may be involved in synaptic plasticity related to learning and memory, occur densely in the lateral and basolateral amygdaloid nuclei. We therefore examined whether an NMDA receptor-mediated process in the amygdala might underlie aversive conditioning as measured by the fear-potentiated startle paradigm.

Rats were implanted with bilateral cannuli aimed at the basolateral nucleus of the amygdala. Following recovery, animals received two training sessions in which 10 light-footshock (0.6 mA) pairings were presented (ISI=4 min). Immediately prior to each session, rats were infused with the competitive NMDA receptor antagonists DL-2-amino-5-phosphono-valeric acid (AP5; 1.25, 2.5, 5.0, 10.0, or 20.0 μg/0.5 ul), 3-((+-)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP; 2.5, 5.0 μg) or vehicle. One week after training, fear-potentiated startle was assessed by comparing the startle response to a brief noise burst presented alone or in the presence of a light.

Both CPP and AP5 blocked fear-potentiated startle in a dose-dependent manner, with a complete blockade at doses of 5.0 μg or higher. AP5 did not produce catalepsy, nor did it alter sensitivity to shock, perception of the light CS (measured with a visual prepulse inhibition paradigm), or baseline startle amplitude. Anatomical specificity of this effect was demonstrated by the fact that bilateral infusion of AP5 (20 ug) into cerebellar nucleus interpositus prior to training had no effect on potentiated startle.

These data suggest that activation of NMDA receptors in the amygdala is necessary for the acquisition of conditioned fear, as measured by fear-potentiated startle.

72.1

SUBSTITUTION OF SERINE FOR CYSTEINE351 PREVENTS PERTUSSIS TOXIN (PTX)-MEDIATED ADP-RIBSOSYLATION OF THE GTP-BINDING PROTEIN G o_α. T.J. Murphy§*, W.F. Simonds¶, T. M. Storman§, A. M. Spiegel¶ and M. R. Brann§. §LMB, NINDS; ¶NIDDK; NIH, Bethesda, MD 20892.

A mutant Go_α gene created by excision of a DNA restricition fragment from a cloned Go_α gene and replacement of the fragment with a cohesive synthetic linker differs from the wildtype gene by three bases and lacks 288 bases of the 3' untranslated region. The mutant gene encodes Go_αser351 which differs from wildtype Go_α by a single amino acid substitution of serine for cysteine at residue 351. The mutant gene was cloned into the mammalian expression vector pCDps and transiently expressed in COS-7 cells. The size of this mutant, as assessed by SDS-PAGE and immunoblots using two anti-peptide antisera specific for Go_α is indistinquishable from an immunoreactive 39 kDa bovine brain membrane protein or wildtype recombinant Go_α also expressed in COS-7 cells. However, [32P]-ADP-ribosylation of membrane proteins using PTX failed to result in labeling of a 39 kDa species in membranes prepared from mutant transfected cells whereas wildtype recombinant Go_α was labeled. Our results demonstrate that the cysteine residue in the carboxyl terminal region of Go_α is required for ADP-ribosylation by PTX. The mutant gene has been stably transfected into A9L cells expressing recombinant m4 muscarinic receptor. We are currently isolating subclones to study second messenger responses to Go_αser351.

72.2

ISOLATION OF A DROSOPHILA GENE ENCODING A Go α-LIKE PROTEIN. J. Yoon*, R. D. Shortridge*, B. T. Bloomquist*, S. Schneuwly*, M. H. Perdew*, and W. L. Pak, Dept. Biol. Sci., Purdue Univ., West Lafayette, IN 47907.

We have isolated a D. melanogaster gene encoding a Go α subunit homolog by screening genomic and adult head cDNA libraries using bovine transducin α subunit cDNA as probe at reduced stringency. Sequence analysis of cDNAs showed that this gene encodes two α subunits which differ in 7 amino acids in the N-terminal region and that the deduced amino acid sequences for both proteins are highly homologous to a rat Go α subunit (81% identity). Analysis of genomic DNA revealed that two proteins differ in the 5'-noncoding region and the first coding exon, but share the remaining 6 coding exons, and that the expression of the two proteins seems to be regulated by two different promoters. The gene was mapped to 47A on the second chromosome. Three transcripts were detected: a 3.9 kb transcript found in all stages of development, a 5.4 kb transcript present predominantly in adult heads, and a 3.4 kb transcript present only in adult bodies. In situ hybridizations to adult tissue sections showed that this gene is expressed abundantly in neuronal cell bodies in the brain and thoracic ganglia. This work was supported by grants from NIH (EY00033, EY04767, EY02723).

72.3

IMMUNOCYTOCHEMICAL LOCALIZATION OF THE NEURAL-SPECIFIC REGULATORY SUBUNIT OF THE TYPE II cAMP-DEPENDENT PROTEIN KINASE IN RAT BRAINS. N. Ludvig*, C.E. Ribak, S. Glantz* and C.S. Rubin* (spon: R.B. Wuerker). Dept. of Anatomy and Neurobiology, Univ. of Calif., Irvine, CA. 92717 and Dept. of Molecular Pharmacology, Albert Einstein College of Med. Bronx, NY. 10461.

The subcellular localization of the neural-specific regulatory subunit of the type II cAMP-dependent protein kinase (RII-B) was analyzed with electron microscopic immunocytochemical methods in the brains of Sprague-Dawley rats. It was found that: (1) the RII-B immunoreactivity (RII-Bi) was exclusively localized to neurons; glial and endothelial cells did not contain RII-Bi; (2) Some neuronal somata lacked RII-Bi, (3) RII-Bi was concentrated in postsynaptic structures, dendrites and perikarya, and not in axons and axon terminals, (4) RII-Bi was associated with postsynaptic densities opposing both excitatory and inhibitory types of axon terminals.

These data provide morphological support for the electrophysiologically and pharmacologically demonstrated interaction between cAMP-dependent protein kinases and post-synaptic receptors and voltage-gated ion-channels. In fact, the subcellular distribution of RII-B seems to correspond with the subcellular distribution of neurotransmitter-receptors (i.e., $GABA_A$ and mAch) reported by other groups. (Supported by NIH Grants NS-15669 and GM-22792).

72.4

EXTRACELLULAR 3',5'-CYCLIC NUCLEOTIDE PHOSPHODIESTERASE IN RAT CEREBRAL CORTEX IN DISSOCIATED CELL CULTURE. S. Vasquez* and P.A. Rosenberg (SPON: A. Lorenzo). Dept. of Neurol., Children's Hosp., Harvard Med. Sch., Boston, MA 02115

One possible mode of action of norepinephrine as a neuromodulator in cerebral cortex is via stimulation of cAMP efflux from astrocytes, thereby increasing the concentration of cAMP or metabolites in the extracellular space. One or more of these adenine nucleotides may interact with receptors on neurons. Previous experiments demonstrating cAMP efflux from cerebral cortex in culture suggested that extracellular cAMP was not stable but was degraded, suggesting the existence of an extracellular phosphodiesterase. In order to pursue this observation, the fate of extracellular cAMP was studied. Mixed cultures of neurons and glia were incubated with ^{3}H-cAMP, and media was sampled at selected times. Cyclic AMP, AMP, and adenosine were separated by thin layer chromatography. Extracellular ^{3}H-cAMP progressively decayed from its original concentration, and the decay of cAMP was accompanied by the appearance of material that cochromatographed with adenosine and with AMP. The phosphodiesterase inhibitors isobutylmethylxanthine (IBMX) and RO 20-1724 (an imidazolidinone), at 300 μM, blocked the phosphodiesterase activity, demonstrated by the reduction in the formation of AMP. Supported by the Grass Foundation and NS00993.

72.5

EFFECTS OF A SELECTIVE BLOCKER OF A LOW K_M cAMP-PHOSPHODIESTERASE ON RAT NEOCORTEX SLICES.
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The intracellular action of the second messenger cAMP has to be terminated by an effectively regulated inactiviation mechanism. Cyclic AMP is degraded by cAMP-phosphodiesterases (cAMP-PDE). Thus, the regulation of the activity of these enzymes may be of crucial importance for neuronal excitability. The availability of selective inhibitors for cAMP-PDE isozymes offers the possibility to study the influence of different cAMP-PDEs on the excitability of CNS neurons.

In biochemical experiments, the inhibitory efficacy of the selective blocker of a low K_M, Ca^{2+}/calmodulin-independent PDE denbufylline (DBF) on the total cAMP-PDE activity in a crude homogenate from rat frontal cortex slices was compared to that of the non-selective inhibitor IBMX. At concentrations of 100 μM, DBF reduced the PDE activity by 20-25 %, whereas IBMX decreased the enzyme activity by 65-70 %.

In electrophysiological experiments, intracellular recordings were performed from superficial neurons of rat frontal cortex slices. At concentrations of 30-100 nM, DBF led to a pronounced increase in synaptic and direct evoked excitability. Upon an increase in concentration up to 10 μM, a marked prolongation of IPSPs was observed. These changes occurred without significant changes in membrane potential or input resistance.

These experiments demonstrate that the selective inhibition of a cAMP-PDE isoenzyme leads to marked changes in the excitability of rat neocortical neurons, although this isoenzyme represents only a fraction of the total PDE activity in the frontal cortex.

72.6

REGULATION OF TYPE II (cGMP-ACTIVATABLE) PHOSPHODIESTERASE IN PC12 CELLS. M.E. Whalin*, J.G. Scammell*, W.J. Thompson*, and S.J. Strada. Dept. of Pharmacology, Univ. of South Alabama College of Medicine, Mobile, AL 36688.

Characterization of phosphodiesterase (PDE) activity in cell-free preparations of PC12 cells showed that the major cAMP hydrolytic activity was Type II (cGMP-activatable) PDE. This activity was inhibited by the isoquinoline compounds papaverine and HL-725 (trequinsin). cAMP accumulation induced by adenosine (ADO) in intact cells showed a peak response (5-8 fold)at 5 min and removal of ADO by the addition of adenosine deaminase resulted in the decay of cAMP to basal levels in 3 min. Papaverine or HL-725 potentiated the ADO response, whereas preincubation of PC12 cells with nitroprusside (NP) or atrial natriuretic factor (ANF), agents which increase cGMP, attenuated the ADO induced cAMP accumulation and increased the rate of cAMP decay. An attenuation of the ADO response by NP or ANF was not observed in the presence of papaverine or HL-725. We conclude from these studies that, in PC12 cells, agents that increase intracellular cGMP can regulate cAMP metabolism by the activation of Type II PDE. Supported by USPHS GM33538 and a contract from the USAF (49620-85-K-0014).

72.7

INHIBITION OF ADENYLATE CYCLASE IN PERMEABLE NCB-20 CELLS IS COUPLED TO THE STIMULATION OF THAT ENZYME. J.H. Gordon and M.M. Rasenick. Dept of Pharmacol, Loyola Univ Med Sch; Res Svce, VA Hosp, Hines, IL 60141 and Dept Physiol and Biophys, Univ IL College Med, Chicago, IL 60680.

The relationships between receptors, G proteins and the catalytic unit of adenylate cyclase (AC) remain intact in cells made permeable by saponin (FEBS Lett. 207:296,1986), this may not be the case in isolated membranes. In saponin treated NCB-20 cells, GTP S elicited a dose-dependent stimulation (stim) of AC. In forskolin activated cells, GTP S provoked a dose-dependent inhibition (inhib) of the AC. At 0.1 uM GTP S produced a minimal stim of AC; the addition of dopamine (DA) produced a dose-dependent stim of AC. At 1.0 uM GTP S produced a modest stim of AC, which was not modifed by the addition of DA. GTP S concentrations >10 uM produced a substantial stim of AC which was inhibited by DA. In contrast, isoproterenol (ISO) produced a dose dependent stim of AC at all concentrations of GTP S. The stim of AC by ISO was also inhibited in a dose-dependent manner by DA. However, ISO produced a dose-dependent inhib of AC when the reaction mixture contained 1.0 uM GTP S and 100 uM DA. The results suggest the existence of a complex series of interactions between the receptors, the various G proteins, and the catalytic unit of AC. Such interactions may involve nucleotide binding and transfer between the various G alpha subunits. (Supported by NSF BNS 87-19758; VA Med Res; NIH NS-26449; Tourette's Syndrome Assn & Scottish Rite Schizophrenia Res Pgm, NMJ, USA)

72.8

TRANSDUCTION MECHANISMS UNDERLYING THE COUPLING BETWEEN ALPHA1 AND 5-HT1a-LIKE RECEPTORS ON CULTURED PACEMAKER SPINAL CORD NEURONS. P.Legendre*, M.Dubar* and J.D.Vincent (SPON:J. Clements).INSERM U.176,33077 Bordeaux cedex, France.

Intracellular recordings were made from cultured mammalian spinal cord pacemaker neurones and the action of noradrenaline (NA)and serotonin (5-HT) were studied. These compounds acted on alphal and 5-HT1a-like receptors to reduced the same K^+ conductance and this effect was mimicked by IBMX, forskolin and 8-bromo-cAMP applications. Such responses were also mimicked by the application of mellitin, an activator of the phopholipase A2, and by the application of arachidonic acid (AA) and Prostaglandin E2 (PGE2). NA, 5-HT and PGE2 induced responses were inhibited by the protein kinase A and G inhibitor H8 while the guanylate cyclase inhibitor methylen blue had no effect. Only the NA induced depolarization was inhibited by the application of AA metabolism inhibitors such as ETYA and indomethacin. Furthermore, ETYA prevented the AA-induced response but did not alter the PGE2 induced depolarization. These results suggest that 5-HT1a-like and alphal receptors on cultured pacemaker spinal cord neurons, are coupled to the same K^+ conductance through different second messenger pathways. NA evoked responses involves PGE2 production which induces adenylate cyclase activity, while 5-HT1a-like receptors appear to be directly coupled to the adenylate cyclase.

72.9

ROLE OF SECOND MESSENGERS IN ACETYLCHOLINE (ACh)-AND VASOACTIVE INTESTINAL POLYPEPTIDE (VIP)-EVOKED SECRETION OF CATECHOLAMINES (CA). T. Wakade, R.K. Malhotra and Arun R. Wakade (Spon. M.A. Marrazzi) Dept. of Pharm., Wayne State Univ., Detroit, MI 48201.

Perfused rat adrenal gland was used to determine relationship between second messengers and CA secretion evoked by ACh, VIP or stimulation of splanchnic nerves. VIP (10 uM) increased cAMP, ^{3}H-inositol trisphosphate ($^3IP_3$) and protein kinase C (PKC) activity. ACh (30 uM) increased ^{45}Ca uptake, ^{3}H-IP_3 and PKC activity. Since ACh activates nicotinic and muscarinic receptors to evoke CA secretion, effects of nicotine and muscarine were investigated. Muscarine (100 uM) increased ^{3}H-IP_3 and PKC activity. Nicotine (17.5 uM) increased only ^{45}Ca uptake. Stimulation of splanchnic nerves (10 Hz for 10 min), which causes release of ACh and VIP, increased ^{45}Ca uptake, cAMP, ^{3}H-IP_3 and PKC activity. None of the procedures affected cGMP content. We conclude that stimulation of adrenal medulla by its neuronal supply results in activation of 4 second messengers that are coupled to 3 types of receptors of chromaffin cells. These messengers interact with each other to modulate Ca concentration and thereby CA secretion.

72.10

REGULATION OF NICOTINE EVOKED DOPAMINE RELEASE FROM PC12 CELLS. N.D. Courtney, A.C. Howlett and T.C. Westfall. Dept. of Pharmacology St. Louis Univ. Sch. of Med., St. Louis, MO 63104.

Nicotine (NIC) stimulates dopamine (DA) release from PC12 cells presumably by activating nicotinic acetylcholine receptors. It is also known that the NIC induced depolarization results in the opening of L type voltage sensitive Ca^{2+} channels with the subsequent increase in Ca^{2+} influx. Not much, however, is known about the events between Ca^{2+} influx and the ultimate DA release. The present series of experiments were designed to determine the nature of the intracellular signals following activation of nicotinic receptors and Ca^{2+} influx. For release experiments, cells were incubated in Gey's balanced salt solution with or without drugs for 2 min. Released and cellular DA was measured in the incubation buffer and cell lysate respectively, by HPLC-EC. NIC significantly increased DA release above basal release (BSL 5.11% ± 1.6, 100 µ M NIC 11.7% ± 3.9). In the absence of extracellular Ca^{2+}, the NIC evoked DA release was not seen. Calmidazolium - a calmodulin antagonist, blocked NIC evoked DA release (NIC 12.9% ± 4.9, NIC + CDZ 4.5% ± 1.6). 2',3' Dideoxyadenosine, which blocks adenylate cyclase, completely abolished the NIC evoked DA release (NIC 12.9% ± 4.9, NIC + DDA 5.4% ± 0.3). A cAMP analog and agents which elevate intracellular cAMP also enhanced nicotine evoked dopamine release. These results suggest an important role for cAMP and calmodulin in the DA release. (Supported by DA03690, DA02668 and NS07254).

72.11

CHRONIC ANTIDEPRESSANT DRUGS MODIFY cAMP-DEPENDENT PHOSPHORYLATION SYSTEM IN RAT CEREBRAL CORTEX. J. Perez*; D. Tinelli*, E. Bianchi*, N. Brunello and G. Racagni, Center of Neuropharmacology, University of Milan Italy.

We have shown that chronic treatment with desmethylimipramine (DMI), a tricyclic antidepressant drug, induces a specific change in the endogenous phosphorylation of a microtubule associated protein, immunologically recognized as MAP-2. This neuronal phosphoprotein is highly enriched in the dendritic portion of neurons and represents one of the major substrate for the cAMP-dependent PK type II. Since several studies have demonstrated that cAMP-PK can be associated to microtubule fraction suggesting that endogenous substrate proteins and PK have a similar subcellular distribution, we have analysed both the endogenous phosphorylation of MAP-2 in the cerebrocortical crude microtubule fraction and the photoaffinity labelling of cAMP-PK after chronic DMI treatment. An increase in the ^{32}P incorporation was found in the MAP-2 of crude microtubule fraction after DMI treatment without changes in its concentration. Moreover, both in the soluble and in the microtubule fraction, DMI treatment specifically affected the photoaffinity labelling with 8-N_3-^{32}P-cAMP of a protein band of 52 KDa which represents the regulatory subunit of PKII. Chronic administration with other antidepressant drugs such as fluoxetine, a specific serotonin uptake blocker, and (+)oxaprotiline induces a similar pattern of changes in the photoactivated incorporation of cAMP to crude microtubule fraction, thus indicating that this modification may be related to those adaptive changes elicited by prolonged antidepressant treatment at synaptic level.

73.1

ISOLATION OF A PARTIAL RAT BRAIN cDNA CLONE RELATED TO THE β_1 ADRENERGIC RECEPTOR. M.W. Hamblin, K. Ariani, G.L. Tan, J. Eberwine, and R.D. Ciarancllo. Laboratory of Developmental Neurochemistry, Stanford University School of Medicine, Stanford, CA 94305.

We have been using probes based on known sequences of G-protein linked receptors to isolate new members of this gene family. We screened a rat hippocampus pCD cDNA library using a mixture of different ^{32}P end-labeled oligonucleotide probes based on the presumed membrane spanning regions of the human $5HT_{1A}$ receptor (Kobilka et al., Nature 329:75, 1987) A 2.5kb clone, AE5A, strongly hybridized at low stringency to probes from presumed membrane spanning regions VI and VII but only weakly or not at all to probes from other membrane spanning regions. Northern hybridization analysis of rat brain total RNA with AE5A showed labeling of a single message species 3kb in size. Partial sequencing shows greater than 90% sequence identity with the previously identified human β_1 adrenergic receptor sequence (Frielle et al., PNAS 84:7920, 1987) over much of the length of the clone. This clone may thus represent the rat β_1 adrenergic receptor. Sequence in the region of the presumed cytoplasmic loop III, however, is less well conserved. Clone AE5A is truncated near the sequence specifying the presumed membrane spanning region II. Although it is not full length, AE5A may be useful as a probe in the study of regulation of rat β_1 adrenergic receptors.

73.2

EXPRESSION OF A D_2 DOPAMINE RECEPTOR cDNA. O. Civelli, P. Albert*, K. Neve, H.H.M. Van Tol*, D. Grandy, J.A. Salon, C. Machida and J.R. Bunzow*, Vollum Inst., OHSU, Portland, OR 97201.

The dopamine receptor belongs to the family of the G protein-coupled receptors. These receptors are expected to share a significant degree of sequence similarity. We have used the hamster β_2-adrenergic receptor gene as probe under low-stringency hybridization conditions to isolate related genes. We have thus been able to isolate a cDNA which directs the expression of a protein with the characteristics of a rat D_2 dopamine receptor (Bunzow, J. et al., Nature 336:783-787). We have since shown that this receptor is functional. The D_2 dopamine receptor is known to couple to G proteins to inhibit cAMP production and hormone release. We show that the D_2 receptor cDNA when transfected into the rat somatomammotrophic cell, GH_4 C_1, directs the expression of a D_2 receptor which couples to a pertussis toxin-sensitive G protein. We further show that this interaction results in an inhibition of cAMP accumulation, adenylate cyclase and prolactin secretion, thus demonstrating that the cDNA codes for a functional receptor. In addition, site-directed mutagenesis is being used to examine the D_2 receptor sites which are important for ligand-binding, G protein-coupling and receptor desensitization.

73.3

CLONING OF A NEW G-PROTEIN-COUPLED RECEPTOR HOMOLOGOUS TO THE ALPHA-2 ADRENERGIC RECEPTOR R.L. WEINSHANK*, H.M. LICHTBLAU* AND P.R. HARTIG Neurogenetic Corporation, 215 College Road, Paramus, NJ 07652

We have used the genomic clone G21, which encodes the serotonin 5-HT_{1A} receptor, to screen a human spleen genomic library. Restriction map analysis of the clonal isolates indicates that most fall within three related families. Two of these families have been identified as previously characterized clones, but the DNA sequence of one clone, designated 5A, is unique. This human genomic clone contains an uninterrupted long open reading frame encoding a protein with seven putative transmembrane segments. All of the expected structural features of a G-protein coupled receptor were present. A comparison of the amino-acid sequence of this putative G-protein coupled receptor with other members of this family showed greatest homology to the human platelet alpha-2 and human kidney alpha-2 adrenergic receptors. Data will be presented describing the pharmacological binding properties of clone 5A transfected into COS cells along with data on the regional distribution in brain of mRNA corresponding to this clone.

73.4

α_2-ADRENERGIC RECEPTOR (A2AR) STIMULATION OF PHOSPHOLIPASE A_2 (PLA2) AND OF ADENYLATE CYCLASE IN TRANSFECTED CHO CELLS ARE MEDIATED BY DIFFERENT MECHANISMS. D.B. Bylund, S.B. Jones*, S.P. Halenda*, C.M. Fraser* and J.C. Venter*. Dept. Pharmacology, Univ. Missouri, Columbia, MO 65212, and NINDS, NIH, Bethesda, MD 20892.

In CHO cells transfected with the A2AR gene (Fraser et al, in press), the effect of epinephrine (EPI) on forskolin-stimulated cAMP production is biphasic. At low concentrations, EPI inhibits cAMP production, whereas at high concentrations, it is stimulatory. EPI also stimulates arachidonic acid mobilization (AAM) presumably via PLA2. We used 3 approaches to test the hypothesis that the potentiation of forskolin-stimulated cAMP production was mediated by PLA2 1. A Ca^{++} ionophore increased AAM, whereas EGTA blocked both basal and EPI-mediated AAM. Neither affected cAMP. 2. Pretreatment with pertussis toxin blocked AAM by EPI (but not by Ca^{++} ionophore), but increased cAMP. 3. Quinacrine blocked the EPI-mediated AAM, but did not alter cAMP. Thus, it appears that in these cells, the A2AR is coupled through G_i to the inhibition of adenylate cyclase and to the stimulation of PLA2, but increases cAMP production by a different mechanism. Supported by NIH GM37664 and HL38406.

73.5

IN VIVO STIMULATION OF D1 DOPAMINE RECEPTORS INCREASES THE PHOSPHORYLATION OF PROTEINS IN THE STRIATUM. I. Levari*, M.J. Zigmond and R.M. Lewis. Univ. of Pittsburgh, Pittsburgh, PA 15261.

DARPP-32 is a neuron-specific phosphoprotein phosphatase inhibitor which co-localizes with D1 receptors in the mammalian CNS. Phosphorylation of DARPP-32 increases when slices of rat striatum are perfused with dopamine or 8-bromo-cAMP. We tested whether this occurred *in vivo*, if it was specific for D1 receptors, and whether the phosphorylation of other proteins was affected. Rats were injected with L-dopa, or the D1 specific agonist SKF 38393, and the striatum was dissected out. The ratio of phosphorylated to total protein for each distinct band on SDS/acrylamide gels was determined by back-phosphorylation. The phosphorylation of several proteins was stimulated by L-dopa in a dose-dependent manner. The lowest concentration of SKF 38393 tested so far led to complete phosphorylation of some of the proteins. Other phosphoproteins appeared to be unaffected by L-dopa or SKF 38393. One of the bands migrated at a position consistent with the molecular weight of DARPP-32, but none of the bands have been positively identified at this time. These data are consistent with the hypothesis that dopamine agonists stimulate the adenylate cyclase coupled to D1 receptors. The increase in cAMP leads to an increase in the phosphorylation of specific substrates by a cAMP-dependent protein kinase.

73.6

POSTNATAL DEVELOPMENT OF DOPAMINE D-1 RECEPTORS MAY REQUIRE D-1 RECEPTOR STIMULATION. H.A. Gelbard*, M.H. Teicher, A.L. Gallitano*, J. Zorc*, G. Faedda* and R.J. Baldessarini. Laboratory of Developmental Psychopharmacology & Mailman Research Center, McLean Hospital & Harvard Medical School, Belmont, MA 02178.

In man (Seeman et al., *Synapse*, 1:339-404, 1987) as well as rat (Gelbard et al., *Dev. Brain Res.*, in press), there is a marked increase in the density of dopamine (DA) D-1 receptors in forebrain during early postnatal life. The density of striatal D-1 receptors (assayed by binding of the D-1 antagonist ^3H-SCH-23390) increased, relative to levels at postnatal day 8, by 1.5-fold at d10, 3.9-fold at d15, and peaked to 5.7-fold by d40. Early depletion of forebrain DA with intracisternal 6-OH-DA (given after desipramine to protect noradrenergic neurons) markedly attenuated this age-dependent increase in D-1 receptor density. Rats treated with 6-OH-DA on d3 had a 50% *reduction* of striatal D-1 receptor site density (Bmax) at d21. In contrast, more mature rats DA-depleted at d35 and also sacrificed 18d later, showed no change in D-1 receptor Bmax. These results accord with observations by Kostrzewa and Saleh (*Dev. Brain Res.*, 45:95-101, 1989) of a 78% decrease in the development of striatal D-1 receptor site Bmax after daily postnatal treatment with SCH-23390 to d32. We found, further, that daily administration of the D-1 agonist SKF-38393 (3 mg/kg, i.p., at days 6-18) after 6-OH-DA treatment on postnatal d3 restored striatal D-1 sites to 120% of control Bmax at 21d. Furthermore, similar daily treatment with SKF-38393 in unlesioned neonatal rats increased striatal D-1 Bmax to 140% of control at 21d. These findings suggest a requirement for DA agonism at a critical time (evidently before d35) in the neonatal brain to induce development of D-1 receptors. [Supported by USPHS [NIMH] grants MH-34006, MH-43743, MH-47370, and awards from the Bruce J. Anderson, Charles Dana, and Milton Foundations, Marion Ireland Benton Trust, and an award from the Regione Autonoma della Sardegna.]

73.7

DOPAMINE D_2 and D_1 RECEPTOR CHANGES IN MPTP PARKINSONIAN PRIMATES. G.M.Alexander, D.L.Brainard* S.Gordon*, J.Grothusen* and R.J.Schwartzman. Department of Neurology, Jefferson Medical College, Philadelphia, PA 19107.

Twenty one monkeys were used in this study. Ten animals were made parkinsonian with MPTP, eleven were used as controls. The parkinsonian animals demonstrated 90-97% dopamine depletion in the striatum. Three of the MPTP monkeys were treated with PHNO, a dopamine D_2 agonist. The brains were frozen and cut into 20 micron sections. For the D_2 assay total binding was determined using various concentrations of ^{3}H-spiperone in buffer containing 300 nm mianserine. For the D_1 assay, total binding was determined using various concentrations of ^{3}H-SCH-23390. Tissue isotope concentration was determined from the autoradiographs. Ligand binding was evaluated in the caudate and putamen nuclei at the level of the anterior commissure. The untreated parkinsonian monkeys demonstrated an increase in the number of D_2 sites as compared to controls. This increase was greatest at the dorso-lateral putamen. The PHNO treated animals demonstrated a 50% decrease in D_2 sites in both caudate and putamen. There was no change in the number of D_1 binding sites in both the untreated and the PHNO treated monkeys.

73.8

IS DEXMEDETOMIDINE'S ANESTHETIC ACTION DEPENDENT ON ISORECEPTOR SELECTIVITY? J.W.Regan, K.Daniel, and M.Maze (SPON R.Jaffe). Howard Hughes Medical Institute Duke University NC 27710; Dept Anesthesia Stanford University, Stanford CA 94305 and PAVAMC, Palo Alto CA 94304

D-medetomidine (D), an alpha2(a2) adrenergic agonist, produces anesthesia while its L stereoisomer, which also binds with high affinity, is not an anesthetic. We wondered if D's anesthetic properties are due to isoreceptor (either a2C10 or a2C4) selectivity. Binding parameters of the D and L isomers were determined from competition curve analysis using [^{3}H]rauwolscine and membranes prepared from COS 7 cells transiently expressing a2C4 or a2C10. D binds with 7-fold greater agonist affinity to the a2C4(K_i=2nM; Hill coefficient=0.69) compared to a2C10(14nM; 0.92). L binds with similar, albeit weaker affinity to both a2C4 (48 nM; 0.99) and a2C10 (59 nM; 0.92). Isomer function was assessed by their inhibition of forskolin-stimulated cAMP formation in hamster fibroblast cell lines (P120) stably expressing the a2 isoreceptors. Functionally, D is even more effective than epinephrine (epi) at inhibiting forskolin-stimulated cAMP accumulation in a2C4 (110%) while displaying weaker agonist activity at a2C10 (65% of epi). L is either ineffective (<5% of epi in a2C10) or exhibits only partial activity (25% in a2C4). In summary D more selectively binds and activates the a2C4 while L appears to have little isoreceptor selectivity and thus no anesthetic activity. These results are consistent with a mediating role for a2C4 in the anesthetic action of a2 agonists.

73.9

SPECT IMAGING OF DOPAMINE D2 RECEPTORS IN NON-HUMAN PRIMATE BRAIN. R.B. Innis, G.R. Heninger, S. Zoghbi, M. Al-Tikriti, S.W. Woods, E. Johnson, D.S. Charney, K. Koster, I.G. Zubal, E.O. Smith, H. F. Kung*, A. Alavi*, P.B. Hoffer. Depts. Psychiatry and Diagnostic Radiology, Yale University School of Medicine, New Haven CT 06510 and *Division of Nuclear Medicine, University of Pennsylvania.

Single Photon Emission Computed Tomography (SPECT) has the potential for non-invasive measuremnent of in vivo biochemical events in human brain. However,many questions exist about the appropriate methods for SPECT data analysis, in part due to attenuation of the gamma radiation emission and the applicability of kinetic versus equilibrium models. Thus, SPECT imaging of animals (non-human primates) with postmortem examination of the brain will be critical to carefully control experimental interventions and to test the accuracy of SPECT imaging in comparison to direct biochemical measuremnts in brain tissue.

We have studied the distribution of radiolabel uptake into brain following i.v. injection of the specific dopamine D2 receptor probe 123I-labeled IBZM (iodobenzamide). Two baboons (10 kg female Papio anubis) were injected with 1.8 and 12 mCi 123I-IBZM and scanned for approximately 2.5 hr in a Strichman 810X Brain Imager. The distribution of label was monitored in mutiple 2-3 min scans throughout the brain with a focus on slices in striatum (caudate, putamen, and globus pallidus). Radiolabel uptake was concentrated in the two striata (with a clear midline separation) within 30 min and was relatively stable for the remaining 120 min. Uptake in extra-striatal tissue washed out within 90 min and maintained that level. At 2 hr. the ratio of striatal to extra striatal label was 2-2.5 to 1.

The pharmacological specificty of radiolabel uptake was tested by injecting one animal with haloperidol (0.02 mg/kg i.v.). The striatal label was rapidly displaced by greater than 65% within 35 min.

These promising studies demonstrate the feasibility of kinetic SPECT imaging with the dopamine D2 receptor probe 123I-labeled IBZM. Further studies, including correlatiuons with MRI scans and direct postmortem measurements, are in progress.

73.10

BEHAVIORAL AND NEUROCHEMICAL EFFECTS OF INTRASTRIATAL 6-HYDROXYDOPAMINE (6OHDA) INJECTIONS DURING DEVELOPMENT IN THE RAT. J. Franks*, B.S. Neal and J.N. Joyce (SPON: D. Schambron). Departments of Pharmacology and Psychiatry, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

Central administration of 6OHDA during early postnatal development results in different behavioral responses to dopaminergic drugs than do injections given adult rats. The mechanisms underlying these behavioral differences are currently unknown. Using the method of Gerfen and coworkers (J. Neurosci. 7:3935, 1987), we gave intrastriatal 6OHDA injections to rat pups on postnatal Day 1. Experiments with these rats during adulthood revealed a potentiation of dopamine (DA) D1 agonist-mediated behaviors, including increased abnormal perioral movements after administration of the D1 agonist SKF 38393, and self-biting behavior after SKF 38393 and the nonselective DA agonists L-DOPA and apomorphine. Animals receiving lesions as neonates also exhibited a decreased sensitivity to D1 antagonist (SCH23390)-induced catalepsy. However, there were no differences in the cataleptic responses to the D2 antagonist haloperidol. Quantitative autoradiography on sections of brain tissue from rats given unilateral 6OHDA or bilateral 6OHDA injections showed a significant decrease in dopamine uptake sites (^{3}H-mazindol) and in the number of μ-opiate (^{3}H-naloxone) receptor patches in the caudate-putamen. DA D1 (^{3}H-SCH23390) and D2 (^{3}H-spiroperidol) receptor density appeared unchanged. Further analyses of the behavioral and neurochemical changes in these neonatally 6OHDA- treated rats are underway.

This research was supported by USPHS grants MH 43852 and GM 34781.

73.11

ONTOGENY OF THE D-1 AND D-2 SUBTYPES OF DOPAMINE RECEPTOR IN RAT BASAL GANGLIA: A QUANTITATIVE AUTORADIOGRAPHIC STUDY. P.A.Rao, P.B. Molinoff and J.N. Joyce, Departments of Pharmacology and Psychiatry, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

We conducted a quantitative autoradiographic study of the density and distribution of D-1 and D-2 dopamine receptors in rat basal ganglia using the iodinated ligands [125I]-SCH 23982 and [125I]-IBZM, respectively, in an attempt to discern: 1.) whether their pattern of development is dependent or independent of each other; and 2.) what their relationship is to the pattern of development for cellular components of the striatum--viz., presynaptic dopamine terminals as indicated by HPLC analysis for dopamine concentration in homogenates and by [3H]-mazindol binding in sections and presynaptic choline terminals as indicated by [3H]-hemicholinium-3 binding in sections. Results indicate that both dopamine concentration and [3H]-mazindol binding in whole striatum increase linearly with age (r=.95). D-1 receptors were found at earliest ages studied (P1-P5), with a patchy distribution consistent with previous work (Murrin, L.C. et al., Brain Res., 480, 1989); by P10 this binding was homogeneously distributed. This patterning was similar to that of [3H]-mazindol binding at the same ages. Both D-2 receptors and [3H]-hemicholinium-3 binding, first evident in appreciable levels at day P5, developed in a lateral-to-medial gradient. D-1 receptors were found in substantia nigra from age P1 through adulthood, increasing 9-fold in density over this period; Bmax for pars reticulata was consistently greater than that for pars compacta. D-2 receptors were not found at appreciable levels in substantia nigra until age P7; from that time on Bmax for pars compacta was greater than that for pars reticulata. These findings are consistent with the hypothesis that D-1 receptors, being noted primarily in patches at the earliest ages studied, are located on the processes of the medium spiny neuron, which is first seen developing in these patches. Also, the synchrony of D-2 receptors and presynaptic choline uptake sites suggests that a large percentage of D-2 receptors may be associated with neurons in the matrix or on the presynaptic processes of the large cholinergic interneuron.

Funded by Grants MH43852 and GM34781 and by an Am. Heart Assoc. fellowship.

73.12

RESPONSE OF STRIATAL DOPAMINERGIC RECEPTORS TO INTROCEREBROVENTRICULAR 6-HYDROXYDOPAMINE

J. N. Joyce and S.B. Caine*, Departments of Pharmacology and Psychiatry, University of Pennsylvania School of Medicine, Philadelphia, PA.

Loss of dopamine (DA) terminals following intranigral injections of 6-hydroxdopaminen (6-OHDA) results in up-regulation of the DA D2 receptor subtype, but decreases in the density of D1 receptor density. In contrast, chronic reserpine injection produces an up-regulation of both receptor subtypes. In order to determine if a critical degree of DA loss is required to see independent regulation of the receptor subtypes, different doses of 6-OHDA or vehicle (VEH) were administered introcerebroventricularly (ICV) to rats. Two weeks later the brains were removed and processed for quantitative autoradiography or for HPLC analysis of DA and NE levels. The lowest dose of 6-OHDA (150 ug/ventricle) produced a 56% reduction in DA concentration, higher doses (250 and 300 ug) produced an 85% and 92% reduction in DA levels in the striatum. Similar reductions in the density of [^{3}H]mazindol labeling of DA uptake sites were observed in animals from the same groups, but the loss was heterogeneous. No 6-OHDA treated animals showed an increase in D1 receptor density ([^{3}H]SCH 23390), the lower doses of 6-OHDA produced consistent declines (15%) in D1 receptor density. The ICV injection of 6-OHDA resulted in an increase in the density of DA D2 receptors ([^{3}H]spiroperidol) at the higher doses but a reduction in density at the lowest dose. These data suggest that ICV and intranigral injections of 6-OHDA that remove DA terminals produce a loss of D1 receptors that is not mimiced by removal of DA or D1 receptor blockade

Supported by grants MH 43852 and GM 34781.

73.13

BEHAVIORAL EFFECTS OF DOPAMINE D_1 AND D_2 AGONISTS ALONE AND IN COMBINATION WITH THE NOVEL D_1 ANTAGONIST SCH39166.
J. Bergman, S. Johnson*, B.K. Madras, and R.D. Spealman*. Harvard Medical School; N.E. Reg. Primate Res. Ctr., Southborough, MA 01772.

Cocaine and selected dopamine agonists were studied alone and in combination with the D_1 antagonist SCH39166 in squirrel monkeys responding under a fixed-interval (FI) schedule of stimulus-shock termination. Intermediate doses of cocaine (0.1-1.0 mg/kg, i.m.), the selective D_2 agonist quinpirole (0.03-0.3 mg/kg, i.m.), and the nonselective D_1/D_2 agonist (-)-apomorphine (0.03-0.3 mg/kg, i.m.) increased FI responding, whereas higher doses increased responding less or decreased it. In contrast, the D_1 agonists R-SKF38393 (0.3-3.0 mg/kg, i.m.) and SKF81297 (0.3-3.0 mg/kg, i.m.) only decreased responding in a dose-related manner. Pretreatment with SCH39166 (0.03 mg/kg, i.m.) antagonized the rate-increasing effects of cocaine in a surmountable fashion. SCH39166 also attenuated the rate-increasing effects of quinpirole, but did not shift the dose-effect curve rightward. Unexpectedly the effects of R-SKF38393 generally were unchanged by SCH39166, suggesting that mechanisms other than or in addition to those associated with the actions of dopamine may be involved in the behavioral effects of the dopamine D_1 agonist.

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CONTROL OF POSTURE AND MOVEMENT III

74.1

CHRONIC EMG IMPLANTS IN MONKEYS USED TO STUDY THE RELATIONS BETWEEN RED NUCLEUS DISCHARGE AND LIMB MUSCLE ACTIVITY DURING A VARIETY OF TASKS
L.E. Miller, G.D. Harris *, and J.C. Houk Departments of Physiology and Surgery, Northwestern University Medical School, 303 East Chicago Ave., Chicago, IL

The red nucleus (RNm) is thought by many to be a source of motor commands directing movements of the distal limbs. As a means of understanding the nature of these putative control signals, we have been studying the spatial and temporal relationships between firing rate of single RNm units and electromyographic (EMG) signals from a large group of forelimb muscles in the alert monkey. We are now able to make long term recordings from the intrinsic hand muscles, as well as those of the shoulder, arm, and forearm.

Prior to surgery, we fabricate and sterilize an assembly of 16 EMG electrodes leading to a miniature connector mounted in surgical dacron mesh. The dacron lies above the muscle and is sutured to the skin in an incision just off the midline of the animal's back. Skin and subcutaneous tissue (but virtually no muscle) readily grows into the dacron, greatly enhancing wound healing and resistance to infection. This technique, and the use of time series analysis algorithms, has allowed us to make objective comparisons of these relations during a number of different behaviors.

Our results suggest that significant differences may exist in the timing between RNm and EMG during execution of a visually guided tracking task, and reaching and grasping tasks. RNm unit activity led EMG modulation during the visually guided tracking for the great majority of well related unit/EMG pairs. By contrast, during the reaching tasks, approximately as many units followed well related EMG activity as led it. The longer lags observed during tracking could occur if neurons in the descending pathways were further from threshold during the tracking task. Alternatively, task dependent differences in the sequence of activation of well correlated RNm units or muscles could be important. Although unlikely, the timing difference might underlie different roles of the RNm in these two types of tasks. We are currently investigating these and other hypotheses, which we expect to lead to a better understanding of the role of the RNm in many types of limb movements.

74.2

FOUR INDEPENDENT SACCADE GENERATORS CONTROL VERTICAL AND HORIZONTAL COMPONENTS OF ORIENTING HEAD MOVEMENT IN OWLS.
T. Masino and E.I. Knudsen. Dept. Neurobiology, Stanford Univ., Stanford CA. 94305

Barn owls make saccadic head turns in response both to natural stimuli and to electrical stimulation of the optic tectum. In the oculomotor system, the optic tectum issues a place-coded command for an eye saccade to four component generators, each coding for movement in one direction: upward, downward, leftward or rightward. Robinson (Vision Res. 12:1795, 1972) demonstrated the independence of these generators by electrically stimulating in rapid succession two tectal sites eliciting two differently directed eye movements. When a particular component of the first movement was shared with the second movement, the shared component disappeared from the second movement. Since the directions that decremented coincided with pull-directions of the extraocular muscles, it seemed reasonable that the underlying generators conformed to the orthogonal arrangement of the eye muscles.

We sought to determine if distinct component generators also exist in a complex movement system and, therefore, actually represent an intermediate spatial code for motor control. We assayed head turns in the barn owl, which involve multiple articulations and geometrically complex musculature. Electrical microstimulation was applied to different sites in the tectum at various intervals, and head movements were measured with a search coil. Results show an effect similar to that in the oculomotor system. For example, while an up-left movement had no effect on a subsequent up-right movement at long interstimulus intervals (>500 msecs), at short intervals (80-100 msecs), the second movement was purely rightward. Examination of many combinations of movement directions yielded four discrete directions that could be made refractory indicating four independent component generators, one each for upward, downward, leftward, and rightward directions. Component decrement occured even when stimulation sites were in opposite tectal lobes, suggesting that the circuits that were made refractory are post-tectal. Thus, even in the control of a complex motor system, between the place-coded representation of motor space in the optic tectum and the motorneuron code for muscle tension, there exists four component generators coding for orthogonal directions of movement.

74.3

HEAD STABILITY DURING NATURAL POSTURAL AND LOCOMOTOR ACTIVITIES.
J. Goldberg and J. Chimenti*. Clayton Laboratory, Otorhinolaryngology Dept., Baylor College of Medicine, Houston, TX 70030.

Spontaneous head movements about the pitch axis were measured during sitting, standing, walking and other activities in 11 normal subjects. Angular head velocity was recorded by a lightweight sensor carried on a headband, digitized at 50 Hz and Fourier-transformed. Root mean square velocity (RMS) was computed over 0-15 Hz bandwidth. Vision could be eliminated by opaque goggles, and pitch axis neck rotation restricted by a Philadelphia collar.

Head movements recorded during sitting and standing exhibited peak velocities of several °/sec, most of the energy within 5 Hz bandwidth, and Fourier components corresponding to harmonics of the pulse rate well above 5 Hz. Low-frequency components were larger during standing than sitting presumably due to the addition of whole body sway. Walking induced peak head velocities on the order of 50 °/sec with Fourier components over 15 Hz.

Median RMS velocity during sitting and standing while fixating a target 2m away was 0.82 (range: 0.66-1.25) and 1.20 (0.90-1.56) °/sec, respectively. Excluding vision (eyes open) while sitting and having the subjects imagine the target **reduced** RMS significantly both with the neck free and fixed. During standing, excluding vision had little overall effect on RMS with neck free but **increased** it significantly with neck fixed. During walking, median RMS head velocity was 12.9 (range: 10.0-25.2) and 14.5 (11.3-22.1) °/sec with neck free and fixed, respectively.

The head motion observed during sitting and standing is sufficient to cause visual acuity loss when the vestibulo-ocular reflex is impaired or high-magnification optical aids are worn. Oscillopsia and balance loss can result from these conditions during walking. Effects of vision observed during postural activities suggest that it contributes to stabilization of the trunk in space but has a destabilizing effect on the head relative to the trunk.

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74.4

ORGANIZING PRINCIPLES FOR SINGLE JOINT ISOMETRIC CONTRACTIONS.
G. L. Gottlieb, D. M. Corcos, G. C. Agarwal and B. Flaherty*. Rush-Presbyterian.-St. Luke's Medical Center, Chicago, IL 60612 & University of Illinois at Chicago, IL 60680.

The dual-strategy hypothesis of motor control postulates certain classes of movements that are controlled by regulating the duration of an "excitation pulse" to the motoneuron pool. They are tasks that may be performed over a range of speeds and as such, are "speed insensitive" (SI). Other classes of movements are generated by regulating the intensity of the excitation pulse. They are "speed sensitive" (SS) and must be performed in a specified duration or at a specific speed. This "dual-strategy" hypothesis has emerged from studies of inertially loaded movements about the elbow. Our purpose is to extend these concepts to isometric contractions about the elbow.

Subjects made isometric elbow contractions (both pulses and steps) of different amplitudes and rates. We measured joint torque and electromyograms from two agonist and two antagonist muscles. The SS strategy is employed when task requires the subject to explicitly regulate the rate at which torque is generated. The SI strategy is employed whenever the SS strategy is not required, such as in generating different isometric torque amplitudes.

The SS strategy is implemented by the nervous system by increasing the excitation intensity to both agonist and antagonist motoneuron pools when the rate of rise of torque must be increased. Increases in the initial slope, peak and area of the EMG bursts accompany increases in the rate of torque rise. The SI strategy is implemented by controlling the duration of motoneuron excitation pulses of uniform intensity. The result is that the initial slope of the EMGs and the torque rise uniformly, regardless of the target torque. Pulse duration proportionally increases with torque in the agonist muscle.

The rules defining SS and SI strategies are broadened to include steps of excitation for sustained isometric contractions and a gradually rising patterns of excitation for contractions occurring over several hundreds of ms.

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74.5

PERCEIVED PROPERTIES OF OBJECTS USING KINESTHETIC SENSE DEPEND ON WORKSPACE LOCATION. B.A. Kay*, N. Hogan*, F.A. Mussa-Ivaldi*, and E. Fassé* (SPON: W. Richards). Depts. of Brain and Cognitive Sciences and Mechanical Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139.

Mussa-Ivaldi, Hogan, & Bizzi (1985, J. Neuroscience, 5, 2732-2743) found that the functional stiffness of the arm, as measured at the hand, varies systematically with changes in location in a two-dimensional workspace. Do these changes in impedance of the arm have any consequences for (1) the production of movement or (2) the perception of the environment using the kinesthetic sense? We conducted experiments in which subjects performed movements of the shoulder and elbow in the horizontal plane, in the absence of visual cues, in order to make perceptual judgements of two simple object properties, size and compliance. The stimulus objects were mechanically simulated two-dimensional rectangles, created using a two-link robot arm under computer control. In judging the relative lengths of the sides of the rectangles, subjects overestimated lengths directed outward from the body relative to lengths directed parallel to the trunk axis, and this overestimation increased with increasing distance from the shoulder. A linear fit of the data indicates that no differential length judgement should occur at the shoulder itself. In judging stiffness of the sides (preliminary results) of the rectangles, stiffness in the trunk axis direction was overestimated relative to the outward direction.

74.6

THE EQUILIBRIUM POINT MODEL FOR TWO-JOINT ARM MOVEMENT CONTROL. J.R. Flanagan*, A.G. Feldman*, and D.J. Ostry* (SPON: L. Bernier). McGill University, Canada and Institute for Information Transmission Problems, USSR.

The present model is based on a recent version of the equilibrium point hypothesis (Berkinblit et al, BBS, 9:585-638). This hypothesis states that the level of activation of a muscle depends on the difference between its actual length and its threshold length and the rate of length change. Central commands control two parameters (R and C) which determine threshold lengths. These parameters, along with external loads, specify the equilibrium position (R) and the level of co-concentration (C) respectively.

In the two-joint model, these are separate R and C commands associated with single-joint muscles spanning the shoulder and elbow and two-joint muscles spanning both joints. The R commands shift the threshold lengths of flexors and extensors in opposite directions; C commands shift them in the same direction.

A simple geometric model and Newton-Euler equations of motion are used to compute joint accelerations and velocities from which muscle lengths and velocities are derived. In this paper, the kinematics predicted by the model will be compared to experimental data from studies of two-joint human arm movements.

74.7

CONTROL OF HINDLIMB MOVEMENT IN FROGS. D.J. Ostry*, A.G. Feldman*, J.R. Flanagan*, S.V. Adamovich*, A. Karpovich*, and L.E. Sergio* (SPON: L.A. Jones). McGill University, Montreal, Canada and Institute of Information Transmission Problems, Academy of Sciences, Moscow, U.S.S.R.

Hindlimb movements were examined in both intact and spinal frogs. The aim was to map out the intrinsic capabilities of the spinal apparatus and the effects of supraspinal input on spinal organization. In studies of hindlimb withdrawal movements, initial joint angles at the hip, knee and ankle were varied systematically. This enabled us to determine the influence of the position of single joints on the coordination of remaining joints. Characteristics of the movement path in joint space and the form of the joint angular velocity profile were examined in both withdrawal and crossed extension reflexes. Our findings to date indicate that the slope of the path in joint space is dependent on the initial joint configuration. Joint angular velocity functions are generally unimodal but differ from one another in skew in some of the joint configurations we have studied. In swimming movements in the intact frog we have observed that the joints do not begin or end moving at the same time but have similarly shaped velocity functions at the joints. Our data are compared to model data based on the equilibrium point hypothesis.

74.8

MOVEMENT CUES ARE NECESSARY FOR LOAD STABILIZATION AGAINST LOW FRICTION. P.R. Burgess. Dept. of Physiol., Univ. of Utah Sch. of Med., Salt Lake City, UT 84108

In order to hold a weight steady it is necessary that muscular force not fluctuate beyond the range of static friction. Subjects with eyes closed pushed on a force transducer mounted on a horizontal track with the right hand. They were asked to support a 50 N weight against 3 N of static friction for 10 sec with the instruction that if the weight moved, the trial would be considered a failure. In some trials, unknown to the subject, the friction was abruptly increased so that no movement could occur. The force did not fluctuate much beyond the range of static friction during the low friction trials because whenever the weight moved 1-2 mm, a force correction occurred even when the subject was not aware of the movement. When the friction was increased so that movement cues were eliminated, the force typically wandered enough that the weight would have traveled many cm during a single low friction trial. Thus, even when maintaining a steady force is critical to success, subjects cannot keep their force sufficiently constant to reliably hold a weight steady for 10 sec in this task unless the range of static friction amounts to about 25% of the weight. However, when movement cues are available, force fluctuations are markedly reduced and the weight rarely moved more than a cm during a 10 sec trial.

74.9

ISOMETRIC FORCES EXERTED BY HUMAN SUBJECTS WITH AND WITHOUT BIAS FORCES. J.T. Massey, R.A. Drake and A.P. Georgopoulos. Bard Labs., Dept. of Neuroscience, The Johns Hopkins Univ. School of Medicine, Baltimore, MD 21205.

The position of a cursor in a visual display was proportional to the net X-Y force exerted on an isometric handle. Nine human subjects were instructed to exert forces on the handle such that the cursor moved in a stimulus direction in a reaction time task. In the absence of a constant bias force the direction of force exerted by the subject on the handle coincided with the direction of motion of the cursor in the display. In contrast, in the presence of a constant bias force subjects exerted forces of varying direction and magnitude throughout a trial such that the vector sum of the subject's force and the bias force was in the stimulus direction. Good performance in this condition was achieved without significant change in reaction time or information transmitted, compared to the performance without a bias force. These results indicate that human subjects can specify the direction of an intended net force effectively and efficiently even when that requires continuous change of the direction and magnitude of their own forces.

74.10

CONTRIBUTION OF PRIOR EXPECTATION TO CORRECTIVE MOVEMENTS. W.E.McIlroy and J.D.Brooke. Human Biology /Biophysics, Univ. Guelph, Guelph, CANADA. N1G 2W1.

The purpose of this study was to identify a role played by prior expectation during corrective movements. Three loads, 100%, 200% and 300% of a control load (12 Kg), were used to perturb the movement which consisted of isometric leg extension. Subjects received trials of 'predictable' magnitude (10 consecutively for each load) and an 'un-predictable' trial of 30 (10 of each load randomly ordered). They were instructed to return to the starting position as accurately as possible. All subjects restored position accurately, for all loads, when they could predict the magnitude. However during 'unpredictable' loading they made errors. These were easily measured as a change in peak velocity during the corrective extension. Three of four subjects consistently predicted high loads, seen by higher velocities at 100% and 200% as compared to the load-matched 'predictable' trials. The fourth subject appeared to predict low loads. Several factors, which we are currently evaluating, likely determine this level of dependence on prior expectation (including the speed/task, range of loads, the number of decisions required, and the probability of disturbance). Interestingly, from this study, 1) subjects selected this strategy with no imposed constraints on speed (accuracy was stressed) and 2) the correction strategy involving prior expectation could not be detected using 'predictable' loading (funded by NSERC).

74.11

AN ANALYSIS OF LOWER LEG MUSCLE ACTIVITY IN HUMAN POSTURAL CONTROL. V.P. Panzer*, D.J. Conti**, S. Thomas* and M. Hallett,
Human Motor Control Section, NINDS, NIH, Bethesda, MD 20892 and
**Select Engineered Systems, Inc., Hialeah, FL 33016.

Previous investigations of human postural control have described the temporal relationships of muscle response onset to perturbations, attributing primary control of upright stance to muscles surrounding the ankle joint. However, results from our laboratory have demonstrated significant EMG activity at the knee and hip associated with postural adjustments. The purpose of this study was to evaluate EMG activity relative to maximum voluntary contractions (MVC) and temporal relationships among these muscles.

Surface EMG was recorded from Tibialis Anterior (TA), Soleus (SO) and Gastrocnemius (GA), while subjects stood quietly for 30 sec. EMG was sampled at 200 Hz, bandpassed, rectified and low pass filtered to obtain the envelope of the signal. Three evenly spaced 500 msec windows were evaluated for each trial. Temporal relationships were obtained by cross correlating muscle pairs and subsequently sliding each signal in 5 msec steps. The muscle pairs were correlated in this manner for 500 msec in each direction. When muscles are in phase, a high positive correlation is observed, while a high negative correlation would indicate reciprocal activity. Zero or low correlation values suggest independent EMG activity.

TA exhibited low amplitude activity (1-5% MVC), while SO showed sustained activity (10-20% MVC). GA was characterized by bursting (10-20% MVC) and relatively quiet periods (1-6% MVC). GA bursts appear to brake forward sway. Low level tonic activity in SO and TA probably provide support to the ankle. GA and SO were temporally correlated with no lag, although a slightly higher correlation sometimes occured with a 20 msec GA lag. High negative correlations between TA and triceps surae were not observed. The relationship between TA and triceps surae lacked the reciprocal characteristics which the inverted pendulum model of postural sway would predict. Ongoing control of upright stance does not appear to be dominated by the ankle joint muscles as has been previously suggested.

74.12

DISCRIMINATORY ANALYSES OF STABILIZING REACTIONS IN YOUNG, ELDERLY, AND PARKINSONIAN POPULATIONS. J.H.J. Allum, E.A. Keshner, F. Honegger* (SPON: D. McCrimmon). ORL Dept., Basel Univ., Basel, CH, and Dept. of Physiology, Northwestern Univ. Med. School, Chicago, IL 60611.

Postural strategies during rotational perturbations at the ankle tend to be replicable within a population. The temporal organization and magnitude of EMG responses, as well as resultant torques are significant indicators of vestibulospinal dysfunction (Allum et al., 1988). Examining the same parameters in other populations should help identify mechanisms that underlie diminished stability. In this study, we have compared latencies and areas under ankle and neck muscle EMG responses, and torque exerted on the support surface during platform dorsiflexion perturbations with eyes open and closed in 3 populations: young normals (20-40 years), elderly normals (50-80 years), and Parkinsonian patients (50-80 years). A stepwise discriminant analysis clearly distinguished between the 3 groups on the basis of the variables in Table 1.

Table 1: Significant Classifying Variables and F Values

	Eyes Open	F	Eyes Closed	F
Latencies				
	SL tibialis anterior (TA)	18.9	SL TA	15.9
	ML trapezius (TRAP)	5.4	ML TA	11.1
Areas				
	ML TRAP	8.0	ML TRAP	6.7
	ML torque	6.3	LL torque	4.4
	SLb soleus (SOL)	5.0	SLb SOL	3.7
	LL TRAP	3.7		

N.B. SL = short latency; ML = medium latency; LL = long latency.

Bonferroni t-tests revealed that elderly normals had significantly longer latencies of SL TA than both other groups. Longer ML SOL latencies, increased areas under SLb (80-120 ms) responses in TA and SOL, and decreased magnitudes of ML torque as compared to young normals suggest impaired balance in the elderly is produced by delayed vestibulospinal and propriospinal reflex responses that are poorly compensated by enhanced response magnitudes. Balance problems in age matched Parkinsonian patients is a function of both age and disease related variables.

74.13

EVIDENCE FOR A TRANSCORTICAL STRETCH REFLEX FROM THE STUDY OF PATIENTS WITH CONGENITAL MIRROR MOVEMENTS. C. Capaday, R. Fraser*, R. Forget* and Y. Lamarre. Centre de recherche en sciences neurologiques, Ecole de réadaptation et Hôpital Hôtel-Dieu, Université de Montréal, C.P. 6128, Succ. A, Montréal, Canada, H3C 3J7

When a patient with congenital mirror movements is asked to flex the distal phalanx of his left thumb he also involuntarily flexes the right distal thumb phalanx and vice versa. Our hypothesis is that the involuntary mirror movement is due to the same motor cortex command reaching the motoneuron pools on both sides of the body. If the long latency EMG responses to muscle stretch were produced by a transmotorcortical pathway, then stretching a muscle should produce long latency EMG responses in the ipsi and contralateral muscles. We tested this idea in three patients by stretching the flexor pollicis longus (FPL) muscle by 10 degrees of arc at a speed of 200 degrees/s using a servocontrolled DC-motor. The patients were required to maintain a tonic level of activity of about 10-20% of their maximum on the stretched side and between 0-20% with the contralateral FPL. Stretch of the distal phalanx of the thumb produced occasional short latency EMG responses (25 ms) in the ipsilateral FPL and longer latency EMG responses consisting of a small burst at about 40 ms and a second much larger response at 55 ms. In the contralateral side of all three patients, short latency responses were never observed but a long latency response was always present at about 55 msec. It was, however, smaller than on the ipsilateral side. No contralateral response, of any kind, was ever observed in any of ten normal human subjects in the same paradigm. The latency of the 55 ms EMG response is considerably shorter than the fastest voluntary reaction to the same stretch which is about 110 ms. To determine if the motor cortex ouput reached the motor pools on both sides, we used transcranial magnetic stimulation of the motor cortex focus for activating hand muscles. This produced responses on both sides in the two patients tested but not in four normal subjects. These results are consistent with the idea that long latency EMG responses to muscle stretch are, at least in part, mediated by the motor cortex. Supported by MRC of Canada.

74.14

PHASE ATTRACTIVE DYNAMICS AND PATTERN SELECTION IN COMPLEX MULTIFREQUENCY BEHAVIORS. J.A.S. Kelso and G.C. deGuzman, Program in Complex Systems and Brain Science, Center for Complex Systems, Florida Atlantic University, Boca Raton, FL 33431.

Frequency and phase-locking are ubiquitous features of rhythmic behavior in neurobiological dynamical systems and crucial to temporal self-organization in general (Kuramoto, Y. *Chemical Oscillations, Waves and Turbulence* Springer, 1984). Previous empirical and theoretical work has pointed to the significance of *relative phase* as an order parameter (collective variable) characterizing coordinated behaviors. Less well-understood are cases of multifrequency behaviors in which the component frequencies are not 1:1. We report results of bimanual experiments in which humans are requested to sustain one frequency (1.5 Hz or 2.0 Hz) with one hand, while the other is passively driven by an external force at another frequency. Phase attraction to 0 or π (in-phase and anti-phase patterns) is demonstrated to persist especially in lower order frequency ratios (1:2, 1:3), along with short-term transitions from one phase-locking to another within a given frequency ratio. Jumping from less stable to more stable frequency ratios, e.g. 4:3 to 1:1 or 5:2 to 2:1 also occurs. A modified sine circle map with built-in phase attractive dynamics accommodates these results and generates new predictions. Observed patterns arise from the *competition* between the extrinsic driving force and the intrinsic, phase attractive dynamics. The relative strength of extrinsic and intrinsic dynamics determines the width of the Arnold tongues (stable mode-locked regions) and consequently the delay or acceleration of irregular behavior (quasiperiodicity and chaos). Universal features of a system's ability to generate multifrequency behaviors are governed by the differential stability of mode-locked states, as seen through the width of Arnold tongues. Behavioral complexity is inversely proportional to this measure, thereby rationalizing the relative difficulty of performing different patterns as well as their frequency of appearance..

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SUBCORTICAL VISUAL PATHWAYS I

75.1

ILLUMINATION OF THE RECEPTIVE FIELD SURROUND CONTROLS THE CONTRAST GAIN OF MACAQUE P RETINAL GANGLION CELLS. *Ehud Kaplan*[1] *and R.M. Shapley*[2], [1]The Rockefeller University and [2]New York University, N.Y.

The retino-cortical pathway of the primate visual system comprises two streams, the Magnocellular (M) and the Parvocellular (P) streams. The contrast gain (response per % contrast) of the M pathway is approximately 8 times higher than that of the P pathway.

We investigated the source of this sensitivity difference in anesthetized and paralyzed macaque monkeys. Retinal ganglion cell activity was recorded in the LGN as synaptic (S) potentials. Sinusoidally modulated (4 Hz) spots and steady annuli were generated on the face of a white CRT. The response measure was the fundamental Fourier component of the average response.

We discovered that steady illumination of the receptive field surround lowered dramatically the gain of all ON-center P ganglion cells, and raised the gain or had no effect on OFF-center P cells. M cells were not significantly affected. The effect on Parvo- and Magnocellular LGN units was similar to the effects observed in their retinal afferents.

The fact that steady (DC) illumination can affect the response to modulated stimuli indicates the presence of a non-linear process, probably shunting inhibition at the level of the inner plexiform layer of the retina.

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75.2

CONVERGENCE AND INPUT-OUTPUT RELATIONS IN THE CAT DORSAL LATERAL GENICULATE NUCLEUS (LGNd) ASSESSED BY S POTENTIAL RECORDINGS. J.S. Tootle, L.-A. Coleman* and M.J. Friedlander. Neurobiology Research Center, University of Alabama at Birmingham, Birmingham, AL 35294.

Our previous work suggests that in neonatal kittens the convergence of retinal ganglion cells onto single LGNd neurons differs from their adult counterparts. Moreover, many kitten LGNd cells have weaker inhibitory surrounds than in adult cats. (Tootle, J.S. & M.J. Friedlander, J.Neurosci. 9:1325, 1989). Here we address these issues directly in adult cats by simultaneously recording the activity of single A-laminae LGNd neurons and their retinal drive - the latter being recorded as geniculate S potentials. Convergence ratios and input-output relations were derived for spontaneous activity, size-response functions and contrast sensitivity functions. S and action potentials were digitized and frequency histograms of S potential amplitude were constructed. Analysis of the amplitude distribution of the S potentials revealed that from 1 to 3 retinal ganglion cells converge onto individual LGNd X-(n-14) and Y- (n=4) cells. For both X- and Y-cells the input-output ratio (LGNd spikes/S potentials) was highest for small spots (high spatial frequencies) and was reduced for large spots (low spatial frequencies). These data demonstrate an intrageniculate contribution to the receptive field inhibitory surrounds of adult LGNd neurons. Supported by NSF grant BNS-8720069.

75.3

BRAINSTEM MODULATION OF GENICULATE CELLS IN CATS D.J. Uhlrich, N. Tamamaki*, P.C. Murphy* & S.M. Sherman. Dept. of Neurobiology, SUNY, Stony Brook, NY 11794.

The projection from the midbrain parabrachial region (PBR) to the lateral geniculate nucleus (LGN) modulates the gain of retinogeniculate transmission. To examine this more closely in cats, we compared the responses of LGN cells to drifting sinusoidal gratings with and without accompanying electrical stimulation of the PBR. The effects of PBR stimulation on the visually evoked responses of LGN cells were generally much greater than predicted from effects on spontaneous activity alone. PBR stimulation sharply increased the depth of visually modulated responses of most geniculate X and Y cells. The enhancement was greatest at roughly 100ms after PBR stimulation and lasted 200-700ms. Some cells also showed evidence of modest response suppression with complex time-courses. For both X and Y cells, the size of the PBR response enhancement usually varied with the grating's spatial frequency and was coupled with a general increase in contrast sensitivity. Y cells showed the greatest enhancement at low spatial frequencies, while the effects on X cells were more variable. Furthermore, PBR activation made Y cells less transient to the grating stimuli, while no such temporal effects were seen for X cells. Thus not only can PBR activity enhance retinogeniculate transmission, it also seems to affect the spatiotemporal tuning characteristics of the visual information relayed to cortex.

(Supported by USPHS grants EY06610, TW04032, and EY03038 plus the Japan Ministry of Education)

75.4

BRAINSTEM MODULATION OF PERIGENICULATE CELLS IN CATS. P.C. Murphy*, D.J. Uhlrich, N. Tamamaki* & S.M. Sherman. (SPON: H. Petry). Dept. of Neurobiology, SUNY, Stony Brook, NY 11794.

Activation of the afferent pathway from the midbrain parabrachial region (PBR) to the perigeniculate nucleus (PGN) is thought mainly to inhibit PGN cells via muscarinic action. However, some evidence suggests that PGN cells are excited by PBR activation. We studied this further in anesthetized, paralyzed cats by recording the effects in PGN cells of activation of the PBR. We found that spontaneous activity is simply decreased by PBR stimulation. However, for most cells, the visually evoked response was strongly enhanced, with or without a decrease in background activity. In each cell, this PBR-induced enhancement followed a similar time course to the inhibitory effect on spontaneous activity, lasting 0.25-1.25s after stimulating the PBR. The inhibition of spontaneous activity may reflect a direct muscarinic action, and the enhancement of visually evoked responses may reflect an indirect facilitation of PGN cells via a direct enhancement of geniculate relay cell responses (see Uhlrich et al., this volume). In any case, the presence or absence of effective visual stimuli strongly influences the effect of PBR activation on PGN cells. Through the role of the PGN in gating retinogeniculate transmission, the PBR thus disinhibits geniculate relay cells in the absence of visual stimuli but enhances the visually driven inhibition of these cells.

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75.5

LAGGED AND NON-LAGGED X-CELLS IN THE CAT LATERAL GENICULATE NUCLEUS RECEIVE RETINAL INPUT THROUGH DIFFERENT GLUTAMATE RECEPTORS. P. Heggelund and E. Hartveit*. Inst. of Neurophysiology, Univ. of Oslo, Oslo, Norway.

The lagged and non-lagged X-cells in LGN are two types of relay cells which have similar spatial receptive field properties but markedly different temporal response properties (1-2). We have compared the effect of the NMDA receptor antagonist CPP on the visual response of the two cell types. The response of single cells in the A-laminae to a stationary light spot was recorded before, during and after iontophoretical application of CPP through a multibarrel glass pipette glued to the recording electrode.

CPP dramatically reduced, or completely abolished the response of all the recorded lagged cells (N=21). The non-lagged cells (N=40) were not or only weakly suppressed by CPP. This selective effect of CPP indicates that lagged and non-lagged cells are excited by retinal afferents through different glutamate receptors; the lagged cells mainly through NMDA receptors and the non-lagged cells mainly through non-NMDA receptors.

(1) Mastronarde J Neurophysiol 1987 57:357.
(2) Humphrey & Weller J Comp Neurol 1988 268:429

75.6

OFF-CENTER CELLS IN THE CAT LATERAL GENICULATE NUCLEUS ARE SENSITIVE TO NMDA RECEPTOR BLOCKADE. Y.H.Kwon, M.Esguerra and M.Sur. Dept. of Brain and Cognitive Sciences, M.I.T., Cambridge, MA 02139.

The putative neurotransmitter at the retinogeniculate as well as the corticogeniculate synapse in the cat visual system is glutamate (Kemp and Sillito, J. Physiol. 323, 377-391, '82; Baughman and Gilbert, J Neurosci. 1, 4:427-439, '81). We have examined the role of NMDA and non-NMDA receptors in the visual responses of lateral geniculate nucleus (LGN) cells in adult cats.

We used six-barrel glass electrodes for extracellular recording as well as for microiontophoresis of the agonists NMDA and kainate, the NMDA-antagonist DL-2-amino-5-phosphonovaleric acid (APV) and the non-NMDA antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX). After we identified each cell as On- or Off-center and as X or Y, we presented a flashing (1Hz) spot of light in the cell's receptive field center and collected post-stimulus time histograms before, during and after application of the drugs. We report on a total of 29 LGN cells from 7 cats. CNQX attenuated visual responses of both On- and Off-center X cells by 33% (n=4) and 38% (n=7) respectively. APV, on the other hand, reduced the responses of Off-center X cells to a much greater extent (52%, n=4) than On-center X cells (7%, n=7). The effect of CNQX on both On and Off-center Y cells was small (<6%, n=8), while the effect of APV may be greater for Off-center Y cells (38%, n=1) than for On-center Y cells (3%, n=6). However, a few On-center Y cells (n=2), as well as Off-center X and Y cells, were excited by NMDA, while On-center X cells were not.

These results demonstrate that glutamate receptors are involved in the transmission of visual information through the cat LGN. Under our recording conditions, X cells are more sensitive to the glutamate receptor antagonists than Y cells. For both X and Y cells however, visual responses of Off-center cells are more sensitive to blockade of NMDA receptors. This suggests that On- and Off pathways are not only segregated physiologically through the LGN but pharmacologically as well.

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75.7

NMDA AND NON-NMDA RECEPTORS MEDIATE RETINOGENICULATE TRANSMISSION IN CAT AND FERRET LGN *IN VITRO*. M. Esguerra, Y.H. Kwon and M. Sur. Dept. of Brain and Cognitive Sciences, MIT, Cambridge,MA 02139.

In the cat lateral geniculate nucleus (LGN), *in vivo* iontophoresis of the specific NMDA receptor antagonist APV (dl-2-amino-5-phosphonovalerate) partially blocks visual responses of OFF-center cells, while CNQX (6-cyano-7-nitroquinoxaline-2,3-dione) antagonizes non-NMDA receptor mediated responses of both ON and OFF center cells (Kwon et al., this volume). In order to determine the site of these drug actions, we made single-unit extracellular recordings from slices of cat and ferret LGN while electrically stimulating the retinal or cortical afferents. We studied the effects of APV and CNQX on responses to single shocks and 30Hz trains delivered to the two pathways.

In normal medium, APV (40μM) attenuated the optic tract-evoked response to single shocks and trains by an average of 72% in 6 of 12 cells. In 10 of the 12 cells, CNQX (5μM) reduced the frequency of responses to optic tract stimulation by an average of 71%. CNQX blocked responses similarly in 4 cells driven by optic radiation stimulation, while APV attenuated this response in 2 of the 4. These results are consistent with our results in the cat LGN *in vivo* and indicate that both NMDA and non-NMDA receptors mediate retinogeniculate and corticogeniculate transmission. Further, the APV sensitivity of single-shock responses suggests that at the retino-geniculate postsynaptic site, non-NMDA receptor mediated EPSPs are necessary and sufficient to activate NMDA receptors, which are in turn necessary for suprathreshold depolarization.

In preliminary experiments, we have studied the effects of APV on paired sub-threshold stimulation of cortical and retinal afferents. The results suggest that conjoint retinal and cortical afferent activity can increase the retinogeniculate transfer ratio by depolarizing LGN cells and activating NMDA receptors postsynaptic to retinal terminals.

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75.8

ORIENTATION SENSITIVITY OF RELAY CELLS IN THE CAT LATERAL GENICULATE NUCLEUS (LGND) Kirk G. Thompson*, Tiande Shou, Yifeng Zhou*, and Audie G. Leventhal. Dept. of Anat., Univ. of Utah Sch. of Med., Salt Lake City, Utah 84132

We studied the physiological orientation biases of over 800 LGNd relay cells as well as of a matched sample of over 1,000 retinal ganglion cell dendritic fields.

Most retinal ganglion cells were orientation biased and there was a strong tendency for cells to prefer stimuli oriented radially relative to the area centralis. Ganglion cells were not clustered according to preferred orientation.

Most LGNd cells were orientation biased and preferred radial stimuli. However, unlike in the retina, there was a relative over-representation of LGNd cells preferring stimuli oriented tangentially. Cells preferring radial and tangential orientations were clustered separately. The change in preferred orientation with distance was non-random along our penetrations (see Shou and Leventhal, Neurosci. Abs. 1988).

While the orientation tuning of most retinal ganglion cells and LGNd cells studied could be accounted for by an elliptical center/surround receptive field (see Soodak et al., J. Neurophysiol. 58:267, 1987), some could not. These cells were strongly biased and overall did not prefer radial stimuli. Their tuning curves were 'butterfly' shaped suggesting inhibition at the orientation orthogonal to the optimal.

We conclude that the orientation biases of most LGNd cells reflect those of their retinal inputs but that significant transformations are occurring in the LGNd.

75.9

CORTICAL CONTRIBUTION TO THE ORIENTATION SENSITIVITY OF RELAY CELLS IN THE CAT LATERAL GENICULATE NUCLEUS (LGND) Audie G. Leventhal, Yifeng Zhou*, Steven J. Ault, and Kirk G. Thompson*, Anat. Dept., Univ. of Utah Sch. of Med., Salt Lake City, Utah 84132

The orientation sensitivity of LGNd relay cells was studied in cats in which Areas 17, 18, 19, and LS were inactivated.

In decorticate cats most LGNd cells were orientation biased, clustered according to preferred orientation and preferred radial stimuli. Unlike in normal cats, in decorticate cats there was no over-representation of LGNd cells preferring tangential stimuli.

As in normal cats, in decorticate cats the responses of some cells could not be modeled by an elliptical receptive field. These cells were similar to the 'butterfly' cells in the LGNd of the normal cat (Thompson et al., Neurosci. Abs., 1989).

We conclude that most LGNd cells prefer radial stimuli as a result of their retinal inputs. Inputs from visual cortex serve to specify the preferred orientation of a minority of cells, most of which prefer tangential orientations. Since LGNd cells are grouped according to preferred orientation in normal and decorticate cats, it appears that sorting according to preferred orientation is occurring during the development of both the retinogeniculate and corticogeniculate projections. The presence of 'butterfly' cells in both normal and decorticate cats suggests that the orientation sensitivity of some LGNd relay cells are enhanced or determined by intrageniculate mechanisms.

75.10

COOLING STRIATE CORTEX ALTERS THE TEMPORAL ENCODING OF SPATIAL PATTERNS BY GENICULATE NEURONS.

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Last year, we reported here that parvocellular LGN neurons encode multiple, simultaneous messages about visual stimuli in multiplexed temporal codes. These temporal codes could arise from differential delays of retinal inputs, interactions within the LGN, and/or feedback from other brain areas such as V1. This study assessed the contribution of cortical feedback on the temporal encoding.

Visual stimuli based on a complete, orthogonal 2D set of Walsh patterns were presented on a video monitor. The visual responses of LGN neurons during reversible inactivation of V1 by cooling (when the feedback was presumably blocked), were compared with their responses when V1 was at normal temperature (when the feedback was presumably present).

In the control condition, the shapes of the response waveforms varied with stimulus pattern and had a 60 Hz component that was obviously driven by the raster of the video monitor. Cooling V1 produced both global and specific effects on the temporal structure of responses. First, cooling V1 reduced the magnitude of the 60Hz component of all of the responses. Second, cooling V1 caused marked changes in the shapes and amplitudes of the response waveforms to some stimulus patterns but not to others. Thus V1 feedback both facilitates high frequency responsiveness of LGN neurons and is critical for generating the normal temporal codes related to individual stimuli.

75.11

ORIENTATION BIAS OF NEURONS IN THE LATERAL GENICULATE NUCLEUS OF MACAQUE MONKEYS. E.L. Smith III, Y.M. Chino, W.R. Bidder III, A. Langston, and K. Kitagawa. College of Optometry, University of Houston, Houston, Texas 77204-6052.

The purpose of this investigation was to analyze the influence of orientation on the responses of neurons in the lateral geniculate nucleus (LGN) of macaque monkeys (n=10). For the majority of both parvo- and magnocellular neurons, response varied in a systematic fashion with stimulus orientation (mean orientation bias = 0.19, range = 0-0.54; Levick and Thibos, 1982). Typically, the preferred stimulus orientations were either parallel to the line connecting the cell's receptive field and the fovea or were rotated slightly toward horizontal. Spatial frequency response functions measured at a cell's optimal and orthogonal orientations were used to derive the response profile for the receptive field center mechanism using a two-dimensional difference of Gaussians model (Soodak et al., 1987). The orientation response bias appears to primarily reflect an elongation of the receptive field center mechanism.

75.12

THREE-DIMENSIONAL FORM AND SIMPLE, ACCURATE FLAT MAPS OF SINGLE LAYERS IN THE LATERAL GENICULATE NUCLEUS (LGN) OF THE CAT. D.B. Bowling and J.I. Caverhill* Department of Medical Physiology and The Lion's Sight Centre, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

Recently, there has been an increase in understanding of how the geniculate in the cat is organized in terms of afferent geometry, single cell responses, cell classification, sublaminar organization and projections to and from the visual cortex. We are interested in modelling these features to suggest how they may contribute to cortical function. As a first step we have considered the three-dimensional structure of single layers in the nucleus (a video image will be presented) and how the retinotopic maps in the layers change through depth as a consequence of three-dimensional form. In this we have needed a way to make accurate flat maps of curved surfaces to model and compare the topographic organization at different depths. We chose to make maps in which the representations of the surfaces are interrupted to form a series of map lunes that can be flattened individually with minimal distortion. Though discontinuous, the resultant maps are close approximations to the ideal of faithfully representing the total areas of the original surfaces, providing constant scales throughout the maps for comparing different regions, and being conformal over small regions. Supported by MRC (Canada) and AHFMR.

ION CHANNEL MODULATION AND REGULATION I

76.1

PROPERTIES OF THE INWARDLY RECTIFYING CHANNELS MODULATED BY SUBSTANCE P. S. Nakajima, Y. Nakajima, P.R. Stanfield* and K. Yamaguchi. Dept. of Biol. Sci., Purdue Univ., West Lafayette, IN 47907.

Previous studies showed that substance P produces neuronal excitation by inhibiting inwardly rectifying K-channels (1). This modulation is mediated through a G-protein which is resistant to pertussis toxin (2). We have investigated this K-channel modulation in more detail. Primary cultured neurons from the nucleus basalis of Meynert were prepared from rat brains. The whole-cell patch clamp was used. The dose-response curve of this substance P effect suggested that the half-effective concentration is roughly 40nM. Continuous presence of substance P (30 nM - 3μM), applied through exchanging the superfusing solution, produced desensitization.

The substance P-sensitive current was obtained by subtracting the current during the action of substance P from the control current. The time course of the substance P-sensitive current showed that there is no time-dependent inactivation even at large hyperpolarizing potentials. As already reported (1), the substance P-sensitive current had properties very similar to the inward rectification of skeletal muscles or oocytes. Application of Cs^+ (0.1 mM) blocked the substance P-sensitive current. This inhibition is consistent with a model which assumes voltage dependent binding of Cs^+ to a site causing channel blockage. The effective valence of this Cs^+ effect was 1.9, suggesting that Cs^+ blockage occurs at a multi-ion pore. Ba^{2+} also blocked the substance P-sensitive current. The effect, however, was less voltage-dependent. Application of Na-nitroprusside neither affected the action of substance P, nor consistently mimicked the substance P effect, suggesting that cyclic GMP does not play a role in mediating the substance P effect. (Supported by NIH grant AG06093, and a grant from Wellcome Trust.)

(1) Stanfield, P.R., Nakajima, Y. and Yamaguchi, K. (1985). Nature, 315:498.
(2) Nakajima, Y., Nakajima, S., and Inoue, M. (1988). PNAS, 85:3643.

76.2

RAS ALTERS PROPERTIES OF A VOLTAGE-DEPENDENT K+ CURRENT IN AtT-20 CELLS. R.E. Flamm, N.C. Birnberg*, & L.K. Kaczmarek. Dept. Pharmacology, Yale Univ. Sch. Med., New Haven, CT 06510.

We have found that transfection of an activated cHa-*ras* oncogene into an excitable pituitary cell line, AtT-20, alters its electrical properties. Control AtT-20 cells generate spontaneous, long duration action potentials, whereas *ras* AtT-20 cells are not usually spontaneously active but when depolarized generate narrow action potentials. We have compared K+ currents recorded under whole-cell voltage clamp. Cells were held at -90mV and stepped to depolarized potentials to activate the current. Both control and *ras* AtT-20 cells generate a delayed rectifier-like potassium current. Threshold for current activation is -15mV. The current differs from the classical delayed rectifier current by showing significant inactivation at potentials depolarized from +15mV. The current differs in the two clones in two ways. First, peak current density (step to +40mV) in *ras* cells is double that of control cells, 183$\pm$57 pA/pF (n=10) to 97$\pm$48 pA/pF (n=14) respectively (mean$\pm$SD). Secondly, the rate and amount of inactivation during a 300ms depolarizing pulse is greater in control cells than in *ras* cells. In control cells inactivation occurred with a time constant of 83.7$\pm$46.4ms whereas the time constant for inactivation of *ras* AtT-20 cells was 167.7$\pm$42.9ms. The control cell current is reduced by 44$\pm$10% over the same pulse but *ras* cells showed only a 30$\pm$11% reduction in current. These differences in properties of the K+ current are consistent with the differences we observe in electrical activity. Work is ongoing to determine whether other voltage-dependent currents are differentially expressed in the two clones.

76.3

EFFECTS OF 2-CL-ADENOSINE ON MEMBRANE CURRENTS AND SYNAPTIC TRANSMISSION IN CULTURED RAT HIPPOCAMPAL PYRAMIDAL NEURONS. Kenneth P. Scholz, Wendy K. Scholz and Richard J. Miller. Dept. of Pharm. and Physiol., Univ. of Chicago, Chicago, IL 60637.

Activation of adenosine receptors has been shown to induce presynaptic inhibition in a number of systems, including excitatory synapses onto CA1 hippocampal pyramidal neurons. We have begun to examine the roles of Ca2+ and K+ currents as well as G-proteins in mediating this effect. Rat pyramidal neurons were cultured by the methods of Banker and colleagues (1977) as modified by Scholz et al., (1988). Preliminary experiments indicate that 1 uM 2-Cl-adenosine (2-CA) produces presynaptic inhibition between synaptically coupled pairs of cells. To begin assessing the role of Ca2+ currents (ICa) in the modulation of synaptic transmission by 2-CA, ICa was recorded using whole-cell patch clamp techniques. Addition of 1 uM 2-CA to the bath caused a 24±1.5% reduction in ICa. This effect was completely reversible and was blocked by addition of 200 uM theophylline to the bath. In addition, preincubation of the cells with pertussis toxin (250 ng/ml) for 16 to 42 hours abolished the ability of 2-CA to reduce ICa. GTP-γ-S augmented the effects of 2-CA while GDP-β-S reduced the efficacy of 2-CA. Measurements of [Ca2+]i using fura-2 microfluorimetry have also shown that 2-CA can reduce Ca2+ entry triggered by depolarization in 50 mM K+. These results indicate that activation of adenosine receptors can regulate ICa in these cells by a mechanism dependent upon a G-protein. We have also found that 2-CA activates a K+ current in these cells, as shown by Trussell and Jackson (1987).

76.4

ADP-RIBOSYLATION AND IMMUNOLOGICAL CHARACTERIZATION OF G PROTEINS THAT MEDIATE PRESYNAPTIC INHIBITION. G.G. Holz IV, T.J. Turner and K. Dunlap. Dept. of Physiology, Tufts University School of Medicine, Boston, MA 02111.

Regulation of voltage-dependent calcium channels by G proteins is a likely means by which inhibitory transmitters suppress excitation-secretion coupling in presynaptic nerve endings. We previously reported that in chick DRG neurons, presynaptic inhibition is blocked by prior exposure of cultures to pertussis toxin (PTX). Here we report that in these same cells, PTX catalyzes ADP-ribosylation of G proteins migrating on SDS-PAGE (1-D gel electrophoresis) as a doublet of M_r 40-41 kDa. Isoelectric focusing combined with SDS-PAGE (2-D gel electrophoresis) demonstrates that this doublet is resolvable as four distinct spots with pI of ca. 5-6. Western immunoblot analysis of 1-D gels using antisera directed against synthetic peptides corresponding to amino acid sequences predicted from cDNAs for PTX-sensitive G protein α subunits reveals a *minimum* of two G_i-like proteins (M_r 40 and 41 kDa) and a third G_o-like protein (40 kDa). Subcellular fractionation of chick cerebral cortical homogenates by sucrose density gradient centrifugation indicates that these ribosylatable, immunoreactive G proteins are included in the synaptosomal plasma membrane and synaptic vesicle fractions. We conclude that G_i and G_o may regulate transmitter release not only at the level of calcium entry, but also at subsequent steps in the secretory pathway.

76.5

PERTUSSIS TOXIN BLOCKS A FAMILY OF NEUROTRANSMITTER RESPONSES, CONSISTING OF ACTIVATION OF 'S'-LIKE K CURRENT AND SUPPRESSION OF Ca CURRENT, IN *APLYSIA* NEURONS. V. Březina*, S.S. Vogel, G.J. Chin & J.H. Schwartz. Howard Hughes Medical Institute, Columbia University, New York.

Aplysia neurons have been proposed (*J. Physiol. 407*:15; *Soc. Neurosci. Abstr. 14*:754) to possess a family of 'slow' neurotransmitter responses, i.e. a distinct receptor for each of a number of transmitters that simultaneously mediates activation of a slow K current resembling the 'S' current and suppression of the Ca current. We have studied such responses in neurons L2-L6, R2 and LP1 (to ACh and the neuropeptide FMRFamide), and L10 (to ACh, FMRFamide and histamine). G-protein involvement is suggested by the finding that GTP-γ-S mimics both the K- and the Ca-current component of the responses. Another probe of G-protein function, pertussis toxin (PTX), blocks similar transmitter-activated slow K currents in other *Aplysia* neurons (*Nature 325*:259; *Jap. J. Physiol. 37*:551; *PNAS 85*: 7810), and indeed, under current clamp, the histamine-induced hyperpolarization in L10 (*Soc. Neurosci. Abstr. 13*:597). We now show that injection of PTX blocks both the activation of 'S'-like K current and the suppression of Ca current due to each of ACh, FMRFamide and histamine. Characterization and quantitation of G proteins in single isolated L10 and R2 cells using PTX-catalyzed [^{32}P]ADP-ribosylation and Western blotting reveals a single PTX-sensitive band, presumably the G protein mediating the transmitter responses.

76.6

12-LIPOXYGENASE METABOLITES OF ARACHIDONIC ACID OPEN SINGLE S-K^+ CHANNELS: ROLE FOR AN EXTERNAL MEMBRANE RECEPTOR? N. Buttner, S.A. Siegelbaum and A. Volterra. Ctr for Neurobiology and Behavior, Dept. of Pharmacology, Columbia University. 722 W. 168 St. New York, NY 10032.

FMRFamide increases the S K^+ channel open probability (p_o) through the 12-lipoxygenase metabolite of arachidonic acid (a.a.), 12-HPETE (Piomelli et al., Nature, 1987). This may be due to a direct action on the S channel (or some other membrane protein) since 20 uM 12-HPETE modulates S channel activity in cell-free inside-out (i.o.) patches (Buttner et al., Biophys. J., 1989; see also Belardetti et al., Soc. Neurosci. Abstr., 1988). Since a.a. metabolites are released from cells and can act at external receptors, we have tested the action of 12-HPETE on S channels in cell-free outside-out (o.o.) patches.

Surprisingly, 12-HPETE is more effective in modulating S channel opening in o.o. patches compared to its action in i.o. patches. 20 uM 12-HPETE causes a 14 fold increase in p_o in o.o. patches (n=4), compared to a 2.9 fold increase in p_o in i.o. patches (n=5). In o.o. patches, 12-HPETE is effective at low concentrations, with 500 nM 12-HPETE causing a 4.6 fold increase in p_o (n=6). 500 nM 12-HPETE has little effect in i.o. patches. Neither a.a. nor 12-HETE, a stable metabolite of 12-HPETE, have any significant effect in i.o. or o.o. patches (at 20 uM). These results confirm the ability of 12-HPETE to modulate S channel activity in cell-free conditions (no ATP or GTP) and suggest that the lipoxygenase metabolite receptor may be at the external surface of the membrane.

76.7

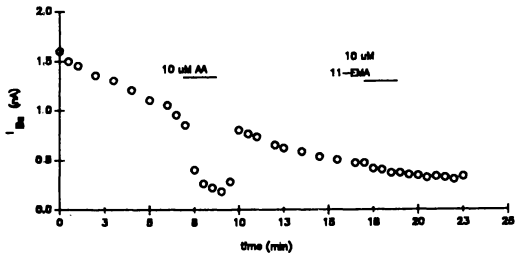
IS PROTEIN-PHOSPHATASE ACTIVATION INVOLVED IN FMRFa RESPONSE IN APLYSIA? G.Demontis*, S.-I.Yang*, M.Mumby* and F.Belardetti Department of Pharmacology, UT Southwestern Medical Center at Dallas, Dallas, TX 75235-9041

In Aplysia sensory neurons (SNs) 5-HT closes the S-K^+ channel through cAMP-dependent phosphorylation. FMRFa: 1. increases the opening of this channel through lipoxygenase metabolism of arachidonic acid, and 2. reopens S channels closed by 5-HT or cAMP. We assayed the protein phosphatase activity in the soluble fraction of homogenates from whole Aplysia nervous systems using phosphorylated histone-H1 as a substrate (12 animals). Under basal conditions, we found a prominent phosphatase activity (7.9 pmoles ^{32}P/μg prot/min) which was suppressed (0.3 pmoles ^{32}P/μg prot/min) by 0.2 μM okadaic acid (OA), a protein phosphatase inhibitor. We then studied the action of OA on the FMRFa response. Brief puff application of FMRFa (2μM) elicited a slow hyperpolarization (8.8±3.3 mV, mean±s.e.m., n=4) in current-clamped SNs or a slow outward current (140 pA, Vh=-40 mV, n=1) in a whole-cell voltage-clamped SN. Then OA was applied to the bath for 20 min (3-8 μM) and the response to FMRFa tested again: the FMRFa response was not changed from the control level (117.6±21.3%, n=5). These experiments suggest that the FMRFa basal action on the S channel does not require the activation of an okadaic acid-sensitive protein phosphatase.

76.8

ARACHIDONIC ACID INHIBITS CALCIUM CURRENT IN CHICK SYMPATHETIC NEURONS. W. Bug, L. Role, S.A. Siegelbaum and L. Simmons. Ctr. Neurobiol. and Behavior. 722 W. 168 St. NY, NY 10032

Arachidonic acid (a.a.) metabolites mediate presynaptic inhibition in Aplysia sensory neurons (Piomelli et al., Nature, 1987). To investigate whether the a.a. cascade plays a similar role in vertebrates, we studied the effects of exogenously applied a.a. on inward calcium current (I_{Ca}) in chick sympathetic neurons using whole cell recording. Arachidonic acid (10 uM) inhibited I_{Ca} by 54% +- 27% (mean, +- S.D., n=5). This effect was reversible in three experiments (Fig.). As a control for non-specific effects of fatty acids, eicosa-11-monoenoic acid (10 uM;11-ema) was shown to have no significant effect on I_{Ca} (n=2). The question now is whether a.a. plays a physiological role in these neurons.



76.9

ARACHIDONIC ACID DEPRESSES HIPPOCAMPAL CALCIUM CHANNEL CURRENT UNDER WHOLE-CELL VOLTAGE CLAMP. D.O. Keyser and B.E. Alger. Dept. of Physiology, School of Medicine, Univ. of Maryland at Baltimore, Baltimore, MD, 21201.

Recent evidence suggests that unsaturated fatty acids (FA) including arachidonic acid (AA) act as second messengers to modulate ionic currents and can activate protein kinase C (PKC). This hypothesis predicts that: 1) the biologically active FA should mimic known PKC activators such as phorbol esters, 2) they should be active in the dose range at which the FA activate PKC and 3) inactive FA should have no effect. To begin to test the hypothesis we used acutely isolated guinea pig hippocampal pyramidal cells and studied calcium channel currents carried by barium under whole-cell voltage clamp.

Bath-applied AA (10-100 uM) decreased I_{Ca} in a dose-dependent manner. Maximal reduction of I_{Ca} was 80% at 100 uM AA. There was no change in holding or leak currents or the voltage dependence of I_{Ca}. The latency of the effect decreased with increasing concentrations of AA. Oleic acid, another active unsaturated FA, also depressed I_{Ca}. The effect of AA and oleic acid resembles that of the phorbol ester PDBu, a PKC activator. The trans isomer of oleic acid, elaidic acid, which does not activate PKC, had no effect. Our results demonstrate for the first time that AA and other FA can modulate calcium channel currents in the hippocampus. The data are consistent with the hypothesis that PKC is involved.

76.10

ACTIVE VS INACTIVE PHORBOL ESTER EFFECTS ON WHOLE-CELL CALCIUM CURRENT IN HIPPOCAMPAL NEURONS. D. Doerner and B. E. Alger. Univ. MD, Sch. Med., Baltimore, MD 21201.

We have reported that phorbol esters that activate protein kinase C (PKC) suppress whole-cell calcium channel current (I_{Ca}) carried by barium in hippocampal neurons acutely-isolated from guinea pig. At the same concentrations inactive phorbol esters had no effect. We now report that high doses of inactive phorbol dibutyrate, 4-α-PDBu, also depress I_{Ca} and have tested the hypothesis that the two isomers act via the same mechanism.

Both 4-α- and 4-β-PDBu reduce I_{Ca} when applied extracellularly, but 4-α-PDBu was less potent by 2-3 orders of magnitude. When applied intracellularly, 4-β-PDBu was effective at doses as low as 20 nM, whereas 4-α-PDBu was inactive up to 100 μM. Besides depressing maximum I_{Ca} conductance, 4-β-PDBu caused a left shift in the voltage dependence of inactivation of I_{Ca} of about 10 mV. 4-α-PDBu did not affect inactivation and neither agent altered voltage dependence of activation. Finally, I_{Ca} suppression due to 4-β-PDBu (50-500 nM) was antagonized by the PKC inhibitor H-7 (20-50 μM), while H-7 did not affect comparable depressant effects of 4-α-PDBu (20-50 μM). We conclude that active and inactive PDBu isomers differ according to potency, cellular site and biophysical mode of action and susceptibility to PKC inhibitors. Apparently 4-β-PDBu depresses hippocampal I_{Ca} via PKC while 4-α-PDBu acts through another mechanism.

76.11

cAMP DEPENDENT MODULATION OF TRANSIENT Ca^{2+} CURRENT IN RAT THALAMIC RELAY NEURONS

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A low-threshold, Ca-dependent spike underlies burst generation in thalamic relay neurons and has an important function in regulating thalamocortical excitability during changes in behavioral state. The underlying Ca^{2+} conductance is affected by agents which alter resting membrane potential, however little information is available regarding the direct effects of intrinsic modulatory substances on the Ca^{2+} currents. Since phosphorylation by cAMP-dependent protein kinase (A-kinase) is known to mediate a number of modulatory cellular responses, we examined the effects of agents which activate A-kinase on the transient (T-type) Ca^{2+} current which is responsible for bursting in these cells.

Using whole-cell clamp procedures, Ca^{2+} currents were recorded from acutely isolated rat ventrobasal complex neurons (Coulter, Huguenard, & Prince, *J. Physiol.*, 1989). Bath application of a membrane permeable analog of cAMP (db-cAMP, 1mM) reversibly reduced T current by 28-53% and induced smaller decreases in L current. Extracellular cAMP and db-cGMP were ineffective. Forskolin (50μM), a direct stimulator of adenylate cyclase, and isobutylmethylxanthine (0.1-1mM), an inhibitor of phosphodiesterase, produced similar effects, with reduction of 20-50% and 10-50%, respectively. The effects of all of these agents were reversible within 15 minutes.

We conclude that A-kinase dependent reduction of T current occurs in thalamic relay neurons, and that modulators which activate the kinase (e.g. ß-adrenergic agonists) may regulate thalamocortical excitability via this mechanism. Supported by NIH grants NS06477 and NS12151.

76.12

A NEW PATCH CLAMP CONFIGURATION FOR STUDYING SINGLE ION CHANNELS: THE NYSTATIN-PERFORATED VESICLE. E.S. Levitan, R.H. Kramer,and L.K. Kaczmarek, Dept. Pharmacol., Yale U. Sch. Med., New Haven, CT 06510 and HHMI, Columbia U. CPS, 722 W. 168th St., NY, NY 10032.

We have developed a new patch clamp configuration for studying outside-out ion channels while they remain exposed to cytoplasmic factors necessary for normal activity and modulation. First, a giga-ohm seal is formed on a pituitary tumor cell (GH3 or GH4) with a patch electrode containing the pore-forming substance nystatin, along with K, Cl, Mg, SO4, Na, and HEPES ions. After nystatin perforates the patch (Rs<50 Mohm), the cell can be voltage-clamped to measure ionic currents (Horn and Marty, 1988). Withdrawing the pipette results in formation of a vesicle which retains water-soluble or mitochondria-specific fluorescent dyes loaded into the cell, suggesting the vesicle contains cytoplasm. The hemisphere of the vesicle in contact with the pipette solution remains perforated and allows the recording of single channel activity in the nystatin-free hemisphere facing the bath. Single channel currents in such vesicles (Rin ~ 10 Gohm) exhibit rapid kinetics and are unattenuated, unlike those in unperforated vesicles. Depolarization activates a 110 pS K channel that is blocked by 50 nM charybdotoxin. These channels open rarely in intact vesicles, but opening increases if the perforated membrane in the pipette ruptures, suggesting that the vesicle maintains low Ca. The peptide hormone TRH, known to induce IP3-stimulated Ca release, increases the macroscopic Ca-activated K current in perforated cells, and activates the K channel in perforated vesicles. Hence these vesicles can be used to study modulation of ion channels because they contain cytoplasmic components necessary for channel survival and regulation.

76.13

MODULATION OF VOLTAGE-GATED CALCIUM CHANNELS STUDIED USING THE PERFORATED PATCH AND PERFORATED VESICLE CONFIGURATIONS R.H. Kramer, E.S. Levitan and L.K. Kaczmarek, Dept. Pharmacol., Yale Univ. Sch. Med., New Haven, CT 06510 and Howard Hughes Med. Inst., Columbia Univ. CPS, 722 W. 168th St., New York, NY 10032

Ca channels are inactivated by intracellular Ca ions. It is difficult to study this phenomenon in small mammalian cells because both whole-cell and excised patch clamp configurations require replacement of the physiological Ca buffering system with exogenous Ca buffers. Hence, we have used the perforated patch configuration to study modulation of Ca channels by agents known to elevate internal Ca in pituitary tumor cells: caffeine and TRH. Dihydropyridine-sensitive Ca or Ba whole-cell currents measured in the perforated patch configuration are reduced 15-60% by 10 mM caffeine, and less dramatically by 1 µM TRH. T-type currents are not affected. 1 µM ryanodine also decreases Ca current, and reduces the effect of caffeine. In outside-out patches, caffeine does not reduce the conductance of single Bay K-treated L channels, and has no obvious effect on gating. We are further investigating the possibility that caffeine inactivates Ca channels indirectly by mobilizing intracellular Ca. We are also using the perforated vesicle configuration to study this phenomenon. Perforated vesicles exhibit a 20 pS L-type channel whose activity persists for more than 15 min in the absence of added EGTA, ATP, or other agents usually necessary for Ca channel survival in cell-free patches. Since the perforated vesicle may be capable of internal Ca release, it presents an ideal preparation for studying the single channel basis of Ca-dependent inactivation of Ca current.

77.1

Cerebellar Evoked Potentials from the Rat: Cord and Sciatic Nerve Responses. R.J. Hurlbert, C.H. Tator, G. Niznik, M.G. Fehlings, R.D. Linden. Spinal Cord Research Laboratory, Playfair Neuroscience, University of Toronto, Canada M5T 2S8.

We have studied the value of cerebellar evoked potentials in an attempt to better monitor the integrity of the ventral spinal cord. The spinal response to cerebellar stimulation has been characterized in 13 rats and the sciatic responses in 5. Using alpha-chloralose and urethane anaesthesia a burr hole was placed 2mm inferior to the occipital protuberance and 2mm lateral to midline; a platinum ball stimulating electrode was inserted epidurally over the cerebellum. A laminectomy at T9/10 allowed the introduction of recording microelectrodes into the spinal cord. Where sciatic potentials were measured, both sciatic nerves were exposed and bipolar recording needle electrodes inserted. A Cadwell 8400 Signal Averager was used to record the waveforms.

The responses have been characterized using intensities of 10 - 25 mA at a rate of 8.1 Hz and a pulse width of 50 us. Lesion studies indicated that the cord potentials were carried primarily in the ventral funiculus while the sciatic responses were conducted in the dorsal columns. This technique is potentially of great value in experimental and clinical spinal cord monitoring. Further studies are needed to clarify the anatomical pathways involved.

77.2

RESPONSES OF CAT EXTERNAL CUNEATE NEURONS DURING PASSIVE AND ACTIVE MOVEMENTS. K.M. Horn, P.L.E. van Kan*, and A.R. Gibson. Division of Neurobiology, Barrow Neurological Inst., Phoenix, AZ 85013.

Previous work (Van Kan et al., Soc. Neurosc. Abst., 12(1):578, '86) has identified sensory and motor components in proprioceptive signals of mossy fibers in intermediate cerebellum of awake monkey. The present study is part of a series designed to identify and characterize sensory and motor response components in the sources of mossy fiber inputs. External cuneate (ECN) neurons were recorded in decerebrate, decerebrate-paralyzed, anesthetized, or awake cats. Units recorded in the decerebrate preparations before and after paralysis demonstrated comparable response magnitudes and characteristics. The overall response characteristics in the decerebrate, anesthetized and awake preparations were similar. Units were responsive to movements of single forelimb joints and contained tonic and phasic response components. Some units showed largely tonic firing rates that were proportional to joint angle. Units with largely phasic components showed increased and decreased responses dependent upon the direction and velocity of joint movement. Preliminary comparisons of the neural responses during passive and active movements indicate that active movement participation may be a significant factor in determining response characteristics of some ECN units.

Supported by NS27373 (P.v.K.) and NS24042 (A.R.G.).

77.3

RED NUCLEUS MEDIATED INHIBITION OCCURS WITHIN THE INFERIOR OLIVE. A.R. Gibson and T. M. Hamm, Barrow Neurological Inst., Phoenix, AZ 85013.

Weiss et al. (Soc. Neurosci., '85) reported that stimulation of the cat magnocellular red nucleus (RNm) inhibits responses of the rostral dorsal accessory olive (rDAO) to peripheral stimuli. We investigated the locus of the inhibition by studying responses of hindlimb rDAO cells. These cells receive hindlimb afference from the gracile nucleus and the spinal cord. We sectioned the dorsal half of the spinal cord at midthoracic levels leaving only spinal input via the ventral funiculus. The section also removed RNm influences on lumbar cord. Olivary responses to hindlimb shocks were still inhibited by RNm stimulation indicating that the inhibition occurred in the olive. As a further control, we bypassed the lumbar cord by directly stimulating either the dorsal columns or ventral funiculus. In both cases the olivary responses were inhibited following RNm stimulation. Although it is likely that afferent pathways are modulated at many levels, the specific action of RNm stimulation on rDAO responsiveness may play an important role in preventing olivary responses to active movement. (Supported by USPHS grants NS24042, NS22454)

77.4

REGULATION OF SIMPLE SPIKE RESPONSES OF PURKINJE CELLS BY THE CLIMBING FIBER SYSTEM IN CEREBELLAR SAGITTAL ZONES. T.M. Kelly, J.D. McAlduff*, J.R. Bloedel. Div. of Neurobiol., Barrow Neurological Inst., Phoenix, AZ 85013, and Dept. of Physiol., U. of A., Tucson, AZ 85724.

Our previous studies demonstrated that perturbations of a forelimb movement activate climbing fiber inputs to sagittal strips of Purkinje cells and that this input is associated with increased simple spike responses of the same neurons. The present experiments were performed to test the hypothesis that this interaction is used to specify which populations of Purkinje cells will be modulated by a distributed mossy fiber input. Two five-electrode arrays were placed sagittally in zones B-C_3 of lobule V in acutely-prepared decerebrate, ambulating cats. The zones were identified electrophysiologically. Purkinje cells recorded with each array were treated as a population of neurons, and the RTPR algorithm was employed to characterize each population's response to a perturbation of the step cycle. The synchronous activation of climbing fiber inputs was quantified in each zone by calculating the synchrony index: the fraction of Purkinje cells receiving evoked climbing fiber inputs within an 80 msec window. The data indicate that the modulation of simple spike activity was much greater in those sagittal strips receiving synchronously activated climbing fiber inputs. The findings support the dynamic selection hypothesis which argues that the sagittal distribution of activated climbing fiber inputs can specify sagittally-oriented populations of Purkinje cells whose simple spike activity is most highly modulated by mossy fiber inputs responding to the same conditions. (Supported by NIH Grant NS21958)

77.5

INTRAOLIVARY INJECTION OF PICROTOXIN CAUSES REORGANIZATION OF COMPLEX SPIKE ACTIVITY. E. J. Lang, M. Chou, I. Sugihara, and R. Llinás. Dept. of Physiol. & Biophys., New York Univ. Med. Ctr., 550 First Ave., New York, NY 10016.

Intravenous injection of picrotoxin, a GABA antagonist, changes the pattern of synchronization of complex spike (CS) activity in cerebellar Purkinje cells (Sasaki & Llinás, Soc. Neurosci. Abst. 11:181, 1985). These results have lent support to the hypothesis that release of GABA within the inferior olive (IO) controls the pattern of electrical coupling of olivary cells (Llinás, R. The Physiologist 17:19-46, 1974).

In order to test this hypothesis in a more rigorous manner picrotoxin was directly injected into the IO of anesthetized rats. A single barreled pipette was carefully lowered through the brain towards the IO while an electrical microstimulation was delivered. Detection of the climbing fiber activation of Purkinje cells on the suface of the Crus IIa of the cerebellum was the criteria for having reached the IO. Following this procedure simultaneous extracellular recordings of up to 22 Purkinje cells in Crus IIa was implemented using the multiple extracellular microelectrode technique. The distance between electrodes was 250 μm and they were organized in a rectangle over the surface of the folium. Pressure injection (1 μl/min) of picrotoxin (2-5 μl of 1 μg/μl) produced a distinct change in the organization of the climbing fiber activity over the cortex. Thus, in the rostro-caudal direction the degree of synchronicity of CS was typically increased by 3 to 4 fold. More significantly however, the degree of synchronicity in the medio-lateral direction as determined by cross-correlation, which is particularly poor under control condition, rose from between 0.03-0.01 to 0.1-0.06. On occasions cross correlations as high as 0.16 were observed after the picrotoxin microinjections. These increases in CS cross-correlation were qualitatively and quantitatively similar to that seen with I.V. injection. In control experiments intraolivary injections of Ringers solution did not produce changes in the pattern of synchronization of CS activity.

These results indicate blockage of intraolivary inhibition is sufficient to reorganize the spatial distribution of CS synchronicity.

77.6

REAL TIME VISUALIZATION OF CALCIUM ENTRY INTO GUINEA PIG PURKINJE CELLS *IN VITRO*. M. Sugimori and R. Llinás, Dept. of Physiology & Biophysics, NYU Med. Ctr., NY 10016.

Study of calcium concentration changes in the soma and dendrites of mammalian Purkinje cells during spike activity have been determined with the use of calcium-sensitive dyes such as Arsenazo III (Ross & Werman, J. Physiol 389:319, 1986) and Fura II (Tank et al., Science 242:633, 1988). In the latter study, a distinction was made between the calcium entry which occurs at the onset of plateau potentials in the peripheral dendritic branchlets and that which occurs in the main branches of the dendritic arbor. The suggestion was made that the spontaneous electrical oscillatory activation of Purkinje cells are initiated by inward calcium current at peripheral dendritic level which upon reaching sufficient amplitude evoked full calcium-dependent action potenials in the main dendritic tree. The measurements reported in this study were obtained at a maximum speed of 250 msec. Presently with improved imaging techniques (a photon counting camera and a high speed video receiver capable of recording 400 frames per second) the actual time course and the distribution of calcium entry during single action potentials in Purkinje cells was directly determined. Because the fluorescence measurements using Fura II were made at only one light frequency (340 μm) the measurements indicate only relative calcium concentration changes in the Purkinje cell cytosol. Simultaneous recordings of light absorption by Fura II and of intracellular Purkinje cells voltage clearly indicates that the plateau potentials which proceed the activation of Purkinje cells occur in the spiny branchlets. The full action potentials are then observed in the main dendrites is followed by a synchronous, antidromic invasion into the fine dendritic tree. This oscillation in light absorption occurs at frequencies of 5-10 Hz per second and return to baseline within 50-100 msec in cells in good condition.

Finally, activation of the climbing fiber system demonstrated a rapid activation of both the main dendritic tree as well as the peripheral branches with the main dendritic tree being the first to demonstrate calcium entry. Support: NS13742.

77.7

TOPOGRAPHY AND ANALYSIS OF VESTIBULAR-VISUAL CLIMBING FIBER SIGNALS IN THE RABBIT CEREBELLAR NODULUS. N.H. Barmack and H. Shojaku*, R.S. Dow Neurological Sciences Institute, Good Samaritan Hospital and Medical Center, Portland, OR, 97209.

The cerebellar nodulus receives primary and secondary vestibular afferent mossy fiber inputs, a vestibular climbing fiber input and a visual climbing fiber input. We have recorded the responses of single Purkinje cells evoked by vestibular and optokinetic stimulation in lobules 9c,9d and 10 of rabbits anesthetized with chloralose-urethane. About 60% of all nodular Purkinje cells received climbing fiber signals (CFRs) which were modulated by dynamic and/or static vertical vestibular stimulation. CFRs which were evoked by stimulation in the plane of the left posterior-right anterior semicircular canals (Lpc-Rac) were found in Purkinje cells distributed over the medial 1.5 mm of the nodulus. CFRs in these Purkinje cells were also evoked by downward vertical optokinetic stimulation in the posterior field of the left eye. These optokinetic responses were synergistic with rotation of the head in the plane of the Lpc-Rac. CFRs in Purkinje cells located in the ventral layer of the nodulus (.5-1 mm from the midline) and with no vestibular sensitivity, were evoked by posterior-anterior stimulation of the left eye. CFRs evoked by stimulation in the plane of the Lac-Rpc were found 1-2 mm laterally in the nodulus. The topographic distribution of these vestibularly and optokinetically evoked CFRs might reflect a medio-lateral gradient of postural response to these signals.

77.8

VISUAL PONTOCEREBELLAR PROJECTIONS IN THE MACAQUE. M. Glickstein, A. Gibson, C. Legg, B. Mercier, J. Stein & J.Voogd. Dept of Anatomy, University College London, London WC1, England.

The pontine nuclei receive visual inputs from cortical and midbrain visual areas of monkeys. The great majority of visual fibers terminate dorsolaterally in the rostral pons. Where do pontine visual cells project on the cerebellar cortex? We first recorded from this region of the pontine nuclei to identify cells responding to visual stimuli. We injected a small amount of wheatgerm agglutinin horseradish peroxidase (WGA HRP) amongst visually driven cells. After two days survival the brains were prepared for HRP histochemistry. We confirmed the visual input by plotting the location of retrogradely filled cells in cortex and midbrain. In addition to projections from areas 19 and 7, this region also receives fibers from area 8 and from the superior colliculus and pretectum. Orthograde projections were distributed bilaterally to caudal cerebellar vermis and hemispheres. The principal target was the contralateral paraflocculus, paramedian lobe and the adjacent ansiform lobe. There were lighter ipsilateral projections to these same targets and a vermian projection which was maximal on the uvula, but which extended rostrally to Lobule VI. Surprisingly, there were few or no efferent fibers from this region of the pontine nuclei to the flocculus of either side.

77.9

ORIENTATION SPECIFICITY OF OPTOKINETICALLY MODULATED NEURONS IN THE GOLDFISH CEREBELLUM. J.F. McGurk and W.M. Graf, The Rockefeller University, New York, NY 10021

Responses of individual neurons in the corpus cerebelli of the goldfish (*Carassius auratus*) during optokinetic stimulation revealed that they are sensitive to visual field rotation. Extracellular recordings were made from 67 neurons in the corpus cerebelli during visual field rotation with a planetarium projector with the axis of rotation located in the horizontal plane (producing vertically directed movement). Neurons were characterized by the orientation of the axis of visual field rotation that produced the greatest modulation in firing rate (the preferred axis). Two response types were found. One population (N = 21) was excited by visual field rotation around the preferred axis in one direction and inhibited by rotation in the opposite direction. Visual field rotation about an axis 90^{o} apart from the preferred axis produced little modulation in firing rate. These neurons were sensitive to both the orientation of the axis of rotation and the direction of movement of the visual field, and were termed **direction-specific.** The second population (N = 17) showed an increase or decrease in firing rate during rotation in either direction around the preferred axis and no modulation in firing rate during visual field rotation about an axis 90^{o} apart from the preferred axis. This population of neurons was called **axis-specific.** In addition, 67% of tested direction-specific neurons and 50% of tested axis-specific neurons were also modulated during visual field rotation about a vertical axis (horizontal visual field motion). This suggests that, for these neurons, the preferred axis is tilted up or down from the horizontal plane.

The preferred axes were widely distributed, and had no tendency to cluster. This is unlike the situation that obtains in the rabbit, where the preferred axes of Purkinje cells in the cerebellar flocculus tend to be close to the sensory axes of the labyrinthine semicircular canals and the axes of rotation of the extraocular muscles.

77.10

FRACTAL GEOMETRY OF PURKINJE NEURONS: RELATIONSHIPS AMONG METRICAL AND NON-METRICAL NEURAL GEOMETRIES. *A.J. Pellionisz* (Dept. of Physiology and Biophysics; New York University Medical Center, New York, NY, 10016).

In one decade since inception of a geometrical approach to brain theory, an alternative was established to the untenable dogma that Euclidean geometry (with a Kronecker delta as the metric tensor) is sufficient to account for structurofunctional features of the CNS. In time, implications of *multidimensional geometrical concepts and formalisms* will be absorbed, intrinsic coordinate systems will be experimentally measured and thus features of neural geometries that are spun over such frames will be established (e.g. spacetime geometry of sensorimotor transformations). *Sensorimotor system neuroscience* may lead this progress since metrical properties of skeletomuscular systems and their governing neural nets can be measured and multidimensional computational properties are to be used in neurocomputing, rehabilitation medicine and (neuro)robotics. Also, representation of distances (and judgements and decisions based on them) in functional spaces with metric tensors that are characteristic to non-Euclidean spaces may become a conceptual and formal basis in *cognitive science* (theory of association of generalized vectors).

Raising sights above Euclidean spaces it is apparent that metrical properties of (quasi)linear, derivable multidimensional manifolds (e.g. those governing gaze) are but the simplest features of neural geometry. It is known that if neural systems revert to a nonlinear non-metrical domain, strange attractors emerge, revealing chaotic geometry. It is postulated here that beneath a chaotic geometry (e.g. of EEG) and the functional "smooth" metrical spaces it may relapse to, a third kind of neural "microstructure" exists: *The growth of cellular (neural) elements reveals a fractal geometry* that is a direct manifestation of the process based on repeated access to the genetic code during growth.

The presentation demonstrates the fractal growth of dendritic trees of Purkinje cells, displaying self-similarity of micro- and macro-features of the arbor, showing that the bifurcation-rule of branching expresses a fractal dimension, and revealing codes responsible for generating normal or pathological arbors. Relation of growth- and functional models of neurons and neural nets is discussed; since fractal, metrical and chaotic neural geometries of the micro-, medium- and macro-domains of the CNS are interdependent (just as relativistic-, Newtonian- and quantum-mechanics apply together to the external world).

77.11

THE CEREBELLUM AS AN ADAPTIVE SMITH-PREDICTOR IN VISUO-MOTOR CONTROL. (SPON: European Neuroscience Assoc.) R.C. Miall*, J.F. Stein* and D.J. Weir*. King's College Research Centre, King's College, Cambridge CB2 1ST, U.K. & Univ. Lab. of Physiology, Oxford OX1 3PT, U.K.

To help us understand the role of the cerebellum in the visual guidance of movements, we have been analysing manual tracking in human subjects, cerebellar patients and rhesus monkeys with temporary cerebellar lesions. We present here a model of visuo-motor tracking based on these experiments. The main features of the model are: i) that tracking is controlled by intermittently sampled negative feedback; ii) that both short- and long-term predictions of target motion are employed; and iii) that an internal representation of the visuo-motor control system helps the system to predict its own performance.

This internal model has two components: The first represents the dynamic behaviour of the visuo-motor system. This allows rapid prediction of the expected outcome of a motor command. The second component models only the time delays in the feedback loop (Smith, 1957), determined by sensory, computational and execution delays. This enables any discrepancies between the predicted and actual movement to be processed as a feedback signal without de-stabilizing the high performance achieved by the dynamic component of the model. Both the dynamic and temporal components of the model must be plastic to adapt to changes in the visuo-motor system or in external visual feedback.

We suggest that the generation and adaption of these two components of the Smith Predictor take place in the cerebellum, lateral regions being responsible for a visuo-motor model, operating in visual coordinates, and medial regions for an executive model, operating in motor coordinates. (Reference: O.J.M. Smith, *Chem. Eng. Prog. 53*, 1957, pp. 217-219)

78.1

THE α_2 AGONIST MEDETOMIDINE POTENTIATES THE ANTINOCICEPTIVE ACTION OF OPIATES. M.H. Ossipov, E. Messineo*, B.-S. Lin, and S. Harris* Anaquest, Division of BOC, 100 Mountain Ave, Murray Hill, NJ 07974, U.S.A.

We examined the antinociceptive interaction between morphine, fentanyl, and meperidine with medetomidine (medet). Male S-D rats received fixed combinations of medet to fentanyl (10:1) or meperidine (1:3) i.t. and i.v., or morphine (10:1) i.t. and (1:10) i.v. Antinociceptive tests were the tail-flick (TF) and hot plate (HP). Response latencies were measured before (Control) and several times after (Post) drug injection. Cut-off (CO) times of 10 (TF, i.v.), 7 (TF, i.t.) or 30 (HP) sec were used. Data were converted to % maximal possible effect (%MPE) by: 100 x [(Post - Control)/(CO - Control)]. The A_{50} (50 %MPE dose) at time of peak effect for each treatment was determined.

A_{50} with/no medet:	fentanyl	morphine	meperidine	medet.
TF i.v. (mg/kg)	.0017/.0031	.183/1.54	.093/1.66	0.034
HP i.v. (mg/kg)	.0027/.0072	.42/4.73	.179/3.23	0.042
TF i.t. (µg)	.158/1.20	.104/1.37	4.75/432	3.06
HP i.t. (µg)	.535/1.59	.351/.72	20.1/none	13.7

Medet consistently increased the potency of each opiate tested. Isobolographic analysis indicated that the interaction between medet and the opiates was synergistic for spinal reflexes when given i.t. and was additive otherwise.

78.2

HIGHLY POTENT INHIBITORY EFFECTS OF 5-HT_3 ANTAGONIST, GR38032F, ON DEFEAT ANALGESIA IN MALE MICE. R.J. Rodgers, J.K. Shepherd* and J.I. Randall*, Department of Psychology, University of Bradford, BD7 1DP, U.K.

Social defeat in male mice is associated with an acute non-opioid form of analgesia. Behavioural and pharmacological studies have strongly suggested that anxiety is a critical factor in the generation of the reaction (Rodgers R.J. and Randall J.I., Psychopharmacology 96: 45, 1988). In this context, recent evidence indicates that selective 5-HT_3 receptor antagonists exert highly potent anxiolytic effects in rodents and primates (Jones B.J. et al., Br. J. Pharmacol. 93: 985, 1988). In the present study, we assessed the effects of one such compound, GR38032F, on basal nociception and defeat analgesia in male DBA/2 mice. GR38032F was studied over the dose range 0.00001-1000 microgm/kg, and administered 45 min before testing. Results show that, while devoid of intrinsic effects on the tail-flick assay, GR38032F potently inhibited the analgetic consequences of social defeat. Total inhibition was evident over the range 1.0-1000 microgm/kg; partial inhibition at 0.0001-0.1 microgms/kg; lower doses were ineffective. These data suggest that 5-HT_3 receptor mechanisms are involved in the mediation of defeat analgesia and further support the putative anxiolytic potential of this compound.

We thank Glaxo Group Research for the gift of GR38032F. This work was supported by a YRHA research grant.

78.3

STIMULATION OF NUCLEUS RAPHE MAGNUS (nRM) REDUCES THE NUMBER OF LUMBAR SPINAL CORD NEURONS DEMONSTRATING *FOS* ONCOPROTEIN-LIKE IMMUNOREACTIVITY FOLLOWING NOXIOUS HEAT STIMULATION OF THE FOOT IN THE RAT. A.R.Light and S.L.Jones. Dept of Physiology, UNC-Chapel Hill, Chapel Hill, NC 27599-7545

The proto-oncogene *c-fos* is expressed by lumbar spinal cord neurons following noxious stimulation of the hindlimb (Hunt, 1987). In the present study we confirmed the presence of *fos*-oncoprotein-like immunolabeling in the nuclei of lumbar spinal grey neurons following noxious heat stimulation of the foot of sodium pentobarbitol-anesthetized rats. The foot of the rat was stimulated with a feedback controlled thermal stimulator. The probe was placed in contact with the plantar surface of the foot, and the probe was heated rapidly to 70°C. Stimulation was terminated when a flexion reflex was evoked. After 10-20 repetitions, the rat was allowed to survive for four hours, following which it was perfused with paraformaldehyde. The lumbar cord was removed, cut transversely with a vibratome into 50µm thick sections and reacted for *fos*-immunoreactivity with an antibody to *fos* proteins (Cambridge). In addition, we demonstrated that stimulation in nRM at an intensity sufficient to inhibit flexion reflexes evoked by noxious heat did not lead to *fos* immunolabeling in the spinal grey. However, nRM stimulation did cause a 50% reduction in the number of neurons demonstrated by *fos*-immunolabeling following noxious heat stimulation of the rat's foot. Supported by PHS grants #DA04420, #DA05341, and #NS16433.

78.4

EFFECTS OF OPIOIDS ON PHYSIOLOGICALLY CHARACTERIZED SUPERFICIAL DORSAL HORN NEURONS IN THE CAT. S.L. Jones, M.J. Sedivec and A.R. Light. Department of Physiology, University of North Carolina, Chapel Hill, NC 27599.

The mechanism(s) and site(s) of action of opioids and opioid peptides are not clearly understood. The objectives of the present study were 1) to determine the effects of opioids and opioid peptides applied locally via microiontophoresis on physiologically characterized laminae I and II dorsal horn neurons and 2) to examine the morphological characteristics of dorsal horn neurons influenced by opioids after intracellularly filling them with horseradish peroxidase. Pentobarbital-anesthetized and decerebrate, spinally transected cats were used. In the absence of spontaneous unit activity, the excitatory amino acid, DL-homocysteic acid (DLH), was used to evoke background unit activity. In pentobarbital-anesthetized cats, morphine (MOR) produced mixed effects on DLH-evoked unit activity; to date, 22/45 units were inhibited, 12/45 were excited and 11/45 were unchanged. Similarly, naloxone (NALO) inhibited 24/36 units, excited 4/36 units and produced no change in the DLH-evoked unit activity of 8/36 units. In decerebrate, spinally transected cats both MOR and DAGO (μ agonist) inhibited DLH-evoked activity in the majority of units examined to date(7/9 units); 2/9 units were excited. Whether MOR, DAGO or NALO exert excitatory or inhibitory influences on DLH-evoked unit activity does not appear to depend on the modality or morphological characteristics of the dorsal horn neuron. Supported by DA04420 and DA05341.

78.5

DESCENDING INHIBITION ON SPINAL NEURONS WITH INPUT FROM NORMAL AND ACUTELY INFLAMED KNEES IN THE CAT. H.-G. Schaible*, V. Neugebauer*, F. Cervero*, R.F. Schmidt. Physiologisches Institut der Universität, D-8700 Würzburg, F.R.G.

In order to study modifications of the descending inhibition onto the spinal cord during an inflammatory lesion in the periphery we recorded from spinal neurons with inputs from normal and acutely inflamed knee joints in chloralose-anesthetized cats. Descending inhibition was estimated from the effects of reversible cold blocks in the lower thoracic cord on the resting and evoked discharges of the spinal neurons.

The cold blocks led in most neurons with joint input to increases of background activity and enhanced responses to A- and/or C-volleys. Descending inhibition was more pronounced on neurons in the deep dorsal and ventral horn than in the superficial dorsal horn. There was a tendency in the whole sample for increased effects of the blocks on neurons with input from inflamed knees suggesting increased descending inhibition in animals with arthritis.

Continuous observation of individual neurons with joint input during developing arthritis showed directly that not only the responses of these cells to stimulation of the joint were enhanced but that descending inhibition onto most of these neurons increased as well showing up as progressive increases of background activity and/or enhanced responses to stimulation of the knee joint during cold blocks.

We conclude that during acute arthritis descending inhibition on spinal neurons is becoming more pronounced. But increase of descending inhibition is not sufficient to block enhancement of responsiveness of spinal neurons during developing inflammation.

78.6

PROPRIOSPINAL ANTINOCICEPTION INDUCED BY HETEROSEGMENTAL NOXIOUS STIMULATION BUT NOT BY SPINAL SUBSTANCE P SUPERFUSION.

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Propriospinal antinociceptive neurones, which inhibit nociceptive neurones some segments apart, can be activated by spinal glutamate application. We now report that noxious cutaneous stimuli may also trigger propriospinal antinociception, while superfusion of related spinal cord segments with substance P, a candidate for an excitatory neurotransmitter in nociceptive afferent nerve terminals, or bicuculline, a $GABA_A$-receptor antagonist, failed to do so.

Single dorsal horn neuronal responses to noxious skin heating were recorded in the lumbar spinal cord of pentobarbital anaesthetized cats after high cervical spinal transection. Mean heat-evoked responses of 6 of 11 neurones tested were reduced to 61.3 ± 21.2 % of control during a conditioning immersion of the ipsilateral front paw in 54°C hot water. Superfusion of the same cervical segments which processed this conditioning stimulus, or superfusion of lower thoracic or sacral spinal segments with substance P (1-100 µM) or bicuculline (0.1-10 mM) failed to likewise trigger propriospinal antinociception.

Thus, propriospinal antinociceptive neurones are apparently not tonically inhibited via $GABA_A$-receptors and spinal substance P does not mimick the effect of conditioning cutaneous noxious stimulation.

78.7

ANALGESIA FROM THE PERIAQUEDUCTAL GREY (PAG) IN DEVELOPING RATS IS REVERSED BY INTRATHECAL ADMINISTRATION OF SEROTONERGIC AND NORADRENERGIC ANTAGONISTS. L.A. Tive and G.A. Barr Biopsychology Dept., Hunter College of the City University of New York, New York, N.Y. 10021 and Dept. of Psychiatry, Albert Einstein School of Medicine, Bronx, N.Y.

To compare stimulation produced analgesia (SPA) to opiate induced analgesia (OA) mediated by the PAG, the analgesic effects of glutamate and morphine administration to this area were compared in developing rats. It was found that OA and SPA develop differentially but are present by 14 days of age. A possible explanation for the differences in development is that OA and SPA are supported by distinct bulbospinal monoaminergic systems. The goal of this study was to determine the degree to which the analgesia produced by glutamate and morphine in the PAG could be antagonized by intrathecal administration of the serotonergic antagonist methysergide and the noradrenergic antagonist phentolamine. Fourteen day old rat pups were implanted with cannulae aimed at the PAG and with intrathecal cannulae. Pups received glutamate microinjections (180 mM/0.2 µl) to the dorsal or the ventral PAG or morphine microinjections (6 µg/0.2 µl) to the ventral PAG only. PAG injections were immediately followed by intrathecal injections of either methysergide (0, 15, or 45 µg) or phentolamine (0, 15, or 30 µg) and withdrawal latencies to noxious thermal and mechanical stimuli was measured. Both morphine and glutamate produced robust analgesia that was attenuated by methysergide and phentolamine in a dose dependent manner. Phentolamine attenuated analgesia against the mechanical stimulus to a greater degree for all PAG injections. These results indicate that although there are developmental differences between OA and SPA, the analgesia resulting from morphine and glutamate administration to the PAG are supported by the same bulbospinal monoaminergic systems. Furthermore, the analgesic effects of spinal serotonin and norepinephrine may be selective for specific stimulus types.

78.8

STIMULATION OF THORACIC VAGAL AFFERENTS INHIBITS THE JAW-OPENING REFLEX IN CATS. W. Maixner, D.F. Bossut and E.A. Whitsel*. Dental Research Center, U. of North Carolina, Chapel Hill, N.C. 27514.

In the present study, we have examined the relative ability of cervical, thoracic, and subcardiac vagal afferent stimulation to modulate the jaw-opening reflex (JOR) produced by tooth pulp (TP) stimulation.

In anesthetized cats, the right maxillary TP was stimulated at 1X to 5X threshold and the JOR was recorded from the right digastric muscle. Conditioning stimuli (7 pulses: 333 Hz, 1.0 msec, 0-10 mamp) were applied to vagal segments at conditioning-test intervals (CTI's) ranging form 5 to 1000 msec.

In general, cervical and thoracic vagal stimulation inhibited the JOR at CTI's greater than 50 msec with maximum inhibition observed at 200 msec. In contrast, subcardiac vagal stimulation produced a weaker inhibition of the JOR. The relative ability of different vagal segments to inhibit the JOR was: Thoracic > Cervical >> Subcardiac.

These findings suggest thoracic vagal afferents may be the primary source of vagal afferents which modulate the JOR in the cat.

Supported by NIDR grant DE08013 (W.M.)

78.9

HYPERTENSION-INDUCED ANTINOCICEPTION: CHARACTERIZATION WITH RESPECT TO CENTRIFUGAL PAIN MODULATION., C.L. Thurston, & A. Randich, Dept. of Psychology, Univ. of Iowa, Iowa City, IA 52242

Previous investigators have shown that hypertension is correlated with antinociception. The purpose of the present series of experiments was to examine the antinociception produced by an acute, drug-independent, hypertension. We found that occlusion of the abdominal aorta just proximal to the renal arteries produces a pressor response (135 % control) and bradycardia (90 % control). In addition, occlusion results in antinociception as measured by the tail flick test. This antinociception is blocked by pretreatment with intrathecal phentolamine (30 ug), but not with intrathecal methysergide (30 ug) or naloxone (30 ug). Cold block applied to the spinal cord at the level of the second thoracic vertebra also prevents the tail flick suppression produced by occlusion. We also showed that sinoaortic denervation, but not bilateral vagotomy, blocks the tail flick suppression. Since lumbar spinal cord blood flow may be impaired with aortic occlusion, we are currently expanding the experiments to include forepaw and hindpaw pinch as a measure of nociception. Aortic occlusion decreases the response to pinch in both the forepaw and hindpaw suggesting that our measure of antinociception may not be a consequence of spinal ischemia. This research was supported by NIH grants NS24958, NS22966, and HL07121.

78.10

CONTRIBUTION OF SUBDIAPHRAGMATIC VAGAL AFFERENTS TO THE ANTINOCICEPTION PRODUCED BY ELECTRICAL STIMULATION OF THE CERVICAL VAGUS. S. A. Aicher, S. J. Lewis,* and A. Randich. Depts. of Psych. & Pharm., U. Iowa, Iowa City, IA 52242.

Electrical stimulation of either the dorsal or ventral subdiaphragmatic vagus produces antinociception via afferents travelling in either the right or left cervical vagus, respectively. This experiment examined the contribution of subdiaphragmatic vagal afferents to the antinociception produced by electrical stimulation of the cervical vagus. In order to completely remove subdiaphragmatic fibers from the cervical region, a unilateral subdiaphragmatic vagotomy was performed by intraneural microinjection of ricinus comminus (100 ng) into the dorsal branch of the subdiaphragmatic vagus. 72 hrs prior to testing, all animals received an i.p. injection of Fast Blue to allow visualization of intact afferent cell bodies in the nodose ganglion and confirm the vagotomy in ricin-treated rats. The minimum intensity of electrical stimulation of the right cervical vagus necessary to produce inhibition of the nociceptive tail-flick reflex was determined for each rat. There were no differences between sham- and ricin-treated groups (n=10 per group) in the ability to produce antinociception during electrical stimulation of the cervical vagus. These data indicate that although subdiaphragmatic fibers can support antinociception, they do not significantly contribute to the antinociception seen during electrical stimulation of the cervical vagus. This work supported by NIH Grants NS24958 & NS22966.

78.11

EFFECTS OF ACTIVATION OF CAROTID SINUS BARORECEPTORS (CSB) ON HUMAN PAIN RATINGS. K.-D. Kniffki, A.V. Apkarian, M.K.C. Mengel* and A. Stiefenhofer*. Physiologisches Institut, Universität Würzburg, D-8700 Würzburg, F.R.G.

In 6 female and 7 male healthy adult volunteers pain was induced by electrical tooth stimulation and by constant pressure on a 2 mm² dorsal area of a finger for 20 s. The pain thresholds (T) were determined and pain sensations were rated (R) on a 50 point category scale. The CSB were stimulated by applying negative pressure (-40 to -100 mmHg) bilaterally to the neck, starting 15 s before the application of the noxious stimulus. In control trials the electrical stimuli resulted in reproducible Ts and the mechanical stimuli evoked continuously increasing Rs. During neck suction trials the mean T as well as the magnitude of the average R were reduced as compared to control. However, clear individual differences were observed. The reductions of T and R were insignificant for 2/10 and for 3/11 subjects, respectively. The mean absolute difference in the Rs between test and control trials increased, and the mean relative difference in the Rs was constant (16%).

The data show that, for at least 20 s, activation of carotid sinus baroreceptors in most subjects results in a reduction of pain perception.

78.12

ACETYLCHOLINE (ACh), GLUTAMATE (Glu), GABA, AND ELECTRICAL STIMULATION (ES) IN THE INTERPEDUNCULAR NUCLEUS (IPN) SYSTEMATICALLY MODIFY ON- AND OFF-CELLS OF THE NUCLEUS RAPHE MAGNUS (NRM). V. Budhrani* and I.D. Hentall. Univ. Puerto Rico Med. Sci. Campus, Dept. Physiol., San Juan, 00936

To explore limbic effects on pain-modulating NRM neurons, pentobarbital-anesthetized female Wistar rats received stereotaxic microinjections of 50mM ACh, Glu, or GABA, or ES (20Hz pulse trains) in their IPN. Cutaneous hindlimb ES evoked responses in extracellularly recorded on- and off-cells in the NRM (about 70 cells in all). Brain stimulation and recording sites were corroborated histologically.

From various IPN locations, ES and Glu ($5x10^{-7}$ l) mostly inhibited off-cells and excited on-cells, but GABA ($5x10^{-7}$ l) showed mixed results. ACh (10^{-6} l), at the optimum IPN location, inhibited all off-cells and mainly excited on-cells.

Since nociception is probably reduced by off-cells and augmented by on-cells, these results fit with the reported lessened acquisition of passive learned avoidance following IPN lesions, and implicate the cholinergic habenulo-interpeduncular pathway in the amplification of pain.

(Supported by NIH grants NS26116 and RR08224.)

79.1

SEROTONIN BLOCKS THE M-CURRENT IN HUMAN CORTICAL NEURONS. D.A. McCormick, A. Williamson, and D. Spencer. Sect. Neuroanatomy and Neurosurgery Yale Univ Sch Med, New Haven, CT

Excitability of neurons in the cerebral cortex is under the influence of ascending serotonergic and cholinergic inputs. We investigated here the postsynaptic actions of ACh and 5HT in human neocortical pyramidal neurons maintained with the *in vitro* slice technique. Tissue was a small portion of that normally removed for the treatment of intractable epilepsy.

Local application of 5HT (300 μM) resulted in three responses: 1) hyperpolarization and increase in membrane conductance. Under voltage clamp conditions (SEVC) this response reversed near E_K, and exhibited non-additivity with outward currrents induced by adenosine or the $GABA_B$ agonist baclofen and therefore probably represents an increase in potassium conductance. Local application of 8-OH-DPAT resulted in a small hyperpolarization and blocked further hyperpolarizing responses to 5-HT, indicting that the 5-HT-induced increase in potassium conductance was mediated by $5HT_{1A}$ receptors; 2) slow depolarization and decrease in input conductance. Under SEVC this slow depolarization appeared as a suppression of the M-current, which was also blocked by stimulation of muscarinic receptors. This current was not a Ca^{++} or Na^+ activated potassium current since neither intracellular injection of EGTA (500 mM) or extracellular application of TTX blocked this effect; Finally, 3) reduction in spike frequency adaptation, block of the slow afterhyperpolarization and the current which underlies it, I_{AHP} (histamine, norepinephrine, and acetylcholine also had these effects).

Serotonin, therefore, has multiple actions in human cerebral cortical neurons and these actions are shared with other putative neurotransmitters. Supported by NINCDS & Jacob Javits Center.

79.2

BETA-ADRENERGIC RECEPTOR-MEDIATED EFFECTS ON CORTICOTECTAL NEURONS. J.S. Solomon & J.M. Nerbonne. Dept. of Pharmacology, Washington Univ., St. Louis, MO 63110.

The actions of norepinephrine (NE) in the mammalian visual cortex are complex: single unit recordings during adrenoreceptor stimulation have revealed excitatory, inhibitory, biphasic and null responses. Because this variety may reflect the diversity of cell types or adrenergic receptor subtypes (α_1,α_2,β_1,β_2) in visual cortex, we are examining the effects of selective adrenergic agents on isolated corticotectal (CT) neurons from rat primary visual cortex (area 17).

To identify CT cells, fluorescent "bead" injections were made into the superior colliculus of P4-7 Long-Evans rats. Area 17 was dissociated 1-8 days later and whole-cell voltage-clamp recordings were obtained from labeled cells. Bath solution contained (mM): 140 NaCl, 10 HEPES, 5 glucose, 4 KCl, 2.5 $CaCl_2$, 2 $MgCl_2$, 1μM TTX. Pipets contained (mM): 140 KCl, 10 HEPES, 5 EGTA, 5 glucose, 3 Mg*ATP, 0.1 Tris*GTP. In 28/65 CT cells, 1 sec applications of 10μM isoproterenol (ISO), a selective β-agonist, evoked small (10-45pA) outward currents at a HP of -90 mV. The ISO-sensitive currents peaked within 1-2 sec, decayed over 10-30 sec and were attenuated ≥50% in the presence of 50μM propranolol (n=2). Similar currents were evoked in all 28 cells by 1 sec exposures to 500μM dibutyryl-cAMP. Current-voltage relations suggest that these outward current shifts result from a decrease in resting inward current due to cation influx or anion efflux; however, the participation of an electrogenic pump cannot be ruled out. These results suggest that the inhibitory effects of NE in visual cortex may be mediated by β-adrenergic receptors. (Support: NSF #BNS 8809823;NIH #5T32 GM07805)

79.3

MECHANISM OF ADENOSINE INHIBITION AND REDUCTION OF CALCIUM CURRENT. R.W. Greene, U. Gerber, D.R. Stevens & H.L. Haas*. Harvard Med. Sch./ VAMC, Brockton, MA 02401; Johannes Gutenberg-Universitat, Mainz

Endogenous adenosine (AD) tonically inhibits CA1 neurons in vitro. Application of exogenous AD either in the perfusate (50uM) or by pressure injection (1mM) was employed with intracellular recordings under voltage clamp control to examine the mechanism of the inhibition. AD increased a voltage insensitive conductance. The ratio of the AD evoked current (ΔI) to the change in conductance (ΔG) varied with the extracellular potassium ($[K^+]_o$) as predicted for a K^+ permeability change, $[K^+]_o=[K^+]_i\exp(E_m/26)\exp-(\Delta I/26\Delta G)$ where E_m is the holding potential. AD also reduced Ca^{++} currents. This reduction correlated both in time and magnitude with the increase in the AD evoked K^+ conductance (G_K). When the G_K was blocked by the replacement of Ca^{++} with Ba^{++} no reduction in Ca^{++} currents was observed.

We conclude AD inhibition is mediated by an increase in a G_K distinct from the voltage sensitive G_K elicited by carbachol, norepinephrine, opioids and serotonin. The observed reduction in Ca^{++} currents results from a shunt by the AD evoked G_K.

79.4

GLUTAMATE BLOCKS PROTEIN-KINASE C MEDIATED REDUCTION OF $GABA_A$ - CURRENTS IN HIPPOCAMPAL NEURONS.

A.Stelzer* and R.K.S.Wong (SPON: R. Miles). Department of Neurology, Columbia University, New York, N.Y. 10032.

The regulation of $GABA_A$ receptor function by protein phosphorylation was examined using acutely dissociated CA1 hippocampal neurons of adult guinea-pigs. Under whole-cell voltage-clamp recording conditions stimulators of protein kinase C (PKC) - phorbol 12-13 dibutyrate (200-500 nM) and 1-Oleolyl-2-Acetyl-rad-Glycerol (OAG; 200-400 nM) - reduced $GABA_A$-mediated chloride currents (Soc. Neurosci. Abstr. 14, 369.2). Experiments were performed at room temperature. GABA (200 μM) was applied by short pressure pulses (10-80 ms). When glutamate (50 μM) was added to the extracellular solution, the GABA-current suppressing effect of both PKC stimulators, phorbol 12-13 dibutyrate and OAG, was blocked. This action of glutamate was mimicked by glutamate analogues quisqualate and N-methyl-D-aspartate (NMDA) at similar concentrations (50 μM). The NMDA-receptor antagonist APV (50 μM) did not prevent the NMDA-mediated protection of GABA currents against PKC stimulators. It remains to be seen whether the PKC antagonizing action of glutamate and its analogues is specific for the $GABA_A$-current. Supported by grants from Klingenstein Foundation, Epilepsy Foundation of America and N.I.H.

79.5

MONOAMINES MODULATE THALAMIC NEURONAL FUNCTION THROUGH INCREASE OF A HYPERPOLARIZATION-ACTIVATED CATION CURRENT

H.-Ch. Pape and D.A. McCormick. Section of Neuroanatomy, Yale University School of Medicine, New Haven, CT 06510. (SPON: M. Schwartz)

Ascending noradrenergic and serotonergic fibers from the brainstem are involved in the control of thalamic neuronal excitability. Here we describe a new mechanism of action of norepinephrine (NE) and serotonin (5HT) in the dorsal lateral and medial geniculate nuclei of cat and guinea pig. Application of NE (0.5mM) to geniculate cells maintained in vitro resulted in a α_1-mediated decrease in K^+ conductance (McCormick & Prince, J.Neurophysiol.,59: 978,1988). Blockade of α-receptors unmasked a NE-induced slow increase in apparent input conductance at potentials negative to -60 mV that was mimicked by the ß-agonist isoproterenol (50μM) and blocked by local application of the ß-antagonist propranolol (100μM). Application of 5HT (0.3mM) elicited a similar conductance increase that was blocked by methysergide (10μM) but not propranolol. Monoaminergic responses persisted in the absence of synaptic transmission, were blocked by Cs^+ (2mM) and were unaffected by Ba^{++} (0.5mM). The block by Cs^+ suggested a enhancement of a hyperpolarization-activated cation current. Indeed, hyperpolarizing voltage pulses from a holding potential of -50mV to beyond -60 mV demonstrated a slowly developing (time constant 0.2 to 1.5 s) inward current, termed I_h. Application of NE or 5HT enhanced I_h with little change in "leak" current. Changing the extracellular concentration of Na^+ or K^+, but not Cl^-, shifted the NE/5HT-induced current in parallel to I_h on the voltage axis by an amount expected for a mixed Na^+/K^+ conductance. The NE and 5-HT induced increase in I_h may be mediated by activation of adenylate cyclase, since local application of 8-bromo-cAMP (500 μM) or forskolin (25 μM), but not 1,9-dideoxy-forskolin (100 μM), resulted in a marked and selective enhancement of I_h.

We conclude that 5HT and NE increase a hyperpolarizing-activated Na^+/K^+ current in thalamic neurons. In this way the ascending noradrenergic and serotonergic systems may inhibit the generation of rhythmic burst activity and promote the accurate transfer of synaptic inputs during increased arousal and attentiveness.

79.6

CALCIUM ACCUMULATION IN ENDOPLASMIC RETICULUM OF PURKINJE CELL DENDRITES. S.B. Andrews*, D.M.D. Landis and T.S. Reese. Lab. Neurobiology, NINDS/NIH, Bethesda, MD 20892 and Dept. Neurology, Case Western Reserve University, Cleveland, OH 44106

We have previously used electron probe microanalysis to show that cisterns of endoplasmic reticulum (ER) in dendritic spines of Purkinje cells accumulate substantial amounts of calcium in response to depolarization of parallel fiber synapses (PNAS 85:1682 (1988)). Based on evidence that the sequestered calcium was derived partly or entirely from the extracellular fluid, we suggested that it entered through ligand- or voltage-gated channels on the spine membrane. Here we examine calcium sequestration in Purkinje dendritic shafts, where cytosolic calcium is regulated by neuron-specific, voltage-gated calcium channels in the dendritic membrane (Tank et al, (1988) Science 24:773; Llinas et al, (1989) PNAS 86:1689). Electron probe microanalysis of cryosections from freshly excised, directly frozen mouse cerebellar cortex reveals that some ER in spine-bearing dendrites accumulates large amounts of calcium (mean concentration 11.2 mmol/kg dry weight) as compared with other dendritic organelles, *e.g.*, mitochondria (1.9 mmol/kg), and with the cytosolic compartment (2.6 mmol/kg). The calcium increase in dendritic ER appears to be at least as large as in spine cisterns (previously reported as 6.7 mmol/kg). While loading of dendritic ER is consistent with calcium entry through voltage-gated channels on dendritic membranes, it leaves open the route of calcium accumulation in spines. Plausible explanations are: 1) voltage-gated calcium channels are also present on the spine membrane; 2) calcium influx is directly coupled to glutamate binding to spine receptors; or 3) spine cisterns backfill by diffusion (some of these are in continuity with the dendritic ER). However calcium enters spines, the spine ER appears to be a component of a larger sequestration system which includes the ER in dendrites.

79.7

INHIBITION ON DENDRITIC SPINES AND THIN DENDRITES MAY BE INEFFECTIVE BECAUSE OF IONIC CONCENTRATION CHANGES. N. Qian and T. J. Sejnowski. Laboratory for Computational Neurobiology, The Salk Institute, P. O. Box 85800, San Diego, CA 92138

The Nernst-Planck equation for electro-diffusion was applied to axons, dendrites and spines. For thick processes (greater than 1 micron) the results of computer simulation agreed accurately with the cable model for passive conduction and for propagating action potentials. For thin processes and spines, however, the cable model may fail during transient events such as synaptic potentials. First, ionic equilibrium potentials assumed to be constant in the cable model can change and alter the driving forces for movement of ions across the membrane. Second, longitudinal diffusion, not considered in the cable model, may dominate over electrical forces when ionic concentration gradients become large. In particular, our model predicts that inhibition is effective only on cell bodies or large processes, but not on small structures such as spines and thin processes (less than 0.1 micron). Large inhibitory input on a spine head may in fact give an excitatory response, because of the shift of the reversal potential caused by large concentration changes. We suggest modifications to the cable model that lead to better agreement with the electro-diffusion model.

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79.8

MUSCARINIC EXCITATION IN THE GUINEA PIG INFERIOR MESENTERIC GANGLION. T.L. Anthony* and D.L. Kreulen (SPON: P. Pickens). Department of Pharmacology, University of Arizona, College of Medicine, Tucson, AZ. 85724.

Sensory neurons (mechanoreceptors) located in the wall of the guinea pig large intestine are both cholinergic and noncholinergic. Colonic distension evokes excitatory cholinergic and noncholinergic synaptic potentials in neurons in the inferior mesenteric ganglion, the cholinergic component has been shown to be partly muscarinic in nature. Our goal was to characterize the cholinergic muscarinic responses. Intracellular recordings were made in principal ganglionic neurons through glass micropipettes (tip resistance: 45-110 Mohms). Bethanechol (100 μM), administered by pressure-ejection, elicited a slow depolarization with an average amplitude and duration of 7.2 $\pm$ 0.6 mV, 348 $\pm$ 15.9 seconds (n=6, 3 preparations). In one cell the depolarization was associated with a 27% increase in conductance. Atropine (1 μM) diminished the depolarization in 84% of cells tested without affecting the resting membrane potential. Low Na^+ (9.6 mM) caused an 40% reduction of the depolarization (n=2, 2 preparations). These results demonstrate a muscarinic depolarization which is mediated in part by sodium influx in a subpopulation of neurons in the IMG. Supported by DK36289, HL27781.

79.9

MUSCARINIC RESPONSES FROM CELIAC GANGLION NEURONS IN PRIMARY CULTURE. J.S. Coggan*, D.L. Kreulen, S. G. Matsumoto, R. Gruener (SPON: P. Consroe). Department of Pharmacology, University of Arizona, College of Medicine, Tucson, AZ. 85724.

The electrophysiologic responses of celiac ganglion neurons in primary culture to acetylcholine were investigated. Neurons dissociated from adult guinea pigs were incubated in serum-containing medium (modified MEM, CO_2) for 30 days prior to recording. Intracellular recordings of E_m, spike amplitude, after spike hyperpolarization, time constant, and rheobase, were similar to those found in whole ganglion. Superfusion of acetylcholine (10 μM) evoked slow depolarizations with superimposed bursts of action potentials (20-30 per cluster). These depolarizations could be distinguished from one another by their amplitude, duration and burst content and each neuron gave one characteristic response. In 10 neurons the depolarizations ranged from 8-20 mV and were from 4-8 min in duration. Each response was abolished by atropine (1μM), but none was sensitive to hexamethonium (500μM). In 1 cell low Ca^{2+}/high Mg^{2+} had no effect on the depolarization. These results suggest that there are cell subtypes in the celiac cultures each with a characteristic muscarinic response. Support: DK36289, HL27781, NS25644.

79.10

SUBSTANCE P INCREASES $[Ca^{2+}]_i$ IN DORSAL HORN NEURONS VIA TWO DISTINCT MECHANISMS. M.D. Womack*, A.B. MacDermott, and T.M. Jessell. Center for Neurobiology and Behavior and Howard Hughes Medical Institute, Columbia University, New York, NY 10032.

Substance P (SP) functions as a transmitter at primary afferent terminals in the dorsal horn of the spinal cord. However the mechanisms by which SP regulates the excitability of dorsal horn neurons (DHN) is not understood. To address this question, we have monitored the effects of SP on the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) of acutely dissociated postnatal rat DHN using the Ca^{2+} indicator fura-2. SP (>/= 1 nM) evoked increases in $[Ca^{2+}]_i$ in 25% of DHN. In about half the SP-sensitive DHN (type E), responses to SP were inhibited by 0.5 mM $LaCl_3$ or in Ca^{2+}-free medium indicating that the increase in $[Ca^{2+}]_i$ is dependent on the entry of extracellular Ca^{2+}. SP responses in type E neurons were also blocked by TTX (0.5 μM) suggesting that SP promotes Ca^{2+} entry via voltage-dependent Ca^{2+} channels. In the remaining SP-sensitive DHN (type R), SP-evoked increases in $[Ca^{2+}]_i$ were detected in the presence of $LaCl_3$ or in Ca^{2+}-free medium indicating that SP promotes Ca^{2+} release from an intracellular store. Caffeine-sensitive Ca^{2+} stores were found in 30% of type R neurons and in 95% of type E neurons. In type R neurons, depletion of caffeine-sensitive stores did not abolish subsequent SP-evoked increases in $[Ca^{2+}]_i$, however the release of $[Ca^{2+}]_i$ by SP abolished subsequent caffeine-induced responses. The SP-sensitive Ca^{2+} stores are therefore at least partially distinct from caffeine-sensitive Ca^{2+} stores. In both type R and type E neurons, responses to SP were mediated by the NK1 class of tachykinin receptors. Thus, the same class of tachykinin receptors regulates $[Ca^{2+}]_i$ by two distinct mechanisms in different populations of DHN.

79.11

PROCTOLIN AND ACETYLCHOLINE ACTIVATE A VOLTAGE-DEPENDENT INWARD CURRENT IN MOTONEURONS OF THE LOBSTER CARDIAC GANGLION. J.E. Freschi and D.R. Livengood. Neurology Dept., Emory Univ. Sch. of Med., Atlanta, GA 30322.

We used the two-electrode voltage clamp technique to study the ionic basis of the slow depolarization caused by the neuropeptide proctolin and by muscarinic cholinergic agonists on motoneurons in the cardiac ganglion of the lobster, Homarus americanus. Both proctolin (10 µm and muscarinic agonists (e.g., methacholine, 1 mM) caused a dose-dependent slow inward current. The agonist-induced current was voltage-dependent: with membrane hyperpolarization the response declined; with membrane depolarization up to 0 mV the response enlarged. With further depolarization the agonist-induced current declined and then reversed at potentials around +20 mV. The response to proctolin and muscarinic agonists depended on $[Na]_o$, and was blocked in Na^+-free solutions. The response also varied with $[K]_o$, but did not show a strong dependence on this ion. Various blockers of Na^+, K^+, and Ca^{++} channels did not appreciably affect the evoked current, nor did changes in E_{Cl}. In summary, the agonist-induced current reversed at positive membrane potentials, strongly depended on $[Na]_o$, and remained inward at membrane potentials negative to E_K. These data indicate tht both proctolin and acetylcholine activate a voltage-dependent inward current that is mainly carried by Na^+.

79.12

SYNAPTIC VARIABILITY OF FOUR-CELL NETWORKS IN THE BUCCAL GANGLIA OF *APLYSIA*. Daniel Gardner. Department of Physiology and Biophysics, Cornell University Medical College, New York NY 10021.

Identified neurons of *Aplysia* ganglia permit recording from several cells in parallel and analyzing differences between similar cells, as well as between the same cells in different individuals. In buccal ganglia, paired presynaptic neurons B4 and B5 each produce inhibitory postsynaptic conductances (PSCs) in several neurons. Synaptic efficacy here depends upon peak conductance (g_{peak}) and decay time constant (τ), each of which is readily determinable and independent of V_m. Previous work in these ganglia analyzed variability at single synapses and in 3-cell networks. I now report data obtained by simultaneous recording from the 4-cell network of B4, B5, and a pair of common postsynaptic cells, with four similar inhibitory synapses whose g_{peak} averaged 0.43 µS and τ = 25 ms. Sequential stimulation of B4 and B5 gives 4 PSCs; pairing by common presynaptic or postsynaptic cell, or cross-pairing, yields 6 comparisons, in which similarities within pairs suggest general presynaptic or postsynaptic determinants of efficacy, while lack of paired similarity implies synaptic independence. I compared both individual PSCs and their means at each synapse.

PSC-TO-PSC COMPARISON: Cross-correlograms of PSC amplitude compare fluctuations between sequences of PSCs paired by pre- or postsynaptic neuron. Correlation of PSCs from the same presynaptic neuron is significantly greater than unpaired, both with ($\rho(0)$ = 0.87 paired, 0.01 unpaired; $P < 0.001$) and without ($\rho(0)$ = 0.56 paired, 0.26 unpaired; $P < 0.05$) deliberate presynaptic modulation.

SYNAPSE-TO-SYNAPSE COMPARISON: Each synapse is summarized by its mean g_{peak} and τ, permitting synapse-to-synapse comparisons. Both g_{peak} and τ of synapses sharing a common postsynaptic neuron are significantly more similar (P = 0.0001) than synapses unpaired by pre- or postsynaptic neuron. Correspondingly, postsynaptically-paired $|\log(g_{peak}/g_{peak})|$ and $|\log(\tau/\langle\tau\rangle)|$ are less than half the unpaired case. In contrast, pairing the same data by common presynaptic neuron reveals no similarity, and log ratios are no smaller than the unpaired case.

I conclude that similarities of synapses on the same postsynaptic neuron are consistent with a postsynaptic site for long-term specificity of synaptic efficacy, while similar fluctuations of PSCs produced by the same presynaptic neuron suggest short-term variability is presynaptically specified.

Supported by NS11555 from NINCDS.

80.1

DO HALOPERIDOL AND SWIM STRESS ACT THROUGH A COMMON MECHANISM IN INTERMEDIATE LOBE POMC REGULATION? E.A. Young, D.M. Bronstein and H. Akil. Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109-0720.

Thirty minutes of swim stress at 30°C is accompanied by release into plasma of NAcβ-endorphin$_{1-31}$ from the intermediate lobe of the rat. Previous work had demonstrated that this release can be antagonized by apomorphine, a dopaminergic agonist. When administered for 30 minutes daily for 14 days, swim leads to an increase in POMC mRNA and an increase NAcβ-endorphin-IR in the intermediate lobe as well as a two-fold increase in plasma levels of NAcβ-endorphin.

To explore dopaminergic regulation, rats were given both apomorphine and haloperidol in conjunction with swim. Daily apomorphine had no effect on intermediate lobe NAcβ-endorphin content or on plasma levels of NAcβ-endorphin. Daily apomorphine concurrent with daily swim stress did not antagonize the swim stress induced increase in NAcβ-endorphin stress. However, apomorphine induced down-regulation of dopaminergic receptors cannot be excluded. Studies with haloperidol indicated that both chronic haloperidol and chronic swim stress lead to a 30% increase in NAcβ-endorphin stores. There were no further increases in the swim stress plus haloperidol group. POMC mRNA levels in the intermediate lobe of these same animals demonstrated a 1.8-fold increase in POMC mRNA levels in the chronic swim and chronic haloperidol groups. The combined haloperidol and swim group demonstrated a 2.8-fold increase in POMC mRNA levels. The mRNA data suggest that these two stressors are acting through independent mechanisms. Studies are currently underway evaluating why this increased mRNA level in the haloperidol swim group does not lead to a greater increase in peptide stores.

80.2

CHRONIC HALOPERIDOL ALTERS REGIONAL B-ENDORPHIN METABOLISM IN THE RAT BRAIN. T. P. Davis and T. J. Gillespie* Dept. of Pharmacology, Univ. of Arizona, Health Sciences Center, Tucson, AZ 85724.

Our laboratory has previously shown that chronically administered haloperidol (HL) can alter the in vitro metabolism of B-endorphin (B-E) and neurotensin (NT) by twice washed, whole rat brain homogenates or purified synaptosomal membranes leading to the accumulation of behaviorally active peptide fragments. The present study examined the effect of chronic haloperidol on the regional metabolism of B-E by using rat brain micro slices from caudate putamen, nucleus accumbens, hypothalamus and hippocampus. Male Sprague-Dawley rats (189-236g) were dosed I.P. for 9 days with HL (3mg/kg/day) or vehicle. Rats were sacrificed and the brain was sliced into 2mm coronal slabs using a rat brain matrix. Caudate putamen and n. accumbens were stereotaxically identified and micropunched (2mm). Hippocampus and hypothalamus were also dissected and 2mm punched. Punches were sliced to a thickness of 250u, using a newly developed tissue slicer and time-course incubated with B-endorphin (43uM) for 0 to 240 minutes. B-endorphin remaining and major peptide fragments produced were isolated and identified by amino acid analysis after HPLC. Major B-E fragments formed were B-E 1-17, 18-31, 2-17, 1-18, and 2-18. B-E half-lives for vehicle treated rats versus HL treated rats were: hypothalamus 603 vs. 485 mins., hippocampus 281 vs. 137 mins., n. accumbens 360 vs. 332 mins., and caudate putamen 367 vs. 462 mins. These data agree with our previous studies of NT in whole brain which showed a shorter half-life for HL treated rats. In contrast, the accumulation of specific behaviorally active fragments was significantly lower in HL treated rats. Since brain slices retain a greater degree of morphology, metabolic properties and intact physiological systems than homogenates, these data suggest that HL can selectively alter the half-life of B-E in specific brain regions of the rat leading to a shift in peptide fragment accumulation. (Supported by NIMH Grant 42600).

80.3

EFFECT OF PEPTIDE STRUCTURE ON PEPTIDE DEGRADATION:DES-ENKEPHALIN-γ-ENDORPHIN (DEγE) METABOLISM BY RAT BRAIN SLICE. Z.W. Li*, K. Brendel* and T. P. Davis (Spon: R. Gruener). Dept. of Pharmacology, Univ. of Arizona, Health Sciences Center, Tucson, AZ 85724.

The enzymatic metabolism of Des-enkephalin-γ-endorphin (DEγE) and its cyclized analogue [cyclo-6α-Glu^{11}]-6-17 was examined by using regionally dissected rat brain slice time-course incubation in vitro. DEγE has been shown to have antipsychotic activity that mimics classic neuroleptic drugs, but does not cause extrapyramidal side effects. However, the rapid in vivo degradation of DEγE limits its clinical use. Therefore, cyclo-DEγE was synthesized by Organon Sci. Dev. Group (Oss, Netherlands) in an attempt to improve resistance to enzymatic degradation. Peptide metabolism was studied by time-course incubations with rat brain regions. The half-life of DEγE was 110.6 min vs 59.3 min for [cyclo-6α-Glu^{11}]-6-17 in caudate-putamen, nucleus accumbens was 183.6 min vs 129.1 min, hypothalamus was 161.7 min vs 209.3 min, and hippocampus 154.9 min vs 118.4 min, respectively. In addition, there were 4 major peptide fragments formed from DEγE metabolism, and 2 from [cyclo-6α-Glu^{11}]-6-17. The data imply a structural effect on peptide degradation. Since both peptides incubated yielded their shortest half-lives in caudate-putamen and the longest half-lives in nucleus accumbens (DEγE) and hypothalamus ([cyclo-6α-Glu^{11}]-6-17), the data also suggest a regional specificity of enzyme population, which has been confirmed in our laboratory by measuring levels of endopeptidase 24.11, 24.15 and aminopeptidase. Slice viability was also examined by measuring ^{3}H-GABA uptake and release. The results showed that ^{3}H-GABA uptake and release was maintained stable for at least 5 hr. The data demonstrate that the brain slice technique is a valid and unique approach for studying peptide structure dependent metabolism, but cyclization of DEγE does not improve enzymatic stability except in the hypothalamus. (Supported by NIH Grant MH 42600)

80.4

AN ECTOENZYME FORM OF ENDOPEPTIDASE-24.15 RAPIDLY DEGRADES INTRAVENTRICULARLY (ICV) ADMINISTERED DYNORPHIN A-(1-8). C.J. Molineaux and J. Ayala.* Department of Pharmacology, Mount Sinai School of Medicine, CUNY, New York, NY 10029.

Aminopeptidases, angiotensin converting enzyme, and EP-24.11 ("enkephalinase") contribute to the rapid degradation of ICV injected leu- (LE) and met-enkephalin (ME). The larger LE and ME containing peptides such as the dynorphin (Dyn)-like peptides, and ME-Arg-Gly-Leu are more resistant to the actions of these enzymes, but are excellent substrates of the enzyme endopeptidase-24.15 (EP-24.15), being rapidly converted to LE or ME by purified enzyme in vitro (Chu and Orlowski,Endocrinol. 116: 1418,1985). In the present studies, cannulae were placed in the lateral ventricle of urethane-anesthetized rats, and peptides were administered in the presence or absence of the EP-24.15 inhibitor N-[1-carboxy-3-phenylpropyl]-Ala-Ala-Phe-pAB (cFP-AAF-pAB). The concentration and identity of Dyn-like peptides within the CSF was then monitored by RIA and HPLC in CSF taken from the cisterna at various times. When Dyn A-(1-8) was administered ICV in the presence of cFP-AAF-pAB, the CSF concentration of the ir-Dyn A-(1-8) after 5 min was found to be 40 times higher when compared with administration of Dyn A-(1-8) alone. The inhibitor of EP-24.15 also afforded protection of full length Dyn A and of α-neo-endorphin, but was of a smaller magnitude, corresponding to the affinity (K_m) of the enzyme for each peptide. No EP-24.15 activity was found in rat CSF. Taken together, these data clearly indicate that an ectoenzyme form of EP-24.15 rapidly converts ICV-administered Dyn-like peptides within the extracellular spaces of the brain. This work was supported by NIDA grant DA04218.

80.5

ENKEPHALIN PRECURSOR PROCESSING ENZYME ACTIVITY IN BOVINE CHROMAFFIN GRANULES: DIFFERENTIAL PROCESSING IN VITRO. V.Y.H. Hook, D. Hegerle*, and H.-U. Affolter*+.(SPON: N.Lee). Dept. of Biochem., Uniformed Services University, Bethesda,MD.,&Brain Research Institute, Univ. of Zurich.

The processing enzyme(s) responsible for conversion of proenkephalin to intermediates and small active opiate peptides has not been definitively identified. The lack of available enkephalin precursor for use as substrate has hindered progress in this area. Using authentic substrate ^{35}S-(Met)-prepro-enkephalin (PPE), synthesized from the rat PPE cDNA by in vitro transcription with SP6 polymerase (cDNA in Riboprobe vector) followed by cell-free translation of the PPE mRNA, we have identified enkephalin precursor processing activity in purified bovine chromaffin granules. PPE products generated in vitro were similar to known endogenous enkephalin intermediates present in bovine chromaffin granules. The pH optimum was 4.5 and there was no activity at pH 7.0 or above. Different PPE-derived products were formed at pH 4.5-5.0 compared to pH 5.5-6.0. In addition, stimulation of activity by DTT resulted in the formation of different enkephalin products. The differential processing of PPE seen in vitro suggest that pH, reducing conditions, and perhaps other factors may regulate the extent of enkephalin precursor processing, which can vary from tissue to tissue. Inhibition by pepstatin and stimulation by DTT show that aspartyl and thiol-dependent proteolytic activity is responsible for enkephalin precursor processing.

80.6

ENKEPHALIN AND TACHYKININ PRECURSORS ARE CLEAVED BY SPECIFIC PROCESSING ENZYME ACTIVITIES IN BOVINE CHROMAFFIN GRANULES. T.J. Krieger*, H.-U. Affolter*+, and V.Y.H. Hook. Dept. of Biochem., Uniformed Services University of the Health Sciences, Bethesda, MD. and +Brain Research Institute, University of Zurich.

Bovine chromaffin granules contain prohormone processing enzyme activities that cleave ^{35}S-(Met)-preproenkephalin (PPE) and ^{35}S-(Met)-preprotachykinin (PPT) into appropriate products. Purification of these activities present in the soluble component of these granules was initiated. Conconavalin-A Sepharose bound both PPE- and PPT-cleaving activities. Two distinct activities were then separated by size exclusion on Sephacryl S200. The first peak cleaves both PPE and PPT. The second peak cleaves PPT, but not PPE under the conditions employed. The PPE-cleaving activity was further purified on thiopropyl Sepharose at pH 5.0. It was completely inactivated during the purification, but was reactivated by treatment with DTT, suggesting that it is a thiol protease. This PPE-cleaving activity exhibits a pH optimum at 6.0 and is strongly inhibited by leupeptin and chymostatin. In contrast, PPT-cleaving activity exhibits a pH optimum of 5.0 and is inhibited by pepstatin indicating that it is an aspartyl protease. These preliminary studies show that at least two distinct proteases with substrate selectivity are involved in chromaffin granule prohormone processing.

80.7

NOVEL SUBSTRATES AND INHIBITORS FOR PEPTIDYLGLYCINE a-AMIDATING MONOOXYGENASE: IMPLICATIONS FOR THE MECHANISM OF ACTION. A.G. Katopodis and S.W. May, School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA 30332.

Peptidylglycine a-amidating monooxygenase (PAM; EC 1.14.17.3) is a copper and ascorbate requiring monooxygenase, responsible for the bio-activation of numerous neuropeptides by catalyzing the cleavage of a C-terminal glycine to produce the active amidated peptide. The mechanism of action of PAM is of great interest, because of the attractiveness of this enzyme as a target for effectors, and because of its similarities to dopamine beta hydroxylase (DBH). We now report that N-benzoyl glycine and several p-substituted derivatives are substrates for purified PAM, producing the corresponding benzamides and stoichiometric amounts of glyoxylic acid in a copper and ascorbate dependent manner. Km for N-benzoyl glycine is 7 mM, and Vmax is about 1/3 of that for the tripeptide trinitrophenyl-D-Tyr-Val-Gly. The oxygen analog 4-methoxy benzoxy acetic acid, however, is not a PAM substrate and is instead a good competitive inhibitor exhibiting a Ki of 0.6 mM. Thus, while 4-methoxy-N-benzoyl glycine is a good PAM substrate, the ester analog cannot be oxidized by this enzyme, suggesting that the amide nitrogen is essential for turnover. S-(4-nitrobenzyl)-2-thioacetic acid is also a PAM substrate producing the corresponding sulfoxide product. In addition a-dideuterio 4-nitro-N-benzoyl glycine exhibits a high deuterium isotope effect on apparent Vmax, which is similar to that reported by others for a tripeptide substrate. Our results therefore suggest, that while alpha C-H bond cleavage contributes substantially to the rate determining step in catalytic turnover, this step is not the initiating step in the mechanism of action of PAM.

80.8

CHARACTERIZATION OF HIGH AFFINITY DIGITONIN-SOLUBILIZED CORTICOTROPIN-RELEASING FACTOR (CRF) RECEPTORS IN RAT CEREBRAL CORTEX. D.E. Grigoriadis, D. Pearsall*, and E.B. De Souza. Neurobiology Lab., NIDA Addiction Research Center, Baltimore, MD 21224.

Corticotropin-releasing factor (CRF) is the primary physiologic regulator of proopiomelanocortin-derived peptides from the pituitary. In addition, CRF has been identified as a neurotransmitter in the central nervous system where it acts to coordinate the endocrine, autonomic and behavioral responses to stress. CRF receptors have been characterized in both brain and pituitary membrane homogenates and found to have identical binding characteristics but different molecular weights. In this study, we have determined the binding characteristics of CRF receptors in rat frontal cerebral cortex membranes solubilized in 1% digitonin. The binding of ^{125}I-Tyr^{0}-oCRF (^{125}I-oCRF) to solubilized membrane proteins was dependent on incubation time, temperature, and protein concentration, was saturable and of high affinity. The solubilized receptors retained their high affinity for ^{125}I-oCRF in the solubilized state exhibiting a dissociation constant (K_D) of 200 pM as determined by direct binding saturation isotherms. Association/dissociation kinetics also revealed high affinity binding of ^{125}I-oCRF with a calculated K_D of 50 pM. Solubilized CRF receptors maintained the rank order of potencies for various related and unrelated CRF peptides characteristic of the membrane CRF receptor: r/h CRF ≥ oCRF ≥ Nle^{21,38} r/hCRF > α-helical(9-41)oCRF > oCRF(7-41) >>> VIP, AVP or substance P antagonist. Interestingly, the absolute potencies (K_i) for the various CRF-related peptides were almost identical to those observed in the membrane preparations suggesting that the CRF receptor retains its high-affinity binding capacity in the digitonin-solubilized state.

80.9

DISULFIRAM, A METAL CHELATOR, LOWERS BRAIN CONCENTRATIONS OF CHOLECYSTOKININ OCTAPEPTIDE (CCK-8). G.P. Mueller, A. Singh* and P.A. Deuster*. Depts. of Physiology and Military Medicine, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814.

Evidence that metals control gene expression and enzyme activities in vitro indicates that these metals probably have essential roles in the synthesis of peptide neurotransmitters in vivo. In this study we sought to determine if concentrations of CCK-8, an abundant neuropeptide in brain, are altered by the administration of disulfiram, a recognized metal chelator. Groups of male rats received daily injections of disulfiram (400 mg/kg, sc) for 1,3,7 or 10 days. Plasma levels of Zn were reduced ($p<0.05$) by 28% and 32% after 3 and 10 days treatment, respectively. Plasma Cu and Mg concentrations were not altered as markedly as Zn. Concentrations of immunoreactive CCK-8 in cerebral cortex, hippocampus and whole brain were significantly reduced ($p<0.05$) by disulfiram with a maximal 44% decrease observed in the hippocampus by 7 days. Gel filtration chromatography revealed that virtually all of the immunoreactivity resembled CCK-8 in molecular size. This suggests that disulfiram preferentially inhibits transcription of the CCK gene and/or translation of CCK mRNA rather than inhibiting the enzymes involved in the posttranslational processing of pro-CCK. This effect of disulfiram may reflect its ability to chelate Zn or other metals involved in the processes of transcription or translation. (Supported by USUHS grant RO 7644 to GPM)

80.10

MICRODIALYSIS OF CCK AND NEUROTENSIN IN THE RAT BRAIN: PRACTICAL CONSIDERATIONS AND PRELIMINARY RESULTS. N.T. Maidment*, D.R. Brumbaugh*, E. Erdelyi*, Y.D. Rudolph*, J.D. Barchas and C.J. Evans. (SPON: K.F.Faull). Pritzker Laboratory, Dept. of Psychiatry, Stanford University School of Medicine, Stanford, CA 94305.

We have previously demonstrated the feasibility of using microdialysis to monitor opioid peptide release in the rat globus pallidus/ventral pallidum (Maidment et al., Soc.Neurosci. Abstr.1988). The present study is directed towards the development of a system for measuring extracellular levels of CCK and neurotensin in the mesocorticolimbic system of the rat. The recovery of radiolabelled forms of these peptides across several types of dialysis membranes was assessed in solution. Polyacrylonitrile membranes (4mm) exhibited recoveries of approx. 8% and 5% for CCK-8 and neurotensin respectively at a flow rate of 2.7μl/min. A novel and highly sensitive radioimmunoassay was developed which enables the serial measurement of sub-fmol quantities of CCK-8 and neurotensin in the same sample. The assay takes place on solid phase with antibody bound via protein G to 96-well plates. This results in negligible non-specific binding such that at the end of the CCK-8 assay the samples can be transferred to a second plate for assay of neurotensin. Preliminary in vivo experiments in the medial nucleus accumbens suggest that basal extracellular concentrations of these peptides are at the limit of detection of the assay and that these levels are elevated by incorporation of high potassium in the perfusion medium.

80.11

POSTTRANSLATIONAL PROCESSING PRODUCTS OF DBI IN HUMAN BRAIN. P. Guarneri*, M.L. Barbaccia[1], E. Costa and A. Guidotti. FGIN, Georgetown Univ., Wash., DC 20007, [1]Dep. Exp. Med., 2nd Univ. Rome Med. School, Italy.

DBI (diazepam binding inhibitor) is an endogenous polypeptide present in rat and human brain. In rat brain the polypeptide (86 residues) is posttranslationally processed into at least two fragments biologically active at different $GABA_A$ receptor subtypes (DBI 17-50 and DBI 32-50). In human brain, tryptic digestion of DBI (104 residues) yields DBI 51-70 (ENP) having high homolgy with rat DBI 32-50 (ODN). We monitored the emergence from reverse phase HPLC of DBI posttranslational processing products in post-mortem human brain extracts with an antibody raised in rabbits against synthetic ENP. Six peaks of ENP-like immunoreactivity were identified; one of them corresponded to ENP itself suggesting that like rat ODN, ENP might naturally exist in human brain and might possess a similar biological activity. PAGE analysis of the other ENP-like immunoreactivities revealed that one of them has a molecular weight of approximately 8kDa and the others appear to have a similar size (3-4kDa). All these ENP-like immunoreactivites were observed in CSF suggesting that they are natural processing products of DBI. Similar to ENP, at least three of these peaks could be obtained by in vitro tryptic digestion of DBI. By using antibodies directed against the synthetic peptides DBI 37-50 and DBI 81-100, we suggest that the 8kDa fragment may include DBI 37-100, while the other 3-4kDa peptides might include DBI 37-80. These inferences are being verified by amino acid sequencing.

80.12

BRADYKININ-EVOKED RELEASE OF CALCITONIN GENE-RELATED PEPTIDE (CGRP) AND SUBSTANCE P (SP) FROM CULTURED VAGAL SENSORY NEURONS: INHIBITION BY INDOMETHACIN. D.B. MacLean and L. Hayes*. Endocrinology Div. Brown Univ. Rhode Island Hospital, Providence, RI 02903.

In this study, the effect of bradykinin (BK) on neuropeptide release from cultured vagal sensory neurons was further characterized. Neonatal rat vagal sensory ganglia were enzymatically dispersed and maintained in L-15, 10% FBS and NGF. Release studies were performed in KRB, 1% FBS and 5mM captopril between 10-20d in culture. Peptides were measured by RIA. 35mM K+ and 1 uM capsaicin evoked SP and CGRP release 6-8 times basal release, i.e. 8-10% of total peptide content during 15-20 min epochs. BK, maximum effect at .01uM, evoked release 2.5-4x basal, similar to phorbol ester (3-5x basal) and forskolin (2-3x). Cobalt, 3.5 mM, blocked K+ and capsaicin-evoked release, whereas the response to BK was not reduced. Pertussis toxin, lug/ml for 16h, had no effect on basal or BK-stimulated CGRP release but increased SP release by 40-50% ($P<.01$). Prior (20 min) and concurrent exposure to indomethacin, 10uM, did not affect basal release but abrogated BK-evoked release of both peptides by 70-80%. In preliminary studies, indomethacin did not block CGRP release (2x basal) by prostaglandin E1. Conclusion: BK evokes submaximal release of CGRP and SP from cultured vagal neurons by pathways independent of extracelluar Ca^{++} flux and may be mediated by prostaglandins.

81.1

LOCALIZATION OF ENDOTHELIN-1 MRNA AND IMMUNOREACTIVITY IN NEURONS OF BRAIN, SPINAL CORD AND DORSAL ROOT GANGLIA IN MAN

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Endothelin is a 21 amino acid peptide recently isolated from the supernatant of porcine aortic endothelial cell cultures. The localization of endothelin-1 mRNA and endothelin-like immunoreactivity was investigated in ten cases of neurologically normal human nervous system (4-7 h post-mortem) using in situ hybridization and immunocytochemistry. Following hybridization with a ^{35}S-complementary (c) RNA human endothelin-1 probe, positively labeled neurons were present in the spinal cord, in laminae IV-VI, thoracic intermediolateral cell columns, with considerably denser labeling in laminae IX, where many motoneurons expressed endothelin transcripts. Sensory neurons of both large and small size were labeled after hybridization in the dorsal root ganglia. In the brain, labeled neurons were seen in laminae III-VI of the frontal and parietal cortices, the hypothalamus and in the Purkinje cell layer of the cerebellum. Antisera raised to endothelin revealed a similar distribution of endothelin-immunoreactive neuronal cell bodies, but fewer positively stained cells were seen when compared with the hybridization. In the motoneurons and dorsal root ganglia, endothelin and CGRP mRNAs or their respective immunoreactivities were often colocalized. In addition a subset of dorsal root ganglion cells coexpressed endothelin and ß-preprotachykinin mRNAs or endothelin and substance P immunoreactivities. The data provide evidence for the presence of endothelin-1 in neurons and suggests that this peptide, in addition to its known vascular-related actions, may play a part in neural transmission/modulation.

81.2

ENDOTHELIN: IN SITU HYBRIDIZATION AND BINDING STUDIES IN THE BRAIN AND PERIPHERY. M. W. MacCumber, C. A. Ross and S. H. Snyder. Neuroscience and Ophthalmology, Johns Hopkins Sch. of Med., Baltimore, MD 21205

Endothelin (ET) is a recently discovered potent vasomodulatory peptide which is synthesized by cultured vascular endothelial cells (Yanagisawa, et al. Nature 332:411, 1988). It has binding sites in the rat brain which are densest in the cerebellum (Jones, et al. Neurosci. Lett. 97:276,1989). We have examined ET synthesis by in situ hybridization and binding sites for ^{125}I-porcine ET in membrane homogenates and tissue sections. In situ experiments reveal ET message that is highest in vessels in densely vascularized organs (e.g. the lung, kidney, intestine and eye). Preliminary experiments in the brain suggest lower message levels, highest in the cerebellar granule cells and less in the thalamus and other brain regions.

The measured K_D in binding experiments is approximately 1 nM in cortex, cerebellum and heart homogenates. Binding is markedly enhanced by monovalent and divalent cations at physiologic concentrations and by low pH (optimum near 6.0). In tissue sections, the distribution of binding sites resembles that of mRNA message with the highest levels in densely vascularized tissues.

These studies suggest a vasomodulatory role for ET throughout the body and a possible neuromodulatory role in the brain.

81.3

GALANIN-LIKE IMMUNOREACTIVITY IS LOCALIZED TO THE CORTICOTROPHS OF THE HUMAN PITUITARY. D.W. Hsu*, M. El-Azouzi*, E.T. Hedley-Whyte, P.McL. Black, & L.M. Kaplan. Massachusetts General Hospital, Brigham & Women's Hospital, and Harvard Medical School, Boston, MA 02114.

Galanin is a neuropeptide which regulates the secretion of several pituitary hormones, including prolactin, GH, TSH, and ACTH. We have previously demonstrated that galanin-like immunoreactivity (Gal-IR) and galanin mRNA in the rat anterior pituitary is located primarily in GH- and prolactin-secreting cells. We examined the cellular distribution of Gal-IR in normal postmortem human pituitaries and pituitary tumors. Paraffin sections were subjected to immunoperoxidase staining using antisera directed against porcine galanin which has been shown to bind human galanin-like peptides specifically. In normal pituitaries, Gal-IR was present in scattered cells throughout the gland. Co-localization studies using antisera directed against GH, prolactin, LH, TSH, and ACTH revealed that Gal-IR is present predominantly in corticotrophs. There was no appreciable staining of other identifiable cell types. Gal-IR was also present in hyperplastic and neoplastic corticotrophs of 5/10 patients with Cushing's disease. Neoplastic pituitary cells exhibited less stringent cell-type specificity: Gal-IR was present in 4/7 GH-secreting tumors and 3/11 nonfunctioning pituitary gonadotrophic tumors. In contrast, we did not detect Gal-IR in any of 5 prolactinomas examined. These results demonstrate that the cellular distribution of Gal-IR in the pituitary is species-dependent. Its restriction to corticotrophs suggests that *human* galanin may participate in the regulation of the hypothalamic-pituitary-adrenal axis. The altered cellular distribution in pituitary adenomas suggests that galanin gene expression may also be a useful marker of pituitary cell dedifferentiation.

81.4

DISTRIBUTION OF GALANIN-LIKE IMMUNOREACTIVITY AND ^{125}I-GALANIN BINDING SITES IN THE CAT SPINAL CORD. B.Ulfhake[1*], T.Hökfelt[2], U.Arvidsson[1*], S.Cullheim[1*] and E.Theodorson[3*]. Departments of 1) Anatomy,2) Histology and Neurobiology and 3) Clinical Chemistry, Karolinska Institutet, S-104 01 Stockholm, Sweden.

The distribution of galanin(GAL)-like immunoreactivity (LI) in the lumbo-sacral spinal cord was studied by use of indirect immunofluorescence technique in normal cats and in cats subjected to chronic spinal cord transection (SCT) alone, or in combination with unilateral dorsal root (DR) rhizotomy below the SCT. GAL-LI axons in the ventral horn (VH) were most frequent in the ventral part of lamina IX. Double-labeling showed that GAL-LI coexisted with serotonin-LI in the axons of lamina IX. Following SCT, all GAL-immunoreactive(IR) fibres in lamina IX disappeared. Together, these two findings implicate that the GAL-IR fibres of lamina IX belong to the serotonergic bulbospinal pathway. In the superficial laminae of the dorsal horn (DH) a dense GAL-IR axonal plexus was present, which did not show any serotonin-LI. Furthermore, SCT did not alter the pattern of GAL-LI within this plexus. Here the vast majority of GAL-IR axonal swellings, however, disappeared following dorsal root sectioning. Small-to-medium sized GAL-IR cell bodies were found in laminae II-III, X as well as in the dorsal root ganglia (DRG). In the DRG, GAL-IR cell bodies in many cases also expressed CGRP-LI. The fine structure of the GAL-IR axonal boutons is described as revealed by the peroxidase-antiperoxidase technique.

Radioimmunoassay analysis revealed that the content of GAL-LI was about 3 times higher in the DH than in the VH, while the DRGs showed values between these. The lumbar and, in particular, the sacral spinal cord had a higher content of GAL than the cervical and the thoracic spinal cord segments investigated. A high density of ^{125}I-GAL binding sites was present in the superficial laminae of the DH.

In conclusion, the GAL-IR axons of spinal cord lamina IX is of supraspinal origin, probably exclusively belonging to the serotonergic bulbospinal pathway. GAL-IR axons in the DH is mainly of primary afferent origin, while a minor portion derives from supraspinal and segmental sources. There was a fairly good concordance between location of GAL-IR axons, tissue content of GAL and distribution of ^{125}I-GAL binding sites.

81.5

NEUROPEPTIDE Y AND LHRH IN THE RAT HYPOTHALAMUS: ANATOMICAL RELATIONSHIPS. J. Calka and J.K. McDonald, Dept. of Anatomy & Cell Biology, Emory University School of Medicine, Atlanta, GA 30322

Neuropeptide Y (NPY) affects LH and LHRH secretion *in vivo* and *in vitro* (for a review see McDonald, 1988: *CRC Crit. Rev. Neurobiol.* 4:97-135). We have investigated relationships between NPY and LHRH immunoreactive neurons in the rat hypothalamus to explore the anatomical basis for the effects of NPY on LHRH secretion. Ovariectomized rats were provided with Silastic capsules containing estrogen (235 µg/ml) or vehicle (Sabatino et al., 1989: *Endo.* 124:2089-98). Three days later 100 µg of colchicine was injected into the lateral ventricle. Rats were perfused with fixative the next day and their brains sectioned coronally at 40 µm. Serial sections were incubated in NPY or LHRH antisera and processed using conventional immunohistochemical techniques. Sections from other animals were treated sequentially with LHRH and NPY antisera to visualize both peptides in the same section. The results show extensive overlap of NPY and LHRH fibers in the ventral retrochiasmatic area and in the subependymal, internal zone and lateral aspect of the median eminence. NPY fibers were concentrated in close proximity to LHRH perikarya and dendrites in the medial preoptic area and the horizontal limb of the diagonal band. NPY containing fibers may influence LHRH secretion via effects on LHRH fibers and perikarya. Supported by HD-19731, HD-00727, March of Dimes 5-524 and the Fulbright Foundation.

81.6

PROJECTIONS OF PEPTIDE-CONTAINING NEURONS INTO THE MEDIAL PREOPTIC AREA OF THE SYRIAN HAMSTER. C. R. Neal, Jr. and S. W. Newman. Department of Anatomy and Cell Biology. University of Michigan Medical School. Ann Arbor, Michigan. 48109

Fibers and terminals containing dynorphin B (rimorphin and leumorphin), adrenocorticotropin (ACTH), beta-endorphin (ßE) and substance P (SP) have been reported in the medial preoptic area (MPOA) of the rat and hamster. The dynorphins, SP and ßE have been shown to affect male sexual behavior in these species, and lesions in the caudal MPOA completely disrupt this behavior. To identify the cells of origin of these fiber systems, we placed Fluoro-gold (iontophoresis) or rhodamine-impregnated latex microspheres (pressure injection) into the caudal MPOA seven days prior to sacrifice. Two days prior to sacrifice, colchicine was injected into the lateral ventricle. The animals were perfused and their brains processed for fluorescence immunohistochemistry. Numerous retrogradely labelled cell bodies were observed in the ipsilateral lateral septum, medial bed nucleus of the stria terminalis (BNSTm), midline thalamic nuclei, several hypothalamic nuclei, the peripeduncular nucleus, medial nucleus of the amygdala (AMe) and amygdalo-hippocampal area. A small, ventral sub-population of ßE- and ACTH-containing neurons in the arcuate nucleus accumulated retrogradely transported tracer. In addition, cells containing both tracer and C-terminus leumorphin immunofluorescent labelling were observed in the preoptic part of the BNSTm and in the ventral part of posterodorsal AMe. However, no SP-immunolabelling was observed in retrogradely labelled cells in BNSTm or AMe. Supported by NIH NS20629 to SWN and NIGMS GM10341-04 to CRNJ.

81.7

HISTOCHEMICAL LOCALIZATION OF NEUROKININ B IMMUNOREACTIVITY IN RAT BRAINSTEM. R.E. Harlan, L.R. Lucas* and J.E. Krause. Dept. Anatomy and Neuroscience Training Program, Tulane Univ. Sch. Med., New Orleans, LA 70122 and Dept. Anatomy and Neurobiology, Washington Univ. Sch. Med., St. Louis, MO 63110.

Two genes encode tachykinins in mammals: preprotachykinin (PPT) A, encoding substance P (SP) and neurokinin A (NKA), and PPT B, encoding neurokinin B (NKB). All three peptides derived from these two genes have identical C-terminal sequences. Thus, immunocytochemical differentiation among the peptides has been difficult. We have utilized an antiserum apparently specific for NKB for immunocytochemical localization of NKB immunoreactive fibers in the brainstem of the female rat. The antiserum, raised in a rabbit against conjugated cys^{10}-NKB, does not cross react with SP or NKA in a competitive binding assay. In the brainstem of animals perfused with Zamboni's fixative, prominent immunostained fibers were seen with antibody dilutions ranging from 1:1000-1:10,000. Immunoreactivity was completely abolished by preabsorption of the antiserum with 10uM NKB, but not with 10uM SP or NKA. Bundles of immunoreactive fibers were found in the spinal trigeminal tract and coursing into and through the nucleus of the spinal trigeminal tract. Extremely dense immunoreactivity was seen in the lateral portions of the interpeduncular nucleus. Scattered immunoreactive fibers were found in many other regions of the brainstem.

NEUROENDOCRINE REGULATION I

82.1

PROENKEPHALIN MESSENGER RNA IS EXPRESSED IN RAT PITUICYTES. M.K.-H. Schafer*, R. Day, H. Akil and S.J. Watson. Mental Health Research Inst., University of Michigan, Ann Arbor, MI 48109-0720.

The existence of opioid peptides in the posterior pituitary is well-known. Their synthesis occurs in hypothalamic magnocellular neurons and in smaller neurons projecting to the posterior pituitary. To date there has been no evidence to suggest synthesis of opioid peptides in the neural lobe itself. To determine potential sites for opioid peptide synthesis in the neurointermediate lobe, the presence of proenkephalin (proENK) and prodynorphin (proDYN) mRNA was examined using *in situ* hybridization histochemistry with [^{35}S] labeled cRNA probes on 10 µm frozen pituitary sections of untreated rats. Cells containing proENK mRNA were found exclusively in the posterior pituitary, but not in the intermediate lobe. No proDYN mRNA could be detected in the neural lobe. The size of proENK mRNA was determined by Northern blot analysis of neurointermediate lobe tissue extracts and compared to the proENK mRNA of rat striatum. Both bands showed the same size of ca. 1.5 kb. Further characterization with a cDNA complementary to glial fibrillary acidic protein (GFAP), a marker for astroglial cells, and an antibody raised against GFAP revealed that the proENK mRNA containing cells are pituicytes, a special class of astroglial cells in the neural lobe. Using antibodies specific for proENK fragments we were not able to detect enkephalin-like immunoreactive material in pituicytes, only in nerve terminals. From these studies we conclude that pituicytes in the posterior pituitary of untreated adult rats express proENK mRNA *in situ* and could be a potential source for proENK derived peptides in this tissue. The regulation of proenkephalin mRNA during different physiological stages of pituicytes and its functional significance is currently being investigated. [cDNA clones were provided by S. Sabol (ENK) and J. Douglass (DYN). Supported by DA02265, MH422251, Theophile Raphael Fund; R.D. is a fellow of the Medical Research Council (MRC) of Canada].

82.2

TESTOSTERONE REGULATES POMC mRNA LEVELS IN A REGIONALLY SPECIFIC MANNER IN THE ARCUATE NUCLEUS OF THE ADULT MALE MACAQUE. L.A. Adams*, L. Vician*, D.K. Clifton*, R.A. Steiner. Depts of Ob/Gyn, and PBIO, Univ. of Washington, Seattle, WA 98195.

Endogenous opioid peptides (EOP) are involved in the steroid-mediated negative feedback control of gonadotropin secretion, and steroid hormones have been shown to influence EOP synthesis and activity. In the primate brain, neurons containing POMC (the precursor for the EOP ß-endorphin) are located in the arcuate nucleus; given the diversity of POMC projections and the variety of physiological actions ascribed to POMC products, these neurons are likely to be a functionally heterogeneous population. We tested whether testosterone (T) influences POMC gene expression in a regionally specific manner in the male primate brain. Using *in situ* hybridization we compared POMC mRNA levels in the arcuate nucleus among intact (n=3), castrated (n=4), and castrated, T-treated (n=4) adult male macaques, *Macaca fascicularis*. Frontal sections (20µm) through the arcuate nucleus were processed for *in situ* hybridization with an ^{35}S-labeled ribonucleotide probe, and the autoradiographic signal (grains/cell) was quantified with a computerized image analysis system. Castration resulted in a small (12%), but statistically significant, decrease in grains/cell when the arcuate nucleus as a whole was considered, and T treatment reversed this effect ($p<0.05$). Analyzing the anterior and posterior halves of the nucleus independently, we saw that the castration-induced decrease in grains/cell was greater in the anterior half of the nucleus (32%; $p<0.03$), whereas the decrease in the posterior half was not statistically significant. T treatment reversed the effects of castration in both halves of the nucleus. Thus, castration decreases, and T treatment restores to intact values, cellular POMC mRNA levels in the arcuate nucleus of the male primate, and this effect of T on POMC mRNA levels is most pronounced in the anterior aspect of the nucleus. The augmentation of POMC gene expression by T provides a mechanism for increasing EOP activity, which in turn subserves the steroid-mediated negative feedback control of gonadotropin secretion.

82.3

NEUROPEPTIDE Y MODULATION OF GONADOTROPIN-RELEASING HORMONE INDUCED CALCIUM ELEVATIONS IN SINGLE IDENTIFIED GONADOTROPES. Gary A. Shangold*, Everett Y. Lee* and Richard J. Miller (SPON: P.R. Huttenlocher). Depts. of Pharm./Phys. and Ob./Gyn., Univ. of Chicago, Chicago, IL 60637.

Neuropeptide Y (NPY) has been shown to inhibit gonadotropin-releasing hormone (GnRH)-induced luteinizing hormone (LH) release *in vivo*. In order to determine whether these effects on gonadotropin secretion are achieved by a direct action on gonadotropes, we studied $[Ca^{++}]_i$ signals in single gonadotropes from 60-day old female rats, plated on etched-grid coverslips, identified via a reverse hemolytic plaque assay for LH, and loaded with the Ca^{++}-sensitive fluorescent indicator Fura-2, with a microspectrofluorimeter. Perifusion of cells with GnRH (1 nM) elicited an initial brisk rise in $[Ca^{++}]_i$, which then returned toward basal levels (~100-150 nM), only to rise again to a lower and more sustained secondary plateau phase. The first peak of $[Ca^{++}]_i$ has been previously shown to be independent of extracellular Ca^{++}, and thus appears to reflect mobilization of Ca^{++} from intracellular stores. The secondary plateau could be prevented by either removal of extracellular Ca^{++} or by L-calcium channel blockade with nitrendipine, and is thus felt to represent influx of extracellular Ca^{++} via voltage-sensitive Ca^{++} channels. Perifusion of cells with NPY (100 nM or 1 µM) during the second phase of the GnRH-induced elevation in $[Ca^{++}]_i$ brought about suppression of this secondary plateau in $[Ca^{++}]_i$. However, no apparent effect on the first (intracellularly-generated) part of the GnRH-induced $[Ca^{++}]_i$ signal could be so demonstrated. Furthermore, NPY did not block $[Ca^{++}]_i$ transients evoked by depolarization with 50 mM K^+. Thus, NPY inhibits Ca^{++} influx into gonadotropes through L-type Ca^{++} channels, but an indirect mechanism appears to be involved.

82.4

***IN VIVO* RELEASE OF NEUROPEPTIDE-Y IN THE STALK-MEDIAN EMINENCE OF CASTRATED RHESUS MONKEYS IS PULSATILE.** M.J. Woller*, J.K. McDonald†, E. Terasawa. Wis. Reg. Primate Res. Ctr., Univ. Wisconsin, Madison, WI 53715 & †Dept. Anat. & Cell Biol., Emory Univ., Atlanta, GA 30322.

It has been shown in rodents that neuropeptide-Y (NPY) modulates LH release, and presumably LHRH release. However, whether NPY has any role in control of LHRH release in primates is unknown. As a first step in investigating this, we have examined *in vivo* release of NPY in conscious rhesus monkeys using a previously described push-pull perfusion method . Long-term castrate male (n=3) and female (n=3) monkeys were used. A modified Krebs-Ringer phosphate buffer solution was perfused through the stalk-median eminence (S-ME) at a rate of 20 µl/min and 10 min fractions were collected for 12-15 h. NPY concentration in perfusates was measured in duplicate by RIA. Results: 1) NPY release in the S-ME of both male and female monkeys was pulsatile. 2) NPY pulses detected using strict criteria with the PULSAR algorithm indicated that NPY pulses occur at 40-62 min (48.1 ± 3.5 min) intervals. 3) Basal NPY release (trough value) ranged from 40-160 pg/ml (103.6 ± 20.9 pg/ml), and NPY pulse amplitude (difference between peak and trough) ranged from 50-125 pg/ml (90.2 ± 14.8 pg/ml). These results suggest that NPY release in the S-ME is pulsatile and that the frequency of NPY release is similar to that reported for LHRH release. Whether any correlation between NPY and LHRH release exists remains to be determined. (Supported by NIH Grants HD15433 & HD11355).

82.5

BRAIN STEM PROJECTIONS ARE REQUIRED FOR THE TESTOSTERONE (T)-INDUCED AUGMENTATION OF MEDIAN EMINENCE NEUROPEPTIDE Y (NPY). A. Sahu, P.S. Kalra, W.R. Crowley[1] and S.P. Kalra, Dept. OB-Gyn, Univ. Fla. Col. Med., Gainesville, FL and [1]Dept. Pharmacol. Univ. TN Col. Med., Memphis, TN.

Various hypothalamic nuclei are innervated by two populations of NPY producing cells - one resident in the arcuate nucleus (ARC) and the other in the brain stem, the latter also display coexistence with adrenergic transmitters. We observed that two weeks after castration, hypothalamic NPY release in vitro in response to KCl decreased and, of the 6 microdissected sites in the hypothalamus, NPY levels were reduced selectively in the median eminence (ME), ARC and ventromedial hypothalamus (VMH). T replacement in the physiological range to castrated rats, restored the NPY release response as well as levels in these three sites, implying a crucial participation of steroid concentrating cells in the brain stem and hypothalamus. Therefore, brain stem projections to the hypothalamus were severed in the next experiment with a 2 mm wide knife lowered on either side of the sagittal sinus to the depth of the dorsal tegmentum in the mesencephalon. In these neurally transected (NT) gonad-intact rats, NPY levels decreased significantly in the ME and not in the ARC or VMH. When castration was performed simultaneously with NT, NPY levels fell in all three nuclei. Replacement of physiological T prevented the decline in NPY levels in the VMH and ARC regardless of whether rats previously underwent NT or not. In contrast, T replacement in the NT group was ineffective as it failed to prevent the castration-induced decrease in the ME NPY levels. These studies show that T facilitates NPY neurosecretion selectively in three hypothalamic sites, but the site of T action is different. To augment NPY levels in the ME, T may require an intact brain stem-hypothalamic link, whereas in the VMH and ARC, the site of T action may be resident locally in the hypothalamus. (Supported by NIH HD 11362 & HD 08634).

82.6

THYROID HORMONE HAS A PERMISSIVE EFFECT IN THE REGULATION OF GALANIN GENE EXPRESSION IN THE RAT ANTERIOR PITUITARY. S.C. Hooi*, J.I. Koenig, J.B. Martin, L.M. Kaplan (SPON: N.T. Zervas). Gastrointestinal Unit and Dept. of Neurology, Massachusetts General Hospital, Boston, MA 02114.

Galanin is a neuropeptide which regulates the secretion of several pituitary hormones, including GH, prolactin, TSH, and ACTH. We have previously shown that galanin gene expression and peptide concentrations in the rat anterior pituitary (AP) are dramatically stimulated by high circulating estrogen levels. In addition, thyroid hormones are required for the maintenance of normal concentrations of galanin-like immunoreactivity in the AP. We used Northern blot analysis to examine the effects of thyroid hormone depletion and replacement on basal and estrogen-stimulated galanin gene expression in normal male rats. Treatment for 4 weeks with propylthiouracil (PTU; a single i.p. injection of 10 mg/kg followed by addition of 0.05% in drinking water) led to a 6-fold reduction in galanin mRNA levels in the AP. Concurrent administration of thyroxine (50 μg/kg/day s.c.) fully reversed this effect. Thyroxine treatment of normal animals had no effect. Pretreatment with PTU for 5 weeks also decreased galanin gene expression in the AP of animals exposed to high doses of 17β-estradiol benzoate (100 μg/kg/day s.c.) for 2 days. Galanin mRNA levels in the AP of these animals was reduced 3-fold compared with animals treated with estrogen alone. Thyroxine administration during the final 2 weeks of PTU treatment partially reversed (to 56% of control levels) the effect of PTU. These observations suggest that thyroid hormones are necessary for normal pituitary expression of the rat galanin gene and that they exert a permissive effect on the estrogen-induced stimulation of galanin gene expression.

82.7

EXPRESSION OF C-FOS IN MAGNOCELLULAR HYPOTHALAMIC NEURONS: SPECIFICITY FOR OXYTOCIN AND VASOPRESSIN. G.E. Hoffman, M. M.Roberts*, J. G. Verbalis, F. Grant*, A.G. Robinson. Dept. Physiology and Div. Endocrinology, Dept. of Medicine, Univ. Pittsburgh, Sch. of Med., Pittsburgh, PA 15261.

Upon activation, the hypothalamic magnocellular neurons express c-fos, an oncogene product implicated in gene regulation. In these experiments, we related changes in c-fos immunoreactivity in oxytocin (oxy) and vasopressin (vp) neurons to plasma levels of these hormones after stimulation under several different conditions. Magnocellular neurons expressing c-fos were identified with standard double label immunocytochemistry (ICC) by first staining with a c-fos specific antibody, followed by staining with antibodies generated specifically against the 14 amino acids of the c-terminus of oxytocin or vasopressin neurophysin (NP). The VPNP and OXYNP antibodies showed no crossreactivity with each other either in RIA or with ICC. The results are shown below.

	plasma levels vs. baseline		c-fos staining	
Treatment	oxy	vp	oxy	vp
H2O deprivation, (24 hr.)	2-3x	5-7x	+/-	++
NaCl, 9.0M (4.0cc, i.p.)	>50x	>50x	++++	++++
CCK (100μg/kg, i.p.)	8-10x	1x	++++	-

These results demonstrate a significant relationship between c-fos immunoreactivity and stimulus-induced neurohypophyseal secretion. C-fos staining accurately reflects the relative magnitude of the secretory response as well as the specificity of the peptide secreted. Consequently, c-fos expression can be used to study functional anatomy of the hypothalamus in response to specific physiological and pharmacological stimuli.

82.8

CHOLECYSTOKININ ACTIVATES C-FOS EXPRESSION IN PARAVENTRICULAR OXYTOCIN AND CORTICOTROPIN RELEASING HORMONE NEURONS. J.G. Verbalis, G.E. Hoffman, E.M. Stricker and A.G. Robinson. Depts. of Medicine, Physiology and Behavioral Neuroscience, University of Pittsburgh, Pittsburgh, PA 15261

Previous studies from our laboratories have shown that in rats cholecystokinin (CCK) causes pituitary secretion of oxytocin (OT), but not vasopressin (AVP), by stimulating hypothalamic magnocellular neurons. However, because OT secretion is also correlated with inhibition of food intake and gastric motility produced by CCK, we have hypothesized that centrally-projecting parvocellular OT neurons may be activated in concert with the pituitary-projecting magnocellular neurons to stimulate these responses. To evaluate which types of neurons in the paraventricular nucleus (PVN) are activated by CCK in rats, we utilized immunohistochemical techniques to identify cells activated to express c-fos antigen 1h after treatment with CCK-8 (100 μg/kg ip). Neurons expressing nuclear c-fos were then further characterized by dual-labelling using specific antisera to OT and AVP neurophysins and corticotropin releasing hormone (CRH, courtesy A. Silverman). C-fos expression was easily detected in magnocellular OT but not AVP neurons, and also in OT neurons located in the lateral parvocellular subdivision of the PVN known to project to the brainstem. Another group of non-OT neurons in the medial parvocellular subdivision known to project to the median eminence was found to contain CRH. These results *demonstrate that CCK stimulates selective groups of neurons in both* the magnocellular and parvocellular subdivisions of the PVN, and suggests a potential mechanism whereby centrally-projecting OT and CRH neurons might act to coordinate the autonomic, behavioral and neuroendocrine effects produced by CCK.

82.9

Blockade of Pulsatile LH Secretion in Ovariectomized Rats by an Alpha1-Adrenergic Receptor Antagonist. J. E. Levine, S. Sun*, and L. Conaghan*. Department of Neurobiology & Physiology, Northwestern University, Evanston, IL 60208.

These experiments were conducted to determine if alpha1-adrenergic receptor activation is required for generation of luteinizing hormone (LH) pulses in ovariectomized (OVX) rats. Female rats were fitted with atrial catheters on day 7 following OVX, and blood sampling sessions were begun at 1200h on the next day. Samples were obtained in each animal at 5min intervals for 4-5h. After 2h of sampling, rats received injections of saline vehicle or the alphal-antagonist, prazosin, at 0.5 *mg/kg* or 1.0 *mg/kg, i.p.. Plasma LH levels were* determined by RIA. Secretion of LH in all animals prior to injections was pulsatile with interpulse interval = 25.9+ 3.0min (mean + SE) and pulse amplitude = 2.23+ .26 ng/ml. While control injections were without effect, administration of prazosin at both doses either abolished pulsatile LH secretion for the remainder of sessions or completely blocked LH pulses for a period of 1/2-2h. Virtually no LH pulses of reduced amplitude were observed throughout the onset, duration, or recovery from drug effects. We conclude that the patency of at least one population of noradrenergic synapses is required for the normal operation of the LHRH pulse generator. Noradrenergic transmission via alpha1 receptors may either regulate the frequency of the LHRH pulse generator or function as a component of the pulse generator itself. Supported by NIH HD20677.

82.10

LHRH mRNA: NEURONAL DISTRIBUTION AND STEROID HORMONE REGULATION. K.F. Malik, A.-J. Silverman, and J.I. Morrell. Institute of Animal Behavior, Rutgers University, Newark, NJ 07102

We are able to identify cells producing LHRH mRNA in rat brain with in situ hybridization cytochemistry using previously described methodology (Zoeller et al., Endocrinol., 122:2570, 1988) and a 51 base cDNA probe 3'-end labeled with ^{35}S-dATP. The distribution of labeled cells is limited to loci previously demonstrated to contain LHRH immunoreactive neurons. The majority of cells identified to contain LHRH mRNA were found in the medial septum, vertical and horizontal limbs of the diagonal bands of broca, and rostral portions of the preoptic area. In these areas we have counted as many as 96 very robustly labeled cells per 10 micron section. No cells in the MBH have been identified to contain LHRH mRNA.

Preliminary results from these experiments suggest that the number of labeled cells is stable between 7d OVX rats and 7d OVX rats that receive 10 micrograms EB and are sacrificed 40h later. We are also currently performing in situ hybridization studies investigating the regulation of LHRH mRNA over the rat estrous cycle. (Supported by HD 22983 to JIM, HD 10665 to A-JS, and Sigma Xi, Grant-in-Aid of Research to KFM.)

82.11

COLOCALIZATION OF LUTEINIZING HORMONE-RELEASING HORMONE IN NEURONS CONTAINING PROGESTIN RECEPTORS IN GUINEA PIG BRAIN. J.C.King, J.D. Blaustein and G. R. Seiler*. Dept. of Anatomy and Cell Biology, Tufts University Health Sciences Campus, Boston, MA 02111 and Neuroscience and Behavior Program, Univ. Mass., Amherst, MA 01003.

Secretion of hypothalamic LHRH, central to successful reproduction, is regulated by multiple modulators, including gonadal steroids. It has been suggested that neurons which synthesize LHRH in female rats do not concentrate estradiol. We have reexamined this issue using double-label immunocytochemistry to simultaneously localize progestin receptors [PR] and LHRH, within Vibratome acrolein-fixed sections through the preoptic area/ hypothalamus. Ovariectomized adult guinea pigs received estradiol-replacement to maximize antigen detection. A subpopulation of LHRH immunopositive [+] neurons displayed PR+ nuclei. These double labelled neurons were confined to regions that were rich in both LHRH+ and PR+ neurons, specifically in the midline nuclei of the preoptic area. No double labelled neurons were detected in the basal preoptic area overlying the optic chiasm or in the more caudal anterior hypothalamic area. PR+ reaction product is only detected within estrogen receptor+ neurons and is enhanced in the preoptic/hypothalamus by estradiol treatment (Blaustein and Turcott, in press). Thus, the PR+ neurons in this study also represent ER+ neurons. Although the number of LHRH-PR double labelled neurons appears to be small [<10% of the population], these neurons may play a critical role in mobilizing the entire population of LHRH neurons. We hypothesize that the influence of this subpopulation is mediated by interconnections within the microarchitecture of the total population of LHRH neurons. Finally, these data are consistent with the hypothesis that a sub-population of LHRH neurons does contain gonadal steroid receptors. NIH HD19803, NS19327, RCDA NS00970.

82.12

CHARACTERIZATION OF A GONADOTROPIN-RELEASING HORMONE (GnRH) CELL LINE OBTAINED FROM A TUMOR IN TRANSGENIC MICE. R.I. Weiner, P.C. Goldsmith, J.J. Windle[1]*, C.A. Padula*, J.L. Roberts[2] and P.L. Mellon[1]*. Reproductive Endocrinology Center, Univ. Calif., San Franicsco, CA 94143, [1]Salk Institute, La Jolla, CA 92037, and [2]Mt. Sinai, Med. Ctr., New York, NY 10029.

Expression of the oncogene SV40 T antigen (TAG) was directed to GnRH neurons of transgenic mice using the GnRH promoter. A tumor in the anterior preoptic area was obtained which expressed GnRH and TAG mRNAs. The tumor was dispersed and cultured. Over a six month period a cell line (GT-1) was established and cloned by limiting dilution. We have characterized the GT-1 cells at the light and electron microscopic level using PAP immunostaining. GT-1 cells produced and released radioimmunoassayable GnRH. Cultures contained flattened cells adhering to the surface of the dish as well as rounded cells layered above. The attached cells have a neuronal appearance and immunostain for GnRH and neuron specific enolase, but not for glial fibrillary acidic protein. GnRH staining was observed within the cytoplasm of the cell bodies as well as in processes. Characteristic protein producing neurons were seen with well-formed secretory organelles. GnRH was localized in dense granules. At sites of contact there was evidence for cell to cell communication. Rounded cells containing mitotic figures were intensely stained for GnRH. These findings demonstrate for the first time the feasibility of producing a neural cell line by expression of TAG under the control of a promoter for a gene normally expressed tissue specifically in terminally differentiated neurons. Supported by NIH grants HD20377 (P.M.), HD08924 (R.W.) and the Keck Foundation (R.W.).

PRESYNAPTIC MECHANISMS I

83.1

VESICULAR CORELEASE OF ACETYLCHOLINE AND ATP FROM THE ELECTROMOTOR SYSTEM OF "NARCINE BRASILIENSIS". C.D. Unsworth* and R.G. Johnson* (SPON: J. Trojanowski). HHMI and Depts. of Med. and Physiol. Univ. of Pa., Phila., PA. 19104

Despite the acceptance of the exocytotic theory of quantal vesicular acetylcholine (ACh) release, reports concluding that ACh is released directly from the cytosol continue to challenge vesicular release. Two independent studies were undertaken to establish the mechanism of ACh release from the cholinergic electromotor system of "Narcine brasiliensis". Since the ATP is colocalized with ACh in the cholinergic vesicle, the exocytotic theory would predict the corelease of these two components with a stoichiometry identical to that of the vesicle contents. Quantitation of the release of ATP from synaptosomes could be accurately quantitated only if the ATPase inhibitor α,β-methylene ATP was included in the medium. A variety of secretagogues including KCl, veratridine, and ionomycin all induced the corelease of ACh and ATP in a constant molar ratio of 5.6:1, a stochiometry consistent with that of the vesicle content. In parallel to these studies, the compound 2-(4-phenylpiperidino)cyclohexanol (AH5183) was used to specifically inhibit vesicular accumulation of newly synthesized (radiolabelled ACh) without affecting cytosolic levels of newly synthesized Ach in cholinergic nerve terminals. Treatment with AH5183 specifically inhibited the release of newly synthesized ACh without markedly affecting total ACh release. We conclude that ACh released upon stimulation originates exclusively from the vesicular pool.

83.2

Amplitude and frequency gradients of unitary EPPs along the frog NMJ. J. P. Tremblay, R. Robitaille and S. Beauregard, Neurobiology Lab., Laval University, Québec, Canada, G1K 7P4.

The frog NMJ was recorded in a low Ca^{++} Ringer simultaneously with two intracellular electrodes placed at its distal ends. The motor nerve was stimulated at 10Hz. The ratio of the intracellular amplitudes of unitary EPPs and MEPPs was used to calculate their site of origin using the Spatial Decay Method. When the junction is divided in 10 equal regions, the evoked release activity was more frequent in the proximal regions than in the distal regions, as previously observed for the spontaneous release activity (Tremblay et al. Neur. Lett. 51, 247, 1984; Robitaille et al. Br. Res. 408, 353, 1987). The mean amplitude of unitary EPPs was also significantly higher in the proximal than in the distal regions (analysis variance) as previously observed for MEPPs (Robitaille and Tremblay, Br Res 408, 353, 1987). In fact the mean amplitude of unitary EPPs in a given region matched exactly the mean amplitude of MEPPs in the same region. This suggests that the same mechanisms are responsible for the nonuniform amplitude distributions. A gradient in the length of postjunctional folds along the frog NMJ could be one of the contributing factors (Tremblay et al., Neurosc., in press).

83.3

EFFECTS OF ANTICHOLINESTERASE AGENTS ON QUANTAL CONTENT IN THE RAT. C. Prior*, W.C. Bowman* and I.G. Marshall*. (SPON: Brain Research Association). Dept. Physiology and Pharmacology, University of Strathclyde, Glasgow, U.K.

The effects 1μM neostigmine (NEO), 10μM edrophonium (EDR, submaximal dose) and 3-4μg/ml of the irreversible anti-cholinesterase (anti-AChE) snake toxin, fasciculin 2 (FS2) have been studied on endplate currents (EPCs, 0.1-0.5Hz) and miniature endplate currents (MEPCs) in cut fibers of rat diaphragms at 32°C. All produced similar moderate increases in peak EPC and MEPC amplitudes (maximum increase 27%). EPC quantal content (direct method) was not significantly altered by the agents (NEO, 91 ± 4% control (n=5); EDR, 102 ± 8% (n=3) and FS2, 105 ± 8% (n=3). However, all three agents increased the decay time constant of EPCs (τ_{EPC}) significantly more than they increased τ_{MEPC} (control: τ_{EPC}, 0.53msec; τ_{MEPC}, 0.45msec; 1μM NEO: τ_{EPC}, 5.4msec; τ_{MEPC}, 1.2msec). Doubling $[Ca]_o$ accentuated this effect. All the effects of FS2 persisted even after prolonged wash in control solution. The observed changes in decay time constants can be accounted for by a cooperative interaction of ACh molecules at the receptor level (Magleby, K.L. and Terrar D.A., *J.Physiol.*, 244:467, 1975). The absence of a selective effect on peak EPC amplitude suggests that neither anti-AChEs *per se*, nor the increased [ACh] in the synaptic cleft associated with their use, have any major effect on the peak quantal release of ACh.

83.4

ACTIONS OF L-VESAMICOL ON NEUROMUSCULAR TRANSMISSION IN THE SNAKE. I.G. Marshall*, T. Searl* and C. Prior* (SPON: N.N. Durant). Dept. of Physiology and Pharmacology, University of Strathclyde, Glasgow, Scotland. U.K.

The actions of the active l-isomer of vesamicol, an inhibitor of the vesicular storage of acetylcholine, has been studied on miniature endplate currents (MEPCs) and endplate currents (EPCs) in cut muscle fibers of the garter snake. In controls, 5 min of 10Hz nerve stimulation produced two populations of MEPCs with mean amplitudes of 37% and 97% of the prestimulation MEPC. Preincubation in 2μM vesamicol reduced the small MEPC amplitude to 24% and 5μM completely abolished them. However, vesamicol had no affect on the larger, normal sized MEPC. After 15 min of 10Hz nerve stimulation EPC amplitude was markedly reduced in both controls (to 6%) and 5μM vesamicol (to 2%). Analysis of EPC amplitude variance showed that in control the decrease was due to reductions in both quantal content (to 18%) and quantal size (to 46%) while in vesamicol the decrease was due entirely to a change in quantal content (to 2%) with no change in quantal size (97%). Thus vesamicol selectively inhibits the release of stimulation-induced small-mode quanta both as MEPCs and as components of EPCs. These quanta are possibly derived from a highly active, readily releasable pool. The selective action of vesamicol on this pool is consistent with its effects to inhibit the vesicular storage of acetylcholine.

Work supported by a grant from the Wellcome Trust.

83.5

LONG LASTING ENHANCEMENT OF SPONTANEOUS M.E.P.S.P.S. BY MAST CELL DEGRANULATING PEPTIDE (MCD) IN CA3 HIPPOCAMPAL REGION. E. Cherubini, Y. Ben-Ari and R.S. Neuman (SPON. C. Polosa). INSERM U029, 123 Blvd. de Port Royal, 75014 Paris, France.

MCD, isolated from bee venom, produces LTP in the CA1 region (Cherubini et al., Nature, 1987) and persistent bursts in the CA3 region of the hippocampus (Cherubini et al., Brain Res., 1988). We have now studied the action of MCD on the transient outward current (I_A) and on minature excitatory postsynaptic potentials (m.e.p.s.p.s.) in rat hippocampal slices.

Neurones were recorded with KCl electrodes and voltage clamped in the presence of TTX (1 μM) to block synaptic transmission and bicuculline (30 μM) to block m.i.p.s.p.s. The m.e.p.s.p.s. appeared to fit a Poisson distribution. Bath application of MCD (200-500 nM) for 3-5 min, blocked I_A and increased the amplitude and frequency of m.e.p.s.p.s. Enhancement of the m.e.p.s.p.s. persisted for several hrs (>2) after wash of MCD and outlasted the blockade of I_A. Application of 4-aminopyridine (1-2 mM) blocked I_A but only transiently increased the m.e.p.s.p.s. It is concluded that a transient blockade of I_A is not sufficient to produce the long lasting enhancement of m.e.p.s.p.s. induced by MCD.

83.6

RECEPTOR-MEDIATED POLYPHOSPHOINOSITIDE HYDROLYSIS DOES NOT STIMULATE CALCIUM MOBILIZATION IN PRESYNAPTIC NERVE TERMINALS. T.L. Smith and E.C. Hamlin. Veterans Administration Medical Center, Tucson, AZ 85723 and Department of Pharmacology, University of Arizona, Tucson, AZ 85724.

Although polyphosphoinositide (PPI) hydrolysis has been linked to Ca^{2+} mobilization in peripheral tissues and neurotumor cells, the function of PPI hydrolysis in nerve endings is unclear. It was of interest, therefore, to determine both receptor-stimulated PPI hydrolysis as well as intracellular free calcium, $[Ca_i^{2+}]$, in a purified synaptosomal preparation. $[Ca_i^{2+}]$ was determined fluorometrically using the new calcium indicator, Fluo 3/AM. PPI hydrolysis was determined by monitoring $[^3H]$inositol phosphate production in the presence of Li^+. While both 1mM norepinephrine and carbachol stimulated inositol phosphate production, neither was able to elevate $[Ca_i^{2+}]$ above its resting level. Furthermore, KCl - induced Ca^{2+} influx was completely abolished in the presence of either 1mM norepinephrine or carbachol. It is concluded that stimulation of PPI hydrolysis through $alpha_1$ - adrenergic and M_1 muscarinic receptors is not coupled to presynaptic calcium mobilization. Furthermore, activation of presynaptic adrenergic and muscarinic receptors (possibly $alpha_2$ and M_2, respectively) inhibit voltage-dependent Ca^{2+} influx.
(Supported by a Vet. Adm. Medical Research Grant).

83.7

PRESYNAPTIC INHIBITION IN THE CRAYFISH OPENER NEUROMUSCULAR JUNCTION INCLUDING EFFECTS OF ETHANOL. J.A. Blundon and G.D. Bittner. Dept. Zoology and Inst. for Neurol. Sci. Res., Univ. TX, Austin, TX 78712.

We have published data which suggest that crayfish (Procambarus clarkii) show behavioral changes in righting time and the escape reflex within 12 - 24 hours of exposure to concentrations of ethanol (EtOH) as low as 75mM (Friedman et al. 1988, JPET 246:125-131). The excitor axon-opener muscle junction in the crayfish walking leg provides synapses whereby we can study changes in synaptic plasticities that may be linked with these behavioral changes due to EtOH exposure. EtOH in concentrations as great as 150 mM had no effect on synaptic facilitation, presynaptic action potentials (APs), or excitatory postsynaptic potentials in the muscle fiber. Other researchers have found synaptic plasticities involving the neurotransmitter γ-aminobutyric acid (GABA) to be especially sensitive to low concentrations of EtOH. The opener inhibitor axon provides presynaptic inhibition via axo-axonal contact with the opener excitor axon and also provides postsynaptic inhibition via synapses on muscle fibers. Both pre- and postsynaptic inhibition are mediated by GABA. Hence, we have begun to study effects of low concentrations (< 100 mM) of EtOH on presynaptic inhibition.

Baxter and Bittner (1981, Brain Res. 223:422-428) reported that presynaptic inhibition decreased excitor axon AP amplitude via an increase in chloride conductance in the nerve terminal which in turn decreased the release of excitatory neurotransmitter. However, our observations using lower frequency stimulus paradigms than used by Baxter and Bittner have shown no decrease in excitor AP amplitude during presynaptic inhibition. We are now studying presynaptic inhibition using a variety of stimulus paradigms including that used by Baxter and Bittner in order to clarify the mechanism of presynaptic inhibition.
Supported by NIAAA grant # AA007746.

83.8

PHACLOFEN-INSENSITIVE PRESYNAPTIC INHIBITORY ACTION OF (±) BACLOFEN IN NEONATAL RAT MOTONEURONS. M. Y. Wang* and N. J. Dun (SPON: M. Kiraly). Dept. of Pharmacol. Loyola Univ. Med. Ctr., Maywood, Il 60153

Intracellular recordings were made from antidromically identified motoneurons in transverse spinal cord slices of neonatal (12-16 days) rats. Baclofen (0.5-50 μM) concentration-dependently depressed the excitatory postsynaptic potentials (EPSPs) and inhibitory postsynaptic potentials (IPSPs) evoked by dorsal root entry zone stimulation. While depressing the synaptic potentials, baclofen (<5 μM) caused little or no change of membrane potential and input resistance. At higher concentrations (>5 μM), baclofen caused a hyperpolarization associated with increased membrane conductance. Spontaneously occurring EPSPs and IPSPs were also reversibly eliminated by baclofen. While reducing the EPSPs evoked synaptically, baclofen (5 μM) did no affect significantly the depolarization induced by exogenously applied glutamate. GABA (1 mM) in the presence of uptake inhibitor nipecotic acid (1 mM) reduced the synaptic potentials as well. Pretreating the slices with phaclofen (0.5-1 mM) or bicuculline (50 μM) did not nullify the inhibitory action of baclofen in any of the motoneurons tested. The results suggest that baclofen reduces the excitatory and inhibitory transmission in motoneurons primarily by a presynaptic action that is mediated by a phaclofen-insensitive $GABA_B$ receptor. (Supported by NS24226)

83.9

PREJUNCTIONAL INHIBITION BY D-2 DOPAMINE RECEPTORS: DEPENDENCE ON STIMULATION INTENSITY. D.J. Friedman*, D.N. Krause and S.P. Duckles (Spon: J. Belluzzi). Dept. of Pharmacology, College of Medicine, Univ. of California, Irvine, CA 92717.

Activation of prejunctional D-2 dopamine receptors inhibits norepinephrine release from vascular adrenergic nerves. The selective D-2 dopamine agonist, ±N-0437; the non-selective dopamine agonist, apomorphine; and the negative isomer of N-0437 inhibited contractile responses to transmural nerve stimulation (1 Hz, 100 pulses) in the rat tail artery in vitro with ED_{50} values of 1.6, 30 and 0.4 nM, respectively. Most striking, however, was the profound dependence of the inhibitory effect of all three agonists on the intensity of nerve stimulation. When stimulated at 1 Hz for 100 pulses, ±N-0437 (100 nM) produced an inhibition of 73 ± 7%. However as frequency was increased to 2, 4 and 6 Hz, ±N-0437 produced progressively less inhibition so that no significant inhibition was produced at 6 Hz. In addition, variation in stimulation train length (at a frequency of 3 Hz) produced a similar effect. 100 nM ±N-0437 caused a 96 ± 3% inhibition with stimulation trains of 9 pulses but only 34 ± 11% inhibition with 90 pulse trains. Thus, ±N-0437, its (-) enantiomer and apomorphine produce an inhibition of contractile responses to adrenergic nerve stimulation in the rat tail artery that is dependent on the intensity of nerve stimulation. This phenomenon may be inherent to the mechanism by which the prejunctional D-2 dopamine receptor inhibits norepinephrine release from adrenergic nerves.
Supported by NIH grant #DK36289.

83.10

GABAERGIC NEURONS CULTURED FROM RAT HIPPOCAMPUS FORM AUTO-INHIBITORY SYNAPSES. M. Benveniste*, N. L. Harrison, D. Bekelman*, and J. L. Barker. Lab. of Neurophysiology, NINDS, NIH, Bethesda, MD 20892.

Simultaneous pre- and postsynaptic whole cell recordings (145 mM K gluconate intracellularly) have been made on 2 week cultures of E19 rat embryonic hippocampal neurons. Action potentials (AP) evoked in the presynaptic cell produced a short-latency, long-lasting, bicuculline-sensitive hyperpolarizing potential in the presynaptic cell in 30% of recordings where an inhibitory postsynaptic current (IPSC) was also observed. Under voltage clamp, a brief step depolarization of the presynaptic cell to 0 mV produced an unclamped AP that elicited an IPSC in the postsynaptic cell and simultaneously evoked a bicuculline-sensitive inhibitory current in the presynaptic cell. The latter reversed between -65 mV and -85 mV, had peak conductances ranging from 2 to 20 nS and decayed exponentially with a decay time (τ) ≈ 10 to 50 msec at -40 mV. These inhibitory synaptic currents recorded from the presynaptic cell had latencies similar to those recorded from the postsynaptic cell. Hyperpolarizing pulses in current clamp to either the presynaptic or postsynaptic cell elicited no response from the corresponding cell of the pair indicating that the cells were not electrically coupled. Recordings with a 145 mM KCl-filled electrode yielded a reversal potential of ≈ 0 mV for these events and clearly showed that the bicuculline-sensitive potential is distinct from, and follows the repolarization of the presynaptic AP due to K^+ currents. Thus, the bicuculline-sensitive potential appears to be mediated by a $GABA_A$ receptor-activated Cl^- conductance. These bicuculline-sensitive potentials functionally inhibit AP generation in the presynaptic cell for 20 to 150 msec. These observations indicate that GABAergic neurons from rat hippocampus are capable of forming autoinhibitory synapses in dissociated cultures. The morphological basis for this phenomenon has also been studied using HRP-filled pipettes during the recordings.

M.B is supported by a National Research Council Fellowship.

83.11

TWO STRUCTURAL MECHANISMS FOR REGULATING NEUROTRANSMITTER RELEASE. S. E. Huestis*J. P. Walrond* and C. K. Govind. Dept. Anatomy and Neurobiology, Colorado State University, Ft. Collins, CO 80523 and Life Sciences Division, University of Toronto,1265 Military Trail, Scarborough, Ontario, M1C 1A4 Canada.

To investigate the structural mechanisms which may regulate the amount of neurotransmitter released at the synapse, excitatory and inhibitory axon terminals on the lobster (*Homarus americanus*) Distal Accessory Flexor Muscle (DAFM) were examined with light microscopic immunohistochemistry and freeze-fracture electron microscopy. The DAFM consists of 5 muscle fiber bundles innervated by one excitatory and one inhibitory axon. The excitatory axon releases 3-10 times more transmitter from synapses on the most distal bundle than from synapses on the most proximal bundle. Serial thin section studies suggest that muscle fibers in the distal and proximal bundles contain a similar number of excitatory synapses. However, freeze-fracture studies show that the difference in the amount of excitatory transmitter released from synapses in the two bundles correlates with the number of large intramembrane particles clustered at the active zones. The inhibitor releases 3 to 4 times more transmitter from synapses on the distal bundle than from synapses on the proximal bundle. Immunohistochemical staining for the inhibitory transmitter, γ- aminobutyric acid, labels the inhibitory axon terminals and outlines varicosities where most of the synapses are located. These studies show that there are 3 to 4 times more varicosities on muscle fibers in the distal bundle than on fibers in the proximal bundle. Since freeze-fracture and thin section studies suggest that active zones at inhibitory synapses on distal and proximal muscle fibers are similar, the inhibitor appears to regulate the amount of transmitter it releases by regulating the number of synapses formed in the two bundles. In contrast, the excitor appears to regulate the amount of transmitter it releases by regulating active zone structure.

83.12

NH_4^+ DECREASES CHOLINERGIC EXCITATION IN CAT SPINAL CORD. W. Raabe. Neurology, VA Med. Ctr. and University of Minnesota, Minneapolis, MN 55417.

NH_4^+ decreases monosynaptic and glutamate mediated excitatory synaptic transmission in cat spinal cord. This effect of NH_4^+ is not due to inhibition of the glutamine cycle by $NH4^+$, but due to a depolarization, which decreases and eventually blocks action potential conduction in presynaptic terminals [low threshold (Ia-) afferents]. This study examines whether NH_4^+ also decreases nicotinic, cholinergic excitatory synaptic transmission from motoneuron axon collaterals to Renshaw cells.

In deeply barbiturate anesthetized cats with spinal section at L_2, an extracellular electrode in the ventral horn recorded the discharge of Renshaw cells in response to stimulation of the L_7 or S_1 ventral roots. Stimulation of a peripheral nerve (PBST, MG or LG) elicited a monosynaptic, glutamatergic motoneuron pool EPSP which was recorded from the L_7 or S_1 ventral roots (VR-EPSP). Ammonium acetate i.v., 6 mmol/kg, abolished the discharge of Renshaw cells when the VR-EPSP decreased to less than 20% of control.

These observations show that NH_4^+ rather unspecifically decreases glutamatergic and cholinergic excitatory synaptic transmission in cat spinal cord. Most likely, NH_4^+ depolarizes and blocks conduction of action potentials in all small diameter, unmyelinated presynaptic terminals. However, effects of NH_4^+ on the presynaptic release of ACh and ACh-receptors on Renshaw cells need to be ruled out.

83.13

A PHYSIOLOGICAL BASIS FOR MAC. S.A. Raymond. Anesthesia Research Laboratories, Brigham & Women's Hospital, Harvard Medical School, Boston, MA 02115.

MAC, originated by Eger et al (Anesthesiology 26:756-763, 1965), is now the most common index of anesthetic potency. MAC is the minimum alveolar concentration (partial pressure at 1 atmosphere) of anesthetic that, at steady state, will ensure immobility during noxious stimulation such as a surgical skin incision. MAC has been shown to be: 1) consistent in serial measures in the same subject; 2) similar (within 20% or so) for many agents across species; 3) essentially unchanged when ½MAC concentrations of 2 different agents are added; and 4) the point where probability of coordinated movement during a noxious event depends most steeply on dose for inhalational agents.

We have measured changes in the threshold of axon membrane for electrical stimulation following conditioning impulses. Anesthetic agents disrupt these activity-dependent changes in threshold in ways that parallel the properties of MAC. In particular, 1) activity-dependent excitability changes were suppressed consistently in serial tests by 3 inhalational agents; 2) pump inhibitors and pulse patterns altered the excitability changes so as to suggest that they depend on common ionic and metabolic mechanisms shared broadly among species; 3) inhalational agents are known to reduce membrane currents in voltage clamp, implying a reduction of net ion transfer across the membrane per impulse during activity, which would be expected to have additive, not synergic or antagonistic interaction on the excitability changes; 4) the dose-response relation for suppression of aftereffects is steepest at concentrations corresponding to MAC for 3 inhalational agents tested. These correlations support the proposition that activity-dependent excitability changes in the membrane, not synaptic processes or impulse conduction itself, are the physiological target of anesthetic action.

83.14

COMPUTER SIMULATION OF ACTION POTENTIAL PROPAGATION IN COMPLEX TERMINAL ARBORIZATIONS. H.-R. Lüscher und J. Shiner*. Dept. of Physiology, University of Berne, CH-3012 Berne, Switzerland.

Nerve fibers reaching their central destination usually branch repeatedly forming complex arborizations before synapsing upon their target neurons. Axon branch points and boutons en passant may be regions of low safety for impulse propagation. Therefore, the propagated action potential may die out before reaching all the synaptic endings. Such a mechanism could explain why some central synapses do not release transmitter after activation of the parent fiber, leading to transmission failure.

In order to study the consequences of conduction failure in axon terminations, impulse propagation in arbitrarily complex arborizations was simulated using SPICE, a general purpose network analysis program (Bunow et al., Biol.Cybern.43:1985). A software package was developed which takes as input a list of the axon segments along with their properties: whether excitable or passive, dimensions, and connections to other segments. In addition, information on how the action potentials are to be initiated at the initial compartment of the branching scheme must be input. From this input list the SPICE code is generated and SPICE called as a subroutine. Output consists of the time course of the membrane potential and various currents in each compartment.

The simulations revealed that the action potential may fail to propagate through boutons en passant and branch points in complex arborizations. Propagation improved at lower temperature. For a regular train of action potentials, impulses may fail either irregularly or at constant ratios. Propagation into only one of two branches was common. Synapses beyond branch points are not depolarized after conduction failures, even on very short collaterals. The serial and parallel organization of branch points in complex arborizations can lead to an unpredictable behavior of the propagated action potential. These results are consistent with the hypothsis that incomplete invasion of the terminal arborizations by the propagated action potentials may be responsible for transmission failure at central synapses. (Supported by SNF and the Swiss Multiple Sclerosis Society.)

83.15

Conduction block at CNS axon bifurcations: a central synaptic switch. X. Gu and K.J. Muller. Dept. of Physiol. & Biophys., U. of Miami School of Med., Miami, FL 33101

Synaptic transmission between neurons can be influenced by neuronal geometry. One mechanism for this is axonal conduction block (CB), whereby impulses in the presynaptic neuron fail to propagate beyond points of low safety factor, such as axon bifurcations. We found that naturally occurring CB in one presynaptic neuron in leech ganglia, the medial pressure (mP) sensory cell, affects transmission to different postsynaptic neurons. The mP cell has three cutaneous axons, converging at a single point within the ganglion. The anterior axon appears to contact the Longitudinal (L) motoneuron and Anterior Pagoda (AP) cell, while the posterior contacts the Annulus Erector (AE) motoneuron. Typically, impulses originating in either of these axons block at the central bifurcation. During anterior CB in the mP cell, transmission to the AE cell stops (70% of cases) or is reduced (30%) while it is maintained to AP and L cells. The reverse occurs for impulses arising in the posterior axon, with transmission continuing only to the AE cell. When a laser microbeam was used to cut either the anterior or posterior axon at the bifurcation, transmission to AP and L or to AE cells was interrupted, confirming the CB results. Thus, CB acts as a switch at central synapses and can be used experimentally to analyze the distribution of effective contacts between neurons. (Supported by NIH Grant NS20607.)

83.16

O-GLYCOSYLATION OF SYNAPSIN I T.Lüthi*, M.Bähler* & P.Greengard (SPON: K.Tsou) The Rockefeller University, New York,NY 10021.

Synapsin I is a neuron-specific phosphoprotein which is highly concentrated in the presynaptic nerve-terminal and is associated with the cytoplasmic surface of synaptic vesicles. Electrophysiological studies on the squid giant synapse suggest that phosphorylation of this protein plays a role in neurotransmitter release. Recent reports indicate the existence of a novel form of cytoplasmic protein glycosylation:N-acetylglucosamine (GlcNAc) in O-linkage to serine and threonine residues. We have investigated the possibility of such a protein modification on synapsin I. Our results have shown that the GlcNAc-specific lectin WGA binds a protein doublet in rat brain homogenate which is pH 3 soluble and of the same molecular weight as synapsin I. The WGA reactivity is associated with a subpopulation of synapsin I. Purified synapsin I is labeled with UDP-[3H] galactose upon incubation with bovine milk galactosyl transferase. Both collagenase treatment and cysteine-specific cleavage with NTCB localized the sugar moiety to its head fragment. One of the proposed functions for O-GlcNAc residues involves a "block" for protein phosphorylation. We are currently testing the relationship between phosphorylation and O-glycosylation of synapsin I.

83.17

AMILORIDE INCREASES THE UPTAKE OF ZINC INTO HIPPOCAMPAL SLICES. M.K. SLY, Y. WANG, & I.L. CRAWFORD*. Departments of Neurology and Pharmacology, The University of Texas Southwestern Medical School at Dallas and Epilepsy Center, VA Medical Center, Dallas, Tx 75216.

The molecular mechanisms by which the hippocampus accumulates and regulates endogenous concentrations of zinc are largely unknown. We tested the hypothesis that the uptake of zinc is linked to an antiporter mediating Na+/H+ flux. Amiloride binds to and inhibits the membrane exchanger.

Guinea pig hippocampal slices (400u) were incubated (37 C) in Krebs solution bubbled with O2/CO2 (95/5%). After 30 min in control or experimental media, radiolabelled zinc-65 was added to each teflon chamber. Uptake was terminated 30 to 60 min latter by transfer to cold Krebs (4 C).

Data for each slice (cpm/ug dry weigh) was normalized for media cpm. Amiloride (1.0 mM) significantly increased uptake by 20% (N=44) compared to control slices (N=49). The results raise the possibility that zinc transport into hippocampal tissue is affected by Na+/H+ exchange.

PHARMACOLOGY OF SYNAPTIC TRANSMISSION

84.1

SYNAPTIC "FATIGUE" OF EMBRYONIC AVIAN CILIARY GANGLION IS MEDIATED BY A NOVEL MUSCARINIC RECEPTOR. C.W. Bowers, C.J. Schmidt*, G. Pilar. Dept. Physiology and Neurobiology, Univ. of Connecticut, Storrs, CT 06269.

The avian ciliary ganglion contains two populations of neurons: choroid and ciliary. Although both populations are driven via nicotinic cholinergic transmission in ovo, choroid but not ciliary synaptic transmission fails at stimulation frequencies of 5-10 Hz (embryonic days 12-17). This frequency-dependent block (FDB) is much less pronounced after hatching. We demonstrate that the FDC in embryonic ganglia is prevented by 0.3μM atropine, indicating the involvement of a muscarinic receptor rather than a non-specific/metabolic inability to follow high-frequency stimulation. Furthermore, 10μM oxotremorine-M, a relativly new muscarinic agonist, mimics the FDB by specifically blocking choroid but not ciliary transmission. This effect of oxotremorine-M is blocked by atropine. Two other muscarinic agonists, bethanecol (10μM) and oxotremorine (0.1-10μM), do not block synaptic transmission. This pharmacology is similar to that reported in the CNS for a muscarinic receptor involved in phosphatidyl-inositol turnover (Freedman et al., Trends Pharmacol. Suppl. Feb. 1988: 54). Intracellular studies are in progress to determine whether the muscarinic inhibition is pre- or postsynaptic.

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84.2

MUSCARINIC RESPONSES OF BASOLATERAL AND LATERAL AMYGDALOID NEURONS RECORDED *IN VITRO*. M. S. Washburn* and H. C. Moises. Dept. of Physiology and Neuroscience Program, University of Michigan, Ann Arbor, MI 48109.

The amygdala is known to receive a massive cholinergic input from the nucleus basalis of Meynert (NB) in the basal forebrain. To characterize the role of this cholinergic input in regulating amygdaloid neuronal activity we compared the cellular actions produced by exogenous application of cholinergic drugs to neuronal responses elicited by synaptic release of endogenous ACh *in vitro*. Intracellular recordings were obtained from cells in the basolateral and lateral nuclei in horizontal or coronal slice preparations of the rat amygdala to examine cholinergic effects on passive membrane properties and synaptic responses elicited by activation of the external capsule or NB. Drugs were applied by bath application or by pressure ejection from a pipet positioned above the slice.

Superfusion of carbachol (300 nM-10 μM) produced a dose-dependent block of the slow AHP and, when tested in cells depolarized to just below threshold (positive to -63 mV), elicited a voltage-dependent depolarization accompanied by an increase in membrane input resistance and elevations in spontaneous firing. Carbachol also blocked the epsp and the early and late ipsp evoked by stimulation of amygdaloid afferents. Pressure ejection of carbachol (1-10 mM) produced the same profile of effects and, in addition, elicited a membrane hyperpolarization which was associated with a decrease in input resistance and reversed near -75 mV. All of the repsonses to carbachol were blocked by atropine (1 μM). Based on the actions of the M_1 antagonist pirenzipine and the M_2 antagonist gallamine, it appeared that an M_1 receptor mediated the reduction in the AHP and late ipsp as well as the changes in membrane potential, whereas the carbachol-induced attenuation of the epsp and early ipsp was mediated by an M_2 receptor. Upon repetitive NB activation, the epsp/ipsp sequence gave way to a series of epsps followed by an ipsp and a slow epsp associated with an increase in neuronal excitability. These responses to tetanic NB stimulation were potentiated in eserine and blocked by muscarinic antagonists. They also mimicked some of the effects elicited by exogenous application of carbachol.

These results are the first to demonstrate muscarinic actions in areas of the amygdala known to receive cholinergic innervation. These effects appear to involve both direct and indirect mechanisms and to result from the activation of both the M_1 and M_2 classes of receptors.

(Supported by NIDA grant DA-03365)

84.3

WITHDRAWN

84.4

TRIFLUOPERAZINE CAUSES A SLOW DEPOLARIZATION IN HIPPOCAMPAL SLICES. N. Agopyan and K. Krnjević. Anaesthesia Research Department, McGill University, Montréal, Québec, Canada, H3G 1Y6.

Trifluoperazine (TFP), a neuroleptic and a proconvulsant, is a potent calmodulin antagonist. In previous experiments TFP, like ACh, reduced dendritic field responses reversibly. Hence we studied the effect of TFP on membrane properties and synaptic potentials in hippocampus in vitro.

At a concentration of 50-100 μM TFP consistently depolarized CA1 pyramidal cells by 10-35 mV, one min after the start of its superfusion. Full recovery of membrane potential was seen within thirty min of wash. This effect was accompanied by a reduction in input resistance and a marked depression of slow afterhyperpolarization (both measured at initial resting potential). The depolarization induced by TFP was affected neither by TTX, nor by 4 mM Cs^+, suggesting that it is not mediated by Na^+ current or anomalous rectification. The involvement of Ca^{2+} currents is presently being investigated.

Synaptic potentials were not significantly affected by TFP, suggesting that the reduction seen in dendritic field responses in both in vitro and in situ experiments is due to the depolarization induced by TFP.

Supported by SAVOY Foundation and MRC.

84.5

BINDING CHARACTERISTICS OF OXYTOCIN RECEPTORS IN THE VENTROMEDIAL HYPOTHALAMIC NUCLEUS OF STEROID TREATED RATS. A.E. Johnson and T.R. Insel, Section on Comparative Studies of Brain and Behavior, Laboratory of Clinical Sciences, NIMH, Poolesville, MD 20837.

Ovarian steroids modulate oxytocin (OT) receptor binding in both brain and uterus. In the hypothalamus, estradiol (E_2) increases [^{3}H]OT binding in portions of the ventromedial hypothalamic nucleus (VMN) that contain the greatest density of E_2 concentrating cells. Recently we have shown that the binding of the selective OT receptor antagonist ^{125}I-d(CH_2)$_5$[Tyr(Me)^2,Thr^4,Tyr-NH_2^{9}]OVT (^{125}I-OVTA; Elands et al., 1987) is similarly regulated by E_2 (Johnson et al., in press). The experiments reported here describe the binding characteristics of ^{125}I-OVTA to sites in the VMN of steroid treated rats with receptor autoradiographic procedures.

Adult female rats were ovariectomized and treated one week later with either E_2 (10μg), E_2 and Progesterone (P, 500μg) or Oil for two days and killed 24h after the last injection. Brains were removed, frozen on dry ice and stored at -60°C. For saturation studies, brain slices (20μm) through the caudal VMN were exposed to a range of ^{125}I-OVTA concentrations (1 to 100pM) with unlabeled OT (5μM) used to define nonspecific binding. Several concentrations of OT and AVP as well as selective OT and AVP ligands were used to characterize ^{125}I-OVTA binding in competition studies. Brain slices and ^{125}I-brain paste standards were apposed to film for 3 days and autoradiograms were analyzed by conventional densitometric methods.

Saturation analysis revealed that ^{125}I-OVTA labeled a single class of high affinity binding sites (K_d=60pM) in the VMN. Competition profiles confirmed that as in hippocampal membranes (Elands et al., 1987), sites labeled by ^{125}I-OVTA are characteristic of OT receptors. Differences in ^{125}I-OVTA binding between steroid treatment conditions were due to changes in the number of ^{125}I-OVTA binding sites rather than binding affinity. ^{125}I-OVTA binding was greatest in E_2 treated animals (70 fmol/mg prot) compared to animals treated with either E_2+P (45 fmol/mg prot) or Oil (15 fmol/mg prot). Data from these experiments confirm previous results on the interaction of ovarian steroids and hypothalamic OT receptors and show that the steroid-dependent increase in OT receptor binding in the VMN is due to an increase in the number of specific OT receptors rather than a change in binding affinity.

84.6

RECEPTOR CHARACTERIZATION OF ADRENERGIC EFFECTS IN THE MEDIAL PONTINE RETICULAR FORMATION IN VITRO. U. Gerber, R.W. Greene, H.L. Haas*, and R.W. McCarley. Harvard Medical School/VAMC, Brockton, MA 02401; Johannes Gutenberg-Universitat, Mainz, FRG

Anatomical and physiological evidence indicates that the medial pontine reticular formation (mPRF) receives a noradrenergic projection from the locus coeruleus. Adrenergic binding sites have been demonstrated autoradiographically in the reticular formation and an extracellular microiontophoretic study (Greene and Carpenter, 1985) has shown that norepinephrine (NE) decreases glutamate-evoked neuronal firing in mPRF. The in vitro slice preparation was employed to investigate the mechanism of action and to characterize the receptors mediating adrenergic responses in mPRF. Adrenergic agonists were bath applied (10^{-6}-10^{-5}M) or pressure ejected with a "puffer" micropipette. NE depolarized 18 of 24 cells by 3 to 10 mV and increased PSP frequency; 5 cells were hyperpolarized. Depolarization seen with NE could be mimicked with phenylephrine in 17 of 20 cells and with isoproterenol in 2 of 9 cells. Voltage clamp in 3 of these cells demonstrated an inward current associated with decreased chord conductance. Clonidine hyperpolarized 5 out of 8 cells (>3mV in 2 cells;<3mV in 3 cells). Responses were unchanged by TTX in 4 cells consistent with a direct, non-synaptic action. These results provide functional evidence for the presence of α_1 and α_2 receptors mediating opposing responses in the mPRF.

84.7

MODULATION OF NEOCORTICAL NORADRENALINE RELEASE BY ACTIVATORS AND INHIBITORS OF PROTEIN KINASE C. D.J. Dooley, C. Schächtele*, C. Rudolph*, H. Osswald* and G. Satzinger*. Gödecke Research Institute, D-7800 Freiburg, F.R.G.

Protein kinase C (PKC) is hypothesized to have a role in the process of neurotransmitter release. The characterization of this role has, however, been hindered by the nonavailability of potent and selective PKC inhibitors. We tested several PKC inhibitors (PKCI; e.g., staurosporine, UCN-01 (7-hydroxystaurosporine)) and activators (PKCA; e.g., 4ß-phorbol-12,13-dibutyrate (4ß-PDB)) for effects on [^{3}H]-noradrenaline release evoked from superfused rat neocortical slices by electrical stimulation, or on such release enhanced or inhibited by various pharmacological or electrical means. Additionally, we established the in vitro potency and selectivity of the PKCI to inhibit this enzyme relative to three other protein kinases (cAMP, cGMP, MLC). The PKCA markedly increased depolarization-evoked [^{3}H] overflow without substantially altering basal [^{3}H] outflow. The PKCI, exemplified by UCN-01 having a desirable potency and selectivity, generally lacked effects on control [^{3}H] overflow or outflow; only enhanced [^{3}H] overflow, esp. in response to direct PKC activation (e.g., 4ß-PDB), was attenuated by these substances. These results indicate that PKC modulates neocortical noradrenaline release under quasi-physiological conditions. PKCI can conceivably normalize excessive neurotransmitter release induced by a dysregulation of the phosphatidylinositol/PKC system.

84.8

CALMODULIN ANTAGONISTS MODULATE SYNAPTIC ACTIVATION OF HIPPOCAMPAL PYRAMIDAL CELLS. M.D. Mauk, P.T. Kelly, R.J. Cormier, and M.N. Waxham. Univ. Texas Medical School, Houston, TX 77225.

Recent studies suggest possible roles for calmodulin (CaM) in synaptic transmission (Llinas et al, **PNAS 82**, 1985) and synaptic plasticity (Perkel et al, **Neurosci Abstr. 15**, 1989). We have observed that bath application of CaM antagonists to hippocampal slices can alter EPSPs elicited in CA1 pyramidal cells. Two of these antagonists (CBP & CBP-3) are peptides based on the CaM binding region of CaM kinase-II and reversibly attenuate EPSPs (100uM, 10 min). The naphthalenesulfonamide CaM antagonists W7 and W5 (0.1-1.0 mM) also reversibly attenuate EPSPs with a potency that parallels their CaM antagonism (W7 > W5). In addition, W5 produces a delayed, robust, and long-lasting (1-3 hrs) facilitation of EPSPs. W7 can produce this effect, although variably and much less potently. We have observed no facilitation with the peptide CaM antagonists. Preliminary experiments suggest that the facilitation of EPSPs produced by W5 is attenuated by the protein kinase antagonist H7.

There are several known CaM-dependent processes which could be mutually antagonistic with respect to synaptic transmission. For example, there are CaM-dependent protein kinases as well as CaM dependent phosphatases. Also CaM can serve to activate some kinases and inhibit the activation of others. In future studies we hope to distinguish among such possibilities.

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84.9

SYNAPTIC PLASMA MEMBRANE PHOSPHOPROTEIN PHOSPHATASE 2Ao MAY REGULATE AUTOPHOSPHORYLATION OF cAMP AND Ca^{+2}/CAM DEPENDENT KINASES. G.N.Barnes*, J.T.Slevin and T.C.Vanaman* (SPON: M.McQuillen) VAMC & Univ.of KY, Lexington, KY 40536

The hippocampus is enriched in protein kinase and phosphoprotein phosphatase activities which appear to play a central role in controlling hippocampal neuronal excitability. We have partially purified a high molecular weight phosphoprotein phosphatase which appears to redistribute from the cytosol to synaptic plasma membranes (SPM) of rat hippocampus after electroconvulsive shock. Here we report purification of the phosphatase by a combination of ion exchange and gel filtration chromatography. This purified phosphatase from both cytosol and SPM contains 63, 55, and 38 Kd polypeptides. Tentatively identified as phosphoprotein phosphatase 2Ao (pp2Ao), the enzyme catalyses the release of $^{32}P_i$ from the purified catalytic and regulatory subunits of cAMP-dependent protein kinase in vitro, and is able to release $^{32}P_i$ from an endogenously phosphorylated 50 Kd band in SPM tentatively identified as the α-subunit of Ca^{+2}/Cam-dependent kinase II. Pp2Ao is a major phosphatase in hippocampus, whose membrane association is enhanced by K^+-depolarization of hippocampal neurons in vitro (Barnes etal, Soc.Neurosci.Abstr, 1989). These data suggest that pp2Ao may regulate kinases actuated by transmitter-triggered operation of cAMP and Ca^{+2}-activated second messengers. Supported by the VA Res.Ser. & the NIH (5-R01-NS21868).

84.10

EXTRACELLULAR BARIUM INCREASES THE AMPLITUDE OF SOMATIC POPULATION SPIKE AND DENDRITIC FIELD POTENTIALS IN THE RAT HIPPOCAMPUS *IN VITRO*. J.P. Harris. Department of Pharmacology and Therapeutics, McGill University, Montreal, Quebec H3G 1Y6, Canada and Department of Computer and Information Sciences, S.U.N.Y. at Potsdam, Potsdam, N.Y. 13676.

The effect of the potassium channel blocker barium (Ba^{2+}) on synaptic transmission was studied in the hippocampal slice *in vitro*. Somatic population spike and dendritic field potentials were simultaneously recorded from the CA1 region following delivery of constant current stimuli to the stratum radiatum. The input current intensity was systematically varied in 0.1 mA steps from I_0, the lowest level at which some degree of neural response was recognizable, to I_{100}, the level that produced a maximal spike amplitude.

Addition of Ba^{2+} (1 mM) to the perfusing medium resulted in i) production of a large population spike at I_0, ii) an approximately 50% increase in the amplitude of the population spike at I_{100} and other intermediate current intensities, and iii) a corresponding increase in the amplitude of the dendritic field potential. The presynaptic volley of the dendritic field potential was also more pronounced during perfusion with Ba^{2+}. The observed results may be due in part to enhanced transmitter release. (Supported by MRC Canada grant to R. Capek and B. Esplin.)

84.11

EFFECTS OF 1,10-o-PHENANTHROLINE ON ELECTROPHYSIOLOGICAL RESPONSES IN HIPPOCAMPAL SLICES. Y. WANG, M.K. Sly, H.J. DOLLER, & I.L. CRAWFORD* (SPON: R.W. Honan). Department of Neurology, The University of Texas Southwestern Medical School at Dallas and Epilepsy Center, VA Medical Center, Dallas, Tx 75216.

The distribution of zinc in the hippocampus is highly localized. Since the chelate or competitve metal affinity and binding properties of many drugs posit zinc as a locus for drug action , we investigated the effect of different chelators on hippocampal physiology. 1,10-o-phenanthroline is a well established chelator used widely in biochemical studies of zinc metalloenzymes. The 1,7 isomer has been shown to be a useful nonchelating control.

Granular cell population spike responses to perforant pathway stimulation were recorded from guinea pig hippocampal slices. Glass recording electrodes of 1.4-1.8 Meg-ohms were used. Responses were measured as area of the population spike. Stimulating voltages ranged from 5 to 70 volts. Inter-stimulation intervals were 20 sec to minimize stimulation interaction.

We found that 5 to 200 uM 1,10-o-phenanthroline inhibited evoke responses. The log-dose response curve indicated the ED50 was around 30 uM. The 1,7 isomer had no effect over this same range. The results provide more suggestive evidence that pertubation of zinc affects neural transmission.

84.12

INHIBITION OF HIPPOCAMPAL AND NEUROMUSCULAR SYNAPTIC TRANSMISSION BY *CAUSUS RHOMBEATUS* VENOM. Nancy L. Peterson and James F. Koerner. Dept of Biochemistry and Neuroscience Grad. Program, Univ. of Minn., Minneapolis, MN 55455.

Eight venoms were screened for their ability to inhibit synaptic transmission in the rat hippocampal slice preparation. Of these venoms, only venom from the snake *Causus rhombeatus* (rhombic night adder) inhibited synaptic transmission. Upon purification by gel filtration, two active venom components were isolated. One component (F-II) was identified as adenosine monophosphate via HPLC elution and enzymatic assays. The identity of the other component (F-I) has not been determined. A molecular weight of 1100-1600 was estimated from Sephadex G-25 elution. Assuming mw=1400, and the total protein concentration of F-I, the IC_{50} in the CA1 pathway would be less than 3 μM if F-I is a peptide. F-I reversibly inhibited both hippocampal CA1 synaptic responses which were recorded extracellularly and frog neuromuscular synaptic responses which were recorded intracellularly. During the intracellular assay, the membrane potential, capacitance and time constant remained unchanged. Furthermore, it was possible to intracellularly stimulate action potentials in the presence of F-I. These data suggest that F-I may be a potent, reversible presynaptic inhibitor.
Supported by NIH NS 17944.

84.13

URETHANE ATTENUATES PHORBOL ESTER-INDUCED INCREASES IN HIPPOCAMPAL CA3 RESPONSES IN VIVO. D.N. Lieberman*, B.E. Derrick, & J.L. Martinez Jr. (SPON: S. Roberts) Dept. of Psych., Univ. of California, Berkeley CA 94720

We previously reported that the mechanisms of induction and maintenance of mossy fiber LTP appear distinct from the NMDA-dependent form of LTP found at commissural-CA3 synapses (Neurosci. Abstr. 13:565, 1988). Urethane anesthesia distinguishes these two forms of LTP since it blocks LTP of mossy fiber-evoked (MF), but not commissurally-evoked (COM), CA3 responses. In this study, phorbol 12,13-diacetate (PDAc) was used to examine the possible roles of protein kinase C (PKC) in these two hippocampal pathways. PDAc pressure-ejected into the CA3 region induced transient increases in both MF- and COM-evoked CA3 responses in both pentobarbital and urethane anesthetized animals. The PDAc-induced increases were significantly greater than in either vehicle or inactive phorbol ester (4-alpha-phorbol 12,13 didecanoate) controls, suggesting these increases were due to PKC activation. While the PDAc-induced excitation under urethane was less overall than that observed under pentobarbital anesthesia, urethane significantly attenuated increases in the MF field EPSP, but not the COM field EPSP or COM population spike. This parallels the earlier results in which urethane blocks LTP of MF but not COM responses.

Supported by NIDA #DA 04195 and the Rennie Fund.

84.14

EFFECT OF REACTIVE OXYGEN SPECIES ON THE SQUID GIANT SYNAPSE. Gilbert, D. L., Yao, J.*, C. A. Colton, and M. Spencer*. Lab. of Biophysics, NINDS, NIH, Bethesda, MD 20892, Dept. of Physiol. Biophys., Georgetown Univ. Med. Sch., Washington, D.C. 20007, and Marine Biological Lab., Woods Hole, MA. 02543.

The effect of H_2O_2 on synaptic transmission in the neuromuscular junction in the synapse of the lobster (Colton, C., Colton, J., and Gilbert, D., J. Free Radical Biol. Med. 2:141-148, 1986) and in the hippocampus slice preparation (Colton, C.A., Fagni, L., and Gilbert, D., Free Radical Biol. Med. 6:, in press) has been reported. We now report on the effect of 0.01 mM, 0.1 mM, and 1 mM H_2O_2 on the perfused squid giant synapse. Excitatory post synaptic potentials were depressed or not changed at 0.01 mM or 0.1 mM. However, a significant depression was seen at the high dose of 1 mM. Addition of Fe^{++} or Cu^{++} ion did not enhance the depression induced by H_2O_2.

Superoxide anion was generated by the enzymatic reaction of xanthine with xanthine oxidase. The reaction mixture was prepared in artifical sea water at a temperature of 22 degrees C, 20 min prior to exposure of the synaptic preparation to allow for the superoxide production. Superoxide conc. was analyzed on separate samples using a cytochrome C reduction assay and was about 0.01 mM. Again, excitatory post synaptic potentials were generally depressed by this reactive oxygen intermediate.

84.15

END-PLATE (EPC) AND MINIATURE END-PLATE CURRENT (MEPC) KINETICS IN VOLTAGE-CLAMPED RAT HEMIDIAPHRAGM FOLLOWING ACUTE EXPOSURE TO THE PARALYTIC AGENT 2,4-DITHIOBIURET (DTB). J.M. Spitsbergen and W.D. Atchison. Dept. Pharmacol./Toxicol., Neurosci. Program, Mich. State Univ., E. Lansing, MI 48824.

Previous microelectrode recording studies have revealed an increased rise and decay time for end-plate potentials (EPPs) and miniature end-plate potentials (MEPPs) following exposure to DTB. To determine if DTB alters kinetics of current flow through the acetylcholine (ACh)-channel at the neuromuscular junction, EPCs and MEPCs were recorded from hemidiaphragms of control rats or 1 hr after treatment of rats with DTB (25 mg/kg, ip) using a two-microelectrode voltage clamp. Only MEPCs with fast rise and decay were used for determination of rise and decay kinetics. Rise and decay times of EPCs appeared to be decreased by DTB. This was even more apparent for MEPCs for which the rise time (10-90%) decreased from 507±41 to 431±28 μsec and the half time of decay decreased from 1.23±0.09 to 0.94±0.03 msec following treatment. Although the rise and decay for normal MEPCs appeared faster in muscles from treated rats, the number of abnormal large slow MEPCs increased from 11 to 20% of recorded MEPCs. Thus, the increase in EPP and MEPP rise and decay times cannot be explained by an increase in the duration of current flowing through the ACh-channel following DTB exposure. One possible explanation for the observed slowing is the increase in the number of abnormal MEPCs following DTB exposure. (Supported by NIH grant NS20683 and a Muscular Dystrophy Association grant.)

84.16

METHYLMERCURY (MeHg) INHIBITS [^{3}H]CHOLINE UPTAKE AND INDUCES [^{3}H]ACETYLCHOLINE (ACh) RELEASE FROM RAT BRAIN SYNAPTOSOMES INDEPENDENTLY OF EXTRACELLULAR CALCIUM CONCENTRATION. P.C. Levesque and W.D. Atchison. Dept. Pharmacol./Toxicol., Mich. State Univ., E. Lansing, MI 48824.

Effects of MeHg on transmitter release from central nerve terminals were tested by labeling ACh in rat brain synaptosomes during a 30-min incubation with 1 μM [^{3}H]choline. [^{3}H]choline accumulation occurred via the high-affinity uptake carrier since uptake was reduced greatly by 20 μM hemicholinium-3 or by Na^+-free medium. MeHg reduced synaptosomal [^{3}H]choline uptake by 20-25% at 10 μM and 35-40% at 100 μM. ACh release was tested by incubating [^{3}H]choline-loaded synaptosomes in the absence or presence of MeHg and Ca^{2+}, filtering and then measuring [^{3}H]ACh in the filtrate by liquid cation exchange chromatography. [^{3}H]ACh release increased by 10-15% when synaptosomes were incubated with 10 μM MeHg for 5 sec and 1 min and by 20-25% when incubated for 3 min. 100 μM MeHg increased [^{3}H]ACh release by 20-25% at 5 sec and 1 min and by 35-40% at 3 min. Excluding Ca^{2+} from the incubation medium moderately attenuated the release of [^{3}H]ACh by 10 μM MeHg and slightly diminished the release induced by 100 μM MeHg. These results indicate that the MeHg-induced increase of spontaneous release of ACh from synaptosomes is only partially dependent upon external Ca^{2+} and corroborate the results of an earlier study (J. Pharm. Exp. Ther. 237: 672, 1986) which utilized a peripheral synapse. (Supported by NIEHS grant ES03299.)

84.17

IN VIVO ELECTROCHEMICAL AND ELECTROPHYSIOLOGICAL MEASUREMENTS USING MULTICHANNEL SOLID-STATE RECORDING ELECTRODES. C.G. Van Horne*,1, S. BeMent*,3, B.J. Hoffer1,2 and G.A. Gerhardt1,2 (SPON: R. Freedman). Depts. of Pharmacology1 and Psychiatry2, Univ. of Colorado Health Sci. Ctr., Denver, CO 80262 and Dept of Electrical Engineering and Computer Science3, Univ. of Michigan, Ann Arbor, Michigan 48109.

Solid-state multi-channel recording electrodes have numerous potential advantages over existing "laboratory-fabricated" designs, such as precise spatially-resolved multiple recording sites, reproducible electrophysiological and electrochemical recording characteristics, and increased mechanical strength. In the present study, five-channel silicon-based microprobes were sputter-coated with carbon, coated with Nafion, and used for both in vivo electrochemical and single unit electrophysiological recordings. High-speed electrochemical studies were performed in vivo and showed that these multi-site probes were capable of monitoring the evoked overflow of monoamines in selected brain regions of the rat. In addition, action potentials from Purkinje cells in the rat cerebellum, identified electrophysiologically, were recorded from different sites on the same probe. The change in firing rate in response to systemic administration of PCP was found to decrease in a dose-dependent manner. These results provide preliminary evidence that solid-state multi-site probes can be utilized for in vivo electrochemical and electrophysiological studies in the rat brain. Supported by USPHS grants AG06434, AG00441, NS09199 and the PMAF.

SODIUM CHANNELS I

85.1

GENETIC ANALYSIS OF THE *SEIZURE* LOCUS IN *DROSOPHILA MELANOGASTER*. P. Deak and L.M. Hall Dept. of Molecular Genetics, Albert Einstein College of Medicine, Bronx, NY 10461. Current Address: Department of Biochemical Pharmacology, SUNY at Buffalo, Buffalo, NY 14260.

Previous genetic, biochemical and electrophysiological studies of two temperature-sensitive paralytic alleles (sei^{ts1} and sei^{ts2}) suggested that the *seizure* locus may be involved in the formation of functional voltage-sensitive sodium channels. In order to understand the molecular nature of the *seizure* gene product and its role in the functioning of sodium channels, we have isolated new alleles in this locus. We report here a genetic and cytological characterization of these mutants as well as complementation analysis with existing deficiencies in this region. Some of these new alleles represent visible deficiences in region 60 of salivary gland chromosomes. They are viable over each other and produce a paralytic phenotype at 38°C. This strongly suggests that the null phenotype (no gene product present) for *seizure* is temperature-induced paralysis. These deficiency alleles can be used as cytogenetic landmarks for cloning the locus. Furthermore, the new alleles interact with other temperature-sensitive mutations affecting membrane excitability providing further evidence that *seizure* shares related function with these genes.

Supported by a Jacob Javits Neuroscience Investigator Award NS 16204 from National Institutes of Health.

85.2

Nested Genes Inside a Putative Drosophila Sodium Channel Gene are Conserved. C. Smith*, A. Butler*, and L. Salkoff. Dept. of Anatomy & Neurobiology, Washington Univ. Sch. Med., St. Louis, MO 63110.

Two putative sodium channel genes have been cloned in Drosophila (Salkoff et. al., Science 237: 744, 1987; Ramaswami and Tanouye, PNAS, USA 86: 2079, 1989). An interspecies comparison of the first sodium channel gene is underway in D. melanogaster and in D. virilis (melanogaster and virilis diverged 50-80 million years ago). We find that the sequence and genomic organization of the gene is highly conserved between virilis and melanogaster. Significantly, two other small genes, with multiple introns and exons, are encoded in separate 5′ introns of the sodium channel gene. This creates multiple overlapping transcription units. Remarkably, these nested genes are conserved between species, with respect to their sequence, intron/exon configuration, and position. It is possible that these nested genes are conserved inside the sodium channel gene because of a functional relationship to the sodium channel, perhaps involving control of gene expression.

85.3

ALTERNATIVE SPLICING GENERATES DISTINCT SODIUM CHANNEL SUBTYPES IN *DROSOPHILA*. B. Ganetzky and K. Loughney*. Lab. of Genetics, University of Wisconsin, Madison, WI 53562.

By use of P element transposon tagging and chromosome walking, we have cloned the *Drosophila para* locus, mutations of which cause defects in action potential propagation. Overlapping cDNAs representing essentially all of the *para* coding region have now been sequenced. The *para* polypeptide is extremely similar to rat brain sodium channels in overall structure and amino acid sequence. The deduced *para* polypeptide shares 46% identity with rat brain sodium channels; within membrane spanning domains there are 60-70% amino acid identities. Thus, genetic, molecular, and electrophysiological data all indicate that *para* encodes a functionally predominant class of sodium channels. Three independently isolated cDNAs encoding the amino terminal half of the *para* polypeptide were analyzed. Each of these has a unique structure and each encodes a slightly different amino acid sequence as a consequence of alternative splicing. These alternative splice events result in the optional inclusion of three microexons of 21, 8 and 13 amino acids respectively. Two microexons are located within the first cytoplasmic linking region and the other is located within the second cytoplasmic linking region. The first optional microexon includes an additional potential site for cAMP-dependent phosphorylation. Another splicing variation results in a choice between two exons, both encoding a 56 amino acid segment spanning a portion of region IIS4 and all of IIS5. Although these two alternative exons are only 80% identical at the nucleotide sequence level, they encode amino acid sequences that are identical at all but two positions. One of the changes results in the creation of an additional potential site for glycosylation in the extracellular region between IIS5 and IIS6. Our data suggest a minimum of three, and potentially more, structually distinct forms of sodium channel may be expressed from the *para* locus by alternative splicing. Whether any of these different forms are also functionally distinct remains to be determined.

85.4

NAP^{TS} AND *MLE*: MUTATIONS IN ONE GENE AFFECTING SODIUM CHANNEL ACTIVITY AND TRANSCRIPTION CONTROL IN *DROSOPHILA*. M. Kernan*, M.Kuroda*, B. S. Baker* and B. Ganetzky (SPON: M. D. Bownds). Lab. of Genetics, Univ. of Wisconsin, Madison, WI 53706 and Dept. of Biol. Sci., Stanford Univ., CA 94305

We have shown by genetic complementation and mutagenesis that nap^{ts1}, a mutation causing temperature-sensitive block of action potentials by affecting sodium channel activity, is a gain-of-function mutation in the *mle* gene. Loss-of-function mutations in this gene eliminate the male-specific hyperactivation of X chromosome transcription and are male-lethal. nap^{ts1} appears to act via $para^{+}$, an X-linked sodium channel structural gene, but its effect cannot be explained as a simple defect in male X transcription.

We have cloned the *nap/mle* gene by chromosome walking and have rescued both *nap* and *mle* effects with 10 kb of wild type genomic DNA in a germline transformation experiment. Analysis of cDNAs from this region shows at least two different transcripts, with different deduced protein products, produced by alternative splicing. To distinguish the nap^{+}, mle^{+} and nap^{ts} activities at a molecular level, and to investigate the nature of the nap^{ts} effect on sodium channels, we are using a nuclease assay to locate and characterize the different types of point mutation. First results indicate that four independently isolated nap^{ts} mutations show a similar, possibly identical alteration. DNA sequence analysis and transformation with mutant and chimaeric mutant/wild-type DNA will be used to confirm the identity of changes giving rise to the mutations.

85.5

STRUCTURAL AND FUNCTIONAL ANALYSIS OF THE VOLTAGE GATED SODIUM CHANNEL. V. Auld#*, T.Hebert#*, A. Goldin+, D.Krafte@, N.Davidson@, and R.Dunn#. #Dept. Medical Genetics, University of Toronto, Toronto, M5S 1A8. +Dept. Molecular Genetics, U.C. Irvine, CA, 92717. @Church Chemical Labs and Division of Biology, Caltech, Pasadena, CA, 91125.

We have cloned and constructed a full length cDNA that encodes a rat brain Na^+ channel α subunit termed Rat IIA. In vitro transcribed RNA from this clone produces a functional sodium channel. The properties of the Rat IIA channel differ with respect to its current/voltage relationship from the Rat II and III channels. We are interested in correlating predicted structural features with this difference in the channel's activation as well as with general functional characteristics of the channel protein. Our approach has been to produce mutations in the channel cDNA by i) site-directed mutagenesis and by ii) random linker-insertion mutagenesis. Functional analysis of these mutant channels in *Xenopus* oocytes will provide clues as to the components that are responsible for the sodium channel's electrophysiological properties.

85.6

EXPRESSION OF SODIUM CHANNEL II AND IIA GENES IN RAT BRAIN. C.M.I.Ahmed*, H.Lester and N.Davidson. Division of Biology California Institute of Technology, Pasadena, CA 91125

The alpha subunit of voltage-sensitive sodium channels expressed in rat brain has been shown to exist in four different isoforms,which are designated as I,II,IIA and III(Noda,M. et al.,1986 Nature 320,188-192;Auld et al.1988 Neuron 1,449-461). The isoforms II and IIA differ in only 6 of 2005 aminoacids.Comparison of cloned subtype II and IIA alpha subunits,as expressed in Xenopus oocytes, shows that the current-voltage relatioship of IIA is shifted by 20-25 mV in the depolarizing direction(Stuhmer,W.et al. 1987 Eur.Biophys.J. 14, 131-138; Auld,V. et al.,1988). The polymerase chain reaction(PCR) technique permits specific and very sensitive detection of mRNA and genomic DNA sequences. Using PCR with primers that are specific for and distinguishable between II and IIA sequences, we find that both II and IIA subunits occur in brain, in a 10:1 ratio. When the polymerase chain reaction was carried out with genomic DNA, using again primers specific for II or IIA subunits, a difference in the intron/exon arrangement for these two subunits was observed. This indicates either alternative splicing or the presence of two different genes for the two polypeptides,most probably the latter.

Research Supported by NIH Grants GM-20927 and NS-11756, and by Klingenstein Foundation.

85.7

CHARACTERIZATION OF A FULL-LENGTH cDNA FOR A SODIUM CHANNEL DIFFERENTIALLY EXPRESSED IN DENERVATED RAT SKELETAL MUSCLE. R.G. Kallen*, Z. Sheng*, L.Q. Chen*, K. Fischbeck and R.L. Barchi. (SPON:A. Rosenquist). University of Pennsylvania School of Medicine, Philadelphia, PA 19104-6059

Pharmacological, electrophysiological, biochemical and immunological data favor the existence of multiple voltage-sensitive sodium channel subtypes in rat skeletal muscle. We now report the complete nucleotide sequencing of a second channel subtype from rat muscle that is preferentially expressed in adult denervated muscle, in fetal muscle, and in rat primary muscle cells in culture. It exhibits a tetra-domain repeat sequence with 6 putative transmembrane alpha-helical subdomains per domain as do all previous sodium channels reported. It differs from skeletal muscle subtype I (SkM-I) as follows: overall length is about 100 amino acids greater, the length of the interdomain I-II is 174 amino acids greater, the putative inter-subdomain 5-6 extracellular loop within Domain I is 28 amino acids shorter and has far fewer prototypical glycosylation sites. The cellular and subcellular localization of the mRNAs encoding individual subtypes have been examined in normal and denervated adult skeletal muscle with in situ hybridization. For the SkM-II probe, grain density increases dramatically with denervation, is concentrated around the subsarcolemmal nuclei, and does not appear fiber type specific. Supported by NIH NS-08075 and NS-18013 and the Muscular Dystrophy Association.

85.8

DIFFERENTIAL EXPRESSION OF mRNAs ENCODING TWO SUBTYPES OF VOLTAGE-DEPENDENT SODIUM CHANNEL IN SKELETAL MUSCLE. J. Yang, Z. Sheng, R.G. Kallen, and R.L. Barchi. University of Pennsylvania School of Medicine, Philadelphia PA, 19104.

The cDNAs of two voltage-dependent sodium channel isoforms found in rat skeletal muscle have recently been cloned and sequenced (Trimmer, J. et al. Neuron, in press, 1989; Kallen, R.G. et al., Biophysical J. 55, 319a, 1989). We have studied the differential expression of these channel messages during myogenesis and under various physiological states in adult muscle. [^{32}P]-antisense RNA probes specific for each subtype were prepared from appropriate restriction enzyme cleaved cDNA fragments cloned in the Bluescript expression vector. Total RNA was isolated from relevant muscle samples, separated on formaldehyde-agarose gels and quantitated both by UV absorbance and by hybridization with a -actin probe. Electrophoretic transfers of identical quantities of total RNA were then hybridized with the subtype-specific RNA probes. The two sodium channel subtype messages show reciprocal temporal regulation during development of skeletal muscle in vivo. Type I message is very low at birth but increases as a sigmoid function of age, rising 10-fold between 1 and 35 days post partum. The expression of type II is transient, being most prominent at birth and dropping below detectable levels by 7 days post partum. In primary muscle cells derived from rat embryos, on the other hand, the type II channel message is expressed at a much higher level than the message for the type I channel.

To investigate the influence of neuronal factor(s) on the differential regulation of type I and II mRNA expression, we examined the effect of surgical denervation of hind leg muscles of adult rats on the steady-state level of subtype-specific message. Type I mRNA is prominent in innervated muscle and appears to increase slightly following denervation. Type II message is undetectable in innervated adult muscle but exhibits at least a 10-fold increase in expression following denervation to levels comparable to that of the type I mRNA. This work was supported in part by NIH grants NS-08075 and NS-18013 and by a grant from the Muscular Dystrophy Association.

85.9

EXTRACELLULAR REGIONS OF HOMOLOGOUS DOMAINS I AND IV OF THE RAT BRAIN SODIUM CHANNEL α-SUBUNIT CONSTITUTE THE RECEPTOR FOR α-SCORPION TOXINS. W.J. Thomsen*, L. Maechler*, S. Rossie*, and W.A. Catterall. Dept. of Pharmacology, Univ. of Washington, Seattle, WA 98195.

Site-directed and monoclonal antibodies that recognize diverse regions of the rat brain Na^+ channel α-subunit have been used to determine the amino acid sequence(s) that comprise the receptor for α-scorpion toxins. To achieve this goal, the influence of these different antibodies on the voltage-dependent binding of an α-scorpion toxin isolated from *Leiurus quinquestriatus* (LqTx) was evaluated. Of five site-directed antibodies (IgG) tested, two which recognize residues 355 - 371 and 382 - 400 located between the S5 and S6 segments of homologous domain I, respectively, and one antibody which recognizes residues 1736 - 1753 located between the S5 and S6 segments of homologous domain IV decreased specific binding of [^{125}I]LqTx to either rat brain synaptosomes or purified rat brain Na^+ channel reconstituted in phospholipid vesicles. This decrease was time- and concentration- dependent and similar half maximally effective concentrations (0.5 μM) and maximal levels of inhibition (40 - 50%) were observed for three antibodies. Fab fragments of all three site-directed antibodies also inhibited binding to similar levels as IgG. Three monoclonal antibodies which all specifically recognize an amino acid sequence located in the extracellular loop between segments S5 and S6 of homologous domain I also reduce toxin binding in a concentration-dependent manner with a maximal inhibition of 80%. These results suggest that proposed extracellular regions of homologous domains I and IV of the rat brain Na^+ channel α-subunit are in close spatial proximity and comprise at least part of the receptor for α-scorpion toxins.

85.10

ISOLATION OF NOVEL NA CHANNEL GENES BY PCR GENE AMPLIFICATION. J.H. Caldwell and K.L. Schaller*, Dept. of Cellular and Structural Biology, Univ. of Colorado Health Sciences Center, Denver, CO 80262.

Gene amplification is ideally suited for isolating members of multigene families. Total RNA was isolated and cDNA made from rat brain, skeletal and cardiac muscle. Oligomers (a 29-mer and 30-mer) that are virtually identical for the three published Na channel brain cDNAs were used in the Polymerase Chain Reaction (PCR) to amplify a region that is highly variable between the brain genes. The different sizes of the products were obvious on EtBr-stained polyacrylamide gels. Southern analysis with oligomers to the expected region confirmed that these were from Na channel genes. The amplified products were gel purified and subcloned into M13; they are now being sequenced.

85.11

PROBING THE TOPOGRAPHY OF THE MUSCLE VOLTAGE-DEPENDENT SODIUM CHANNEL BY PROTEOLYTIC CLEAVAGE. S. Kraner*, S. Zwerling* and R.L. Barchi, University of Pennsylvania Medical School, Philadelphia PA, 19104.

The alpha subunit (260 kDa) of the rat skeletal muscle sodium channel is sensitive to limited cleavage by endogenous and exogenous proteases. Using antisera against five oligopeptides spaced along the primary structure, cleavage fragments generated by endogenous muscle proteases during membrane isolation were first examined to define the location of protease-sensitive regions of the structure in its native environment. Major components that included the amino terminus were 130 and 90 kDa with lesser contributions from other peptides extending to 12 kDa. Antisera to epitopes within the carboxy terminal half recognized two fragments of 110 and 78 kDa.

The effects of exogenous trypsin, chymotrypsin, and V-8 protease on the intact 260 kDa alpha subunit in isolated membranes and in solubilized channel preparations were then examined using the same antibodies. All three exogenous proteases produced amino terminal fragments identical to those seen with endogenous proteolysis. The 90 kDa fragment was particularly resistant to further cleavage by these enzymes. The C-terminal antibodies identified an initial cleavage fragment of 110 kDa, but a 78 kDa fragment was not seen; rather several different smaller peptides were labeled by the three antisera in this group.

The similarity in cleavage patterns suggests that sites of proteolysis are located in loops easily accessible from the solvent in the tertiary structure. Comparison of the cleavage map to the primary structure indicates that these sites fall within putative cytoplasmic loops linking the homologous repeat domains; the domains themselves appear to be protected from cleavage by their compact transmembrane structure regardless of the protease involved. The region between D-III and D-IV is resistant to the endogenous protease, yielding a 78,000 MW limit fragment, but easily cleaved by exogenous proteases. The overall distribution of regions of protease sensitivity and resistance supports the predictions of current structural models for the channel. This work was supported in part by NIH grants NS-08075 and NS-18013 and by a grant from the Muscular Dystrophy Association.

85.12

N-ALKYLAMIDE NEUROTOXINS ACT AT TWO BINDING DOMAINS ON THE VOLTAGE-SENSITIVE SODIUM CHANNEL. J. A. Ottea* and D. M. Soderlund. Dept. of Entomology, NYSAES, Cornell University, Geneva, NY 14456.

Synthetic analogs of naturally-occurring *N*-alkylamide neurotoxins activated sodium flux through mouse brain sodium channels in the presence of scorpion (*Leiurus quinquestriatus*) venom. For some compounds, concentration-dependent activation was monophasic and was correlated with the inhibition of both batrachotoxin-dependent sodium uptake and [^{3}H]batrachotoxinin A-20-α-benzoate (BTX-B) binding. These compounds appear to act as partial agonists at the activator recognition site (Site 2) of the sodium channel. Other analogs exhibited biphasic concentration-response curves; in these cases, only the larger, low affinity response was correlated with inhibition of BTX-B binding, whereas the smaller, high affinity response occurred at concentrations that did not affect BTX-B binding. The latter effect therefore involves an action at another binding domain of the sodium channel. Among the analogs examined, only those with insecticidal activity were found to stimulate sodium uptake. Some nontoxic analogs also partially inhibited BTX-B binding but did not produce a corresponding activation of sodium flux; these compounds may therefore act as weak antagonists at Site 2. Our findings suggest that *N*-alkylamides may act at two distinct binding domains of the voltage-sensitive sodium channel but that the neurotoxic effects of these compounds in insects are correlated with their actions at Site 2. [Supported by NIH grant ES02160]

EXCITATORY AMINO ACIDS: RECEPTORS I

86.1

THE MULTIPLE EFFECTS OF MAGNESIUM ON THE GLUTAMATE- AND GLYCINF-STIMULATED ^{3}H-TCP BINDING IN RAT CEREBRAL CORTEX. T. Hori*, T. Yamamoto* and T. Moroji*(SPON: K. Yoshikawa). Dept. of Psychopharmacology, Psychiatric Research Institute of Tokyo, Tokyo 156, JAPAN.

Phencyclidine(PCP) has been well known to elicite psychotic condition in human. An accumlating evidence indicates the existence of a PCP receptor complex, consisting of the PCP binding site, the NMDA receptor, glycine receptor and K^+-channel molecules in the brain.

In this study, we have shown multiple regulatory effects of Mg^{2+} on the ^{3}H-TCP binding sites. The binding of ^{3}H-TCP to the well washed membrane was determined as described by Bonhaus and McNamara (Mol. Pharmacol., 34: 250-255,1988).

Mg^{2+} has a biphasic effect on ^{3}H-TCP binding, e.g.a stimulatory effect at low concentrations (EC_{50}= 5 uM) and an inhibitory effect at high concentrations (IC_{50}= 5 mM). In the presence of glutamate (10 uM) or glycine (10 uM), the EC_{50} value for Mg^{2+} was lowered by one fifths or one half, respectively. On the contrary, the IC_{50} value for Mg^{2+} was reduced by one tenths in the presence of glutamate but not glycine. On the other hand, K^+ inhibited dose-dependently both glutamate- and glycine-stimulated ^{3}H-TCP binding in the same manner. The present findings indicate the presence of multiple sites for Mg^{2+} of the PCP receptor complex associated with both glutamate and glycine (high affinity site) and only with glutamate (low affinity site).

86.2

CHRONIC MK-801 TREATMENT ENHANCES NMDA RECOGNITION SITE BINDING IN PERINATAL RATS. J. Uckele*, J.W. McDonald, K. O'Mara*, F.S. Silverstein, & M.V. Johnston. Neuroscience Program, Depts. of Pediatrics & Neurology, University of Michigan, Ann Arbor, MI 48104 & Johns Hopkins University, Baltimore MD 21205.

We previously reported that MK-801, a non-competitive NMDA antagonist, induces a rapid up-regulation of NMDA recognition sites (30-50% increase)when administered (i.p., 1 mg/kg)to postnatal day (PND)7 rats 2 or 24 hrs prior to sacrifice (McDonald et al., Soc. Neurosci. Abst., 1988). This study examines the effect of chronic treatment with MK-801 on binding to the NMDA receptor/channel complex & quisqualate-type glutamate receptors. Beginning on PND 5, rats received 4 i.p. doses of MK-801 (1 mg/kg, n=5) or saline (n=5)at 24 hr intervals. Animals were sacrificed on PND 13, 5 days after the last treatment. MK-801 treatment produced a 38±10% to 66±13% mean increase in NMDA recognition site binding in 3 hippocampal regions, 78±17% & 90±15% increase in cingulate cortex & striatum, & a 265±60% increase in the granule cell layer of the cerebellum compared to saline treatment ($p < 0.001$, ANOVA). Binding to glycine modulatory sites & PCP receptors associated with the NMDA receptor complex were unchanged. Quisqualate receptor binding was increased in all regions by 13±5% to 29±10% by MK-801 compared to saline($p < 0.001$,ANOVA). The data indicate that chronic treatment with the NMDA antagonist MK-801 produces a selective & marked increase in NMDA recognition site binding without altering glycine or PCP receptor/channel binding; MK-801 may alter the relationship between the receptor components of the NMDA receptor complex.

86.3

ONTOGENY OF THE RECEPTORS COMPRISING THE NMDA RECEPTOR COMPLEX. J.W. McDonald, M.V. Johnston and A.B. Young, Dept. Neurology, Univ. of Michigan, Ann Arbor, MI 48109.

The ontogeny of 3 binding sites on the NMDA receptor complex was examined in rat hippocampus by quantitative autoradiography. The NMDA recognition site was labeled using [3H]glutamate. [3H]Glycine was used to label the strychnine-insensitive glycine modulatory site and [3H]TCP to label the PCP receptor. Binding to each receptor was examined in adjacent brain sections from postnatal day (PND) 1, 4, 7, 10, 14, 21, 28, 90 rats (n=4/age). Values represent mean±SEM and are expressed as % of adult binding. A different developmental pattern was observed for each receptor component of the NMDA receptor complex. In stratum oriens of area CA1, NMDA receptor binding rose from 20% to 40% from PND 1 to 7, then increased rapidly to maximal densities at PND 14 (145% of adult), stabilized through PND 28 and then decreased to adult levels. Glycine and PCP receptor binding developed more slowly than NMDA receptor binding; densities rose in a linear fashion from PND 1 through PND 21 and maximal densities were at PNDs 21 (110%, PCP) and 28 (150%, glycine). Age of maximal binding and age at which binding began to decrease to adult levels varied between hippocampal areas for each receptor. Data suggest that the relationship between the component binding sites of the NMDA receptor complex changes markedly during development. Supported by USPHS grant NS 19613.

86.4

NMDA AND QUISQUALATE/AMPA RECEPTORS: DIFFERENTIAL REGULATION BY PHOSPHOLIPASE C (PLC). G. Massicotte*, M. Kessler, G. Lynch, and M. Baudry. Center for the Neurobiology of Learning and Memory, University of California, Irvine, CA 92717, U.S.A.

Activation of membrane-associated phospholipases has been proposed to participate in the regulation of synaptic transmission at glutamatergic synapses. The present study was directed at determining the effects of PLC on the binding properties of different subclasses of glutamate receptors. Treatment of membranes with PLC (from Clostridium perfringens) produced an increased affinity of the quisqualate/AMPA receptor for [^{3}H]-AMPA, while kainate receptor binding was not affected. Both kinetic analysis and equilibrium saturation experiments indicate that PLC treatment produced a decrease in affinity for [^{3}H]-TCP (a ligand for the NMDA receptor associated ionic channel) when the channel was fully activated by high concentration of glutamate and glycine. The binding of [^{3}H]-glutamate and [^{3}H]-glycine was not modified by PLC treatment, but the binding affinity for the NMDA antagonist [^{3}H]-CPP was decreased by about 2 fold. The decrease in [^{3}H]-CPP binding was accompanied by a loss of the stimulation by glycine of [^{3}H]-glutamate binding. These data suggest that modification of glutamate receptors resulting from phospholipase activation might be involved in various forms of synaptic plasticity.

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86.5

TWO MOLECULES OF AGONIST ARE REQUIRED FOR NMDA RECEPTOR ACTIVATION. D.C. Javitt, M.J. Frusciante* and S.R. Zukin. Departments of Psychiatry and Neuroscience, Bronx, NY 10461.

PCP and related psychotomimetic agents induce unique neurobehavioral effects by binding to a site (PCP receptor) located within the ion channel of the *N*-methyl-D-aspartate (NMDA) receptor complex. Access of PCP-like agents to the PCP receptor depends upon the functional state of the NMDA receptor complex. Binding of the PCP receptor ligand [^{3}H]MK-801 was studied in order to determine mechanisms of NMDA receptor functioning.

Kinetics of [^{3}H]MK-801 binding fit best to a biexponential model, suggesting the presence of two discrete components of association. The time course for the fast component of association ($t_{1/2}$=5 min) was similar to the predicted time course for MK-801-induced open channel blockade. Fast association of [^{3}H]MK-801 thus appears to represent association via the open channel of the activated conformation of the NMDA receptor complex. Addition of L-glutamate induced a dose-dependent 40-fold increase in steady-state binding to the fast component of [^{3}H]MK-801 association. The Hill coefficient for stimulation of fast binding, 2.06 ± 0.08, was statistically indistinguishable from 2, suggesting that the cooperative binding of 2 molecules of agonist may be required for NMDA receptor activation. Hill coefficients of 2 have been reported for agonist-induced activation of nACh and $GABA_A$ receptors, both of which are members of the Class I superfamily of neurotransmitter receptor. The existence of functional homology between NMDA and Class I receptors may suggest structural homology as well. (Support: PHS DA03383, Ritter Foundation and David Berg Family Fund (SRZ); MH00631 (DCJ); AECOM Department of Psychiatry, H.M. van Praag, M.D. Ph.D., Chairman.)

86.6

TWO CLASSES AND TWO STATES OF NMDA RECEPTORS. D.T. Monaghan[1], H.S. Lin*[1], and C.W. Cotman[2]. Div. Neurosurgery[1] and Dept. Psychobiology[2], University of California, Irvine, CA 92717.

We have previously shown that there are two forms of the NMDA receptor (Monaghan et al., 1988; PNAS 85: 9836-9840). These two subtypes display differing regional distributions and differing relative affinities for agonists and antagonists. Furthermore, glycine increases L-[^{3}H]glutamate binding while decreasing radiolabelled antagonist binding.

In this study we have evaluated the effect of glycine and the glycine antagonist HA-966 upon the distribution of binding sites for L-[^{3}H]glutamate and the NMDA antagonist [^{3}H]CPP. If the anatomically-distinct forms of NMDA sites simply represent different states of the NMDA receptor that are regulated by glycine, then addition of glycine or glycine antagonists should result in identical L-[^{3}H]glutamate and [^{3}H]CPP binding site autoradiograms. (Autoradiographic methods described in PNAS 85: 9836) Glycine stimulates L-[^{3}H]glutamate binding but not [^{3}H]CPP binding; HA-966 stimulates [^{3}H]CPP binding, but not L-[^{3}H]glutamate binding. However, in the presence of 5 μM glycine or 100 μM HA-966, NMDA-displacable L-[^{3}H]glutamate and [^{3}H]CPP binding sites retain their differing regional distributions. Glycine exhibits a greater percent stimulation of L-[^{3}H]glutamate binding in antagonist-preferring regions than in agonist-preferring regions; however, the regional distribution of glycine stimulation of L-[^{3}H]glutamate binding appears to be the average of both the agonist-preferring and the antagonist-preferring distributions. Together these results suggest that there are two distinct NMDA receptor subtypes and that each has two states regulated by glycine.

86.7

(+)3-PPP BINDING SITES IN PC12 CELLS. G.A. Paleos, Z.W. Yang, and J.C. Byrd. Developmental Neurobiology Program, University of Pittsburgh School of Medicine, W.P.I.C., Pittsburgh, PA 15213.

Phencyclidine (PCP) binds with high affinity to PCP and sigma receptors. The clonal cell line PC12 is known to express (+)3-PPP binding sites but no PCP receptors (Yang et al., Eur. J. Pharmacol., in press). Neither nerve growth factor nor sodium butyrate treatment affected the expression of either binding site. The binding of (+)3-PPP was linearly dependent upon protein concentration and was protease sensitive. the pH maximum of (+)3-PPP binding was 9.5, at which the K_D of (+)3-PPP was 27 nM. The time required for binding to reach equilibrium was moderately temperature sensitive. This (+)3-PPP binding site differs from the CNS sigma receptor in two respects. First, divalent cations cause a greater decrease in the affinity of (+)3-PPP for its PC12 binding site than for the sigma receptor. Secondly, the stereoselectivity for benzomorphan opiates is reversed, with the (-) isomer being favored (cf. Bowen et al., Neurosci. Abst., 1988); however, classical opiate antagonists are inactive at this site. These data suggest that the (+)3-PPP binding site in PC12 cells is either a sigma receptor subtype, or, alternatively, a binding site unrelated to the CNS sigma receptor.

86.8

(+)3-PPP AND TCP BINDING SITES IN RAT AND BOVINE ADRENAL MEDULLA. F. DePietro*, G.A. Paleos, and J.C. Byrd. Developmental Neurobiology Program, University of Pittsburgh School of Medicine, W.P.I.C., Pittsburgh, PA 15213.

The radioligand (+)[^{3}H]3-PPP is often used to label CNS sigma receptors. We have recently shown (Yang et al., Eur. J. Pharmacol., in press) that the rat pheochromocytoma cell line PC12 contains (+)3-PPP binding sites, but no PCP receptors, which have a similar pharmacological profile to sigma receptors. The (+)3-PPP site in PC12 cells differs from the CNS sigma receptor, however, with regard to the mechanism of binding inhibition by cations as well as its stereoselectivity for benzomorphan opiates. Since PC12 cells resemble primitive neuroblasts, we investigated whether adult rat and bovine chromaffin cells express either PCP or sigma-like receptors. Rat adrenal glands were found to contain both a large number (B_{max} = 900 fmol/mg protein) of high affinity (K_D = 26 nM) (+)3-PPP sites, as well as a significant number (B_{max} = 300 fmol/mg) of high affinity [^{3}H]TCP binding sites. Bovine adrenal medullae showed a higher number (B_{max} = 4380 fmol/mg) of high affinity (K_D = 69 nM) TCP binding sites, as well as a large number (B_{max} = 1250 fmol/mg) of high affinity (K_D = 48 nM) (+)3-PPP binding sites. In both species, there was a 10-fold stereo-selectivity for (+)3-PPP vs. (-)3-PPP. These data suggest that immature chromaffin cells may express only (+)3-PPP sites, while mature cells express both (+)3-PPP and TCP binding sites.

86.9

Evidence for PCP Site 2 (Biogenic Amine-Reuptake Complex Coupled) in Membranes Prepared from Human Cortex and the Brains of Other Species. H. Akunne[1], A. Reid[2], M.P. Heyes[1], A.Thurkauf[2], J.A. Monn[2] A.E. Jacobson*[2], K.C. Rice[2] and R.B. Rothman[1]

[1]Unit on Receptor Studies, LCS, NIMH and [2]Laboratory of Medicinal Chemistry, NIDDK, Bethesda, MD 20892.

Previous work demonstrated two high affinity PCP binding sites in guinea pig brain labeled by [^{3}H]TCP: site 1 (NMDA-coupled) and site 2 (DA-reuptake complex coupled). The present study examined brain membranes prepared from various species, including human, for the presence of site 2, defined as binding in the presence of 1 μM (+)MK801 minus binding in the presence of 10 μM TCP. The results demonstrated detectable levels of site 2 in brain membranes of rabbit, chicken, mouse, sheep, human, but not rat. Using human cortical membranes, high affinity DA (GBR12935, GBR12909) and 5-HT reuptake inhibitors (paroxetine, fluoxetine) produced a wash-resistant inhibiton of [^{3}H]TCP binding to site 2, but not site 1. Site 2 was the predominant binding site in the human cortex. These results demonstrate that human cortex, possesses a high affinity PCP binding site associated with biogenic amine reuptake binding sites, and that guinea pig , but not rat brain, may be an appropriate animal model for studying the PCP binding sites of human brain.

86.10

ALLOSTERIC REGULATION OF NMDA-COUPLED AND DOPAMINE UPTAKE CARRIER COUPLED PHENCYCLIDINE BINDING SITES IN GUINEA PIG BRAIN. A.A. Reid, J.A. Monn*, A.E. Jacobson*, K.C. Rice and R.B. Rothman. Lab of Medicinal Chemistry, NIDDK and Unit on Receptor Studies, LCS, NIMH, Bethesda, MD 20892.

Recently, we described the presence of two high affinity PCP binding sites in guinea pig brain, with one site coupled to the NMDA receptor (site 1) and the other site associated with the dopamine reuptake carrier (site 2). (+)MK801 is 129-fold selective for site 1. In this study, we conducted kinetic experiments to examine the ability of PCP and (+)MK801 to allosterically modulate the two PCP binding sites. [3H]TCP (5 nM) was utilized to assess effects at site 1 (total binding - binding in the presence of 1 μM (+)MK801) and site 2 [binding in the presence of 1μM (+)MK801-nonspecific binding (1 μM TCP)]. [3H]MK801 (2.5 nM) was used to determine effects at site 1. Guinea pig brain homogenates were incubated with the ligands in 5 mM Tris-HCl pH 8.2 buffer for 2 h at 0°C. The reaction was terminated by centrifugation, the pellets were resuspended with buffer and then filtered (1 ml aliquots) at 15 min intervals to assess the degree of dissociation of the ligands over time. PCP (1 μM) and (+)MK801 (500 nM) were each added to the suspensions at the 30 min time point.

	% OF CONTROL BINDING AT 2.5 HOURS		
	[3H]TCP		[3H]MK801
	SITE 1	SITE 2	SITE 1
CONTROL	74.2±7.6	95.4±3.4	91.4±4.2
PCP	46.5±2.2*	55.4±3.3*	80.0±1.5
(+)MK801	39.3±1.9*	92.0±0.6	78.7±1.6

These data provide evidence for allosteric binding sites associated with PCP site 1 and 2 and indicate that [3H]MK801 predominantly labels the allosteric site associated with PCP site 1.

86.11

CHARACTERIZATION OF SIGMA AND PHENCYCLIDINE BINDING SITES ON NEURAL CELL LINES

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The psychotomimetic effects of benzomorphane opiates and phencyclidine (PCP) in man are hypothetized to be mediated by both haloperidol sensitive sigma and PCP receptors. In the present study several cell lines including the NCB-20 hybrid, rat pheochromocytoma PC-12, neuroblastoma x glioma hybrid NG 108-15, rat glioma C6-BU-1 lines were investigated for the presence of these receptors by radioligand binding studies. For these assays, the sigma receptor ligands ^{3}H-DTG (di-o-tolylguanidine), ^{3}H-(+)-3-PPP-(3-(3-hydroxyphenyl)-N-(1-propyl)piperidine), the sigma/PCP-receptor ligand ^{3}H-(+)$_5$SKF 10047 (N-allylnormetazocine) and the PCP-receptor ligand ^{3}H-TCP (n-(1-(2-thienyl)cyclohexyl))piperidine were used. Binding sites for the sigma ligands were found on membranes of all cell lines investigated the K_D- and B_{max}-values varying between 20 and 120 nM and 2 and 20 pmol/mg protein, respectively. TCP-specific sites could only be detected in membranes of NCB-20 and NG 108-15 cells. The various cell lines differed in their profile of expressed binding sites. Furthermore, the pharmacological characterization of these sites revealed at least in part marked differences as compared to corresponding sites in rat brain. The present results suggest in some neural cell lines the existence of several types of sigma and PCP receptors. Thus, these cell lines may serve as useful tools for the further characterization of these sites and for the investigation of their functional role.

86.12

IFENPRODIL, A NOVEL NMDA ANTAGONIST, BINDS TO A POLYAMINE-SENSITIVE SITE. H. Schoemaker*, J. Allen* and S.Z. Langer, Synthélabo Recherche (L.E.R.S.), 58, rue de la Glacière, 75013 Paris, France.

It has been suggested that ifenprodil exerts its cerebral anti-ischaemic activity through a noncompetitive antagonism at the NMDA-sensitive glutamate receptor. Recent evidence suggests that it acts through the polyamine modulatory site of the NMDA receptor complex (Carter et al., Eur. J. Pharmacol., in press). We therefore studied the binding of [^{3}H]ifenprodil (spec. act. 31 Ci/mmol) to thrice-washed membranes of the male rat cerebral cortex. Following 120 min of incubation in 50 mM Tris-HCl buffer (pH 7.4) at 0°C, membranes were recovered by filtration over 0.05% polyethylenimine-treated Whatman GF/B filters. Specific [^{3}H]ifenprodil binding, defined using 10 µM unlabeled ifenprodil, was of high affinity (K_d = 45 nM, B_{max} = 5.5 pmol/mg protein) and sensitive to the polyamines spermine (IC_{50} = 7.3 µM) and spermidine (IC_{50} = 87 µM), but not putrescine (IC_{50} > 1000 µM). The ifenprodil analog SL 82.0715 inhibits specific [^{3}H]ifenprodil binding with an IC_{50} of 136 nM. Saturation analysis indicates that spermine decreases the affinity of [^{3}H]ifenprodil binding. Thus, the present experiments indicate that [^{3}H]ifenprodil is a novel ligand that labels a polyamine-sensitive site possibly associated with the NMDA receptor in the rat brain.

86.13

IS IFENPRODIL AN NMDA ANTAGONIST? L. J. Robichaud, L. J. Brahce*, L. L. Coughenour*, and P. A. Boxer. Parke-Davis Pharm. Res. Div., Warner-Lambert Co., Ann Arbor, MI 48105.

Ifenprodil has been reported to be an atypical NMDA receptor antagonist and to reduce ischemic damage in the cat and rat (Gotti et al., 1988). In the rat cortical wedge model bathed with Mg^{++}-free buffer ifenprodil reduced the amplitude of spontaneous epileptiform discharges (SED) by 70% at a concentration (10 µM) that increased the SED rate (39%). At 30 µM, ifenprodil blocked all SED reversibly, as did CPP (1 µM) and ketamine (10 µM). However, ifenprodil differs from CPP in that it binds very weakly to the NMDA recognition site (44% inhibition of [^{3}H]CPP binding at 100 µM vs an IC_{50} of 0.08 µM for CPP. Similarly ifenprodil displaces [^{3}H]TCP binding (IC_{50}=57 µM vs 0.86 µM for ketamine). Another difference is that ifenprodil (300 µM) had no effect on repeated depolarizations induced by NMDA in the cortical wedge. In contrast, CPP and ketamine inhibited NMDA responses $\geq$ 50% at concentrations which blocked SED. Ifenprodil (100 µM) also failed to inhibit quisqualate or kainate induced depolarizations. In summary ifenprodil weakly inhibits the binding to at least 2 sites on the NMDA receptor channel complex, but fails to inhibit a direct response to NMDA. However, the inhibition of SED indicates that ifenprodil can modulate a response which is apparently mediated by endogenous activators of NMDA receptors, perhaps through an interaction at another modulatory site.

86.14

DIFFERENTIAL NEUROPSYCHOPHARMACOLOGICAL PROFILES OF PCP-LIKE AND IFENPRODIL-LIKE NMDA ANTAGONISTS. B. Zivkovic, Gh. Perrault*, E. Morel* and D.J. Sanger*. Synthélabo Recherche (L.E.R.S.), 31, ave P.V. Couturier, 92220 - Bagneux, France.

Neuroprotective agents ifenprodil and its congener SL 82.0715 have been shown to inhibit NMDA receptor function by acting at a site distinct from those involved in the actions of competitive antagonists such as APV and CPP or non-competitive agents like PCP and MK-801. The present studies show that the neuropsychopharmacological profiles of ifenprodil and SL 82.0715 differ from those of both the competitive and PCP-like NMDA antagonists. In mice, PCP and MK-801 produce stimulant effects whereas ifenprodil and SL 82.0715, like the competitive antagonists, decrease locomotor activity. In contrast to these latter drugs ifenprodil-like and PCP-like antagonists do not induce myorelaxation. The anticonvulsant spectrum of activity of all three groups of NMDA antagonists is similar. However, rats trained to discriminate PCP or MK-801 do not show drug lever responding after ifenprodil or SL 82.0715. Thus, the differences in the mode by which NMDA antagonists interact with the receptor appear important for their pharmacological profiles.

86.15

CHARACTERIZATION OF POLYAMINE EFFECTS ON ^{3}H-TCP AND NMDA-SPECIFIC ^{3}H-GLUTAMATE BINDING. **A. I. Sacaan and K. M. Johnson. Department of Pharmacology and Toxicology, Univ. of Texas Med. Br., Galveston, TX 77550.**

Reciprocal interactions between glutamate, glycine and spermidine on ^{3}H-TCP binding to rat brain membranes were previously reported (Ransom and Stec, 1988). This study was conducted to further investigate these interactions. Putrescine (N-4C-N) and cadaverine (N-5C-N) which alone did not alter ^{3}H-TCP binding, blocked both spermidine (N-3C-N-4C-N) and spermine (N-3C-N-4C-N-3C-N) enhancement of ^{3}H-TCP binding. Moreover, putrescine blocked glutamate- and glycine-induced ^{3}H-TCP binding. Putrescine blockade of both spermidine- and glutamate-induced ^{3}H-TCP binding was of an uncompetitive nature, i.e. the receptor density and affinity of ^{3}H-TCP for its receptor decreased and increased, respectively, with increasing concentrations of putrescine. This result not only suggests a unique mechanism of action of putrescine on glutamate- and spermidine-induced ^{3}H-TCP binding, but also points out that spermidine and putrescine are acting via two different sites to alter ^{3}H-TCP binding. Spermidine, spermine and putrescine exhibited little displacement of NMDA-specific ^{3}H-glutamate binding, suggesting that the polyamines do not exert their effects on ^{3}H-TCP binding via direct action on the NMDA receptor. In addition, magnesium and spermidine had opposite effects on ^{3}H-TCP binding when tested in the presence of glutamate and glycine, suggesting that spermidine does not act at the voltage dependent site for divalent cations. Supported by DA-02073.

86.16

CHARACTERIZATION OF POLYAMINE EFFECTS ON THE STRYCHNINE-INSENSITIVE GLYCINE RECEPTOR. **K. M. Johnson and A. I. Sacaan. Department of Pharmacology and Toxicology, Univ. of Texas Med. Br., Galveston, TX 77550.**

Spermine (N-3C-N-4C-N-3C-N) and the propanediamine derivative (N-3C-N-3C-N-3C-N) enhanced strychnine-insensitive ^{3}H-glycine binding to rat cortical membranes with EC_{50} values of 27 + 3.1 and 18 + 0.7 µM respectively. The ethylenediamine derivative (N-3C-N-2C-N-3C-N) exhibited a partial agonist effect of similar potency. Spermidine (N-3C-N-4C-N) and putrescine (N-4C-N) were without effect. Neither spermidine nor the partial agonist inhibited the effect of spermine. Eadie-Hofstee analysis revealed that spermine increased the affinity of glycine for its receptor without a significant change in receptor density. This effect of spermine is thought to be distinct from that reported on ^{3}H-TCP binding (see Sacaan & Johnson, this volume). First, spermidine mimicked the effects of spermine on ^{3}H-TCP binding but was without effect on ^{3}H-glycine binding. Secondly, spermine enhancement of ^{3}H-TCP binding was blocked by putrescine, whereas its enhancement of ^{3}H-glycine binding was not altered by putrescine. Moreover, spermine's effect on ^{3}H-glycine binding persisted in the presence of glycine or NMDA receptor antagonists, whereas spermine's effect on ^{3}H-TCP binding was blocked by NMDA and glycine receptor antagonists. Inasmuch as the effect of spermine on ^{3}H-TCP binding, but not on ^{3}H-glycine binding, is blocked by putrescine, the significance of the effect of spermine on NMDA receptor function mediated through the glycine receptor remains to be determined. Supported by DA-02073.

86.17

POLYAMINE STIMULATION OF [^{3}H]MK-801 BINDING: IMPLICATIONS FOR NMDA RECEPTOR FUNCTIONING. M.-L. Wong*, M.J. Frusciante*, D.C. Javitt and S.R. Zukin, Departments of Psychiatry and Neuroscience, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461.

Both polyamines and Mg^{2+} have been reported to stimulate the functioning of *N*-methyl-D-aspartate (NMDA) receptors. In order to study the mechanisms of these effects, the actions of spermidine upon [^{3}H]MK-801 binding to the phencyclidine (PCP) receptor of the NMDA-gated ion channel were compared with those of Mg^{2+}. In extensively washed rat cortical membranes, both agents produced concentration-dependent increases in specific binding of [^{3}H]MK-801. The maximal stimulatory effect produced by spermidine was significantly greater than that produced by Mg^{2+}. In the presence of a maximally-stimulatory concentration of spermidine, Mg^{2+} produced no further increase in [^{3}H]MK-801 binding. By contrast, in the presence of a maximally-stimulatory concentration of Mg^{2+}, spermidine dose-dependently produced a further four-fold increase in specific [^{3}H]MK-801 binding. Above their maximally stimulatory doses, both spermidine and Mg^{2+} caused dose-dependent inhibition of [^{3}H]MK-801 binding. The dose-response curve and maximal stimulatory effects of spermidine were not significantly altered in the presence of glycine. In the presence of a saturating concentration of L-glutamate, the spermidine dose-response curve was shifted to the left compared to the absence of L-glutamate. In the presence of L-glutamate, the addition of glycine shifted the dose-response curve of spermidine to the left but did not alter the maximal spermidine effect. Association studies indicate that the stimulatory effects of spermidine involve alterations in the kinetics of [^{3}H]MK-801 binding.

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86.18

POLYAMINES ENHANCE N-METHYL-D-ASPARTATE RECEPTOR FUNCTION IN VITRO AND IN VIVO. L.M. Pullan, L.C. Litwin*, R.J. Stumpo*, J.M. Goldstein, M. Britt*, and A.I. Salama. Dept. of Pharmacology, ICI Pharmaceuticals Group, ICI Americas Inc., Wilmington, DE 19897.

The polyamines spermine and spermidine have recently been reported to enhance [^{3}H] MK-801 binding. We report here the correlation of polyamine effects on [^{3}H] TCP binding in rat brain synaptosomes and on NMDA excitation in vivo in the red nucleus. The polyamines enhance the binding of [^{3}H] TCP, but do not displace [^{3}H] glycine binding to the strychnine insensitive glycine site associated with the N-methyl-D-aspartate receptor complex. The enhancement of TCP binding is additive to that seen with glycine and NMDA. The inhibition of TCP binding by the glycine antagonist 7-chlorokynurenic acid (7-Cl-Kyn) is not reversed by spermine or spermidine. Iontophoretically applied spermidine enhances the excitation induced by NMDA as seen with extracellular single unit recordings. The potentiation by spermidine of NMDA excitation is additive to maximally effective applications of D-serine. Thus, both in vitro and in the intact adult brain, polyamines enhance the function of the NMDA receptor, apparently at a unique site.

86.19

EFFECTS OF POLYAMINES ON THE BINDING OF [^{3}H]MK-801 TO THE NMDA RECEPTOR: AGONIST AND ANTAGONIST EFFECTS AT A PUTATIVE POLYAMINE RECOGNITION SITE. K. Williams, C. Romano and P.B. Molinoff. Dept. of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104-6084.

Experiments were carried out to examine the effects of polyamines on the binding of [^{3}H]MK-801 to membranes prepared from rat brain. Spermine and spermidine increase the affinity of NMDA receptors for [^{3}H]MK-801 in the presence of 100 μM L-glu and 100 μM gly. The effects of other polyamines on the binding of [^{3}H]MK-801 were determined in the presence of L-glu and gly in the absence and presence of 10 μM spermine. In the absence of spermine, $NH_2(CH_2)_3NH_2$, $NH_2(CH_2)_3NH(CH_2)_2NH_2$, and $NH_2(CH_2)_3NH(CH_2)_3NH_2$ (1-300 μM) increased the binding of [^{3}H]MK-801 in a manner similar to that of spermidine. It is concluded that these compounds are agonists at the polyamine site. Putrescine, cadaverine, $NH_2(CH_2)_2NH(CH_2)_2NH_2$, and $CH_3NH(CH_2)_3NHCH_3$, at concentrations of 1-100 μM, inhibited the binding of [^{3}H]MK-801 in the presence of spermine down to the level seen with only L-glu and gly, but did not affect binding of [^{3}H]MK-801 in the absence of spermine. The data indicate that these compounds are selective antagonists of the effects of spermine at the NMDA receptor. Several long-chain diamines including $NH_2(CH_2)_{10}NH_2$ were nonspecific inhibitors of the binding of [^{3}H]MK-801 in the absence or presence of spermine. The results suggest that there is a specific polyamine recognition site associated with the NMDA receptor complex and that this site is distinct from the binding sites for glutamate and glycine. *(Supported by a grant from the PEW Charitable Trusts).*

EXCITATORY AMINO ACIDS: RECEPTORS II

87.1

PRESYNAPTIC MODULATION OF GABA MEDIATED SYNAPTIC EVENTS BY GLYCINE IN IMMATURE RAT HIPPOCAMPAL SLICES
by I.L. Gaiarsa*, A. Coradetti*, E. Cherubini & Y. Ben Ari (SPON: G. Barbin. INSERM U-29, 123 Bd de Port-Royal, 75014 PARIS (France)

In neuronal cultures, glycine in micromolar concentrations potentiates NMDA currents by acting on an allosteric site of the receptor (Johnston and Ascher, Nature, 325, 529,1987). Although strychnine insensitive glycine binding sites are present in the hippocampus, bath applications of glycine have no effect on NMDA mediated events in adult hippocampal slices ; this is likely due to concentrations of endogenous glycine in the slice which saturate this site. We report now a powerful effect of glycine on synaptic mediated events in immature slices.

Intracellular recordings made from CA3 neurons in slices revealed during the first week of life the presence of spontaneous recurrent network driven -giant depolarizing potentials (GDPs), these are mediated by GABA which has an exclusively depolarizing action at this age -and modulated presynaptically by NMDA receptors (Ben Ari et al, J. Physiol. (London) 1989, In Press). Glycine (10-30 μM) and D serine (5-20 μM) considerably augmented the synaptic noise and frequency of GDPs. Glycine and D serine also considerably potentiated the effects of bath applications of NMDA, thus in presence of these agents, a subtreshold application of NMDA (1 μM) produced a large response. These effect was blocked by APV (30 μM), kynurenate (100-200 μM), 7 cl· kynurenate (10μM) but not by strychnine (1 μM). Glycine (up to 100 μM) had no effect on spontaneous activity or NMDA responses after P7. We conclude that glycine during the first week of life has a powerful potentiating effect on GABA mediated GDPs through the allosteric modulation NMDA receptor.

87.2

Glycine and glutamate regulation of the NMDA receptor-gated ion channel: allosteric interactions. Douglas W. Bonhaus, Geng-Chang Yeh* and James O. McNamara V.A. and Duke Medical Centers, Durham, NC 27705

Sustained activation of the N-methyl-D-aspartate (NMDA) receptor-gated ion channel requires the simultaneous presence of agonists for both the NMDA and glycine recognition sites. To investigate the mechanism by which glycine and NMDA receptor ligands interact to control channel activation we measured [3H]glycine and [3H]L-glutamate binding in a hippocampal membrane preparation. To examine the consequences of glutamate and glycine interactions on channel activation we measured the binding of TCP {[3H]N-(1-[thienyl] cyclohexyl) piperidine} (a ligand whose binding is dependent upon activation of the NMDA channel). This allowed direct comparison of receptor occupancy and channel activation in an identical preparation. As previously reported glycine was virtually an absolute requirement for NMDA stimulation of TCP binding. However, in this preparation, glycine had no effect on glutamate binding. These findings suggest that the mechanism by which glycine maintains NMDA evoked currents is not by regulating agonist binding to the NMDA recognition site. Rather, NMDA and glycine receptor ligands act by independent and mutually required mechanisms.

87.3

DIFFERENTIAL MODULATION OF THE NMDA RECEPTOR ASSOCIATED GLYCINE RECOGNITION SITE BY COMPETITIVE NMDA ANTAGONISTS.
W.F. Hood, R.P. Compton, J. Biesterfeldt* and J.B. Monahan. G.D. Searle R & D, CNS Diseases Research, St. Louis, MO 63198.

Recent studies have shown that NMDA responses may be modulated through an associated strychnine-insensitive [^{3}H]glycine binding site. Additionally, several reports indicate that competitive NMDA antagonists may negatively modulate interactions at this glycine recognition site. Using rat forebrain synaptic plasma membranes (SPM), we found that the binding of [^{3}H]glycine to the NMDA associated strychnine-insensitive glycine recognition site was differentially inhibited to a maximum of 35-50% by competitive NMDA antagonists. Those antagonists with a 5-carbon chain backbone (eg. CGS 19755, D-AP5, and 3-(2-carboxypiperazin-4-yl)-methyl-1-phosphonate) were potent displacers of [^{3}H]glycine binding whereas the 7-carbon analogs (eg. 4-methylphosphono phenylglycine, D-AP7, and CPP) were ineffective. The potency of 5-carbon antagonists for displacing [^{3}H]glycine binding correlated with their affinity at the NMDA recognition site. In addition, NMDA recognition site agonists (eg. L-glutamate and NMDA) were shown to reverse this antagonist induced inhibition of [^{3}H]glycine. In conclusion, the data suggest that competitive NMDA antagonists with 5-carbon and 7-carbon chain backbones differentially modulate the glycine recognition site. The difference may be due to either their binding at dissimilar pharmacophores or inducing distinct conformational changes through interactions at a common site.

87.4

EVIDENCE FOR FUNCTIONAL COUPLING OF THE GLYCINE AND [^{3}H]CGS 19755 RECOGNITION SITES.
R.P. Compton, W.F. Hood and J.B. Monahan. CNS Diseases Research, G.D. Searle & Co., St. Louis, MO 63198.

We report a functional coupling between the [^{3}H]CGS 19755 recognition site and the NMDA receptor associated strychnine-insensitive glycine recognition site. Glycine agonists (glycine, 1-aminocyclopropyl-1-carboxylate (ACC) and D-serine) inhibit [^{3}H]CGS 19755 binding to a maximum of 70% in a dose dependent manner over a range of 1-100 μM. This inhibition is reversible by glycine antagonists (1-aminocyclobutyl-1-carboxylate (ACBC) and HA-966) which alone have no effect on [^{3}H]CGS 19755 binding over the range of 1-100 μM.

These results complement our earlier findings concerning the interactions between the glycine and [^{3}H]3-(2-carboxypiperazin-4-yl)propyl-1-phosphonate ([^{3}H]CPP) recognition sites (Compton et al., Miami Winter Symposium 9:35, 1989). [^{3}H]CPP binding is stimulated by glycine antagonists (ACBC and HA-966) and is reversible by glycine agonists (ACC, D-serine and glycine). These findings add support for the functional coupling of the glycine and NMDA recognition sites. Furthermore, differences in the coupling of glycine agonist and antagonist recognition site(s) to the 5-(CGS 19755) and 7-carbon (CPP) NMDA antagonist recognition sites may exist.

87.5

[^{3}H]CGS-19755 BINDING TO NMDA RECEPTORS: INTERACTIONS WITH THE GLYCINE MODULATORY SITE.
W.F. White, P.B. Senatus*, S.A. Lipton, R.H. Loring, and E. Aizenman. Department of Neurology, The Children's Hospital & Harvard Medical School, and Department of Pharmacology, Northeastern University, Boston, MA 02115.

The binding of the NMDA receptor antagonist [^{3}H]CGS-19755 was studied in extensively washed membranes prepared from rat telencephalon. Fifteen minute incubations containing 10 nM [^{3}H]CGS-19755 were performed in 50 mM Tris-HCl pH 8.0 at 4°C. Non-specific binding was determined using 1 mM L-glutamate. Specific binding represented about 80% of total binding. Equilibrium binding analyses revealed a single population of sites with a $K_D = 51 \pm 6$ nM and a Bmax = 4.71 ± 0.71 pmoles/mg protein. Glutamate displacement curves were complex with an IC_{50} of 138 ± 84 nM. Glycine displacement curves were shallow suggesting multiple sites. Nonlinear least-squares curve fitting analyses suggested to populations of sites with IC_{50}'s of 139 ± 39 nM and 1.18 ± 0.04 mM; 37 ± 3% of the total [^{3}H]CGS-19755 sites were displaced with high affinity, and the remaining sites were displaced with low affinity. 7-chlorokynurenate (5 µM), an antagonist at glycine modulatory site, blocked the displacement produced by 1 µM glycine. These results suggest that [^{3}H]CGS-19755 binding is to a site or sites which are sensitive to modulation by compounds active at the glycine modulatory site but which appear to be distinct from the site(s) recognized by glycine and 7-chlorokynurenate. Supported by Morgan Memorial.

87.6

GLYCINE SITE AGONISTS OF THE NMDA RECEPTOR; THE ROLE OF HYDROGEN BONDING.
C.J.McBain*, N.W.Kleckner, S.Wyrick* and R.Dingledine, Department of Pharmacology and School of Pharmacy, University of North Carolina, NC, 27599-7365.

Voltage-clamp and molecular modelling studies highlighted five structural features important for activation of the glycine modulatory site on NMDA receptors in *Xenopus* oocytes injected with mRNA isolated from rat brain. First, the presence of sterically unhindered carboxyl- and amino-termini were essential. Second, an increase in the inter-terminal separation by greater than one carbon markedly attenuated potency. Third, activity at the modulatory site was stereoselective. Fourth, only small, sterically unobtrusive substitutions at the α- carbon could be tolerated. Finally, hydrogen bonding of the β-carbon substituent promotes activity. The hydroxyl of D-serine is envisioned as hydrogen bonding to an additional site on the receptor, since isosteric substitutions on the β-carbon incapable of hydrogen bonding are inactive. The position and size of the hydroxyl containing group is critical for activity. Glycine analogs capable only of proton acceptance (β-F-D-alanine and β-Cl-D-alanine) possess modulatory action, while those capable of only proton donation (D-cysteine, 1,2 diamino propionic acid) are inactive. Full dose-response curves were constructed for those analogs displaying >25% of the effect of glycine. Most compounds were nearly full agonists and had Hill coefficients ~1. The potency order was 1-amino-cyclopropane-carboxylic acid > glycine > D-serine > D-alanine > β-F-D-alanine > R(+)-cycloserine. These data offer compelling evidence that the active site of the glycine modulatory site is a small pocket containing at least three points of possible attachment; negative and positive ionic sites and an hydrogen bond donating site.

87.7

GLYCINE-LIKE MODULATION OF NMDA RECEPTORS BY A MONOCLONAL ANTIBODY THAT ENHANCES LONG-TERM POTENTIATION.
J.R. Moskal, P.K. Stanton and R. Haring, Dept. Neurosurgery, Neurology and Neuroscience, Albert Einstein College of Medicine, Bronx, N.Y. 10461.

A monoclonal antibody, B6, that modulates long-term potentiation in rat hippocampal slices was examined for its ability to interact with NMDA receptor-ionophore complexes (NMDAR). [^{3}H]-TCP (1-[1-(2-thienyl)cyclohexyl]-piperidine) binding to extensively washed hippocampal membranes has been shown to be specifically enhanced by NMDAR agonists and thus can serve as an index of functional activation of the NMDAR. 5 µg of affinity purified B6 per 100 µg of membranes gave a 2 to 3-fold elevation in TCP binding. No significant elevation in TCP binding was observed using rabbit IgG, bovine serum albumin or another affinity purified monoclonal antibody from the same panel as B6. When TCP binding was stimulated by the combined addition of maximal concentrations (50 µM) of glutamate, glycine and magnesium, no further stimulation of TCP binding was found upon the addition of B6. Like glycine, B6 enhanced the effect of NMDA and glutamate in stimulating TCP binding but it had no effect on the inhibition of TCP binding by AP5. Similar to the effect of NMDAR agonists, B6 increased the rate of association and dissociation of TCP binding but had no effect on the equilibrium binding of TCP. AP5 prevented these antibody effects. However, glutamate enhanced the effect of B6 whereas there was no effect of glycine on B6. Taken together these results strongly suggest that B6 binds directly to the NMDAR and that B6 displays properties similar to glycine.

87.8

GLYCINE ANTAGONIZES ETHANOL-MEDIATED INHIBITION OF NMDA-STIMULATED CALCIUM UPTAKE INTO PRIMARY CULTURES OF CEREBELLAR NEURONS.
C.S.Rabe and B.Tabakoff, Unit for Special Projects, NIAAA, Rockville, MD 20852.

We have shown that NMDA-stimulated $^{45}Ca^{2+}$ uptake in cultures of cerebellar neurons is quite sensitive to inhibition by ethanol (EtOH). Examination of the effects of EtOH on NMDA-stimulated cGMP formation in these cultures suggested that glycine-NMDA interactions may be particularly sensitive to inhibition by EtOH (Hoffman et al., in press). Since NMDA-stimulated cGMP formation is believed to be the result of NMDA-stimulated calcium influx, we have now directly examined the effects of glycine on EtOH-mediated inhibition of NMDA-induced $^{45}Ca^{2+}$ uptake. In the absence of added glycine, EtOH (50 mM) caused approximately a 40% decrease in NMDA (50 µM) stimulated (Mg^{2+}-free buffer) $^{45}Ca^{2+}$ uptake. As progressively larger concentrations of glycine were added to the assay medium, the amount of inhibition of NMDA-stimulated $^{45}Ca^{2+}$ uptake produced by 50 mM EtOH decreased. The threshold for antagonism of the EtOH (50 mM) mediated inhibition was approximately 300 nM glycine and essentially complete antagonism was observed at 100 µM glycine. These results suggest that endogenous levels of glycine acting as a cotransmitter may affect the amount of EtOH-induced inhibition of NMDA-mediated events evidenced in the brain.

Additional studies examining the selective effects of sedative/hypnotics on NMDA and kainate responses revealed that EtOH but not the barbiturates (pentobarbital or phenobarbital) or the benzodiazepine, flurazepam, demonstrated preferential inhibition of NMDA- over kainate-stimulated $^{45}Ca^{2+}$ uptake into cerebellar neurons in culture.

87.9

GLYCINE AND SEROTONIN MODULATE NMDA-INDUCED EXCITATION IN CEREBELLAR PURKINJE CELLS. J.G. Netzeband*, J.C. Strahlendorf and H.K. Strahlendorf. Depts. of Physiology and Neurology, Texas Tech Univ. Health Sciences Center, School of Medicine, Lubbock, TX 79430.

Actions of glutamate have been linked via the N-methyl-D-aspartate (NMDA) receptor to learning, neurotoxicity and epilepsy. Glycine has been shown to allosterically modulate the NMDA receptor in *in vitro* preparations. We have used extracellular recording and iontophoretic drug application in urethane-anesthetized male rats to support recent findings that cerebellar Purkinje cells (PCs) contain NMDA receptors. Glycine (+5nA) potentiated (36±6%; mean+S.E.M.) NMDA-induced excitations in 9 of 17 cells tested and attenuated (43±9%) the response in the remaining 8 cells. Cells in which glycine potentiated the NMDA-induced excitation showed a significantly higher predrug firing frequency (44±4 Hz) than those responding with attenuation (18±3 Hz) as shown by a t-test ($p<0.05$). In addition, analysis by linear regression showed that with increasing firing rates, higher percentages of cells responded to glycine with potentiation of the NMDA-induced excitation ($r=0.98$). In other experiments, we have shown that serotonin (5-HT) potently attenuates (41±7%; 22 of 25 cells) NMDA-induced excitation in PCs. These results represent a functional demonstration of glycine modulation of the NMDA response in an *in situ* preparation and further supports that NMDA receptors do exist in PCs. This is the first demonstration that glycine modulation of the NMDA response is rate-dependent and together with the 5-HT data provides strong evidence that the NMDA receptor is under complex, multiple control mechanisms. Supported by NS19296.

87.10

CHANGES IN BINDING DENSITY OF N-METHYL-D-ASPARTATE (NMDA) RECEPTORS PRODUCED BY GLYCINE AND 7-CHLORO-KYNURENATE. **S.M. Jones, L.D. Snell* and K.M. Johnson. Dept. Pharmacol. & Toxicol., Univ. Texas Med. Branch, Galveston, TX 77550.**

Recent reports have stated that agonists at the strychnine-insensitive glycine site associated with the NMDA receptor increase the apparent affinity of ^{3}H-L-glutamate binding (GLU; Fada et al., Neuropharm. 27: 1183, 1989) and, conversely, that agonists at the NMDA recognition site increase the apparent affinity of ^{3}H-glycine binding (GLY; Kessler et al., J. Neurochem. 52: 1319, 1989). We have found that the binding of NMDA-specific ^{3}H-L-GLU and ^{3}H-GLY can both be assayed in a "buffy coat" preparation of rat cerebral cortices. Membranes were incubated in the presence of 10 nM ^{3}H-GLU or 20 nM ^{3}H-GLY for 30 min at 4°C in 50 mM Tris acetate (pH 7.4) and then rapidly filtered over GF/C filters using a Brandel M-24 cell harvester.

The selective NMDA antagonist 3-((±)-2-carboxypiperazin-4-yl)propyl-1-phosphonate (CPP), displaced ^{3}H-GLY binding with similar affinity as that reported for the NMDA site (K_i=2μM; Hill slope (nH)=1.0) and at 100μM produced a 24% decrease in the density of ^{3}H-GLY binding sites while not altering the apparent affinity. Agonists at the NMDA receptor (L-GLU, L-homocysteate) did not produce a significant change in ^{3}H-GLY binding but were able to reverse the effect of CPP. Glycine (10μM) produced a 20% increase in the B_{max} of ^{3}H-GLU binding while 10μM 7-chlorokynurenate (7Cl-KYN) produced a 37% decrease in B_{max}. Neither compound significantly changed the apparent K_D. 7Cl-KYN was a selective antagonist at the glycine site (K_i=0.49μM; nH=0.74) relative to the NMDA site (K_i=113μM; nH=0.88). Addition of high concentrations of CPP or L-GLU did not alter the K_i value or the nH for 7Cl-KYN displacement of ^{3}H-GLY binding. These data are consistent with, but do not directly support, the hypothesis that glycine converts a subpopulation of antagonist preferring receptors in the cortex to agonist preferring receptors (Monaghan et al., PNAS 85: 9836, 1988). Supported by DA-02073.

87.11

D-CYCLOSERINE: A PARTIAL AGONIST AT THE GLYCINE SITE OF THE RAT N-METHYL-D-ASPARTATE (NMDA) RECEPTOR. T. H. Lanthorn, G. B. Watson, M. P. Baganoff*#, C. L. Deppeler*#, and M. A. Bolanowski*# (SPON: S. D. Hess) CNS Disease Research, G. D. Searle & Company, and #Biological Sciences, Monsanto Company, 700 Chesterfield Village Parkway, St. Louis, MO 63198.

D-cycloserine (DCS) has been reported to exhibit the binding profile of a partial agonist at the glycine modulatory site of the NMDA receptor/channel complex (Hood, *et al.*, 1988). Others have reported that DCS behaves as a full agonist at this site (Johnson, *et al.*, 1988). We have examined the effect of DCS on functional NMDA receptors expressed in *Xenopus laevis* oocytes injected with rat brain mRNA. In voltage clamped oocytes, responses to 100 μM NMDA were potentiated by glycine (EC50=0.60±0.16 μM). NMDA responses were also potentiated by DCS (half-maximal response at 4.8±1.2 μM), however, the amplitude of these responses plateaued at only 40% of that observed in the presence of glycine. Furthermore, in the presence of 3 μM glycine, DCS dose-dependently antagonized NMDA responses to approximately 40% of levels found in glycine alone. From these observations, we conclude that DCS has the characteristics expected for a partial agonist at the glycine site of the NMDA receptor /channel complex.

Hood, *et al.*, (1988), Neuroscience Letters, **98**, 91.
Johnson, *et al.*, (1988), Front. Exc. Amino Acid Res., pg. 551.

87.12

CYCLOLEUCINE BLOCKS NMDA RESPONSES IN CULTURED HIPPOCAMPAL NEURONS UNDER VOLTAGE CLAMP: ANTAGONISM AT THE STRYCHNINE-INSENSITIVE GLYCINE RECEPTOR. N. Hershkowitz and M.A. Rogawski (SPON: K.L. Kelner). Medical Neurology Branch, NINDS, NIH, Bethesda, MD 20892.

Radioligand binding studies have demonstrated that the neutral amino acid cycloleucine (CL) may act as a competitive antagonist at the glycine modulatory site on the *N*-methyl-D-aspartate (NMDA) receptor complex. In the present study we examined the effects of cycloleucine on NMDA-evoked inward current responses in dissociated hippocampal neuronal cultures using the whole cell voltage-clamp technique. In the presence of 1 μM glycine, CL caused a reversible, dose-dependent inhibition of NMDA responses with an IC_{50} of 24 μM. An increase in glycine to 100 μM resulted in a shift to the right of the CL concentration-effect curve (IC_{50}, 1.4 mM). However, with CL concentrations less than or equal to 100 μM, a fraction of the block could not be overcome by glycine even at concentrations as high as 1 mM. The CL block was unaffected by shifts in the holding potential (-60 to +60 mV), and there was no effect of CL on the reversal potential of the NMDA-evoked current. CL failed to affect kainic acid and quisqualic acid evoked currents at concentrations which inhibited NMDA responses. We conclude that cycloleucine is a potent and selective antagonist of NMDA-receptor mediated responses. Although this effect occurs in part via competitive antagonism at the glycine modulatory site, the CL block cannot be completely reversed by glycine indicating an interaction with an additional site on the receptor-channel complex.

Dr. Hershkowitz was a research associate of the NRC.

87.13

GLYCINE ANTAGONISTS CHARACTERIZED AT THE N-METHYL-D-ASPARTATE RECEPTOR COMPLEX. B.A. Meiners, L.M. Pullan, R.A. Keith, M. Britt*, A.B. Klika*, T.J. Mangano*, R.J. Stumpo* and A.I. Salama. Dept. of Pharmacology, ICI Pharmaceuticals Group, ICI Americas Inc., Wilmington, DE 19897.

The antagonism of N-methyl-D-aspartate (NMDA) responses by 7-chlorokynurenic acid (7-Cl-Kyn) and HA-966 (1-hydroxy-3-amino-pyrrolidone-2) has been suggested to occur via the glycine modulatory site of the NMDA receptor complex. We explore here glycine antagonism and the interactions between sites of the receptor complex. HA-966 and 7-Cl-Kyn displace [3H]glycine binding to a strychnine insensitive site. Glycine stimulated [3H]TCP binding is inhibited by 7-Cl-Kyn and HA-966 with potencies greater than on [3H]TCP binding in the absence of added glycine. 7-Cl-Kyn and HA-966 also inhibit NMDA evoked [3H]norepinephrine release from hippocampal slices. The inhibition by 7-Cl-Kyn or HA-966 of glycine stimulated [3H]TCP binding and of NMDA evoked [3H]norepinephrine release is reversible by glycine, D-serine, or L-serine. HA-966 caused an enhancement (up to 2 fold) of the binding of the NMDA antagonist [3H]CPP. The increase in [3H]CPP binding could be reversed by added glycine, suggesting mediation via the glycine recognition site. Thus, 7-Cl-Kyn and HA-966 appear to function as glycine antagonists on the NMDA receptor complex, presumably displacing endogenous glycine and modulating the recognition site for NMDA agonists and antagonists.

87.14

ACTIONS OF GLYCINE ANTAGONISTS ON RAT N-METHYL-D-ASPARTATE (NMDA) RECEPTORS EXPRESSED IN *XENOPUS LAEVIS* OOCYTES. G. B. Watson, M. A. Bolanowski*#, M. P. Baganoff*#, C. L. Deppeler*#, and T. H. Lanthorn, CNS Disease Research, G. D. Searle & Company, and #Biological Sciences, Monsanto Company, 700 Chesterfield Village Parkway, St. Louis, MO 63198.

1-amino-cyclobutyl-1-carboxylate (ACBC) and 1-hydroxy-3-aminopyrrolid-2-one (HA-966) have the binding profile of specific ligands at the glycine modulatory site of the NMDA receptor/channel complex. Additionally, HA-966 has been shown to be a functional glycine antagonist in slice and cultured neuron preparations. We have extended this observation by showing that ACBC and HA-966 antagonize functional NMDA receptors expressed in *Xenopus laevis* oocytes injected with rat brain mRNA. Voltage clamped oocytes responded to NMDA, in the presence of 10 μM glycine, in a dose-dependent manner (EC50=27±2 μM). Responses to NMDA were blocked competitively (EC50 shifted to127±21 μM) by 10 μM D-2-amino-7-phosphonoheptanoate (D-AP7). ACBC and HA-966 noncompetitively blocked responses to 100 μM NMDA, as evidenced by the inability of increasing concentrations of NMDA to overcome antagonism by 300 μM ACBC or 100 μM HA-966. Potentiation of responses to 100 μM NMDA by glycine was dose-dependent (EC50=0.60±0.16 μM). This potentiation was blocked in a competitive manner by 100 μM ACBC (EC50=3.2±0.8 μM) or 100 μM HA-966 (EC50=6.1±2.1 μM). We conclude that ACBC and HA-966 block NMDA mediated responses in a noncompetitive manner through a competitive interaction at the glycine modulatory site of the NMDA receptor complex.

87.15

CHARACTERIZATION OF MDL 28,469 AND MDL 29,695 AS SELECTIVE GLYCINE ANTAGONISTS WITH POTENT ANTICONVULSANT ACTIVITY.

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HA-966 and 7-Cl kynurenic acid have been described as selective glycine antagonists. IC50 values vs. [^{3}H]CPP were > 100 μM and 75 μM, and vs. [^{3}H]glycine were 18 μM and 0.4 μM, respectively. MDL 28,469 and MDL 29,695 were synthesized as selective glycine antagonists. Respective IC50 values vs. [^{3}H]CPP were 709 μM and 4500 μM and vs. [^{3}H]glycine were 25.1 μM and 16 μM. All compounds inhibited NMDA-stimulated [^{3}H]NE release and cGMP accumulation in rat brain slices. The inhibition was not competitive with respect to NMDA but was reversible with glycine. Upon i.c.v. administration, HA-966, 7-Cl kynurenic acid, MDL 28,469 and MDL 29,695 antagonized quinolinic acid-induced seizures in mice (ED50 values were 276, 13.8, ~ 81, 16.7 nmoles, respectively) and antagonized sound-induced seizures in DBA/2J mice (ED50 values were 43.2, 6.4, 26.6, 13.3 nmoles, respectively). These results demonstrate that the selective glycine antagonists MDL 28,469 and MDL 29,695 may have potential therapeutic value in the treatment of seizure disorders.

87.16

GLYCINE ANTAGONISTS AND ZINC INHIBIT [^{3}H]MK-801 BINDING IN RAT BRAIN. S.Y. Sakurai, J.B. Penney and A.B. Young. Neuroscience Program and Dept. Neurology, Univ. Michigan, Ann Arbor, MI 48104-1687.

The N-methyl-D-aspartate (NMDA) receptor-ion channel complex is thought to possess regulatory sites for magnesium, phencyclidine (PCP), glycine and zinc (Zn^{2+}). [^{3}H]MK-801 binds to the PCP site within the associated cation channel. The modulation of [^{3}H]MK-801 binding to the PCP site by ligands which bind to several of these regulatory sites was investigated in rat brain using an in vitro quantitative autoradiographic assay.

[^{3}H]MK-801 binding was inhibited by compounds thought to be acting at the strychnine-insensitive glycine binding site in all brain regions examined. The glycine antagonists, 7-chlorokynurenic acid and kynurenic acid, inhibited [^{3}H]MK-801 binding with IC_{50} values of approximately 10 μM and 100 μM respectively, while HA-966 (1 mM) was not an effective inhibitor of [^{3}H]MK-801 binding. The quinoxalinedione compounds, DNQX and CNQX inhibited [^{3}H]MK-801 binding with IC_{50} values of 10 μM and 30 μM, respectively. The inhibition of [^{3}H]MK-801 binding by CNQX and DNQX was reversed by glycine. Finally, Zn^{2+} inhibited [^{3}H]MK-801 binding with an approximate IC_{50} value of 100 μM in all brain regions examined. Supported by USPHS grants NS 19613 and 15655 and a Merck Faculty Development Award.

87.17

ANTAGONIST ACTIONS OF KYNURENATE AND QUINOXALINE DERIVATIVES AT THE STRYCHNINE-INSENSITIVE GLYCINE SITE OF THE N-METHYL-D-ASPARTATE RECEPTOR. S.L. Pedrotti*, R.A. Zubrowski*, J.W. Ferkany and P.V. Kaplita. Nova Pharmaceutical Corp., Baltimore., MD 21224.

Glycine enhances the actions of glutamate at the N-methyl-D-aspartate (NMDA) receptor, including the induced binding of [^{3}H]N-(1-[2-thienyl]-cyclohexyl)-3,4-piperidine ([^{3}H]TCP) to the phencyclidine (PCP) site, via an interaction with a strychnine-insensitive site. We have evaluated the abilities of several kynurenate and quinoxaline derivatives to inhibit both [^{3}H]glycine binding and glycine-enhanced, NMDA-induced [^{3}H]TCP binding in rat cortical membranes. [^{3}H]Glycine binding was inhibited by 7-chlorokynurenate (7-ClKY, K_i=1.1 μM), 6,7-dichloro-3-hydroxy-2-quinoxaline carboxylic acid (DCHQX, K_i=1.4 μM), 6,7-dinitroquinoxaline-2,3-dione (DNQX, K_i=3.9 μM) and 6,7-dichloroquinoxaline-2,3-dione (DCQX, K_i=6.4 μM). 7-ClKY, DCHQX, DNQX and DCQX also antagonized glycine-enhanced, NMDA-induced [^{3}H]TCP binding (IC_{50}s=30-100 μM). Increasing concentrations of 7-ClKY and DCHQX (1-30 μM) right-shifted the concentration response curves for glycine in enhancing [^{3}H]TCP binding in a parallel fashion, with no effect on the maximum response. DNQX and DCQX (3-100 μM) attenuated the maximum response to glycine. The quinoxalines did, however, exhibit affinity for kainate and quisqualate receptors as well. Thus the data suggest that kynurenate and quinoxaline derivatives are novel, albeit nonselective, antagonists at the NMDA receptor-associated glycine site.

RETINA II

88.1

LIGHT INDUCED MEMBRANE CONDUCTANCE CHANGES OF PINEAL PHOTORECEPTORS. P.L.Marchiafava*, C.Kusmic* and E.Strettoi*. (SPON: G. Berlucchi). Dip. Fisiol. e Biochim., Univ. Pisa; Ist. Neurofis. CNR, Pisa, 56100-Italy.

As an initial step to investigate the phototransduction mechanism of pineal photoreceptors we have measured the membrane conductance changes associated with their hyperpolarizing response to a 50 msec flash. A single electrode voltage-clamp technique was used on pineal photoreceptors of the trout Salmo irideus. Current-voltage relations measured at the peak of the photocurrent between +/-40 mV holding potential indicated a decrease of membrane conductance of about 20 per cent relative to the dark values (membrane resistance: 460 MΩ +/- 120 s.d.). The reversal potential of the photocurrent was extrapolated at ca. 50 mV above the dark membrane potential (-19 mV +/- 4 s.d.). These results suggest that an ionic mechanism similar to that in retinal photoreceptors may be involved. The recorded cells were identified at the electron microscope by intracellular injection of Lucifer yellow followed by photoconversion.

88.2

EXTRAOCULAR PHOTORECEPTORS IN THE PERIOCELLAR EPITHELIUM OF LOCUSTA MIGRATORIA ?

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The median ocellus of is Locusta migratoria is surrounded by a two-layered epithelium, the periocellar epithelium (POE), which forms two elongated horns reaching to the lateral ocelli. In this POE numerous tiny structures stain intensely with an antiserum against histamine. They have an elongated bean-like shape with a diameter of ca. 0.5 um, length ca. 3 um, and contain a nucleus. They are directed towards the cuticle and connected with finger-like processes to large cells of the inner row. Upon potassium induced depolarisation they release histamine, this is partially blocked in the presence of cobalt. Intraocellarly injected Lucifer yellow is selectively taken up by them which shows a connection between them and the ocellus. Further evidence for a possible role as additional photoreceptors derives from the following: 1. Histamine is also the transmitter of the ocellar photoreceptors, 2. The POE is restricted to areas where the cuticle forms lens-like structures and is transparent, 3. Pigment migration could be observed in the POE.

88.3

A MUTATION AT THE DROSOPHILA fused rhabdomeres (fur) LOCUS AFFECTS RHABDOMERE MORPHOLOGY AND PHOTORECEPTOR FUNCTION A. Blake*, P.M. O'Day, T.R. Venkatesh*, Institutes of Neuroscience and Molecular Biology, University of Oregon, Eugene, Oregon 97403

In the Drosophila compound eye the plasma membrane of each photoreceptor cell is elaborately infolded to form the rhabdomeres. These structures are studded with the primary photopigments, run the length of each retinal cell, and are the region in which photo-transduction occurs. We are interested in the development of the rhabdomeres and their role in retinal function. We have characterized fur, a new P element-induced mutation that causes fusion of the rhabdomeres in neighboring photoreceptor cells and compaction of the rhabdomere structure on the longitudinal axis. fur has been localized to the proximal region of the X chromosome by recombination and deficiency mapping. Mutations at fur also produce several distinct physiological abnormalities, including a 10-fold decrease in retinal sensitivity to light relative to wild-type and the absence of a prolonged depolarizing afterpotential. The loss of sensitivity is wavelength-independent. It has been possible to generate partial and complete revertants of fur. Complete revertants show restoration of both structural and functional deficits, suggesting that the mutant phenotype is indeed the result of a P element insertion. We are in the process of cloning the fur locus via its P element tag.

88.4

PROPERTIES OF RETINAL GUANYLATE CYCLASE. Y. Horio* and F. Murad** (SPON: R. S. Eisenberg). Dept.of Pharmacology, Northwestern Univ. Med. Sch., Chicago, IL 60011, *Pharmaceutical Division, Abbott Laboratories, Abbott Park, IL 60064.

Light triggers a cascade of reactions in retinal rod cells that leads to the amplified breakdown of cyclic GMP (cGMP) and the generation of a neural impulse. Guanylate cyclase (GC) of bovine rod outer segments (ROS) is entirely a particulate isoenzyme associated with membranes. Previous attempts to solubilize ROS GC have been unsuccessful. We dissociated this enzyme from ROS using high concentrations of KCl and Triton X-100. More than 80 % of total GC activity was recovered in the supernatant fraction after centrifugation (104,000g, 1hr), when ROS GC was treated with 1 M KCl and 0.5% Triton X-100. The dissociation by KCl and Triton X-100 was not observed with lung particulate GC (1) and intestine particulate GC (1). Additionally, atrial natriuretic factor(ANF), E.coli heat stable enterotoxin(ST) and ATPγS that activate lung and intestine particulate GC could not activate ROS GC. These results show that ROS GC is a different isoenzyme from that associated with ANF receptor and ST receptor. When dissociated ROS GC was analyzed by gel filtration TSK SW-4000 column, three peaks (1,200 kD, 1,000 kD and 710 kD) and one minor peak (580 kD) were observed, which indicates that the dissociated ROS GC is part of large complexes with other proteins, sugars and/or lipids.

(1) S. A. Waldman and F. Murad Pharmacol Rev., 39:163, 1987.

88.5

CYCLIC GMP ANALOGUES BLOCK LIGHT-INDUCED ELONGATION IN ISOLATED TELEOST ROD INNER AND OUTER SEGMENTS. B. A. Liepe* and B. Burnside*(SPON: G. Jacobs). Dept. of Physiology-Anatomy. University of California at Berkeley, Berkeley, California. 94720

Teleost rod photoreceptors change cell length in response to changing light intensities. In the light rods elongate and in the dark they contract. These movements are mediated by the inner segment myoid. To examine intracellular signals that mediate the signal transduction process from light absorption to the onset of motility we are studying mechanically detached rod fragments containing inner and outer segments (RIS-ROS) from retinas of the fish, *Lepomis cyanellus*. RIS-ROS consist of outer segment, ellipsoid, and myoid. When cultured in the light RIS-ROS myoids elongate 16.1 ± 0.9μ (Mean ± SEM, n=12). Dark cultured RIS-ROS myoids elongate only 3.6 ± 0.5μ (n=12). We show light-induced RIS-ROS elongation can be blocked 70-80% by millimolar concentrations of 8-Bromo-cyclic GMP or Dibutyryl-cyclic GMP in the presence of 0.25mM 3-isobutyl-1-methylxanthine (IBMX). In 0.25mM IBMX RIS-ROS elongation was not affected; however at higher dosages (0.5 and 1.0mM) IBMX produced a 40-60% block of light-induced elongation in RIS-ROS. Cyclic GMP analogues and phosphodiesterase inhibitors have been shown to depolarize vertebrate rods. Thus we tested the effects on rod motility of K^+ concentrations shown to block light-induced hyperpolarization in vertebrate rods (27-54mM). These concentrations of K^+ produce a 50-60% block of light-induced RIS-ROS elongation. Our results show that light-induced rod motility is blocked both by agents which prevent light-induced fall in cGMP and by agents which prevent light-induced hyperpolarization. These observations suggest that light-activation of rod motility requires membrane hyperpolarization. (supported by NIH grant EY03575).

88.6

CALCIUM/CALMODULIN-DEPENDENT PROTEIN KINASE IN LIMULUS PHOTORECEPTORS. B.G. Calman*, S.C. Edwards, A.C. Wishart*, and B.-A. Battelle, Whitney Laboratory, University of Florida, St Augustine, FL 32086.

A 46 kD protein in Limulus photoreceptors is a substrate for light- and Ca-dependent phosphorylation (Edwards et al., 1989; Wiebe et al., 1989). Here we report that the light-stimulated phosphorylation of this protein is blocked by the calmodulin inhibitor calmidazolium (CZ) at 30 μM, and present an initial characterization of calcium-calmodulin dependent protein kinase (Ca/CAM PK) in Limulus photoreceptors.

^{125}I-calmodulin binds in a Ca-dependent manner to 8 bands in Western blots of photoreceptor proteins. One of these bands, M_r=55 kD, is recognized by a monoclonal anti-body against vertebrate Ca/CAM PK II (from Dr. H.S. LeVine III) and runs in the same position in SDS-PAGE as a Ca-dependent phosphoprotein. Ca/CAM PK activity was assayed in homogenates of lateral eye and ventral photo-receptors using an exogenous peptide substrate, syntide-2. The calmodulin inhibitors CZ (10 μM), mastoparan (3 μM), and CGS 9343B (30 μM) block >90% of Ca/CAM-stimulated activity in this assay. The protein kinase C inhibitor H-7 does not lower activity at concentrations up to 100 μM.

These studies indicate that a Ca/CAM PK is present in Limulus photoreceptors and suggest it may mediate some of the effects of calcium on photoreceptor function. Supported by NSF (BNS 86-07660) and the Whitney Laboratory.

88.7

RECOVERY OF BLEACHING ADAPTATION IN PHOTORECEPTORS AND THE cGMP TRANSDUCTION CYCLE. K.N. Leibovic and J. Bandarchi*. Dept. of Biophysics, SUNY/Buffalo, Buffalo, NY 14214.

The objective of this study concerns the mechanisms of bleaching adaptation in vertebrate rods and their effects on response threshold and amplitude. Our method uses isolated rods-in which there is no regeneration of bleached pigment-from the retina of Bufo marinus. The rods are held in a suction electrode and the current responses to flashes of increasing intensity are recorded both in the dark adapted state and after bleaching a fraction of the native rhodopsin. The responses are obtained in Ringer solution and compared with those in a maintainance medium (KNL medium). We have found that the dark adapted responses are the same in Ringer and in medium. After bleaching 30% of the pigment the cell recovers to a steady state in about 45 minutes when in Ringer. Its threshold is elevated by about 1.7 log units above the dark adapted value and its response amplitude is reduced to about 50%. By contrast, after 30% bleaching in medium it takes some 90 minutes to reach a steady state; the response amplitude recovers completely and the threshold is elevated only about 0.7 log units. We have determined the necessary and sufficient components in our medium which produce these effects. They are biotin, pyruvate and elevated dextrose. Our results imply that adaptive metabolic processes exert control at focal points in the transduction cycle, restoring free cGMP after bleaching and increasing sensitivity.

88.8

SPECIFICITY AND SELECTIVITY OF POLYCLONAL ANTISERA AGAINST COMPONENTS OF THE NEURAL AND VISUAL TRANSDUCTION PATHWAYS IN RAT AND HUMAN RETINA HISTOLOGIC SECTIONS. J.J. Erdos*, H. Tamir*, C.J. Barnstable*, and J.K. Northup*. (SPON: J.R. Cooper) Depts. of Pharmacology and Opthalmology, Yale Univ. Sch. of Med., New Haven, CT. 06510

Polyclonal antisera were raised in rabbits specific for purified components of the visual and neuronal signal transduction pathways; the α and β/γ subunits of transducin, (G_t-2, G_tβ-11), retinal cyclic GMP phosphodiesterase, (PDE-4), the α subunits of the GTP binding proteins G_o and G_p (G_o-1, G_p-1) and the β/γ subunits common to these proteins, (β-6). Dissected retinas were fixed, sectioned, and preincubated in 5% goat serum before washing and incubation with the above primary antisera at dilutions of 1:500. The retinas were washed and reincubated in secondary antibody as described Hicks, D., and Barnstable, C.J., J. Histochem. Cytochem. 35:1317-1328, 1987.

In rat and human retina Gt-2 specifically labelled the photoreceptor (PR) while β-11 was selectively reactive with the PR and rod outer segments (ROS). In rat PDE-4 only labelled ROS. G_o-1 labelled bipolar cells preferentially while β-6 reacted with cell bodies, axons, synaptic terminals but not ROS. Similarly in rat retina G_p-1 labelled the synaptic layer as well as bipolar cells.

At day three of development rat retinal photoreceptors were reactive for G_t-2 but not β-11 suggesting that in development the α and β subunits of transducin may be differentially expressed. The presence of G_p-1 immunoreactivity in cells of the retinal synaptic layer suggests that the low molecular weight protein G_p is a component of visual neuronal transduction cells and may be involved in the visual transduction process.

88.9

EFFECTS OF MILD SYSTEMIC HYPOXIA ON $[H^+]_o$ OUTSIDE ROD PHOTORECEPTORS IN CAT. F. Yamamoto* and R.H. Steinberg. Depts. of Physiol. and Ophthalmol., Univ. of Calif., San Francisco, CA 94143.

Measurements of $[H^+]_o$ by intraretinal microelectrodes showed that pH outside dark-adapted rods (subretinal space) is relatively acidic and is elevated by as much as 0.2 pH units by light (Borgula, G. A., and Steinberg, R. H., Suppl. IOVS, p.289, 1984). We studied the effect of mild systemic hypoxia (P_aO_2 40-90 mmHg) produced by adding N_2 to the inspired gas. In the dark, any measurable drop in P_aO_2 below the normoxic level (range 90-110 mmHg) further decreased subretinal pH. Depth profiles located the maximum decrease to rod inner segments (about 70-85% retinal depth). Its magnitude increased with degree of hypoxia, reaching .06 pH units at a P_aO_2 of 46 mmHg. Light reduced the size of this maximum acidification, and completely blocked it at about 0.5 log units above rod saturation. These data suggest that glycolysis occurs in dark-adapted cat rods, and is increased by any drop in P_aO_2 below the normoxic level, and is decreased by light. The data can be viewed in the context of other evidence indicating that to support the dark current the actively metabolizing rod inner segments exist in an hypoxic environment, even during systemic normoxia (Linsenmeier, R. A. and Steinberg, R. H., J. Gen. Physiol., 84:945, 1984; Linsenmeier, R. A., J. Gen. Physiol., 88:521, 1986).

In the dark, addition of oxygen to the breathing mixture led to an increase in subretinal pH, which was maximal at a hyperoxic P_aO_2 of 150-200 mmHg. This effect is interpreted as an inhibition of glycolysis by the local increase in PO_2 outside rods (Pasteur effect), which has been measured in hyperoxia (Linsenmeier, R.A. and Yancey, C. M., IOVS, 30:612, 1989).

88.10

IONIC MECHANISMS OF THE PROLONGED DEPOLARIZATION IN CONES AND RODS. W. B. Thoreson, D. A. Burkhardt, J. Gottesman & S. Zhang. Departments of Physiology & Psychology, Neuroscience Program, Univ. of Minnesota, Minneapolis, MN 55455.

The "prolonged depolarization" is a regenerative, voltage-sensitive response, accompanied by a conductance increase, which can be evoked by the injection of depolarizing current. We have investigated the ionic mechanisms which underlie the prolonged depolarization in intact turtle cones using intracellular recording in the superfused eyecup.

The prolonged depolarization involves activation of a voltage-sensitive Ca channel. It is enhanced by high Ca or Sr, depressed by low Ca, and blocked by Co. The organic Ca channel blockers diltiazem and D600 do not affect the prolonged depolarization. In contrast, D600 blocks Ca-dependent spikes in turtle cones. This suggests that turtle cones possess more than one type of Ca channel.

The prolonged depolarization also requires the presence of intracellular Ca. EGTA (250mM) in the recording electrode abolishes the response, whereas it persists when the electrode contains 100mM CaCl2. TEA, 4-AP, and ion substitution experiments indicate that K and Na fluxes are not responsible for the prolonged depolarization.

The response depends on the chloride gradient. It is enhanced by superfusion with Cl-free media and more evident when using KCl, rather than K methylsulfate or K acetate, as an electrolyte.

Rods in the toad generate a prolonged depolarization which is clearly similar to that of turtle cones. The response may thus reflect conductance mechanisms common to the inner segments of both rods and cones. Our evidence suggests that the prolonged depolarization is initiated by the activation of voltage-sensitive Ca channels, and that the resulting Ca influx activates a long-lasting Cl conductance.

88.11

DEVELOPMENT OF CALCIUM CURRENTS IN CULTURED PHOTORECEPTORS. E.L. Gleason, P. Mobbs* and M. Wilson. Dept of Zoology. University of California, Davis, Ca 95616.

Ribbon synapses are the major synapse type found between photoreceptor and second order cells in the vertebrate retina. We have previously shown that cone cells dissociated from embryonic day 8 chick retinae begin to form ribbon synapses by embryonic equivalent (E.E) day 12 (Gleason and Wilson, Soc. Neurosci. Abstr. 14: 1988). In this study, we asked when calcium currents develop in cone cells put in culture on day 8.

Patch clamp recordings obtained from cultured cone cells (E.E. 9 - E.E.18) in the whole-cell configuration showed large outward currents activated positive to -20 mV which were almost completely blocked by an internal solution containing Cs^+ (160 mM) and TEA (5 mM) and were therefore thought to be carried by K^+. These outward K^+ currents were present from E.E. 9 onwards. When outward currents were blocked, a small inward current could be detected between - 30 and +50 mV. The inward current we believed to be a calcium current as it was enhanced in 10 mM $[Ba^{++}]_o$ and blocked completely by 2 mM $[Co^{++}]_o$. The calcium current was extremely rare (2.7% of cells examined) on E.E. 9 and 10 but became more common with time in culture (E.E. 12, 30%; E.E. 14, 64% and E.E. 16, 88%). At all ages, the calcium current activated positive to -30 mV and reached a peak between +7 and +35 mV. Full activation of the current occurred within 8 msec and no inactivation was observed during a 50 msec voltage step. The mean maximum of the subtracted current ($I_{Ba++} - I_{Co++}$) was 31.3 pA but varied greatly from cell to cell (S.D.=15.1 pA). Neither the age nor the size of the cell (on average, capacitance values of cone cells increased with time in culture) was correlated with the magnitude of a cell's peak inward current. We conclude that the full complement of a cell's Ca^{++} channels become functional over a brief period, probably less than one day.

88.12

CRITICAL ANALYSIS OF A KINETIC MODEL FOR PHOTOTRANSDUCTION IN CONES. [1,2]D. Tranchina* and [2]J. Sneyd* (SPON: K. Purpura). [1]Dept. of Biology, [2]Courant Institute, New York University, New York, NY 10012.

We evaluate a recently introduced kinetic model for phototransduction in cones by testing its ability to simulate photoreceptor behavior in several experiments involving manipulation of light input, injected current, and the concentration of free internal calcium, $[Ca^{2+}]_{in}$. In the basic model, light adaptation is mediated through only one site of biochemical feedback: the activity of guanylate cyclase depends sigmoidally on $[Ca^{2+}]_{in}$. The model accounts quantitatively for changes in sensitivity and dynamics of cone responses to light with increasing background level, I_0, and provides a good fit to families of temporal frequency responses measured at 5 mean light levels spanning 4 log units. The model also gives a shift in the stimulus-response function along the log-intensity axis with a change in background light level. However, the fact that injection of depolarizing current in the model does not increase flash sensitivity (as in real cones) suggests that the model be embellished with a voltage-dependent conductance or a voltage dependence of the Na/Ca exchange pump. When $[Ca^{2+}]_{in}$ is frozen at its dark level, sensitivity of the model cone decreases more rapidly with I_0 (compared to the rate of decrease when $[Ca^{2+}]_{in}$ is left free to vary), because [cGMP] decreases more rapidly with I_0 in the absence of guanylate cyclase feedback. Even with fixed $[Ca^{2+}]_{in}$, the time to peak and recovery phase of the model cone's impulse response still shorten with increasing I_0; we believe that both features are mediated by saturation of activated phosphodiesterase. The theoretical behavior of sensitivity and dynamics with fixed $[Ca^{2+}]_{in}$ is similar to that measured by others in rods.

88.13

CALCIUM CURRENT IN CONE PHOTORECEPTOR SYNAPTIC TERMINALS. E.M. Lasater and P. Witkovsky. Dept. Physiol., Univ. Utah, Salt Lake City, UT 84108 and Dept. Ophthalmol., NYU Medical Center, New York, NY 10016.

Cone photoreceptors were isolated from the retinas of the turtle, Pseudemys. Whole cell patch clamp recordings were made from cone synaptic terminals. No difference in membrane currents was noted between cone classes. A putative calcium current was isolated with a blocker Ringer, pH 7.6, consisting of (in mM) TEA, 20; $CaCl_2$, 20; 4-AP, 10; NaCl, 130; KCl 2.9; $MgCl_2$, 1.2; HEPES, 8.4; glucose, 10. The electrode contained CsCl, 120; NaCl, 4; EGTA, 5, HEPES, 84; $CaCl_2$, 0.5, trace Mg-ATP. The average calcium current (N=38) activated near -40 mV, reached its peak value of -70 pA near +10 mV and reversed near +80 mV. The calculated reversal potential for i_{Ca} is +200 mV. The measured current reversed at a more hyperpolarized potential, a finding attributed to residual outward currents. The time constant of activation was about 4 msec. The current relaxed to about 90% peak value with tau=1 sec and was sustained thereafter (longest test step 16 sec). In 20 mM $[Ca]_o$ the calcium current was blocked partially by 4 mM Co and completely by 20 mM Co (N=5). It was reduced 50% by 10 uM nifedipine (N=5), unaffected by the nifedipine carrier solution (N=2) and enhanced 2x by 10 uM Bay K 8644 (N=4). The current was unaltered by the $GABA_b$ agonist baclofen (10 uM, N=4) or by dopamine (10 uM, N=2) or serotonin (10 uM, N=2). Supported by EY05972 (E.M.L.) and EY03570 (P.W.).

88.14

PHARMACOLOGICAL CHARACTERIZATION OF CALCIUM CHANNELS MEDIATING SYNAPTIC TRANSMISSION FROM PHOTORECEPTORS TO HORIZONTAL CELLS. J. Kleinschmidt, Dept. of Ophthal., New York Univ. Med. Ctr., New York, NY 10016.

An L-type Ca current has been described in amphibian rods and cones and in turtle cones. The properties of this current raise doubts about its involvement in photoreceptor transmitter release. To characterize the Ca channels which support transmitter release, I have examined the effect of agents which modify Ca currents on horizontal cell membrane potential and light-evoked responses in the isolated superfused salamander retina. Synaptic transmission was functional in nominally Ca-free medium (0 Ca) as well as in 0 Ca medium containing 1 mM Sr or Ba. The potency of inorganic Ca channel blockers in hyperpolarizing H cells and blocking their responses decreased in the following order: La > Cd > Ni = Zn > Co > Mn > Mg. Omega-conotoxin (10-100 uM) had no effect in either salamander or goldfish retina. At very high concentrations (10-100 uM), nifedipine reduced but did not block H cell responses. Bay K 8644 (5 uM) had complex effects on H cells, reducing responses and eventually inducing prolonged regenerative responses, presumably Ca spikes. D600 at 40 uM had no effect. These results will be discussed in relation to the 6 or 7 distinct types of Ca channels so far identified in other cell types.
Supported by NIH grant EY05213.

88.15

DO RODS AND CONES ACTIVATE DIFFERENT EXCITATORY AMINO ACID (EAA) RECEPTORS? H. G. Kim and R. F. Miller. Washington Univ. Sch. of Med., St. Louis, MO 63110 and Dept. of Physiology, Univ. of Minnesota, Mpls, MN 55455.

We have studied rod and cone neurotransmission onto 2nd-order neurons, by dual whole-cell recordings from synaptically coupled pairs, using direct visualization in a perfused slice preparation of the mudpuppy retina. In addition, we examined rod- and cone-mediated synaptic responses in the superfused retina-eyecup preparation of the same species, using cone-matched stimuli to determine the relative rod and cone inputs into horizontal cells (HCs). Both experimental paradigms were used to evaluate the sensitivity of rod- and cone-mediated transmission to a variety of known EAA antagonists.

In the presence of comparatively weak concentrations of EAA antagonists (kynurenic acid, CB-PzDA, BB-PzDA, CNQX, and D-O-Phosphoserine), HC recordings revealed that cone-mediated responses were significantly more attenuated than rod-mediated ones. This distinction was made by comparing the antagonist action on high vs. low-intensity stimuli, response waveform, response to flickering light and synaptic responses evoked by current injection into rods and cones. These observations suggest that the postsynaptic receptors on the HCs are different for rods vs. cones. (Supported by NEI grant R01EY03014, and R01EY07376)

88.16

WAVELENGTH-RELATED SYNAPTIC MULTIPLICITY IN THE TRANSMISSION FROM CONE PHOTORECEPTORS TO H1 HORIZONTAL CELLS M. Yamada*, S. Yasui* and M.B.A. Djamgoz*. (SPON: M. Murakami). Imperial College of Science, Tech. and Med., London, UK.

Horizontal cells of the H1 type as well as vertebrate photoreceptors of every kind respond with graded hyperpolarization to all visible lights. This sign-conserving synaptic transmission has been accounted for by assuming that the light-induced presynaptic hyperpolarization reduces the secretion of an excitatory neurotransmitter from photoreceptor terminals. Recently, however, we have demonstrated that short- and long-wavelength (λ) signals are transmmited differently to H1 cells in the carp retina (Yasui, S. & Yamada, M. *Exp. Brain. Res.*, **74:256**, 1989). Such spectrally segregated synaptic multiplicity was further examined here. Application of 5 μM dopamine to the retina enhanced preferentially the responses to long-λ stimuli, whereas application of 1mM 2-amino-4-phosphonobutyrate (APB) potentiated preferentially the short-λ responses. Also, dopamine depolarized and APB hyperpolarized the resting membrane potential in the dark. These effects are consistent with the idea that the short-λ specific synaptic mechanism is of a conductance (g_m)-decreasing and sign-reversing type, as opposed to the classical g_m-increasing paradigm of excitatory synapse mediating mainly long-λ signals; probablly APB acts as an agonist of the g_m-decreasing endogenuos transmitter (as in the transmission from rod photoreceptors to ON-center bipolar cells), whereas dopamine potentiates the postsynaptic chemosensitivity to the g_m-increasing excitatory transmitter which may be glutamic acid (*cf.* Knapp, A.G. & Dowling, J.E., *Nature*, **325:437**, 1987).

88.17

ELECTROPHYSIOLOGICAL EVIDENCE OF CONE INHIBITION OF RODS IN NORMALS-VS-ACHROMATS. K.W. Wright* and B.E.S. Fox. Childrens Hospital Los Angeles & Univ. Southern California Sch. of Med., Dept. of Ophthalmology, Los Angeles, CA, 90027.

Previous psychophysical experiments have indicated a probable cone inhibition of rods under photopic conditions. This cone-rod interaction, however, has not been verified electrophysiologically. This study examines cone-rod interaction using standard ERG under special conditions in normal humans and patients with congenital absence of cones (ie, complete achromatopsia). All subjects were dark adapted for 15 minutes. A blue (467nm) flash (23 ft-L) ERG was obtained in the dark adapted state. The blue flash ERG was repeated with red ambient illumination (660nm, 550 ft-L). Dark adapted blue flash ERGs, which evoke primarily rod responses, showed robust responses in both normals and achromats. Ambient red illumination, which primarily activates the cone photoreceptors, caused an approximate 2/3 reduction in the blue flash ERG in normals. Achromats however, showed no reduction in blue flash ERG under red ambient illumination.

These results suggest active inhibition of rods by cones when cones are stimulated in normals. The blue flash responses were not decreased in achromats, who have normal rod function, which indicates that the red ambient light did not bleach the rod photopigment. Blue flash responses in achromats were not decreased because achromats do not have cones. Thus, this mechanism is different from the inactivation of rods by bleaching of rhodopsin and may help explain why patients with cone dysfunction (eg. achromatopsia) are photophobic.

AUDITORY SYSTEM: HAIR CELLS

89.1

ELECTROPHORETIC ANALYSIS OF PROTEINS OF THE TROUT SACCULAR OTOLITHIC MEMBRANE. K.M. Khan* and D.G. Drescher. (SPON: J.A. Kaltenbach) Lab. of Bio-otology, Wayne State Univ. Sch. of Med., Detroit, MI 48201.

The ultrastructure of the otolithic membrane has been described for various vertebrate species. However, the chemical composition and properties of this entity are virtually unknown. We have analyzed proteins of the otolithic membrane of the trout saccule (considered to be an organ of hearing in fish) by SDS-PAGE. Endolymphatic sacs were dissected and placed in cold trout saline. The lateral saccular walls were excised, the otoliths removed, and the otolithic membranes carefully peeled from the sensory epithelium. Ten membranes were pooled and homogenized in 100 μl SDS buffer (62.5 mM Tris chloride, 2.3% SDS, 12.5% glycerol, 5% 2-mercaptoethanol, pH 7.0). Nine major proteins were detected in the homogenate, with molecular weights ranging from 35 to >300 kDa. Periodic acid-Schiff staining of gels indicated that the 43, 94, 100, and 160 kDa bands were glycoproteins. Lectin binding on western blots further suggested that the 53 and 65 kDa bands may be glycoproteins. Incubation of blots with collagen type II antibodies suggested that the 94 kDa band was collagen type II or a related protein. The general similarites in chemical composition of the trout saccular otolithic membrane and the mammalian tectorial membrane are consistent with the theory that the two structures may perform similar functions. Supported by NIH Grant NS 16166 to DGD.

89.2

MEMBRANE CURRENTS IN DISSOCIATED HAIR CELLS FROM THE INNER EAR OF *RANA PIPIENS*. E.E.Serrano and P.M. Narins. Dept. of Physiology and Dept. of Biology, University of California, Los Angeles, CA 90024.

We are interested in the effects of temperature on membrane properties of hair cells from the frog, *Rana pipiens*. In these ectothermic animals, changes in daily core temperature of 20°C are not uncommon. We have used the patch clamp technique in the whole cell configuration to characterize the membrane currents normally present in these cells. Hair cells were mechanically dissociated from the sacculus after sequential treatment with a)0.05% papain in low-divalent saline (LDS) for 5 min b)0.1% bovine serum albumin in LDS for 1 hour. Standard patch clamp technique was used to obtain recordings from dissociated hair cells at 12°C. We observed the following currents under voltage clamp in the saccular hair cells: inward current at potentials more positive than -30 mV; a fast transient outward current; delayed outward current; delayed inward current at potentials more negative than -70 mV. We noted that while all cells contained the inward and delayed outward current, the delayed inward and fast transient outward current were present in a subpopulation of hair cells. We examined the effect of temperature on the delayed outward current. Outward current magnitude increased with temperature with a Q_{10} of 2.0 to 2.5 in the temperature range 12-24°C. (Supported by NIH grants NS19725 and NS09012-20)

89.3

COCHLEAR CYTOGENESIS AND MIGRATION VISUALIZED THROUGH PULSE LABELING OF CHICK EMBRYOS IN CULTURE. A. Katayama and J.T. Corwin. Depts. of Neuroscience and Otolaryngology, University of Virginia Med. Ctr., Charlottesville, VA 22908.

Cytogenesis in the cochlear sensory epithelium in chickens was investigated through pulsed ^{3}H-thymidine (^{3}H-TdR). Embryos were removed from their shells at E3, and cultured in petri dishes until E7, when ^{3}H-TdR was injected i.v. via a glass micropipette. This method resulted in a fast systemic delivery and clearance of the DNA precursor as shown by measurements of total tritium and ^{3}H-TdR in plasma. Many progenitor cells in this organ are in the synthesis (S) phase at E7. The fates of those cells were determined by examining cochleae from embryos fixed for autoradiography after progressively longer survival periods (between 0.5 and 192 hr) following the pulse.

Preliminary results indicate that heavily labeled nuclei are mainly in the basal two thirds of the thickness of the sensory epithelium 30 min. after ^{3}H-TdR injection. These cells incorporated ^{3}H-TdR in S phase, and remained in S phase or proceeded to the G2 phase by the time of fixation. Longer survival periods allowed the cells to proceed further in the cell cycle; label started to appear in the nuclei at the luminal surface, where mitotic figures were occasionally observed. These observations suggest that the nuclei of proliferating cells synthesize DNA while in the basal strata, then migrate through the thickness of the sensory epithelium toward the luminal surface and divide. This intermitotic migration of the cell nuclei is similar to that observed in cytogenesis in the neural tube.

Across the basilar papilla, the spatial pattern of unlabeled cells observed minutes and hours after pulse labeling at E7 is very similar to the pattern observed after cumulative labeling of mitotic cells from E7 to E15 (Katayama, A and Corwin, J. T., J. Comp. Neurol., 281:129, 1989). New hair cells and supporting cells do not invade the space already occupied by older postmitotic cells, instead they differentiated in the same areas where they were produced.

(Supported by NINCDS and NIDCD)

89.4

REGENERATED HAIR CELLS CAN ORIGINATE FROM SUPPORTING CELL PROGENY: EVIDENCE FROM LASER ABLATIONS, ACOUSTIC TRAUMA, AND DNA LABELING. J.T. Corwin, J.E. Jones, K.J. Balak, and D.A. Cotanche. Depts. of Neuroscience and Otolaryngology, Univ. of Virginia Med. Ctr., Charlottesville, VA 22908.

We have reported that supporting cell mitoses can give rise to new cells that differentiate as hair cells during regeneration, but that has been challenged. Here we present additional evidence.

In one series of experiments, chickens were exposed to 40 hrs of acoustic overstimulation followed by a varied length of recovery and administration of ^{3}H-thymidine shortly before fixation. The earliest thymidine labeling of nuclei was found at the sites of acoustic lesions in cells beneath the basilar membrane, in cells at the inferior edge of the epithelium, and in the supporting cells. Later labeling involved both supporting cells and hair cells at those sites. In some lesions early labeling was clearly restricted to the supporting cell strata.

In another series of experiments, a laser microbeam was used to cleanly and individually kill all of the hair cells in identified lateral line epithelia in the tails of anesthetized axolotl salamander larvae. Individual 3 ns pulses of 355 nm (UV) wavelength from a neodymium-YAG laser were sufficient to kill hair cells without causing noticeable damage to surrounding cells. After the laser ablation these epithelia contained only supporting cells, but they still regenerated hair cells. Regeneration was monitored by a series of procedures, which included nearly continuous time-lapse video microscopy from the time of ablation through to the time of new hair cell formation.

We conclude that epithelia that are capable of regenerating hair cells contain differentiated cells that respond to trauma-induced changes in the sensory epithelia by reentering the mitotic cycle. Supporting cells are one of the cell types that can serve as multipotent progenitors producing progeny that can differentiate as replacement hair cells.

(Supported by NINCDS and NIDCD)

89.5

THE FORMATION OF MATURE KINOCILIA IN AMPHIBIAN HAIR CELLS. C.K.Ochiai*, M.W.Kelley* and J.T.Corwin. (SPON: N. Lenn) Depts. of Otolaryngology and Neuroscience, Univ. of Virginia Med. Ctr., Charlottesville, VA 22908.

In the bullfrog sacculus, new hair cells are added at the edge of the sensory epithelium. The cilia bundles of cells in this region differ from more centrally located cells in the size, spacing and length of their stereocilia and in the size of their kinocilia. Over time, these immature cells develop the morphology of the central, mature cells. Two of the morphometric changes that occur during this development are growth of the kinocilium and the formation of a kinociliary bulb. We have hypothesized that kinocilium growth is followed by kinocilium shortening, and that the kinociliary bulb forms as a result of depolymerization of microtubules at the apical end of the kinocilium during this (shortening) phase of maturation.

In order to investigate this hypothesis, sacculi from adult bullfrogs were removed and prepared for scanning electron microscopy. The length of the kinocilium and the width of the bulb were measured and plotted against the length of the longest stereocilia for 82 cells.

The results indicate that kinocilium length changes biphasically. An initial, apparently rapid, growth phase is followed by shortening of the kinocilium. Increase in kinocilium bulb width correlates with shortening of the kinocilium length. A linear relationship exists between increasing bulb width and increasing stereocilia length.

These findings indicate that kinocilia develop biphasically. They are consistent with the hypothesis that increasing bulb width results from an accumulation of cytoskeletal elements during the kinocilium shortening phase. It seems likely that this shortening results from depolymerization of microtubules at the apical end of the kinocilium.

(Supported by funds from NINCDS and NIDCD)

89.6

HAIR CELL STEREOCILIA BEND AT THEIR BASES AND TOUCH AT THEIR TIPS. D.P. Corey, N.Hacohen*, P.L. Huang and J.A. Assad. Massachusetts General Hospital Dept of Neurology, Harvard Medical School, and Howard Hughes Medical Institute, Boston, MA 02114

If the tip-links hypothesis for hair cell transduction is correct, then a detailed description of the mechanics of the transduction elements requires a good understanding of the geometry of bundle motion. Two consistent but qualitative observations from direct visualization of moving hair bundles are that the stereocilia move as rigid rods that pivot at their bases, and that the bundle moves as a unit--*i.e.*, the stereocilia seem to stick together (Flock *et al*, 1977; Hudspeth, 1983).

We have used high-resolution video imaging of single bundles on dissociated bullfrog hair cells to quantify the relative movement of parts of the bundle, at a sensitivity of about 10 nm. These measurements are consistent with a geometrical model that follows from two assumptions: moving stereocilia bend at their bases and touch at their tips. The measurements are inconsistent with any appreciable degree of spreading or splay with displacement.

The geometrical model then makes two predictions: First, the tip links are mechanically in parallel and not in series, so they act as independent elements when the bundle is driven. Second, for a given displacement, the stretch on the tip link is nearly the same for all links, so they receive identical stimuli. These consequences greatly simplify biophysical analyses.

The model also provides a geometry factor, γ, relating tip-link stretch to bundle displacement, which is 0.1 relative to the tip of the bundle or about 0.14 relative to the bulb of the kinocilium, confirming the calculation of Howard *et al.* (1988) from a similar model.

89.7

MECHANICAL AND ANATOMICAL CORRELATES OF OUTER HAIR CELL VULNERABILITY. B.N.Evans * (SPON:P.Dallos). Dept. of Neurobio. and Physiol., Northwestern U., Evanston Il, 60201.

A hallmark of outer hair cell (OHC) morphology is the prominent cisternal complex associated with its plasma membrane. When "whole-organ" tissue is examined by conventional fixation techniques, the subsurface cisternae have a fenestrated appearance. As part of an on-going study of the structure/function relationships involved in hair cell motility, OHCs were isolated from guinea pig cochleae with mild enzymatic and mechanical treatments (Brownell et al., Science:1984, 227, pp.194-196). The cells were fixed and prepared for electron microscopy. An unexpected finding was the occasional occurrance of multiple, non-fenestrated cisternae lining the perimeter of the cytoplasmic compartment.

To examine whether the non-fenestrated form more closely represents the morphology of this complex under in-vivo conditions, individual cells were recovered following mechanical challenges induced by transmembranous application of electrical current waveforms. Peak displacements greater than 500 nm led to a slow decrease in the resting length (10-20%) of cells and as well as a loss in movement threshold sensitivity and response asymmetry. The ultrastructural correlates of recovered cells revealed vesiculation patterns which were graded with the magnitude of the change in their resting length. Such findings suggest that the "fenestrated" system may be an artifactual remnant of a more highly organized and non-fenestrated network in-vivo.

89.8

GADOLINIUM IONS REVERSIBLY BLOCK VOLTAGE DEPENDENT MOVEMENTS OF ISOLATED OUTER HAIR CELLS. J. Santos-Sacchi* (SPON:H. Sapru), Laboratory of Otolaryngology, UMDNJ - New Jersey Medical School, Newark, NJ, 07103.

Whole cell voltage clamp was used to study the voltage dependent changes in the length of outer hair cells (OHC). OHCs were non-enzymatically isolated from the the guinea pig organ of Corti, and were placed in a chamber containing modified Leibovitz medium (NaCl 137 mM, KCl 5.37 mM, $MgCl_2$ 1.8 mM, $CaCl_2$ 1.25 mM, glucose 5 mM, HEPES 5 mM, pH 7.0-7.2). In Ca++-free media EGTA (1 mM) replaced Ca++. Cells were perfused via a multi-barreled pipette with various test solutions. Patch electrodes contained 140 mM KCl or CsCl, 2 mM $MgCl_2$, 1, 5, or 10 mM EGTA, and 5 mM HEPES pH 7.0- 7.2. Giga-ohm seals were made at the nuclear level on OHCs prior to cell entry. Under voltage clamp, cells were held near -70 n.V and stepped to hyperpolarized and depolarized potentials for 200 msec, and current records saved for analysis. Simultaneous video recordings at 2000x were made and the mechanical movements of the cuticular plate were subsequently measured with a differential optoresistor off the monitor.

OHCs exhibit an outward rectification upon depolarization which is carried by K+. The outward current can be blocked by a variety of treatments, including intracellular Cs, or extracellular 20 mM TEA, 20 mM 4- AP, 10 mM Ba++, 1 mM Cd++, or 0.5 mM Gd+3. Intracellular CsCl unmasks an inward Ca++ current which peaks at potentials of + 30 to +40 mV. Although 200 nM ChTX blocks some of the outward K+ current, Ca++ - free media is ineffective. This suggests that an intracellular store of Ca++ is responsible for the activation of Ca++ - activated K currents.

OHCs contract when depolarized from the holding potential. Responses as large as 29 nm/ mV have been observed. The average response is 15 nm/ mV in the depolarizing direction, but hyperpolarization causes an elongation which rapidly saturates above the holding potential; above -70 mV the response is about 2 nm/mV. All treatments which block the various currents in OHCs do not interfere with the mechanical responses, except Gd+3. Gadolinium rapidly and reversibly reduces or abolishes OHC contractions due to depolarization, and its effect is probably not due to an ionic current block as none of the other blocking agents were effective. It is interesting to note that gadolinium is a potent blocker of stretch activated channels which also are voltage activated, and that recent evidence indicates that stretch activated channels exist in the OHC lateral membrane.

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89.9

EVIDENCE FOR KAINIC ACID RECEPTOR ON NON-NEURONAL CELLS IN THE FROG LABYRINTH. C.J. Dechesne, D.R. Hampson, G. Goping*, K. Wheaton* and R.J. Wenthold Lab. Mol. Otology, NIDCD, NIH, Bethesda, MD 20892.

In previous studies, we examined the presence of kainic acid (KA) receptor (KAR) in the frog utricle using monoclonal and polyclonal antibodies against purified KAR isolated from frog brain (Hampson et al, in prep). The results (Neurosci Abst, 321.10, 1988) showed immunostaining of afferent terminals making synapses with hair cells. In addition, oblong fibroblast-like (Flo) cells, situated in the inner ear connective tissue, were intensely stained on their cytoplasmic membranes. Here, we further investigate the presence of KAR in these cells.

Three sets of experiments were performed on frog inner ear. 1) Immunoblots of 1- and 2-dimensional gels, with monoclonal and polyclonal antibodies, 2) 3H-KA binding studies with in vitro autoradiography 3) Neurotoxic effects of in situ injections of KA.

On immunoblots, immunoreactive proteins were detected, with both types of antibody, at the same isoelectric point but slightly different molecular weight than the purified KAR. High density of 3H-KA binding was observed in connective tissue of the inner ear. Flo cells showed signs of degeneration after injections of 10μM KA. The presence of KAR on Flo cells is then confirmed but its role at this location remains hypothetical.

AUDITORY SYSTEM: COCHLEA

90.1

LASER DOPPLER VIBROMETER MEASUREMENTS OF BASILAR MEMBRANE MOTION IN THE GUINEA PIG. Alfred L. Nuttall, David F. Dolan, and Gopal Avinash, Kresge Hearing Research Institute, The University of Michigan Medical School, Ann Arbor, MI 48109.

Basilar membrane (BM) motion has previously been studied using five different methods; Optical microscopy, Mössbaurer velocimetry, capacative probe, laser speckle, and laser interferometry. Each of these methods had major drawbacks concerning the difficulty of placing objects on the basilar membrane (their areal size, mass, or toxicity), keeping the analysis area small, or maintaining cochlear hydromechanical integrity. We present here a new approach which solves most of these problems. Using an optical compound-microscope, the laser beam of a laser Doppler vibrometer (Polytec OVF1000) was focused to a diffraction limited spot. The focused beam was directed onto a 10-15 micrometer solid glass bead which was previously gently placed on, and sticks to, the basilar membrane. Placement of the microbead and visual observation to focus the laser beam are done through a 0.5 mm diameter hole made in the bony wall of the cochlea. The hole in the cochlea and the microbead do not cause obvious inner ear sensitivity change. Isodisplacement functions at 0.5 nanometer were obtained for the frequency variable. These functions showed appropriate frequency tuning as has been shown by previous studies. Intensity functions of displacement vs. sound level showed compressive-type nonlinear behavior at frequencies near the best or characteristic frequency for the measured location on BM (about 17 kHz). Qualitative observations of both propagated intermodulation traveling waves and an apparent slow shift in BM position during sound stimulation were made. The method offers the important advantage of multiple point measurements of basilar membrane vibration. *(Supported by NIH grant #NS15107)*

90.2

SPONTANEOUS OTOACOUSTIC EMISSION INCIDENCE IN HUMAN EARS: GENDER DIFFERENCES AND BILATERAL SYMMETRY. M.L. Whitehead* (SPON: G.K. Martin). Dept. of Otolaryngol. and Communicative Sci., Baylor Col. of Med., Houston, TX 77030.

Strickland et al. (*J. Acoust. Soc. Am.*, 78:931-935, 1985), in a survey of both ears of 50 prepubescent children, found a similar incidence of spontaneous otoacoustic emissions (31% of ears possessed SOAEs) to that previously reported for adults using similar microphones. They also reported that significantly more female than male children possessed SOAEs (52% vs. 24%, a ratio of 2.2:1). Their data further demonstrates a clear correlation of the number of SOAEs detected in the two ears of each emitting subject ($r=0.76$, $p<0.001$). These findings may be interpreted as evidence for a genetic involvement in the determination of SOAE incidence. To explore this possibility further, both ears of 38 normally hearing adults (mean age 24.7 yrs) were surveyed for SOAEs with a custom-modified, low-noise microphone (Wilson, J.P. *J. Physiol.*, London, 298:8-9P, 1980). A mean of 3.7 SOAEs per emitting ear were found in 50% of ears. This incidence is significantly greater than that reported by Strickland et al. However, there was no significant difference between the two studies after correction for the lower noise-floor of the present microphone system. Significantly more female than male subjects possessed SOAEs (77% vs. 38%, a ratio of 2.0:1). There was a clear correlation of the number of SOAEs detected in the two ears of each emitting subject ($r=0.63$, $p<0.005$). These findings provide further support for the hypothesis of a genetic involvement in the determination of SOAE incidence by corroborating in adults the gender difference and between ear correlation of SOAE incidence described in prepubescent children.

90.3

RESPONSES OF THE MAMMALIAN COCHLEA TO WAVEFORM SINGULARITIES. E.R.Lewis and K.R.Henry*. Electronics Research Lab., Univ. of Calif., Berkeley, CA 94720 and Dept. of Psychology, Univ. of Calif., Davis, CA 95616.

Whenever an acoustic waveform either begins or changes its trajectory, there will be an abrupt change either in sound pressure or in one or more of the time derivatives of sound pressure. The resulting singularity in the acoustic waveform has the potential of being a precise timing cue for acoustic image processing. Waveform singularities are ubiquitous among biologically significant sounds, such as footfalls and vocalizations. Appropriate filters, with high-order dynamics, can respond selectively to singularities; and over a bank of such filters, variously tuned, different singularities will produce different signatures (Lewis, E.R. and Henry, K.R., *Hearing Res.* 37:219, 1989). The typical mid- and low-frequency filter channels of the cochlea are especially well suited to this task. In an experimental study to determine whether or not the cochlea of the gerbil (*Meriones unguiculatus*) is selectively responsive to singularities, we employed ramp-modulated sine waves with stepwise changes in the first or second time derivatives of sound pressure. We report that these singularities can trigger nearly synchronous spikes over substantial populations of afferent axons, and that markedly different populations are excited by different singularities. Thus, in addition to its celebrated role as frequency analyzer, the cochlea is an excellent analyzer of temporal events.

90.4

ALDOSTERONE MEDIATES AN INCREASE OF INNER-EAR OUABAIN BINDING SITES. T.P. Kerr*, D.Z. Pitovski* and D.G. Drescher. Lab. of Bio-otology, Dept. of Otolaryngology, Wayne State Univ. Sch. of Med., Detroit, MI 48201

The various chambers of the membranous labyrinth contain endolymph, an extracellular fluid with high $[K^+]$, low $[Na^+]$, and a positive DC potential. These properties are thought to be maintained by active transport mechanisms in which the enzyme Na^+, K^+-ATPase participates. We have isolated inner-ear tissues of Hartley guinea pigs by microdissection. The specimens were then incubated with ^3H-ouabain (a specific Na^+, K^+-ATPase inhibitor), and binding was normalized to tissue dry weight. Scatchard analysis of cochlear lateral wall from normal animals revealed a single population of ouabain binding sites, with K_d of 2.82 uM. We hypothesized that aldosterone ("aldo," a mineralocorticoid hormone) might increase the population of Na^+, K^+-ATPase sites. Therefore, guinea pigs were maintained on a high Na^+/low K^+ diet for 4-5 days (to suppress endogenous aldo secretion). Twenty-two hours prior to sacrifice, the experimental group received an aldo injection (10 ugm/100 gm); paired controls were sham-injected. At 5 uM ^3H-ouabain concentration, binding in ampullae from the aldo group was 171% higher than controls (but failed to attain statistical significance with the sample size employed). Binding in lateral wall of the basal cochlear turn from the aldo group was 127% higher than controls (p .05 by two-tailed t-test). [Supported by NASA NAG 2-404, ONR N00014-88-K-006, and NIH T32-NS-07305].

90.5

EFFECTS OF ARGININE VASOPRESSIN ON COCHLEAR BLOOD FLOW IN THE NORMOTENSIVE RAT. Grant M. McLaren*, H.A. Dengerink, and John W. Wright.(SPON: J. Wright). Washington State University, Pullman, WA.,99164. Previous studies in our laboratory have revealed modifications of cochlear blood flow (CoBF) measurements via laser Doppler flow meter following both intra-arterial and local infusion of the neuroactive hormone arginine vasopressin (AVP)(Laugel et al.,1988). To test the hypothesis that AVP would yield dose-dependent alterations in CoBF, ten minute intra-arter-ial infusions of 0, 0.1, 1, 10, and 100 pmol/50µl 0.15 M NaCl/ min were utilized in anesthetized male adult Sprague-Dawley (SD) rats. Results indicate that systemic blood pressure (BP) responds to AVP in a dose-dependent fashion. Significant changes in CoBF were observed only in animals that received the 100 pmol/min AVP infusion while animals that received doses at and below 10 pmol/min evidenced significant elevations in BP but no alterations in CoBF. Laser Doppler measurements of skin blood flow (SBF) were conducted in eight additional SD rats that received the intra-arterial infusions of AVP (10 pmol/min for ten min). Significant elevations in BP and decreases in SBF were observed under these experimental conditions.

Dose (pmol AVP)	ΔBP(mm Hg)	ΔCoBF(%)	ΔSBF(%)
0	0.5±0.5 *	-0.34±0.3	-0.87± 0.2
0.1	2.5±1.9	-4.5±2.8	
1	9.3±2.0	-2.9±4.4	
10	52.2±5.0	3.6±3.9	-18.5±1.6
100	83.6±4.7	47.4±4.4	*[mean±SEM]

90.6

DISTRIBUTION OF FIBRONECTIN IN THE RAT COCHLEA DURING INNER EAR DEVELOPMENT. N. K. Woolf, F. J. Koehrn* and A. F. Ryan. Division of Otolaryngology, UCSD Medical School and Veterans Administration Medical Center, La Jolla, CA 92161.

Fibronectin stimulates the growth of neuronal processes in many neural systems by altering cell surface adhesive properties. Immunocytochemistry was employed to document the distribution of fibronectin in the inner ear of Sprague-Dawley rats from embryonic day 8 (E-8) to post-natal day 120 (P-120).

From E-20 to P-4, dense fibronectin immunoreactivity was observed immediately beneath the cochlear hair cells. At E-20, this reactivity was restricted primarily to the region beneath the inner hair cells. At P-0 to P-4, reactivity was observed beneath both outer and inner hair cells. Immunoreactivity beneath hair cells was not present or was much less intense at other ages. During the developmental period from E-20 to P-4, the region beneath the cochlear hair cells is being actively innervated by both afferent and efferent nerve fibers. The results of the present study suggest that fibronectin is positioned to play a role in guiding and regulating neural outgrowth within the developing organ of Corti.

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90.7

NIMODIPINE, A CALCIUM CHANNEL ANTAGONIST, REVERSES THE SUMMATING POTENTIAL. R.P. Bobbin, P.J. Jastreboff, M. Fallon* and T. Littman*. Kresge Hearing Res. Lab., LSU Med. Cntr., New Orleans, LA 70112; Dept. of Surgery, Yale Univ., New Haven, CT 06510.

This study examined the effect of nimodipine (BAY E 9736; Miles), an L-type of calcium channel antagonist on cochlear potentials recorded from anesthetized guinea pigs. Perilymph spaces of guinea pig cochleae were perfused with Ringer solutions containing up to 10 µM concentrations of nimodipine at a rate of 2.5 µl/min for 10 min. Immediately after each period of perfusion the compound action potential of the auditory nerve (CAP), cochlear microphonics (CM) and the summating potential (SP) evoked by 10 kHz tone bursts of varying intensities were recorded from a wire inserted in the basal turn scala vestibuli. Nimodipine suppressed the CAP at about 0.1 µM and the CM and SP at 0.3 µM. SP evoked by low intensity tone bursts (62 dB SPL) changed from negative to positive at 3 µM nimodipine. At higher intensity (98 dB SPL) the positive SP induced by nimodipine remained while a negative SP reappeared during the positive SP. L-type calcium channels appear to be involved in hair cell function and generation of the negative SP.

(Supported by NIH grants NS-22024 and NS-24238, Kresge Foundation and the Louisiana Lions Eye Foundation.)

90.8

SENSORINEURAL HEARING LOSS AND TEMPORARY THRESHOLD SHIFTS IN AN ANIMAL MODEL OF CALCITONIN INDUCED HYPOCALCEMIA, HYPERMAGNESEMIA AND HYPERPHOSPHATEMIA. A.M. Shapiro*, C.L. Campese*, A.T. Cacace* S.M. Parnes* and N.L. Strominger (SPON: L. Nelson). Depts. of Surgery and Anatomy, Albany Medical College, Albany, N.Y. 12208.

Hypocalcemia induced sensorineural hearing loss is thought to involve active processing at the cellular level. Immunoreactive synthetic salmon calcitonin (iCT) was used to produce temporary threshold shifts (TTS) in the New Zealand white rabbit which correlated with maximal decreases in serum calcium (Shapiro et al., Assoc. Res. Otolaryn., 98, 1989). We have now studied this effect prospectively in four macaque monkeys utilizing intravenous iCT. Similar to the rabbit, decreases in serum calcium correlated with increases in auditory thresholds during the first 14 days post-iCT administration determined by brainstem auditory evoked potentials (BAEPs). Temporary threshold shifts, approx 28 dB, were maximal between one and two hrs post-iCT infusion. Increases in phosphate and magnesium were consecutive with the observed calcium decreases, supporting a possible role for these divalent ions.

90.9

DOSE-DEPENDENT SERUM CALCIUM DECREASE AFTER SALICYLATE. P. J. Jastreboff, R. Hansen* and C. T. Sasaki. Dept. of Surgery, Yale Univ. Sch. of Med., New Haven, CT 06510.

Salicylate administration results in increased hearing threshold and tinnitus but mechanisms of its action remain unknown. After salicylate, used to induce tinnitus in rats, decreased serum and CSF calcium levels were observed. Since modification of cochlear calcium might cause changes in auditory nerve activity, salicylate's effect on calcium in serum was investigated initially. Pigmented rats were injected i.p. with sodium salicylate in doses 57-350 mg/kg. Blood was taken from the tail just before and 2 h after injection, and salicylate and total calcium were evaluated.

Salicylate level was linearly dependent on the dose injected and ranged from 15 to 60 mg/dL. Salicylate resulted in a significant decrease of calcium ($p<0.01$) to about 90% of control values for the highest doses used. Nonlinear regression analysis revealed that this decrease was significantly related to the dose injected and to salicylate level in serum. Interestingly, calcium levels exhibited rapid decrease once serum salicylate levels rose above 20 mg/dL, corresponding to the threshold level for evoking tinnitus in humans, followed by a plateau reached for salicylate levels of about 40 mg/dL. The results suggest the possibility of calcium-related mechanisms of salicylate action on the cochlea, since a correlation between changes of calcium level in serum and in CSF has been reported. (Supported by NIH Grant NS22024)

90.10

THE LATERAL OLIVOCOCHLEAR SYSTEM IN RATS AS REVEALED BY CHOLERA TOXIN-HRP. D.E. Vetter and E. Mugnaini, Lab. of Neuromorphology, Univ. of Conn., Storrs, CT. 06269-4154.

HRP conjugated to cholera toxin B chain (CT-HRP) is an excellent neuroanatomical tract tracer that produces distinct delineation of the dendritic arbors of retrogradely labelled neurons. Using CT-HRP, we have re-examined the olivocochlear (OC) system in adult rats and found that the lateral OC system is more complex than originally presumed. There are at least two lateral OC neuron groups that differ in the size and location of the cell bodies and in dendritic features. Small spherical cells, 12-13um in diameter, are located primarily in the body of the lateral superior olive (LSO). Large fusiform cells, 20um in long diameter, are located in the fiber capsule surrounding LSO, and rostrally they merge into RPO. Small OC cells generally have dendritic arbors perpendicular to the LSO turns, but in the tips of the LSO limbs, the cells exhibit curving dendrites. The dendrites of small cells do not extend beyond LSO borders. The large OC neurons have straight, long, and thick dendrites, some of which project into LSO. CT-HRP intracochlear injections consistently reveal over 400 lateral OC neurons in the superior olive ipsilateral to the injected cochlea. Few medial OC cells are revealed after intracochlear CT-HRP injections, possibly reflecting a paucity of binding sites for the CT B-subunit on the medial system OC terminals.

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90.11

INNERVATION OF THE DEVELOPING COCHLEA *IN VIVO AND IN VITRO* AS REVEALED BY GAP-43. H.M. Sobkowicz, M.R. Emmerling*, and D.J. Schreyer. Dept of Neurology, Univ. of Wisconsin, Madison, WI 53706 and Dept of Neurobiology, Stanford Univ. School of Medicine, Stanford, CA 94305.

Monoclonal antibody to GAP-43, a protein associated with growing nerve fibers, was used to study immunocytochemically the innervation of the intact and cultured cochlea from ICR mice. GAP-43-like immunoreactivity *in vivo* is present in the spiral ganglion cells and their growth cones on embryonic day 13 (E13); on E16 in radial bundles; and on E18 in radial bundles, in neurofibrillar cups on inner and outer hair cells, in inner spiral plexus and bundle, in tunnel fibers, and in three outer spiral bundles. The staining in the 1-day old fades away, but reappears in the 2 to 3-day old in endings around the inner hair cells and the inner spiral bundle. From 4 to 10 days, GAP-43 in the inner spiral bundle disappears and is expressed mainly in neurofibrillar cups on the outer hair cells of the 1st and 2nd rows, and finally of the 3rd row. By 10 days, GAP-43 is re-expressed in part of the inner spiral bundle, the only structure that stains in the adult. Comparing GAP-43 stainings with that of efferents by AChE and of afferents by neurofilament antibody suggests that GAP-43 is transiently expressed in the afferents mostly prenatally and in the efferents early postnatally. The apparent perinatal loss of GAP-43 staining in the afferents may be reversed in culture. GAP-43 may be induced by mechanical injury or by treatment with the ototoxic drug gentamicin both in free-growing and synaptically engaged fibers. The free growing fibers express GAP-43 distally, but in synaptically engaged fibers, growing by elongation, GAP-43 is expressed proximally. Thus early postnatally, spiral ganglion cells are able to synthesize GAP-43 for growth and repair. (NSF Grant BNS-8719716 and NIH-NINCDS NS26513-01)

90.12

DEGENERATION OF EFFERENT TERMINALS IN THE CHINCHILLA COCHLEA BY A CHOLINERGIC NEUROTOXIN. Barbara J. Morley, Kevin Spangler, Bobbie L. Schneider*, and Eric Javel. Boys Town National Institute, Omaha, NE 68131 and the Department of Anatomy, Creighton University Medical School, Omaha, NE 68178.

Ethylcholine aziridinium ion (AF64) is a choline analog that blocks high-affinity choline transport in cholinergic neurons and produces specific degeneration of cholinergic terminals and axons. The effects of AF64 are biphasic, producing specific effects on cholinergic terminals at low concentrations (<22 μM) and non-specific effects at higher concentrations (Amir, A. et al., *Brain Res.* 454:298, 1988).

For these studies, chinchillas were anesthesized and the cochlear fenestrae were exposed. A solution of 1 μM of AF64 diluted in artificial perilymph was infused through the cochlear scalae. Control animals were injected with artificial perilymph only. Seven days after the infusion the animals were anesthesized and perfused with mixed aldehyde fixative. The cochleas were removed and processed for transmission electron microscopy.

Organs of Corti in AF64-treated animals exhibited loss of synaptic terminals in the region of the inner spiral bundle while many thin, presumably efferent axons remained in both the inner and tunnel spiral bundles. Preliminary observations indicate that only lateral efferent terminals degenerated. These results indicate that AF64 could be a useful pharmacological agent in studying cholinergic efferent terminals in the cochlea.

This research was supported by grants from the Deafness Research Foundation (KS), State of Nebraska (BJM), and NIH (EJ).

90.13

EFFECTS OF PURE TONE ACOUSTIC TRAUMA ON COCHLEAR WHOLE-NERVE RESPONSES TO ELECTRICAL STIMULATION OF THE CROSSED OLIVOCOCHLEAR BUNDLE. D.F. Dolan* and A.L. Nuttall, (SPON: S. Shore). Kresge Hearing Research Hearing Institute, Univ. of Mich. Med Sch., Ann Arbor, MI 48109.

Acoustic overstimulation is known to produce either temporary (TTS) or permanent threshold shift (PTS). The acoustic overstimulation has differential effects on the inner (IHC) and outer hair cells (OHCs) depending on stimulus duration and intensity. Electrical stimulation of the crossed olivocochlear bundle (COCB) reduces the cochlear whole-nerve response (CAP) to low to moderate acoustic stimuli also by differential effects on hair cells. In this study we present data on the effects of electrical stimulation of the COCB on the CAP to tone bursts after acoustic trauma.

Anesthetized guinea pigs were surgically prepared for placement of a round window (RW) recording electrode and stereotaxic placement of a bipolar stimulating electrode at the floor of the 4th ventricle. Acoustic overstimulation, in the form of pure tones, were presented from 90 - 120 dB SPL for 1.0 to 10.0 minutes. When overstimulation produced only small changes in sensitivity less than 20dB (3-15 dB), there was either a similar (proportional) change in the effects of COCB activity or an increase in CAP reduction amounting to 3-8 dB in excess of control levels. Similarly short, intense overstimulation, producing changes in sensitivity greater than 20dB, tended to cause little or no effect on COCB action. Long duration, less intense overstimulation producing changes in sensitivity greater than 20dB tended to reduce the effect of COCB activation. The results will be discussed in terms of possible anatomical cites of damage. (Supported by NIH grant #NS15107).

90.14

DEVELOPMENTAL CHANGES IN THE CHARACTERISTIC FREQUENCY OF SINGLE SPIRAL GANGLION NEURONS AT A FIXED LOCATION WITHIN THE MAMMALIAN COCHLEA. S.M. Echteler, E.M. Arjmand* and P. Dallos. Auditory Physiology Lab. and Dept. of Neurobiol. and Physiol. Northwestern Univer., Evanston, IL 60208.

To ascertain the process by which the mammalian cochlear frequency-place map develops, we have examined the emergence of frequency tuning in single spiral ganglion neurons. Recordings were made at a constant location within the basal cochlea in an age-graded series of anesthetized Mongolian gerbils.

Our surgical approach to the spiral ganglion was patterned after that of Robertson and Manley (J. Comp. Physiol. 91:363, 1974). Frequency tuning curves were obtained from individual spiral ganglion neurons with a computer-automated paradigm. Following electrophysiological characterization, neural recording sites were marked by iontophoretic injection of horseradish peroxidase.

We find that between postnatal days 14 and 17, neural tuning curves measured from gerbil cochlear neurons supplying the same region of the organ of Corti (3.0 mm $\pm$ 200 μm from the basal end of the basilar membrane) undergo a systematic increase in characteristic frequency (CF) from 7.3 to 16.5 kHz. During this same time period, neural threshold at CF decreases by 80 dB. As cochlear length is fully developed in the gerbil by postnatal day 12 and functional maturation of the gerbil middle ear is essentially complete by postnatal day 14 (Woolf and Ryan, Hearing Res. 35:131, 1988), our findings provide direct support for the hypothesis that mature tonotopy arises within the inner ear through changes in the spatial encoding of frequency along the cochlear duct (Rubel and Ryals, Science, 219:512, 1983; Harris and Dallos, Science, 225:741, 1984). (Supported by NIH grant NS08635).

90.15

ACOUSTIC NEURONS AND THEIR PAPILLAR INNERVATION PATTERNS IN THE FROG: AN HRP STUDY. D. D. Simmons[1] and P. M. Narins[2]. [1]Natural Science Division, Pepperdine University, Malibu, CA and [2]Dept. of Biology, UCLA, Los Angeles, CA.

Horseradish peroxidase injections were made into the eighth nerve of the frog *Rana pipiens*. These injections retrogradely labeled ganglion neurons in both divisions of the eighth nerve. The anterior branch of the nerve innervated vestibular organs and the posterior branch innervated two distinct acoustic organs: the amphibian papilla (AP) and the basilar papilla (BP).

In the ganglion, all labeled neuronal somas had bipolar cell shapes. Acoustic GCs had the smallest average soma areas (roughly 120 μm^2) and, near the cell body, had dendritic (Dp) fibers that were much thinner than axonal (Dc) fibers (Dc/Dp ratio > 2). Vestibular GCs had larger average soma areas (> 250 μm^2) and roughly equal diameter dendritic and axonal fibers.

In the AP and BP, labeled fibers demonstrated different patterns of innervation. Labeled fibers terminating in the caudal-most region of the AP were thin (< 0.7 μm in diameter), and traveled long distances (> 1 mm) whereas fibers terminating in the rostral-most region tended to be thicker (up to 1 μm), traveled shorter distances, and were more highly branched. Fibers in the BP were more homogeneous: they traveled relatively short distances (< 0.5 μm) underneath the supporting cells before rising to terminate directly on single hair cells. As of yet, no branching has been observed within the BP. Possible functional significance of these anatomical features will be discussed.

This research was supported by grants [1]BNS 8719610 (NSF), a grant from the Ralph M. Parson's Foundation, and [2]NS 19725 (NIH).

90.16

ISOINTENSITY TONAL RESPONSES OF AUDITORY FIBERS IN THE GREEN TREEFROG (*Hyla cinerea*) AS A MEASURE OF SELECTIVITY TO COMPLEX SOUNDS. D.Lim* and R.Capranica. Section of Neurobiology and Behavior and School of Electrical Engineering, Cornell University, Ithaca, NY 14853.

At a fixed sound intensity, the firing rate in spikes/sec of a single auditory nerve fiber was measured as the tonal frequency was varied incrementally across the entire extent of the excitatory tuning curve, thus generating a spike rate contour as a function of frequency. A family of such isointensity contours was compiled at several different intensities within each fiber's tuning curve.

The isointensity contours fall into three distinct classes: (1) single-peaked profiles, (2) flat-shaped profiles, and (3) multipeaked profiles. For a single fiber, it's family of profiles represents a set of tonal response transfer functions from which a transfer matrix can be constructed. This matrix is characterized by a singular value decomposition method in which the fiber's selectivity in complex signal space can be predicted, namely it is most sensitive to signal components containing the highest singular values. Preliminary results using this approach to studies of encoding of complex sounds in the green treefrog's peripheral auditory system will be discussed. (Supported by NIH grant NS-09244.)

90.17

COCHLEAR DYSFUNCTION IN A TRANSGENIC MOUSE FAMILY. C.D. Katz*[1], S.J. Madors*[1], C.M. Henley*[1], P.A. Overbeek*[2], M. Kovak*[2], G.K. Martin[1], and B.L. Lonsbury-Martin[1] (SPON. A.C. COATS). [1]Dept. of Otolaryngol. Commun. Sci. and [2]Dept. of Cell Biol. and Howard Hughes Med. Inst., Baylor Col. of Med., Houston, TX 77030.

Transgenic mice were generated by microinjection of one-cell stage FVB/N mouse embryos with a construct containing the human beta-actin promoter and the bacterial neomycin resistance gene. When F1 mice from one (OVE7) of three transgenic families were inbred, nearly 1/4 of the offspring showed pronounced hyperactivity, head-bobbing, and circling. Further studies indicated that homozygous transgenic mice exhibited head-bobbing, while some heterozygotes were hyperactive and others were behaviorally normal. Cochlear activity was tested in control and heterozygous and homozygous OVE7 mice using response-growth functions (2-8 kHz, 30-85 dB SPL, 5-dB steps) of $2f_1$-f_2 distortion-product emissions (DPEs). Control mice showed normal DPE thresholds of ~37 dB SPL and maximum amplitudes in response to 75-dB SPL primaries of ~35 dB SPL at 6 and 8 kHz. In general, heterozygous mice displayed either normal or poor (thresholds >45, amplitudes ~20 dB SPL) DPEs that were relatively independent of their behavioral phenotype, while homozygous subjects had essentially no DPEs. For these latter mice, histologic examination of the cochlea indicated that inner-ear development was arrested at an early embryologic stage. Follow-up experiments are attempting to clone the transgenic insert from a genomic library so that the molecular basis of the suppression of inner-ear development in transgenic mutants can be established. [Supported by NS22278, ES03500, HD25340, DRF].

90.18

RESISTANCE TO REPEATED PURE-TONE EXPOSURE IN ANESTHETIZED RABBIT. B.D. Mensh*[1,2], B.L. Lonsbury-Martin[1,3] and G.K. Martin[3]. [1]Div. of Neurosci., [2]Med. Sci. Tr. Prog. and [3]Dept. of Otolaryngol. Commun. Sci., Baylor Col. of Med., Houston, TX 77030.

Previous findings in our laboratory (Lonsbury-Martin et al., *Soc. Neurosci. Abstr.*, 18:1099, 1988) showed that repeated exposure of awake rabbits to moderate sounds resulted in reduced susceptibility to cochlear dysfunction that was measured physiologically using distortion product emissions (DPEs). The primary goal of the present work was to develop a "resistance" model in anesthetized rabbit so that experiments investigating the role of the cochlear efferent system could be performed acutely. The paradigm consisted of: (1) establishing baseline 2f1-f2 DPEs at the geometric-mean frequency (2.3 kHz) 1/2-octave above exposure, in response to 50-dB SPL primaries, (2) exposing the ear to 1.7 kHz at 100-dB SPL until 10-20 dB reductions in DPEs occurred, and (3) tracking recovery to within 2 dB of control DPE levels. For each animal, after the first exposure, the paradigm was repeated at regular intervals for 8-11 days. Exposing four rabbits demonstrated that repeated exposure initially decreased the susceptibility of the cochlea to the effects of loud sound. That is, the duration of the exposure needed to reach the criterion reduction in DPE magnitude increased. Further exposures eventually reversed this trend, probably due to cochlear damage. Future experiments in the acute "resistance" model will use pharmacologic, contralateral acoustic stimulation, and lesioning techniques to define the contribution that cochlear efferents make to the reduced susceptibility process. [EY02520, ES03500, DRF].

PEPTIDES: PHYSIOLOGICAL EFFECTS I

91.1

EFFECTS OF CHOLECYSTOKININ ON NEURONS OF CAT PANCREATIC GANGLIA. R. C. Ma* and J. H. Szurszewski. Dept. of Physiology and Biophysics, Mayo Medical School, Rochester, MN 55905.

Immunocytochemical studies have shown that cholecystokinin-like material is present in pancreatic ganglia. To study the effects of cholecystokinin octapeptide (CCK), intrapancreatic ganglia were pinned out in vitro and perfused with oxygenated Krebs solution (35°C). During intracellular recordings, CCK (10^{-4} M) was applied extracellularly by pressure ejection. Sulfated and desulfated CCK evoked a slow depolarization in 76 of 94 and in 29 of 36 cells, respectively. The mean (± SEM) depolarization was 6.5±0.5 mV and 6.8±0.8 mV, respectively. In 23 of 30 cells, both forms of CCK evoked similar changes in membrane potential. During depolarization evoked by both forms of CCK, membrane input resistance increased in 28 cells and decreased in 7. No change occurred in 13 cells. Hexamethonium and a low Ca^{2+} solution did not effect CCK induced changes in membrane potential and input resistance indicating a post-synaptic action. In 20 of 105 cells, both forms of CCK evoked periods (10 to 120 min long) of spontaneous activity which consisted of fast excitatory postsynaptic potentials and action potentials indicating CCK can also affect some neurons through presynaptic cholinergic terminals. In summary, sulfated and desulfated CCK can act at presynaptic and postsynaptic sites. (DK 17632.)

91.2

ELECTROPHYSIOLOGICAL EFFECTS OF CHOLECYSTOKININ ON AMYGDALA NEURONS. C. ROUILLARD, L.A. CHIODO and A.S. FREEMAN. Lab. of Neurophysiology, Center for Cell Biology, Sinai Research Institute and the Cell. & Clin. Neurobiology Prog., Wayne State Univ., Detroit, MI 48235.

Cholecystokinin (CCK) is found in high concentrations in the limbic system of mammalian CNS. Large numbers of CCK cell bodies and CCK-containing fibres are located throughout the amygdaloid complex. The effects of CCK-8S on amygdala neurons were studied using standard extracellular single-unit and iontophoretic recording techniques in locally anesthetized, gallamine-immobilized, artificially respired rats. Intravenous injection of CCK-8S (cumulative dose: 2-32 ug/kg) excited most of the amygdala neurons tested (8/10). In some of these neurons (n=4), the excitatory effect of CCK progressed to apparent depolarization inactivation as indicated by the widening of the wave form and the decrease of its amplitude even with relatively modest firing rates increases. CCK exerted an inhibitory effect on one cell and the last cell was not affected by the peptide. Iontophoretic application of CCK-8S produced similar results: the peptide excited 3 of the 6 cells tested, partially inhibited one and had no effect on the two other cells. These results strongly suggest that CCK acts as a neurotransmitter or a neuromodulator in the amygdaloid complex.

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91.3

CHRONIC INTRAVENTRICULAR ADMINISTRATION OF CHOLECYSTOKININ MARKEDLY REDUCES THE NUMBER OF SPONTANEOUSLY ACTIVE MIDBRAIN DOPAMINE NEURONS. L.H. Jiang and R.Y. Wang. Department of Psychiatry and Behavioral Sciences, SUNY Stony Brook, Stony Brook, NY 11794-8790.

Ample evidence suggests that cholecystokinin (CCK) functionally opposes dopamine (DA). The aim of the present study was to decide whether chronic CCK mimics the effect of antipsychotic drugs on midbrain DA cells. Since CCK does not readily cross the blood brain barrier, it was administered directly to the lateral ventricle (icv) of Sprague-Dawley rats via a cannula connected to an osmotic minipump with a polyethylene tubing. CCK was delivered continuously by the minipump (132 ng/day) for 2 to 3 weeks. Standard extracellular single cell recording techniques was used to determine the number of spontaneously active DA cells/electrode track in anatomically defined A9 and A10 areas. Acute icv administration of CCK-8S (252.1 ± 49 ng in 2-4 µl, n=7) decreased the number of spontaneously active DA cells in both the A9 (0.46 ± 0.1, n=7) and A10 (0.8 ± 0.2, n=7) regions as compared to the control values (A10. 1.33 ± 0 .1; A9, 0.71 ± 0.1). Intravenous (i.v.) administration of the CCK-A antagonist lorglumide (4.8 µg/kg) reversed CCK-induced effects (n=3). Similarly, chronic icv administration of CCK, but not saline, also significantly reduced the number of spontaneously active A9 (0.33 ± 0.03, n=8) and A10 (0.53 ± 0.06, n=8) DA cells. The CCK-A receptor antagonist L-364,718 (40 ng/kg, i.v.) but not CCK-B receptor antagonist L-365,260 (40 ng/ng, i.v., n=4) reversed the chronic CCK-induced effects, returning the number of A9 and A10 DA cells to control values (A9, 0.8 ± 0.09; A10, 1.16 ± 0.1, n=5). These results suggest that chronic CCK mimics the actions of antipsychotic drugs and this effect is mediated by CCK-A receptors in the rat brain. (Supported by USPHS Grants MH-41440 and MH-00378)

91.4

CHOLECYSTOKININ (CCK) EVOKES OXYTOCIN AND VASOPRESSIN RELEASE FROM PERFUSED EXPLANTS OF RAT HYPOTHALAMUS. C.R. Jarvis, B.J.M. van de Heijning*, and L.P. Renaud, Centre for Research in Neuroscience, Montreal General Hospital and McGill University, Montreal, Canada H3G 1A4.

The supraoptic nucleus displays both CCK-immunoreactive cells and high affinity CCK-binding. The observation that CCK also induces a receptor-mediated depolarization of rat supraoptic neurons prompted an in vitro study of the ability of CCK to evoke the release of vasopressin (VP) and/or oxytocin (OT) from the neurointermediate lobe of hypothalamic explants. Explants (with the anterior pituitary removed) were perfused with oxygenated artificial cerebrospinal fluid via the internal carotid arteries. Perfusate was collected by a suction pipette positioned over the neurointermediate lobe and levels of OT and VP were assessed by RIA. Three minute infusions of 0.1-1.0 µM sulfated CCK-8 evoked a two to three-fold increase in the basal levels of both OT and VP. Test pulses of norepinephrine (60 µM), which induce VP release, verified the viability of the explant. These data suggest a role for CCK in OT and VP secretion.

Supported by FCAR, FRSQ, and MRC.

91.5

CHRONIC CHOLECYSTOKININ OCTAPEPTIDE (CCK8) ALTERS THE NUMBER OF SPONTANEOUSLY ACTIVE DOPAMINE NEURONS IN THE MESENCEPHALON OF THE RAT. J.D. Stittsworth, Jr.[1] and A.L. Mueller[2] 1)Neurosci. Res. Div., Abbott Labs., Abbott Park, IL 60064 and 2)Natural Product Sciences,Inc.,420 Chipeta Way,Salt Lake City,Utah 84108

Chronic administration of antipsychotic drugs results in a time dependent depolarization inactivation of dopamine (DA)-containing neurons in the ventral tegmental area (VTA, A10) and/or the substantia nigra (SN, A9). This phenomenon can be observed electrophysiologically as a decrease in the number of DA-containing neurons encountered with extracellular recording. Since previous experiments have demonstrated that CCK8 might modulate DA actions in the VTA and the SN, we decided to investigate CCK8's antipsychotic potential utilizing this in vivo model. Recordings were made in chloral hydrate anesthetized, male Sprague-Dawley rats. The recording electrodes were passed through stereotaxically defined areas of the SN and the VTA and the number of spontaneously active DA-containing neurons were counted and averaged per track (10 tracks/area). The number of DA-containing neurons encountered in vehicle treated animals were 0.70 +/-0.07 for the VTA (n=!8) and 0.71 +/-0.05 for the SN (n=18). After 21 days of daily subcutaneous administration of either haloperidol(HAL, 0.5 mg/kg), clozapine (CLOZ, 20 mg/kg) or CCK8 (0.03 mg/kg or 0.1 mg/kg), reductions in number of spontaneously active DA-containing neurons were observed. The reductions seen with HAL and CLOZ were consistent with previous reports. CCK8 reduced the number of neurons in both SN (0.03 mg/kg: 0.28 +/-0.07, n=8 and 0.1 mg/kg: 0.33 +/-0.08, n=11) and VTA (0.03 mg/kg: 0.43 +/-0.11, n=7 and 0.1 mg/kg: 0.36 +/-0.14 n=9).

91.6

OPIOID-LIKE ACTIONS IN VITRO AND IN VIVO OF A NOVEL CHOLECYSTOKININ ANALOG WITH HIGH SELECTIVITY FOR BRAIN CCK RECEPTORS. T.H. Kramer*, S.N. Fang*, P. Davis*, E.A. Ayres*, P.K. Lemcke*, V.J. Hruby*, and T.F. Burks. Depts. of Pharmacology and Chemistry, University of Arizona, Tucson AZ 85724.

$[CH_3\text{-}Nle^{28},CH_3\text{-}Nle^{31}]CCK_{26\text{-}33}$ (F8702), an analog of sulfated $CCK_{26\text{-}33}$ (CCK) synthesized in our laboratories, has high affinity for brain CCK receptors and low affinity for pancreatic (peripheral) CCK sites. We have studied F8702 and CCK actions in guinea pig ileum (GPI) whole segment (GPI-WS), longitudinal muscle/myenteric plexus (GPI-LM), and mouse vas deferens (MVD) bioassays, as well as hotplate analgesia (HP) and gastrointestinal transit (GIT) tests in mice. Whereas CCK was a full agonist in GPI-WS (A_{50}=2.56nM), F8702 had no (contractile) activity. In GPI-LM, F8702 was a partial (inhibitory) agonist (IC_{50}=10μM), sensitive to naloxone (NLX) and the μ antagonist CTAP. In MVD, F8702 was a full agonist (IC_{50}=1μM), with measurable but low sensitivity to the antagonists NLX, CTAP, ICI 174864, and nor-BNI. CCK was devoid of opioid actions in both GPI-LM and MVD. After i.c.v. injection in mice, F8702 caused analgesia (A_{50}=3μg) which was blocked by high dose (10 mg/kg) NLX, and inhibited GIT (A_{50}=2μg); CCK had minimal activity in both tests. F8702 may stimulate endogenous opioid release or nonselectively act at opioid as well as brain-type CCK receptors. (Supported by USPHS grants DA04248 and DK36289.)

91.7

PHARMACOLOGICAL AND FOOD INTAKE SUPPRESSING ACTIONS OF A-68552, A CONFORMATIONALLY-CONSTRAINED ANALOG OF CCK7. A.M. Nadzan*, M.D. Tufano*, C.W. Lin*, K. Asin, T. Miller*, D. Witte*, L. Bednarz*, M.J. Cullen*, W. Montana (SPON: W. Giardina). Neuroscience Research Division, Pharmaceutical Discovery, D-47H, Abbott Laboratories, Abbott Park, IL 60064.

A metabolically-stable and conformationally-constrained analog of CCK7, A-68552, was compared with CCK8 for its ability to bind to type A and B CCK receptors, stimulate phosphoinositol turnover and amylase release from pancreatic acinar cells and suppress food intake. A-68552 exhibited high affinity binding to pancreatic acinar cells (type A, IC50 = 4 nM) and to cortical receptors (type B, IC50 = 0.3 nM). In studies of phosphoinositol turnover in pancreatic acinar cells, A-68552 stimulated only 79% that of CCK8: however, it proved to be a full angonist in the stimulation of amylase release (EC50 = 1 nM). Both actions were blocked by the type A CCK antagonists, A-65186 and L-364,718. In food-deprived rats, A-68552 suppressed feeding in a dose dependent manner (ED50 = 3 ug/kg) and exhibited a prolonged duration of action over that of CCK8. This analog represents a valuable probe for the study of CCK's involvement in pancreatic function and in feeding.

91.8

COMPARISON OF MICRODIALYSIS AND PUSH-PULL PERFUSION TECHNIQUES FOR IN VIVO STUDIES OF NEUROPEPTIDE RELEASE. S.DeMesquita[1], K.Laitinen*, M.Beinfeld[2], A.Rokaeus*[3], J.N.Crawley. Clinical Neuroscience Branch, NIMH, Bethesda, MD 20892; [1]Dept. of Physiology, Marshall Univ., Huntington, WV 25755; [2]Dept. of Pharmacology, St. Louis Univ., St. Louis, MO 63104; [3]Dept. of Biochemistry, Karolinska Institutet, Stockholm, Sweden.

The release of neuropeptides was studied using intracerebral microdialysis and push-pull perfusion techniques. The coexistences of cholecystokinin (CCK) and dopamine in the mesolimbic pathway, and of galanin (GAL) and acetylcholine in the septohippocampal pathway, provide model systems for investigating peptide release in vivo. Microdialysis and push-pull perfusion experiments were compared for sensitivity and accuracy in sampling CCK from the posterior nucleus accumbens and GAL from the ventral hippocampus. Dialysis probes (3mm) or concentric push-pull cannulae (1mm) were perfused with aCSF or bovine serum albumin and bacitracin in aCSF at flow rates of 1 or 10 μL/min respectively. RIA was performed on samples obtained before and during perfusion with 100 mM KCl or 150 μM veratridine. Push-pull perfusion yielded a much greater percent recovery in vitro for both peptides as compared to microdialysis.

91.9

NEUROPEPTIDES MICROINJECTED INTO THE VENTRAL TEGMENTUM MODULATE DOPAMINE AND METABOLITES IN THE NUCLEUS ACCUMBENS AS MEASURED BY IN VIVO MICRODIALYSIS. K.Laitinen*, S.DeMesquita, J.N.Crawley (SPON: L.R.Skirboll). Unit on Behavioral Neuropharmacology, Clinical Neuroscience Branch, NIMH, Bethesda, MD 20892.

Microinjection of neuropeptides, including neurotensin, cholecystokinin, substance P, substance K, and oxytocin, into the ventral tegmental area (VTA), produce behavioral effects such as locomotion and grooming in the rat. The relative importance of each peptide on its receptors in the VTA in regulating postsynaptic dopamine (DA) release is unknown. In vivo microdialysis was employed to quantitate DA and its metabolites from the posterior nucleus accumbens (NA) before and after neuropeptide administration into the VTA. Anesthetized male Sprague-Dawley rats were unilaterally infused with each of the above neuropeptides at 1 nmole or 1 pmole/0.5 ul saline, over a one minute period, at 5.8 mm posterior and 0.5 mm lateral to bregma, 8.3 mm ventral to the surface of the skull (VTA). A Carnegie Medicine microdialysis probe, 2 mm length, was positioned ipsilaterally at 1.2 mm anterior and 1.2 mm lateral to bregma, 8.5 mm from the surface of the skull (NA), perfused with Ringers's solution at a flow rate of 1.0 ul/min. Perfusate was analyzed by HPLC with electrochemical detection for DA, DOPAC, and HVA. Neurotensin, which increases locomotion, increased DA, DOPAC and HVA. Cholecystokinin, substance P and substance K, which have complex behavioral actions on locomotion and grooming, produced small increases in DOPAC. These data suggest a correlation between behavioral effects of neuropeptides and their actions on mesolimbic dopamine release.

91.10

VENTRAL SEPTAL NEURONS MAY MEDIATE VASOPRESSIN ANTIPYRESIS BY ALTERING ACTIVITY OF ARCUATE AND DORSOMEDIAL HYPOTHALAMIC NEURONS. W.B. Mathieson, Q.J. Pittman and W.L. Veale. Dept. Med. Physiol., Univ. Calgary, Alberta T2N 4N1.

During fever, arginine vasopressin (AVP) is released from nerve terminals in the ventral septum to limit the magnitude of the hyperthermic response. To investigate potential pathways which may mediate AVP's antipyretic effects, a retrograde neuronal tracer, Fluorogold, was iontophoretically injected into hypothalamic sites suspected to receive efferent projections from the ventral septum. Following Fluorogold injections in the arcuate and dorsomedial hypothalamic nuclei, retrogradely labeled neurons were visualized in septal sites previously associated with AVP release and highly sensitive to microinfusion of AVP during fever. In separate experiments, single unit recordings in the arcuate and dorsomedial hypothalamic nuclei demonstrated both excitatory and inhibitory synaptic potentials following electrical stimulation of the ventral septal area. Since the arcuate nucleus contains a-MSH synthesizing neurons which may regulate the early phase of fever (Samson et al., Peptides 2:419, 1981) and the dorsomedial hypothalamic may activate heat production in brown adipose tissue (Freeman and Wellman, Br.Res.Bull.18:7, 1987), these results suggest two pathways by which AVP release in the ventral septum could initiate antipyresis.

91.11

LIGHT-DARK RHYTHMS OF VASOPRESSIN (AVP) IN DISCRETE HYPOTHALAMIC NUCLEI: RELATIONSHIP TO FEEDING. M.Jhanwar-Uniyal, M. Chapleur, A.J.Burlet and S.F.Leibowitz. The Rockefeller Univ., N.Y., and Laboratoire de Biologie Cellulaire, Faculte de Medecine, INSERM U 308, Nancy, France.

The present study examined AVP content in discrete hypothalamic areas at 5 different times of the 12/12 hr light/dark cycle (0700, 1300, 1800, 2000, 0100), with lights out at 1900 hr. Nine nuclei, namely, paraventricular (magnocellular PVN-M or parvocellular PVN-P), dorsomedial (DMN), median eminence (ME), arcuate (ARC), perifornical lateral (PLH), supraoptic (SON), suprachiasmatic (SCN), and ventromedial (VMN), were assayed for AVP content via RIA.

The results revealed a significant shift in AVP content across the light/dark cycle, in 5 hypothalamic nuclei. In the PVN-P and PVN-M, opposite temporal patterns were observed. The PVN-P exhibited lowest AVP levels (4.2 ng/mg P) 1 hr after dark onset and peak AVP content (9.6 ng/mg P) at light onset. In contrast, AVP in the PVN-M peaked in the early dark period (21.0 ng/mg P) and declined towards light onset (7.1 ng/mg P). Moreover, peptide levels in the SCN shifted in a similar manner to those in the PVN-M, whereas the ARC and PLH responded similarly, but not identically, to the PVN-P. The AVP content of the PVN-P showed a sharp decline from 1 hr prior to dark onset to 1 hr after dark onset. This may be related to the onset of natural feeding at this time, since our previous studies have shown a similar change after refeeding in food-deprived rats.

91.12

ARGININE VASOPRESSIN AND ATRIAL NATRIURETIC FACTOR: EVIDENCE FOR A HOMEOSTATIC SYSTEM SET AT THE THRESHOLD OF VOLUME-MEDIATED NATRIURESIS. P.A. Mason, K.M. Chu*, D. Bhaskaran, L.G. Ganousis*, A.S. Nies*, C.R. Freed, and J.A. Durr*. Depts. of Med. and Pharm., Univ. of Colo. Health Sci. Ctr. Denver, CO 80262.

Arginine vasopressin (AVP) and atrial natriuretic factor (ANF) have opposite vascular and renal effects and are reciprocally controlled by blood volume (BV). The influences of BV and plasma osmolality (Posm) on the plasma concentrations of AVP and ANF were investigated using rats. Chronic changes in BV and Posm were produced by fluid deprivation. Acute changes in BV and Posm were produced by intraperitoneal injections of 35% polyethylene glycol and/or saline solutions (200, 600, 1000 mOsm/kg). Regression analysis showed that AVP correlated directly with Posm and inversely with BV. Posm was the major predictor of baseline AVP. Conversely, ANF correlated directly with BV and inversely with Posm. BV was the major predictor of baseline ANF. A 25% decrease in BV would be required for complete ANF suppression. Increasing BV by only 2% stimulates ANF release and natriuresis until BV normalizes and the ANF returns to its basal level. Since the ANF release threshold is 25% below normal BV and ANF secretion is not suppressible without AVP stimulation, natriuresis appears to be continuously used to maintain circulating volume. These results suggest that sodium conservation is not the primary regulator of circulating volume.

91.13

VASOPRESSIN PRODUCES A CHOLINERGICALLY-MEDIATED AROUSAL (ANALEPTIC) EFFECT IN PENTOBARBITALIZED RATS. A. Horita and M.A. Carino (SPON: H. Lai). Depts. of Pharmacology and Psychiatry, Univ. Wash. Sch. of Med., Seattle, WA 98195.

Arginine vasopressin (AVP), in addition to possessing antidiuretic and vasopressor activities, may play a role in behavioral, memory and temperature regulatory processes. We now describe a cholinergically-mediated analeptic effect of AVP that is produced in pentobarbital (PB)-narcotized rats. Icv doses of 100 ng, but not 50 ng, of AVP significantly shortened the duration of loss of righting reflex produced by PB. Atropine, but not methylatropine, completely blocked the analeptic effect, indicating the cholinergic nature of the response. Sodium dependent, high affinity choline uptake (HACU) activity was reduced in cortical and hippocampal synaptosomes prepared from PB-treated rat brains (as compared to conscious controls). AVP (100 ng, icv) restored HACU activity to normal in hippocampus, but not in cortex. The desglycinamide analog of AVP was devoid of analeptic activity. [Mcp^1,Tyr(OMe)^2]-AVP (10 ug, icv), the vasopressor antagonist, produced complete and specific block of the AVP-induced analeptic response. [d(CH$_2$)^5, D-Ile^2,Ile^4]-AVP, an antagonist of the antidiuretic effect of AVP, only partially blocked the AVP response at 10 ug, icv. (Supported by Rehabilitation Research and Training Grant, H133B80081).

91.14

HIPPOCAMPAL INHIBITION PRODUCED BY STIMULATING VASOPRESSIN CELLS IN THE MEDIAL AMYGDALA. D. Albeck*, T. Smock, P. McMechen*, D. Purves* and L. Floyd*. (SPON: Herbert P. Alpern). Center for Neurosciences, Dept. of Psychology, Univ. Colorado, Boulder, CO 80309.

Arginine vasopressin (AVP) acts directly on two target cell types in the rat hippocampus; inhibitory interneurons and small blood vessels. Excitation of the interneurons produces secondary inhibition of pyramidal cells (Albeck and Smock, 1988, Brain Res. 463:394). Recently the source of hippocampal AVP-like peptide has been located in the medial amygdala (Caffé et al., 1987, J. Comp. Neurol. 261:237). Here we report that electrical stimulation of this nucleus produces inhibition of pyramidal cells that is indistinguishable from the action of applied AVP.

Experiments were performed on 44 male Sprague-Dawley rats acutely anesthetized with urethane. When 100-700 ng AVP was applied to the central ventricle, a large and reversible inhibition of the evoked field population spike in CA$_1$ was observed (n = 6). The characteristics of the inhibition were the same as that obtained with 1 μM AVP in the hippocampal slice preparation. Furthermore, when brief trains of electrical stimuli (5-15 v, 0.5 msec at 100 Hz for 100 msec) were applied to the medial amygdaloid nucleus, presumably releasing endogenous AVP, a reversible inhibition of the hippocampal population spike was also obtained (n = 32). Histology showed that the inhibition could only be obtained from electrode positions in the medial amygdaloid nucleus, shown by immunocytochemistry and tract-tracing techniques to be the source of hippocampal AVP. Like peptidergic potentials in simple preparations, the response to amygdala stimulation was slow to develop (15 sec) and lasted a long time (10-15 min).

Since AVP applied to the slice preparation, applied to the ventricle in the whole animal, or presumably released endogenously by stimulation of the medial amygdala has in each case the same impact on pyramidal cells in the hippocampus, we propose that each result is a reflection of a common peptidergic mechanism in the mammalian brain. Since specific structural antagonists can be used to block the action of AVP in the hippocampus, the results present the prospect that the nature of the native transmitter can be determined based on susceptibility to antagonist blockade.

91.15

HIPPOCAMPAL INHIBITION BLOCKED BY A VASOPRESSIN/OXYTOCIN ANTAGONIST. T. Smock, D. Albeck*, P. McMechen*, and D. Purves*. Center for Neurosciences, Department of Psychology, University of Colorado, Boulder, CO 80309.

Stimulation of the source of hippocampal vasopressin-like peptide produces an inhibition of pyramidal cells that is identical to that obtained by application of vasopressin in the slice preparation or the whole animal (Albeck et al., this meeting). Here we show that the action of the endogenously released peptide can be blocked by an antagonist specific to brain vasopressin/oxytocin receptors. Taken together, the results constitute strong evidence for peptidergic transmission in the rat hippocampus.

Experiments were done on 17 male Sprague-Dawley rats. As for the action of vasopressin in the slice, the action of i.c.v. vasopressin (100-500 ng) was blocked by the structural analog d(CH$_2$)$_5$ Tyr (Me) AVP (4 of 5 trials). The fact that the hippocampal response to stimulation of peptidergic fibers from the amygdala is repeatable within animals was exploited to test for vasopressin mediation in an internally controlled paradigm. In ten trials, the specific V$_1$ pressor antagonist was ineffective in blocking the action of the endogenous peptide.

However, when the experiments were repeated with the oxytocin/vasopressin analog [des-Gly-NH$_2$ d(CH$_2$)$_5$ Tyr (Me)^2 Thr^4] OVT, the action of the endogenously released peptide was invariably blocked (n = 4).

The resemblance of the endogenous signal to the action of exogenous vasopressin, together with evidence for synthesis, transport and secretion of vasopressin-like material by neurons in the medial amygdala constitute evidence for peptidergic transmission mediated by a vasopressin-like peptide. The evidence, presented here, for blockade of the action of the native peptide with a specific antagonist for the brain vasopressin/oxytocin receptor makes the case very strong. The additional fact that an antagonist that blocks the action of exogenous vasopressin fails to block the action of the endogenously released substance suggests that this endogenous substance may be similar, but not identical, to pituitary vasopressin.

Supported by NSF BNS 8520622 and a gift of antagonists from Dr. Maurice Manning.

91.16

Atrial natriuretic peptide (ANP) decreases glucose utilization in the subfornical organ (SFO). E. Nermo-Lindquist*, M.L. Terrell, J. Nassar*, H. Lekan, S. Freeman* and M. Kadekaro. Div.of Neurosurgery, Univ. of Texas Med.Branch, Galveston, Tx. 77550.

Previous studies have shown that glucose utilization in the SFO is increased in several rat models in which angiotensin II (AII) levels are elevated. The objective of this study was to investigate with the deoxyglucose method if ANP acts at the SFO to antagonize the effects of AII. Male adult homozygous Brattleboro (DI) and Sprague-Dawley (SD) rats were used. DI rats, who are characterized by high plasma levels of AII, were infused intravenously with saline (n=12) or ANP (0.1 ug/ min) (n=12) for 30 min. ANP decreased blood pressure but did not change glucose utilization in the SFO or neural lobe (NL). Because hypotension could mask the effect of ANP, DI rats were infused with albumin (n=6), or with albumin+ANP (n=6) to prevent hypotension. Glucose utilization tended to decrease in the SFO (p=0.08) but did not change in the NL. SD rats were infused intravenously with saline (n=8), AII (2.5 ug/ min) (n=8) or AII (2.5 ug/ min)+ANP (0.15 ug/min) (n=8) for 45 min. AII stimulated water intake and increased glucose utilization in the SFO and NL. ANP reduced the drinking response to AII and decreased glucose utilization in the SFO (p=0.003), but did not in the NL. The results suggest that ANP acts at the SFO to antagonize the effects of AII.

91.17

INTRACEREBROVENTRICULAR CORTICOTROPIN-RELEASING FACTOR INCREASES BRAIN CONCENTRATIONS OF PRO-OPIOMELANOCORTIN-RELATED PEPTIDES L.P. Kapcala, C.F. Weng* and R.L. Hauger. University of Maryland School of Medicine, Dept. Med., Baltimore, MD 21201 and VAMC and UCSD School of Medicine, Dept. Psych., La Jolla, CA 92093

Corticotropin-releasing factor (CRF) is considered an important brain neuroregulator in view of its widespread brain distribution and its diverse biochemical and behavioral effects on brain. Despite sugggestions that CRF stimulates hypothalamic secretion of pro-opiomelanocortin (POMC)-related peptides in vitro, effects of CRF on brain levels of POMC-derivatives are not known. We tested the hypothesis that central administration of CRF alters brain concentrations of POMC-related peptides. CRF (1μg) or placebo was administered into the lateral ventricle of adult rats and brain concentrations of IR-POMC peptides were measured after 60 min. IR-α-MSH was measurable only in hypothalamus. Concentration changes are shown below.

* $p<0.05$ ** $p<0.01$

Region	IR-	% Change Relative to Control ACTH	β-endorphin	α-MSH
Hypothalamus		+ 42	+ 51*	+376**
Hippocampus		+128	+300*	-
Amygdala		+ 25	+ 91**	-
Cortex		+463**	+692**	-

CRF also produced significant decrements in molar ratios of IR-ACTH/IR-β-endorphin in amygdala and hippocampus and an increment of hypothalamic IR-α-MSH/IR-β-endorphin.

CONCLUSIONS: 1) Intracerebroventricular CRF produces potent behavioral effects and increases brain concentrations of POMC-related peptides particularly in cortex which contains highest CRF receptor concentrations. 2) Changes in molar ratios of POMC-related peptides suggest a possible differential regulation of different POMC derivatives. 3) Some of CRF's behavioral effects may be mediated via changes in brain POMC-related peptides.

91.18

PARTICIPATION OF AMYGDALOID CENTRAL NUCLEUS (ACE) IN CRF INDUCED INCREASES IN IN VITRO TRYPTOPHAN HYDROXYLASE ACTIVITY (TrpH). **V.B.Singh, T-H.Phan and Margaret Boadle-Biber. Dept. of Physiology, Medical Coll. of Virginia, Virginia Commonwealth University, Richmond, Va-23298-0551.**

Corticotropin-releasing-factor (CRF) may regulate behavioral, visceral and neuroendocrine responses to stress. We reported that ICV infusions of CRF (3 ug total in 6 uL vehicle or vehicle alone given over 30 sec) into awake male Sprague Dawley rats, increase in vitro cortical and midbrain TrpH activity by 50-60% over that of vehicle infused control rats (Singh et al. Faseb J. 3: A729. 1989). We have now examined whether CRF infusion into amygdaloid central nucleus (ACE) or dorsal raphe nucleus (DR) also increases the in vitro activity of cortical and midbrain TrpH. CRF (3 ug/6 u L vehicle) or vehicle (6 u L) alone was infused into ACE or DR over 30 sec via guide cannulae implanted 3-4 days earlier under surgical anesthesia. Rats were killed by decapitation at different time intervals after infusion and cortex and midbrain were carefully removed on ice and stored at -70°C until assayed for TrpH activity (Boadle-Biber et al. Brain Res. 482: 306, 1989). A rapid (within 15 min) increase in TrpH activity was noticed with CRF infused into ACE and a slow increase (over 45 min) with CRF infusion to DR. This increase in TrpH activity was comparable with that of sound-stress-induced changes and direct electrical stimulation to dorsal raphe nucleus. It was reversed by alkaline phosphatase treatment and was nonadditive with the increase in the activity obtained under phosphorylating condition. The above findings suggest a direct role of ACE in stress induced changes in TrpH activity. Supported by NIH GRANT NS14090 to MCB-B.

91.19

CORTICOTROPIN RELEASING FACTOR (CRF) DECREASES A MEDIUM/SLOW AFTERHYPERPOLARISATION (m/sAHP) AND EVOKES A HYPERPOLARISATION IN AMYGDALOID NEURONES. **D.G. Rainnie*, B.J.H. Fernhout*, M.E. Abreu and P. Shinnick-Gallagher** (SPON: O.S. Steinsland). Dept. of Pharmacology, Univ. of Texas Medical Branch, Galveston, TX 77550.

The amygdaloid complex has been shown to contain a high density of CRF receptors and CRF-immunoreactive neurones. In the present in vitro study we examined the effect of superfusing CRF (10-250nM) on neuronal firing activity in the basolateral (BLA) and central (ACe) amygdala of the rat by conventional intracellular recording techniques. In those BLA neurones that showed a m/sAHP CRF (250nM) caused a voltage independent reduction (33-90%) in the m/sAHP amplitude observed following evoked burst firing activity (6/6 cells tested). In ACe neurones CRF (10-250nM) caused a similar reduction (41-77%) in 4/6 cells tested. Additionally in ACe neurones CRF evoked a 2-14mV hyperpolarisation (6/6 cells tested) which was associated with an increase in membrane input resistance (R_m) (3/6 cells) and a reduction in anomalous rectification. Conversely in the BLA CRF caused no apparent change in the membrane potential but decreased R_m in 4/7 cells tested. Hence CRF has differential effects in individual nuclei of the amygdaloid complex which may reflect their physiological requirements during times of stress. (Supported in part by NS24643).

91.20

ACTIVATION OF RAT RETINA ADENYLATE CYCLASE BY CORTICOTROPIN-RELEASING FACTOR. P. Onali and M. C. Olianas. Dept. of Neurosciences, Univ. of Cagliari, Italy.

Corticotropin-releasing factor (CRF), a peptide widely distributed in the CNS, has also been detected in the retina of different animal species, where it may act as a neurotransmitter. In the present study we report that human-rat CRF stimulates adenylate cyclase activity of rat retina homogenate. The CRF stimulation is concentration-dependent with an EC50 value of 50 nM. Maximal stimulation corresponds to 90% increase of basal enzyme activity. The CRF effect is counteracted by the CRF antagonist α-helical CRF 9-41 with a Ki value of 40 nM. Other CRF-like peptides such as sauvagine (Sauv) and urotensin I (Urot) are as effective as CRF with the rank order of potency: Urot ≥ Sauv > CRF. The Sauv and Urot stimulations are not additive with that elicited by CRF. The CRF effect is independent of the concentration of Ca^{++}, is enhanced by increasing the Mg^{++}concentration up to 5-10 mM and requires GTP. These results indicate that in rat retina CRF and CRF-like peptides may increase cyclic AMP formation by activating a receptor site linked to adenylate cyclase. This biochemical response may constitute an important mechanism for the action of CRF in the retina.

91.21

Ca^{++}-DEPENDENT ACTIVATION OF STRIATAL TYROSINE HYDROXYLASE BY CRF : INHIBITION BY PRESYNAPTIC DOPAMINE AUTORECEPTORS. M.C. Olianas and P. Onali. Department of Neurosciences, University of Cagliari, Italy.

In mouse striatal synaptosomes corticotropin- releasing factor (CRF), a putative neurotransmitter in the CNS, stimulated dopamine (DA) synthesis by activating tyrosine hydroxylase (TH). TH activity was assayed in extracts prepared by centrifugation and sonication of mouse striatal synaptosomes preincubated with the test agents. CRF maximally increased TH activity by 60% with an EC50 value of 15 nM. The CRF stimulation was antagonized by α-helical CRF 9-41 (Ki 100 nM) and was mimicked by sauvagine and urotensin I, two peptides homologous to CRF. The stimulation of TH activity was not associated with a stimulation of cyclic AMP production by CRF in striatal homogenates. The CRF stimulation was strictly dependent on the concentration of Ca^{++}in the incubation medium. Quinpirole (1 μM), a selective D2 DA receptor agonist, reduced the maximal CRF stimulation by 47% without changing the EC50 of the peptide. These results indicate that striatal dopaminergic terminals contain a receptor system for CRF that activates TH via a Ca^{++}-dependent pathway which is sensitive to modulation by presynaptic DA autoreceptors.

92.1

A NOVEL ANTAGONIST TO VASOACTIVE INTESTINAL PEPTIDE.
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To better understand the interactions of vasoactive intestinal peptide (VIP) with its receptor, we have devised a novel VIP antagonist by a hybrid peptide strategy. A molecule was synthesized that combines a portion of VIP with a portion of neurotensin, peptides of opposite pharmacological action. The antagonistic nature of the new molecule was tested in cell cultures derived from the central nervous system. The hybrid antagonist displaced 85-90% of VIP binding to glial cell cultures. This displacement had a biphasic nature indicating two binding sites, one with an IC50 of 30 pM and another with an IC50 of 0.1 μM. Moreover, the formation of VIP-stimulated cAMP in these cell cultures was decreased by the peptide with an IC50 of 0.08 μM. The inhibition in all assays was more effective than that achieved in the presence of other putative VIP antagonists. The hybrid antagonist (10 μM) did not stimulate cAMP formation. Finally, previous studies have shown that nonneuronal cells stimulated by VIP secrete neuronal survival factors. Indeed, the addition 1 nM hybrid antagonist to dissociated spinal cord cultures resulted in neuronal cell death (42% decrease from controls). The availability of highly potent VIP antagonists may offer a route to study the molecular characteristics of the multiple VIP receptors as well as help delineate other biological activities attributable to VIP. (IG is on sabbatical from the Weizmann Inst.)

92.2

VAGINOCERVICAL STIMULATION RELEASES VASOACTIVE INTESTINAL PEPTIDE-LIKE IMMUNOREACTIVITY (VIP) INTO SPINAL CORD SUPERFUSATES IN RATS.
B.R. Komisaruk[1], A.R. Gintzler[2], C. Banas[1*] and M.A. Blank[3*] Institute of Animal Behavior, Rutgers Univ., Newark, NJ 07102[1], Depts. Biochem.[2] and Surgery[3], SUNY Medical Center Brooklyn, NY 11203

It has been shown previously that vaginocervical mechanostimulation (VS) produces analgesia and elicits afferent activity in the pelvic nerve, transection of which significantly reduces VS-produced analgesia. Other laboratories provide evidence that the primary afferent terminals of the pelvic nerve contain VIP, and electrical stimulation of the pelvic nerve releases VIP. VIP administration to the spinal cord produces analgesia. The present study ascertained whether VS releases VIP into superfusates of the lumbosacral spinal cord. Samples (2.5 min each) were collected on ice and frozen at -70°C immediately. VIP was assayed by RIA before, during and after VS or foot pinch (FP). The peak level of VIP in the baseline period was compared with that in the stimulation period. Mean ± sem level of VIP (fmol/ml superfusate) was 12.1 ± 2.4. This was significantly greater ($p<0.03, n=10$) than the prestimulation baseline (7.6 ± 1.2) or the FP (6.8 ± 1.1) control levels, which did not differ significantly from each other. These data support our hypothesis that VIP released from pelvic nerve primary afferent terminals in the spinal cord may mediate, at least in part, VS-produced analgesia.
Supported by NIH NLS-2-1RO1-NS22948 to BRK.

92.3

VAGINO-CERVICAL STIMULATION (VS) REDUCES SUBSTANCE P RELEASE INTO SPINAL CORD SUPERFUSATES IN RATS.
JL Steinman, C Banas*, SW Hoffman* & BR Komisaruk, Institute of Animal Behavior, Rutgers Univ., Newark, NJ 07102.

In the present study, levels of substance P (sP) measured in superfusates of the lumbosacral region of the spinal cord were significantly reduced to 61.6% below basal levels during and immediately after VS. Superfusate samples, collected on ice and frozen at -70C, were analyzed by RIA. Six basal samples (3 min ea.) were collected before and after VS trials. Electric shock to the hindpaw evoked a 64.2% increase in sP levels. In 4 experiments, the levels of sP were significantly lower after VS plus shock (52%) than basal levels or than levels measured after shock alone ($p<.04$ Mann-Whitney U test).

These results indicate that VS, which produces analgesia in rats, reduces the basal release of sP and attenuates hindpaw shock-induced sP release from the spinal cord.
(Supported by NIH NLS-2-1RO1-NS22948 to BRK).

92.4

LHRH Modulates Nociceptive Responses in Female Rats.
A. Ratka* and J.W. Simpkins. Dept. of Pharmacodynamics, U. of Florida, Gainesville, FL 32610.

We have shown that responsiveness to painful stimuli changes during the estrous cycle (Neuroendocrinol. 48:394, 1988). In the present study, we evaluated the role of LHRH in modulating nociceptive responses in female rats. In ovariectomized (OVX) rats, an LHRH agonist ([Des-Gly10]LHRH Ethyl Amide; 1 ng/rat/μl) given icv at either 90, 60, 30 min before the hot-plate test caused a time-dependent, four-fold, increase in pain perception (hyperalgesia) vs. saline treated controls. Further, the LHRH agonist (1 ng/rat/μl; icv) attenuated morphine (5 mg/Kg, sc)-induced antinociception. The injection of an LHRH antagonist ([D-Phe2,Pro3,D-Phe6]LHRH) to OVX rats in doses of 0.1, 1 or 10 ng/rat 30 min prior to morphine, potentiated and prolonged morphine-induced antinociception in a dose-dependent manner. Moreover, the potent hyperalgesia observed in OVX rats treated with naloxone (1 mg/kg, sc) was reversed by pre-injection of the LHRH antagonist (0.1 ng/rat, icv). OVX rats primed with estradiol (EB) and progesterone (P) were less sensitive to the antinociceptive effect of morphine than OVX rats. When EBP treated rats received the LHRH antagonist prior to morphine, a two-fold increase in morphine-induced antinociception was observed. In conclusion, LHRH may interact with central opioid systems causing an increased sensitivity to pain (hyperalgesia) and a reduction of the antinociceptive effect of morphine. (Supported by AG 02021).

92.5

Response of the Normal and Arreflexic Bladder to Spinally Administered Primary Afferent Neurotransmitters in the Intact and Chronic Spinal Rat.
Paul J. Tiseo, and Tony L. Yaksh*. (Spon. L.-Y. Liu-Chen)*
Univ. of California, San Diego Medical Center, Dept. of Anesthesiology.

Following spinal cord lesion, there is a reliable loss of the ability of the bladder to store and efficiently expel urine. Normally, information relevant to communicating the state of bladder distention is part of a supraspinally mediated reflex and is carried by large afferent fibers. Following spinal trauma, it appears that smaller (type C) afferents may play a more dominant role. As the identity of many C-fiber transmitters is well documented and known to include amino acids (glutamate) as well as neuropeptides (SP, CCK, VIP, CGRP and SST), the present study systematically examined the effect of these agents and several selective antagonists administered into the lumbar intrathecal space on the volume-evoked micturition reflex (VEMR) of intact and spinally transected rats. Male S-D rats were surgically implanted with chronic catheters in the urinary bladder (PE-90) and the lumbar intrathecal space (PE-10). At the time of testing, the VEMR was studied during a constant infusion of saline (200 µl/min) into the bladder before and after intrathecal (i.t.) injection of an agent. In the intact animal, i.t. administration of glutamate, SP, CCK, VIP, or SST produced *no* alterations in the VEMR. However, administration of antagonists for glutamate (MK-801), and VIP ([4-Cl-D-Phe^6, Leu^{17}]-VIP), but *not* CCK(L-364, 718-000), produced a dose-dependent increase in frequency of detrusor muscle contraction and sphincter opening pressure with a corresponding decrease in volume to VEMR. Preliminary studies in transected animals (T-12) have shown that both glutamate and VIP can generate detrusor muscle contractions and VEMR's in arreflexic bladders beginning 5-10 days post-transection. These studies suggest that it may be possible to pharmacologically drive several systems mediating the VEMR, following the loss of this reflex in transected animals.
(Supported by the American Paralysis Association)

92.6

PARAVENTRICULAR NUCLEUS INJECTION OF TACHYKININS CAUSES HYPERTENSION AND TACHYCARDIA VIA AVP DESCENDING PATHWAY.
D-P. Tan* and K. Tsou. (SPON: G.D. Trisler). Dept. Pharmacol. III, Shanghai Inst. of Materia Medica, Academica Sinica, Shanghai 200031.

Our previous studies have shown that small amounts of intrathecal vasopressin or oxytocin elicited significant hypertension mediated via excitation of the spinal sympathetic system (*Peptides* 6:1191 and 7:569). We sought to determine whether stimulating the hypothalamic paraventricular nucleus (PVN) by tachykinins can regulate cardiovascular function and whether such regulation is modulated by the descending vasopressinergic pathway from the PVN to the spinal cord. In sino-aortic denervated rats, PVN injection of tachykinins elicited hypertension and tachycardia:

	Max. Δ Mean Arterial Pressure (mm Hg)		Max. Δ HR (beats/min)	
	1 µg	3 µg	3 µg	
Saline (1 µl)	-2 ± 2		-4 ± 6	
Substance P	9 ± 3*	18 ± 2**	14 ± 4*	
Physalaemin	n.t.	18 ± 1**	n.t.	
Kassinin	18 ± 3**	36 ± 3**	19 ± 4*	* P<0.05
Eledoisin	24 ± 3**	46 ± 6**	25 ± 4*	**P<0.01

The hypertensive effect began within 2 min after tachykinin injection and lasted 10-20 min. The potency order was eledoisin > kassinin > substance P = physalaemin. Tachycardia began within 4 min after injection and lasted ≥12 min. The potency order was eledoisin > kassinin > substance P. Intrathecal injection of a vasopressin antagonist Pmp^1-O-Me-Tyr^2-Arg^8-vasopressin (but not saline) greatly attenuated the two cardiovascular effects of PVN injection of eledoisin (ΔMAP 46 ±6 vs. 8 ± 2 ($P<0.001$); ΔHR 25 ± 4 vs. -2 ± 2, $P<0.01$)).

These data suggest that (1) tachykinins can stimulate the PVN to elicit significant cardiovascular effects; (2) these effects mostly were via NK-3 receptors in the PVN; and (3) the AVP descending pathway from PVN to spinal cord may be involved in the tachykinin-induced cardiovascular effects.

92.7

SOMATOSTATIN-14 (SOM-14) AND SOMATOSTATIN-28 (SOM-28) INHIBIT CA CURRENTS IN RAT NEOCORTICAL NEURONS. H.L. Wang, T. Reisine, M. Dichter, Departments of Pharmacology and Neurology, University of Pennsylvania School of Medicine and Graduate Hospital, Philadelphia, PA 19104.

The prosomatostatin-derived peptides, SOM-14 and SOM-28, are believed to function as neurotransmitters or neuromodulators in the cerebral cortex. We have examined the effects of these two peptides on ionic conductances in cultured rat neocortical neurons. These cultured neurons express SOM receptors and release both immunoreactive SOM-14 and SOM-28. We previously showed that SOM-14 and SOM-28 differentially regulate delayed rectifier K currents in these neurons. As SOM peptides have also been reported to block voltage-dependent Ca currents in pituitary cell lines and sympathetic ganglionic neurons, we examined their effects on Ca currents in neocortical neurons.

Voltage-dependent Ca currents of rat neocortical neurons were recorded using whole-cell patch clamp techniques under conditions in which K and Na currents were blocked. SOM-14 (100 nM) and SOM-28 (100 nM) did not effect the low voltage activated Ca current, but both peptides reversibly blocked the high voltage activated Ca current and slowed the activation of this current. When patch pipettes contained 100 uM cAMP and 0.5 mM IBMX, SOM-14 and SOM-28 still inhibited Ca currents. Inclusion of the non-hydrolysable GTP analog, GTP-γ-S (500 uM), in the recording electrode produced effects similar to those produced by SOM-14 and SOM-28. Pretreating neurons with pertussis toxin (PTX) prevented the inhibition of Ca currents by SOM-14 and SOM-28. Our results suggest that SOM-14 and SOM-28 inhibit Ca currents of rat neocortical neurons through a cAMP-independent mechanism involving PTX-sensitive G-proteins. Further studies are needed to determine whether SOM-14 and SOM-28 regulate Ca currents through the same population of receptors and/or G-proteins.
Supported by NIH grant GM 34781

92.8

BRADYKININ-INDUCED CURRENTS IN RAT DRG NEURONS AND F-11 CELLS ARE INSENSITIVE TO PERTUSSIS TOXIN. D.S.McGehee* and G.S. Oxford, Dept of Physiology, University of North Carolina, Chapel Hill, NC 27599

Cultured rat DRG neurons will respond to bradykinin (BK) with a transient inward cation current (10-60pA) which desensitizes markedly. Whole cell patch clamp techniques were used to measure BK responses from DRG neurons dissociated from rat pups aged E15 to day 1 postnatal. A subpopulation of these cells responded to BK (10-1000nM), while the age of the animals used had no remarkable influence on either the character of the response or the % of cells responding. Ion replacement studies have shown that the BK-induced current can be carried by Na^+, Li^+ or Cs^+, but not by large impermeant species such as NMG^+ or TMA^+. Varying the chloride reversal potential had no effect on the response. In standard recording solutions ramp voltage clamp analyses have shown that the BK current reverses near 0mV, indicating a non-selective cation conductance. The mouse neuroblastoma x rat DRG hybrid cell line, F-11 responds to BK with a biphasic response, a strong outward current followed by a slow inward current, much like the BK response of the NG108-15 cell line reported by Brown et al. (1987). The responses differ, however, in that the second phase in NG108-15 represents a conductance decrease, whereas the response of DRG and F-11 neurons results from a conductance increase which reverses near 0mV. Overnight pretreatment with *Pertussis* toxin (350ng/ml) had no obvious effect on BK responses in DRG or F-11 cells. We are currently undertaking experiments to determine the role of GTP-binding proteins and second messenger candidates in these responses. Supported by NIH Grant NS23804.

92.9

SUBSTANCE P-MEDIATED SYNAPTIC INTERACTIONS BETWEEN RAT MYENTERIC NEURONS IN CELL CULTURE. A.L. Willard, Dept. of Physiology, University of North Carolina, Chapel Hill, NC 27599-7545.

Subsets of rat myenteric neurons in cell culture evoke slow depolarizations (slow EPSPs) in other myenteric neurons without causing detectable fast EPSPs (Willard & Nishi, *Neuroscience* 16:213, 1985). I have tested the hypothesis that Substance P (SP) mediates these slow EPSPs.

Experiments were performed on 3 to 5 week old cultures. Intracellular recordings were made from individual myenteric neurons while other neurons were stimulated with either a loosely sealed (R_{seal} < 200 MΩ) extracellular patch pipet or with an intracellular electrode.

The following data support the hypothesis that SP mediates a subset of slow EPSPs between cultured myenteric neurons. i. Seventeen of 28 neurons that evoked slow EPSPs contained immunohistochemically detectable SP-LIR. ii. The slow EPSPs, which appear to be due to decreased resting K^+ conductance, were mimicked by synthetic SP or by an analog, [pGlu^6, Pro^9]-Substance P 6-11 (septide), which is reported to be specific for NK-1 tachykinin receptors (Laufer et al, *J. Pharm. Exp. Ther.* 245:639, 1988). iii. Prior application of SP or septide reduced or prevented the slow EPSPs. iv. Specific antisera against SP reduced or blocked the slow EPSPs caused by 8 of 13 neurons. All 8 of the neurons whose postsynaptic actions were affected by anti-SP were found to be SP-positive in the immunohistochemical assay, while 0/4 neurons whose actions were unaffected by anti-SP were SP-positive.

I conclude that a subset of the slow EPSPs are mediated by a Substance P-like molecule. The mediator of the slow EPSPs caused by neurons that lack immunohistochemically detectable SP remains to be determined. Supported by NIH grant NS24362.

92.10

EFFECT OF CENTRALLY ACTIVE NEUROPEPTIDES ON GASTRIC LUMINAL ACID (H^+), SEROTONIN (5-HT) AND HISTAMINE (HIST) RELEASE IN THE RAT. **R.L. Stephens, Jr. and Y. Tache' Department of Physiology, The Ohio State Univ., Columbus, Ohio 43210 and CURE, VA Wadsworth Medical Center, UCLA, Los Angeles, CA 90073.**

Intracisternal (ic) injection of TRH analogue RX 77368 stimulates gastric H^+ and 5-HT secretion into the gastric lumen (Amer. J. Physiol. 256; G377-383, 1989). This study was designed to assess the effect of ic injection of other centrally active neuropeptides on H^+, 5-HT, and HIST secretion into the gastric lumen of 2-hr pylorus ligated rats. 5-HT and HIST were measured by HPLC methodology. Bombesin (500ng) and CRF (5 μg) produced marked (>95%) reduction in H^+ and 5-HT secretion. However, vagal stimulants RX 77368 (100ng) and somatostatin analogue ODT8-SS (1μg), while increasing H^+ secretion, produced stimulation and inhibition, respectively of luminal 5-HT and HIST secretion:

Treatment	N	H^+ Output (μmol/2 hr)	5-HT (ng/2hr)	HIST (ng/2hr)
vehicle	5	189 ± 63	159 ± 33	2.8 ± 1.4
RX 77368	5	348 ± 55[a]	568 ± 65[a]	15.9 ± 6.6[a]
ODT8-SS	5	395 ± 67[a]	14 ± 4[b]	0.4 ± 0.1[b]

[a]: sign. increase $p<0.05$; [b]: sign. decrease $p<0.05$

The data reveals central peptidergic pathways which influence gastric 5-HT and HIST release, and suggests a dissociation between mechanisms of central regulation of gastric H^+ output and luminal 5-HT and HIST release. Supported by Ohio State University Seed Grant 221192.

92.11

OXYTOCIN AGONIST ADMINISTERED CENTRALLY DECREASES FOOD INTAKE IN RATS. B.R. Olson*, M.D. Drutarosky*, M.S. Chow*, V.J. Hruby*, E.M. Stricker, and J.G. Verbalis (SPON: A. Robinson). University of Pittsburgh, Pittsburgh, PA and University of Arizona, Tuscon, AZ.

Activation of central oxytocinergic (OT) pathways is frequently correlated with inhibition of gastric motility (GM) and food intake (FI) in rats. Although systemically administered OT does not decrease GM or FI, OT projections from the paraventricular nucleus to the brainstem have been shown to participate in vagally-mediated inhibition of GM (Rogers et al, Peptides, 8:505, 1987). To ascertain whether central OT projections similarly mediate inhibition of FI, we evaluated the effect of acute icv injections of an OT agonist (MSC(11)51; OTAg) on FI in male rats trained to eat pelleted chow on a twice daily feeding schedule. Various doses of OTAg dissolved in 5 ul of artificial CSF (aCSF) were administered through indwelling lateral ventricular cannulae 30 min before a 1h morning feeding session. Significant inhibition of FI was found with increasing doses of OTAg as compared to each animal's FI after aCSF icv (*$p < 0.001$):

OTAg dose (pmol)	10	25	50	500
FI (% of baseline)	82 ± 33 (n=4)	55 ± 19* (n=4)	53 ± 6* (n=8)	41 ± 12* (n=5)

Our results demonstrate that central injection of an OT agonist decreases food intake in rats, and support the hypothesis that central OT projections may act to inhibit food intake in addition to their already established role of inhibiting gastric motility.

92.12

OXYTOCIN-INDUCED PENILE ERECTION AND YAWNING: ROLE OF CALCIUM AND PROSTAGLANDINS. A. Argiolas and M.R. Melis, Department of Neurosciences, University of Cagliari, Via Porcell 4, 09124 Cagliari (Italy).

The effect of inhibitors of calcium channels verapamil, flunarizine, nimodipine, nicardipine, and nifedipine, and of prostaglandin synthesis indomethacin and aspirin, on penile erection and yawning induced by oxytocin was studied in male rats. All calcium channel inhibitors given i.p. 60 min before i.c.v. oxytocin (30 ng) prevented in a dose-dependent manner oxytocin effect. Nimodipine and nicardipine were the most effective being active at doses between 5 and 20 mg/kg, while nifedipine, verapamil and flunarizine were active at doses higher than 15 mg/kg. Unlike calcium channel inhibitors, indomethacin (10 and 50 mg/kg i.p.) and aspirin (100 mg/kg i.p.) were ineffective. Microinjection of calcium, but not of prostaglandin E_2 and prostaglandin F_2 in the paraventricular nucleus of the hypothalamus, the brain area most sensitive for the induction of the above behavioral responses by oxytocin, induced a symptomatology similar to that induced by oxytocin. The present results suggest that calcium might be the second messanger which mediates the expression of penile erection and yawning induced by oxytocin.

92.13

ANGIOTENSIN II INDUCES OXYTOCIN AND VASOPRESSIN RELEASE FROM PERFUSED HYPOTHALAMIC EXPLANTS. M. J. Sullivan, B. J. M. van de Heijning* and L. P. Renaud. Centre for Research in Neuroscience, Montreal General Hospital and McGill University, Montreal, Quebec, Canada. H3G 1A4.

Intracerebroventricular injections of angiotensin II (Ang II) have been demonstrated to produce increases in circulating levels of vasopressin (VP) and oxytocin (OX) in the rat. Electrophysiological evidence implicates angiotensin in depolarization of putative OX and VP neurosecretory neurons of the supraoptic nucleus. These studies suggest a role for the central angiotensin system in mediating the release of both OX and VP from the neural lobe of the pituitary.

This study examined the effect of Ang II on release of OX and VP from hypothalamic explants prepared from rat brain. Explants were perfused via the internal carotid arteries with approximately 1.5 ml/min oxygenated, warmed artificial cerebrospinal fluid and the anterior pituitary removed. The perfusate was continuously sampled via a suction tube positioned near the neural lobe. Addition of 100 µM Ang II (200 µl/min for 2 min) to the perfusate resulted in increased release of both OX and VP. Attenuation of the angiotensin-induced responses by application of the angiotensin antagonist Sar^1, Thr^8 angiotensin II (1 mM) and by electrolytic lesion of the SON were investigated. The results demonstrate hypothalamic angiotensin is involved in modulation of OX and VP release from the pituitary.

Supported by FRSQ, FCAR and the MRC.

92.14

ANGIOTENSIN II (AII) BUT NOT CARBACHOL DIPSOGENESIS IS RESISTANT TO PERTUSSIS TOXIN (PTX) TREATMENT. R.C. Speth and K.L. Grove* (SPON: R.E. Hruska). Dept. of VCAPP, Washington State University, Pullman, WA 99164-6520.

AII receptor stimulation is transduced via two or more different GTP binding (G) proteins. AII inhibits adenylate cyclase activity via the PTX sensitive G_i, and stimulates phospholipase C activity via PTX sensitive, and insensitive mechanisms. There is little information regarding G protein coupling of brain AII receptors, therefore this study examined whether a PTX sensitive G protein mediates the dipsogenic actions of AII in the brain. Rats were implanted with a stainless steel guide cannula positioned 2 mm above the lateral ventricles (LV). 200 pmol of AII was administered into the LV with an injector cannula to determine the dipsogenic response to AII, after which 0.5-1.0 ug of PTX was administered into the LV. 2-3 days later the dipsogenic response to 200 pmol of AII in the LV was again determined. The same protocol was used to test the effect of PTX on the dipsogenic response to 1.64 nmol of carbachol.

DIPSOGEN	(n)	CONTROL PERIOD	2-3 DAYS post-PTX
AII	(6)	9.2 +/- 2.6	10.0 +/- 2.4
Carbachol	(10)	4.1 +/- 2.1	1.8 +/- 2.2†

† $p = 0.0022$, less than control carbachol response.

These results suggests that the dipsogenic actions of AII in the brain are not mediated by a PTX sensitive G protein, while the muscarinic cholinergic dipsogenic response is mediated by a PTX sensitive G protein.

92.15

EXCITATION OF RAT SPINAL MOTONEURONES BY TRH. G. Lacey* and A. Nistri. Pharmacology Dept., St. Bartholomew's Hosp. Med. Coll., London EC1M 6BQ., England.

The endogenously occurring tripeptide TRH can excite a number of central neurones. We examined the effect of TRH on spinal motoneurones in vitro. Intracellular recordings were obtained from lumbar motoneurones of the isolated spinal cord of 5-6 day old rats. Preparations were continuously superfused at 25°C with Krebs solution. Motoneurones had average membrane potentials of -65 mV and input resistance of 40 MΩ. Bath-applied TRH (protirelin tartrate; 50 µM) elicited slowly developing membrane depolarizations (on average 25 mV) with slow recoveries on washout. During TRH application neurones fired repetitively and displayed intense synaptic activity. In contrast, the putative transmitter glutamate (1 mM) induced rapid depolarizations of smaller amplitude (15 mV on average) with fast recovery. Cell input resistance was consistently increased by TRH, a phenomenon not due to membrane rectification. In the presence of TTX (1 µM) to block fast regenerative activity, 80% of motoneurones were depolarized by TRH and largely increased their input resistance; the latter change was not mimicked by raising extracellular K^+ or by steady depolarizing currents. Our results suggest that TRH produced a long-lasting period of increased motoneuronal excitability during which repetitive spike discharges were observed.

Supported by Cyanamid-Takeda, NATO and Wellcome Trust.

92.16

THE EFFECTS OF INTRATHECAL INJECTION OF NPY ON RENAL SYMPATHETIC NERVE ACTIVITY AND THE INTERACTION WITH OTHER VASOACTIVE AGENTS. X. Chen, M.M. Knuepfer and T.C. Westfall (Spon: M.A. Walz). Dept. of Pharmacology, St. Louis Univ. Med. Ctr., St. Louis, MO 63104

The intrathecal (Int) injection of NPY produces a decrease in BP. In the present study we examined the effect of Int injections of NPY on renal sympathetic nerve and baroreflex activity, and the interaction between NPY and norepinephrine (NE) and vasopressin (AVP). For Int injections, one polyethylene catheter (PE10) was inserted down the spinal subarachnoid space (about T10) through a puncture of the atlantoocipital membrane of anesthetized rats. Drugs were dissolved in saline and slowly injected at a volume of 10 µl-15 µl. A branch of the renal nerve was dissected for recording of renal sympathetic nerve activity. The left femoral (or carotid) artery and vein were cannulated for (BP) measurement and drug infusion, respectively. The Int injection of NPY (4 nmol/kg) significantly decreased renal sympathetic nerve activity (38± 3.6%, $p < .05$) and decreased BP (12± 1.5%, $p < .05$), but did not change the baroreflex sensitivity significantly. The Int injection of NE (5 nmol) significantly decreased BP and heart rate ($p < .05$). Pretreatment with a subthreshold dose of NPY prevented the depressor effect of Int NE ($p < .05$). The Int injection of AVP produced an increase in BP with a peak response at 3 min. A 2nd injection of AVP resulted in attenuation of the peak response. NPY (.01 nmol) 30 min previously did not alter the second AVP response (Supported by HL26319 and 35202).

92.17

INTRAPORTAL INFUSIONS OF NEUROPEPTIDE Y INHIBIT GLUCOSE OUTPUT BY THE LIVER. L. Goehler, L. O'Farrell, G. Boublick*, J. Rivier*, and D. Novin. Dept. Cell. and Struct. Biol., U. of Colorado, Denver, CO, Dept. of Psych., UCLA, Los Angeles, CA, and Salk Institute, La Jolla, CA.

Neuropeptide Y (NPY), the neuronal component of the pancreatic polypeptide family of regulatory peptides, colocalizes with tyrosine hydroxylase in sympathetic nerves innervating the livers of rabbits and guinea pigs. Sympathetic nerves innervating the liver regulate liver glucose production, particularly during stress. To investigate whether NPY modulates stress-induced hyperglycemia, NPY (200 ug/kg) was infused intraportally into the livers of anesthetized, surgically stressed rabbits, and unstressed chronically catheterized rabbits alone or in combination with the hyperglycemic hormones glucagon or norepinephrine (NE).

NPY infusions significantly reduced hyperglycemia compared to vehicle infused controls in surgically stressed animals. In unstressed rabbits, NPY had a mild but significant hypoglycemic effect when infused alone, and attenuated the actions of glucagon and NE by 35% and 60%, respectively, supporting role for NPY in the regulation of liver glucose production.

92.18

Neuropeptide Y (NPY) *does not* inhibit the perforant path-dentate granule cell synapse in rat hippocampal slice. **William F. Colmers and Gloria J. Klapstein* Dept. Pharmacology, Univ. of Alberta, Edmonton, Alberta, T6G 2H7, Canada.**

NPY potently inhibits excitatory synaptic transmission at the stratum radiatum-CA1 and mossy fiber-CA3 synapses in rat hippocampus. The action of NPY was therefore assessed on the perforant path (PP) to dentate granule cell (DGC) synapse, the first major input to the hippocampal formation.

Hippocampal slices (500 µm) were prepared using standard techniques, and held submerged in oxygenated buffer at 35°C. DGC's were impaled with 2M K^+-acetate filled microelectrodes (100-150 MΩ); field potentials were recorded with 2M NaCl-filled pipettes. Drugs were applied via the bath.

DGC's were characterized by their position, resting potential and action potential waveform. Stimulation of PP elicited a short-latency EPSP which could be abolished by application of the (Q/K) glutamate receptor antagonist CNQX (10 µM). Application of 1 µM NPY only caused a reversible reduction (of 20%) in the PP EPSP amplitude in 1 of 6 neurons. Resting and active membrane properties were not affected. 1 µM NPY only reduced (by 16%) the PP-evoked extracellular population spike (PS) recorded in the granule cell layer in 1 of 4 experiments. However, the presynaptic inhibitors baclofen (30 µM), muscarine (10 µM) and 2-chloroadenosine (2-CA; 3 µM) caused large (>60%), reversible reductions in the intracellularly recorded EPSP, and reversibly abolished the PS in all preparations.

The results indicate that NPY does not significantly affect synaptic transmission between PP and DGC's. Because NPY potently inhibits several other major excitatory synapses in hippocampus, NPY's function may be to modulate excitatory transmission only within the hippocampus proper.

Supported by the Medical Research Council of Canada

92.19

TRH STIMULATES INOSITOL PHOSPHATE (IP_X) RELEASE IN RAT HIPPOCAMPAL (HC) SLICES. A. Sattin, N.D. Means*, and M.J. Kubek. Depts. of Psychiatry and Anatomy, VA and Indiana Univ. Med. Centers, Indianapolis, IN 46202.

Our previous work has suggested that HC contains both extrinsic and intrinsic TRH and the latter may be augmented 5-fold following seizures. Our histochemical observations have localized the TRH in cell bodies and dendrites of CA and granule cells. Since HC contains TRH receptors, and since TRH is a potent stimulus of IP_X release in pituitary-derived GH_3 tumor cells, we incubated HC slices (0.3 mm) in an oxygenated artificial CSF (37°). After a 1 h. load-up with myo-(2-^{3}H) inositol followed by a 20 min. chase with cold myo-inositol, and then a 15 min. preincubation (with or without 10 mM Li^+), we exposed the slices to agonists. Following extraction of the ^{3}H-IP_X by grinding the slices in chloroform/methanol, the aqueous IP_X was isolated chromatographically. Results were expressed as the ratio of aqueous soluble dpm (^{3}H-IP_X) vs. the total radioactivity present in the sample (sum of the dpm in both the aqueous and organic phases). A 2 min. exposure to 10 nM TRH in the absence of Li^+ increased the formation of IP_X 18% over baseline (p .02) which was less than the 29% increase observed after exposure to 10 mM L-glutamic acid. In 10 mM Li^+, TRH gave a 5%-25% increase at 10 min. and a 32%-53% increase at 30 min., compared to 33%-82% and 142%-176%, respectively, for 100 uM NE. Results with higher doses of TRH will also be presented. Supported by VA Res. Svc.

92.20

BETHANECHOL AND GALANIN INITIATE HYPERPOLARIZATION OF MUDPUPPY ATRIAL MUSCLE CELLS BY DIFFERENT MECHANISMS. L. A. Merriam, L. M. Konopka and R. L. Parsons. Dept. of Anat. & Neurobiol., Univ. of Vt., Burlington, VT 05405.

Vagal stimulation produces cholinergic and non-cholinergic inhibition of cardiac output in mudpuppy heart (Axelsson and Nilsson, Exp. Biol. 44:229, 1985). A neuropeptide, similar to mammalian galanin, is co-localized with ACh in the postganglionic parasympathetic neurons innervating the heart. ACh and galanin inhibit twitch tension in isolated cardiac muscle preparations (Parsons et al., Neuroscience, 1989). We demonstrate here that enzymatically dissociated mudpuppy atrial muscle cells can be hyperpolarized by both bethanechol and galanin. The onset of hypolarization is much slower and the duration longer with galanin than with bethanechol. The hyperpolarizations by both agonists are voltage dependent, increasing with depolarization and reversing at ~-100mV. Atropine (5uM) blocks the bethanecol hyperpolarization but not the galanin hyperpolarization. Following substitution of Mg^{2+} for extracellular Ca^{2+}, the galanin hyperpolarization is markedly reduced without a significant change in the bethanechol response. We suggest that both agonists initiate hyperpolariation by activating a K^+ conductance, but the membrane receptors and the class of K^+ channels involved appear to be different. Supported by Grants NS 23978 and NS 25973.

92.21

GALANIN STIMULATES PHOSPHATIDYLINOSITOL TURNOVER IN CARDIAC TISSUE OF NECTURUS. J.C. Hardwick, T.W. McKeon, R.E. Carraway*, L. Konopka, and R.L. Parsons. Department of Anatomy and Neurobiology, University of Vermont, Burlington, VT 05405 and Department of Physiology, University of Massachusetts Medical Center, Worcester, MA 01605.

Application of either galanin or bethanechol, a muscarinic agonist, causes hyperpolarization of cardiac muscle cells in Necturus maculosus (mudpuppy). Biochemical and immunohistochemical studies were undertaken to investigate the effects and location of galanin-like peptide in mudpuppy cardiac tissue. Galanin-like peptide is present in high concentration in cardiac tissue as compared to other tissues known to contain substantial amounts of galanin across species. Galanin-immunoreactive processes of postganglionic parasympathetic neurons are present in atrial and ventricular tissue in close association with cardiac muscle fibers. In addition, galanin, but not bethanechol, stimulates phosphatidylinositol (PI) turnover in both atrial and ventricular preparations. A dose-response curve for the galanin-induced stimulation of PI turnover in atrial muscle showed maximal stimulation at a concentration of 10^{-6} M, and an apparent EC_{50} of 7.5 nM. Preincubation with pertussis toxin (50 ng/mL) had no effect on galanin stimulation of PI turnover. From these data, we conclude that a galanin-like peptide is present in fibers innervating cardiac muscle of the mudpuppy. Galanin can then act as a modulator of cardiac muscle via stimulation of the phosphatidylinositol second messenger system. Supported by PHS grants NS 23978 and NS 25973.

92.22

EFFECT OF NEUROTENSIN ON IN VIVO RELEASE OF ACETYLCHOLINE FROM THE RAT STRIATUM. M. Shimoyama and S. Kito. Third Department of Internal Medicine, Hiroshima University School of Medicine, Hiroshima, 734. Japan.

Interaction between neuropeptides and dopamine (DA) has been well documented in certain brain regions. Nevertheless, there have been few papers on neurotensin(NT)'s effect on acetylcholine (ACh) in the central nervous system. We examined the effect of NT on in vivo release of ACh and DA from the rat striatrum using intracerebral dialysis. A dialysis probe (3 mm in length) was implanted into the rat striatum and perfused with a Ringer solution (DA release experiments) or that containing 10 μM physostigmine (ACh release experiments). ACh and DA in the perfusates were measured by HPLC with electrochemical detector. Local administration of NT(10^{-6} M - 10^{-4} M) into the rat striatum stimulated both DA and ACh release. Intraperitoneal administration of NT(25-100 μg/kg) also enhanced spontaneous release of ACh and DA, though less effective than intrastriatal administration. These results demonstrated that intrastriatal and systemic administration of NT caused increases of striatal ACh and DA release. The action of NT resembled the effect of a DA receptor antagosist.

SEROTONIN RECEPTORS I

93.1

TEMPERATURE DEPENDENCY OF QUIPAZINE'S AGONISTIC/ ANTAGONISTIC INTERACTION AT SEROTONIN AUTORECEPTORS. G.M. Williams, D.L. Smith* and D.J. Smith. Anesth. & Pharmac., WVU Health Sci. Ctr., Morgantown, WV 26506.

Some investigators (Neuropharmacol 27:37, 1988) show that quipazine acts as an agonist at autoreceptors, while others (Neuropharmacol 21, 445, 1982; J. Neurochem. 45:1886, 1985) only report an antagonistic action at ≥ 1μM. In the present study, several experimental conditions were tested for their ability to influence quipazine's action. Initially, synaptosomes isolated from rat spinal cord tissue were superfused at 25°C with a Tris-buffered Krebs solution containing 1μM fluoxetine. They were preloaded with ^{3}H-5HT, and release was induced by elevating K^+ to 15mM. Under this condition 1μM quipazine was only antagonistic to autoreceptor function. The addition of 10μM pargyline and/or 5.7mM ascorbic acid to the medium failed to alter this effect. Likewise, an agonistic action was not observed when fluoxetine was removed. In contrast, elevating the temperature of the superfusion medium to 37°C allowed a dose related agonistic action, with 10nM-1μM quipazine reducing the release of ^{3}H-5HT from 90-67% of control. Also, the K^+-induced release of ^{3}H-5HT was doubled and the agonist potencies of 5HT and RU24969 were 1.5 times greater. These data suggest that the processes occurring in response to the interaction of agonists at autoreceptors are sensitive to temperature changes. Supported by NIH 5-T32-GM07039 and the Dept. of Anesth.

93.2

CHARACTERISTICS OF 5-HT_3 RECEPTOR-EVOKED DOPAMINE RELEASE FROM RAT BRAIN. P.Blandina*,J.Goldfarb, B. Craddock-Royal* and J.P.Green. Department of Pharmacology, Mount Sinai School of Medicine, C.U.N.Y., New York, NY 10029.

5-HT3-receptor activation releases dopamine (DA) from superfused rat striatal slices (P.Blandina,J.Goldfarb, and J.P.Green.Eur.J.Pharmacol. 155:349,1988). We now report that the process is Ca^{++}-dependent, sensitive to tetrodotoxin (TTX) and enhances the K^+-evoked DA release. Rat striatal slices were superfused and DA was measured (P.Blandina et al.Eur.J.Pharmacol. 155:349,1988). When the slices were prepared in and superfused with Ca^{++}-free, EGTA-containing medium,5-HT-evoked DA release was completely abolished. A similar result was obtained with 3 min preincubation in Ca^{++}-free,EGTA-containing medium, thus suggesting that 5-HT releases DA by promoting the entrance of Ca^{++} ions into the nerve terminals. Superfusion of the slices with 0.5 μM or 1 μM TTX reduced the 5-HT-evoked release by 40%,implying multiple mechanisms for the 5-HT action. Both 5-HT and the 5-HT3 agonist,2-CH3-5-HT,enhance the K^+-induced DA release by 30%. Activation of 5-HT3 receptor could be the mechanism by which the serotonergic raphé projections modulate striatal dopaminergic activity independently of control of firing of dopaminergic cell bodies in the substantia nigra. Supported by a grant (DA 01875) from the National Institute on Drug Abuse.

93.3

RU 24969-INDUCED EMESIS IN THE CAT: SEROTONIN-1D SITES IMPLICATED. J.B. Lucot. Dept. Pharmacol., Wright State University, Dayton, OH 45435.

This study was part of a series of experiments designed to characterize the role of serotonin (5-HT) receptor subtypes in emetic syndromes. 5-HT agonists were administered subcutaneously and the cats observed for 45 min or for 15 min after the last emetic event, whichever occurred later. RU 24969 elicited emesis with a maximally effective dose of 1.0 mg/kg. 5-Methoxytryptamine was found to have lower efficacy and more nonspecific effects. TFMPP was devoid of emetic effects. Emesis elicited by 1.0 mg/kg of RU 24969 was unaltered by pretreatment with phentolamine, yohimbine, (-)propranolol and haloperidol, suggesting that catecholamines played no role. Emesis was prevented by metergoline but not by ICS 205 930, ketanserin, cyproheptadine, mesulergine or BMY 7378. Evaluation of the results implicates 5-HT_{1D} sites in the emetic response. This conclusion will have to be verified when drugs selective for this site become available. The emesis was also prevented by 8-OH-DPAT, confirming that it has a general antiemetic effect in cats.

93.4

NEURONAL AND MUSCULAR 5-HT RECEPTORS OF THE GUINEA PIG LONGITUDINAL MUSCLE-MYENTERIC PLEXUS (LM-MP). J. Coupet, H. Park, C. E. Rauh, F. Arnold and L.R. Meyerson. American Cyanamid Co., Med. Res. Div., Ramapo College, Mahwah, NJ 07430

Multiple 5-HT receptor subtypes have been implicated in the excitatory and inhibitory responses evoked by 5-HT when applied to ileal preparations. The present study shows that 5-HT_{1A}, 5-HT_2 and 5-HT_3 receptors are functionally involved in these responses based upon electrophysiological and biochemical experiments with LM-MP. Muscle tension in LM-MP strips was monitored in the presence of various agonists and antagonists. Tetrodotoxin (TTX) at 0.2 µM was used to differentiate between direct muscular and neuronal mediated contractile components. Neuronally mediated contractions were completely attenuated by TTX and by the 5-HT_3 antagonists ICS 205-930, GR-38032F or zacopride. Those contractions due to direct stimulation of 5-HT receptors on the smooth muscle were neither affected by TTX nor by 5-HT_3 antagonists but were completely inhibited by ketanserin or cinanserin (200 nM). The binding of [^{3}H]-ketanserin to ileal membranes revealed high and low affinity components with Kd values of 0.5 and 20 nM, respectively. In myenteric plexus synaptosomes, 8-OH-DPAT prevented the evoked release of [^{3}H] acetylcholine and inhibited forskolin-stimulated adenylate cyclase with IC_{50} values of 0.5 and 1.0 µM, respectively. Collectively, the results of these experiments indicate that in the guinea pig ileum, at least three separate 5-HT receptor subtypes are functionally involved in the excitatory and inhibitory responses to 5-HT. An excitatory 5-HT_2 receptor resides directly on the smooth muscle while an excitatory 5-HT_3 as well as an inhibitory 5-HT_{1A} receptor is located on the neuronal network of the LM-MP.

93.5

IMPORTANCE OF 5-HT_{1A} AND 5-HT_2 RECEPTORS IN THE EXPRESSION OF FOREPAW TREADING AND HEAD TWITCHES IN RATS. J. Arnt and J. Hyttel*. H. Lundbeck A/S, Ottiliavej 7-9, DK-2500 Copenhagen-Valby, Denmark.

Serotonin$_{1A}$ (5-HT_{1A}) receptors are considered to mediate the forepaw treading syndrome in rodents, whereas 5-HT_2 receptors are considered to mediate head twitch behaviour (Tricklebank, M., Trends Pharmacol. Sci. 6, 403, 1985). In this study an interaction between these sites in the expression of both behaviours is suggested. The 5-HT_{1A} agonist 8-OHDPAT induced a maximum score of forepaw treading (ED_{50} 8.0 µmol/kg, s.c.). After combination with the 5-HT_2 agonist DOI, which does not induce forepaw treading per se, the potency was increased 20 times. In contrast, 8-OHDPAT inhibited the head twitches induced by DOI. This suggests an opposite interaction between 5-HT_{1A} and 5-HT_2 receptors in expression of these 2 behaviours. In contrast, forepaw treading induced by the mixed $5\text{-HT}_{1A}/5\text{-HT}_2$ agonist 5-methoxy-N,N-dimethyltryptamine was unchanged by DOI. The partial 5-HT_{1A} agonists buspirone, ipsapirone and gepirone did not induce forepaw treading when given alone or in combination with DOI, whereas all inhibited DOI-induced head twitches. The combined effect of 8-OHDPAT and DOI on forepaw treading was inhibited by (-)-alprenolol (a mixed 5-HT_{1A}/β-adrenoceptor antagonist) and also by the 5-HT_2 antagonists ritanserin or ketanserin. These results support the hypothesis that 5-HT_{1A} receptors mediate forepaw treading, but that activation of 5-HT_2 receptors is necessary for expression of the maximum resonse and sensitivity. Further, the results indicate that high 5-HT_{1A} efficacy is necessary for expression of forepaw treading.

93.6

1-(2,5-DIMETHOXY-4-IODOPHENYL)-2-AMINOPROPANE (DOI) ACTIVATION OF THE 5-HT_2 RECEPTOR SUBTYPE INCREASES RAT PLASMA ACTH CONCENTRATION.

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We have investigated the plasma ACTH response to 1-(2,5-Dimethoxy-4-iodophenyl)-2-aminopropane (DOI) in the rat, with the aim of distinguishing the role of 5-HT_2 from 5-HT_{1C} receptors in the mediation of measured changes. Male Sprague-Dawley rats received s.c. antagonist or vehicle at time zero, followed by DOI, 0.8 mg/kg s.c. at 30 minutes, and cardiac puncture under halothane anesthesia at 60 minutes. Plasma ACTH was measured by two-site immunoradiometric assay (Euro-Diagnostics BV, Apeldoorn, Holland). The ACTH response to DOI was blocked by the 5-HT_2 receptor antagonists ICI 170,809, mesulergine, spiperone, and ketanserin, whilst the $5\text{-HT}_{1A}/5\text{-HT}_{1B}$ receptor antagonist, (-)-pindolol, had no effect.

The lack of demonstrable inhibition by (-)-pindolol, a drug with high affinity at $5\text{-HT}_{1A}/5\text{-HT}_{1B}$ sites but not $5\text{-HT}_{1C}/5\text{-HT}_2$ sites effectively minimizes the former receptor subtypes' importance, as well as beta-receptor involvement in the mediation of the DOI effect on ACTH. Additionally, spiperone has approximately 600-fold greater affinity for the 5-HT_2 than for the 5-HT_{1C} site (Hoyer, D, *J. Recept. Res.*, 8:59-81, 1988), and ketanserin, which shares nanomolar affinity at the 5-HT_2 site but not at dopaminergic sites, also inhibited the DOI-mediated ACTH increase. Thus it appears that 5-HT_2 receptor stimulation is more important than that of the 5-HT_{1C} receptor in the production of the DOI-induced ACTH response.

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93.7

ESTROGEN EFFECTS ON 5-HT_{1A} BINDING SITES AND INHIBITION OF FORSKOLIN STIMULATED ADENYLYL CYCLASE ACTIVITY IN HIPPOCAMPUS OF OVARIECTOMIZED RATS. William P. Clarke[1], Saul Maayani[1,2] and Joseph Goldfarb[1]. Depts. of Pharmacology[1] and Anesthesiology[2], Mount Sinai School of Medicine, CUNY, New York, NY 10029.

Activation of the serotonin$_{1A}$ (5-HT_{1A}) receptor elicits two independent effects in rat hippocampal preparations: 1) an increase in K^+ conductance of pyramidal cells, resulting in hyperpolarization and decrease in population spike amplitude, and 2) inhibition of forskolin stimulated adenylyl cyclase (FSAC) activity. Chronic treatment of ovariectomized (OVX) rats with estrogen (3-6 days) enhances 5-HT_{1A} agonist induced decrease in population spike amplitude and hyperpolarization (Clarke and Goldfarb, Eur. J. Pharm. 160:195, 1989; Clarke et al., Neurosci. Abstr 14:553, 1988).

Female Sprague Dawley rats were OVX and two weeks later received silastic capsule implants sc containing either estradiol-17β (ES, 10% or 20% in cholesterol) or cholesterol (CHOL) alone. Rats were sacrificed 4 days later. For 5-HT inhibition of FSAC activity, mean (±SEM) pEC_{50} and E_{max} values for CHOL rats (n=5) were 7.23 ± 0.13 and 21.7% ± 2.08 and for ES (n=5) rats were 7.75 ± 0.24 and 24.1% ± 1.59, respectively. For [^{3}H]8-OH-DPAT binding mean (±SEM) pK_d and B_{max} (fmols/mg protein) values for CHOL rats (n=3) were 8.84 ± 0.07 and 650 ± 49 and for ES rats (n=3) were 9.03 ± 0.08 and 639 ± 48, respectively. Based on these preliminary data, the magnitude of estrogen induced leftward shift in the concentration response curve (CRC) for inhibition of FSAC by 5-HT appears similar to the estrogen induced shift in the CRC for 5-HT_{1A} induced decrease in population spike amplitude.

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93.8

Ca^{++} MOBILIZATION MEDIATED BY 5-HT_2 RECEPTORS IN FURA-2-LOADED HUMAN PLATELETS.

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Recent studies suggest that the activation of 5-HT_2 receptors increases phosphatidylinositol hydrolysis in the CNS and human platelets. It has also been shown that inositol-trisphosphate can mobilize intracellular Ca^{++}. To know the precise relationship between 5-HT_2 receptors and cytoplasmic Ca^{++} mobilization, we have investigated the effects of serotonergic agents on intracellular Ca^{++} concentration in fura-2-loaded human platelets. 5-HT increased intracellular Ca^{++} concentration in a dose dependent manner. A maximal increase in Ca^{++} concentration, which was observed at 10uM 5-HT, was 116±21nM with 5-HT's EC_{50} value of 0.2uM. Ketanserin, a selective 5-HT_2 antagonist, inhibited 5-HT-induced Ca^{++} increase dose-dependently with IC_{50} value of 2nM. DOI, a 5-HT_2 agonist, increased Ca^{++} concentration in a ketanserin-reversible fashion. Since DOI, however, partially antagonized the ability of 5-HT to elevate intracellular Ca^{++} control, DOI may act as a partial agonist at 5-HT_2 receptor. These results indicate that the stimulation of 5-HT_2 receptors increases intracellular Ca^{++} concentration in human platelets, suggesting that platelets can be useful means to investigate 5-HT_2 receptor functions in the CNS.

93.9

SEROTONIN 5-HT$_2$ RECEPTORS ON A CLONAL CELL LINE ARE COUPLED TO PHOSPHOINOSITIDE HYDROLYSIS. K. J. Nicklaus and P. B. Molinoff. Dept. of Pharmacology, Univ. of Penn., Phila., PA 19104.

A clonal cell line was established from the transplantable rat pituitary tumor 7315a. Results of radioligand binding studies indicate that P11 cells express 5-HT$_2$ receptors. Analysis of the binding of [^{125}I]LSD to membranes from P11 cells revealed the presence of a single class of high-affinity sites (K_d=1.6 nM, B_{max}=211 fmol/mg protein). The pharmacological profile of the inhibition of the binding of [^{125}I]LSD by a panel of drugs was consistent with the expected profile of these drugs at 5-HT$_2$ receptors. The affinity of the site for serotonin was in the low micromolar range and was decreased by GTP. Serotonin stimulates phosphoinositide hydrolysis in P11 cells. Increasing concentrations of the 5-HT$_2$ selective antagonist ketanserin block phosphoinositide hydrolysis stimulated by serotonin, and Schild analysis suggests simple competitive inhibition. The K_i for ketanserin derived from Schild analysis is comparable to the K_i for ketanserin measured in binding assays with [^{125}I]LSD. These results suggest that stimulation of phosphoinositide hydrolysis in P11 cells by serotonin is mediated by 5-HT$_2$ receptors. Pretreatment of P11 cells with pertussis toxin resulted in ADP-ribosylation of G_i and G_o, but did not affect the ability of serotonin to stimulate phosphoinositide hydrolysis. Therefore the G protein that couples 5-HT$_2$ receptors in P11 cells to phospholipase C is unlikely to be G_i or G_o. P11 cells will be a useful model system for future studies of the regulation and function of 5-HT$_2$ receptors on cultured cells. *Supported by NS18591 and a N.S.F. fellowship to K.J.N.*

93.10

BLOCKADE OF AN ATYPICAL CENTRAL 5-HT RECEPTOR BY BRL 24924 IN THE RAT HIPPOCAMPUS. Y. CHAPUT, R.C. ARANEDA* AND R. ANDRADE. Dept. of Pharmacology, St. Louis Univ. School of Med., St. Louis, MO 63104.

It has been shown previously that the superfusion of serotonin (5-HT) onto CA1 cells in hippocampal slices elicits a hyperpolarization which is accompanied by a marked reduction of the calcium-activated after-hyperpolarization (AHP). Since this potential is the primary determinant of spike-frequency adaptation in these neurons, this second effect functions to modulate the inhibitory actions of the 5-HT induced hyperpolarization. While the ability of 5-HT to hyperpolarize these cells is known to be mediated via a 5-HT$_{1A}$ receptor, the effects on the AHP are mediated by a different as yet unidentified receptor. Therefore, CA1 pyramidal neurons were recorded intracellularly using an in vitro slice preparation, in order to determine the effectiveness of a series of 5-HT receptor antagonists in blocking this second response to 5-HT.

Calcium spikes and their associated AHPs were recorded in the presence of TTX (0.3 μM) and TEA (5 mM). As previously reported, bath administration of 5-HT (10 μM) reduced the AHP without affecting the calcium spike. BRL 24924 (1 to 30 μM), markedly reduced this effect of 5-HT (10 μM) on the AHP, without either altering the AHP itself or reducing the 5-HT$_{1A}$-mediated hyperpolarization. The ability of 5-HT to reduce spike-frequency adaptation was also blocked by BRL 24924. In contrast to these results, bath administration of the non-selective 5-HT$_1$/5-HT$_2$ receptor antagonists methysergide (30 μM) and metergoline (30 μM), or of the 5-HT$_3$ receptor antagonist ICS 205-930 (up to 50 μM), had little effect on the ability of 5-HT to reduce the AHP.

These data further strengthen the suggestion that the 5-HT receptor mediating the decrease in the AHP of rat CA1 hippocampus pyramidal neurons does not conform to any of the 5-HT receptors defined by binding studies. Moreover, its selective blockade by BRL 24924 indicates that this receptor may resemble the atypical 5-HT receptor present on enteric neurons. This work was supported by the Pharmaceutical Manufacturers Association Foundation and USPHS Grant MH 43985 to R.A., and, by an MRC postdoctoral Fellowship to Y.C.

93.11

THE IDENTIFICATION OF 5-HT$_2$ AND 5-HT$_3$ RECEPTORS IN RAT DORSAL ROOT GANGLION (DRG) CELLS. **S. Todorovic* and E.G. Anderson. Dept. Pharmacology, University of Illinois at Chicago, Chicago, IL 60612.**

The effects of 5-HT on the transmembrane potential of rat DRG somata was studied in 150 A type, 16 C type and 22 unidentified neurons. 5-HT produced a concentration dependant depolarization in 88% of these neurons. Current-voltage curves were used to determine membrane input resistance (Rin) in these cells. In 51% of the responding cells, 5-HT increased Rin, in 41% 5-HT decreased Rin, while in 8% both responses were observed on the same cell. A and C type cells responded similarly. In cells showing an increase Rin, 1-10nM of ketanserin (5-HT 2 and alpha-1 antagonist) shifted the dose response of 5-HT to the right (pA2 =9.5 $\pm$0.26). Alpha-methyl serotonin (a 5-HT 2 agonist) mimicked 5-HT in this action. Norepinephrine (NE) also depolarized many of these cells (77%, N=20) while increasing Rin, an action blocked by 10nM ketanserin or prazosin (a selective alpha-1 antagonist). However, prazosin had no effect on the 5-HT response.

Cells showing depolarization to 5-HT with decreased Rin were unaffected by ketanserin. However, ICS 205-930 and MDL 72222 (selective 5-HT3 antagonists) strongly shifted this dose response curve to the right (pA2 = 10.4 $\pm$ 0.4 and 8 $\pm$0.27, respectively). 2-Methyl 5-HT acted as agonist on this response.

Neither depolarizing response showed desensitisation when exposed to supramaximal concentrations of 5-HT for up to 15 min. Quipazine and MK-212 mimicked the depolarizing response with increased Rin, but antagonized (10 - 100 nM) the depolarizing response with decreased Rin.

These data indicate that 5-HT induced depolarization with increased Rin is mediated by 5-HT 2 receptor and the depolarization with decreased Rin is mediated by 5-HT 3 receptor. Supported by PHS NS 17834-04.

93.12

PROTEIN KINASE C INHIBITORS POTENTIATE SEROTONIN-INDUCED DEPOLARIZATIONS IN PYRAMIDAL LAYER CELLS OF PIRIFORM CORTEX P.W. Sheldon* and G.K. Aghajanian. Departments of Pharmacology and Psychiatry, Yale University, New Haven, CT 06510.

Previously we found that serotonin (5-HT) causes a small depolarization (2-6 mV) in a subpopulation of pyramidal layer cells of the rat piriform cortex. This depolarization is mediated by a 5-HT-2 receptor. It is known that the 5-HT-2 receptor stimulates phosphoinositide (PI) turnover, which can lead to an activation of protein kinase C (PKC). This study explores the effects of PKC inhibitors on 5-HT-2-mediated depolarizations in piriform pyramidal cells.

In intracellular recordings in brain slices of rat piriform cortex, bath application of the isoquinoline sulfonylamide protein kinase C inhibitor H-7 (100μM) had no effect alone but increased the magnitude of the depolarizing effect of 5-HT (100μM) approximately 100% and prolonged the duration of the 5-HT response in all cells tested. Another protein kinase C inhibitor, sphingosine (10μM), had similar effects. In cells which were not depolarized by 5-HT, H-7 produced no effect alone or in the presence of 5-HT. In piriform interneurons which are induced to fire via 5-HT-2 receptors, H-7 also increased the effect of 5-HT. We conclude that inhibition of PKC potentiates 5-HT-2-mediated excitation. Furthermore, we propose that 5-HT activation of PI turnover, by leading to an activation of PKC, could act as a negative feedback loop to regulate 5-HT-induced excitation.

93.13

A 5-HT MEDIATED IPSP IN THE NUCLEUS PREPOSITUS HYPOGLOSSI AND PRESYNAPTIC INHIBITION BY 5-HT$_{1D}$ RECEPTOR AGONISTS. D.H. Bobker* and J.T. Williams (SPON: E.A. Zimmerman). Vollum Institute, OHSU, Portland OR 97201

Intracellular recordings were made from guinea pig prepositus hypoglossi (PH) neurons in vitro. Focal stimulation of the brain slice evoked a slow, inhibitory synaptic potential (IPSP) that was typically 10-30 mV in amplitude. The IPSP reversal potential was -111 $\pm$ 1.4 mV (2.5 mM K^+) and showed a Nernstian dependence on $[K^+]_o$. Spiperone blocked the IPSP with an IC_{50} of 40 nM, while ketanserin (1 μM) and sulpiride (1 μM) had no effect. 5-HT hyperpolarized PH cells with an EC_{50} of 8.5 μM in control, and 135 nM in 10 μM cocaine. Spiperone produced a parallel, right-shift of the 5-HT concentration response curve; Schild analysis yielded a K_d of 10 nM. These results suggest that this IPSP was mediated by an increased conductance to potassium resulting from synaptically released 5-HT acting upon 5-HT$_{1A}$ receptors.

The amplitude of the IPSP was reversibly decreased by TFMPP. The EC_{50} value for TFMPP was 50 nM, with a maximal inhibition of 90% at 300 nM. TFMPP (1 μM) had no effect on the membrane potential and did not change the hyperpolarization induced by 5-HT (10 μM). Quipizine acted as a competitive antagonist, and had an estimated K_d of 164 nM. These data are compatible with the presence of a presynaptic 5-HT$_{1D}$ receptor which modulates 5-HT release. Supported by USDHHS DA 04523 & NIH HL 07596

93.14

EFFECTS OF TYPICAL AND ATYPICAL ANTIPSYCHOTIC DRUGS ON MEDIAL PREFRONTAL CORTEX 5-HT$_2$ RECEPTORS: A MICROIONTOPHORETIC STUDY. C.R.Ashby, Jr., and R.Y.Wang. Department of Psychiatry and Behavioral Sciences, SUNY Stony Brook, Stony Brook, NY 11794-8790.

Previously, we have reported (Neurosci. Abstr., 1988, 14:645) that iontophoresis (ionto) of the 5-HT$_2$ (S2) agonist (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) suppresses medial prefrontal cortex (mPFc) cell firing and this effect can be reversed by ionto of various putative S2 antagonists. DOI's suppressant effect is not blocked by the selective S3 antagonists BRL 43694 or the 5-HT$_{1a,1b}$,ß antagonist (±)-pindolol, suggesting that DOI's action is relatively selective for the S2 receptor. In this abstract, we report the effects of the typical antipsychotic drugs (APDs) l-sulpiride (SUL), spiperone (SPIP) and haloperidol (HAL) and the atypical APD clozapine (CLOZ) on DOI's action in the mPFc. Male -Sprague Dawley rats (200-300g) were anesthetized with chloral hydrate (400mg/kg,i.p.), placed in a stereotaxic instrument and a small burr hole drilled over the mPFc. (±)DOI produced a significant dose-dependent suppression ($p < 0.001$, analysis of variance) of mPFc cell firing. CLOZ (10nA) significantly blocked DOI's effect at 20,40 and 80 nA ($p < 0.01$, paired t-test). HAL (10nA) significantly potentiated DOI's action at 20nA ($p < 0.01$, paired t-test) and had no effect at the other currents. SPIP (10nA) only blocked DOI's suppressant effect at 80nA ($p < 0.01$), whereas SUL did not alter DOI's action. Overall, these results suggest that there is an apparent difference between typical and atypical APDs in blocking DOI's effect. Further studies are needed to determine whether the differential actions between typical and atypical APDs can be explained by their differences in in vivo affinity for S2 receptors or differences in S2/D2 ratios. (Supported by USPHS Grants MH-41440, 00378 to RYW; NRSA 09791 to CRA).

93.15

DIFFERENTIAL EFFECTS OF HALOPERIDOL AND CLOZAPINE IN ANTAGONIZING THE SUPPRESSANT ACTION OF THE 5-HT_3 AGONIST 2-METHYLSEROTONIN ON MEDIAL PREFRONTAL CORTICAL CELLS. R.J. Kasser, C.R. Ashby, Jr. and R.Y. Wang. Department of Psychiatry and Behavioral Sciences, SUNY Stony Brook, Stony Brook, NY 11794-8790.

We have previously reported that the selective 5-HT_3 (S3) agonist 2-methylserotonin (2-Me-5HT) dose-dependently suppresses medial prefrontal cortical (mPFc) cell firing. This effect is antagonized by the S3 antagonist BRL 43694 but not by metergoline (5-$HT_{1a,1b,1c}$, 5-HT_2 antagonist) or (±)-pindolol (5-$HT_{1a,1b}$,ß antagonist). Radioligand binding data indicates that the typical antipsychotic drugs (APDs) fluphenazine, sulpiride and haloperidol (HAL) fail to inhibit ^{3}H-zacopride binding in rat cortical membranes. However, there have been no studies reporting the effects of atypical APDs on S3-mediated functions. We examined the effects of HAL and clozapine (CLOZ) on 2-Me-5HT-induced suppression of mPFc cells using single cell recording and iontophoresis (ionto). Male Sprague-Dawley rats (200-300g) were anesthetized with chloral hydrate (400 mg/kg, i.p.), placed in a stereotaxic instrument and a small burr hole drilled over the mPFc. The concentration of the drugs in the six barrel micropipettes was 10mM. 2-Me-5HT dose-dependently suppressed (ANOVA, $p < 0.001$) mPFc cell firing. Ionto-CLOZ (10nA,) but not HAL (10nA) blocked 2-Me-5HT's suppressant effect at 20, 40 and 80 nA ($p < 0.01$, paired t-test). Overall, these results suggest that APDs may interact with S3 binding sites in the brain. Whether their action on S3 sites is correlated with therapeutic efficacy or side effects remains to be determined. (Supported by USPHS Grants MH-41440 and MH-00378 to RYW, NRSA 09791 to CRA).

93.16

INTRAVENTRICULAR ADMINISTRATION OF THE 5-HT_3 RECEPTOR AGONIST 2-METHYL-5HT RELEASES DOPAMINE IN THE NUCLEUS ACCUMBENS: CHRONOCOULOMETRIC STUDIES. R.Y. Wang, L.H. Jiang, R.J. Kasser and C.R. Ashby, Jr. Department of Psychiatry and Behavioral Sciences, SUNY Stony Brook, Stony Brook, NY 11794-8790.

A high density of 5-HT_3 (S3) sites have been shown to be present in the mesocorticolimbic system. The functional role of (S3) receptors in the CNS remains unclear. It has been reported that activation of S3 receptors causes release of dopamine (DA) from rat striatal slices. The aim of the present study was to determine whether intraventricular administration of the S3 receptor agonist 2-methylserotonin (2-Me-5HT) releases DA in the nucleus accumbens (NAc). Sprague-Dawley rats were anesthetized with chloral hydrate. A nafion coated carbon-fiber electrode was calibrated in vitro and then was implanted in the NAc. A computer-aided in vivo electrochemical recording system was used to monitor the release of DA. Intraventricular (icv) injection of 2-Me-5HT (0.7 ± 0.02 nmole, n=8) significantly enhanced DA release (0.117 ± 0.016 µM). ICV administration of saline, 8-OHDPAT (5-HT_{1a} agonist) or (±)DOI (5-$HT_{1c,2}$ agonist) was without effect. 2-Me-5HT-induced release of DA in NAc was prevented by icv injection of the S3 antagonist BRL 43694 (86 ± 25 nmole), which by itself did not alter the release of DA in the NAc. 6-OHDA injected into the medial forebrain bundle (n=3) abolished 2-Me-5HT-induced effect. These results suggest that 2-Me-5HT activates DA neuronal activity and S3 antagonist reverses the DA hyperactivity; they are in line with the view that S3 antagonists have antipsychotic potential. (Supported by USPHS Grants MH-41440 and MH-00378 to RYW, NRSA 09791 to CRA and MH-43893 to RJK).

93.17

SEROTONERGIC AGENTS REVERSE DECISION-MAKING DEFICITS INDUCED BY A DOPAMINE AGONIST IN NEONATAL RATS. P.M. Whitaker-Azmitia, L. Molino, J. Caruso and A.V. Shemer. Dept. of Psychiatry and Behavioral Science, SUNY, Stony Brook, NY 11794.

Serotonin and dopamine interact in regulating some motor functions, however, an interaction in cognitive behaviors has not been examined.

15D rat pups were injected with 0.3 mg/kg SKF 38393 (a selective D_1 agonist, shown to produce decision-making deficits) or SKF 38393 with the following drugs: SCH 23390 (.075-.5mg/kg), ipsaperone (5-20mg/kg), chlomipramine (6-25mg/kg), buspirone (2-10mg/kg), or diazepam (1-5mg/kg). SKF 38393-induced behaviors were completely reversed in animals injected with the D1 antagonist SCH 23390, the selective 5-HT_{1a} agonists ipsaperone and buspirone and the serotonin uptake inhibitor chlomipramine. All of these groups displayed a significant dose dependent increase in spontaneous alternation and a decrease in vacillatory behavior in comparison to the SKF 38393 group [*$p < .001$]. Diazepam-treated animals showed no reversals.

In conclusion, we have reported what we believe to be the first finding of a serotonin-dopamine interaction in a cognitive behavior.

Supported by grants from NICHHD and NINDS.

93.18

5-HT_{1A}-RECEPTORS IN THE BRAIN OF THE MONGOLIAN GERBIL K.M. Bode-Greuel*, E. Horvàth*, M. de Jonge*, T. Glaser, J. Traber* (SPON: J. TAUTZ), Department of Neurobiology, Troponwerke GmbH & Co. KG, Berliner Strasse 156, D-5000 Köln 80, F.R.G.

The mongolian gerbil (meriones unguiculatus) is widely used as laboratory animal for a variety of experimental studies, especially for work on forebrain ischemia. In the course of our work on characterization of serotonin (5-HT)$_{1A}$-receptors it was of interest to look for the presence of 5-HT_{1A}-receptors into the gerbil brain by means of receptor binding and autoradiographic techniques. Furthermore, it was investigated, whether or not learning paradigms such as the 8-arm radial maze could be applied to gerbils to study cognitive processes in this species. The receptor binding assay revealed specific high affinity binding sites for ^{3}H-Ipsapirone on hippocampal and cortical membranes, the K_D- and B_{max}-values being 7.9 nM, 0.57 pmol/mg protein and 8.3 nM, 0.16 pmol/mg protein, respectively. Binding affinity and capacity were comparable to those detected in several other species. In addition, the autoradiographic distribution of ^{3}H-Ipsapirone binding site did not show any qualitative differences in comparison to rat brain. In the 8-arm radial maze gerbils acquired the learning task even faster than rats. The present study revealed the existence of 5-HT_{1A}-receptors in the gerbil brain and thus provides a basis for further functional studies.

93.19

INVOLVEMENT OF THE DORSAL RAPHE NUCLEUS IN THE DISCRIMINATIVE STIMULUS PROPERTIES OF THE 5-HT1A RECEPTOR AGONIST 8-OH-DPAT. R. Schreiber* and J. De Vry*. (SPON: D.G. Spencer), Department of Neurobiology, Troponwerke, Berliner Strasse 156, D-5000 Köln 80, F.R.G.

Male Wistar rats were trained to discriminate either 8-OH-DPAT [(8-hydroxy-2-(di-n-propylamino)tetralin), DPAT, 0.1 mg/kg], a selective 5-HT1A receptor agonist, or 5-OMe-DMT [(5-methoxy-N,N-dimetyltryptamine), DMT, 1.25 mg/kg], a mixed 5-HT1A/5-HT1B/5-HT2 receptor agonist, from saline (i.p., t-15 min) in a FR-10 food-reinforced two-lever operant procedure. The DPAT cue generalized to DPAT (ED50 in mg/kg: 0.04) and the selective 5-HT1A ligand ipsapirone (1.5), partially to DMT and the non-selective 5-HT ligand quipazine and not to the mixed 5-HT2/5-HT1C ligand DOI [1-(2.5-dimethoxy-4-iodophenyl)-2 aminopropane]. The DMT due generalized to DMT (0.35), DPAT (0.07) and ipsapirone (4.2), and partially to DOI and quipazine. These results suggest that both cues are 5-HT1A receptor mediated, although the latter one may involve additional 5-HT receptor subtypes. Local application of DPAT into the dorsal raphe nucleus (nRD) of DPAT and DMT trained rats resulted in dose-dependent and complete generalization (ED50's: 9.2 and 3.9 µg/kg, respectively, t-15 min); in similarly trained rats the ED50 obtained with DPAT after icv administration was 8.1 µg/kg (DMT cue) and after i.p. administration was 15.5 (DPAT cue) and 10 µg/kg (DMT cue). These results suggest that the nRD mediates at least partially the discriminative effects of DPAT.

93.20

DIFFERENTIAL EFFECT OF 5-HYDROXYTRYPTOPHAN (5-HTP) ON APPROACH AND AVOIDANCE RESPONDING IN THE SAME RAT. W.K. Park, J.N. Hingtgen and M.H. Aprison. Depts. of Psychiatry; Biochem.; Inst. Psychiat. Res.; Program in Medical Neurobiol.; Indiana U. Sch. Med.; Indianapolis, IN 46202.

The hypersensitive serotonin (5-HT) postsynaptic receptor theory of depression has been developed and expanded by Aprison and Hingtgen, based on an approach behavior model. We now wanted to compare the effect of both the L- and D,L-forms of the immediate precursor of 5-HT, 5-HTP (25-50mg/kg. I.P.), on Sidman avoidance (SS10;RS20) as well as on approach (VI 1) behavior in rats trained on both schedules in the same experimental chamber. From our earlier data only 5-HTP-induced suppression of approach behavior appeared to be mediated by 5-HT postsynaptic receptors. In the present study behavioral suppression was found with a food-reinforced approach schedule in a dose-dependent manner with either L- or D,L-5-HTP ($p < 0.001$), whereas no significant suppression was seen with avoidance behavior. We previously showed that 5-HTP increased the release of 5-HT and pretreatment with postsynaptic receptor antagonists blocked the behavioral suppression. Other laboratories have reported that decreases in catecholamine levels have been shown to suppress Sidman avoidance responding. Thus, the results of present study are consistent with our theory of 5-HT postsynaptic receptor involvement in depression. (Supported in part by Indiana Dept. of Mental Health).

93.21

ANTAGONISM OF THE DISCRIMINATIVE STIMULUS (S^D) EFFECTS OF 8-HYDROXY-2(DI-*N*-PROPYLAMINO)TETRALIN (8-OH DPAT) BY THE PUTATIVE SEROTONIN 1A ANTAGONIST 1-(2-METHOXYPHENYL)-4-[4-(2-PHTHALIMMIDO)BUTYL]PIPERAZINE HBR (NAN-190). J.L. Arbuckle*, C.M. Canan*, and J.E. Barrett (SPON: S.T. Ahlers). Uniformed Services Univ. of the Health Sciences, Bethesda, MD 20814-4799.

The serotonin ($5\text{-}HT_{1A}$) agonist 8-OH DPAT has been shown to function as an S^D. This S^D function of 8-OH DPAT has been antagonized by pindolol and alprenolol. Although these ß-antagonists have a high affinity for $5\text{-}HT_1$ rather than $5\text{-}HT_2$ receptors, their affinities for both $5\text{-}HT_{1A}$ and $5\text{-}HT_{1B}$ receptors are similar. Recently the putative $5\text{-}HT_{1A}$-selective ligand NAN-190 been shown to block the 8-OH DPAT stimulus in rats. The present experiment attempted to block the S^D functions of 8-OH DPAT with NAN-190 in pigeons, a species that serves as a good model for novel $5\text{-}HT_{1A}$ compounds. Pigeons were injected with either saline or 0.3 mg/kg 8-OH DPAT before daily experimental sessions. Key-peck responses were reinforced according to a fixed ratio schedule of food presentation for pecks to the right key during 8-OH DPAT sessions and for pecks to the left key during saline sessions. NAN-190 (0.3-5.6 mg/kg) produced a dose-dependent shift to the right of the 8-OH DPAT function. Alone, NAN-190 produced only saline-key responding. NAN-190 appears to be an effective antagonist of the S^D functions of 8-OH DPAT with little intrinsic activity under these conditions. Supported by DA-02873.

SEROTONIN II

94.1

CHARACTERIZATION OF ALPHA-METHYLSEROTONIN IN SYNAPTOSOMES FROM RAT BRAIN. T.J. Montine* and T.L. Sourkes. Departments of Psychiatry and Biochemistry, McGill University, Montreal, Quebec, H3A 1A1.

Alpha-methylserotonin (AM5HT) accumulates in whole brain of rats administered alpha-methyl-DL-tryptophan (AMTP) (Roberge et al., Neuropharmacology 11:197, 1972). The objective of these experiments was to characterize the behavior of AM5HT in nerve terminals. Synaptosomes from rat brain were prepared and intrasynaptosomal concentrations were measured by fluorescence detection after separation of indoles by HPLC. Rats treated with 50 mg/kg AMTP 24 h prior to sacrifice yielded synaptosomes with 505±15 pmol AM5HT/mg protein and 11±2 pmol serotonin (5HT)/mg protein; control rats had 99±6 pmol 5HT/mg protein. K^+ concentrations ranging from 5 to 100 mM in the medium induced fractionally equivalent release of intrasynaptosomal AM5HT and 5HT. This was maximal (72% of the initial content) between 35 and 100 mM K^+. Reserpine or p-chloroamphetamine treatment of rats resulted in synaptosomes with reductions of 58% and 68% resp., in AM5HT. Synaptosomal accumulation of AM5HT from the medium was similar to 5HT and was inhibited by 5HT and fluoxetine. It is concluded that AM5HT is stored in brain nerve terminals and its uptake into and release from synaptosomes corresponds to 5HT. (Research supported by MRC and Merck Frosst, Canada)

94.2

EFFECTS OF TRYPTOPHAN ON NEUROCHEMICAL ESTIMATES OF THE ACTIVITY OF HYPOTHALAMIC 5HT NEURONS FOLLOWING STIMULATION OF THE DORSAL RAPHE NUCLEUS (DNR) OR ADMINISTRATION OF THE $5HT_{1A}$ RECEPTOR AGONIST 8-HYDROXY-2-(DI-n-PROPYLAMINO) TETRALIN (8-OH-DPAT). Y. Tian*, J.L. Goudreau*, K.J. Lookingland and K.E. Moore (SPON: J.E. Thornburg). Dept. of Pharm./Tox., Mich. State Univ., East Lansing, MI 48824.

Neurochemical estimates of the activity of 5HT neurons in the brain are based upon the coupling between neurotransmitter synthesis, release and metabolism. A potential problem with the use of neurochemical estimates of 5HT neuronal activity relates to the availability of substrate for the rate-limiting enzyme tryptophan hydroxylase. In the present study, the effects of tryptophan on the synthesis (5HTP accumulation following administration of a decarboxylase inhibitor) and metabolism (5HIAA concentrations) of 5HT were determined in discrete hypothalamic regions of the male rat following: 1) activation of 5HT neurons by electrical stimulation of the DRN, and 2) inhibition of 5HT neuronal activity by administration of 8-OH-DPAT. Tryptophan caused a two-fold increase in the rate of 5HTP accumulation and a concurrent increase in the concentrations of 5HIAA and 5HT in all hypothalamic regions. DRN stimulation produced similar increases and 8-OH-DPAT produced similar decreases in 5HTP and 5HIAA concentrations in vehicle- and tryptophan-treated rats. These results indicate that precursor administration has little effect on the coupling between the activity of hypothalamic 5HT neurons and the synthesis and metabolism of 5HT. (Supported by a MSU-BRSG grant.)

94.3

ANALYSIS OF TRYPTOPHAN HYDROXYLASE (TPH) mRNA FROM RAT PINEAL GLANDS, DORSAL RAPHE NUCLEI AND BRAINSTEMS BY NORTHERN BLOT AND IN SITU HYBRIDIZATION, USING TPH OLIGONUCLEOTIDE PROBES. D.H. Park, T. Wessel and T.H. Joh, Lab. of Mol. Neurobiol., Cornell Univ. Med. Coll., Burke Rehab. Ctr., White Plains, NY 10605.

In the present study we sought to analyze TPH mRNA from rat pineal glands (PG), dorsal raphe nuclei (DRN) and brainstems (BS) by northern blot and in situ hybridization techniques, using synthetic oligonucleotide probes, and to determine TPH specific activity from PG and DRN. Several TPH oligonucleotides based on published rat pineal TPH nucleotide sequence (Darmon, M. C. et al., J. Neurochem., 51:312,1988) were synthesized. These oligonucleotides were radiolabelled with either α-^{32}P- or ^{35}S-deoxy CTP. Poly A^+ RNAs were prepared from rat PG, DRN and BS. By northern blot all probes hybridized with two TPH mRNA species from PG but none hybridized with mRNA species from DRN and BS. By in situ hybridization ^{35}S - labelled TPH oligonucleotide probes exhibited strong hybridization with pineal TPH mRNA but did not hybridize with DRN and BS. However, TPH activity in DRN (11.3 nmol/5-hydroxytryptophan (5-HTP) formed/region/15 min,37°C; specific activity, 17.2 nmol 5-HTP/mg protein/15 min,37°C) is much higher than in PG (0.304 nmol 5-HTP formed/ PG/15 min,37°C; specific activity, 4.0 nmol 5-HTP formed/mg protein/15 min,37°C). The inability to demonstrate TPH mRNA in DRN using oligonucleotide probes based on pineal sequences, despite higher TPH activity in this brain region, may be attributed to low relative abundance of the mRNA in CNS. However, the present data could also be interpreted to suggest that TPH enzyme in pineal gland differs from that in CNS. Supported by grant # MH 44043.

94.4

MDL 27,777A: A SELECTIVE INHIBITOR OF SEROTONIN UPTAKE. J. Freedman*, M. Dudley, B. Baron, A. Ogden* and T. Holman* (SPON: M. Palfreyman). Merrell Dow Research Institute, Cincinnati, OH 45215.

MDL 27,777A (2,3-dihydro-N-methyl-1-[4-(trifluoromethyl) phenoxy]-1H-indene-2-methanamine·HCl) inhibited [3H]serotonin (5-HT) and [3H]norepinephrine (NE) uptake into rat cortical synaptosomes with IC50 values of 0.2 µM and 8.4 µM, respectively. In vivo, MDL 27,777A and fluoxetine were equipotent as inhibitors of p-chloroamphetamine-induced 5-HT depletion from rat cortex following either intraperitoneal (IC50 values were 1.9 and 2.5 mg/kg, respectively) or oral (IC50 value was 5.0 mg/kg for both) administration. MDL 27,777A at doses up to 40 mg/kg, ip did not inhibit the xylamine-induced depletion of NE from rat cortex or the MPTP-induced depletion of dopamine from mouse striatum. MDL 27,777A was inactive at adrenergic, serotinergic and cholinergic receptors as measured by radioligand binding assays. The combined treatment of desipramine (DMI) (5 mg/kg, ip) and MDL 27,777A (15 mg/kg bid, ip) for 4 days was able to significantly down-regulate rat cortical ß-adrenoceptor density and isoproterenol-stimulated cAMP accumulation in rat cortical slices to an extent similar to the previously reported effect of DMI plus fluoxetine (Baron et al., Eur. J. Pharm. 154:125, 1988). Therefore, MDL 27,777A is a selective inhibitor of 5-HT uptake and may prove effective in the monotherapy of depression; or in combination with a NE uptake inhibitor may provide a novel treatment of depression with a more rapid onset of action.

94.5

SUBCLASSES OF PLATELET ^{3}H-IMIPRAMINE BINDING RESPOND DIFFERENTLY TO CHRONIC IMIPRAMINE TREATMENT. E. M. DeMet, K. Bell, R. Gerner*, C. Reist*, and A. Chicz-DeMet. Dept. Psychiatry and Human Behavior, Univ. of California, Irvine, Irvine, CA 92717.

A number of studies suggest that platelet ^{3}H-imipramine (^{3}H-IMI) binding is decreased in depressed patients and is subsequently increased by chronic imipramine treatment. However, total ^{3}H-IMI binding is thought to consist of 2 separate subclasses of high affinity sites. Binding to one site is trypsin sensitive with properties similar to serotonin (5HT) uptake whereas binding to the other site is not. The present study measured platelet ^{3}H-IMI binding in the presence/absence of 0.25 nM cyanoimipramine (CNIMI), a potent 5HT uptake blocker, and irreversible inhibitor of ^{3}H-IMI binding. At this concentration CNIMI inhibited ca. 50% of total ^{3}H-IMI binding. Both CNIMI resistant and CNIMI sensitive binding sites were decreased in depressed patients although only the latter was significant. Chronic (6 wk) imipramine treatment increased total and CNIMI resistant binding but decreased CNIMI sensitive binding. Post-treatment Bmax values of CNIMI resistant sites were inversely correlated with plasma cortisol levels whereas the Bmax of CNIMI sensitive binding was not. The results suggest that treatment induced changes in the more specific CNIMI sensitive sites are not secondary to improvements in mood state whereas CNIMI resistant binding may fluctuate with plasma cortisol levels.

94.6

STIMULATION AND INHIBITION BY ETHANOL ON THE HIGH-AFFINITY UPTAKE OF [^{3}H]5-HT BY BRAIN SYNAPTOSOMES. T. Alexl, J. Poblete and E.C. Azmitia (SPON: J.A. McCaughran). Department of Biology, New York University, NY, NY 10003.

Ethanol has long been shown to be associated with the CNS serotonin system. Our investigation of the effects of ethanol on the uptake of [^{3}H]5-HT by rat brain synaptosomes shows that acute application of ethanol is stimulatory at 0.15-0.6% and is inhibitory at concentrations above 9.5%. With a 5 minute pre-incubation, stimulation of 5-HT uptake was no longer observed, and the inhibitory concentration 9.5%, which normally decreased uptake 44%, decreased uptake 65%. The serotonergic transporter is probably not involved, since ethanol was unable to alter control [^{3}H]paroxetine binding levels.

To examine whether ethanol altered calcium homeostasis, we tested a variety of drugs known to mobilize internal calcium or to affect calcium channels directly or through NMDA receptors. 0.5mM glutamate was able to protect by 37% the inhibition caused by 12% ethanol. Nimodipine, Bay K8644 and MK801 were without effect. With a 5 minute pre-incubation, 10^{-6} M fluoxetine in combination with 0.3% ethanol caused an inhibition of uptake. Nimodipine, potassium depolarization and MK801 had no effect. External 1mM calcium supplement had no effect on the inhibition by ethanol. Research supported by NIDA 271-87-8144.

94.7

INHIBITION OF 5HT UPTAKE BY THE 5HT1B AGONIST TFMPP. W.A. Wolf* and D.M. Kuhn. Lab. of Neurochemistry, Lafayette Clinic and Cellular and Clinical Neurobiology Program, Dept. Psychiatry, Wayne State Univ. Sch. of Med., Detroit, MI 48207.

1-(m-Trifluoromethylphenyl)piperazine (TFMPP) is an agonist at 5HT receptors of the 1B subtype and it is used frequently to study the pharmacological and physiological properties of 5HT receptors. The in vivo effects of TFMPP on 5HT neurochemistry are reminiscent of the effects of selective uptake inhibitors such as fluoxetine on 5HT and 5HIAA levels. Thus, we considered the possibility that TFMPP could also inhibit the neuronal uptake of 5HT. Synaptosomes from rat brain were incubated in modified Krebs Ringer phosphate buffer and the uptake of exogenous 5HT (50 nM) was determined at 37°C. The addition of TFMPP produced a concentration dependent inhibition of 5HT uptake into synaptosomes. The IC50 for uptake inhibition by TFMPP was estimated to be 350 nM. TFMPP did not cause the release of endogenous synaptosomal 5HT at any concentration which inhibited uptake. TFMPP could be differentiated from a "classical" 5HT uptake inhibitor such as fluoxetine, in that the in vitro 5HT releasing properties of p-chloroamphetamine could not be prevented by TFMPP. Other 5HT1B agonists of the aryl-piperazine class (e.g. quipazine and MK 212) also share 5HT uptake inhibiting properties with TFMPP. The results indicate that the pharmacological properties of TFMPP may reflect, in part, its 5HT uptake inhibiting effects. Furthermore, these results suggest that a relationship may exist between 5HT1B sites and the recognition site which is part of the presynaptic neuronal 5HT uptake complex.

94.8

MOLECULAR MODELING OF SEROTONIN UPTAKE INHIBITORS. L. A. McQuaid*, R. P. Pioch*, and J. A. Nixon* (SPON: M. J. Schmidt). Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana 46285.

A series of serotonin (5HT) uptake inhibitors were examined using MacroModel (C. Still) molecular modeling program in order to develop a three dimensional model for the recognition site of the uptake pump. Citalopram, Indalpine, Paroxetine, CGP-6085A and Fluoxetine were chosen based on their superior potency and selectivity. In addition, a new 5HT uptake inhibitor, LY233708, was evaluated in the resulting model. Using the MULTIC submode of MacroModel, multiple conformations of each compound were generated through the use of a dihedral angle driver and stored. Each conformation was subjected to energy minimization using the blocked diagonal Newton-Raphsson method with a MM2 force field. The lowest energy conformations were further refined using the full matrix Newton-Raphsson method. The distance between the aromatic ring(s) and the nitrogen atom was calculated for the low energy conformations of the 5HT uptake inhibitors. The distance between the aromatic ring(s) and the nitrogen atom was found to vary between 5.14 to 7.71 Å. However, the average distance between the two aromatic rings in Citalopram, Paroxetine and Fluoxetine was found to be very similar, 4.84-5.05Å. A model was developed describing the relative positions of the two aromatic binding sites (Ar and Ar') and a basic nitrogen binding site (N). LY233708 was found to have only one low energy conformation and a comparison of LY233708 with the above series of uptake inhibitors will be presented.

94.9

QUASI- AND TRUE OPIOID WITHDRAWAL IN 14 DAY-OLD CHICKEN EMBRYOS: ASSOCIATION WITH ALTERED BRAIN SEROTONIN METABOLISM. G.Seran,* M.E.Bronson* and S.B.Sparber (SPON: G.Livezey). Dept. of Pharmacology, Univ. of Minnesota, Minneapolis, MN 55455.

Previous attempts to induce and maintain opioid dependence in 14 day-old embryos, expressed as withdrawal-induced increased motility, were unsuccessful (PB&B,30:357,1988). We report here that isobutylmethylxanthine causes increased motility at this age, demonstrating the capacity of the embryo to express quasi-opioid withdrawal, confirming the maturity of the output component (i.e. expression). If appropriate receptors and/or adaptive capacity are sufficiently developed, true opioid dependence/withdrawal should be expressed. Injection of various doses of l-alpha-noracetylmethadol, methadone or morphine, as soon as 1 hr or up to 11 days before injection of various doses of naloxone enabled us to demonstrate that the 14 day-old embryo can be studied for effects of early opioid dependence/withdrawal. Changes in brain 5-HIAA, but not dopamine metabolites, at a time when acute dependence can be demonstrated (i.e. 1 hr) confirms the importance of 5-HT in the adaptive process or its expression (JPET 236:157,1986; Kleven & Sparber, Psychopharmacol., in press). Such studies will determine if sensitive periods exist for long-term consequences of exposure to opioids and/or withdrawal, and to understand the mechanism for such effects. Moreover, strategies for early treatment interventions (detoxification and/or pharmacologic, with non-opioids) might be derived from such studies. It is possible that opioid detoxification at early stages of development can prevent potential long-term neurobehavioral dysfunction, which may attend severe perinatal opioid withdrawal. Supported in part by USPHS grants DA01880 and T32DA07097.

94.10

SEROTONIN RELEASE AND MONOSYNAPTIC RESPONSE AMPLITUDE INCREASES FOLLOWING 5-HYDROXYTRYPTOPHAN INJECTION IN RATS. P. Duffy*, G. Samathanam*, P.W. Kalivas and S.R. White (SPON: D.Sarkar). Dept. of VCAPP, Washington State University, Pullman, WA 99164-6520.

Intravenous administration of the serotonin precursor, 5-hydroxytryptophan (5HTP), markedly increases the amplitude of the lumbar monosynaptic response (MSR) in cats (Anderson and Shibuya, JPET 153:352, 1966). However, a recent report suggests that 5HTP may decrease lumbar MSR amplitude in rats (Nagano et al., Eur. J. Pharmacol. 139:315, 1987). The present study used in vivo dialysis to measure 5HT release in the lumbar spinal cord of urethane-anesthetized rats in response to 5HTP injection. Small dialysis probes were placed in the lumbar enlargement and 5HT and 5HIAA in 15 min dialysate samples were measured using HPLC and electrochemical detection. Lumbar MSRs were measured in L5 dorsal roots in another set of rats. Intravenous injections of a 50 mg/kg dose of 5HTP significantly increased 5HT release and significantly increased the amplitude of the MSR whereas a 25 mg/kg dose had no effect on 5HT release or MSR amplitude. These results indicate that (1) 5HTP enhances the lumbar MSR amplitude in rats as well as cats and (2) the enhancement of MSR amplitude by 5HTP is correlated with increased 5HT release in the lumbar cord. (Supported by NS24388).

94.11

OPPOSITE EFFECTS OF INTRAVENOUS AND IONTOPHORETIC ADMINISTRATION OF THE $5HT_{1A}$ AGONIST, 8-OHDPAT, ON SPINAL MOTONEURON EXCITABILITY. D.A. Jackson and S.R. White. Dept. of VCAPP, Washington State University, Pullman, WA 99164-6520.

$5HT_{1A}$ receptor binding sites are relatively abundant in the rat spinal cord (Huang and Peroutka, Brain Res., 436:173, 1987) but the functional significance of these receptors is not known. The present study compared the effects of intravenous and iontophoretic administration of the $5HT_{1A}$ agonist, 8-hydroxy-2-di (propylamino) tetralin (DPAT), on spinal motoneuron excitability. Lumbar laminectomies were performed on urethane anesthetized rats and multibarrel micropipettes were used to record glutamate-evoked responses from identified spinal motoneurons and to apply serotonin (5HT), DPAT and control solutions. Serotonin ejection increased glutamate-evoked motoneuron firing and the increase was sometimes preceded by brief inhibition. The inhibition was more prominent with higher than with lower 5HT ejection currents and was mimicked by acid control ejections. Iontophoretic application of DPAT decreased glutamate-evoked firing of the motoneurons. However, intravenous injections of DPAT markedly enhanced glutamate-evoked firing of the motoneurons. These results suggest that (1) DPAT indirectly enhances spinal motoneuron firing by acting on as yet unidentified spinal cord or supraspinal neurons and (2) enhancement of spinal motoneuron excitability by microiontophoretically applied 5HT is not mediated by $5HT_{1A}$ receptors. (Supported by NS 24388).

94.12

SEROTONIN RELEASE IN THE MEDIAL AND LATERAL HYPOTHALAMUS DURING FEEDING AND ITS ANTICIPATION. D.H. Schwartz, L. Hernandez* and B.G. Hoebel, Department of Psychology, Princeton University, Princeton, NJ 08544-1010

We have previously used intracerebral microdialysis to show that a large meal increased lateral hypothalamic (LH) serotonin release in food deprived rats (Schwartz et al., 1989). The present experiment was carried out in order to confirm the effect and to test (1) anticipatory release of serotonin, (2) medial vs. lateral hypothalamic release, (3) verification that dialysate serotonin was neural in origin, (4) verification of serotonin chromatographic peaks using a dual potentiostat, (5) assessment of the distance monitored by the microdialysis probe. In this experiment, a serotonergic reuptake blocker (fluoxetine, 10 uM) was added to the Ringer's solution in order to enhance serotonin recovery by microdialysis probes. The sight and smell of food as well as eating food caused extracellular serotonin to increase in both the medial hypothalamus (MH) and the LH. This increase in extracellular serotonin before the meal began suggests that hypothalamic serotonin release was not alone sufficient to cause satiety. The experiment found no difference between LH and MH serotonin release and did not distinguish between serotonin released during feeding and feeding-related arousal. The chromatographic peaks had the same voltage characteristics as serotonin standards. Serotonin levels were reduced after systemic treatment with 8-OH-DPAT (250 ug/kg ip), a serotonin cell body autoreceptor ($5\text{-}HT_{1A}$) agonist suggesting that serotonin observed in this experiment was neurogenic in origin. Large elevations in LH serotonin by local fenfluramine infusion were not detected by MH probes about 1 mm away, suggesting that dialysis probes were in fact sampling from separate regions. This experiment confirms that hypothalamic serotonin is released during feeding behavior as reported earlier, and suggests that preingestive events also increase serotonin release.

Schwartz, D.H., McClane, S., Hernandez, L. & Hoebel, B.G. (1989) Brain Research 479: 349-354.

94.13

THE EFFECT OF GEPIRONE ON SOUND STRESS (SS) INDUCED INCREASES IN TRYPTOPHAN HYDROXYLASE (TRPH) IN RAT. K.C. Corley, V. Singh*, T-H. Phan*, and M.C. Boadle-Biber. Physiol. Dept., Med. Col. of Va., Va Commonwealth University, Richmond, VA 23298

Short term electrical stimulation of the dorsal raphe nucleus and acute SS (1-2 h) produce a reversible increase in in vitro TrpH activity that is non-additive with the increase seen under phosphorylating conditions and is reversed by preincubation with alkaline phosphatase. Repeated SS (1 h/day for 3 days) induces a stable increase in enzyme activity that is insensitive to alkaline phosphatase. Since 5-HT neuronal firing is inhibited by activation of autoreceptors on the cell body, we examined the effect of gepirone, a novel anxiolytic and partial $5\text{-}HT_{1A}$ agonist, on the increase in TrpH of acute and repeated SS in Fischer 344 rats. In chronic experiments, gepirone infused s.c. (40 mg/kg per day over 28 days) by an Alzet Minipump (2ML4; Eison, A.S. and Yocca, F.D. Eur. J. Pharmacol. 111: 389, 1985) completely blocked the effects on TrpH to both repeated and acute SS (110 dB, 2.9 kHz, 100 ms, VI-1, 1 h/day over 3 days). In rats stressed once (acute SS) for 1 h 15 m after dosing (2, 5, or 10 mg/kg i.p.) the increase in TrpH activity was completely blocked by the highest dose tested. Supported by Bristol-Myers.

94.14

ANTIDEPRESSANT-LIKE EFFECTS OF $5\text{-}HT_{1A}$ AGONISTS. D. Z. Press*, S. Wieland and I. Lucki (SPON: O'Connell). Dept. of Psychiatry, University of Pennsylvania, Philadelphia, PA 19104

Recent studies have suggested that $5\text{-}HT_{1A}$ selective agonists may produce antidepressant-like effects in animal models of depression. The selective $5\text{-}HT_{1A}$ agonist 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) has been shown to be effective in both the restraint-hypolocomotion and forced swim test models of depression. We utilized the forced swim test to evaluate a number of $5\text{-}HT_{1A}$ selective agonists for their antidepressant-like properties.

8-OH-DPAT (0.12-1.0 mg/kg) and SM-3997 (5-20 mg/kg) both produced dose-related decreases in immobility time following sub-chronic treatment of rats. These effects were similar to those of the tricyclic antidepressant desmethylimipramine (5-15 mg/kg). In addition, the $5\text{-}HT_{1A}$ agonists, buspirone (20 mg/kg), gepirone (20 mg/kg) and ipsapirone (10 mg/kg) demonstrated antidepressant-like effects by significantly reducing immobility time. Other groups of rats treated sub-chronically with each of the $5\text{-}HT_{1A}$ agonists showed no increase in locomotor activity so that general changes in activity could not account for the effects of $5\text{-}HT_{1A}$ agonists in the forced swim test.

5-HT agonists selective for other receptor subtypes, such as the $5\text{-}HT_{1B/1C}$ agonist m-CPP (5 mg/kg) and the $5\text{-}HT_2$ agonist DOB (1 mg/kg) were not effective in this animal model of depression. Moreover, the benzodiazepine diazepam (5 mg/kg) was also ineffective on this behavior. The stimulant d-amphetamine (2 mg/kg) did significantly reduce immobility time, but also significantly increased locomotor activity, and therefore was considered a false positive as an antidepressant drug in this test.

These results suggest that $5\text{-}HT_{1A}$ agonists may have antidepressant efficacy and act as a novel class of antidepressant drug. In addition, the forced swim test is an effective animal model of depression for studying drugs that are selective for the $5\text{-}HT_{1A}$ receptor.

94.15

PRESYNAPTIC SEROTONIN MECHANISMS IN LEARNED HELPLESSNESS. E. Edwards, W. Kornrich*, K. Harkins*, H. Willmott* and F.A. Henn. Dept. Psychiatry,SUNY, Stony Brook, NY 11794.

We report on changes of presynaptic serotonergic activity in the learned helplessness animal model of depression. The K^+-evoked release of ^{3}H-serotonin from hippocampal slices after shock testing was significantly higher in learned helpless rats (LH) when compared to naive controls (NC) and non-helpless rats (NLH): 17.5 ± .67% vs 11.94 ± .82% and 12.3 ± 1.7%. High affinity uptake of ^{3}H-serotonin was measured in hippocampus and hypothalamus of NC, NLH and LH rats. In the hippocampus, ^{3}H-5HT uptake was significantly increased in LH rats as compared to NC and NLH (+41%). In contrast, it was decreased in the hypothalamus of LH rats as compared to NC and NLH (-20%). Ligand binding studies of the 5-HT uptake site were carried out using ^{3}H-paroxetine in cortex, septum, hippocampus, hypothalamus and striatum of NC, NLH and LH rats. In the hippocampus, uptake binding sites increased significantly in LH rats as compared to NC and NLH rats (B_{max}, fmol/mg: +120% and +40% vs NC and NLH). In the hypothalamus these sites were decreased in LH rats (B_{max}, fmol/mg: -40% and -31% of NC and NLH. No changes in ^{3}H-paroxetine binding were seen in the other brain regions. These data support a major role for serotonin in the neurochemistry of the LH model with a limbic-hypothalamic pathway as a control center for behavioral response to stress. (Supported by BNS 8614098 to E.E.)

94.16

ELECTRICAL STIMULATION OF THE DORSAL RAPHE AS A DISCRIMINATIVE STIMULUS: GENERALIZATION TO (±)-DOI. L. Stambaugh* and D.J. Mokler, Dept. Pharmacol., Univ. New England, Biddeford ME 04005.

Electrical stimulation (ES) of the dorsal raphe nucleus increases the release of 5-hydroxytryptamine (5-HT) in forebrain areas and has been shown to act as a discriminative stimulus which generalizes to the hallucinogen LSD. The purpose of the present experiment was to examine this discrimination using a new discrimination paradigm, the discriminated taste aversion (Lucki, J. Pharmacol. Exp. Ther. 247: 1120, 1988); and to examine the effects of the $5\text{-}HT_2$ agonist DOI in this discrimination. Male rats were trained to discriminate ES of the dorsal raphe by pairing ES with LiCl injection following access to saccharin for half the animals. ES consisted of 100 uA biphasic square wave pulses delivered at 20 Hz. Over 6 sessions animals decreased saccharin drinking during electrical stimulation from 14 to .5 mls in sessions 1 and 6 respectively. In a parallel group of animals, non-stimulation was paired with LiCl injection. A similar decrease in saccharin consumption was seen in this group during non-stimulation sessions. Thus, ES of the dorsal raphe per se did not alter saccharin drinking. Administration of DOI (0.1, 0.5 and 1.0 mg/kg) substituted for ES of the dorsal raphe. Therefore, stimulation of $5\text{-}HT_2$ receptors with the agonist DOI has stimulus properties in common with the effects of ES of the dorsal raphe. (L.S. supported by a UNE Dean's Summer Research Fellowship.)

94.17

DIFFERENTIAL MODULATION OF THE STIMULUS PROPERTIES OF 5-HT AGONISTS BY 5-HT NEURONS. I. Lucki, S. Wieland and R. Andrews*. Departments of Psychiatry and Pharmacology, University of Pennsylvania, Philadelphia, PA 19104

Rats can be trained to discriminate the stimulus effects of 5-HT agonists that are selective for individual 5-HT receptor subtypes using the discriminated taste aversion procedure (Lucki, JPET, 1988, 247:1120-1127). This study examined whether presynaptic or postsynaptic components are involved in the stimulus effects of the 5-HT_{1A} agonist 8-OH-DPAT or the 5-$HT_{1B/1C}$ agonist TFMPP.

Separate groups of rats were trained to discriminate the stimulus effects of the 5-HT_{1A} agonist 8-OH-DPAT (0.4 mg/kg) from saline or the 5-$HT_{1B/1C}$ agonist TFMPP (0.8 mg/kg) from saline. Rats treated with the tryptophan hydroxylase inhibitor PCPA (300 mg/kg 72h prior to testing) and injected with saline demonstrated generalization to 8-OH-DPAT but not to TFMPP.

In other groups of rats, the 5-HT uptake inhibitor sertraline substituted completely for TFMPP but not for 8-OH-DPAT. The effect of sertraline was due to enhancing 5-HT neurotransmission because it failed to generalize in other rats treated with the toxin 5,7-DHT. These results suggest that the stimulus properties of the 5-HT_{1A} agonist 8-OH-DPAT ordinarily involve presynaptic actions on 5-HT neurons whereas those of the 5-$HT_{1B/1C}$ agonist TFMPP involve effects at postsynaptic 5-HT receptors. This research was supported by USPHS grant MH 36262.

INTERACTIONS BETWEEN NEUROTRANSMITTERS I

95.1

GLYCINE (GLY) INDUCES RELEASE OF ACETYLCHOLINE (ACh) FROM NUCLEUS TRACTUS SOLITARIUS. W.T. Talman and L. Wellendorf*. Lab. of Neurobiol., VAMC and Univ. of Iowa, Iowa City, IA 52242.

GLY microinjected into the nucleus tractus solitarius (NTS) of rat decreases blood pressure and heart rate. The effect, which is like that produced by ACh, is blocked by atropine and prolonged by physostigmine. Thus, we sought to determine if GLY induces release of ACh from NTS. Adult male Sprague-Dawley rats were decapitated, the brain was removed, and a 1 mm transverse slice of the brain stem was taken through the "cardiovascular" NTS. [^{3}H]-ACh released from bilateral 1 mm punches of NTS was measured by the method of Arneric and Reis (Brain Res 374:153-161). Release during control studies was compared with that after exposure of tissues to 35 mM KCl or 100 uM or 1 mM GLY. [^{3}H]-ACh released was measured in DPM/mg tissue; data were expressed as mean $\pm$ SEM. Control measures of ACh release were -2.8 $\pm$ 1.2 DPM/mg in contrast to 41.7 $\pm$ 4.3 DPM/mg after KCl, 22.3 $\pm$ 8.0 DPM/mg after 100 uM GLY and 67.4 $\pm$ 9.0 DPM/mg after 1 mM GLY. These data support the hypothesis that GLY induced cardiovascular responses in NTS are mediated through release of ACh. (Support: VA Merit Review, HL32205, HL14388, and NS24621).

95.2

OCCLUSION OF SEROTONIN (5HT-2) EXCITATIONS BY SUBSTANCE P IN THE NUCLEUS TRACTUS SOLITARIUS. J.Champagnat, T.Jacquin and M.Denavit-Saubié (SPON: R.Naquet) Laboratoire de Physiologie Nerveuse, C.N.R.S., 91198 Gif-sur-Yvette, France.

Afferent fibers containing both serotonin and substance P immunoreactivity project to the nucleus tractus solitarius, a brainstem structure controlling respiratory and cardiovascular parameters. We thus have investigated modulation of serotonin actions by substance P in the rat nucleus tractus solitarius using coronal brain stem slices and intracellular recordings. Serotonin (4-100 µM) was excitatory causing depolarization and increasing input resistance. These effects involved postsynaptic receptors of the 5HT-2 type sensitive to methysergide and to the selective 5HT-2 antagonist ketanserin (0.05-0.2 µM).

5HT-induced excitations were reversed using a protocol of conditioning applications of substance P (0.1 µM, >5 min) and shorter (20-60 s) test applications of serotonin: serotonin, which was excitatory during controls, became inhibitory of the steady action potentials discharges induced by conditioning substance P. Prolonged conditioning applications were required since addition or potentiation of the effects, but no reversion, was found combining 20-60 s substance P and serotonin applications. Thus, functional effects of serotonin in the nucleus tractus solitarius can be reversed after occlusion of 5HT2-related excitations by substance P.

95.3

SEROTONIN ATTENUATES GLUTAMATE ELICITED EXCITATION OF CEREBELLAR PURKINJE CELLS AT SOMATIC AND DENDRITIC SITES IN VITRO. H.K. Strahlendorf and J.C. Strahlendorf. Depts. of Neurology and Physiology, Texas Tech University Health Sciences Center, Lubbock, TX 79430.

We previously reported that serotonin (5-HT) potently reduced glutamate (GLU)-induced excitation of cerebellar Purkinje cells (PCs) when both were applied iontophoretically in the in situ anesthetized rat (Lee, et al. Brain Res 361, 1986). To elucidate sites and mechanisms underlying this modulatory effect of 5-HT we have initiated extracellular and intracellular recordings of PCs in the in vitro cerebellar slice. Extracellular recordings of somatic PC action potentials and iontophoresis of GLU and 5-HT were accomplished with multibarrel micropipettes. A second multibarrel pipette was positioned in the molecular layer for dendritic ejection of GLU and 5-HT. 5-HT applied at somatic and dendritic sites caused a 75-100% reduction in GLU elicited excitation. Superfusion with 100-400µm 5-HT failed to affect GLU responses elicited from the soma or dendrites. Intracellular recordings revealed an 8-10 mV depolarization in response to dendritically applied GLU. 5-HT superfusion did not markedly affect the resting potential or input resistance and did not attenuate GLU elicited depolarizations and decreases in input resistance. These results demonstrate that 5-HT can potently modulate GLU actions when both agents are focally applied to either the dendrites or soma of PCs. Additional intracellular studies are aimed at resolving the membrane ionic mechanisms mediating this interaction. Supported by NS 19296.

95.4

ACh - NMDA SYNERGISM IN RAT OESOPHAGEAL MOTONEURONS **Y.T. Wang* and D. Bieger.** Faculty of Medicine, Memorial University of Newfoundland, St. John's, Nlfd. CANADA A1B 3V6

The interaction between acetylcholine (ACh) and glutamate (Glu) at the nucleus ambiguus was investigated in rats anaesthetized with urethane. 1) Micropneumophoretic ejection of either Glu (6-10 pmol) or ACh (20-50 pmol) at the tip of the ambiguus complex produced propulsive or synchronous oesophageal contractions. 2) The Glu-evoked response was dramatically enhanced in a dose-dependent manner by a prepulse of ACh (15-40 pmol) at the same site. This effect was reversibly inhibited by a prepulse of the NMDA receptor antagonist, AP-7 (DL-2-amino-7-phosphono-heptanoic acid, 8-10 pmol). ACh also facilitated NMDA-induced responses at Glu-responsive sites. 3) The excitatory and facilitatory effects of ACh were mimicked by the nicotinic agonist, DMPP (1,1-dimetyl-4-phenyl-piperazinium iodide, 5-8 pmol), but not by muscarine, and reversibly blocked by dihydro-β-erythroidine, 8-10 pmol. These results suggest that there is a synergistic interaction between the two excitatory transmitters, ACh and Glu, at the level of ambiguus oesophageal motoneurons. ACh facilitates the NMDA-mediated response via an action at a nicotinic cholinoceptor site.

95.5

IMMUNOCYTOCHEMICAL EVIDENCE FOR THE CO-LOCALIZATION OF GABA AND GLYCINE IN SYNAPTIC TERMINALS OF THE LOWER AUDITORY BRAINSTEM. R.A. Altschuler, José M. Juiz, R.H. Helfert, J.M Bonneau* and R.J. Wenthold+. Kresge Hearing Research Institute, Univ. of Michigan, Ann Arbor, MI, 48109; +LMO, NIDCD,NIH.

GABA and glycine are believed to be major inhibitory transmitters in the auditory brainstem. While these transmitters are generally in separate neural pathways, coexistence of GABA and glycine has been shown in cerebellar Golgi cell terminals, using immunocytochemical techniques (Ottersen et al. '88). In the cochlear nucleus light microscopic studies have shown GABA and glycine to be co-contained in small cells in the dorsal cochlear nucleus as well as in puncta in all cochlear nuclei (Wenthold et al.'87). Glycine receptor immunoreactivity has been shown to appose GABA and GAD immunoreactive terminals (Oberdorfer et al '88). In the present study we have used post-embedding immunoperoxidase and immunogold techniques on serial sections, at the LM and EM levels respectively, to identify and characterize terminals co-containing GABA and glycine immunoreactivities in the cochlear nuclear complex (CNC) and the superior olivary complex (SOC). These were compared to terminals immunolabeled for GABA only, glycine only or neither.

Terminals co-labeled with GABA **and** glycine were identified in all divisions of the CNC as well as in the SOC. These terminals contained oval/pleomorphic synaptic vesicles and made symmetric axo-somatic or axo-dendritic contacts. The size and shape of the co-labeled terminals appeared to be heterogeneous. They were particularly numerous in the anteroventral and dorsal divisions of the cochlear nucleus. Glycine immunoreactive terminals contained either flattened or oval/pleomorphic vesicles. Terminals labeled for GABA only contained oval/pleomorphic vesicles.

Supported by NS 24369 and a Generalitat Valenciana Fellowship to J.M.J.

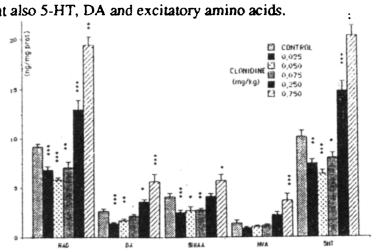
95.6

Interaction between a2 receptors with Noradrenaline, Serotonin, Dopamine, Glutamate and Aspartate. B. Delbarre, G. Delbarre, F. Calinon*, C. Loiret* and A. Ferger*. Faculté de Médecine,37032, Tours, FRANCE.

Interferences of a2 adrenoceptors with 5-HT and DA release had been demonstrated. Recently, J. Mitra (Mitra, J., Br. Res. Bulletin, 18 : 681,1987) found vasomotor effects of excitatory amino acid on ventral medullary surface.

In the brain stem of the young chicken, clonidine, a2 agonist(0.025-0.75 mg/kg) modified levels of NAD, DA, HVA, 5-HT and 5-HIAA (HPLC method). 5-HTP (50 mg/kg) significantly increased NAD, DA, HVA, 5-HT and 5-HIAA. In the anesthetized dogs, 90 min after treatment, clonidine (0,01mg/kg I.V.) decreased mean blood pressure (21%) and in the CSF (lateral ventricle) significantly decreased NAD (60%), HVA (86%), 5-HIAA (81%), glutamate (57%) and aspartate (45%) (HPLC method).

In the brain, small and high doses of clonidine had opposite effects on levels of catecholamines and indoleamines. Decrease of B.P. after clonidine implicated not only NAD but also 5-HT, DA and excitatory amino acids.



95.7

ENDOGENOUS OPIOID AND DOPAMINERGIC INTERACTIONS PRODUCE A HYPERSALIVATION RESPONSE FOLLOWING RADIATION EXPOSURE. D.E. Morse*, and G.A. Mickley, (SPON. J. Frascella), Dept. of Behavioral Sciences, AFRRI, Bethesda, MD 20814-5145

Endogenous opioids and dopamine modulate a wide variety of behaviors, including "appetitive behaviors". Radiation exposure alters release of the endogenous opioids and dopamine, and suppresses appetitive behavior. We observed that opiate receptor blockade (IP inj. of naloxone or naltrexone) in conjunction with radiation exposure resulted in hypersalivation in Sprague-Dawley rats. Saliva production was pronounced 15 minutes following radiation exposure and, was maintained for approximately 15 minutes. Postirradiation administration of an opiate antagonist resulted in hypersalivation among 75% of test animals. Shielding the torso or head from radiation exposure resulted in a reduction in the hypersalivation response. Administration of haloperidol or spiperone in combination with naltrexone, resulted in a reduction in postirradiation saliva production. Apomorphine administration in conjunction with opiate receptor blockade resulted in saliva production at lower doses of apomorphine than would induce salivation independently. The data suggest a role for opioid and dopamine involvement in the regulation of saliva production. Understanding radiation-induced changes in this regulatory system may be useful for biological radiation dosimetry and for design and testing of anti-emetic drugs.

95.8

EFFECTS OF 5HT ON GABA-INDUCED DEPOLARIZATIONS OF FROG AFFERENT FIBERS. A. Gharagozloo*, J.C. Hackman, A.M. Holohean and R.A. Davidoff. Neurophysiology Laboratory, VA Medical Center and Department of Neurology, University of Miami School of Medicine, Miami, FL 33101.

We examined the effects of serotonin (5-HT) and selective 5-HT receptor agonists on dorsal root (DR) depolarizations induced by gamma-aminobutyric acid (GABA). Sucrose gap recordings were made from the DR of the isolated, hemisected frog spinal cord superfused with HCO_3^--buffered Ringer's solution.

All concentrations of 5-HT (1-100 μM) reduced the depolarizations produced by 15-20 sec applications of GABA (0.1 mM). Low concentrations of 5-HT (1 μM) reduced the GABA responses by 12±7% (n=3), while the high doses of 5-HT (100 μM) decreased the GABA responses by 44±3% (n=5). The reductions resulted from a direct action on afferent terminals since comparable results occurred in the presence of TTX (0.625 μM). The 5-HT_{1A} receptor agonists, 8OH-DPAT (0.01 μM) and ipsapirone (1 μM) produced reductions of 22±5% (n=10) and 29±7% (n=6) in GABA-induced depolarizations respectively. These effects were reversed by the addition of 20 μM spiperone, suggesting the reductions are 5-HT_{1A} receptor related. 5-$HT_{1C/2}$ receptors also contributed to the reduction of the GABA depolarizations since α-methyl-5-HT (100 μM) reduced the GABA-induced depolarizations (54±8%, n=3). In addition, the 5-$HT_{1C/2}$ antagonists, methysergide (0.1 μM) and mianserin (10 μM) antagonized the reduction of the GABA responses. The site of interaction may be the $GABA_A$ receptor since 8OH-DPAT and α-methyl-5-HT reduced the depolarization of the DR produced by muscimol (3-10 μM). MDL 72222 (20 μM), a $5HT_3$ antagonist, had no effect on the 5-HT-induced decrease of GABA responses.

These results indicate that activation of both 5-HT_{1A} and 5-HT_{1C}/5-HT_2 act to reduce the depolarization produced by GABA on primary afferent terminals. (Supported by VAMC funds MRIS #1769 and #3369 and USPHS grant #17577).

95.9

SP AND CGRP MODULATE RELEASE OF GLUTAMATE AND ASPARTATE FROM THE RAT SPINAL DORSAL HORN. I. Kangrga, J. S. A. Larew & M. Randic, Depts. of Vet. Physiol. Pharmacol. and Biochem., Iowa State University, Ames, IA 50011, U.S.A.

Glutamate (Glu), aspartate (Asp), substance P (SP) and calcitonin gene-related peptide (CGRP) have been proposed as neuromediators or neuromodulators of primary afferent transmission. Coexistence of SP and CGRP and SP and glutamate in the small rat dorsal root ganglion neurons was shown. The potential interactions between SP or CGRP with endogenous Glu and Asp were investigated by measuring their basal and electrically-evoked efflux using rat spinal slice. A slice was incubated in Krebs solution at 32°C and a lumbar dorsal root stimulated (25V, 0.02-0.2s, 3-5Hz for 5 min) in the absence or the presence of SP or CGRP. Samples were analyzed for amino acids using HPLC with OPA derivatization. Bath-applied SP ($2\text{-}5x10^{-7}$M) produced a 3-fold increase of the basal efflux of Glu and at a higher dose (10^{-6}M) a 2-fold increase in Asp. The effects were present in a zero-Ca^{2+} solution. SP produced also an increase in electrically-evoked efflux of Glu. CGRP (10^{-7}M) increased the basal efflux of Glu and Asp and electrically-evoked efflux of Asp and GABA. Neonatally-applied capsaicine reduced the evoked efflux of Glu and Asp, and modified the basal efflux. The effects of CGRP and SP were reduced in the capsaicine-treated rats. It is suggested that SP and CGRP may be physiologically involved in modulation of Glu and Asp release during primary afferent neuro-transmission. Supported by NIH, NSF and USDA.

95.10

NEUROPEPTIDE Y IS REGULATED DIFFERENTLY THAN SOMATOSTATIN AND SUBSTANCE P IN CULTURED SYMPATHETIC NEURONS. M. Freidin* and J.A. Kessler, Departments of Neurology and Neuroscience, Albert Einstein College of Medicine, Bronx, New York 10461.

The regulation of Neuropeptide Y (NPY) was examined in sympathetic neurons cultured from the neonatal rat superior cervical ganglion. Particular emphasis was placed on defining the effects of environmental factors which are known to regulate other neurotransmitter systems in the same neurons. Levels of NPY increased with time in culture. Co-culture of these neurons with ganglion nonneuronal cells, a condition which stimulates cholinergic and substance P (SP) expression while decreasing noradrenergic development, had no effect on levels of NPY. Similarly, treatment with media conditioned by nonneuronal cells, which has striking effects on cholinergic, somatostatin (SS), and SP expression, had no effect on neuronal content of NPY. Potassium or veratridine induced membrane depolarization which decreased levels of SS, SP, and cholinergic traits and increased noradrenergic traits, but did not alter levels of NPY. Finally, dexamethasone and long term forskolin treatment were similarly without effect on neuronal NPY content. These observations indicate that the mechanisms regulating other neuropeptides and neurotransmitters are not the same as those regulating NPY.

95.11

INTERACTIONS OF ALPHA$_2$ ADRENOCEPTOR ANTAGONISTS WITH THE HYPOTHERMIC AND HYPERGLYCEMIC EFFECTS OF 8-OH-DPAT.

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8-hydroxy-dipropylaminotetralin (8-OH-DPAT), which has a high affinity for 5HT$_{1A}$ sites, produces both hypothermia and hyperglycemia in mice. In this study pretreatment with novel alpha$_2$-adrenoceptor antagonists on 8-OH-DPAT-induced responses was investigated. The antagonists used were: atipamezole (ATZ), which occupies both central and peripheral receptors, and L659,066, which poorly penetrates the blood/brain barrier. NIH Swiss mice were pretreated with either 0, 1 or 3mg/kg i.p. ATZ immediately after body temperatures and blood glucose levels had been recorded. 20min later each treatment group (N = 10-14) received either 0 or 0.25 mg/kg s.c. 8-OH-DPAT and temperature and blood glucose measured every 20 min thereafter for 1hr. ATZ alone had no effect on temperature whereas 8-OH-DPAT induced hypothermia was significantly ($p<.01$) attenuated by both doses of ATZ. The 8-OH-DPAT hyperglycemia was also attenuated ($p<.01$) by ATZ pretreatment. L659,066 (3, 10 or 30mg/kg i.p.) failed to alter 8-OH-DPAT-induced hypothermia but did attenuate 8-OH-DPAT-induced hyperglycemia. The results suggest that the attenuation of 8-OH-DPAT hypothermia caused by alpha$_2$-adrenoceptor antagonists may be centrally mediated whereas the hyperglycemia blockade may involve peripheral mechanisms.

95.12

IMIPRAMINE ANTAGONIZES TRH-INDUCED GASTRIC ULCERS IN RATS.

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Brain thyrotropin-releasing hormone (TRH) may play a role in experimental ulceration. Intracisternally (IC) -administered TRH aggravates stress- and indomethacin -induced gastric lesions and produces gastric ulcers in naive rats [Life Sci 39(4):279-296, 1986]. Other studies have shown that TRH potentiates the antidepressant effect of imipramine in the forced-swimming test (Br J Pharmacol 85:463-470, 1985). However, potential interactions between TRH and imipramine on the development of gastric ulcers have not been investigated. Adult male SD rats (200-250g) were food- but not water deprived for 24h prior to IC injections of vehicle (10µl of 0.9% NaCl) or TRH (1ug) concomitantly with imipramine (0-5mg/Kg, IP). Rats were sacrificed 4h after IC and IP treatments and the stomachs examined for evidence of gastric lesions. In another experiment, the effect of imipramine (5mg/Kg,IP) on TRH-induced acid secretion was examined in rats with pylori ligation. Rats were killed by decapitation after 2h and gastric acid quantitated. IC TRH induced a high (100%) incidence of gastric lesions. Imipramine dose-dependently inhibited TRH-induced gastric glandular lesions and blocked TRH-induced acid secretion. These findings provide evidence for a functional dicotomy between TRH and imipramine in regards to ulcer formation.

95.13

DOPAMINERGIC AND ADRENERGIC REGULATION OF HYPOTHALAMIC PROOPIOMELANOCORTIN RNA.

D.L. Allen, M. Blum AND J.L. Roberts. Fishberg Research Center for Neurobiology, Mt. Sinai School of Medicine, New York, NY 10029.

Monoaminergic neurotransmitters regulate levels of beta-endorphin in the hypothalamus. In order to better understand monoaminergic regulation of beta-endorphin synthesis, levels of proopiomelanocortin (POMC) RNA were measured with a solution hybridization/nuclease assay using a cRNA probe containing 100 bp of exon 1 and 60 bp of intron A.

After treatment with haloperidol (dopaminergic antagonist, 2 mg/kg s.c.) for six days, levels of POMC hnRNA were decreased by 55% ($p < 0.05$) in both intact and ovariectomized female rats. Levels of cytoplasmic mRNA were decreased by 24%, but this was not significant. In two separate experiments, levels of POMC hnRNA were decreased by 23% and 21% ($p = 0.05$ for each experiment) one hour after a single injection of haloperidol.

Treatment with clonidine (alpha-adrenergic agonist, 500 ug/day for 6 days) decreased POMC mRNA levels by 42% ($p < 0.01$). One hour after clonidine, levels of POMC hnRNA were decreased by 60%. These results suggest that POMC-containing neurons in the hypothalamus are regulated by dopaminergic and adrenergic inputs. (Supported by NIH-NICHHD Post-doctoral fellowship HD-07186).

95.14

α_1-RECEPTOR MEDIATED NORADRENERGIC TRANSMISSION IS INVOLVED IN THE INDUCTION OF HYPOTHALAMIC OXYTOCIN RECEPTOR BINDING BY ESTRADIOL.

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Several studies have shown that some of the effects of ovarian steroids on reproduction are mediated by noradrenergic transmission (NAT). For example in rats, α-receptor antagonists attenuate the steroid-dependent display of sexual receptivity and reduce the concentration of cytosolic estrogen receptors in hypothalamus. In guinea pigs, α_1-receptor blockade with prazosin (PRZ) reduces the concentration of estradiol (E_2)-dependent cytosolic progestin (P) receptors in the ventromedial hypothalamic nucleus (VMN), a brain region involved in reproduction. Another neurotransmitter system involved in steroid-dependent reproductive processes is the oxytocin (OT) system. Similar to hypothalamic P receptors, E_2 increases OT receptor binding in regions of the VMN that contain a high density of E_2 concentrating cells. The purpose of this study was to determine if α_1-receptor mediated NAT is involved in the regulation of E_2-dependent OT receptors.

Adult female rats (4/group) were ovariectomized and treated 7 days later with 10µg E_2 and PRZ, E_2 and Veh or Oil and Veh. Animals given PRZ were injected at the time of E_2 treatment and 40 h later (10mg/inj). Animals were killed by decapitation 48h after E_2 treatment, brains were removed and frozen on dry ice. Brain slices (20µm) through the hypothalamus were cut on a cryostat and stored at -60°C. OT receptors were labeled with 70pM ^{125}I-d(CH$_2$)$_5$[Tyr(Me)^2,Thr^4,Tyr-NH$_2^9$]OVT (^{125}I-OVTA) according to published methods (Elands et al., 1987) with unlabeled OT (5µM) used to define nonspecific binding. Receptor binding was measured using standard receptor autoradiographic methods (Kuhar, 1986).

Results of this study showed that PRZ treatment reduced OT receptor binding in caudal regions of the vlVMN compared to animals treated with E_2 alone. These data suggest that NAT is involved in the modulation of OT receptor binding by E_2. Whether the inhibitory effects of PRZ on E_2-dependent OT receptors are the result of a decrease in E_2 sensitivity (due to a decrease in estrogen receptors) or to an inhibition of E_2-stimulated protein synthesis remains to be determined.

95.15

FMRF-NH$_2$-LIKE PEPTIDE IN PITUITARY OF BRATTLEBORO RAT AND SALT TREATMENT.

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FLFQPQRF-NH$_2$ (F-8-F-NH$_2$), a mammmalian FMRF-NH$_2$-like neuropeptide, was isolated from bovine brain. F-8-F-NH$_2$ can decrease rat tail flick latencies and attenuate morphine induced analgesia suggesting a role in opiate mediated antinociception. F-8-F-NH$_2$ immunoreactivity (IR) is most highly concentrated in the rat neurohypophysis, an area rich in vasopressin (VP), oxytocin and dynorphin. In order to explore the role of F-8-F-NH$_2$ in this area, we have studied F-8-F-NH$_2$-IR in the CNS and pituitary of the Brattleboro (DI) and the control Long Evans (LE) rat. In the DI animal, which lacks Arg^8-VP, there is no detectable F-8-F-NH$_2$-IR in pituitary gland while there are normal levels in hypothalamus and spinal cord as compared to the LE. We have also subjected normal rats to both chronic and acute NaCl treatment. Acute IP injection of 2M NaCl failed to change F-8-F-NH$_2$-IR in either pituitary, hypothalamus or spinal cord when compared to controls. Animals who drank water containing 1% NaCl for 13 days also showed no differences in F-8-F-NH$_2$-IR in CNS or pituitary when compared to controls. The mechanism underlying the deficiency of F-8-F-NH$_2$-IR in the DI rat still remains unclear.

95.16

TYROSINE HYDROXYLASE (TH)-IMMUNOREACTIVE NERVE FIBERS IN THE RAT NEUROINTERMEDIATE LOBE: ENHANCED STAINING AFTER PARACHLOROPHENYLALANINE (PCPA) TREATMENT.

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The pituitary neurointermediate lobe (NIL) of adult rats receives both TH and serotonin (5-HT)-immunoreactive nerve fibers from hypothalamic and brainstem cell bodies, respectively. Some fibers in the NIL co-localize both TH and 5-HT (Saland et al.,'88, Neurosci. Lett. 94: 39). PCPA, an inhibitor of tryptophan hydroxylase, has been shown to increase TH synthesis in mammalian brain, and to enhance visualization of TH-positive perikarya in the cat brain (Kitahama et al, '87, Neurosci. Lett. 77:155). Here, adult male Sprague-Dawley rats received three successive daily intraperitoneal (i.p.) doses of PCPA (300, 100, 100 mg/kg). Controls received i.p. saline injections. One week after the first dose, rats were ether-anesthetized, and perfused with saline followed by buffered paraformaldehyde. Paraffin sections incubated with anti-TH (Eugene Tech, 1:300) were treated with avidin-biotin-peroxidase or IgG-5nM gold, and coded slides were examined by two observers. After PCPA treatment, TH fiber staining was enhanced in both neural and intermediate lobes, particularly along the border between the two areas. PCPA, by reducing 5-HT, may increase TH synthesis in hypothalamic neurons, and may enhance catecholamine input to the NIL. Supported by NIH NS-21256 and RR-08139.

96.1

TOPOGRAPHICAL ORGANIZATION OF THE CHOLINERGIC CELLS PROJECTING FROM THE LATERODORSAL TEGMENTAL (LDTg) AND PEDUNCULOPONTINE TEGMENTAL (PPTg) NUCLEI TO THE THALAMUS IN RAT BRAIN.

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The LDTg and the PPTg provide the major cholinergic input to the thalamus. Previous studies have indicated that the LDTg projects only to those thalamic nuclei associated with the limbic system while the PPTg projects to all thalamic nuclei. This study provides a detailed analysis of the entire span of these two cholinergic nuclei and demonstrates that they are better understood as a single nucleus which is topographically organized according to the function of the thalamic nuclei to which it projects.

Injections (30nl) of Fluoro-Gold were placed into anterior, ventrolateral, and posterior thalamic nuclei. Cholinergic projections to these nuclei were traced using simultaneous visualization of retrogradely transported Fluoro-Gold and choline acetyltransferase immunofluorescence. Anterior thalamus is supplied primarily by the LDTg and the caudal PPTg, which are continuous both anatomically and functionally and can be understood as the "limbic pole" of the entire cholinergic group. Ventrolateral and posterior nuclei, involved in sensory-motor processing, are supplied primarily by the rostral part of the cholinergic group, which can be understood as the "sensory-motor relay pole". In the middle portion of this group cells project to both limbic and relay nuclei, but the exact pattern of cells projecting to any nucleus can be predicted by the function of that nucleus. This functional organization is also reflected in the degree of crossover of the projections. Projections from the limbic pole cross more frequently than those from the sensory-motor relay pole.

96.2

SITE-DIRECTED ANTIBODIES DESIGNED FOR THE IMMUNOCYTOCHEMICAL LOCALIZATION OF NICOTINIC ACETYLCHOLINE RECEPTORS IN RAT BRAIN

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Polyclonal antibodies were raised in rabbits against a non-conserved intracellular domain of the predicted sequence of the α4 agonist-binding subunit of rat central nicotinic acetylcholine receptor (Goldman, D. et al., *Cell*, 48:965, 1987). A dodecapeptide corresponding to the segment 535-546 was synthesized and conjugated with glutaraldehyde to BSA and poly-D-Lys for immunization. Several antisera showed high titers in RIA with the iodinated peptide analog. One of these, N-94, bound to a major band of Mr = 65-70 kdaltons in Western blots of rat brain membranes. This binding was insensitive to aldehyde fixation of blotted proteins. In sections from 4% paraformaldehyde-perfused brain, processed for PAP immunohistochemistry with 1/200-800 dilutions of N-94, strong and selective neuronal labeling was observed in anatomical regions where α4 transcripts have been detected by in situ hybridization (Wada, E. et al., *J. Comp. Neurol.*, 1989, in press). This immunostaining appeared confined to neuronal perikarya and dendrites, and spared the nucleus. In cerebral cortex, it was restricted to pyramidal neurons and conspicuously absent from the granular cells of layer IV. Such subtype-specific immunoprobes visualized at light and electron microscopic levels should allow the cellular and ultrastructural localization of nicotinic acetylcholine receptors in rat brain. (Supported by MRC, NRC and FRSQ).

96.3

REGIONAL DISTRIBUTION OF BINDING SITES FOR SELECTIVE MUSCARINIC ANTAGONISTS IN RAT BRAIN.

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The distribution of subtypes of the muscarinic receptor in homogenates of the rat brain was investigated by measuring the competitive inhibition of the binding of [^{3}H]N-methylscopolamine ([^{3}H]NMS) by pirenzepine and AF-DX 116 (11[[2-[(diethylamino)methyl]-1-piperidinyl]acetyl]-5,11-dihydro-6H-pyrido[2,3-b][1,4]benzodiazepine-6-one). Pirenzepine and AF-DX 116 have been shown to exhibit selectivity for M1 and M2 receptor subtypes, respectively. In most brain regions, the pirenzepine/[^{3}H]NMS and AF-DX 116/[^{3}H]NMS competition curves were consistent with a two-site model. The dissociation constant of pirenzepine for its high affinity site (M1 receptor) was approximately 10^{-8} M, whereas the dissociation constant of AF-DX 116 for its high affinity site (M2 receptor) was approximately 10^{-7} M. In many regions, particularly those in the forebrain, the sum of the densities of the M1 and M2 receptors was substantially less than 100% of the total sites, indicating the existence of a third population of sites lacking high affinity for either pirenzepine or AF-DX 116. We have designated these latter sites as non-M1, non-M2 muscarinic receptors. In general, the relative densities of the M1 and non-M1, non-M2 binding sites were highest in cerebral cortex, corpus striatum and hippocampus, intermediate in thalamus and hypothalamus, and lowest in midbrain, medulla-pons and cerebellum. In contrast, the relative density of the M2 binding site was exactly the converse. *Supported by NIH Grant NS26511.*

96.4

MUSCARINIC ACETLYCHOLINE RECEPTORS IN NEURAL AND NON-NEURAL HUMAN EYE STRUCTURES

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The neurotransmitter actions of acetylcholine play an important role in the function of the eye , most notably manifest in the muscarinic cholinergic actions of miotics. We studied the localization of muscarinic cholinergic binding sites in human donor eyes . Autoradiograms were generated after incubation with the tritiated ligands, Quinuclidinyl Benzylate (QNB) and N-Methylscopolamine (NMS). In addition, characterization studies of the iris-ciliary body were done. The results of these experiments demonstrate binding sites in the retina, and of particular interest, the non- neural uveal tract structures, within which marked density differences are present. Characterization studies show normal binding kinetics for the iris-ciliary body , with a Kd value of 0.51 nM. Preliminary studies of the iris-ciliary body point to a predominance of the muscarinic M2 subtype. The present results may be relevant to the future development of more specific ophthalmic drug therapy, and to a greater understanding of its effects on muscarinic cholinergic actions in the eye.

96.5

[^{11}C]-BENZTROPINE AS A POSSIBLE MUSCARINIC CHOLINERGIC RECEPTOR LIGAND IN HUMAN BRAIN FOR PET.

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Involvement of the cholinergic system has been implicated as one of the underlying causes of Alzheimer's disease. Benztropine is a widely prescribed anti-cholinergic agent used to relieve the extrapyramidal side effects of neuroleptics. Preliminary studies in baboons using [^{11}C]-benztropine demonstrated that the regional distribution of binding closely paralled the known distribution of muscarinic receptors in the primate brain (Dewey, et al. Brain 89). We investigated the regional distribution and temporal profile of [^{11}C]-benztropine in human brain with high resolution PET. More than 50% of the brain uptake occurred within the first 5 min. Accumulation continued throughout the 80 min of scanning for all brain regions except for cerebellum. Regional concentration of [^{11}C]-benztropine was striatum > cortex > thalamus > cerebellum. Analysis of [^{11}C]-labelled metabolites revealed no significant metabolism of [^{11}C]-benztropine. Sequential injections of [^{11}C]-benztropine done 2 hrs apart in the same subject revealed a test/retest variability of < 10%. The regional uptake of [^{11}C]-benztropine corresponded to the known distribution of muscarinic receptors in the human brain as measured with postmortem autoradiographic techniques (J. Neurochem. 43, 1984). USDOE, OHER, NIH NS-15638.

96.6

IDENTIFICATION OF B-2 BRADYKININ RECEPTORS IN SHEEP NASAL TURBINATE MEMBRANES.

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Bradykinin (BK) has been implicated as a potential mediator of the nasal symptoms associated with colds and allergy and may exert its effects by interacting with at least two receptor subtypes named B-1 and B-2. B-2 receptors have been studied in a variety of tissues using radioligand binding methods. In order to determine the subtypes of BK receptors present in nasal tissues, we performed in-vitro binding assays using ^{3}H-BK in membranes prepared from middle and inferior nasal turbinates from sheep. Binding was saturable and specific (K_D of 0.02nM) and revealed a low density of sites (Bmax of 0.6 pMoles/g tissue) with a pharmacology characteristics of a B-2 receptor (shown below).

	K_D (nM) +/- SEM	N:
1) BK	.039 +/- .008	7
2) Lys-BK	.09 +/- .03	4
3) des-Arg^9	952 +/- 143	3
4) Leu^8, des-Arg^9-BK	4735 +/- 1160	3

The B-2 receptor agonists BK and Lys-BK competed with high affinity for ^{3}H-BK-labelled sites while the B-1 agonist des-Arg^9-BK and B-1 antagonist Leu^8, des-Arg^9-BK competed in the micromolor range. These data identify the presence of B-2 receptors in sheep nasal turbinate membranes. Saturation, kinetic, displacement and guanine nucleotide analog studies will be presented.

96.7

DIFFERENCES IN HUMAN AND SALMON CALCITONIN BINDING ON RAT BRAIN SECTIONS SUGGEST RECEPTOR SUBTYPES. D.I.Lambie* J.M. Hill A. Harris* J. Barbour* L. Kwart* M.R. Ruff* and C.B. Pert.* (SPON: C. Sharp). NSB, NIMH Bethesda, Md. 20892 and Peptide Design 12321 Middlebrook Rd. Germantown, Md 20874.

Studies of the Scatchard analysis of salmon calcitonin (SCt) binding to brain tissue have reported either a single high affinity site or both high and low affinity.sites. SCt and human calcitonin (HCt) have only 50% of their amino acid composition in common, however, both are assumed to bind to the same brain receptors. In this study the autoradiographic binding patterns of ^{125}I-SCt and ^{125}I-HCt are compared on adjacent rat brain sections as are the patterns achieved with competition with cold SCt, HCt, or PD89.010, a calcitonin analog.

Both HCt and SCt bound in many brain regions, however, the HCt binding pattern was more restricted in its boundaries and encompassed fewer regions than that of SCt. Although HCt revealed dense binding in the hypothalamus and central grey as did SCt, in many areas in which SCt had dense or moderately dense binding HCt apparently did not bind at all. Although SCt at micromolar concentrations displaced both ^{125}I-SCt and ^{125}I-HCt completely, HCt at this concentration, displaced ^{125}I-HCt but reduced ^{125}I-SCt only slightly. At micromolar concentrations PD89.010 displaced ^{125}I-HCt and reduced ^{125}I-SCt binding.

These results are consistent with the hypothesis that SCt may bind to more than one receptor subtype in brain tissue.

96.8

RADIOLABELING OF THE RAT CNS BY BLOOD-BORNE ^{125}I-SAR^{1},ILE^{8} ANGIOTENSIN II (^{125}I-SIAII). B.P. Rowe* and R.C. Speth (SPON: E.A. Daigneault). Dept. Physiol., Coll. Medicine, E. Tenn. State Univ., Johnson City, TN 37614 and Dept. of VCAPP, Washington State Univ., Pullman, WA 99164-6520.

Blood-borne angiotensin II (AII) acts at circumventricular organs (CVO's) in the brain which lack a blood-brain-barrier (BBB). This study examined binding of ^{125}I-SIAII, in the rat CNS after administration into the bloodstream. 65-90 g rats were anesthetized and injected intracardially with 44 pmoles of ^{125}I-SIAII in 1 ml of 0.1 M sodium phosphate (n=3) or ^{125}I-SIAII plus 4 nmoles of AII (n=2). Two min later the rats were perfused with chilled phosphate buffered saline for 2 min and 50 ml of 3% paraformaldehyde. The brain, spinal cord (C-1) and pituitary were removed and frozen. Each entire brain was frozen sectioned at 20 micron thickness. The sections were placed in apposition to X-ray film for 2-3 days to localize ^{125}I-SIAII binding. The ^{125}I SIAII binding was present in the choroid plexus of the lateral, 3rd and 4th ventricles, and the pineal recess, the subfornical organ, area postrema, pineal, anterior and posterior pituitary, pia-arachnoid and organum vasculosum of the lamina terminalis. ^{125}I-SIAII binding was only partially reduced with 100-fold excess of AII. Therefore some of the ^{125}I-SIAII binding may be nonspecific. This study confirms prior studies indicating that AII does not cross the BBB, and suggests that AII acts in the choroid plexus as well as the CVO's in the brain.

96.9

QUANTITATIVE AUTORADIOGRAPHY OF HIGH AFFINITY SOMATOSTATIN BINDING SITES IN RAT BRAIN USING A NOVEL ANALOGUE [125I] L 363,586 AND RECEPTOR DESATURATION PROCEDURES. M. Dugich-Djordjevic, R. Vandlen*, and C.A. Altar, (SPON: B.S. Glaeser) Dept. of Pharmacological Sci. and *Dept. of Protein Chemistry, Genentech, Inc. South San Francisco, CA 94080

Evidence has suggested the existence of somatostatin receptor populations in the rat and human CNS with varying affinities for [125I]-somatostatin-28 (SST-28). [125I]-L 363,586, a potent cyclic hexapeptide somatostatin analogue, binds to rat brain homogenates with high affinity, but differs from SST-28 in its sensitivity to GTP (Vandlen et al., in press). We employed guanine nucleotides (Leroux et al., Neuroendocrinol., 47:533, 1988) or high NaCl to dissociate endogenous somatostatin from CNS binding sites and to characterize binding to unoccupied receptors using quantitative autoradiography. Adjacent sections of rat brain were prewashed in buffer (20 mM Hepes, pH 7.4, 5 mM $MgCl_2$, 0.1% BSA, 0.1% bacitracin) alone or in buffer containing GTP (10 µM) or 120 mM NaCl and then 90 min in buffer only. Sections were incubated 2 h in 5-600 pM [125I]-SST-28 or [125I] L 363,586 with or without 100 nM SST-14 or L 363,586. Either GTP or NaCl prewashes significantly ($p<.001$) increased 100 pM [125I]-SST-28 specific binding 3-5 fold. L 363,586 displaced all specific [125I]-SST-28 binding after GTP desaturation, but only 30-80% after the NaCl prewash. Scatchard analysis revealed saturable binding of comparable affinity for both ligands (Kd=30-150 pM) in the cingulate and entorhinal cortex, claustrum and septum, and lower affinity binding (Kd=350-430 pM) in the CA1 region of hippocampus for [125I] L 363,586. The area of highest binding for [125I]-SST-28 was in neocortical layers V and VI, the cingulate, claustrum, olfactory nuclei, dentate gyrus and CA1, with intermediate levels of binding in the septum and subiculum. The caudate-putamen, n. accumbens, hippocampal CA2 and CA3 regions, outer neocortical layers and cerebellum exhibited low levels of unsaturable binding. 85-90% of [125I] L 363,586 was specifically bound compared to 70% for [125I]-SST-28 binding. The binding of [125I]-SST-28 and [125I] L 363,586 in the rat CNS is differentially affected by NaCl and GTP. L 363,586 and the desaturation procedures described here should be useful in determining the distribution and specificity of decreases in somatostatin binding in Alzheimer's patients (Beal et al., Science, 229:289,1985).

96.10

MU OPIOID RECEPTOR AUTORADIOGRAPHY: RELATIONSHIP TO MIDBRAIN DOPAMINERGIC NEURONS. S.G. Speciale, M. Sadeq* and D.C. German. Dept. of Psychiat., Univ. of Texas Southwestern Med. Cntr., Dallas, TX 75235.

The present study sought to map the distribution of µ opioid receptors within the midbrain and relate the pattern of binding to the location of the midbrain dopaminergic (DA) neurons. Coronal sections were cut from the mammillary bodies to the level of the rostral pons in the rat. Sections were incubated at 4°C for 40 min. in 50 mM Tris HCl (pH 7.4) containing 3 nM [3H] D-Ala2MePhe4Glyol enkephalin (3H-DAGO) to assess total binding. Adjacent sections were incubated under the same conditions with the addition of the opioid agonist levorphanol (1 µM). Every 3rd section was processed for film autoradiography, along with Amersham 3H standards, using LKB Ultrofilm (exposure time = 30 days). Other sections were processed for tyrosine hydroxylase immunohistochemistry. Within 7 brains, 3H-DAGO labeling was ++++ within the rostral substantia nigra (SN) pars compacta, +++ within the SN from rostral to caudal, ++ within the ventral tegmental area and 0 within the retrorubral area. Research supported by DA-05314.

96.11

AUTORADIOGRAPHIC LOCALIZATION OF KAPPA OPIOID BINDING SITES WITH [^{3}H]NalBzoH. D. Paul,* E. Huang* and G.W. Pasternak. (SPON: R. Price). Cotzias Lab. for Neuro-Oncology, Memorial Sloan-Kettering Cancer Center and Depts. of Neurology and Pharmacology Cornell U. Medical College, New York, NY 10021

In brain homogenate binding studies, naloxone benzoylhydrazone, NalBzoH, labels a novel class of κ binding site (κ_3) as well as the classical κ_1 binding site. Using conditions to selectively label κ_3 sites, we directly examined the autoradiographic distribution of κ_3 binding sites labelled by [^{3}H]NalBzoH. Nonfixed thin rat brain sections (9µm) were incubated with 1 nM [^{3}H]NalBzoH (45.1 Ci/mmole) for 1 hr at 0°C in the presence of K_2EDTA (5mM). Nonspecific binding was defined as that remaining in the presence of 1µM unlabeled NalBzoH. The regional distribution of the κ_3 binding sites was quite distinct from previously reported κ distributions. In fact, the distribution of κ_3 binding sites labelled by [^{3}H]NalBzoH more closely resembled the distribution of µ sites, with high levels of binding in layers I and IV of the cerebral cortex, clusters of the striatum, superficial layer of the superior colliculus, the interpeduncular nucleus, and the presubiculum. Moderate levels of binding were also seen in the pyramidal cell layer of the hippocampus, the periaqueductal gray and the lateral thalamus.

96.12

VENTRAL STRIATOPALLIDAL COMPARTMENTS REVEALED BY COMBINING TRACER HISTOCHEMISTRY AND RECEPTOR AUTORADIOGRAPHY. L. Churchill, R.M. Bowker, H. Eberhardt*, R.P. Dilts, & P.W. Kalivas. Dept. of VCAPP, Washington State University, Pullman, WA 99164-6520.

GABA and met-enkephalin coexist in striatopallidal neurons. The receptors for these transmitters, $GABA_A$ and µ-opioid respectively, are localized on neurons in both the ventral striatum and ventral pallidum. Quinolinic acid lesions in the dorsomedial shell of the nucleus accumbens correlate with upregulation of $GABA_A$ receptors in the dorsal rim of the ventral pallidum, without a change in µ-opioid receptors; whereas quinolinic acid lesions that destroy neurons in the lateral nucleus accumbens do not change either the $GABA_A$ or µ-opioid receptors in the ventral pallidum. Since lesions in the ventrolateral caudate decrease $GABA_A$ receptors throughout the entire ventral pallidum, $GABA_A$ receptors may be localized on presynaptic terminals. Fluoro-Gold injections into the dorsal rim of the ventral pallidum retrogradely-labeled neurons from the dorsomedial core of the nucleus accumbens through the ventrolateral caudate; whereas injections in the ventromedial pallidum labeled neurons in the nucleus accumbens shell. Specific [125I]D-Ala-Tyr-Gly-mePhe-Gly(ol) binding to µ-opioid receptors occurred over Fluoro-Gold labeled neurons in the nucleus accumbens shell. The autoradiographic grain distribution suggested that the receptors were localized on the neuropil as well as the cell bodies.

This work was partially supported by NIH program project # NS 24388-03.

96.13

DIFFERENTIAL EXPRESSION OF CALBINDIN IMMUNOREACTIVITY IN THE THALAMOLIMBIC AND THALAMOSTRIATAL CELL POPULATIONS

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Calcium binding protein calbindin D28k (Cb) displays an inhomogeneous distribution, as evidenced by immunocytochemistry (ICC), in the rat thalamus. Cb-positive cell bodies are densely grouped in medial, midline and anterior intralaminar nuclei traditionally included in the 'nonspecific' thalamus whose efferents reach multiple cortical and subcortical targets. The occurrence of Cb-positive neurons was verified in some of these pathways by means of combined retrograde axonal tracing and ICC. The vast majority (75%-95%) of thalamic midline cells projecting to the hippocampus and only 20%-40% of those projecting to the amygdala were found to be Cb-positive. These findings indicate that the flow of information between thalamus, amygdala and hippocampus is characterized by a different neurochemical expression. In the study of midline and intralaminar cells projecting to dorsal striatum and nucleus accumbens a high proportion of neurons was found to be Cb-positive. However, topographic and quantitative differences in the Cb-positivity of thalamo-caudate and thalamo-accumbens cells point to a different neuroregulation of the two circuits.

96.14

PURIFICATION, IDENTIFICATION AND IMMUNOHISTOCHEMICAL LOCALIZATION OF A BRAIN-SPECIFIC CALCIUM BINDING PROTEIN (PROTEIN 10) .

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We previously identified a protein (MW = 29 kD, pI = 5.3) which is particularly abundant in the cochlear nuclei of rabbits, rats and guinea pigs and recently reported the calcium binding property of this protein 10. We report here the complete purification of protein 10 from guinea pig brain, the partial amino acid sequence of proteolytic digests and an immunocytochemical mapping of protein 10.

Protein purification was monitored by two dimensional gel electrophoresis. Protein 10 was precipitated by 60-80% ammonium sulfate in homogenates of guinea pig brain. Complete purification steps included two DEAE cellulose columns followed by gel filtration (TSK 3000) column chromatography on FPLC. Partial amino acid sequence was obtained for fragments of protein 10 following partial digestion with Asp-N, trypsin and V8 proteases. Results indicate a sequence homology of 58% between protein 10 and rat brain calbindin and 82% between protein 10 and chick retinal calretinin. Immunohistochemical analysis of protein 10 revealed specific label in CNS sensory systems (visual, auditory/vestibular, olfactory, nociceptive) and other discrete circuits in brain.

96.15

IMMUNOHISTOCHEMICAL LOCALIZATION OF THE NEURON-SPECIFIC FORM OF THE c-src GENE PRODUCT, pp60$^{c\text{-}src(+)}$, IN RAT BRAIN.

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Neurons express high levels of a variant form of the c-src gene product, denoted pp60$^{c\text{-}src(+)}$, which contains a six amino acid insert in the amino-terminal half of the c-src molecule. We have determined the localization of pp60$^{c\text{-}src(+)}$ in rat brain using a polyclonal antiserum raised against a synthetic peptide containing the six amino acid insert of pp60$^{c\text{-}src(+)}$. The antibodies which specifically recognized the neuron-specific form of the c-src gene product were purified using a pp60$^{c\text{-}src(+)}$-affinity column. pp60$^{c\text{-}src(+)}$ immunoreactivity was localized within the cell soma and processes of specific types of neurons throughout the brain. In the cerebellar cortex the dendritic arbor as well as the cell bodies of Purkinje cells were densely immunoreactive while granule cells exhibited lower levels of pp60$^{c\text{-}src(+)}$. In the pons and in the medulla motor neurons also displayed strong immunoreactivity. Pyramidal cells in layer V of cerebral cortex were densely labeled while some layer VI neurons were moderately immunoreactive. The caudate-putamen also contained a few densely immunoreactive neurons, which appear to be of the large aspiny type. These results indicate that pp60$^{c\text{-}src(+)}$ is differentially expressed in neurons in rat brain, suggesting a role for this variant form of the c-src gene product in specific classes of neurons in the adult central nervous system.

96.16

MAPPING OF ALPHA AND BETA THYROID HORMONE RECEPTOR GENE EXPRESSION IN RAT BRAIN AND PITUITARY BY IN SITU HYBRIDIZATION HISTOCHEMISTRY.

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Thyroid hormone (T3) is essential for normal brain development, and in adults abnormal thyroid hormone levels have been associated with neurologic and behavioral disturbances. Multiple thyroid hormone receptor cDNAs have previously been identified in rat and are classified into alpha and beta subtypes. Alternative splicing of the alpha gene gives rise to the functional receptor, rTRα1, and the non-T3-binding isotype, rTRα2. The beta gene encodes two functional receptors, rTRβ1 and rTRβ2. Using 35S-labelled synthetic DNA probes common to rTRβ transcripts and specific for rTRα1 and rTRα2 transcripts, we mapped the expression of these mRNAs in adult rat brain and pituitary by hybridization histochemistry. rTRα1 and rTRα2 transcripts were widely distributed in a similar, if not identical, pattern. rTRα1 and rTRα2 mRNAs were most abundant in the olfactory bulb, hippocampus, and granular layer of the cerebellar cortex. In contrast, rTRβ transcripts were found in a more restricted pattern with highest levels in the hippocampus, hypothalamic paraventricular nucleus, and anterior pituitary. High levels of rTRβ mRNAs in the parvocellular part of the paraventricular nucleus and the anterior pituitary suggest beta receptors may mediate thyroid hormone feedback regulation of thyrotropin-releasing hormone and thyroid-stimulating hormone. Neither alpha nor beta mRNAs were detected above background in Purkinje cells. Our results identify sites in the mammalian CNS in which regulation of gene expression by specific thyroid hormone receptor subtypes may occur.

96.17

ISOLATION OF A NEW G-PROTEIN COUPLED RECEPTOR FROM NG108 CELLS.

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A variety of neurotransmitter receptors have been shown to exist in the hybrid cell line NG108, including opioid, adrenergic, serotonergic, kinin and prostaglandin receptors. Receptors coupled to the G-protein transduction apparatus display sequence similarity within their predicted membrane topology. This observation has led to a strategy for isolating new receptor cDNA clones. Based on best conserved regions of primary structure, nucleic acid probes can be selected and used to screen for other members of the G-protein receptor family.

Using a hamster β-adrenergic receptor probe we have isolated several clones from an NG108 cDNA library. One of these clones codes for a protein which shares a significant degree of sequence similarity with other G-protein coupled receptors but is sufficiently different to suggest that it is an entirely new receptor. We shall discuss the predicted structure, tissue distribution and ligand specificities of this new receptor.

96.18

REGIONAL DISTRIBUTION OF INSULIN-LIKE GROWTH FACTOR-2 (IGF-2) IN RAT BRAIN.

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The measurement of IGF-2 in brain tissue has been problematic because of its low levels and the presence of binding proteins. We have developed a method for the extraction and quantification of IGF-2 in rat brain. Brains from 6 week old rats were dissected at 4°C and homogenized with 4 vol. of 3.3 M HCOOH/5% Tween-20. After centrifugation, the supernates were treated with acetone (70% final), vortexed and centrifuged. The extracts were diluted and analyzed by a nonequilibrium RIA at 4°C using rat IGF-2 as standard. Separation of bound and free IGF-2 was achieved by precipitation using 6.6% PEG/second Ab. Assay sensitivity was 1 pg/tube with an ED50 of 30 pg/tube.

Rat [IGF-2] (ng/g tissue; mean ± SEM; n=6)

Olfactory Bulb	25.9 ± 2.0	Striatum	13.7 ± 0.8
Pituitary	354.7 ± 29.3	Thalamus	19.6 ± 1.5
Brain Stem	17.5 ± 1.4	Hippocampus	26.5 ± 0.9
Cerebellum	21.8 ± 2.3	Cortex	24.4 ± 1.2
Hypothalamus	21.9 ± 1.5	Serum	13.6 ± 0.6

IGF-2 is heterogeneously distributed in rat brain with the highest concentration in the pituitary. The concentrations of IGF-2 are higher in brain than in the serum, suggesting that it is synthesized there. In summary, we have developed a new method for extracting and quantifying IGF-2 in rat brain tissue, which should facilitate further investigation of its functional role in the CNS.

96.19

IMMUNOHISTOCHEMICAL IDENTIFICATION OF A NOVEL CORTICAL CELL POPULATION. M.N. Gordon, S. Scully*, W.A. Schreier*, S. Kumar*, A. Espinosa de los Monteros* and J. de Vellis. Dept. of Anatomy and MRRC, UCLA Medical School, Los Angeles, CA 90024-1759.

The cytoplasmic enzyme glycerol phosphate dehydrogenase (GPDH) is believed to represent a specific oligodendrocyte marker. During experiments to examine the postnatal appearance of GPDH-like immunoreactivity relative to other specific markers in oligodendrocytes, a novel population of cells was identified in the rat cerebral cortex. Coronal vibratome sections of paraformaldehyde perfused rat brain (aged 1 d to 17 mo) were stained using a rabbit polyclonal antiserum against GPDH and appropriate fluorescent or peroxidase-conjugated secondary antibodies. A population of large cells was observed in the cerebral cortex, with morphology and time course of appearance during ontogeny which was strikingly different from that observed for perineuronal oligodendrocytes. These large cells were bipolar, with a major axis of 15-20 um, and were oriented perpendicular to the pial surface. Long, immunoreactive processes extended across several cortical laminae, and appeared beaded. Bipolar cell soma area was twice that of perineuronal oligodendrocytes. Bipolar cells were present in small numbers on the day of birth (P1), then increased in density to peak at P11-15, before decreasing to adult cell density. Bipolar cells are not astrocytes, radial glia or myelinating oligodendrocytes; sequential immunohistochemistry and in situ hybridization for combinations of cell-type specific markers revealed that GPDH+ cells do not contain protein or RNA encoding glial fibrillary acidic protein, vimentin or myelin basic protein. In addition, the myelin deficient mutant rat brain displayed a 50% reduction in the number of GPDH+ perineuronal oligodendrocytes, but no reduction in bipolar cell density. The morphology, cortical localization and ontogenic appearance of these bipolar cells most resembles that of peptidergic interneurons. Supported by HD06576.

96.20

AROMATASE NEURONS IN THE MONKEY FOREBRAIN DEMONSTRATED BY ANTIBODY AGAINST HUMAN PLACENTAL ANTIGEN X-P_2(hPAX-P_2). K.Shinoda[1], N.Sakamoto*[2], Y.Osawa*[3], J.Pearson[2]. [1]Dpt. Neuroanat. Osaka Univ, Japan; [2]Dpt. Path. NYUMC NY; [3]Dpt. Endo. Biochem., Med. Fdn., Buffalo, NY

Aromatase, which converts androgen into estrogen, appears to be involved in brain sexual differentiion, control of gonadotropin secretion and triggering of puberty. Immunohistochemical study was made of Japanese monkeys with hPAX-P_2 antibody to an antigen associated with aromatase activity. Most hPAX-P_2 immunoreactive cells are in the ventral pallidum extending from the area surrounding the islands of Calleja to the sublenticular substantia innominata (not in the preoptic area as previously reported). Others are in the second layer of the cerebral cortex, in a cluster in the mediocortical zone of the amygdala anteriorally, and in the nucleus of the diagonal band. Structures resembling axons are seen in the medial preoptic area, the hypothalamic periventricular zone, the supraoptic nucleus, the hypothalamic paraventricular nucleus and the median eminence including the arcuate nucleus. These may represent ventral pallidal projections to preoptico-hypothalamic regions involved in sexual differentiation or in the neuroendocrinergic system including the hypothalamo-hypophyseal tract.

96.21

ADENOSINE A_1 RECEPTORS ARE LOCATED ON THE INTRINSIC NEURONS OF THE VENTROBASAL COMPLEX OF THE THALAMUS. J. I. CHOCA, R. D. GREEN*, AND H.K. PROUDFIT. DEPARTMENT OF PHARMACOLOGY, UNIVERSITY OF ILLINOIS AT CHICAGO COLLEGE OF MEDICINE, CHICAGO, IL 60612.

Adenosine agonists modulate nociception when administered intrathecally or centrally. We have previously shown that some spinal cord adenosine A_1 receptors are located on the cell bodies or dendritic branches of the spinothalamic tract (STT) neurons. The following studies were performed to determine if adenosine A_1 receptors of the ventrobasal complex (VBC) of the thalamus are located on the terminals of the STT neurons. In autoradiography studies, unilateral microinjections of kainic acid into the dorsal horn or hemitransections of the thoracic spinal cord failed to alter the A_1 receptor density in the VBC of the thalamus. In contrast, the microinjection of kainic acid into the VBC eliminated greater than 90% of these binding sites. In conclusion, the STT neurons express adenosine A_1 receptors on their cell bodies or dendritic branches in the spinal cord, but not on their terminals in the thalamus. Rather these thalamic adenosine receptors are located on intrinsic neurons. Whether these receptors mediate part of the central antinociceptive action of adenosine analogs remains to be explored. (Supported by PHS Grant 03980).

96.22

IDENTIFICATION OF A SPECIFIC, HIGH AFFINITY PGE BINDING SITE IN GUINEA PIG CEREBELLUM. M. Savage* and M. Reichman* (SPON: D.L. Hammond) CNS Diseases Research, G.D. Searle & Co., Skokie, IL 60077

Prostaglandin E_2 has numerous central effects; however, its exact role in neural communication remains unclear. Progress in this area is hindered in part by the lack of information regarding PGE_2 receptors in the CNS. We report here the identification and characterization of a specific, high affinity, saturable binding site for [^{3}H]PGE_2 in neural membrane suspensions prepared from guinea pig cerebellum. The K_d of the site is 1.28 ± 0.08 nM with a B_{max} of 29.4 ± 0.4 fmole/mg protein. Specific binding represents up to 90% of total binding, exhibits a distinct regional distribution, and is sensitive to heat denaturation at 50°C and to trypsin exposure, as well as to the composition and pH of the incubation buffer. Eicosanoids at low nM concentrations compete effectively with [^{3}H]PGE_2 with differing relative potencies whereas numerous non-eicosanoids are ineffective at concentrations as high as 10 μM. Taken together, the results indicate that the specific binding site has many of the features expected of a pharmacological receptor for PGE in the CNS.

OTHER BIOGENIC AMINES AND PURINES: ADENOSINE AND HISTAMINE

97.1

MECHANISMS UNDERLYING SYNAPTIC MODULATION BY ADENOSINE IN RAT HIPPOCAMPUS: EFFECTS OF K^+ CHANNEL BLOCKERS AND THE ROLE OF ARACHIDONIC ACID METABOLITES. T. V. Dunwiddie, T. S. Worth, C. Lupica and M. Taylor. Dept. of Pharmacology, Univ. of Colo. Health Sciences Center and Veterans Administration Medical Center, Denver, CO 80262

Adenosine modulates the efficacy of synaptic transmission in the CA1 subregion of the hippocampal formation via a presynaptic A1 receptor. This response is elicited by selective A1 receptor agonists such as cyclohexyladenosine, is potently antagonized by A1 antagonists, and the selective A2 receptor agonist CGS 21680 is only weakly active. Arachidonic acid metabolites formed by a lipoxygenase pathway have been proposed as mediators of similar modulatory effects in other systems. To determine whether adenosine acts via this mechanism, we characterized the actions of cyclooxygenase and lipoxygenase inhibitors, and inhibitors of phospholipase A_2; none of these agents had significant effects upon responses to adenosine at appropriate concentrations. A potassium channel blocker (4-aminopyridine; 4AP) also had no significant effect on adenosine responses. We conclude that unlike the adenosine A1 inhibition of DA release in striatum (W.A. Cass et al, this meeting), modulation of hippocampal neurotransmission by adenosine is unlikely to be mediated by arachidonic acid metabolites. Unlike adenosine modulation of excitatory transmission in the olfactory cortex, hippocampal responses to adenosine are not blocked by 4AP. These data suggest that adenosine may act through similar receptors but with a variety of mechanisms in different brain regions to inhibit neurotransmission.
Supported by the Veterans Administration and DA02702.

97.2

LOCAL CEREBRAL BLOOD FLOW RESPONSES TO 2-CHLOROADENOSINE AND CO_2 IN THE PIGLET.
T. S. Park and J.M. Gidday. Dept. of Neurosurgery Univ. of Virginia, Charlottesville, VA 22908.

2-chloroadenosine (2CADO) is a nonmetabolizable adenosine analogue. Topical application of 2CADO was shown to dilate pial arterioles and increase cerebral blood flow (CBF) in adults. This study examined the effect of 2CADO on resting CBF and CO_2 reactivity of CBF in neonatal piglets. Isoflurane-anesthetized piglets (<5 d, n=7) had brain dialysis probes placed in the bilateral frontal cortex. The probe contained a platinum wire for measurement of local CBF by H_2 clearance. One probe was infused with artificial CSF, while the contralateral probe was infused with 10^{-5} M 2CADO in CSF to deliver the substance to tissue. Local CBF was measured under conditions of varied $PaCO_2$.

Results:

MABP (mmHg)	$PaCO_2$ (Torr)	CBF (ml/100 gm/min) control	CBF (ml/100 gm/min) 2CADO
64±4	37±1	28±4	72±10^
63±3	77±4	55±10*	99±13^
62±4	110±5	72±11**	122±16**^
42±2	23±2	20±3*	43± 4*^

*=<0.05,**=<0.01; vs normocapnia in the same group ^=<0.01;control vs 2CADO

This study of neontal piglets indicates that 2CADO is a potent cerebral vasodilator but has little effect on CO_2 reactivity of CBF. Supported by NS00924 & 21045; American Heart Association (Virginia Affiliate).

97.3

BINDING OF BIS(N^6-ADENOSYL)ALKANES TO THE ADENOSINE A_2 RECEPTOR DEPENDS ON THE LENGTH OF THE ALKANE SPACER.
J. B. Wollack. Dept. of Neurology, Div. of Pediatric Neurology, Columbia University, New York, NY 10032.

Bivalent analogs of adenosine (Ado) represent a novel class of ligands for the adenosine receptor which may be useful in elucidating inter-receptor spacing. A series of bis(N^6-adenosyl)alkanes (Ado-$(CH_2)_n$-Ado) was synthesized by reaction of the appropriate alkane diamines with 6 chloropurine riboside. Compounds were thus obtained with spacing groups of 2 to 10 carbons between the adenosyl groups. Binding to A_2 receptors was investigated in rat striatal tissue using ^{3}H-NECA (N-ethyl-carboxamidoadenosine) as a radioligand and 50nM cyclopentyl-adenosine to block the A_1 component of binding. The order of binding affinities was found to be NECA>Ado-$(CH_2)_{10}$-Ado≈ Ado-$(CH_2)_8$-Ado>Ado-$(CH_2)_6$-Ado>Ado-$(CH_2)_2$-Ado. Full evaluation of Ado-$(CH_2)_3$-Ado, was limited by its low solubility, however, it appeared to bind less well than Ado-$(CH_2)_2$-Ado.

Ado-$(CH_2)_2$-Ado and Ado-$(CH_2)_6$-Ado, the compounds with the highest solubilities, were also tested for their ability to inhibit platelet aggregation, a physiologic effect which may depend on the A_2 receptor. At a concentration of 100uM, Ado-$(CH_2)_6$-Ado significantly inhibited platelet aggregation, whereas Ado-$(CH_2)_2$-Ado had only minimal effects. These results suggest that separation of adenosyl moieties by a six or more carbon distance appears to be optimal for A_2 receptor activation.

Supported by the Charles A. Dana Foundation and the Aitken Trust Fund.

97.4

SPECIFIC AUTORADIOGRAPHIC LOCALIZATION OF ADENOSINE A_2 RECEPTORS IN RAT BRAIN USING THE A_2-SELECTIVE AGONIST, [^{3}H]CGS 21680.
M.F. Jarvis and M. Williams, Research Depart. CIBA-GEIGY Corp. Summit, NJ 07901

A specific neuromodulatory role for adenosine is supported by the identification of discrete distributions of adenosine A_1 and A_2 receptor subtypes in the mammalian brain. Characterization of of the biochemical and functional properties of the A_2 receptor has been limited due to the lack of available ligands which have high affinity and selectivity for this receptor subtype. Recently, a highly selective A_2 agonist radioligand, [^{3}H](2-[p-(2-carboxyethyl)phenethylamino)-5'-N-carboxamidoadenosine ([^{3}H]CGS 21680) has been shown to directly label the A_2 receptor (Kd = 16 nM) without the need to block binding to the A_1 receptor (Williams et al., 1989, FASEB J 3: A1047). In the present study, 5 nM [^{3}H]CGS 21680 was used to localize A_2 receptors in the rat brain. Specific [^{3}H]CGS 21680 binding to 20 μm thick sagittal rat brain sections represented approximately 95% of total binding and was highly localized in the striatal region. The highest density of binding was found in the caudate nucleus (80 ± 4 fmol/mg tissue). Specific [^{3}H]CGS 21680 binding was also observed in the nucleus accumbens and olfactory tubercle and represented approximately 80% of the binding found in the caudate nucleus. No significant amounts of specific [^{3}H]CGS 21680 binding were observed in any other brain region. These results are consistent with and extend the localization of brain high affinity A_2 receptors (A_{2a}) obtained with the nonselective agonist, [^{3}H]NECA, in the presence of 50 nM CPA to block binding to the A_1 receptor. The highly selective localization of A_2 receptors in the striatal region provides further support for a specific neuromodulatory role of adenosine in basal ganglia function.

97.5

PURIFICATION OF ADENOSINE A_1 RECEPTOR OF RAT BRAIN MEMBRANES.
H. Nakata. Lab. of Clinical Science, NIMH, Bethesda, MD 20892.

Adenosine receptors have been classified into two subtypes, A_1 and A_2. The A_1 receptor (A_1R) is linked to inhibition and the A_2 receptor is linked to activation of adenylate cyclase. In order to obtain more knowledge of the molecular properties of these receptors, purification of the A_1R has been performed using a newly developed affinity chromatography system (Nakata, H. Mol. Pharmacol. in press). The A_1R was solubilized with digitonin/cholate from rat brain membranes and then purified by sequential use of affinity chromatography on XAC immobilized-agarose, hydroxylapatite chromatography and re-affinity chromatography on XAC-agarose. SDS-PAGE of the final preparation showed a single broad band with silver stain, migrating with a M_r about 34,000. This M_r 34,000 peptide was covalently labeled by affinity labeling reagent, [^{3}H]p-DITC-XAC (Stiles, G. et al., Mol. Pharmacol. 34:724,1988). The A_1R preparation gave a specific binding activity of about 30 nmol[^{3}H]DPCPX/mg of protein (60,000-fold purification) and a K_d of 1.4 nM which is similar to that found for the receptor in crude membrane preparations. The purified receptor also showed essentially the same specificity for adenosine agonists and antagonists as unpurified receptors. The ability to purify the A_1R should facilitate the molecular characterization of this receptor and the production of specific antibodies.

97.6

HETEROGENEITY OF ADENOSINE A_1 RECEPTOR SYSTEMS BUT NOT 5-HT_{1A} RECEPTOR SYSTEMS IN HIPPOCAMPUS ACROSS MAMMALIAN SPECIES,
J. Zgombick, C.D. Mahle, and S. Maayani, Depts. of Pharmacology and Anesthesiology, Mt. Sinai Sch. Med., CUNY, N.Y., N.Y., 10029

Functional and binding assays were conducted in hippocampal membranes of 3 species using the selective adenosine (AD) A_1 agonist, R-phenylisopropyladenosine (R-PIA). R-PIA inhibited forskolin-stimulated adenylyl cyclase (FSAC) activity in these preparations. Cyclopentyltheophylline (CPT) was a potent, competitive antagonist of R-PIA-mediated responses. [^{3}H]-R-PIA labeled an apparent homogeneous population of CPT-sensitive AD A_1 binding sites which were sensitive to 5'-guanylylimidodiphosphate (Gpp(NH)p) and NaCl. In contrast, no species differences were observed in 5-HT_{1A} receptors and related binding sites in mammalian hippocampal membranes. The ensemble of data indicate heterogeneity across species of AD A_1 receptor systems in mammalian hippocampus. [Supported by USPH GM34852 and DA07135]

INHIBITION OF FSAC: R-PIA	COW	RAT	GUINEA PIG
EC_{50} (nM)	2±1	36±4	147±7
E_{max}	32±2	41±1	41±1
CPT Apparent K_b (nM)	3±1	10±1	45±2
[^{3}H]-R-PIA BINDING			
K_d (nM)	0.23±0.01	0.45±0.06	1.16±0.09
B_{max} (pmol/mg prot.)	1.73±0.11	1.88±0.09	2.42±0.34
100 μM Gpp(NH)p (% decrease)	2±3	22±5	47±8
150 mM NaCl (")	8±2	1±2	19±3
Gpp(NH)p+NaCl (")	29±2	38±5	63±4

97.7

A_1 ADENOSINE RECEPTOR REGULATION OF ADENYLYL CYCLASE ACTIVITY IN BRAIN MEMBRANES OF A DEEP-LIVING TELEOST FISH.
M. Leid[1], T.F. Murray[1], P.H. Franklin[1] and J.F. Siebenaller[2]. Oregon State Univ., College of Pharmacy[1], Corvallis, OR, and Louisiana State Univ. Dept. of Zoology and Physiol.[2], Baton Rouge, LA. 70803.

A_1 adenosine receptors in central nervous tissue have a wide phylogenetic distribution among vertebrates (Siebenaller and Murray, *Biochem. Biophys. Res. Commun.* **137**: 182, 1986). We have undertaken a characterization of the A_1 adenosine receptor, G_i protein, adenylyl cyclase system in a deep-living, cold-adapted teleost fish, Antimora rostrata, to determine whether the A_1 receptor of this species is functionally coupled to adenylyl cyclase under the deep-sea conditions of low temperature (0-6°) and high hydrostatic pressures (85-250 atm.) which this species experiences. Adenylyl cyclase activity was assayed using [α-^{32}P]ATP as a substrate. At an incubation temperature of 5°C both the basal and forskolin-stimulated rates of adenylyl cyclase activity were linear for at least 120 min. Cyclopentyladenosine (CPA) had no effect on adenylyl cyclase in the absence of GTP; however, at GTP concentrations of 1-100 μM, the A_1 selective agonist inhibited activity. The maximal percentage of inhibition of basal activity by CPA ranged from 7 to 17%. The basal adenylyl cyclase activity of A. Rostrata brain membranes was unaffected by 272 atm. of pressure which is comparable to the pressures experienced by this species at the lower end of its depth range. The efficacy of CPA as an inhibitor of adenylyl cyclase was not affected by this increased pressure. In contrast, investigation of CPA inhibition of adenylyl cyclase activity in brain membranes of shallow-living marine teleosts has shown that elevated pressure reverses CPA-induced inhibition. (Supported by ONR Contracts N00014-88-K-0426 and N00014-88-K-0432).

97.8

ROLE OF CYCLIC AMP IN RELEASE OF ADENOSINE BY MORPHINE FROM THE SPINAL CORD.
D. Nicholson*, T.D. White, and J. Sawynok (SPON: J. Rutherford).Dept. Pharmacology, Dalhousie University, Halifax, Canada.

Spinal antinociception produced by morphine may involve release of adenosine (JPET 243:657,1987). Recent studies suggest that spinal opioid effects are mediated via inhibition of adenyl cyclase (Brain Res. 370:61, 1986). Pertussis toxin reduces spinal antinociception produced by morphine (EJP 146:65,1988) and morphine-evoked release of adenosine (submitted). This study examined the role of cyclic AMP in release of adenosine induced by morphine. The phosphodiesterase inhibitors, Ro 20-1724 1-100μM and Rolipram 1-100μM, and Forskolin 10uM, a direct activator of adenylate cyclase, increased basal release of adenosine from whole spinal cord synaptosomes by 15-40 pmoles/mg protein/15 min as detected by HPLC. This adenosine may originate from released nucleotide, as it is reduced 40-77% by 5'-nucleotidase inhibitors. Forskolin 10μM and Ro 20-1724 10μM inhibited adenosine release induced by 50μM morphine (30 pmoles/mg protein/15 min) by >90%. Rolipram 1μM and Ro 20 1724 1μM reduced evoked-release from dorsal spinal cord synaptosomes by >90%. Subsequently, isobutylmethylxanthine 500μM was shown to increase basal adenosine release by 50 pmoles/mg protein/15 min and inhibit morphine-induced release by 68%. These data support the involvement of cyclic AMP in spinal actions of morphine. (Supported by MRC).

97.9

2'-DEOXYCOFORMYCIN INHIBITION OF ADENOSINE DEAMINASE IN RAT BRAIN: SPECIFICITY, DOSE DEPENDENCY AND ENZYME RECOVERY. R. Padua*, J.D. Geiger, S. Dambock* and J.I. Nagy. Depts. of Pharmacol. and Physiol., Univ. of Manitoba, Winnipeg, MB., R3E 0W3, CANADA.

2'-Deoxycoformycin (DCF) is a potent inhibitor of adenosine deaminase (ADA) and a clinically effective drug. DCF may prove useful for investigations of adenosine neuromodulation. We determined its inhibitory potency against ADA and adenylate deaminase (AMPDA), the rate of ADA recovery in 10 brain regions after single and double i.p. injections, and the effects of DCF on several neurotransmitter synthetic enzymes. Ki values for inhibition of ADA and AMPDA were 23 pM and 233 μM, respectively. In Vivo, DCF inhibited ADA with ED_{50} values of 155 to 280 μg/kg. AMPDA was not inhibited by doses up to 5 mg/kg. A second dose of DCF was not as effective as a single dose at inhibiting ADA. 40 days after a single dose of 5 mg/kg, ADA activity (% of control) recovered by 68 to 78 in regions with normally high levels of activity and from 44 to 59 in other regions. The activities of CAT, GAD and HDC-an enzyme colocalized with ADA in hypothalamic neurons- were unaffected by DCF treatment. The low doses of DCF required for ADA inhibition in vivo is consistent with its high potency against ADA in vitro and any physiological effects observed at low doses might therefore be ascribed to inhibition of ADA.

97.10

ADENOSINE DEAMINASE FACILITATES GLUTAMATERGIC TRANSMISSION IN CULTURED CEREBELLAR NEURONS. P.L. Canonico, M.V. Catania*, M.A. Sortino and F. Nicoletti*. Department of Pharmacology, University of Catania, Italy.

Addition of adenosine deaminase (ADA, 0.05-2 U/ml) to cultured cerebellar neurons induces large increases in $^{45}Ca^{2+}$ influx and [^{3}H]inositol-monophosphate formation. Both effects appear to be secondary to an enhanced release of endogenous glutamate acting at specific receptors coupled to either ion channels or inositol phospholipid hydrolysis. Stimulation of excitatory amino acid release by ADA requires the presence of extracellular Na^+ and is still present after 7 hours of dialysis to remove any possible ionic contaminant of the enzyme preparation. The effects of ADA are not reversed by a number of adenosine receptor agonists (L-PIA, NECA or 2-CADO) and are not mimicked by isobutylmethylxanthine or theophylline. In addition, the action of ADA is enhanced by exogenously applied adenosine, although the products of adenosine deamination (inosine and ammonia) are inactive. We suggest that, besides removing a tonic inhibition by the endogenous adenosine, ADA interacts with the plasma membrane stimulating excitatory amino acid release from cultured cerebellar neurons.

97.11

PROCONVULSANT ACTIONS OF BOTH CENTRALLY AND PERIPHERALLY ADMINISTERED ADENOSINE AGONISTS ON CAFFEINE-INDUCED SEIZURES. P.F. Morgan and M.J. Durcan. Laboratory of Clinical Studies, Bldg10 3C218, NIAAA, Bethesda, MD20892.

The effect of the adenosine agonists N-ethyl carboxamido-adenosine (NECA), cyclohexyladenosine (CHA) or 2-chloroadenosine (2-CL-ADO) on the seizure activity of the adenosine antagonist caffeine was investigated in NIH Swiss mice. Pretreatment with adenosine agonists (1 mg/kg, i.p., 20 min prior to caffeine infusion into a lateral tail vein) significantly ($p's < 0.05$) decreased the seizure threshold to caffeine whereas a similar pretreatment with the nucleoside transport inhibitors dipyridamole or nitrobenzyladenosine (both 0.25 mg/kg) were without effect. Intracerebroventricular administration, through an indwelling cannula, of 2.5 ug of adenosine agonists in 5ul artificial CSF, 20 min prior to i.p. administration of caffeine, significantly lowered the seizure ED_{50} of caffeine, whereas the same volume of artificial CSF alone was without effect. These results suggest that effects of adenosine agonists and antagonists on seizure activity may not be explained entirely by competitive interactions at adenosine receptors.

97.12

CENTRALLY MEDIATED ANTICONVULSIVE ACTIVITY OF ADENOSINE AGONISTS. J.B. Wiesner, R.M Krieger*, E.P. Rossi*. Gensia Pharmaceuticals, Inc., San Diego, CA 92121

Adenosine agonists have been shown to inhibit pentylenetetrazol (PTZ) induced seizures after systemic injection. In light of a recent suggestion (Brodie et al., 1987) that the behavioral effects of systemically administered agonists may be mediated by a non-CNS action, we wished to determine (1) whether the agonists exert their anticonvulsant activity within the CNS, and (2) whether intraventricularly (ICV)-injected agonists can reach the central site(s) of action required to inhibit convulsions induced by a systemically-administered convulsant.

Male CFW mice were administered either CHA (A_1 agonist) or NECA (mixed A_1/A_2 agonist) via either intraperitoneal (IP) or ICV route of administration at 20 (ICV) to 30 (IP) minutes prior to s.c. injection of PTZ (75 mg/kg). Both CHA and NECA exerted potent anticonvulsive activity following ICV administration. CHA exerted this activity with an approximate ED_{50} of 40 μg (2 mg/kg) for IP administration and 0.07 μg for ICV administration. NECA exerted anticonvulsive activity with an approximate ED_{50} of 2 μg (0.1 mg/kg) for IP administration and 0.04 μg for ICV administration. For both agonists, ICV administration achieved 100% protection at doses which were below threshold for protection with IP administration, indicating that leakage into the periphery cannot account for the efficacy of ICV administered agonists.

Central injections of adenosine agonists have been shown to mitigate seizures induced from localized foci of the rat limbic system that have been kindled (Barraco et al., 1984; Rosen & Burman, 1987) or locally injected with bicuculline (Franklin et al., 1988). The present results demonstrate that adenosine agonists exert anticonvulsant activity in mice, as in rats, via central mechanisms. Furthermore, these results also show that ICV administration of adenosine agonists provides access to all sites necessary to reverse the global, perhaps diffusely triggered seizures induced by systemic PTZ.

97.13

EXTRACELLULAR ATP INDUCES A RISE OF CYTOSOLIC CALCIUM ION IN PHEOCHROMOCYTOMA PC12h CELLS. N.Nakanishi, K.Akoba-Aono*¶, H.Suzuki*¶, and S.Yamada*. Depts of Biochemistry and Pedodontics, Meikai Univ. Sch. Dentistry, Sakado, Saitama 350-02, Japan.

Extracellular ATP has been reported to induce fluxes of ions such as Ca^{2+}, K^+ and Na^+, and to stimulate inositol phospholipid metabolism. This suggests the possibility that some actions of growth factors such as NGF can be affected by extracellular ATP, since the changes in cytosolic Ca^{2+} and the phospholipid metabolism are thought to play an important role in signal transduction system. We have examined by using fluorescent Ca^{2+} indicator, Indo-1, the effect of ATP on the cytosolic Ca^{2+} level of PC12h cells, a subclone of PC12. ATP induced a rise of the cytosolic Ca^{2+} (EC_{50}=1 uM). This rise was predominantly due to an increased influx rather than to intracellular mobilization of the ion, because EGTA inhibited this Ca^{2+} rise by more than 90%. ADP also induced the Ca^{2+} rise but to a lesser extent than ATP, whereas neither AMP nor adenosine showed such effect. Quinidine inhibited the ATP-induced rise of cytosolic Ca^{2+}. These results indicate the presence of P_2-type purinergic receptors whereby ATP could influence the physiology of the cells. In fact, NGF-induced neurite outgrowth from the cells was stimulated by the addition of ATP into the culture medium. Supported by a Grant from the Ministry of Education, Science and Culture of Japan (No.62570841).

97.14

[^{3}H](R)α-METHYLHISTAMINE BINDING TO RAT BRAIN MEMBRANES. R.E. West, Jr.*, A. Zweig*, C. Magatti*, M.I. Siegel*, R.W. Egan* (SPON: D.C. Bolser) Depts. of Allergy and Chemical Research, Schering-Plough Research, 60 Orange St., Bloomfield, NJ 07003.

A novel, high-affinity, histamine receptor, designated H_3, has been described recently as inhibiting depolarization-induced, rat brain histamine synthesis and release. To characterize the receptor we have synthesized the specific agonist [^{3}H](R)α-methylhistamine ([^{3}H]RAMHA). Saturation binding to rat brain membranes was characteristic of a single [^{3}H]RAMHA binding site with K_D=2.0 nM and B_{max} = 186 fmol/mg protein. Kinetic studies yielded k_1 = 0.025 $nM^{-1} \cdot min^{-1}$ and k_{-1}=0.057 min^{-1} for a K_D = 2.3 nM. The rank order of potency among a number of compounds tested in competition binding assays was N^{α}-methylhistamine, RAMHA > histamine, thioperamide > impromidine > burimamide > dimaprit. The k_i value of other H_1 and H_2 compounds was greater than 1 μM. [^{3}H]RAMHA binding was greatest in caudate, midbrain (thalamus and hippocampus), cortex and hypothalamus, less in brainstem, and least in cerebellum. While these data are generally consistent with the current understanding of the histamine H_3 receptor, they differ in that the site density is six times what has been reported and the affinity of the specific antagonist, thioperamide, is ten-fold lower than reported.

97.15

WITHDRAWN

97.16

IN VIVO AND IN VITRO RELEASE OF ENDOGENOUS HISTAMINE FROM RAT HYPOTHALAMUS. A.Yamatodani*, T.Mochizuki*, J.Kishino*, J.Ono*#, M.Takemura*, N.Inagaki*, and H.Wada.* (Spon: Y.Fukuda) Dept. Pharmacology & #Dept. Pediatrics, Faculty of Med., Osaka Univ., Osaka 530, Japan.

In rats, histaminergic neurons are confined to the tuberomammillary nucleus, and the highest concentration of the fibers is found in the hypothalamic nuclei (J.Comp.Neurol., 273: 283, 1988). In this study, the effects of various neuroactive substances on the basal release and high K^+-evoked or electrically stimulated release of endogenous histamine from rat hypothalamus were examined using a brain microdialysis method in vivo and a continuous superfusion method in vitro. Released histamine was determined by a highly sensitive HPLC-fluorometric method (J.Chromatogr., 344: 115, 1985). In the in vivo experiments, the basal release of histamine from the anterior hypothalamic area was 80-100 fmol/20min, and about 1.5- to 2-fold increase in release was observed by dialyzing with high K^+ solution or on electric stimulation of the tuberomammillary nucleus. These evoked releases were Ca^{2+}-dependent. In in vitro superfusion experiments, high K^+-evoked release of endogenous histamine from hypothalamic slices was significantly enhanced by superfusion with glutamate, aspartate, Met-enkephalin, acetylcholine and neuropeptide Y, and suppressed by superfusion with substance P, GABA and adenosine. With these methods, it is possible to investigate the dynamics of histaminergic neurocircuits.

97.17

CHARACTERIZATION OF HISTAMINE H_1-RECEPTORS IN PRIMARY CULTURES OF RAT ASTROCYTES. N.Inagaki*, H.Fukui*, Y.Taguchi*, S.Ito*#, R.Yoshida*#, A.Yamatodani* and H.Wada* (Spon: S.Inagaki) Dept. Pharmacology II, Faculty of Medicine, Osaka University, Osaka 530, and #Osaka Bioscience Institute, Suita 565, Japan.

The characteristics of histamine H_1-receptors in primary cultures of astrocytes were analyzed by [^{3}H]mepyramine binding assay, and compared with those in brain tissue. Astrocytes were prepared from the cerebral cortex of newborn rats and maintained for three weeks in culture. Similar values for the dissociation constant (Kd value) of [^{3}H]mepyramine binding and the inhibition constants (Ki values) of mepyramine, triprolidine, d-chlorpheniramine, diphenhydramine and histamine for [^{3}H]mepyramine binding were obtained from the receptors in cultured astrocytes ($Kd=10.4\times10^{-9}M$) and in brain tissue ($Kd=9.4\times10^{-9}M$). The density of H_1-receptors in cultured astrocytes increased during culture for three weeks and reached a plateau. The pattern of increase in receptor density resembled that in the brain during postnatal development, and the Bmax value (262±60 fmol/mg protein) was comparable to that of 3-week-old rat brain tissue (194±24 fmol/mg protein). Furthermore, histamine elevated the intracellular Ca^{2+} concentration of the cultured astrocytes. These results suggest that the receptors in cultured astrocytes and in brain tissue are identical and that astrocytes are a major target of the central histaminergic system.

97.18

CHANGES IN OCULAR HISTAMINE IN RATS WITH EAU C. H. Lee[1]*, M. Kunkle[2]*, L. A. Smith[2]*, K. R. Aoki[2]*, and E. L. Orr[1], [1]Dept. of Anatomy, Texas College of Osteopathic Medicine and [2]Alcon Laboratories, Inc., Ft. Worth, TX 76107.

Degranulation of choroidal mast cells (MC) is an early event in experimental autoimmune uveoretinitis (EAU), suggesting that MC may participate in this disease. Since histamine (HA) is released from degranulating MC, we monitored the temporal changes of HA levels in various regions of the eye as a measure of MC activation in EAU. Lewis rats were immunized with purified S-antigen emulsified in complete Freund's adjuvant (CFA) or with CFA alone (control). Clinical signs of EAU were detected by direct ophthalmoscopic examination. Rats were killed on days 5, 7, 9, 11, and 13 postinjection (pi), and the retina, choroid, sclera and "rest" of each eye was dissected, weighed, and later assayed for HA. Clinical disease was present from day 10-13 pi. Retinal edema (i.e., increased wet weights) was present on days 9, 11 and 13 pi. Histologically, eyes taken on days 11 and 13 pi exhibited edema in both the outer nuclear layer and at the neural retina-choroid junction. Compared to control rats, EAU rats showed higher choroidal HA on days 9 (27%), 11 (79%) and 13 (84%) pi and higher retinal HA on day 11 (150%) pi; scleral HA was unaltered, but HA was decreased by as much as 57% (on day 13 pi) in the rest of the eye. The timing of these regionally-specific changes in ocular HA are consistent with a role for ocular MC in EAU.

97.19

HISTAMINE-STIMULATED LOW-K_M GTPASE ACTIVITY IN THE RAT BRAIN. S. Ghodsi-Hovsepian, G.J. Durant* and W. Hoss Department of Medicinal and Biological Chemistry, College of Pharmacy, University of Toledo, Toledo OH 43606.

Histamine H_2 receptors stimulate the activity of adenylate cyclase in the brain. This effect is amplified in the presence of H_1 receptor agonists, which stimulate phosphoinositide turnover in the brain. On the other hand, histamine H_3 receptors regulate the release of histamine presynaptically. In order to assess the role of G-proteins in the coupling of histamine receptors to their effectors, the ability of histamine agonists and antagonists to stimulate or inhibit low-K_m GTPase was measured in the rat brain membranes. Histamine produced a dose-dependent stimulation of low-K_m GTPase in rat cortical membranes, reaching a value of 40 percent above control, with an EC_{50} value of 1.5 mM. Cimetidine and diphenylhydramine inhibited histamine-stimulated low-K_m GTPase with IC_{50} values of approximately 1 μM with 1 mM histamine. The muscarinic antagonist atropine was ineffective as an inhibitor of histamine-stimulated low-K_m GTPase. Comparing different regions of the brain, the maximum value of histamine-stimulated GTPase activity displayed the following rank order: cortex > striatum > hippocampus > midbrain. Neither cerebellum nor pons-medulla displayed histamine-stimulated low-K_m GTPase. The distribution of histamine-stimulated low-K_m GTPase is correlated with the distribution of histamine receptors in the brain. The data suggest that at least some histamine receptors are coupled to their effectors through G-proteins in the brain.

97.20

IMMUNOHISTOCHEMICAL AND BEHAVIORAL EVIDENCE FOR SENSORY FUNCTION OF HISTAMINE IN MOLLUSCAN NERVOUS SYSTEM. S. Soinila, G. J. Mpitsos and P. Panula. Hatfield Marine Science Center, Oregon State Univ. Newport, OR 97365, and Deptment of Anatomy, University of Helsinki, Finland.

We conducted correlated immunohistochemical and behavioral studies to make a comparative analysis of histaminergic nervous systems in *Pleurobranchaea californica* and *Aplysia californica*. The distribution of histamine was obtained using an antiserum to histamine-protein conjugate. Histamine-immunoreactive neurons and nerve fibers were present in all major ganglia. Histamine fibers were also found in nerve roots innervating the tentacles, the major chemosensory organs, as well as in the neuropil of the peripheral tentacular ganglion, and in the tentacular subepithelial connective tissue. A behavioral measure of histaminergic function was obtained by injecting into the animals seawater (control), pyrilamine (H_1-receptor blocker) or cimetidine (H_2-receptor blocker) and determining threshold values for L-glutamate or beer solutions that provoke orientation and biting responses. Each blocker produced characteristic postural changes. In both species, pyrilamine increased the threshold values. In *Pleurobranchaea*, cimetidine had no effect on the thresholds, while in *Aplysia* it increased thresholds. These results suggest that histaminergic neurons modulate chemosensory input and that two different histamine receptor sites are involved in this function. The statocyst, the organ of spatial orientation, contained large ciliated histamine-immunoreactive neurons which projected into the statocyst nerve. Pyrilamine, but not cimetidine, inhibited the animals from righting when turned upside down. These results suggest that histamine is the primary sensory transmitter in the molluscan statocyst. In preliminary studies, the behavioral measures that have been ported from *Pleurobranchaea* to *Aplysia* indicate that they may be usefully applied to show rapid associative food-aversion learning in this animal also. Supported by AFOSR 89-0262.

98.1

SEIZURE-LIKE NEURONAL DISCHARGES IN BRAIN SLICES OF THE DENTATE GYRUS FROM EPILEPTIC PATIENTS DURING 1 HZ STIMULATION. L. M. Masukawa and M. O'Connor. Graduate Hospital Research Center, Depts. of Neurology and Surgery, Graduate Hospital, Philadelphia, PA 19146.

Brain slices from human hippocampi removed during surgical treatment of intractable epilepsy were studied at the Graduate Hospital to determine the neuronal activity of tissue obtained under different surgical conditions than used in the previous Yale study. In summary, two types of responses produced in the dentate gyrus by single stimuli presented to the perforant path were observed in the present study as well as the Yale study. That is, the field response was made-up of either 1-2 population spikes (Type I) or was composed of 3 or more population spikes (Type II) at a maximal stimulation intensity. In the present study, we observed a new response to individual stimuli during a short train of 1 Hz that was characteristically more explosive than what we observed routinely in tissue from the Yale study. This response was of long duration, from 200 msec to 1-2 sec, and therefore represented a prolonged afterdischarge. In some slices a refractory period occurred that lasted 1-3 hours during which only mild potentiation could be generated. The refractory period was greatest immediately following the maximal afterdischarge (about 5-10 sec) during which no field responses could be evoked. This new response was observed in both anterior and posterior biopsies of the dentate gyrus. Thus far, this response comes closest to resembling a seizure-like electrical response in its highly potentiated and prolonged afterdischarge, and the persistent refractory period. Supported by NIH grant NS 23077 to LMM.

98.2

AN NMDA-MEDIATED COMPONENT OF EXCITATORY SYNAPTIC INPUT TO DENTATE GRANULE CELLS IN "EPILEPTIC" HUMAN HIPPOCAMPUS STUDIED *IN VITRO*. P.G. Aitken[1,2], L. Urban[1]*, A. Friedman[3]*, G.G. Somjen[1,2]. Departments of Cell Biology[1], Neurobiology[2], and Division of Neurosurgery[3], Duke Medical Center, Durham, NC 27710 USA.

Hippocampi were obtained from patients undergoing temporal lobe resection for treatment of intractable seizures. Transverse 400μM slices were cut by vibratome and kept at 34.4-35.5°C in an interface chamber. Extracellular field potentials in stratum granulosum of gyrus dentatus (GD) were recorded in response to afferent fiber stimulation. The early part of the extracellular response was similar to that seen in rodent hippocampal slices: a positive field EPSP with, at higher stimulation intensities, a superimposed population spike. In some experiments, higher stimulus intensities (100-1200μA) also evoked a late component (LC) that followed the "normal" field EPSP. This LC was negative in 5 slices (30-50msec, 0.6-4.2mV) and positive in 2 slices (30-120msec, 2.0-8.5mV). Both positive and negative LC's were enhanced by lowering extracellular Mg^{2+} from 1.2 to 0.6mM, and were reversibly depressed 50-100% by application of the NMDA receptor antagonists CPP (4-10μM) or d-APV (20μM). The NMDA antagonists had no effect on the early portion of the evoked potentials. These results invite comparison with the NMDA component of synaptic input to GD that appears in kindled rats. Lacking proper controls, however, firm conclusions cannot be drawn. [Supported by NIH grant 17771]

98.3

CORRELATION OF UNIT RECORDINGS WITH REGIONAL CELL LOSS IN EPILEPTOGENIC HUMAN TEMPORAL LOBE. R.C. Frysinger, M.F. Levesque, and R.M. Harper. Dept. of Anatomy and Cell Biology, Division of Neurological Surgery, and the Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

More single cell activity can be recorded contralateral to a seizure focus than ipsilateral in humans undergoing long-term depth electrode recordings as part of their evaluation for surgical treatment of medically intractable epilepsy. This may result from cell loss in hippocampus and related structures. We examined records of single cell recordings and electrode localization in 28 patients who later received anterior temporal lobectomy and had cell count data from hippocampus. We determined the number of microwires showing activity greater than 25 μV in each of the 239 electrode bundles in these patients. Analysis of variance was used to test whether activity varied as a function of site (amygdala; anterior, mid, and posterior hippocampal gyrus; anterior and mid pes hippocampi) and side (ipsilateral or contralateral to the resected lobe). The number of active wires per bundle in resected areas was correlated with the percent cell loss in anterior pes, posterior pes, transitional cortex, or neocortex of the resected lobes. Resected regions had fewer active wires ($p=.045$), and some brain areas had more than others ($p=.004$), with amygdala and hippocampal gyrus having more activity than hippocampus and presubiculum. The ipsilateral mid pes showed 4 times the activity of contralateral mid pes, while contralateral anterior pes had 4 times the activity of the ipsilateral side. The A-P by Side interaction was significant ($p=.012$) for this region. Cell loss correlated with activity for both amygdala and hippocampal gyrus, but not hippocampus. These results suggest that cell loss is only one of several factors determining cell activity recorded from mesial temporal structures.
Supported by NIH Grant NS 02808

98.4

INCREASED PAIRED-PULSE INHIBITION IN THE EPILEPTOGENIC HUMAN TEMPORAL LOBE. S.U. KHAN*, C.L. WILSON, M. ISOKAWA-AKESSON, T.L. BABB, AND M.F. LEVESQUE. Dept. of Anatomy and Cell Biology, Dept. of Neurology, Div. of Neurological Surgery, and Brain Res. Inst. UCLA School of Medicine, Los Angeles, CA 90024.

Paired-pulse tests of perforant path excitability show increased inhibition of the dentate population spike during kindling in the rat (Tuff et al, Brain Res, 1983, 79-90). In order to examine excitability of the human epileptic hippocampal formation (HF) we employed paired stimuli in a study of 21 complex-partial epilepsy patients who were candidates for surgical seizure therapy, with depth electrodes implanted in the HF for diagnostic monitoring. Conditioning and test stimuli were delivered to HF sites individually while recording evoked field potentials from adjacent HF sites. Paired-pulses were delivered at interstimulus intervals (ISI) of 20 to 1600 msec, and response depression or facilitation measured by dividing test by conditioning response amplitude. Interictal recordings were made in epileptogenic HF (side of all recorded seizure onsets) vs. non-epileptogenic HF (no seizure onsets). The amplitude of evoked field potentials in response to the test pulse was significantly suppressed on the epileptogenic side at ISI's of 20 to 100 msec ($p< 0.01$). These results indicate interictal inhibition is significantly stronger in the epileptogenic HF, a functional difference consistent with the anatomical findings of Babb et al (J. Neurosci., in press) showing a major loss of principal neurons in human epileptic HF with no significant loss of GAD+ inhibitory interneurons. Supported by NS02808.

98.5

EXCITABILITY OF SPECIFIC HIPPOCAMPAL FORMATION PATHWAYS IN EPILEPTOGENIC HUMAN TEMPORAL LOBE. C.L. Wilson, S.U. Khan, M. Isokawa-Akesson, T.L. Babb and M.F. Levesque. Dept. of Neurology, Dept. of Anatomy, Div. of Neurological Surgery, and Brain Res. Inst. UCLA School of Medicine, Los Angeles, CA 90024.

Paired pulse stimulation in the human hippocampal formation in complex partial epilepsy patients shows reduced excitability in areas of seizure genesis (Khan et al, Neurosci. Abst., in press), which may be attributed to the synaptic reorganization associated with hippocampal sclerosis (Babb et al, J. Neurosci., in press). We employed paired pulse stimulation of the epileptogenic (EPG) and non-epileptogenic (NEG) hippocampal formation to determine relative excitability in each of three prominent hippocampal pathways: 1) perforant path, 2) retrohippocampal path, and 3) associational path. The results showed significant reductions in excitability in the EPG perforant path with interstimulus intervals (ISI) of 20 to 100 msec and in retrohippocampal pathway at ISIs of 50 msec. On the other hand, stimulation of associational pathways showed no significant differences between EPG and NEG hippocampus. Finally, recordings were made in the anterior hippocampus in an area in which epileptic neuronal loss is documented as greatest (Babb et al., Epilepsia, 1984, 721-740). Stimulation in entorhinal cortex, presubiculum and parahippocampal gyrus showed significantly decreased excitability between 20 and 100 msec in EPG hippocampus. These results indicate no decreases in excitability *within* epileptogenic regions prone to neuronal loss, but significantly increased inhibitory suppression of afferent pathways projecting *into* epileptogenic hippocampus. Supported by NS 02808.

98.6

LIGHT AND ELECTRON MICROSCOPY OF MOSSY FIBER TERMINALS IN HUMAN "EPILEPTIC" FASCIA DENTATA. T.L. Babb, W.R. Kupfer*, J.K. Pretorius* and M.F. Levesque. Department of Neurology, Division of Neurological Surgery and the Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

Reactive synaptogenesis by mossy fiber recurrent innervation of the inner molecular layer (IML) of the fascia dentata (FD) is positively correlated with the extent of hilar cell loss, (9 epileptics, 4 normals, $R = 0.61$, $p < .05$). The present studies have demonstrated mossy fiber recurrent zinc-labelled puncta and axon terminals in the IML. The terminals were typical of mossy fiber terminals because of their large size, large ovoid vesicles, zinc located in vesicles and apparent excitatory-type asymmetric synaptic profiles. These findings suggest that mossy fibers sprout and innervate synaptic zones denervated by mossy cells of the hilus, which are the major neurons projecting to the inner molecular layer (IML). Golgi studies of the hilus in two epileptics and two non-epileptics revealed a 77% loss of mossy cells in epileptics ($2.7/mm^2$ vs. $11.5/mm^2$) which would result in denervation of the IML.

Timm's-positive, zinc-labelled puncta located in the granule cell layer indicated that mossy fibers innervated sites denervated by loss of hilar GABA neurons whose fibers normally synapse on granule cell bodies. In the hilus of seven epileptics, there was a 33% loss of GABA neurons compared to GABA neuron density in normal monkeys. However, to date we have not yet found a typical zinc-labelled mossy fiber terminal synapsing on a granule cell. These results indicate that in the hilus of human hippocampal epilepsy, there is greater damage to mossy cells than GABA neurons, which leads to greater reactive synaptogenesis and recurrent excitation of the IML than on granule cell bodies. These electron microscopic studies also suggest that most of the MF terminals in stratum granulosum (SG) innervate dendrites passing through SG.
NIH Grant NS 02808.

98.7

MORPHOMETRIC ANALYSIS OF HIPPOCAMPAL SYNAPTIC PROFILES IN TEMPORAL LOBE EPILEPSY. J. H. Kim, M. Y. Shen*, P. O. Guimaraes*, N. C. de Lanerolle and D. D. Spencer. Sections of Neuropathology and Neurosurgery, Yale Univ. School of Medicine, New Haven, CT 06510

Our previous quantitative analysis revealed no proportional differences in the number of hippocampal symmetric (putatively inhibitory) and asymmetric (putatively excitatory) synapses between tumor-associated epilepsy (TAE) and non-tumor epilepsy (NTE) (Epilepsia 29:684, 1988). TAE cases served as the control, as they revealed no statistically significant differences in neuronal density from the normal control. A morphometric study of the synaptic structures was conducted on the pyramidal layer of the hippocampal CA1 in 5 TAE and 7 NTE cases with intractable temporal lobe epilepsy. With a computer assisted scanning device the following parameters of symmetric and asymmetric synaptic structures were measured on electron micrographs: length of the postsynaptic density, length of the synaptic membrane, size of presynaptic terminals and postsynaptic profiles. Compared with TAE cases, NTE cases revealed a statistically significant enlargement of postsynaptic profiles associated with both symmetric (261%, $p < 0.02$) and asymmetric (246.5%, $p < 0.005$) synapses, but no significant differences were found in the length of synaptic membranes and postsynaptic densities, length of the postsynaptic density/length of the synaptic membrane, and size of presynaptic terminals. The enlargement of postsynaptic profiles in NTE may represent changes secondary to excessive neuronal stimulation.

98.8

MU AND DELTA OPIOID RECEPTORS AND TCP BINDING SITES ARE UNCHANGED IN THE EPILEPTIC TEMPORAL CORTEX. H.S. Pan, E.E. Weirich*, C. Kufta, and N.S. Nadi. NIH, Bethesda, MD and Bryn Mawr.

Many transmitter systems and their receptors are implicated in epilepsy. We used receptor autoradiography to study mu and delta opioid receptors and TCP binding sites in surgically removed spiking (S) and less spiking (LS) temporal cortices of patients with intractable seizures. Mu and delta opioid receptors were respectively labelled with [^{3}H]DAGO and [^{3}H]DADLE in the presence of morphiceptin. TCP binding sites were labelled with [^{3}H]TCP. The ligands bound with different laminar patterns. In LS, delta and TCP binding sites showed a gradient with layer I most heavily labelled; mu receptors were least numerous in layers I and VI. The binding patterns were not changed in S. Bmax's (fmol/mg tissue) were not different in LS vs S (38.1 ± 1.9, n=13, vs 38.0 ± 1.7, n=6 for mu receptors and 65.5 ± 4.8, n=9, vs 58.0 ± 3.5, n=6 for delta receptors). There were no K_d changes for the two ligands. The amount of [^{3}H]TCP bound (fmol/mg tissue) was not different between LS (39.6 ± 4.1, n=6) and S (32.4 ± 4.0,n=5). The lack of receptor changes along with previously shown changes in transmitter levels suggest the existence of complex changes in epilepsy.

98.9

PEPTIDE IMMUNOREACTIVITY AND mRNA ARE ALTERED IN HUMAN EPILEPTIC HIPPOCAMPAL FORMATION. J.F. McGinty, J.N. Simpson*, D. A. Hosford[1] and B.J. Crain[2]. Dept. of Anatomy & Cell Biol. E. Carolina U. Sch. Med. Greenville, NC 27858 and Depts. of [1]Neurology and [2]Pathology, Duke U. Med. Ctr. Durham, NC 27710.

An increase in dynorphin immunoreactivity (DYN IR) in the supragranular zone of the fascia dentata of temporal lobe epileptics (Houser and Miyashiro, Soc. NS 14:1033, 1988) and loss of somatostatin (SS) hilar neurons in rats after seizures (Sloviter. Science 235:73, 1987) have been reported. We report here immunostaining for DYN, enkephalin (ENK) and somatostatin (SS) in surgically resected human hippocampi with mesial temporal sclerosis. Also, the proDYN and proENK mRNA content of dentate granule and CA4 cells was investigated with in situ hybridization histochemistry (ISHH). Nine of 12 hippocampi immediately frozen after surgical removal for intractable epilepsy exhibited mesial temporal sclerosis. Four normal hippocampi were frozen 3-14 h. after non-neurological deaths. Adjacent frozen sections were cut for Nissl, immunohistochemistry (IHC) and ISHH. Nissl and IHC sections were collected directly into a cold buffered 4% paraformaldehyde solution for 1.5 hr. After rinsing and incubation in primary antisera, the sections were reacted with avidin-biotin Vectastain reagents. Nissl staining revealed a typical gradient of CA3-4 and CA1 neuronal loss and gliosis in sclerotic tissue; the most severely affected had a patchy loss of dentate granule cells. DYN and ENK IR was observed in CA4,CA2-3, and, in epileptic tissue, in the supragranular zone over remaining dentate granule cells. ENK-IR and SS-IR neurons were present in CA4; in epileptic tissue, fewer total cells but more large, pyknotic neurons were seen than in normal tissue. ISHH sections on slides were postfixed and incubated with S^{35}-labeled oligonucleotides followed by stringent washes. A strong DYN mRNA signal in the granule cell layer was present on film autoradiographs. Differences in opioid mRNA among epileptic and normal tissues will be determined by analysis of emulsion coated sections. Supported in part by DA 03982 (JFM).

98.10

SPECTRAL ANALYSIS OF EEG DURING STATUS EPILEPTICUS. N.Y. WALTON and D.M. Treiman. Neurology and Research Services, Wadsworth VAMC, and Department of Neurology, UCLA Sch. of Med., Los Angeles, CA 90024.

EEG changes which occur during the course of status epilepticus, previously described by us (Treiman et al, Epilepsy Res, in press), were studied using computer-generated frequency spectra. Status was induced in rats by injection of lithium chloride followed 20-24 hours later by injection of pilocarpine. EEG recorded from chronic epidural electrodes was digitized and frequency spectra were computed from EEG samples recorded periodically from the end of the last discrete seizure until periodic epileptiform discharges were the principal pattern.

Distribution of EEG power shifted from 2-6 Hz when the EEG pattern was waxing and waning epileptiform discharges to frequencies faster than 14 Hz during the first 30 minutes of continuous spiking. Power then gradually shifted back to 2-14 Hz over the next 90 minutes, as the EEG pattern evolved into periodic epileptiform discharges. Analysis of variance revealed these changes to be statistically significant, $p<.0001$.

The shift in power to frequencies greater than 14 Hz which takes place during the first 30 minutes of continuous spiking occurs at the time status becomes intractable to treatment. This is also the time when brain concentrations of aspartate and glutamate are significantly depressed. Successful treatment of status epilepticus at this time may require blockade of release of these amino acids.

98.11

COLPOCEPHALY: MAGNETIC RESONANCE IMAGING FINDINGS, NEUROPSYCHOLOGIC DATA AND ELECTROENCEPHALOGRAPHIC ABNORMALITIES. I.S.Brown, Ph.D., A.R.Riela, M.D.*, and A.D.Elster, M.D.*, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC.

Colpocephaly is a condition defined as a disproportionate enlargement of the occipital horns of the lateral ventricles. This condition is associated with abnormal motor and mental development, visual abnormalities and seizures. Pathogenetic causes are varied. We report 2 patients with this condition radiographically and clinically. Magnetic Resonance Imaging findings not only demonstrate enlargement of the occipital horns but also demonstrate abnormal signal in the occipital white matter and surprisingly, from the frontal periventricular white matter. Neuropsychological data in these two patients demonstrate diffuse mental retardation that cannot be explained solely on the visual abnormalities found in both patients. Both patients had partial seizures. Electroencephalographically, diffuse slowing and multi-focal epileptiform discharges were noted in both patients. These findings further suggest that colpocephaly is a generalized developmental abnormality most likely occurring in the first 8 weeks of gestation.

99.1

PURSUIT-RELATED, BEHAVIORALLY ENHANCED VISUAL RESPONSES IN MONKEY DORSOLATERAL PONTINE NUCLEUS (DLPN). D.A. Suzuki and R.D. Yee, Dept of Ophthalmology, Indiana Univ. Sch. of Medicine, Indianapolis, IN 46202.

The visual motion responses that have been observed in the DLPN have implicated a role in pursuit eye movement (PEM) control that was limited to PEM initiation. This limitation resulted from the use of visual stimuli moving at speeds that were higher than the retinal error speeds associated with the maintenance of PEMs. The present study compared the responsiveness of DLPN cells during visual motion stimulation with and without PEMs.

Extracellular activity was recorded in alert monkeys that were trained to fixate a 0.5 deg red spot that moved slowly on a tangent screen or was stationary during the presentation of moving visual stimuli.

The activity of DLPN units was studied during PEM when eye velocity lagged target velocity. Attention was focused on DLPN cells that exhibited a clear response to moving visual stimuli. The sensitivity to visual motion increased 50% or more when visual motion was behaviorally significant. The enhanced visual response was observed both at the initiation and during the maintenance of PEMs.

The results implicate a role for the DLPN in processing the visual motion information that is required in regulating both the initiation and the maintenance of PEMs.

99.2

Direction Selectivity of Pontine Burst Neurones L. Ling*, A.F. Fuchs, and C.R.S. Kaneko. Dept. Physiol.& Biophys. and Reg. Primate Research Center, Univ. Wash., Seattle, WA 98195

Amongst eye movement-related neurones in the pons are the short-lead burst neurones, which begin their discharge about 10 msec before the saccade. We have started to re-investigate the details of their response to determine whether their discharge reflects intermediate stages during the transformation from a spatial to a temporal coding of the saccade metrics. Alert Rhesus monkeys were trained to follow a specified pattern of target steps for food reward.

Many short-lead burst neurones exhibited the classical sinusoidal direction tuning curve in which the number of spikes encode linearly the magnitude of the **component** in an optimal direction, although the optimal direction was often oblique and not horizontal. In contrast, we found a class of units that displays no direction preference. Amongst these, one type discharges a few spikes irrespective of the direction and the size of the saccade. A second type increases its number of spikes with the amplitude of the movement regardless of direction. In addition, we encountered occasional previously undescribed short-lead burst neurones with distinct movement fields: the maximal number of spikes occurred for saccades of a specific size and direction, and declined for **both** smaller and larger movements. This rich variety of short-lead burst neurone types suggests that not all of them can act as immediately premotor elements.

This study was supported by National Institues of Health grants EY00745, EY06558, and RR00166

99.3

ANATOMY AND PHYSIOLOGY OF LONG-LEAD BURST NEURONS (LLBNs) C.A.Scudder, S.Highstein, T.Karabelas, A.Moschovakis. Dept. Otol., Washington. Univ. Sch. Med. St. Louis, MO. 63110

In the pons of the alert squirrel monkey, neurons having "long-lead" presaccadic bursts were recorded and stained intraaxonally using HRP filled pipettes. Frequently encountered LLBNs fell in three classes. (1) Cells projecting to the cerebellum. Somata were in the NRTP and the rostral PPRF within and below the MLF. Axons coursed down the midline, laterally above the pontine nuclei, and dorsally in the brachium pontis where staining faded. Cells had ordinary vector and directional long-lead discharges. (2) Presumed efferents of the superior colliculus (SC), identified using electrical stimulation of the SC (somata were not recovered). Decending axons decusated in the predorsal bundle under N.III and branched sparsely until NRPo. Terminal arborizations were found throughout NRPo (esp. medially and dorsally), somewhat in NRPc (but sparse in the EBN area), densely in the raphe nuclei near the abducens (including the OPN area), and densely in the medullary RF (including IBN area). Axons faded near the obex while in the tectospinal tract. All were vector LLBNs, but often had non-saccadic discharges as well. (3) Reticulospinal neurons. Somata were at the level of rostral abducens nucleus. Axons decended in the reticulospinal tract, giving off many fine branches which arborized in the prepositus nucleus dorsally and in many parts of the medulary RF ventrally and laterally. Staining faded before C1. Presaccadic discharges were poorly related to saccade metrics and cells fired spontaneously in a way unrelated to eye movements.

99.4

DYNAMIC RESPONSES OF SACCADE RELATED BURST CELLS IN SUPERIOR COLLICULUS CONTRASTED DURING MEMORY AND DOUBLE JUMP PARADIGMS. D. M. Waitzman, T. P. Ma, L. M. Optican, and R. H. Wurtz. Lab. of Sensorimotor Research, National Eye Institute, Bethesda, MD 20892, USA.

The dynamic responses of superior colliculus neurons were recorded while monkeys (macaca mulatta) performed saccades of the same metrics under visually guided, memory contingent and double jump conditions. In the double jump condition the second saccade matched the preferred movement of the cell, but had no target in its visual receptive field.

Of the cells studied, all but one had visual receptive fields. About 23% of cells responded during the visually guided saccade, but did not fire under either memory or double jump conditions. Another 15% discharged under all three conditions. Interestingly, about twice this number of cells (32%) discharged during the double jump condition and not for memory contingent saccades. In each of these categories the dynamics of the discharge during the saccade were examined. There were at least two types of responses, either a burst that declined rapidly to end with the saccade (e_m), or a more gradual increase in activity which remained above background throughout the saccade and declined from 10 to 100 msec after the saccade(ΔE). In each of the behavioral categories listed, cells with both types of dynamics were identified.

These results show that there is a wide variety of cells in the superior colliculus that can be categorized by the behavioral paradigm and their dynamic characteristics. Different cells participate in identical movements under different behavioral conditions regardless of their dynamic properties (ΔE or e_m). This may indicate that the superior colliculus is a site of convergence for saccadic commands from different sources.

99.5

DIENCEPHALIC AND MESENCEPHALIC NEURONS PROJECTING TO THE ROSTRAL PONTINE NUCLEI IN THE RAT: FLUORESCENCE AND WGA-HRP METHODS. J. Yamada, H. Sato*, T. Kitamura* and K. Yamashita*, Dept. of Anatomy, Nippon Med. Sch., 1-1-5 Sendagi, Bunkyo-ku, Tokyo 113, Japan.

When WGA-HRP was injected into lobule VII of the cerebellum whose microstimulation evoked saccades, a large number of labeled neurons were seen in the medial (MPN) and the lateral (LPN) parts of the rostral pontine nuclei of the monkey. In the present study, first, WGA-HRP was injected into the MPN or the LPN of the rat to determine which neurons send their axons to the MPN and/or LPN in the diencephalon and midbrain. For the data analysis, we used cases in which anterogradely labeled terminals were seen in lobule VII of the vermis. In both injection cases, retrogradely labeled neurons were seen ipsilaterally in the same areas: lateral hypothalamic area, ventral nucleus of the lateral geniculate body, zona incerta, anterior and posterior pretectal areas, prerubral field, perirubral area, medial nucleus of the accessory optic tract and periaqueductal gray. Second, when fluorescent tracers (Diamidino Yellow and Fast Blue) were injected into the MPN and LPN to examine the collateralization of these projections, no double labeled neurons were seen in these areas. These results suggested that oculomotor function of lobule VII was affected by many areas in the diencephalon and midbrain via the LPN and MPN.

99.6

A SEARCH FOR THE NEUROANATOMICAL BASIS FOR UP- AND DOWN-GAZE PARALYSIS. A TRANSSYNAPTIC TRACING STUDY IN THE MONKEY. A.K.E. Horn* and J.A.Büttner-Ennever*, (SPON: U. Büttner). Inst. Neuropathology, LMU Munich, W.Germany

The rostral interstitial nucleus of the medial longitudinal fasciculus (riMLF) contains premotor neurons important for the generation of vertical saccadic eye movements. Burst neurons in the riMLF are activated either during upward or downward saccades. (Büttner-Ennever et al., Brain 105:125, 1982). Although the premotor areas for vertical saccades are known, the anatomical basis for isolated up-, or down-, or combined up- and down-gaze paralysis in humans is not understood.

In this study we investigated, whether in riMLF there is a topographic organization of premotor neurons innervating upward (superior rectus, inferior oblique) or downward (inferior rectus, superior oblique) motoneurons. The injection of a retrograde transsynaptic tracer, non-toxic Tetanus Toxin - Fragment C, into individual vertical eye muscles enabled us to selectively label their premotor neurons in the riMLF of monkeys. We found no striking separation of "upward" and "downward" premotor neuron pools in the riMLF. This result suggests that isolated up- or down-gaze paralysis is not due to differential destruction of an "upward" or "downward" set of premotor neurons, but probably depends on selective damage to efferent pathways.

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99.7

GAZE-RELATED ACTIVITY OF BRAINSTEM OMNIPAUSE NEURONS RECORDED IN THE ALERT HEAD-FREE CAT. M. Paré* and D. Guitton. Montréal Neurol. Inst. and McGill Univ., Montréal H3A 2B4, Canada.

The firing pattern of brainstem omnipause neurons (OPNs) is thought to act as a neural gate controlling the saccadic eye movement generator. In head-restrained animals, these inhibitory preoculomotor neurons fire tonically for stationary eye positions and cease firing just before and during all saccades. Munoz and Guitton (Soc Neurosci Abstr 1988) proposed that, when the head is unrestrained, the superior colliculus and some of its brainstem target elements control the displacement of gaze (= eye + head) rather than ocular motility alone; and that OPNs should be silent when dynamic gaze position error is not null. Here we report the discharge characteristics of OPNs during combined eye-head gaze shifts in the cat. OPNs ceased firing not for the duration of either the saccadic eye movement or head movement components but for the duration of gaze shifts. This relation was particularly evident in large (>50°) gaze shifts where the difference between ocular and gaze saccade durations is striking. The duration of the pause in activity of OPNs was strongly correlated with the duration of saccadic displacements of gaze, irrespective of whether these were accomplished by eye movements alone (head-fixed) or by combined eye-head movements (head-free). We propose that OPNs are part of a local gaze feedback loop that controls gaze shifts.

99.8

DISCHARGES OF FASTIGIAL NEURONS DURING SACCADIC EYE MOVEMENTS IN THE MONKEY. K. Ohtsuka and H. Noda. Sch of Optometry, Indiana Univ. Bloomington, IN 47405.

Discharges of fastigial neurons that receive Purkinje-cell projections from the oculomotor vermis (lobules VIc, VII) were recorded during spontaneous and targeting saccades, and during head rotation in trained monkeys. The units were identified by an inhibition of firing during repetitive microstimulation of vermal lobule VII.

All units were spontaneously active (10 - 110 imp/s) and in some units the tonic levels of firing were highly correlated with steady eye positions (r = 0.90 - 0.94). Preferred direction of eye-position was variable from contralateral horizontal to vertical, but none was ipsilateral. The majority of units also showed bursts during a saccade or a quick phase of nystagmus. Preferred direction for burst was also contralateral. Lead times of bursts during saccades in preferred directions ranged from 0 - 60 msec (group mean = 30 msec). Weak and late bursts or pauses were found during ipsilateral saccades in some units. Burst- and saccade-durations were highly correlated for saccades in the preferred direction (r = 0.91 - 0.97). Thus, a higher activity of neurons in the fastigial oculomotor region was associated with contralateral saccades and fixation. (Supported by NIH grant EY04063)

99.9

PATHWAYS OF AXONS OF OCULOMOTOR NEURONS IN THE FASTIGIAL NUCLEUS OF MACAQUE MONKEY. H. Noda and Y. Ikeda*. Sch of Optometry, Indiana Univ., Bloomington, IN 47405.

Anatomical studies show that cells of the fastigial nucleus (FN) give rise to two distinct efferent bundles: an uncrossed bundle that emerges from the cerebellum via the juxtarestiform body (JB); and a crossed bundle that forms the uncinate fasciculus (UF). A question is asked whether impulses for evoking saccades during microstimulation of the oculomotor vermis are conveyed via JB or UF, or both.

Stereotaxic locations of JB and UF in macaques were identified by anterograde transport of WGA/HRP following injections into the oculomotor region of FN. Mappings of thresholds and saccadic directions were conducted by stimulating an area rostral to FN. Low-threshold areas were correlated with the locations anatomically identified in other monkeys. Ipsilateral saccades were evoked consistently with currents less than 5 μA from the area corresponding to UF. Contralateral saccades were evoked only from FN and no saccade was evoked from the JB area even with currents more than 10 μA. We have already shown that stimulation of oculomotor fastigial neurons (receive Purkinje-cell axons from the oculomotor vermis) elicits saccades with a contralateral horizontal component. Since contralateral saccades were not evoked from the JB area, fastigial oculomotor neurons emerge from the cerebellum via UF. (Supported by NIH grant EY04063)

99.10

CONNECTIONS BETWEEN A VENTRAL, GABAERGIC SUBDIVISION OF ZONA INCERTA AND THE SUPERIOR COLLICULUS. U. Kim*, E. Gregory and W. C. Hall Department of Neurobiology, Duke University, Durham, North Carolina 27710

A ventral subdivision of zona incerta may contribute to gaze mechanisms by virtue of its connections with the intermediate grey layer of the superior colliculus. This ventral subdivision can be distinguished from the rest of zona incerta in both the cat and rat by its dense cytochrome oxidase staining, and by its dense population of medium-sized neurons that are immunoreactive to antibodies against glutamic acid decarboxylase (GAD) and γ-aminobutyric acid (GABA). The connections between this subdivision and the superior colliculus in the rat have been studied using anterograde and retrograde axonal transport techniques. Both horseradish peroxidase and *Phaseolus vulgaris* leucoagglutinin were used as tracers. The results indicate that this subdivision gives rise to a topographically organized projection to the superior colliculus which terminates in dense patches in the intermediate grey layer. Experiments in which the retrograde transport of horseradish peroxidase from the superior colliculus was combined with GAD immunocytochemistry in the same animal indicate that the majority of the zona incerta cells that give rise to this projection are GABAergic. The results also indicate that cells in the intermediate grey layer project to the same ventral subdivision of zona incerta. Thus, the ventral subdivision of zona incerta and the superior colliculus are reciprocally connected by a circuit that provides a major source of GABAergic input to the intermediate grey layer. (Supported by BNS-86-07060, EY0823301)

99.11

LAMINAR ORIGINS OF THE DESCENDING TECTAL PATHWAYS IN MACAQUE MONKEY. Paul J. May and John D. Porter. Depts. of Anatomy and Ophthalmology, Univ. of Mississippi Med. Ctr., Jackson, MS 39216.

The superior colliculus is one of the major brainstem centers active in initiation of saccadic eye movements. It receives inputs from a wide variety of structures, but projects upon gaze-related regions in the midbrain, pons and medulla via only two descending output pathways, the crossed and uncrossed tectobulbar pathways. The distributions of the cells of origin for these two pathways have not been determined in the macaque monkey, despite the fact this is the most widely used species in studies of tectal eye-movement control. The present study retrogradely labelled the collicular cells of origin for these two descending pathways in 5 Macaca fascicularis. Following a large unilateral injection that included the entire pons and extended into the medulla and midbrain tegmentum, the approximately 3,500 contralateral labelled neurons were restricted to the intermediate gray layer. This represents less than 15% of the overall population in this layer, indicating that local circuit neurons may outnumber projection neurons. Only a small number of cells were observed ipsilaterally in the deep and intermediate layers, indicating that few ipsilateral tectobular axons descend caudal to the midbrain. Smaller brainstem injections distinguished the presence of subpopulations within the cells of origin for the predorsal bundle. Specifically, injections centered medially in the pontine reticular formation mainly labelled neurons in the superficial sublamina of the contralateral intermediate gray layer. In contrast, laterally placed injections mainly labelled neurons in the deeper sublamina of this layer. This pattern of lamination is significantly different from that of the cat, where predorsal bundle cells are found throughout the intermediate and deep gray layers, and of rodents, where they are restricted to the deeper sublamina of the intermediate gray. In light of the laminar arrangement of afferent terminations within the intermediate gray layer (e.g., cerebellotectal projections terminate in the deeper sublamina), these results indicate that the crossed tectobulbar pathway may contain at least two axonal populations with differing signals and targets. Supported by NEI grants EY07166 (PJM) and EY05464 (JDP).

99.12

A CHOLINERGIC INPUT TO THE CAT ABDUCENS NUCLEUS. R.F. Spencer and R. Baker. Dept. Anat., Med. Coll. of Virginia 23298, and Dept. Physiol. & Biophys., New York Univ. Med. Ctr., New York, NY 10016.

A potential cholinergic input to the cat abducens nucleus previously was identified on the basis of the ultrastructural localization of acetylcholinesterase. In the present study, the light and electron microscopic immunohistochemical localization of choline acetyltransferase (ChAT) has revealed immunoreactive axons that establish *en passant* and terminal synaptic connections predominantly with the dendrites of ChAT-immunoreactive abducens motoneurones and non-immunoreactive abducens internuclear neurones. ChAT-immunoreactive synaptic endings contained spheroidal synaptic vesicles and synaptic contact zones were characterized by an asymmetrical pre-/postsynaptic membrane profile. Given the absence of axon collaterals of abducens motoneurones, a likely candidate for the source of these cholinergic synaptic endings is a population of ChAT-immunoreactive neurones in the caudal half of the nucleus prepositus hypoglossi. Injections of HRP into the abducens nucleus have retrogradely labelled both ChAT-immunoreactive neurones and non-immunoreactive neurones in the prepositus. The results suggest that the glycinergic inhibitory input to the abducens nucleus from the nucleus prepositus hypoglossi is balanced, at least in part, by a cholinergic excitatory input. Findings from other experiments in which the oculomotor nucleus was injected with HRP furthermore indicate a unique role of the cholinergic neurones in the nucleus prepositus hypoglossi in horizontal eye movement.

Supported by U.S. Public Health Service MERIT Award EY02191 and Research Grant EY02007.

99.13

VESTIBULAR AND CEREBELLAR INPUTS TO ABDUCENS NEURONS IN THE RHESUS MONKEY. D. M. Broussard and S. G. Lisberger. Dept. of Physiology, UCSF, San Francisco, CA 94143.

We have investigated the brainstem circuitry underlying the vestibulo-ocular reflex (VOR) by recording the responses of abducens neurons (AbNs) to current pulses applied to the vestibular labyrinth and the cerebellar flocculus in alert, behaving monkeys. This method characterizes the strengths of synaptic inputs to neurons whose firing patterns can be studied during eye movements. Contralateral or ipsilateral AbNs responded to single shocks to the labyrinth with a transient increase or decrease in firing rate at a latency of 1.68 ± 0.27 or 1.68 ± 0.63 msec, respectively. These latencies are consistent with a disynaptic input. An index of the strength of the neuron's response was calculated by dividing the mean number of spikes in the response by the associated change in eye position. The mean response was 5.98 ± 3.4 spikes/deg for contralateral and -2.25 ± -1.07 spikes/deg for ipsilateral AbNs; the results were similar, with slightly smaller responses, in a second animal. This suggests that the excitatory disynaptic input to an abducens neuron from the contralateral labyrinth is 2 to 3 times as effective as the inhibitory input from the ipsilateral labyrinth. The response to contralateral stimulation had a shorter duration than the ipsilateral response, and often displayed a second, late component. The late component may represent input from multisynaptic VOR pathways. Stimulation of the flocculus caused a transient increase in firing in ipsilateral AbNs. Unexpectedly, a transient decrease in firing was observed in some contralateral AbNs, indicating that the flocculus exerts a modulatory influence on both crossed and uncrossed VOR pathways. (This investigation was supported by NIH grant EY03878.)

99.14

PROCESSING OCULOMOTOR INFORMATION WITHIN THE MONKEY CEREBELLAR FLOCCULUS. L. S. Stone †§ and S.G. Lisberger §, †Vision Group, NASA, Ames Research Center, MS 239-3, Moffett Field, CA 94035, and §Neuroscience Graduate Program, Department of Physiology, Box 0444, University of California, San Francisco, CA 94143.

The simple-spike firing rate of the majority of Purkinje cells (P-cells) in the monkey flocculus encodes gaze velocity (eye velocity in the world) and is only weakly sensitive to eye position in the orbit. However, the firing rate of the oculomotor mossy fiber afferents (OMFs) that are thought to drive these P-cells is very sensitive to eye position. The response of OMFs during the initiation of smooth pursuit eye movements sheds new light on how OMF inputs may be transformed into P-cell responses.

We recorded from 45 OMFs in the flocculi of two monkeys during pursuit of sinusoidal target motion and all 4 cardinal preferred directions were well represented (13 ipsi, 16 contra, 10 down, and 6 up). We examined the response of 26 of these during the initiation of pursuit to 30°/s motion of a small spot in the preferred direction. Ten (38%) had a brisk saturating response that was a near perfect analog of eye velocity. Sixteen (62%) had a sluggish non-saturating response that increased nearly linearly with eye position. In addition, the two groups differed in mean latency (4 ms after the onset on eye movement vs 29 ms), mean static eye-position sensitivity (2.21 vs 3.44 sp/s/°), mean eye-velocity sensitivity (2.28 vs 0.98 sp/s°/s), regularity of firing rate (mean coefficient of variation: 0.27 vs 0.11), and mean phase of the response during sinusoidal pursuit (16 vs 55° lag). A larger sample will be required to determine whether these two types represent two distinct populations or the extremes of a continuum. Nevertheless, within the cerebellar cortex, a weighted sum of inputs from two OMFs of opposite type and opposite directional preference, could produce a response during pursuit with a sensitivity to eye-velocity (~ 1 sp/s/°/s) and eye-position (~ 0.4 sp/s/°) that is appropriate for gaze-velocity P-cells.

99.15

SINGLE UNIT RECORDINGS FROM RAT CEREBELLUM DURING ADAPTIVE GAIN MODIFICATION OF THE BLINK REFLEX. **J. J. Pellegrini* and C. Evinger. (SPON: A.W. Seymour) Dept. Neurobiology & Behavior, SUNY, Stony Brook, NY 11794.**

Since cerebellar lesions prevent or severely impair motor learning, the cerebellum must be essential for some aspect of such learning. Understanding how the cerebellum can perform this function, however, requires an analysis of the activity of individual neurons during learning. We investigated the modification of cerebellar neuron response properties during adaptive gain changes of the blink reflex, a form of motor learning.

Airpuffs or electrical stimulation of the cornea of decerebrate or urethane anesthetized rats evoked a burst of orbicularis oculi activity (OOemg) and a concomitant blink. After upper eyelid restraint, these same stimuli elicited a larger burst of OOemg activity with time. Before restraint, blink stimulation elicited three patterns of responses from neurons found in Crus I & II of cerebellar cortex and the deep cerebellar nuclei (DCN): 1) a complex spike (latency 20-25 ms) followed by a transient increase in simple spike activity in Purkinje cells; 2) a long lasting (up to 1 s) suppression of tonic activity in cerebellar interneurons; and 3) a burst of spikes (latency 15-40 ms) followed by a brief pause in activity in DCN cells. Throughout adaptation and recovery, Purkinje cells' simple spike discharge correlated with the apparent motor error. The response of other cerebellar neurons, however, corresponded to the adapted blink magnitude. DCN units of this type also altered their discharge in a non-learning paradigm in which paired conditioning-test stimuli produced a reduction of long latency OOemg activity to the test pulse. These long latency components are the same ones that undergo modification during adaptation. These data suggest distinct functions for the different cell types of the cerebellum in modulating blink reflex gain. **Supported by grant EY07391.**

99.16

ON THE ROLE OF THE NUCLEUS OF THE OPTIC TRACT (NOT) DURING OPTOKINETIC NYSTAGMUS IN SQUIRREL MONKEYS (SAIMIRI SCIUREUS). J. Kröller*, F. Behrens*, O.-J. Grüsser and B. Schielke*. (SPON: ENA). Dept. of Physiol., Freie Universität Berlin, Arnimallee 22, 1000 Berlin 33, Germany

The aim of the present study was to elucidate the impact of directly activating retinal ganglion cells on neurons of the NOT compared to the situation with an additional loop through the visual cortex. Recordings from single NOT neurons were obtained in 3 awake monkeys. In order to evoke an optokinetic nystagmus (OKN) the animals were subjected to a vertical pattern of black and white stripes, moving horizontally at different velocities (1-400 deg·s^{-1}). Eye movements were monitored by means of search coils.

When the pattern movement was directed towards the recording side, all units but one were activated during the slow phase of the OKN, while the firing ceased abruptly during the backward saccades. Movements in the non-favoured direction maintained the discharge rate at the spontaneous level or, in most cases, lowered it. The receptive fields of NOT units always extended over more than 45x45 deg. and included the fovea. In darkness, the spontaneous activity was either between 50 and 70 or in the order of 20 imp·s^{-1}, and no eye-movement related modulation in discharging occurred during after-nystagmus or spontaneous eye movements. In order to determine the precise receptive field properties of NOT neurons, an open-loop OKN was evoked in one animal when one eye was temporarily immobilized by intraorbital injection of Botulinum toxin (BoTX). Supported by the Deutsche Forschungsgemeinschaft (Gr 161).

99.17

STIMULUS PATTERN DEPENDENCE OF HUMAN OPTOKINETIC AFTERNYSTAGMUS. S. H. Lafortune, D. Ireland*, G. Wei* and R.M. Jell. Depts of Physiol. and Otolaryngol., University of Manitoba, WINNIPEG, MB R3E0W3, CANADA.

Normal subjects were exposed to different optokinetic (OK) stimulus patterns in the following order: A) Vertical stripes, 2° black and 18° white using a cylindrical curtain 85cm diameter; B) Random dots on the inside of a white, plastic sphere, 85cm in diameter, which could be lowered over the subject's upper body; i) Large (1.9cm) and small (1.3cm) black dots (2:1 large/small), mean spacing 13.2° (±4°) ii) as in i) but small dots removed, mean spacing 15.5° (±4.6°); iii) as in i) but with small dots replaced by large dots; iv) as in iii) but with an irregular row of large yellow numbered dots placed at eye height, in which subjects were asked to call out the number on the dots they followed so as to increase mental alertness; C) During constant velocity (60°/s) rotation in a rotating chair, with a striped (2° black, 18° white) stationary surround 180cm diameter. In all conditions except C, stimulus velocity was 40 deg/sec. Subjects were asked to do mental arithmetic during OKAN. Statistical analysis of the results revealed that the parameters A, 1/B, C of the double exponential model (Jell *et al. Acta Otolaryngol* 1984,98:462-471) and the area under the decay curve were independent of stimulus conditions. Long time constant 1/D was found to be significantly higher ($p < 0.05$) in A) than in all other conditions. Whereas the first exposure consistently yielded a double exponential decay with normal parameter values in all subjects, subsequent stimulus conditions often yielded single exponential decays in which the long time constant component was absent [2 Ss in B(i) and 4 Ss in B(ii - iv)], or two-component decays with significantly lower long time constant (1/D) values. In two subjects, testing in C) with chair rotation brought back the long time constant component, but a control repeat of condition A did not. The full expression of OKAN decay is clearly pattern dependent, and most reliable after first exposure to the stimulus. (Supported by MRC and Winnipeg Health Sciences Centre Research Foundation.)

99.18

A UNIQUE SUBPOPULATION OF MEDIAL RECTUS MOTONEURONS AND ITS RELATIONSHIP WITH THE NUCLEUS OF EDINGER WESTPHAL. **J.T. Erichsen and C. Evinger. Dept. of Neurobiology & Behavior, SUNY, Stony Brook, NY 11794.**

A small subpopulation of medial rectus [MR] motoneurons in the pigeon (Columba livia) comprises an immunologically distinct and perhaps functionally unique class of motoneurons. Forming a dorsal crescent in the MR nucleus just medial to the nucleus of Edinger Westphal [nEW], these motoneurons [nMRd] share properties normally associated with neurons in nEW that innervate the ciliary ganglion.

Injections of HRP into the MR muscle label all ipsilateral MR motoneurons, including nMRd. The cells in nMRd, however, are generally smaller than the other MR motoneurons. Injections of a transneuronal tracer, fragment C of tetanus toxin [TTC], into the MR muscle result in direct retrograde labeling of the entire ipsilateral MR nucleus. In addition, the contralateral nMRd contains labeled terminals. This latter result suggests the possibility of an afferent shared by both nMRd's.

In common with cells in nEW, only developing nMRd neurons are transiently immunoreactive for enkephalin and substance P [SP] (Erichsen et al., 1982). In the adult, SP-positive neuropil is localized to nMRd as well as to nEW, but not to any of the other oculomotor nuclei. This result suggests that nMRd shares an input with the ipsilateral nEW. In addition, neuropil immunoreactive for vasoactive intestinal polypeptide [VIP] is found only in nMRd.

Our HRP and TTC results suggest that nMRd in the pigeon is similar to the C subdivision of the MR nucleus in the monkey, and thus may play a role in vergence. The pattern of immunoreactive staining for three peptides further indicates that nMRd, acting in concert with nEW neurons, might be involved in the near response. **Supported by grants EY04587 (JTE) and EY07391 (CE).**

99.19

BEHAVIOR OF IDENTIFIED EDINGER-WESTPHAL NEURONS DURING OCULAR ACCOMMODATION

P.D.R. Gamlin, Y. Zhang*, and L.E. Mays, Department of Physiological Optics, School of Optometry, and the Neurobiology Research Center, University of Alabama at Birmingham, Birmingham, AL. 35294.

The nucleus of Edinger-Westphal (EW) lies dorsal to the oculomotor nucleus and contains the parasympathetic preganglionic neurons that, by way of the oculomotor nerve and ciliary ganglion, innervate the ciliary muscle. Since sympathetic innervation has little influence on accommodation, EW neurons are responsible for controlling ocular accommodation. The goal of the present study was to investigate the behavior of identified EW neurons during accommodation in the primate.

Using a continuous recording infrared optometer, accommodation-related neurons were recorded in one trained rhesus monkey. Nine of these cells could be antidromically activated (current < 400 μA) by a stimulating electrode placed in the intracranial portion of the ipsilateral oculomotor nerve. In all cases antidromic activation was confirmed by collision. All EW neurons were located above the oculomotor nucleus within 1 mm of its dorsal extent. The activity of EW neurons is characterized by a low spontaneous activity (Mean = 11.7 spikes/sec; Range = 5-20 spikes/sec) and a mean relationship to accommodation of 9.4 spikes/sec per diopter (Range = 5-14 spikes/sec per diopter).

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BIOLOGICAL RHYTHMS AND SLEEP: SLEEP

100.1

CIRCADIAN ANALYSIS BY IMAGE PROCESSING: QUANTIFYING WHAT YOU SEE. W.N. Tapp and J. Heller*. Primate Neurobehav. Unit, VA Med. Ctr. and Dept. Neurosciences, New Jersey Med. Sch., East Orange, NJ 07018.

Circadian data is often presented in plots where the basic layout depicts one day's data as a single line with each successive day's data plotted on the line below. The level of the variable being studied has been encoded as pen strokes from chart recorders, darkness on gray scales and many other ingenious methods. While these plots can present circadian data in a format where the important details are easy to extract visually, it is often difficult to extract quantitative information that corresponds to the details seen in the plot. This is troublesome since the plots are the primary data in many cases and since it is often difficult to match the results of conventional time series analyses to the major landmarks on the plots. As an alternative, we have adapted image processing tools as a means of extracting quantitative information about visually important features, such as activity onsets and offsets. Further, we have developed error functions and their associated statistics to permit significance testing on data drawn from these analyses. After systematic and objective identification of image features (e.g. activity onset), period can be estimated by regression or other methods and phase can be determined directly. Supp by VA Res.

100.2

DIFFERENTIAL INVOLVEMENT OF SEROTONIN ACROSS THE SLEEP-WAKING CYCLE. RELATIONSHIP WITH HYPNOGENIC FACTORS. N.Chastrette@, R.Cespuglio@ and M.Jouvet@ (SPON:K.Jorgenson). Department of Experimental Medicine, INSERM U52, Cl.Bernard University, 8 Ave. Rockefeller, 69008 Lyon, FRANCE.

In order to clarify the involvement of serotonin (5-HT) in sleep, as well as its relationship to hypnogenic factors, we used differential pulse voltammetry to measure 5-hydroxyindoleacetic acid (5-HIAA) extracellular concentrations in rats chronically implanted for recording of sleep parameters. In the caudate nucleus, which contains 5-HT nerve endings, the voltammetric signal was maximum during waking state (W) and decreased during slow wave sleep (SWS) and paradoxical sleep (PS; -37%); in this nucleus, 5-HT release is thus diachronous to sleep. Conversely, in the cell bodies of raphe dorsalis nucleus, 5-HIAA concentrations were the lowest during W and markedly increased during SWS (+39%) and PS (+71%); this increase may reflect a dendritic release of 5-HT, synchronic to sleep; such a release could induce the auto-inhibition of the serotoninergic system, a necessary concomitant of sleep occurrence. Furthermore, corticotropin-like intermediate lobe peptide (CLIP, 10 ng/2 ul), a PS-inducing peptide, augmented this 5-HT release. Such an action could be one of the mechanisms through which hypnogenic factors exert their action.

100.3

REM SLEEP GENERATION BY CHOLINERGIC MICRO-STIMULATION AT DIFFERENT PONTINE TEGMENTUM SITES. M.L. Rodrigo-Angulo, I. de Andrés and F. Reinoso-Suárez. Depto. Morfología. F. Medicina. U.A.M. Madrid. Spain.

Injections of cholinomimetics in the brain stem have evidenciated that the effective sites for inducing REM sleep are situated in the pontine tegmentum (Badhoyan et al. 1984,87). However, there is a considerable controversy concerning the specific pontine structures mediating this effect. Given the diverse influence of pontine structures on sleep (De Andrés et al.1985) and the large volume of cholinomimetics injected in previous studies (200-500 nl), we have investigated the role of the locus of the injection by making carbachol microinjections (20-40 nl, 0.8-1.6 μg) in dorsal and ventral areas of oral pontine tegmentum (AP 0, -4) in adult cats. In order to label the stimulated area, HRP was injected together with carbachol.

Desynchronized EEG and atonia (median latency: 3 min.) occurred in all experiments. *Dorsal* injections produced wakefulness (open eyes, midriasis, increased respiration rate) during the initial period of atonia. Scarce or no PGO activity was observed and vertical eye movements were often present. After 10-20 min the cats started to move and active wakefulness prevailed during the next 4 hs.*Ventral* injections produced behavioral sleep simultaneously with atonia. PGO and REMs developed, although with some delay. Later, short periods of movements and arousal appeared and from the 2nd hour, some cats showed extended periods of quietness with the head up and still with PGOs.

The present results indicate that a contribution of dorsal pontine tegmentum to REM sleep atonia cannot be excluded but, the effective site to induce REM sleep by cholinomimetics is the pontine ventral tegmental area.

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100.4

CORTISONE AND IMMUNIZATION ALTER THE SOMNOGENIC EFFECTS OF INFECTIOUS CHALLENGE IN RABBITS. L. A. Toth and J.M. Krueger. Depts. of Comparative Medicine and Physiology and Biophysics, Univ. Tennessee, Memphis, TN 38163.

Infectious challenge of rabbits produces time-dependent changes in sleep. To extend those observations, we examined the influence of prior immunization with killed Candida albicans (CA) (8 weekly IV inoculations) or 24-hr pretreatment with cortisone (40 mg/kg, i.m.) on sleep after IV inoculation with viable CA. CA alone enhanced slow-wave sleep (SWS) and delta wave amplitude (DWA) from 4-16 h after challenge, and later reduced both SWS and DWA. Rapid-eye-movement sleep (REMS) was inhibited for 48 h after challenge. Cortisone alone decreased DWA within 12 h and blocked the enhanced SWS and DWA induced by CA challenge. Immunization attenuated the CA-induced SWS increase. CA-induced attenuation of REMS occurred in both cortisone-treated and immunized rabbits. We conclude that somnogenic effects of infectious challenge can be differentially altered by manipulation of the host's immune system. Supported by NIH-NS-25378 and NS 26429.

100.5

VIP INDUCED REM SLEEP IS BLOCKED BY CLONIDINE AND ENHANCED BY ATROPINE IN PCPA PRETREATED CATS. A. Jiménez-Anguiano*, O. Prospéro-García and R. Drucker-Colín. Depto. Neurociencias, Instituto de Fisiología Celular, Universidad Nacional Autónoma de México, Apdo. Postal 70-600, México 04510, D.F. México.

Vasoactive Intestinal Polypeptide (VIP) may be a REM sleep factor. The purpose of this work is to study the interaction of VIP with acetylcholine (Ach) and norepinephrine (NE) to induce REM sleep. Adult cats (2.5-3.5 kg) implanted for standard sleep-wake cycle recordings were used in this study. A stainless steel cannula (22 gauge) was additionally implanted into the 4th ventricle to administer VIP or saline. All cats were treated with 2 i.p. injections of parachlorophenylaline (PCPA) to induce insomnia, then were divided into 6 groups (n=6) and additionally each 2 groups were treated with either: atropine (0.5 mg/kg i.p.); clonidine (0.01 mg/kg p.o.) or maintained only under PCPA effect. One hour after, each group of one drug received 50 ul of saline or 200 ng of VIP into the 4th ventricle. Cats were recorded during 11 hrs. Results showed that VIP induces REM sleep, but such an effect was abolished by clonidine but enhanced by atropine. Interestingly, atropine by itself induces REM sleep. These results suggest that VIP is interacting with Ach and NE to induce REM sleep in the PCPA insomniac cats.

100.6

THE SLEEP/WAKE ACTIVITY OF THALAMIC RETICULAR NEURONS IN THE RAT. G.A. Marks and H.P. Roffwarg. UT - Southwestern, Dallas, TX 75235-9171.

As a prelude to a study of the synaptic interactions between the thalamic reticular nucleus (TRN) and principal cells of the thalamic relay nuclei, spontaneous activity across the sleep/wake cycle of TRN cells was studied in unanesthetized, freely moving rats and compared to the activity of relay cells expressed respectively during the different states of arousal. TRN cell discharge was extracellularly monitored by a remotely driven etched tungsten electrode mounted to the animal's skull. Somatosensory responsive cells were identified by shocks to the medial lemniscus. The EEG, EMG and gross hippocampal activity served to identify state. Over 31 cells were examined and all were found to exhibit similar and pronounced changes in discharge pattern across states. Most of SW sleep is characterized by rhythmic trains of high rate bursting followed by pauses. At the transition from SW sleep to all REM sleep periods and many spontaneous arousals, activity assumes a tonic pattern with a higher mean discharge rate. Within the REM period pattern remains tonic, though more bursty, at a reduced mean rate. Upon arousal, there is either a low rate single spike pattern or complete cessation of firing. During waking, however, with specific somatosensory stimulation the cell can fire at its highest rate. In as much as the activity of TRN cells is not the reciprocal of that exhibited by thalamic relay cells across the sleep/wake cycle, these results do not support the concept of TRN mediation of sleep/wake activity in relay cells by inhibition and disinhibition. It does not, however, rule out partial control of this behavior. In the future it will be necessary to determine what factors modulate the action of TRN Gaba release as well as to determine the influence of other afferents to thalamic relay cells.

100.7

SLEEP AND RESPIRATORY PATTERNS IN LEAN AND OBESE ZUCKER RATS. K.A.Burgess* and S.DeMesquita (SPON:M.A Simmons). Dept. of Physiology Marshall Univ. School of Medicine, Huntington, WV 25755

Obese Zucker rats have been found to have altered levels of dopamine, serotonin and norepinephrine when compared to their lean litter mates (Orosco, M. et al. Physiol Behav 36:853, 1986). This study was undertaken to determine what effect the altered neuroendocrine levels would have on the Zucker sleep and respiration pattern. Nine lean (475±6g, mean±SEM) and six obese (743±20g) Zucker rats, instrumented with EEG and EMG electrodes; fitted with chest pneumographs were allowed to sleep for 4hr on five consecutive days

The obese rats had a significantly shorter latency to both Rapid Eye Movement (REM) and Non-REM sleep. Although there was no difference in total sleep time or REM sleep percent between the lean and obese rats, the latter had significantly ($p<0.05$) more frequent and shorter Wake and Non-REM periods. The obese rats had a significantly ($p<0.05$) faster breathing rate in Non-REM sleep preceeding REM sleep.

	obese	lean	signif.
Non-REM Latency(min)	13.9±1.9	50.9±5.2	p<0.05
REM Latency(min)	24.4±3.6	57.7±9.7	p<0.01

The obese Zucker rat had a more fragmented sleep pattern and an earlier sleep onset than the lean Zucker rat.

100.8

SEROTONINERGIC RECEPTORS BLOCKADE ENHANCES SLOW WAVE SLEEP IN PCPA PRE-TREATED CATS. O. Prospéro-García, and A. Jiménez-Anguiano*. Depto. de Neurociencias, Instituto de Fisiología Celular. Universidad Nacional Autónoma de México. Apdo. Postal 70-600, México, 04510, D.F. México.

The serotoninergic antagonists Ritanserin (5-HT_2 blocker) and Piremperone (5-HT unspecific blocker) induce slow wave sleep (SWS) in animals and humans. The purpose of this study is to test their effect on the sleep-wake cycle of cats with 5-HT depleted by parachlorophenylalanine (PCPA). Two groups A and B (n=15) of cats implanted for sleep-wake cycle recordings were used. Group B received a PCPA pre-treatment (2 i.p. injections of 400 mg/kg separated by 24 h.); whereas group A had no pre-treatment. Each group was subdivided (n=5) in 3 groups A1 and B1 that received piremperone (1 mg/kg p.o.); A2 and B2 that received ritanserin, (10 mg/kg p.o.), and A3 and B3 that received an empty capsule. Results showed that in group A, both drugs increase wakefulness and have a tendency to increase SWS II, whereas REM sleep is strongly reduced. In group B, both drugs reduced the wakefulness induced by PCPA enhancing SWS I and II. Piremperone preferentially increased SWS I whereas ritanserin SWS II. As for REM sleep, only piremperone enhanced it. These results suggest that serotoninergic receptors are differentially involved in the modulation of the different SWS phases.

100.9

EFFECTS ON SLEEP OF ADENOSINE, ADENOSINE ANALOGS, TRIAZOLAM, PENTOBARBITAL AND GABA MICROINJECTED INTO THE PREOPTIC AREA OF RATS. **S.R.Ticho and M. Radulovacki. Dept. of Pharmacology, University of Illinois Coll. of Medicine, Chicago, IL 60612.**

We have examined the effects on sleep of bilateral microinjections of adenosine (ADO), ADO analogs, and drugs effecting the GABA receptor complex in the preoptic area of the rat. The cannulae placements and the effects of the drugs on the sleep-wake cycle were analysed by a two-way ANOVA. EEG recordings were examined during a six hour period of the light cycle. Rats treated with a selective ADO A1 receptor agonist (CPA, 6.25 nmoles) had no changes in the sleep-wake cycle as compared to controls. However, microinjection of ADO (12.5 nmoles) and NECA, 0.5 nmoles, (an ADO A1/A2 receptor agonist) significantly increased deep slow wave sleep (SWS2) by 22% ($p<0.05$) and 28% ($p<0.05$) respectively. Furthermore, NECA caused a significant increase in total sleep (15%, $p<0.05$). In contrast, no changes in sleep parameters were observed with the drugs effecting the GABA receptor complex: Triazolam (0.125 ug), pentobarbital (3 nmoles), and GABA (250 nmoles). Although, we observed a 10%, 26%, and 13%, respectively, increase in light slow wave sleep (SWS1), this increase did not attain statistical significance.

100.10

EVOKED PONTO-GENICULO-OCCIPITAL (PGO) WAVES AND ORIENTING. W.A. Ball*, L.D. Sanford*, A.R. Morrison, R.J. Ross, W.H. Hunt*, G.L.Mann*. Lab. of Anatomy, Sch. of Vet. Med., U. of Pa., Phila., Pa. 19104.

PGO waves in cats' lateral geniculate bodies (LGB) occur spontaneously during paradoxical sleep (PS). In waking (W) tones produced head orienting and evoked PGO-like waves (PGO_E), perhaps modifying visual processing in the LGB. Orienting habituates readily and is inhibited during sleep; habituation of PGO_E is less well studied. We evaluated whether: 1) overt orienting and PGO_E covary; 2) a change of state to slow wave sleep (SWS) or PS inhibits behavior and PGO_E after exposure to tones in W. Nine blocks of 48 tones (90 ms, 90 dB, 1000 Hz, 2s ISI) were given to 6 cats in W on two days a week apart. Then, dishabituation tones were presented in SWS or PS on alternate weeks. PGO_E were recorded in the LGB on 45% of W trials, but overt behavior rapidly declined and occurred on fewer than 23% of trials. In PS PGO_E amplitude and probability increased despite 442 W trials; both declined significantly across the PS episode. SWS showed less recovery. Thus, behavior shows rapid habituation in W and inhibition in PS, while PGO_E are consistent in W and prominent in PS. The latter is consistent with the idea that PGO waves are indicators of the activation of alerting mechanisms. Supported by MH-42903, MH-09584, MH-14654.

100.11

TOWARD A PATHOPHYSIOLOGICAL MODEL OF NARCOLEPSY-CATAPLEXY. J. Quattrochi, J. Macklis, A. Mamelak*, J. Feldman*, and J.A. Hobson. Laboratory of Neurophysiology and Department of Neurology, Harvard Medical School, Boston, MA 02115.

To help understand the mechanism by which neuronal populations are organized so as to induce a cataplectic behavioral state, microinjections of carbachol-fluorescent microspheres (13μg/250nl) were performed at 4 sites in the anterodorsal pons in 2 unanesthetized adult male cats via cannulae inserted through stereotaxically implanted guide tubes. Polygraphic and behavioral data were collected using surgically implanted EMG, EOG, subcortical PGO, and EEG electrodes. Cataplectic behavior was assessed by monitoring the onset and time course of atonia in the EMG and desychronized patterns in the EEG. Results indicate a mean latency of 1.5±0.3 min. to onset of cataplexy with a mean duration of 55±2.7 min. Histological verification of cannula placement identified injection sites to be within the locus coeruleus α - a nuclear region associated with REM sleep atonia. It is now possible to map and define retrogradely fluorescent labeled neurons projecting to target sites, thereby ascertaining the neuronal network involved in the induction of this cataplectic behavioral state. These findings directly inform conceptualization of the human disease and may provide a model for understanding the pathogenesis of narcolepsy-cataplexy behavior.

100.12

THE DIMENSIONALITY OF EEG-RHYTHMS DURING SLEEP J. ROESCHKE*, H. SCHWARZE*, J.B. ALDENHOFF, Dept. of Psychiatry, University of Mainz, F.R.G.

According to Rechtschaffen and Kales five different states of EEG-activity can be defined. Conventional approaches of system theory have been useful for analyzing the brain waves. However, the highly nonlinear character of the brain's dynamic behaviour suggests to use phase space analysis: Every instantaneous functional state of the brain, such as represented by the EEG-signal, corresponds to one point in the phase space. The sequence of such states over the time scale defines a curve called a trajectory. As time increases the trajectory either penetrates the entire phase space or it converges to a lower dimensional subset called an attractor which can be identified in some cases with properties of deterministic chaos. Following a proposal of Grassberger and Procaccia (Physica D 9:183, 1983) we evaluated the (correlation-) dimension D_2 of the attractor. D_2 is a measure for the complexity and an estimation of the information content of the EEG. We investigated ten healthy male probands (20-25 years old) and find different values for D_2 between 4.50 (REM) and 7.10 (stage 1). This means, that the unpredictability of the brain waves might be caused by a finite dimensional dynamical system. The degrees of freedom during sleep vary within a wide range and are highly correlated to clinical observations and physiological approaches of information processing.

100.13

GENDER-DIFFERENCES IN CIRCADIAN TEMPERATURE RHYTHMS OF HEALTHY ELDERLY SUBJECTS. M.V. Vitiello*, P.N. Prinz, L.H. Larsen* and A.L. Marks* (SPON: A.C.N. Chen). Dept. of Psychiatry, Univ. of Washington and American Lake VAMC, Seattle, WA 98195.

Previously we and others have reported age-related diminished amplitudes in circadian core body temperature rhythms primarily as a result of elevated temperature nadir. These reports based on males only have not taken into account possible gender effects on temperature. 14 female (67.5±8.5 yrs, Mean±SD) and 12 male (65.0±5.3 yrs) healthy aged normal subjects were studied. Core body temperature of each subject was monitored continuously for ≈36 hours using a Vitalog/Yellowsprings system during a 72 hour Clinical Research Center stay. Data was edited to remove artifacts and entered into a cosinor analysis. Group analyses of cosinor-derived measures revealed significantly higher 24 hour mean temperature (36.9±.15 vs. 36.8±.08 °C; F=4.3, p<.05) and a non significant trend toward a higher amplitude (.9±.10 vs. .84±.09; F=3.7, p=.06) in females compared to males. No significant difference in group acrophases was observed. Analyses of raw (non-cosinor) temperature showed that females were warmer than males at mid-day but were similar at other times including nadir. We conclude that gender effects are not present near the circadian nadir. It is at the nadir when age effects on temperature are most notable, and are most likely to interact with sleep quality.
Supported by PHS Grants MH33688, RR37 and the VA.

100.14

CIRCADIAN RHYTHMS IN CARDIOVASCULAR FUNCTION DURING TOTAL SLEEP DEPRIVATION J.B. Fertig, D.M. Penetar*, and P.A. Newhouse*. Walter Reed Army Institute of Research, Washington, D.C., 20307

In an effort to adequately characterize circadian changes in cardiovascular activity that occur during Total Sleep Deprivation (TSD) we studied 91 healthy males during 48 hours of TSD. Measurements of heart rate (HR), diastolic (DBP) and systolic (SBP) blood pressure were taken every two hours throughout the 48 hours of TSD. Time series data for each of these measures were subjected to linear regression analysis. Following extraction of linear trends, residuals were submitted to rhythmic analysis using a complex demodulation technique. Although significant linear trends were found for HR (increasing) and DBP (decreasing) the changes were of small magnitude and accounted for a relatively minor portion of the variance. Circadian rhythms, however, accounted for 39% of the variance in HR, 72% in DBP and 35% in SBP. Further complex demodulation of HR and SBP data also revealed higher frequency cycles accounting for significant portions of the variance in those measures. Circadian rhythms for HR and DBP were somewhat altered by 48 hours of TSD and showed increased peak amplitudes and moderately decreased periods during the second 24 hour cycle. These results suggest that sleep deprivation effects on cardiovascular functioning can best be described by rhythmic analysis and that the overall effect of TSD is in the direction of amplifying circadian rhythms and reducing circadian periods.

100.15

CIRCADIAN RHYTHM AND SLEEP DEPRIVATION EFFECTS ON COGNITIVE PERFORMANCE D.M. Penetar*, D.R. Thorne*, J.B. Fertig, and H.C. Sing* (SPON: Joyce C. Cochran). Walter Reed Army Institute of Research, Washington, D.C. 20307

Four cognitive tasks from a computerized performance battery were studied during 48 hours of Total Sleep Deprivation (TSD) and were analyzed for those changes due to TSD and those due to circadian rhythms. Normal male subjects (N=91) participated in studies where measurements of reaction time, sustained attention, and spatial and logical reasoning were made every 2 hours. Measurements of each task included accuracy of performance, speed (responses/time) and throughput (% correct/averaged reaction time). Time series data for each of these measures were subjected to linear regression analysis. Following extraction of linear trends, residuals were submitted to rhythmic analysis using a complex demodulation technique. Accuracy on the spatial and logical reasoning tasks was not affected by TSD. Accuracy on the sustained attention and reaction time tasks, however, was affected by TSD. Circadian rhythms accounted for a large portion of the variance in all tasks. The relative amplitude of the circadian component was smallest for the spatial rotation task with the other tasks having similar amplitudes. For all tasks, significant decreases in speed were found. Throughput measures largely reflected the changes in speed. Results indicate that accuracy is maintained at the expense of speed for complex cognitive tasks and that performance on the logical reasoning, sustained attention, and reaction time tasks are influenced strongly by circadian factors.

100.16

THE EFFECTS OF M1 AND M2 MUSCARINIC RECEPTOR AGONISTS AND ANTAGONISTS ON REM SLEEP GENERATION Javier Velazquez-Moctezuma, J. C. Gillin and P. Shiromani. Dept Psychiatry, San Diego VAMC and UCSD, La Jolla, CA 92161 and UAM-Iztapalapa, Mexico (JVM)

Specific agonists and antagonists for muscarinic receptor subtypes were infused into the medial pontine reticular formation of freely moving cats and sleep was recorded for five hours. M2 muscarinic receptor agonists, *oxotremorine-M* and *cis-methyl-dioxolane*, significantly increased REM sleep, decreased the percentage of slow wave sleep and did not affect wakefulness, when compared to Ringer's control. These effects were similar to that obtained with *carbachol*, a mixed M1 and M2 agonist. The M1 agonist *McN-A-343* did not show any effect on the sleep-wake percentage. The powerful effects of *cis-methyl-dioxolane* were blocked by *atropine*. The M1 antagonist *pirenzepine* did not block the effects of *cis-methyl-dioxolane*. Preliminary results using *gallamine*, a neuromuscular nicotine blocker which is also an M2 receptor antagonist, indicated that it blocked the effects of dioxolane in 3 of 7 trials.

These results strongly indicate that REM sleep generation by cholinergic stimulation in the medial pontine reticular formation is mediated by M2 muscarinic receptors. Now pharmacological treatments can be targeted at the M2 receptor to block the inadvertent intrusions of REM sleep in diseases such as narcolepsy.

100.17

EFFECTS OF THE NEW D-1 DOPAMINE RECEPTOR ANTAGONIST SCH 39166 ON THE SLEEP-WAKING CYCLE IN THE RAT. M. Trampus *, A. Monopoli * and E. Ongini. Research Labs, Essex Italia (Subsidiary of Schering-Plough), I-20060 Comazzo, Milan, Italy.

Sedation is an undesired effect of neuroleptic therapy. This action seems to depend upon concomitant blockade of both D-1 and D-2 receptor subtypes (Neuropharmacology, 27: 799, 1988). Sedation is best evaluated by measuring electroencephalographic (EEG) activity and drug effects on stages of the sleep-waking cycle. To investigate whether selective blockade of D-1 receptors induces similar effects, we studied the new selective D-1 antagonist, SCH 39166 (J. Pharmacol. exp. Ther., 247: 1093, 1988) in rats chronically implanted for the recording of the EEG. For comparison, SCH 23390 and haloperidol were used. Haloperidol (0.1-3 mg/kg po) typically induced a synchronization of the EEG, with a dose-dependent increase of slow-wave sleep. SCH 23390 (0.03-0.3 mg/kg sc) and SCH 39166 (3-30 mg/kg po) did not modify significantly the various features of the sleep-waking cycle. The data suggest that the new D-1 antagonist SCH 39166, which is active orally, may be devoid of sedation liability at clinically useful doses.

100.18

IS PERIODIC BREATHING "AROUSAL-STATE" DEPENDENT? W. Milsom, B. Krilowicz*, D. Grahn*, C. Radeke* and H. Heller*, Dept. of Zoology, Univ. of British Columbia, Vancouver, B.C. and Dept. of Biology, Stanford Univ., Stanford CA.

Recordings of the EEG, EMG, ECG, ventilation and the discharge from respiratory neurons in the ventral medullary respiratory center (nucleus retroambiguous, made from chronically implanted stainless steel microwire (25 micron) electrodes), were obtained from unanesthetized, unrestrained golden-mantled ground squirrels. Recordings were obtained from animals during wakefulness, slow-wave sleep, and rapid eye movement sleep as well as during entrance into, and arousal from hibernation. While many neurons with respiratory related discharge showed little state dependence, recruitment/derecruitment of other respiratory neurons was often seen during changes in arousal state and was commonly associated with changes in the repiratory pattern. These changes included waxing and waning of ventilation during sleep and entrance into and arousal from hibernation, and Cheyne-Stokes type breathing during deep hibernation (which is believed to be an extension of slow-wave sleep). Taken together, these data suggest that state dependent changes in the activity of the central respiratory neurons, rather than changes in peripheral inputs to the respiratory centers, underlie the genesis of periodic breathing during both sleep and hibernation. Supported by the NSERC of Canada.

100.19

LATE MATURATIONAL DECLINE IN 0-3 HZ EEG AMPLITUDE DURING SLEEP: A REFLECTION OF SYNAPTIC ELIMINATION? I. Feinberg, MD, J. D. March*#, K. Flach*, T. Maloney*, W.-J. Chern*, MA and F. Travis*, PhD, VA Medical Center, Martinez, CA and University of California at Davis; #Delta Software, San Francisco, CA

We used period-amplitude analysis (1) to measure average sample amplitude (integrated amplitude/time in band) over the first 6 hours of sleep in 45 children (CH), 23 young adults (YA) and 36 normal elderly (NE) with mean ages 11.7, 23.6 and 71.9 yrs resp. ASA declined by 50% between CH and YA (38.3 vs 19.0, $p<.0001$) and by 26% between YA and NE (19.0 vs 14.0 $p<.0001$) (means are uV x 10^{-3}.) Thus, the absolute amplitude decline over twelve years of maturation was almost four times that produced by almost 50 yrs of aging. These maturational data confirm previous findings with hand measurement of EEG (see (2)). The 50% decline between CH and YA groups parallels the 50% reduction recently found for cortical metabolic rate over the same age range (3). Taken together, these new metabolic and EEG findings strongly support the hypothesis (2) that late, pervasive maturational changes take place in the human cortex as a result of the (?programmed) synaptic elimination discovered by Huttenlocher (4). A fault in these widespread regressive events may cause or permit the expression of the "functional" psychoses (notably schizophrenia); these subtle brain disorders rarely occur in typical form before puberty. Lastly, the sleep EEG may provide a non-invasive tool for tracking the maturational process longitudinally; deviations from the normal rate of decline may indicate vulnerability to mental disorder.(1) I Feinberg, et al. EEG Clin Neurophys, 1978:44,202. (2) I Feinberg, J Psy Res 1982/83:17,319. (3) HT Chugani, et al Ann Neurol 1987:22,487. (4) PR Huttenlocher, Br Res, 1979: 163, 195.

HUMAN BEHAVIORAL NEUROBIOLOGY: MEMORY AND LANGUAGE

101.1

COGNITVE SKILL LEARNING IN AMNESICS. E.A. Phelps*, W. Hirst*, M.K. Johnson*, B.T. Volpe and (Spon. F. McDowell). Dept.of Psych., Princeton Univ., Princeton, N.J. 08544, Dept. of Neurology, Cornell Univ. Medical School, The Burke Rehabilitation Ctr., White Plains, N.Y. 10605.

Evidence for skill learning in amnesics has been important for theories of amnesia (Schacter, 1988; Squire, 1987) as well useful for understanding normal skill learning (Anderson, 1983). However, there are very few instances of amnesics learning purely cognitive skills, that is, skills without a perceptual or motor component. The present work extends the domain of preserved functioning in amnesics to certain complex cognitive skills. Specifically, it demonstrates that amnesics can learn to use a rule system based on two linear equations. This is similar to the rule system Broadbent, Fitzgerald and Broadbent (1986) used to demonstrate that normals can learn rule systems in the absence of explicit knowledge of the rules. This finding is contrasted with findings of impaired cognitive skill learning in amnesics and an additional learning condition is added to demonstrate some specific components of cognitive skills that may impair performance. When amnesics are required to form relations between learning exemplars that are not simultaneously available they are no longer able to learn the cognitive skill. This method of examining the processes involved in different cognitive skills is a new approach common to both Johnson's Multiple Entry Modular Memory System (1983, 1988) and Hirst's Coherence Model of Amnesia (1988). The present work demonstrates that the use of this approach may help resolve some of the discrepancies in the skill learning literature.

101.2

FRONTAL LOBE AND VISUOSPATIAL MEMORY FUNCTION IN KORSAKOFF AND NON-KORSAKOFF ALCOHOLICS. E.M. Joyce* and T.W. Robbins.+(SPON: P.Dean). Institute of Psychiatry, London SE5 8AF, U.K and +Dept. of Expt. Psychology, Univ. of Cambridge, U.K.

Groups of subjects with the alcoholic Korsakoff syndrome (AKS), abstinent non-Korsakoff alcoholics (NKA) and age- and IQ-matched controls were compared on computerised tests of memory and frontal lobe function. Using a delayed matching-to-sample procedure (delays 0-16s) the NKA group showed a monotonic delay-dependent impairment in choice accuracy, with no deficit at 0s. By contrast, the AKS group was impaired at 0s and impaired to the same extent as the NKA group at 16s. In a test of spatial working memory the AKS group was significantly worse than the NKA group, which in turn was impaired relative to controls. Despite their mnemonic deficit, the NKA subjects were as accurate as controls on a test of planning (Tower of London), but were impaired at visual search and attentional set-shifting, to the same degree as the AKS group. The AKS group, however, showed some impairment in the test of planning, in terms of accuracy and thinking time. The results are considered in terms of differing degrees of diencephalic and frontal lobe pathology in the two test groups.

101.3

SPATIAL MEMORY PERFORMANCE IN TWO YEAR OLD HUMAN INFANTS. P. Mangan* and L. Nadel (SPON: A. W. Kaszniak). Dept. of Psychology, Univ. Arizona., Tucson, AZ 85721.

The maturation of the hippocampal formation has been implicated in the ontogeny of spatial learning in the rat. Prior to weaning rats perform in the water maze task at the level of adult rats with hippocampal lesions; several days after weaning they perform at the level of intact adult rats. Similar results should be obtained if human infants are tested on spatial tasks at comparable ages.

The hippocampal formation appears to mature between 18 and 24 months in humans. Thus, by 2 years of age the human infant should be able to perform tasks involving hippocampal-dependent spatial memory tasks. Recent work with infants performing 8-arm maze tasks and search tasks in the natural environment have not shown this; infants less than 26 months performed at or near chance levels on these tasks.

The present study further evaluated performance on spatial memory tasks of children aged 24 or 36 months. They were tested in a circular room and required to locate a toy hidden under one of eight evenly spaced identical covers. Subjects had to use cues located on the room walls, placed so as to not be contiguous with any of the eight possible targets. During the test trial subjects entered the room at a point 180° from that used during training. Both groups limited their search to the correct quadrant of the room, indicating that they recognized the location of the hidden toy.

We conclude that 24-month old infants can perform tasks requiring spatial memory skills when memory for only a single location is involved. Poor results obtained in the 8-arm maze could be due to the increased memory load of that task.

101.4

CORTICAL LESIONS DISSOCIATE SHORT AND LONG TERM COMPONENTS OF REPETITION PRIMING. Z.A.Kersteen-Tucker* & R.T. Knight (SPON: K. Sigvardt). Dept. of Neurology, Univ. of California, Davis, VAMC, Martinez, CA 94553.

Presentation of a word facilitates the subsequent identification of that word. This shortening of response latency has two components. A short term component is observed when two words are presented sequentially. The long term component is observed when different stimuli are interposed between the first and second presentation and results in a lesser but significant degree of response facilitation. We investigated the repetition effect in patients with unilateral lesions in dorsolateral prefrontal cortex (PFCx, N=7, Mean lesion volume=41 cc.), in temporal-parietal cortex (T-PCx, N=6, Mean lesion volume=50 cc.) and in age matched controls (N=7). In comparison to controls and T-PCx patients, PFCx patients showed a selective impairment in short term priming (Controls=102 ms, T-PCx=124 ms, PFCx=57 ms) In contrast to the short term priming effect, T-PCx patients exhibited a complete absence of long term facilitation in comparison to controls and PFCx patients (Controls=51 ms, PFCx= 45 ms, T-PCx=2 ms). The dissociation of short and long term priming by brain lesions supports the view that priming is not a unitary phenomena from either a behavioral or neuroanatomical perspective. (Supported by NIH grant NS21135).

101.5

EFFECTS OF SCOPOLAMINE ON THE MODULATION OF EVENT RELATED BRAIN POTENTIALS BY WORD REPETITION. M.D.Rugg*,D.D.Potter*,C.D.Pickles* & R.C.Roberts*. (SPON: Brain Research Association). Wellcome Brain Research Group, Dept Psychol, Univ St Andrews, and Dept Med, Univ Dundee, U.K.

The effects of scopolamine on event-related potentials (ERPs) were studied in three visual tasks. The incidental memory task required the detection of non-words interspersed in a series of words, some of which were repeats of previous items. The recognition task required subjects to discriminate words shown for the first or second time. The oddball task required a response to a low probability target letter occurring in a series of non-targets. 12 subjects received an IV injection of scopolamine (5.7 µg/Kg) before one session, and saline before another.

Scopolamine attenuated the positive-going ERP modulation evoked by repeated words in the incidental task. It had no effect on the size of either the positive shift evoked by repeated words in the recognition task, or the P300 component to targets in the oddball task.

The ERP word repetition effect in the incidental task may be more dependent upon cholinergic systems than either the apparently similar effect in the recognition task, or the 'oddball' P300.

101.6

COMPREHENSION AND RETENTION OF SCRAMBLED AND REGULAR TEXTS BY PATIENTS WITH UNILATERAL CORTICAL EXCISIONS. V. Frisk* (SPON: M. Moscovitch). Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada H3A 2B4.

Damage to the anterior temporal region in the dominant left hemisphere impairs the ability to remember stories (Milner, 1958, 1967). To investigate whether such an impairment results from difficulty in integrating information across sentences, patients with unilateral cortical excisions (28 left temporal [LT], 24 right temporal [RT], 6 left frontal [LF], 11 right frontal [RF]) and 13 normal control (NC) subjects listened to four stories, matched for difficulty. In two passages sentence order was scrambled so that textual continuity was disrupted. Immediately after presentation, subjects completed a free recall and answered questions. To rule out deficient comprehension as a reason for poor performance on the memory task, subjects later answered the same questions again, using the stories for reference. Retention of the stories by the LT group was expected to be impaired relative to that of other subject groups, but the disruption of textual continuity was anticipated to have a lesser effect on their recall. No group differences were expected in the ability to answer the questions when using the stories for reference. The LT group remembered substantially less of the stories than did the NC or RF groups, irrespective of the type of story. Only the LF group was impaired in locating the correct answers to the questions when using the stories for reference. These findings suggest that left temporal-lobe lesions do not affect the ability to integrate information across sentences, but do influence the amount of information retained. The impaired performance by the LF group is interpreted as another indication of difficulty in generating appropriate responses and in verifying the accuracy of these responses.

101.7

PLANNING AND SPATIAL WORKING MEMORY FOLLOWING FRONTAL LOBE LESIONS IN MAN. A.M.Owen,* J.J.Downes,* B.J.Sahakian,* C.E.Polkey,*† T.W.Robbins (SPON: B.J.Sahakian). Department of Experimental Psychology, University of Cambridge, Cambridge, CB2 3EB, U.K. †Institute of Psychiatry, University of London, London, SE5 8AF.

Thirty patients with unilateral or bilateral frontal lobe excisions primarily for epilepsy, were compared with age and IQ matched controls on a computerised battery of tests of spatial working memory and planning. A test of spatial short term memory capacity revealed no significant impairment in the patients' ability to execute a given sequence of visuo-spatial moves. In contrast, a computerised paradigm, designed to assess spatial working memory capacity, revealed significant impairments in the patient group in both possible types of search errors.

Higher level planning function was also investigated, using a test based on the 'Tower of London' problem. Frontal lobe cases required more moves to complete the problem and a yoked control condition revealed increased motor initiation times in these patients. Taking both of these factors into consideration, initial thinking (planning) time was unimpaired in the patient group although the subsequent thinking time, following the first move, was significantly prolonged. These data are compared to previous findings from patients with idiopathic Parkinson's Disease and will be discussed in terms of an impairment of higher cognitive functioning following frontal lobe damage.

101.8

THE PERFORMANCE OF AUTISTIC ADULTS ON TESTS SENSITIVE TO HIPPOCAMPAL AND PREFRONTAL (PF) DAMAGE. M. Rasmussen† E. Courchesne (SPON: R. Johnson). Neuropsychology Research Laboratory, Children's Hospital Research Center, 8001 Frost St., San Diego, CA 92123.

On the basis of behavioral findings, Boucher & Warrington (1976) and DeLong (1978) drew attention to a hippocampal theory of autism. However, evidence from anatomical studies has been mixed (Hauser, et al., 1975; Bauman & Kemper, 1985; 1988; Creasey, et al., (1986). Damasio & Mauer (1978) implicated the PF system in autism and Rumsey & Hamburger (1988) concluded that problem-solving deficits in autism are consistent with PF system dysfunction. There is neurophysiological evidence that the fronto-central P300 is reduced in amplitude in autistic persons (Courchesne, et al., 1984), yet no anatomical evidence of PF involvement has been found (Williams, et al., 1980; Bauman, et al., 1985; Coleman, et al., 1985).

As part of a neuropsychological/neurophysiological investigation of autism, a battery of behavioral tests found to be differentially sensitive to hippocampal and/or PF damage was used. We report here, the results from 4 high-functioning autistic adults matched to controls on the basis of age and Performance IQ.

The autistic group was most impaired on tests of spatial memory. They were less impaired on conditional associative learning and unimpaired on price estimation and recency discrimination. A similar pattern of impairment has been reported for people with hippocampal damage (Milner, 1985).

101.9

MAGNETIC FIELDS ASSOCIATED WITH SEMANTICALLY INCONGRUOUS SENTENCES. D. L. Arthur, A. Schmidt*[1], M. Kutas*[2] and E. Flynn, Biophysics Group, Los Alamos National Laboratory, Los Alamos, NM 87545; [1] Westinghouse, Pittsburgh, PA; [2] Dept. of Neurosciences, Univ. of Calif.-San Diego, La Jolla, CA.

Kutas and Hillyard (1980) reported a centro-parietal negative-going ERP component that occurred approximately 400 ms after subjects read sentences presented one word at a time which ended in a semantically anomalous fashion. Subsequent studies found that this "N4" component is slightly larger when recorded over the right compared to the left hemisphere. The present study is aimed at defining the magnetic field pattern which occurs over left and right hemispheres in a typical "N4" paradigm.

Subjects were right-handed adults with no left-handed relatives in their immediate families. Simultaneous magnetic and electrical data were recorded from various positions over left and right temporal and temporo-parietal areas; additional electrical data were also recorded from midline sites. Thirty congruent and thirty incongruent sentences were presented one word at a time at each neuromagnetic recording location. The subject's task was to read the sentences. To determine if a N4 was present, the responses to the last words of the congruent and incongruent words were compared. This difference was then compared to the electrical N4.

Preliminary recordings demonstrate a magnetic N4 response within the same latency range as the electrical N4. Complete field maps will be presented.

101.10

MAGNETOENCEPHALOGRAPHIC LOCALIZATION OF LANGUAGE CORTEX ADJACENT TO A CEREBRAL ARTERIOVENOUS MALFORMATION. R.A. Johnson[1], J. Beatty[1], N.A. Martin*[3], M.L. Collaer*[1], S. Jordan*[2], F. Vinuela*[4], J. Dion*[4], and D. Becker[3]. Departments of Psychology[1] and Neurology[2], Divisions of Neurosurgery[3] and Neuroradiology[4], UCLA, Los Angeles, CA 90024.

A case report is presented in which magnetoencephalography (MEG) was used to preoperatively and non-invasively map the intracortical source of speech-receptive cortex in RS, a 25 year old right handed male with a dominant left temporal lobe arteriovenous malformation (AVM). The speech-evoked magnetic field was analysed at 36 positions over the left hemisphere in response to presentations of the CV syllables /da/ and /ga/. The 250 msec duration syllables were generated by the DECtalk speech synthesizer and delivered binaurally with an interstimulus interval of 1.5 seconds.

A topographical map of the magnetic component 110 msec after stimulus onset, which was negative going at the vertex in concurrent electrical recordings, was congruent with a superficial cortical neuronal current source. This source was displaced from that usually observed in normals to tonal or click stimuli, being superior to the probable location of auditory cortex, and superior and anterior to the probable location of Wernicke's area as conventionally described.

The MEG results were in accord with the determination of position of language cortex as assessed by direct electrical stimulation of the cortex during surgery under local anesthesia. MEG and exposed brain stimulation sites were coordinated by cranial measurements and angiography. Investigations such as this, that compare MEG findings with those from established clinical procedures, are an essential step in determining the physiological and anatomical utility of magnetoencephalography. (Research supported by NIMH MH-37430-07 and NSF BBS-871057)

101.11

DISTINCT NEURAL SYSTEMS FOR LEXICAL AND EPISODIC REPRESENTATIONS OF WORDS. H.J. Neville, M.E. Pratarelli*, K.I. Forster*. The Salk Institute, La Jolla, CA 92037, The University of Arizona.

We investigated the hypothesis that specialized, linguistic or "lexical" representations of words exist and can be distinguished from general, non-linguistic or "episodic" representations of words. Adults rapidly decided whether a string of letters ("target") was or was not a word. On one half of the trials the targets were preceded by identity "primes" e.g. sun - SUN, gif - GIF, or by semantically related primes e.g. cat - DOG. Event-related brain potentials (ERPs) and behavior were examined for evidence that distinct patterns of priming occurred when episodic memory systems were normally present and subjects (N=10) were aware of the primes, as contrasted to when episodic traces were minimized by a masking procedure that rendered subjects (N=10) unaware of the primes. The results for the unmasked presentation displayed typical behavioral and ERP effects for semantic priming and for word and non-word identity priming. The ERP effect consisted of a negative peak maximal at 400 msec (N400) that onset at 300 msec, was 300 msec in duration and displayed a centro-posterior distribution. Remarkably, under masked conditions there was also a consistent ERP effect of semantic priming. However this onset at 200 msec, was 100 msec in duration and displayed a centro-anterior distribution. Moreover whereas both experiments provided evidence of identity priming for words, nonwords were primed only in the unmasked version. These data raise the hypotheses that the N400 indexes access to or decisions about general, episodic representations of words and nonwords. In contrast, we propose that the early negativity from 200-300 msec indexes access to a specialized representation of a word in a lexicon that does not include non-words. Most generally these data suggest that there exist separate lexical and episodic representation of words that are organized within non-identical brain systems.

101.12

COMPREHENSION OF COREFERENCE IN ALZHEIMER'S DISEASE. S.T. Smith, (SPON: C.R. Hamilton). Neurolinguistics Laboratory, Massachusetts General Hospital, Boston, MA 02114.

A two-choice picture verification test (PVT) was administered to Alzheimer's patients (AD) and age-matched controls to examine comprehension of sentences with forms of coreference. Correct interpretation of coreference between lexical items requires a complex system of grammatical rules, and thus provides a test for retention of complex aspects of syntactic ability. Subjects heard sentences that contained reflexive pronouns and reciprocals. Simple and complex sentences were tested to examine the effect of complexity. Good performance on simple sentences would suggest a processing limitation, not a syntactic deficit. The effect of task complexity was examined with a four-choice PVT in some subjects.

AD patients and controls performed nearly perfectly on simple sentences. On complex sentences, AD patients made more errors than controls. Their pattern of errors, however, was similar to that of controls. Task complexity affected both groups as indicated by increased errors and requests for repetitions with greater task difficulty. These findings are consistent with the interpretation that AD patients' performance reflects a limitation in processing, not a syntactic deficit. As processing demands increase, AD patients' ability to demonstrate retained syntactic ability is diminished.

101.13

DYSCALCULIA FOLLOWING IDIOPATHIC HYPOKALEMIA: A CASE STUDY. S.M. Sokol. Neurolinguistics Laboratory, Massachusetts General Hospital, Boston, MA 02114.

A case study is reported of a 55 year old female (B.B.) who complained of significant difficulty manipulating and remembering numbers. Twenty-three years prior to the present complaint, B.B. had been hospitalized for idiopathic hypokalemia and secondary hyperaldosteronism. At the time of original admission, she was quadriplegic; however, her condition soon improved, leaving her with a right hemiplegia which itself abated after two months. In addition to motor impairments, B.B. was severely dyslexic, dysgraphic and dyscalculic. Recovery of language processing skills occurred rapidly with minimal remediation. However, B.B. reports that her difficulties with numbers have persisted, despite excellent pre-morbid skill.

The present study examined the nature and extent of B.B.'s cognitive impairments. On standardized and experimental tests of language processing and general cognitive ability, she scored quite well. However, she demonstrated significant impairments in digit span, number processing and calculation, memory for biographical details involving numbers and general number knowledge. For example, she made a number of errors reading aloud Arabic digits and was severely impaired in the retrieval of basic multiplication facts (e.g., 7 x 6 = 42). She was unsure of her own age as well as those of her closest family members. Further, she could not recall a single digit of her brother's phone number, a number she calls at least once a week.

The relationship between potassium deficiency and cognitive impairment has received scant attention in the neurological and neuropsychological literatures. This report provides preliminary evidence that such impairments do occur; however, the precise mechanisms (either neural or cognitive) which could give rise to such circumscribed behavioral sequelae require further study.

101.14

ORGANIZATION OF HUMAN LANGUAGE CORTEX. G.A. Ojemann, Univ. of Washington Sch. of Medicine, Seattle, WA 98195.

Investigations of language during neurosurgical operations under local anesthesia include identification of essential areas, using electrical stimulation mapping, and of physiologic correlates in the electrocorticogram, and in subsequently resected areas, extracellularly recorded neuronal activity. Changes observed with these techniques during different language behaviors provide evidence on the organization of human dominant hemisphere lateral cortical language association cortex. That cortex includes separate systems for different language behaviors and languages. These systems are distributed widely across nonessential cortex, but separated at a neuronal level. Essential areas for different language functions and languages are in part grossly separated; usually two or more separate essential areas of 1-2 cm^2 extent with sharp boundaries function in parallel for each language behavior. Behaviorally specific selective attention characterizes activity in each system, of greater intensity at essential sites. Different subjects show substantial variability in the location of those sites, varying in part with sex and verbal ability. Essential areas are localized at age 4, but, in adults, may become more localized with increased languge skill.

Supported by NIH Grants NS17111, 21724, and 20482.

101.15

NEURONAL ACTIVITY RELATED TO FACES AND MATCHING IN HUMAN RIGHT, NONDOMINANT TEMPORAL CORTEX. J.C.Ojemann*, G.A.Ojemann, O.D.Creutzfeldt*, Ettore Lettich*. (SPON: J. Loeser.) Univ. of Washington Sch. of Med.,Seattle, WA, 98195 and Max Planck Institute of Biophysical Chemistry, Göttingen, West Germany.

During craniotomy under local anesthesia, extracellular neuronal recordings were obtained from subsequently resected right, nondominant temporal cortex during measures of face matching (FM), labeling of facial emotional expression (FE), object naming (N) and complex figure matching (CM) using overt speech responses. Recordings from 21 neuronal populations in 12 patients showed statistically significant changes in activity during FM in 67% of populations, FE 48%, N 19%, CM 33%. The pattern of changes identified several roles for these neurons: 33% related to perception of faces, changed activity with both FM and FE, confirming in man the finding in animals of temporal lobe neurons related to face perception; 29% were related to the generic act of matching, changing activity with FM and CM; 14% located only in middle temporal gyrus were related only to FE, providing independent confirmation of the role for this gyrus in FE, previously identified by stimulation mapping (Brain 105:349, 1982). Two of 6 neuronal populations that increased activity during speech showed a greater increase when speech was part of a spatial task (FE in one, matching in the other), compared to N, indicating that nondominant hemisphere visuospatial neuronal systems include speech effector neurons.

Supported by NIH Grants NS17111, 21724, and 20482.

BEHAVIORAL PHARMACOLOGY: ANALGESICS AND NMDA

102.1

Conditioned Stimulus Control of the Heart-rate Effects of Morphine. K.S. Schwarz and C.L. Cunningham, Dept. of Med. Psychology, Oregon Health Sci. Univ., Portland OR 97201.

The present experiment was designed to determine whether drug responses could be conditioned when intravenous morphine (5mg/kg) was paired with a 15-min light/noise conditioned stimulus (CS). Adult male rats were implanted with a jugular vein cannula and heart rate electrodes. Rats were housed 24 hrs/day in the experimental chambers. The Paired (P) group received morphine 30 sec after CS onset; therefore, the CS remained on for 14.5 min after morphine administration. The Unpaired (U) group received explicitly unpaired presentations of the CS and morphine, i.e., morphine was administered 75 min after CS offset.

Morphine produced a biphasic heart-rate response in both groups: bradycardia followed by tachycardia. After several CS-morphine exposures, the P group showed tolerance to the bradycardia, whereas the U group continued to show the biphasic response. When the P group received morphine without the CS, loss of tolerance was evident, in that it showed a biphasic response. Furthermore, presentation of the CS without morphine elicited a tachycardic response in the P group. No change in heart rate to the CS was observed in the U group. These data suggest that conditioned tachycardia contributes to tolerance of morphine bradycardia. In contrast to previous studies, conditioned bradycardia was elicited by a discrete CS in a situation where the response was not confounded by handling or the stress of injection.

102.2

BRAIN SITES INVOLVED IN KETOPROFEN-INDUCED ANALGESIA ON THE HOT PLATE TEST. R. de Beaurepaire, C. Suaudeau*, C. Cimetière* and A. Chait*. Laboratoire de Pharmacologie C.H.U. Côte de Nacre, 14032, Caen, France.

Ketoprofen is a non-steroidal anti-inflammatory drug (NSAID) used in the treatment of arthritis for its anti-inflammatory, analgesic and antipyretic properties. NSAID analgesic properties have a peripheral as well as a central origin since intracerebroventricular injections of NSAID in the rat produce analgesia. Male Sprague-Dawley rats were chronically implanted with fixed guide cannulae terminating 1.5mm over 20 different brain sites. Ketoprofen (10ug in 0.3ul) was injected into the 20 brain sites through an injection cannula descended 1.5mm below the guide cannula. Eight minutes after injection the pain thresholds were tested on a hot plate set to a constant temperature of 55±0.5°c. Latency to the first hindpaw lick were recorded in treated animals and controls injected with saline. An average of 8 treated and 8 control animals per site was used and each animal was only used for one measure. Student t-test for unmatched pairs was used for statistics. Significant analgesia was obtained in the 9 following sites: central gray, raphé magnus, centro-medial nucleus of the thalamus, nucleus reuniens, dorso-lateral geniculate nucleus, medial geniculate nucleus, dorso-medial/ventro-medial nuclei of the hypothalamus, posterior hypothalamic nucleus, lateral vestibular nucleus. A slight analgesia which did not reach significance ($p<0.2$) was observed in two structures: dorsal raphé and ventral postero-medial thalamic nucleus. In the following 7 sites, which included several thalamic structures, no analgesia was observed: centro-lateral nucleus of the thalamus, posterior thalamus nuclear group, parafascicular nucleus, bed nucleus of the stria terminalis, central tegmental tract, gigantocellular reticular nucleus, parvocellular reticular nucleus, nucleus of the tractus solitarius, spinal trigeminal nucleus oralis. Therefore the central analgesic action of ketoprofen is mediated by several different brain structures.

102.3

MU- AND DELTA-OPIOID RECEPTOR MODULATION OF AFFECTIVE DEFENSE BEHAVIOR ELICITED FROM MIDBRAIN PERIAQUEDUCTAL GRAY IN THE CAT. **C.L. Lu*, M.B. Shaikh and A. Siegel. Dept. of Neurosciences, UMDNJ, Newark, N.J. 07103.**

Recently, we have demonstrated that opioid peptides can regulate affective defense behavior (AD) elicited from the midbrain periaqueductal gray (PAG) in the cat. The present study sought to identify specific opioid receptor subtypes at the level of the PAG involved in the modulation of AD.

Cannula-electrodes were utilized for eliciting AD from the PAG as well as for microinjecting mu and delta agonists (morphiceptin and D-Pen^2,D-Pen^5 enkephalin [DPDPE]) and naloxone. Prior to drug infusion, stable baseline threshold values were determined over 3-4 days. Following microinjections of DPDPE (0.2- 1.5nmol/0.25ul) or morphiceptin (0.2-0.4nmol/0.25ul) into the sites from which AD was elicited, threshold values were determined.

The results indicated that DPDPE and morphiceptin suppressed AD in a dose and time-dependent manner. However, the magnitude and duration of suppression of AD was far greater following morphiceptin administration (i.e., morphiceptin [0.2nmol] suppressed AD for 180 min, while DPDPE [0.8nmol] suppressed AD for 60 min), indicating a preferential role of mu receptors in modulating AD. Pretreatment with naloxone blocked the suppressive effects of DPDPE. Administration of vehicle alone (saline/0.25ul) did not alter the threshold of AD. [Supported by NIH (NS07941) and the Fogarty Internat. Center (FO5TWO4110)].

102.4

INVOLVEMENT OF DEEP PREPIRIFORM CORTEX (AREA TEMPESTAS) IN MORPHINE-INDUCED ANALGESIA IN RATS. A. D'Amore*, P.Lorenzini* and M.Massotti.(SPON:R.Del Carmine). Lab. Farmacologia, Istituto Superiore di Sanità, 00161 - Roma, Italy.

Microinfusion of morphine into Area Tempestas (AT) delays the responses in tail-flick and hot-plate tests in rats (D'Amore and Massotti,17th Ann.Meet.Neurosci.,New Orleans,LA, 1987,Abst.n.-64.3). In this study we present evidences that AT can contribute to the antinociceptive effect of systemic administration of morphine.

In rats, injection of morphine-HCl (5 mg/kg sc) delays the responses in tail-flick (from 1 to 10 sec) and hot-plate (from 6 to 45 sec) tests. This effect initiates 10-20 min after injection and lasted about 2 hours.

These effects are antagonized by microinfusion into AT of the μ antagonist naltrexone (10-30 ng) and of δ antagonist ICI 154,129 (15-120 ng). In contrast, no significant changes can be observed after microinfusion of WIN 44,441-3 (120 ng) or saline.

These findings confirm that AT participates of the endogenous pain suppression system.

102.5

CHRONIC NEUROLEPTIC TREATMENT INDUCES A SUPERSENSITIVE FEEDING RESPONSE TO MORPHINE. J.S. Mogil[1], F.J. Vaccarino[1] and L. Stinus[2]. [1]Department of Psychology, University of Toronto, Toronto, Ontario, M5S 1A1. [2] INSERM Unite 259, Bordeaux, France.

The locomotor and feeding-stimulatory effects of systemically-administered opiates have been extensively studied. Current evidence indicates that dopaminergic (DAergic) influences modulate the behavioural effectiveness of opiates. It has been found that chronic DAergic blockade enhances the effects of opiates with regard to locomotor activation. The present study was aimed at examining the extent to which this "supersensitive" opiate response is also associated with feeding behaviour. To this end, the effects of chronic neuroleptic treatment on morphine-induced feeding were investigated.

Male Wistar rats were administered the long-acting neuroleptic, flupenthixol decanoate (12 mg/kg, s.c.), approximately every 10 days for 40 days. Rats were tested for their 2 hour feeding response to morphine (0.5 mg/kg, s.c.; a threshold dose) or saline on days 8, 16, 24 and 32.

The results indicated that chronic DAergic blockade enhanced the effectiveness of morphine. Rats receiving flupenthixol showed a progressive increase in the feeding response to morphine over time. This increase in feeding was statistically significant after the third week of neuroleptic treatment as evidenced by a 65% increase in food intake over controls. The similar time course of this effect and the therapeutic efficacy of neuroleptics supports a possible opiate involvement in the antipsychotic actions of neuroleptics.

This research was supported by a NSERC grant to FJV.

102.6

EVIDENCE THAT SIGMA RECEPTORS DO NOT MEDIATE FEEDING BEHAVIOR IN NONHUMAN PRIMATES. **N. L. Katz, R. F. Schlemmer, Jr., D. McGinness* and J. M. Davis. Univ. of Illinois at Chicago & Illinois State Psychiatric Institute, Chicago, IL 60612.**

Antipsychotic drugs frequently induce movement disorders in treated patients. Efforts to discover drugs devoid of this distressing adverse reaction have led to speculations about new mechanisms of psychotic behavior and to new 'atypical' antipsychotic drugs. A less recognized disturbing effect of most typical and atypical antipsychotic drugs is weight gain. In an animal model of psychosis developed in this laboratory using Stumptail macaque (Macaca artoides) monkeys housed in social colonies, we are able to observe many solitary, social, and drug-induced behaviors among which are several food-related behaviors. In the present study in which the effect of the selective sigma antagonist (±)-BMY14802, an antipsychotic candidate, was being studied in the model, several observations were made which suggested that the drug may have little or no effect on appetite and weight gain. Given acutely or chronically in oral doses up to 5 mg/kg, (±)-BMY14802 did not affect the frequency or time that the animals handled, chewed, or foraged food. The amount of time spent drinking was not affected. All of these behaviors were significantly decreased by chronically administered amphetamine and could not be reversed by (±)-BMY14802. Subsequently, we observed the effect of the prototype sigma agonist N-allylnormetazocine (NANM) on these same food-related behaviors and found them to be decreased in a dose-related fashion by racemic NANM. However, the decrease could be accounted for mainly by the (-)-NANM isomer, a ligand primarily for mu and kappa opiate receptors, and not by the (+)-isomer, a ligand for sigma receptors. These data support our previous studies in rats and do not suggest a role for sigma receptors in feeding. (Supported in part by Bristol Myers Company).

102.7

ISOMERIC SEPARATION OF THE MU, KAPPA AND SIGMA-MEDIATED STIMULUS EFFECTS OF METAZOCINE IN THE PIGEON; S. S. Negus, M. J. Picker* and L. A. Dykstra*; Department of Psychology; University of North Carolina; Chapel Hill, NC 27599

Metazocine is a benzomorphan related in structure to the prototype kappa agonist ketocyclazocine and the prototype sigma agonist N-allylnormetazocine (NANM). Like these other benzomorphans, metazocine exists as a racemic mixture of (-) and (+) isomers. Previous studies have demonstrated that (-)metazocine acts as an agonist at mu opioid receptors, whereas (+)metazocine appears to have sigma agonist activity. The present study aimed to extend this characterization of metazocine by evaluating both the agonist and antagonist properties of the (-) and (+) isomers of metazocine (0.3-10.0 mg/kg for both isomers) in pigeons trained to discriminate the kappa agonist bremazocine (0.01 mg/kg; N=3), the mu agonist fentanyl (0.05 mg/kg; N=4) or the sigma agonist (+)NANM from water in a two-lever, food-reinforced, drug-discrimination procedure. As expected, (-)metazocine generalized completely to the fentanyl (mu) stimulus, whereas (+)metazocine generalized to the (+)NANM (sigma) stimulus. Neither isomer generalized to the bremazocine (kappa) stimulus. In the antagonism studies, (-)metazocine was found to antagonize the bremazocine stimulus. In conclusion, these results suggest that (-)metazocine acts as both a mu agonist and as a kappa antagonist in the pigeon drug discrimination procedure, while producing neither agonist nor antagonist effects via the sigma receptor. (+)Metazocine, in contrast, acts as a sigma agonist with neither agonist nor antagonist activity at either the mu or kappa opioid receptors. (Supported by U.S. Public Service Grants DA05355 and DA02749)

102.8

NALOXONE DRUG DISCRIMINATION SUBSTITUTION BY NALTREXONE, DIPRENORPHINE AND NALORPHINE.
B. Geter, S. Smurthwaite*, M. Kautz and A. Riley. Psychopharmacology Laboratory, The American University, Washington, D.C. 20016.

Recently, Kautz and her colleagues reported rapid drug discrimination with low doses of naloxone (1 and 3 mg/kg) in non opiate-dependent animals, suggesting an opiate receptor mediation of the stimulus (Kautz et al., Drug Dev. Res., 16: 317, 1989). To further examine naloxone drug discrimination and its receptor mediation, in the present experiment a range of opiate antagonists were substituted for naloxone (1 mg/kg) in a test of their generalization. Specifically, rats were given naloxone 15 min prior to a saccharin - LiCl pairing. On intervening days, they were injected with distilled water prior to a nonpoisoned exposure to saccharin. This cycle was repeated until subjects were avoiding saccharin following naloxone and consuming saccharin following distilled water. Subjects were then given one of a range of doses of naltrexone (0.018 - 5.6 mg/kg), diprenorphine (0.1 - 18.0 mg/kg) and nalorphine (0.56 - 32.0 mg/kg) prior to saccharin. All drugs substituted for naloxone, producing dose-related decreases in saccharin consumption. That a range of opiate antagonists substitute for naloxone provides further evidence that the naloxone stimulus is mediated by the opiate receptor.

102.9

NONCONTINGENT TOLERANCE TO MORPHINE-INDUCED ANOREXIA IN RATS. D.L. Wolgin and H. Benscn*. Dept. of Psychology, Florida Atlantic Univ., Boca Raton, FL 33431.

In order to determine whether tolerance to morphine-induced anorexia is contingent on access to food during drug intoxication, rats were given one of two doses of morphine sulfate (10 or 20 mg/kg) either before (Before Groups) or after (After Groups) access to sweetened milk for 30 min. on alternative days. Control groups were given injections of saline. Dose-response curves were determined before and after the period of chronic treatment.

On the initial doses-response determination, morphine produced similar dose-dependent decreases in milk intake in all groups. Following chronic administration of the drug, there was a rightward shift in the dose-response curves of rats given chronic morphine, but not for rats given chronic saline. The extent of the shift was slightly higher in the Before Groups (2.5- and 1.8-fold) than in the After Groups (1.9- and 1.5-fold). We conclude that tolerance to morphine-induced anorexia is only minimally affected by access to milk in the drugged state.

102.10

SHORT- AND LONG-TERM FACTORS IN MORPHINE TOLERANCE: SINGLE OR MULTIPLE MECHANISMS? C. R. McLaughlin & M. S. Fanselow*. Depts. of Psychol., Dartmouth Col., Hanover, NH 03755 and University of California-Los Angeles, Los Angeles, CA 90024

Data from several laboratories indicate that Pavlovian conditioning plays an important role in the formation of tolerance to the analgesic effect of morphine. One account of these data, provided by Baker & Tiffany (1985), suggests that there are two forms of tolerance, long-term and short-term, which are a result of associative and nonassociative mechanisms, respectively. According to their model, the short-term and long-term processes should develop at the expense of one another, and only the long-term tolerance should be long-lasting. We tested these predictions.

Following the procedure described by Tiffany and Maude-Griffin (1988), adult male rats were administered either morphine sulphate (30 mg/kg, ip) or isotonic saline at intervals of either 24 hr (short-term acquisition) or 96 hr (long-term acquisition) for a total of 7 exposures. The animals were then reassigned to either short-term (24 hr after final acquisition exposure) or long-term test (96 hr after final acquisition exposure). The animals were tested with morphine (10 mg/kg, ip) in the hotplate (51.5 ± 0.5 °C) test of nociception.

All animals pre-exposed to morphine displayed shorter pawlick latencies than the saline controls, indicating tolerance, regardless of acquisition or test IDI. Contrary to the prediction of the Baker and Tiffany model, these results indicate that tolerance resulting from both short- and long-term acquisition parameters is long-lasting.

102.11

DEXTROMETHORPHAN, A NONCOMPETITIVE NMDA ANTAGONIST, IS RELATIVELY INEFFECTIVE AS A NEUROPROTECTANT IN A GERBIL ISCHEMIA MODEL. C.A. Boast, R.R. Notvest, C.A. Hoffman*, T.E. Emrey*, K. McMonagle-Strucko*, and T.G. Demetriou*, Wyeth-Ayerst Research, Princeton, NJ 08543-8000

Dextromethorphan (DM), like MK-801, is an NMDA antagonist. DM and MK-801 (ip) were compared in several *in vivo* tests related to NMDA modulation and neuroprotection. In mice, DM was less potent than MK-801 in blocking NMDA-induced lethality (ED50-mg/kg = 21 vs 0.2) and did not as readily disrupt traction reflex (ED50 = 45 vs 0.2). In gerbils, distinct changes in spontaneous locomotor activity were produced by both drugs, however, DM was weaker (MED = 30 vs 0.3). In gerbil ischemia studies MK-801 was active at 3 mg/kg (15 x ED50 for NMDA lethality). In normal gerbils DM was 100% lethal at 54 mg/kg (2.5x ED50 for NMDA lethality). DM (30 mg/kg) prevented ischemia-induced hyperactivity but not hippocampal brain damage. Relative to the NMDA lethality ED50, high doses of MK-801 are needed for neuroprotection. Even at proportionally lower doses, DM is lethal in gerbils and at still lower doses, is ineffective against ischemia. Thus, DM has a narrow therapeutic window.

102.12

THE EFFECTS OF GLYCINE AGONISTS AND ANTAGONISTS ON PHENCYCLIDINE-INDUCED STEREOTYPY AND ATAXIA. P. C. Contreras and I. Daly*. CNSDR, G.D. Searle & Co., St. Louis, MO 63198.

Glycine agonists of the strychnine-insensitive site associated with the NMDA complex potentiate the actions of NMDA. In contrast, agonists of the PCP receptor associated with the NMDA complex are noncompetitive antagonists of NMDA. The purpose of this study was to assess whether glycine ligands at the glycine-NMDA receptor modify the actions of PCP. Glycinamide (2 umol/rat, icv) and glycine (1 and 2 umol/rat, icv) antagonized PCP-induction of stereotypy and ataxia. Unlike glycine, d-serine (1.0 and 0.5 umol/rat) is a selective agonist at the strychnine-insensitive glycine site and was a more potent antagonist of PCP than glycine. D-Serine also antagonized MK-801-induced stereotypy and ataxia. Two glycine antagonists, HA-966 and ACBC (1-aminocyclobutane-1-carboxylate), at doses that did not produce ataxia did not significantly affect the behavioral actions of PCP. It is clear that glycine agonists at the strychnine-insensitive site antagonized the behavioral effects of PCP, but a more selective and potent glycine antagonist may be needed to demonstrate any effect on PCP behaviors.

102.13

BLOCKADE OF NMDA-INDUCED CONVULSIONS AND TASTE AVERSION BY ANTAGONISTS OF NMDA AND GLYCINE: RELATIONSHIP TO BEHAVIORAL EFFECTS INDUCED BY THE PUTATIVE ANTAGONISTS. A. Jackson* W. Koek* and F.C. Colpaert* (Spon: M. Marien). FONDAX-Groupe de Recherche SERVIER, 7, rue Ampère, 92800 Puteaux, France.

Antagonism of N-methyl-D-aspartate (NMDA)-induced convulsions by a variety of drugs was compared with their ability to produce phencyclidine (PCP)-like behavioral activity (locomotor stimulation and falling) in mice. Convulsions produced by i.c.v. administration of NMDA were antagonized, at doses that did not block kainate- and quisqualate-induced convulsions, by competitive NMDA-antagonists (e.g., AP5, CGS 19755), non-competitive antagonists (e.g., PCP, MK-801) and also by some putative glycine antagonists (e.g., 7-chloro-kynurenic acid,, HA-966). Only the competitive and the non-competitive NMDA antagonists produced locomotor stimulation and falling, and their potencies to do so were highly correlated with their relative potencies to antagonize NMDA-induced convulsions (r = 0.92). However, with the competitive antagonists, PCP-like behavioral effects were of a lesser magnitude than those seen with non-competitive antagonists and were only seen at doses higher than those required to block NMDA-induced convulsions. In a different series of experiments, the ability of some of these compounds to block taste aversion induced by i.p. administration of NMDA was studied in rats. Effects of these drugs as antagonists of NMDA-induced taste aversion were investigated as well as their ability to produce taste aversion when given alone.

102.14

STUDIES OF THE DISCRIMINATIVE CUE OF (+)-MBDB, THE ALPHA-ETHYL HOMOLOGUE OF MDMA. R. Oberlender* and D.E.Nichols (SPON. R. P. Maickel) Dept. of Medicinal Chemistry and Pharmacognosy, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, IN 47907.

In 1988, we reported that rats trained to discriminate (+)-MBDB from saline responded on the drug lever when tested with the entactogens MDMA or MDA, but not the hallucinogens LSD, DOM, or mescaline, or the stimulants amphetamine, methamphetamine, or cocaine. We now report the results of further studies directed toward the analysis of the (+)-MBDB cue.

Complete substitution for the training stimulus was observed with drugs which are known to induce the release of serotonin (PCA, (±)-fenfluramine, (+)-fenfluramine) and drugs with 5-HT_{1B} agonist activity (TFMPP, RU-24969). The latter drugs also produced significant numbers of disruptions. No substitution was observed for compounds with selective 5-HT_{1A} agonist activity (8-OH-DPAT, buspirone).

The (+)-MBDB cue was effectively antagonized by the nonselective serotonin antagonist metergoline (0.5 mg/kg, 90 min prior to testing). No significant effect on the perception of the training stimulus resulted from pretreatment with the 5-HT_2 antagonist ketanserin, or the dopamine antagonist, haloperidol.

These results suggest that the cue produced by entactogens (MBDB, MDMA) may be primarily mediated by 5-HT_{1B} agonist effects of released serotonin.

DRUGS OF ABUSE: BIOGENIC AMINES

103.1

EFFECTS OF CHRONIC COCAINE TREATMENT ON THE STRIATAL DOPAMINE AND ACETYLCHOLINE RELEASE. S.-J. Yi and K.M. Johnson (SPON: G. Greeley). Dept. of Pharmacol. & Toxicol., Univ. of Texas Medical Branch, Galveston, TX 77550.

Repetitive administration of cocaine is associated with both an enhanced behavioral effect upon acute challenge (behavioral sensitization) and an enhanced effect of amphetamine (Amph) on DA release in vitro. We tested the effects of chronically administered cocaine on Amph-induced potentiation of basal and K^+-stimulated ^{3}H-DA release and Amph inhibition of K^+-stimulated ^{14}C-ACh release from striatal slices in order to determine the relationship between dopaminergic transmission and the behavioral augmentation produced by chronic cocaine. Female Sprague-Dawley rats were injected with either saline or 15 mg/kg cocaine (i.p.) twice a day for 7 days. After 7 days of withdrawal, rats were injected with saline or cocaine and were sacrificed 30 min later. Brains were removed and striata were dissected to assay ^{3}H-DA and ^{14}C-ACh release in a dual K^+ stimulus paradigm. K^+-stimulated ^{3}H-DA release from striatal slices was not affected by acute or chronic cocaine; however, acute cocaine challenge after chronic cocaine injection potentiated Amph's effect on K^+-induced ^{3}H-DA release. This effect was mirrored by an increase in K^+-stimulated ^{14}C-ACh release in the absence of Amph. This may be due to a decreased dopaminergic inhibitory tone caused by an increase in DA uptake (Yi and Johnson, Soc. Neurosci. Abst. 14: 392, 1988). This increased capacity of the DA carrier could also account for the enhanced ability of Amph to potentiate K^+-stimulated ^{3}H-DA release. However, the latter effect is difficult to reconcile with the observation that chronic cocaine administration (with or without a cocaine challenge) resulted in a diminished effect of Amph on spontaneous ^{3}H-DA release. This may be the result of the paradigm used, in which Amph was added after a depolarizing stimulus, or may be associated with similar effects of Amph observed previously on the compartmentalization of ^{3}H-DA in striatal synaptosomes (ibid). These data suggest that repetitive administration of cocaine in a regimen that elicits behavioral sensitization alters the substrates through which Amph exerts its effects on release of ^{3}H-DA.

103.2

PERSISTENT COCAINE-INDUCED CHANGES IN ELECTRICALLY-STIMULATED DOPAMINE RELEASE FROM RAT STRIATAL SLICES. N.R. Zahniser, P. Curella and G. Larson. Dept. Pharmacology, Univ. Colorado Hlth. Sci. Ctr., Denver, CO 80262.

Previously we found that a single injection of cocaine (C) results in a persistent 30-50% increase in amphetamine-stimulated release of [^{3}H]dopamine ([^{3}H]DA) from rat striatal slices (Peris and Zahniser, Pharmacol. Biochem. Behav. 27:533-535, 1987). To test whether endogenous DA release is similarly changed, rats were pretreated with either saline (S) or C (10 mg/kg, i.p.) 1 day before release was measured. In contrast with [^{3}H]DA release, amphetamine-stimulated release (6 or 20 uM for 2.5 min) of endogenous DA or dihydroxyphenylacetic acid (DOPAC) did not differ between the two pretreatment groups. These results, together with the augmented amphetamine-stimulated [^{3}H]DA release, suggest that the specific activity of the pool of DA released by amphetamine, rather than release per se, may be increased by C pretreatment. Next, release evoked by 60 electrical pulses (1Hz) was measured in the presence of two uptake blockers. When 10 uM C was used to inhibit uptake, C pretreatment significantly decreased stimulated release of DOPAC by 30% from 150 ± 22 pmol/mg but not of DA [S: 27 ± 8.0, C: 22 ± 6.5]. In the presence 10 uM nomifensine, a more potent uptake blocker, evoked release of DA was higher (S: 86 ± 5.4 pmol/mg); and both DA and DOPAC release were diminished by 30-40% in response to C pretreatment. Regardless of the uptake blocker, evoked tritium release was also reduced by 35-40% following C pretreatment. Taken together, the results suggest that C can induce persistent alterations in both nonvesicular and vesicular release of DA in striatum and are more consistent with the development of dopaminergic tolerance than sensitization. Supported by USPHS DA04216.

103.3

THE STIMULUS EFFECTS OF COCAINE: ROLE OF MONOAMINE REUPTAKE INHIBITION. P.M. Callahan, S.O. Idemudia*, J.M. Lakoski and K.A. Cunningham. Dept Pharmacology/Toxicology, Univ Texas Medical Branch, Galveston, Texas 77550.

Cocaine inhibits the reuptake of dopamine (DA), norepinephrine (NE) and serotonin (5-HT). To investigate the relative role of such processes in the stimulus properties of cocaine, male rats (N=16) were trained to discriminate cocaine (10 mg/kg, ip) from saline in a two-lever, water-reinforced task and were given various neuroactive compounds during substitution (generalization) tests. The DA reuptake inhibitor GBR 12909 (4-16 mg/kg) completely mimicked cocaine. The reuptake inhibitors for NE (desipramine, 2-8 mg/kg) and 5-HT (fluoxetine, 0.625-5 mg/kg) did not substitute for the training drug. In contrast to the lack of substitution engendered by these agents, a low dose of desipramine (3.0 mg/kg) or fluoxetine (1.25 mg/kg) significantly potentiated the stimulus effects of low doses of cocaine (0.313-2.5 mg/kg). The hypothesis that the stimulus properties of cocaine are mediated predominantly by DA systems is corroborated by the finding that GBR 12909 mimics cocaine. Reuptake inhibitors for NE and 5-HT systems may enhance the subjective (euphoric?) effects of cocaine possibly through increasing brain cocaine concentrations (Misra et al, Res Comm Subs Abuse 7: 85, 1986).

Supported by DA 04296, J. Sealy Memorial Foundation.

103.4

POTENTIATION OF THE COCAINE-LIKE EFFECTS OF DOPAMINE RECEPTOR AGONISTS. R.L. Barrett* and J.B. Appel. *Behavioral Pharmacology Laboratory, Department of Psychology, University of South Carolina, Columbia, SC 29208.*

The effects of several centrally acting dopamine (DA) agonists were studied in rats trained to discriminate cocaine (10 mg/kg, *i.p.*) from saline. When given alone over a wide range of doses, neither bromocriptine nor SKF 38393 substituted for the training drug; pergolide substituted only partially. However, combinations of a non-discriminable dose of cocaine (2.5 mg/kg) with each of these DA agonists resulted in significant amounts of cocaine-appropriate responding. These data suggest that central DA systems are involved in the behaviorally relevant actions of cocaine and that at least some inhibition of DA reuptake (e.g., by the 2.5 mg/kg dose of cocaine) may be essential to the induction of cocaine-like discriminable (subjective) effects. This hypothesis is being investigated further with several directly (LY-171555, apomorphine) and indirectly acting (L-DOPA) DA agonists.

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103.5

REPEATED COCAINE ADMINISTRATION POTENTIATES THE HYPOMOTILITY EFFECTS OF APOMORPHINE. F. Weiss, Y.L. Hurd,* U. Ungerstedt*, and G.F. Koob. Research Institute of Scripps Clinic. La Jolla, CA 92037. Dept. of Pharmacology, Karolinska Institute, Stockholm, Sweden.

Several lines of evidence indicate that repeated cocaine (COC) administration may produce functional changes in dopamine (DA) neurotransmission including alterations in receptor sensitivity, DA synthesis and metabolism. Using apomorphine (APO)-induced changes in motility as a behavioral assay, we have further explored the nature and direction of changes in DA receptor function associated with repeated COC exposure in COC self-administering rats. Locomotor activity in response to APO (0.1 mg/kg) was assessed 24 h prior to (PRETEST), and 24h after POSTTEST) 10 daily 3h COC SA sessions. To determine the persistence of the observed changes, a second POSTTEST was given 7 days after the first. APO produced only slight reductions in motility during the PRETEST. In contrast, locomotor activity was strongly reduced in the POSTTEST (30-40% of PRETEST levels). This hypomotility was no longer observed after one week (POSTTEST 2). Identical results were obtained in a replication of the experiment after 10 daily IP injections of COC (30 mg/kg). Consistent with the behavioral results, preliminary in vivo microdialysis data from animals tested under the same experimental protocol suggest the APO-induced inhibition of DA release in the nucleus accumbens is enhanced by repeated COC pretreatment. Together, these results suggest that presynaptic DA receptor sensitization may be associated with repeated cocaine use.

103.6

SELECTIVE D1 AND D2 RECEPTOR ANTAGONISTS BLOCK THE REINFORCING EFFECTS OF COCAINE IN THE RAT. C.B. Hubner and J.E. Moreton. University of Maryland School of Pharmacy, Baltimore, MD 21201.

A role for dopamine in mediating the reinforcing effects of cocaine has been suggested from studies in which dopamine antagonists increase rates of responding maintained by cocaine under fixed-ratio (FR) schedules. Since rate of responding maintained by drug can be influenced by factors other than its reinforcing efficacy, progressive-ratio (PR) schedules have been used to compare the reinforcing efficacy of different drugs. The purpose of the present study was to investigate the effect of the D1 antagonist, SCH 23390, and the D2 antagonist, spiperone, in rats self-administering cocaine on a FR and PR schedule. Sprague-Dawley rats, trained to self-administer intravenous cocaine (0.75 mg/kg/inj) on a FR 5 or PR schedule, were pretreated 30 minutes prior to the session with s.c. 2.0 - 20.0 ug/kg SCH 23390 (n=12) or 2.0 - 40.0 ug/kg spiperone (n=13). Under the FR 5 schedule, the dose-effect curves for SCH 23390 and spiperone each had an inverted-U shape, with maximal increases in responding at 10 ug/kg. Under the PR schedule, both SCH 23390 and spiperone produced dose-dependent decreases in the highest ratio completed, with SCH 23390 having a greater potency. These results suggest that both D1 and D2 receptors are involved in mediating the reinforcing effects of cocaine. [Supported in part by DA05292 (CBH) and BRS grant 2S07RR05770-10 (CBH & JEM)].

103.7

PERSISTENCE AND DOPAMINE IN MORPHINE-INDUCED STEREOTYPY. J. Pollock and C. Kornetsky. Boston University School of Medicine, Boston, MA 02118.

Administration of morphine (MS) induces an oral stereotypy in rats characterized by compulsive gnawing behavior, a behavior believed to be mediated by the activation of the mesostriatal dopamine system. This study was designed to determine the persistence of this stereotypy. Male F-344 rats (N=5) received three injections of MS (10, 20, 20 mg/kg, sc), 12 hours apart. By the third MS dose all rats showed the oral stereotypy, lasting 2-3 hours. After the last high dose of MS, animals were challenged with 4 mg/kg (sc) of MS, a dose that does not induce the oral stereotypy in drug naive animals, at 30, 90, 180, 270, and 360 days. After each challenge dose all animals exhibited the stereotypy. Since the acute stereotypy can be blocked by naloxone (NX) (1 mg/kg, ip) or the D1 antagonist SCH 23390 (0.08 mg/kg, ip), but not by the D2 antagonist raclopride (0.25 mg/kg, ip) (Pollock and Kornetsky, 1989), we challenged the persistent stereotypy at approximately 30 and 270 days following the last high dose of MS. Raclopride antagonized the stereotypy at 270 days, while NX and SCH 23390 were effective antagonists at both test days. The results indicate that the stereotypy caused by brief high dose MS persists for at least 12 months, and after what appears to be a prolonged "kindling" period, the D2 as well as the D1 receptor system appears to be involved in the behavior. (Supported in part by NIDA grant DA02326 and Research Scientist Award DA00099 to CK).

103.8

DIFFERENTIAL DOPAMINE RECEPTORS AND PHENCYCLIDINE-INDUCED BEHAVIORS IN RATS. P.A. Beers* and A.P. Leccese. Psychology Department, Kenyon College, Gambier, OH 43022.

The roles of D_1 and D_2 dopamine receptors in phencyclidine (PCP)-induced hyperlocomotion and stereotypy were investigated. Rats were injected with the D_1 antagonists SCH 23390 (0.05 or 0.25 mg/kg ip) or the D_2 antagonist, metoclopromide (1.0 or 5.0 mg/kg ip). These injections were immediately followed by 0.0, 4.0, or 16.0 mg/kg ip PCP. PCP-induced hyperlocomotion and stereotypy were measured by an automated activity apparatus. The D_1 antagonist, SCH23390, potentiated the depressant effects associated with the larger doses of PCP. Conversely, the D_2 antagonist, metoclopromide, blocked the behavioral effects of PCP. These findings demonstrate the importance of D_2 receptors in PCP-induced hyperlocomotion and stereotypy, and have implications for the pharmacotherapy of PCP-induced psychosis.

103.9

REASSESSMENT OF THE INVOLVEMENT OF CATECHOLAMINERGIC MECHANISMS IN THE STIMULUS EFFECTS OF COCAINE. J. Broadbent, E.K. Michael,* E. Riddle* and J.B. Appel. *Behavioral Pharmacology Laboratory, Department of Psychology, University of South Carolina, Columbia, SC 29208.*

Recent reports that several antidepressants, which are known to inhibit the reuptake of norepinephrine (NE), are clinically effective in the treatment of cocaine "craving," accentuate the possibility that adrenergic as well as dopaminergic (DA) neuronal systems play a role in the subjective effects of cocaine. Thus, the present study was concerned with the extent to which the discriminative stimulus properties of this substance (in rats) resemble (generalize to) those of several compounds that act directly or indirectly as NE or DA agonists. Neither the relatively selective NE reuptake inhibitor nisoxetine nor the less selective uptake inhibitors desipramine and imipramine substituted for cocaine (10 mg/kg, *i.p.*) in a two-lever, water-reinforced (FR 20), drug discrimination procedure. However, the cocaine cue generalized to buproprion and nomifensine, both of which selectively inhibit DA reuptake. These data suggest that DA is involved in the subjective effects of cocaine; additional tests, which indicate that neither the direct D_2 agonist bromocriptine nor the indirect DA agonist amantadine substitute for cocaine, suggest that these mechanisms are more likely to concern DA reuptake than direct receptor activation.

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103.10

THE EFFECTS OF AMPHETAMINE AND CATHINONE ON IN VIVO DOPAMINE RELEASE AND METABOLISM. E.A. Pehek, B.K. Yamamoto and M.D. Schechter. Department of Pharmacology, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272.

The major psychoactive alkaloid of the Khat plant is (-)cathinone (CATH). This plant grows in Africa and Asia where the leaves are chewed by some residents, producing an amphetamine (AMPH)-like euphoric state. Previous studies have shown that CATH's mechanism of action is similar to that of AMPH, but findings regarding the potency of CATH relative to AMPH are equivocal.

The present study employed *in vivo* microdialysis to compare the actions of equimolar doses of CATH and AMPH on extracellular DA, DOPAC, HVA, and 5-HIAA levels in the urethane-anesthetized rat. Both drugs increased DA and decreased DOPAC and HVA levels. However, CATH had a longer lasting effect on these amines in the caudate nucleus whereas AMPH had a greater effect in the nucleus accumbens. These findings demonstrate the importance of examining different brain areas when comparing psychostimulant drug effects.

103.11

CHARACTERIZATION OF COCAINE WITHDRAWAL: Dopamine function. Authors: Palumbo JM, Price LH, Woods SW, Kosten T, Krystal JH, Charney DS, Klebèr H (Sponsor: Sewitch,DE) Dept. of Psychiatry, Yale Univ. Sch. of Med., 34 Park St., New Haven, CT 06508

Between 15 and 22 million Americans have used cocaine at least once . 17% of all college students are said to use cocaine. To our knowledge, this study represents the first systematic description of dopamine metabolism following discontinuation of cocaine in hospitalized adult human chronic cocaine users.**Methods:** Men and women, ages 18 to 45, with a history of continuous cocaine abuse for a period of at least the 12 months immediately preceding entrance into the study, and a minimum consumption of at least 5 grams of cocaine per week were entered into the study provided that they demonstrated no significant medical or psychiatric disorders. Also excluded were patients with intercurrent use of other substances. 12 patients were entered into the study. All tested positive for cocaine, and negative for other substances, at the time of admission. All patients had used cocaine during the 24-36 hrs immediately prior to admission. Neurochemical studies: On admission, and at 8 AM Monday, Wednesday, and Friday, blood was drawn for assays of prolactin (PRL), growth hormone (GH), and homovanillic acid (HVA). **RESULTS:** Data were analyzed using Wilcoxon signed ranks tests with measurements made with respect to admission values for PRL, GH, and HVA. Significant increases from admission values were noted for the meaned interval day 10-12, and the meaned interval day 13-15 for HVA. Similar statistically significantly increases in PRL were noted during each of the three day intervals from day 13 through 21.**CONCLUSION:** Changes in dopamine function occur during cocaine discontinuation. Significant increases in HVA and PRL are both noted after day 12 of admission. These data do not support the conclusions of Mendelson, et al, who demonstrated persistent hyperprolactinemia during cocaine abstinence (1). Continued data analysis is ongoing in our facility. It is likely that additional patients will need to be studies to further understand the findings of the current study.

(1).Mendelson JK, et al.: *American Journal of Psychiatry* 145:9/1988

103.12

DOPAMINERGIC TREATMENTS IN COCAINE WITHDRAWAL. J.A. Cocores,* M.S. Gold, A.L.C. Pottash. Fair Oaks Hospital, Summit, NJ 07901 & Delray Beach, FL 33484.

Cocaine smoking is the most difficult to treat and addictive form of cocaine abuse (Verebey K & Gold MS, Psych Annals 18(9):513-520, 1988). After cocaine smoking very high brain concentrations are reached faster, resulting in greater behavioral effects than those experienced after cocaine snorting. Rapid mood alternation between positive and negative effects makes continued use or relapse likely. Forty consecutive crack cocaine addicts were assigned in groups to structured, outpatient treatment (S.O.T.) alone, S.O.T. with bromocriptine (1.25 mg bid x 21 days) and S.O.T. with levodopa (0.1gm tid x 21 days) to determine whether the high dropout rates for cocaine smokers could be reduced. We have previously reported S.O.T. dropout rates of 200% greater for cocaine smokers when compared to a sociodemographic matched group of snorters (Cocores JA & Gold MS, AMSAODD, 1989). Treatment with bromo or l-dopa during the earliest phases of cocaine rehabilitation may decrease relapse and improve treatment success rates. Further studies are necessary.

Treatment	Drug free at 28 days	Drug free at 120 days
S.O.T./Bromocriptine	8/10 = 80%	6/10 = 60%
S.O.T./Levodopa	8/10 = 80%	7/10 = 70%
S.O.T. alone	11/20 = 55%	6/20 - 30%

103.13

EFFECTS OF A NOVEL DOPAMINE AGONIST, SANDOZ 205-152, ON ETHANOL SELF-ADMINISTRATION. S. Rassnick*, L. Pulvirenti, and G.F. Koob (SPON, K. T. Britton). Research Institute of Scripps Clinic, La Jolla, CA 92037.

Previous evidence suggests an important role for the brain dopamine system in drug reinforcement. To examine whether pharmacological manipulation of the dopamine system would affect ethanol (EtOH) reinforcement in rats, we studied the effect of SDZ 205-152, a novel dopamine receptor agonist on oral EtOH self-administration in rats. A two-lever free choice task was used: responses at one lever delivered EtOH (10% w/v), while responses at the other lever delivered water during a 30 min. daily session. This model of oral self-adminstration of EtOH with limited access in non dependent animals represents a behavioral analogue of EtOH drinking in humans and is a valuable tool to examine CNS pharmacological actions without caloric value, taste or smell as confounds. SDZ 205-152 was administered at doses of 0.5, 1.0, 2.0 and 5.0 mg/kg sc 30 minutes prior to testing. The drug produced differential effects on responding for EtOH and water. Responding for EtOH was reduced by the treatment [$F(1,54) = 63.84$, $p<.001$], and there was a significant treatment vs dose interaction [$F(4,54)=4.498$, $p<0.01$]. Post hoc Newman-Keuls tests revealed that SDZ 205-152 at all doses tested significantly decreased EtOH drinking compared to baseline ($p<0.001$ in all cases). The lack of significance of SDZ 205-152 on responses for water suggests that nonspecific effects of this drug on motor performance cannot account for the reduction of responses for EtOH. These results contribute further support for the role of dopamine as a possible neurochemical substrate mediating EtOH reinforcement (This research was supported in part by NIDA 06420 and Sandoz Pharmaceuticals Corporation).

103.14

d-AMPHETAMINE AND MORPHINE WITHDRAWAL AGGRESSION: ROLE OF DOPAMINE RECEPTORS. J. W. Tidey* and K. A. Miczek (SPON: L. Erinoff). Dept. of Psych., Tufts University, Medford MA 02155.

Opiate withdrawal causes profound physiological and behavioral effects, including disruptions of motor activity and heightened aggressivity. Each of these effects is most sensitive to *d*-amphetamine at different times of opiate withdrawal. Male mice which had been pair-housed with females for 3 weeks were implanted s.c. with 75 mg morphine or placebo pellets for 3 days. Mice were challenged 5, 48 or 96 hours after pellet removal with *d*-amphetamine (0.3-3.0 mg/kg), SKF38393 (3.0-100 mg/kg) or quinpirole (0.1-1.0 mg/kg). The mice were videotaped alone in their home cages for five minutes, followed by a five minute period of interaction with a group-housed male "intruder". Rearing and walking were decreased at 5 hours but restored to control levels by 48 hours after pellet removal. Increases in threat and attack behaviors, however, were most enhanced at 48 hours and were still higher than control levels 96 hours after pellet removal. In placebo-implanted mice, *d*-amphetamine dose-dependently decreased grooming, increased walking and decreased or did not affect threats and attacks. In mice undergoing morphine withdrawal, *d*-amphetamine restored the suppressed rearing and walking seen at 5 hours of withdrawal to control levels and dose-dependently decreased grooming. Heightened aggressivity during morphine withdrawal was increased by 0.3 and 1.0 mg/kg and decreased by 3.0 mg/kg *d*-amphetamine at 5 and 48 hours after pellet removal. These effects are qualitatively similar to results with quinpirole at 5 hours after pellet removal, implicating D2 dopamine receptor mediation.

103.15

ENDOGENOUS CRF: ROLE IN STRESS- AND AMPHETAMINE- INDUCED SENSITIZATION OF FOREBRAIN DOPAMINE SYSTEMS. B J Cole[1]*M Cador[2]*L Stinus[2] G F Koob[1]M LeMoal[2]. (SPON: D A MacNiel). [1]Scripps Clinic and Research Foundation La Jolla, CA 92037. [2]INSERM U259 Bordeaux France.

Some aspects of affective disorders show a pattern of increasing pathology over time, and the animal model of behavioral sensitization has been suggested to provide a useful analogy to this longitudinal aspect of affective disorders. Behavioral sensitization refers to the observation that repeated administration of certain drugs produces a progressive increase in their effects on behavior. As CRF has been implicated in behavioral responses to stress, and stress shows cross sensitization with amphetamine (AMPH), we investigated the role of endogenous CRF in behavioral sensitization, using the CRF antagonist, alpha-helical CRF. In Experiment 1, rats were injected ICV with either 25µg alpha-helical CRF or vehicle, and then either restrained or returned to their home cage, once per day for 5 consecutive days. Starting 7 days later, they were tested for their locomotor response to systemic administration of saline and 0.75 mg/kg AMPH, and their stereotyped response to 3.0 mg/kg AMPH. The vehicle treated restrained rats showed a significantly greater locomotor response to saline, and a significantly greater stereotyped response to 3.0 mg/kg AMPH. These effects were reversed by the CRF antagonist. However, there was no effect of prior restraint on AMPH (0.75 mg/kg) induced locomotor activity. In Experiment 2, rats were initially injected ICV with either 25 µg alpha-helical CRF or vehicle, and systemically with 3.0 mg/kg AMPH or saline. They were then tested as in Experiment 1. The CRF antagonist was found to severely attenuate the AMPH-induced sensitization of both locomotor activity and stereotypy. These results suggest that although there are some behavioral differences between the nature of restraint stress- and AMPH-induced sensitization, central administration of the CRF antagonist significantly attenuates the development of both forms of sensitization.

103.16

CENTRAL ADMINISTRATION OF CRF PRODUCES SENSITIZATION OF AMPHETAMINE-INDUCED BEHAVIOR. M Cador[1]*B J Cole[2]*G F Koob[2] L Stinus[1]M LeMoal[1]. (SPON: M A Geyer). [1]INSERM U259 Bordeaux France. [2]Scripps Clinic and Research Foundation La Jolla, CA 92037.

The polypeptide corticotropin releasing factor (CRF) is the primary hypothalamic releasing factor involved in mobilizing the pituitary-adrenal axis response to stress. CRF has also been localized in extrahypothalamic regions of the CNS, where it is throught to play a crucial role in initiating behavioral responses to stress. The purpose of the present experiments was to examine whether central administration of CRF, like stress, would produce sensitization of the forebrain dopamine systems. Rats were initially given 5 ICV injections of CRF (0, 0.5 or 2.5µg). The injections were given on consecutive days and the rats locomotor activity was measured in photocell cages for 120 min after each injection. Seven days after the last CRF infusion, the rats locomotor response to systemically administered saline was measured. Two days later, half the rats were tested for their locomotor response to 0.75 mg/kg amphetamine (AMPH), and the other half were tested for their stereotyped response to 3 mg/kg AMPH. The rats treated with 2.5µg CRF showed a progressive increase in their locomotor activity over the 5 days of testing, while rapid tolerance developed to the hyperactivity induced by 0.5µg CRF. Furthermore, in comparison to rats pretreated with 0 and 0.5µg CRF, the rats pretreated with 2.5µg CRF showed a greater locomotor response to saline and 0.75 mg/kg AMPH, and a more intense stereotyped response to 3 mg/kg AMPH. The implications of the results for individual differences in the propensity to show stress-induced sensitization will be discussed.

DRUGS OF ABUSE: COCAINE I

104.1

COCAINE TOXICITY AFTER PRE- AND POST-NATAL VALPROIC ACID (VPA) EXPOSURE. S.K. Sobrian, N.K. Robinson*, L.E. Burton*, H. James* and A.K.N. Nandedkar*. Dept. of Pharm. Howard Univ. Coll. of Med., Washington, D.C. 20059.

We have reported that VPA pretreatment (VPA-P) prevents cocaine-induced convulsions (CICs) but increases cocaine fatalities two-fold. Prenatal VPA protects offspring against VPA/cocaine fatalities but reduces the anticonvulsant's postnatal efficacy. Offspring of rats exposed to VPA (PV) or water (control:PC) on gestation days 15-21 were treated at 60 or 180 days of age with 200 mg/kg of VPA, or vehicle, 30 min prior to a 60mg/kg i.p. dose of cocaine. Fatalities and convulsions were scored and compared with EKG and enzyme activity from serum taken immediately after behavioral testing. Total lactate dehydrogenase (LD) was elevated in animals that died without CICs. Liver enzymes (alanine aminotransferase: ALT) were increased in animals that convulsed but decreased in animals that died (gamma glutamyl transpeptidase: GGT). Neither total creatinine kinase (CK), CK-MM, (muscle), CK-MB (cardiac) nor EKG parameters correlated with cocaine toxicity. However, CK-MB was increased in all cocaine rats and CK-BB (brain) was increased by CICs. Results suggest that cardiac involvement in cocaine fatalities may be secondary to derangement in liver function. The increase in CK-BB following CICs implicates the CNS as a major mediator of cocaine toxicity, and introduces this isoenzyme as a serum marker for abnormal brain activity.

104.2

REGIONAL BINDING OF THE HIGH AFFINITY COCAINE ANALOG, [^{3}H]CFT, IN MONKEY BRAIN. D.R. Canfield*, R.D. Spealman*, B.K. Madras. (SPON: J.L. Neumeyer) Harvard Medical School, New England Regional Primate Research Center, Southborough, MA 01772.

[^{3}H]CFT (also designated WIN 35,428) binds with high affinity to cocaine receptors in monkey caudate-putamen. Regional distribution of [^{3}H]CFT binding was assessed on slide-mounted brain tissue (6 µm sections) of an adult monkey (Saguinus oedipus). Sections were selected from three areas: most anteriorly from the level of the nucleus accumbens, 2) just posterior to the optic chiasm at the level of the amygdala, 3) most posteriorly just caudal to the globus pallidus. Alternate sections from each area were incubated for 2 h at 4°C in the presence of [^{3}H]CFT (6 nM) alone or with unlabeled (-)-cocaine (50 µM). After washing, the slides were dipped in emulsion, stored in the dark for 4 weeks, developed, and examined for regional distribution of cocaine-specific binding. Binding of [^{3}H]CFT, which was specifically inhibited by (-)-cocaine, was most convincingly demonstrated in caudate nucleus, putamen, and nucleus accumbens septi. Binding was considerably lower in cortex, thalamic nuclei and white matter tracts. These findings indicate that [^{3}H]CFT binding is prominent in brain areas implicated in the stimulant effects and abuse liability of cocaine. (Supported by DA05648, DA00499, DA00088, RR00168, RR07000)

104.3

LONG-SLEEP AND SHORT-SLEEP MICE DIFFER IN RESPONSE TO HIGH DOSE EFFECTS OF COCAINE. C.M. de Fiebre, J.A. Ruth, and A.C. Collins. Inst. for Behavioral Genetics and Sch. of Pharmacy, Univ. of Colorado, Boulder, CO 80309.

Cocaine is often used simultaneously with ethanol. In an attempt to test whether common genes regulate sensitivity to both these drugs, we have measured the cocaine sensitivity of the long-sleep (LS) and short-sleep (SS) mouse lines which were selectively bred for differential ethanol-induced "sleep-time". In a battery of behavioral and physiological tests, differences between these mouse lines were subtle except at high doses where SS mice displayed greater sensitivity. Consistent with this finding, SS mice were found to be more sensitive to the seizure-producing effects of cocaine (ED_{50}'s: SS: 43 ± 3; LS: 65 ± 9 mg/kg). While the LS and SS mouse lines did not differ in sensitivity to the inhibition by cocaine of uptake of the monoamine neurotransmitters [^{3}H]dopamine, [^{3}H]norepinephrine and [^{3}H]5-hydroxytryptamine, SS mice were found to be more sensitive to the seizure-producing effects of lidocaine, a local anesthetic (ED_{50}'s: SS: 51 ± 4; LS: 76 ± 11 mg/kg). Therefore, it is concluded that the differential sensitivity of these mice to cocaine is due to differential sensitivity to cocaine's local anesthetic effects.
Supported by AA-06391, MH-16880 and DA-00116.

104.4

MODULATION OF THE LETHAL EFFECTS OF COCAINE BY CHOLINOMIMETICS. S. R. Goldberg*, M. J. Kuhar, J. L. Katz*, J. M. Witkin* (SPON: S. Bird). NIDA Addiction Research Center, Baltimore, MD 21224.

The finding that (-)-cocaine has antimuscarinic effects at high doses led to the suggestion that these actions may contribute to cocaine toxicity (Sharkey et al., J. Pharmacol. Exp. Ther. 246: 1048, 1988). This study was designed to elaborate on the possible involvement of muscarinic receptors in the lethal effects of cocaine. Cocaine HCl had an LD 50 of 81.7 mg/kg (95% CL: 77.3 - 86.4), ip, in male F344 rats. Atropine (1 - 30 mg/kg) did not alter the lethal effects of cocaine at doses that were effective in preventing oxotremorine-induced death. Physostigmine and oxotremorine markedly potentiated lethality of cocaine at doses devoid of convulsive or lethal effects of their own (1 and 0.3 mg/kg, respectively). At lower doses, pretreatment with physostigmine (0.3 mg/kg) but not oxotremorine increased the LD 50 of cocaine to 99.8 mg/kg (95% CL: 87.9 - 113.3). This prophylactic effect of physostigmine was not prevented by atropine. Protection against lethality was also conferred by 0.1 mg/kg neostigmine. These results suggest that whereas cocaine lethality may be related to muscarinic receptors, protection against death appears to involve nonmuscarinic receptors in the periphery.

104.5

DISCRIMINATIVE STIMULUS EFFECTS OF INHALED COCAINE IN SQUIRREL MONKEYS. J.L. Katz*, L. Sharpe, J.H. Jaffe*, and J.M. Witkin*. (SPON: J.E. Moreton). NIDA Addiction Research Center, POB 5180, Baltimore, MD 21224.

Squirrel monkeys (N=4) were trained with food reinforcement to press one of two levers after administration of i.v. cocaine (0.3 or 1.0 mg/kg) or the other lever after saline. After training, i.v. cocaine (0.03 - 3.0 mg/kg) produced dose-related increases in the percentage of responses on the cocaine lever (ED 50=0.15 mg/kg). Cocaine, delivered i.m. also produced dose-related increases in cocaine-appropriate responding (ED 50=0.32 mg/kg), but was less than half as potent (RP=0.42) as i.v. cocaine. Similar relative potency relations were obtained for decreases in response rates produced by cocaine. Prior to some sessions subjects were placed in a Plexiglas chamber and exposed for 60 sec to cocaine vapor created with an ultrasonic nebulizer. Exposure to vapor from cocaine solutions produced concentration-dependent increases in cocaine-appropriate responding and decreases in response rates. Exposure to vapor from a 30 mg/ml concentration produced virtually exclusive cocaine-appropriate responding. Concentration-effect curves for inhaled cocaine were not appreciably different from dose-effect curves obtained when cocaine was administered by the other routes. The minimally effective concentration of inhaled cocaine was examined at different times after exposure in one subject. In this subject, inhaled cocaine had a duration of action longer than i.v. cocaine. The results indicate that inhaled cocaine vapor has effects qualitatively similar to those of i.v. cocaine, and may have a duration of action longer than that of a functionally equivalent i.v. dose of cocaine.

104.6

DOPAMINE-1 RECEPTOR SPECIFIC INVOLVEMENT IN THE LETHAL EFFECTS OF COCAINE. J. M. Witkin,* S. R. Goldberg,* J. H. Jaffe,* and J. L. Katz* (SPON: C. W. Schindler). NIDA Addiction Research Center, POB 5180, Baltimore, MD 21224.

The D1 antagonist, SCH 23390, protects against the lethal effects of cocaine in rats; the LD 50 of cocaine HCl is increased by 20 mg/kg. In contrast, the D2 antagonist, haloperidol, is inactive against cocaine at doses which protect against amphetamine-induced lethality (Witkin et al., Life Sci. 44: 1285, 1989). The present experiments were designed to evaluate further the role of dopaminergic neurotransmission in cocaine-induced lethality. In male Swiss Webster mice, cocaine i.p. produced convulsions (ED 50 = 53 mg/kg) and lethality (LD 50 = 73 mg/kg). The dopamine uptake inhibitor, mazindol, shifted the dose-effect functions for both convulsions and lethality to the left and produced a dose-dependent potentiation of the toxic effects of cocaine. In male F344 rats, SCH 23388 which binds to D1 receptors with a 600-fold lower affinity than SCH 23390, did not alter the lethality of cocaine when given in doses 30 times higher than required for effective protection by the active enantiomer. The related D1-selective antagonist, SKF 83566, was also effective in increasing the LD 50 of cocaine. Lidocaine, a local anesthetic devoid of dopaminergic activity, also produced dose-dependent lethality. However, death induced by lidocaine was not altered by pretreatment with SCH 23390. The results of these experiments provide further support for a selective role of D1 receptors in the lethal effects of cocaine.

104.7

AN INVESTIGATION OF SENSITIZATION AS A PROCESS UNDERLYING ACQUISITION OF COCAINE SELF-ADMINISTRATION B. Horger, K. Shelton*, H. Bonavera*, and S. Schenk, Texas A&M University, Dept. of Psychology, College Station, TX 77843.

This experiment examined the role of sensitization in the acquisition of cocaine self-administration. Rats were injected daily with either cocaine HCl (10 mg/kg. i.p.) or the saline vehicle. Some were tested for the motor activating effects of cocaine during a 15 day treatment regimen. Others were tested for acquisition of intravenous cocaine self-administration following 9 days of chronic pre-treatment. Sensitization to the activating effects of cocaine in the locomotor tests was apparent after 3 days and became more pronounced with repeated exposure. In the self-administration tests, a higher percentage of cocaine pre-treated rats acquired the operant when compared to the saline pre-treated rats. Several of the cocaine pre-treated animals did not acquire until the original dose of 1.0 mg/kg/infusion was decreased. However, it is not clear as to whether this effect was do to a shift to the left in the dose response curve or simply a delay in latency to acquisition. Experiments are now being conducted to investigate these possibilities. HPLC analysis of the prefrontal cortex, nucleus accumbens and striatum failed to reveal differences in turnover or levels of dopamine between cocaine and saline treated rats. Thus, the sensitization effect may be due to postsynaptic rather than presynaptic alterations in dopamine functioning.

104.8

PRENATAL COCAINE EXPOSURE IN RATS: SENSORINEURAL HEARING LOSS AS EVIDENCED BY THE BRAINSTEM AUDITORY EVOKED POTENTIAL. M. W. Church and G. W. Overbeck.* Fetal Alcohol Research Center, Wayne State Univ. Sch. Med., Detroit, MI 48201.

Pregnant Long-Evans rats were injected daily with 60, 80 or 100 mg/kg cocaine HCl (s.c., 2% solution) from gestational days 7-20 (sperm positive = day 0). Daily doses were split evenly with half given between 9-10 a.m. and half between 3-4 p.m. An Ad Lib-fed group as well as nutritional control groups that were Pair-Fed to the 80 and 100 mg/kg Cocaine dams were also evaluated. One male and one female offspring from each litter were evaluated by brainstem auditory evoked potentials (BAEPs). Stimuli were clicks (0.1 msec) and tone pips (2000, 4000, 8000 Hz).

None of the animals in the Ad Lib, Pair-Fed, or the 60 mg/kg/day Cocaine groups had hearing loss (N = 12-15/group). So far, 4 of 20 offspring (20%) in the 80 mg/kg/day Cocaine group have tested positive for sensorineural hearing loss as evidenced by significant elevations in BAEP thresholds (ranging from 12-23 dB) and recruitment-type latency-intensity profiles. BAEPs in response to tone pips revealed a mixed picture of hearing loss in regards to pitch. One animal had the greatest hearing loss at 8000 Hz; another had the greatest hearing loss at 2000 Hz; the remaining two animals had roughly equal hearing losses at 2000, 4000 and 8000 Hz. Elevated BAEP thresholds are also being observed in the 100 mg/kg/day Cocaine group. (Supported by DA05536).

104.9

PRENATAL COCAINE EXPOSURE IN RATS: NEUROBEHAVIORAL EFFECTS. G. W. Overbeck,* M. W. Church, and A. L. Andrzejczak.* (SPON: C. S. Zajac), Fetal Alcohol Research Center, Wayne State Univ. Sch. Med., Detroit, MI 48201.

Pregnant Long-Evans rats were injected daily with 40, 60, 80 or 100 mg/kg cocaine HCl (s.c., 2% solution) from gestational days 7-20 (sperm positive = Day 0). Daily doses were split evenly with half given between 9-10 a.m. and half between 3-4 p.m. An Ad Lib-fed group as well as nutritional control groups that were Pair-Fed to the 80 and 100 mg/kg Cocaine dams were also evaluated (N=11-18 litters/group).

The **negative geotaxic reaction** of the offspring, evaluated from Day 2-14 (birth = Day 0), showed no group differences. **Spontaneous alternation** behavior showed no evidence of perseveration in any group on either Day 21 or Day 80. The Cocaine-Treated offspring generally showed shorter starting latencies on Day 21. **Activity monitor** behavior showed that the Cocaine-Treated and Pair-Fed offspring were hypoactive on Day 20. Some degree of hypoactivity was still evident on Day 49, but absent on Day 80. The **passive avoidance** behavior of Day 19 offspring showed no group differences in acquisition of task learning. The 100 mg/kg Cocaine offspring did show significantly poorer retention of task learning 48 hrs later. On Day 80, no group differences were seen in passive avoidance behavior. Acquisition of an **active avoidance** behavior on Day 80 was significantly poorer in the 100 mg/kg Cocaine group. (Supported by DA05536).

104.10

TIME COURSE OF COCAINE-INDUCED CHANGES IN BRAIN STIMULATION REWARD. R.A. Frank, P. Manderscheid & H.P. Williams*. Dept. of Psychology & Psychiatry, Univ. of Cincinnati, Cincinnati, OH 45221.

The time course of cocaine's mood altering effects was evaluated by assessing changes in self-stimulation thresholds over a period of chronic cocaine administration. Self-stimulation train-duration thresholds were determined for rats implanted with ventral tegmental area electrodes following injection of saline or 15-30 mg/kg cocaine HCl (IP). Testing times ranged from 15-435 min post-injection, and the animals were tested with cocaine for 18 consecutive days. Cocaine lowered thresholds during the initial post-injection tests across the entire 18 days of drug administration, a finding that supports the view that cocaine-induced euphoria does not exhibit tolerance with repeated drug use. In addition, cocaine treatment increased thresholds 6 to 7 hr post-injection, but only after 9 days of drug administration. This finding suggest that cocaine use can lead to rebound dysphoria, but only after an extended period of drug use.

This research was supported by NIDA grant # DA 04483 to R.A. Frank.

104.11

DIAZEPAM PRETREATMENT REDUCES THE CONFLICT BEHAVIOR OBSERVED IN RATS TRAVERSING A STRAIGHT-ALLEY FOR INTRAVENOUS COCAINE REWARD. T.Geist* and A.Ettenberg (SPON:G.Austin). Dept. Psychology, Univ. California, Santa Barbara, CA 93106.

Male rats implanted with jugular cannulae were trained to traverse a 5 ft runway for intravenous cocaine reward. Each animal was tested on a single trial per day during which the following dependent measures were recorded: the latency to initiate responding (i.e. to leave the start box), the latency to enter the goal box (once response initiation had occurred), the number of "retreats" (i.e. reversals in course back toward the start box), and the location within the alley where such retreats occurred. Once in the goal box, the passage back into the alley was blocked and a reward of five IV injections (5 s duration) of 0.75 mg/kg cocaine were administered at 30 s intervals. The reinforcing value of this treatment was determined by reductions in start latencies over trials and confirmed by conditioned place preference upon completion of the experiment. Although animals continued to run for cocaine reward, "retreat" behavior increased over trials with the probability of a "retreat" rising dramatically as a positive function of an animal's proximity to the goal box. This behavior was dose-dependently attenuated by pretreatments with IP diazepam (0.5, 1.0, 2.0 mg/kg). Our results suggest that animals experience a state of "conflict" when placed into the runway prior to reward presentation -- an effect presumably resulting from concurrent positive and negative consequences of cocaine administration.

104.12

ATYPICAL FETAL BREATHING PATTERNS ASSOCIATED WITH IN UTERO COCAINE EXPOSURE. J Gingras, R Hume*, K O'Donnell*, C Stanger* Dept of Peds, OB/GYN, Psychiatry, Duke U Med Ctr, Durham, NC 27710.

Fetal breathing demonstrates a specific ontogenetic pattern, is episodic, and is characterized by irregular rate with periods of apnea. In utero cocaine exposure is associated with poor obstetrical and neonatal outcome; increased incidence of SIDS has been suggested by some. We have observed a high incidence of abnormal breathing patterns in cocaine exposed fetuses, supporting the hypothesis that cocaine affects respiratory control. 30 cocaine using women were identified prenatally; serial ultrasound evaluations of fetal growth and behavior were recorded, analyzed with attention to patterns of breathing, and compared to controls. In 7/30 fetuses, fetal breathing was abnormal, characterized by persistent, rhythmic, hyperpneic respirations or by repetitive yawning that crossed behavioral states. The abnormal patterns were seen as early as 26 weeks of gestation and as late as 38 weeks in the exposed group, were not observed in controls, and were not associated with poor growth. All infants demonstrating abnormal breathing patterns were scored as abnormal in neurobehavioral exams in the neonatal period. These observations suggest that in utero cocaine exposure disrupts central neural mechanisms important in respiratory regulation.

104.13

VIOLENT BEHAVIORS AND COCAINE. N.S. Miller,* M.S. Gold, P. Herridge.* Cornell University Medical School, White Plains, NY 10605; Fair Oaks Hospital, Summit, NJ 07901.

Violent behaviors have been associated with cocaine use. The violent behaviors ranged from psychological aggressions to physical acts including murder of current cocaine users. A study examined by structured telephone interview 452 consecutive male cocaine users' reports of violent behaviors associated with cocaine. The mean age was 28.3 years. The route of administration for cocaine was intranasal=159, intravenous=32, freebase=136, crack=66, combination=66. As a group without regard to route of administration, the following effects induced by cocaine were confirmed. Cocaine made them: angry (42%), violent (32%), suspicious/paranoid (84%), stronger (32%), commit violent crimes (46%) (types of violent crimes; physical fights=23%, attempted murder=1%, armed robbery=22%, violent arguments=25%, verbal arguments=33%, child abuse=1%, wife abuse=7%, murder= less than 1%, rape=1%, robbery=14%); time of last use in relation to committing crime; during or immediately after use (30%), during withdrawal (19%); cocaine causes- to carry a weapon (17%), to see an ER or MD (22%), to commit violence (83%), violence to get money to pay for cocaine (82%). Crack users report more violent acts than intranasal users. Other characteristics of violence by route of administration are presented (Verebey K, Gold MS, Psych Annals 18(9):513-520, 1988).

104.14

ENVIRONMENTAL CUES INFLUENCE COCAINE- AND PROCAINE-MEDIATED CONDITIONED PLACE PREFERENCE. M. Coxen*, J.M. Carney and T.W. Seale (Spon: M.E. O'Connor). Univ. of Oklahoma Hlth. Sci. Ctr., Oklahoma City, OK 73190.

The conditioned place preference (CPP) paradigm is a behavioral assay for drug-seeking behavior in animals. Specific context variables - i.e. environmental manipulations used to provide visual, olfactory and tactile cues - were characterized in 8 week-old male BALB/cByJ inbred mice. Mice were conditioned in 3 compartment chambers in which the compartment cues were hue (black, white, or gray) floor texture (wire mesh, smooth floor or wood chips), and scent (pine, cedar or none). Animals were conditioned for three daily trials with vehicle in one compartment and an optimal dose of cocaine (32 mg/kg ip) or procaine (100 mg/kg ip) in the other compartment. In one cue configuration, positive CPP of large magnitude (84% of total time spent in the drug-paired compartment; 1517 $\pm$ 94 seconds in cocaine treated animals versus 441 $\pm$ 70 seconds in vehicle treated animals) was appropriately paired with either compartment associated with drug administration. In another cue configuration, positive CPP of comparable magnitude occurred in one compartment but no CPP was observed when cocaine dosing was paired with cues in a second compartment. Tactile cues appeared to have the most salience. Cocaine and procaine induced identical CPP behavior across the various cue configurations. These data establish that environmental stimuli can markedly alter drug-induced CPP.

104.15

ELECTROPHYSIOLOGICAL ACTIONS OF COCAINE ON RAT DENTATE GRANULE NEURONS MEASURED INTRACELLULARLY IN VITRO. S.S. Jahromi,* and P.L. Carlen. Playfair Neuroscience Unit, Addiction Research Foundation, Departments of Physiology and Medicine (Neurology), The Toronto Western Hospital, University of Toronto, Toronto, Ontario, M5T 2S8.

Cocaine, a widely abused drug, has local anesthetic actions at high doses, and at low doses, it acts as a central nervous system stimulant. Having already studied the excitatory actions on dentate granule (DG) neurons of amphetamine (Jahromi et al, Soc. Neurosci. 14, 809) which is supposed to act similarly to cocaine, we have now studied the actions of cocaine on these neurons.

Cocaine (10 or 20 µM) was perfused onto DG neurons at 36°C. In the majority of cells, a few mV hyperpolarization occurred within about 20 min. The amplitudes of EPSPs and IPSPs elicited from perforant path stimulation were both decreased from 10% up to 40%. All effects were reversible upon drug washout. There was no significant reduction of the post-spike train afterhyperpolarization or spike frequency adaptation by cocaine perfusion. Contrary to what was expected, the above measured inhibitory actions of cocaine were essentially opposite from the excitatory actions of amphetamine on these neurons.

Supported by the Hospital for Sick Children Research Foundation and the MRC.

104.16

ALTERATIONS IN RAT BRAIN METABOLIC ACTIVITY FOLLOWING ACUTE AND CHRONIC COCAINE. A. Pert, S. Weiss, and R. Post. Biological Psychiatry Branch, NIMH, Bethesda MD 20892

The purpose of this study was to compare the effects of acute and chronic cocaine injections on the regional metabolic activity of rat brain. Different groups of rats were injected with either saline (i.p.), a single dose of cocaine (40 mg/kg), this dose for 15 days, or this dose of cocaine until the development of motor seizures. Standard 2-DG autoradiographic procedures were used to visualize ^{14}C-2DG uptake. Acute injections of cocaine increased metabolic activity in the anterior olfactory nuclei, frontoparietal motor cortex, striatum, lateral septum, globus pallidus, dorsolateral and ventrolateral thalamic nuclei, ZR and ZC of the substantia nigra, red n. and various pontine reticular nuclei. Activity decreased, on the other hand, in the dorsomedial thalamic n., lateral habenula and VTA. Chronic injections of cocaine produced a relatively similar profile. In addition, increases in activity were also seen in the ventral pallidum, subthalamic nucleus, PAG, superior colliculus and cerebellum. Alterations in behavior following chronic cocaine injections are likely related to these alterations in regional metabolic activity. The most striking changes noted during cocaine seizures were increases in metabolic activity of the n. accumbens, olfactory nuclei, and ventral hippocampus in some rats.

104.17

FACILITATORY EFFECT OF POSTTRAINING COCAINE ON MEMORY
I.B.Introini-Collison and J.L.McGaugh, Center for the Neurobiology of Learning and Memory and Department of Psychobiology, University of California, Irvine, CA 92717.

In previous studies we have found that retention is influenced by treatments affecting monoaminergic systems. The present experiments examined the effect of cocaine on retention. Mice were trained in a one-trial step-through inhibitory avoidance task and given immediate posttraining injections of cocaine (0.03-1.0 mg/kg). On a retention test 24 hr later, the retention latencies of mice given the 0.10 mg/kg dose were significantly higher than those of the controls. The effect of cocaine on retention was time-dependent: retention latencies were not altered in animals given cocaine 60 minutes after training, ruling out a proactive effect of cocaine on retention. Administration of cocaine (0.1 mg/kg) prior to the retention test did not modify the retention performance of mice that received either saline or cocaine (0.1 mg/kg) immediately posttraining. The findings suggest that cocaine affects retention by influencing posttraining processes involved in memory storage.

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104.18

EFFECTS OF PRENATAL EXPOSURE TO COCAINE OR RELATED DRUGS ON RAT DEVELOPMENTAL AND NEUROLOGICAL INDICES. M. G. Henderson* and B. A. McMillen. (Spon. by J. P. DaVanzo), Dept. of Pharmacol., School of Medicine, East Carolina Univ., Greenville, NC 27858.

Gravid rats were injected s.c. with either cocaine (15 mg/kg b.i.d.), amitryptiline (10 mg/kg), amfonelic acid (1.5 mg/kg) or saline from gestational day 2 until parturition. Upon birth, 20 male pups from 3 mothers were cross-fostered to 2 surrogates. The pups were tested at birth and at 30, 60 and 180 days postnatal. Prenatal cocaine resulted in 4 of the 6 stillbirths. The AFA groups had a significantly smaller litter size while the other groups showed no differences. There were no differences in the ratio of male to female pups as compared to controls. Righting reflex was attenuated in the cocaine exposed pups as a group, but there was no difference in the median score, which suggests that the delay was due to outliers. The activity data showed that the cocaine pups had significantly increased spontaneous locomotor activity at 30 days: both during lights on and off. Beta 1 and 2 binding in the cortex revealed no changes at 30 days in any groups as compared to control. Preliminary results on cortex membrane Na/K ATPase activity show a trend towards increased activity in the cocaine exposed pups at 30 days. These data indicate that prenatal cocaine may cause some subtle abnormalities in rat development. (Supported by DA-04895)

104.19

EFFECT OF COCAINE ON ACETYLCHOLINE AND CHOLINE LEVELS FOLLOWING CHOLINESTERASE BLOCKADE. R.B. MILLER*, S.G. HOWARD AND C.L. BLANK, Dept. of Chemistry and Biochemistry, U. of Oklahoma, Norman, OK 73019, and Dept. of Pharmacology, UCLA, Los Angeles, CA 95432.

The normal human metabolism of cocaine involves ester cleavage by serum and other cholinesterases. Individuals at risk in the commonly encountered multiple dosage utilization found in the abuse of cocaine, thus, would be expected to include those which are homozygous for the so-called 'silent' gene as well as those which are exposed to pesticides such as diisopropylfluoryl phosphate, DFP. In one set of experiments, mice were pretreated with either isotonic saline or DFP (6.3 mg/kg, i.p.) 24 hours prior to a series of multiple cocaine injections (15 mg/kg, i.p., every 5 min). The mean number of cocaine injections required to cause expiration was: controls, 8.5±1.8, and DFP-pretreated experimentals, 3.9±1.1 ($P<0.001$). The decrease in serum cholinesterase activity resulting from DFP was also determined employing the same DFP or saline pretreatment alone. Using acetylcholine as substrate and liquid chromatography with electrochemical detection, the mean serum cholinesterase activities for controls and DFP-pretreated experimentals was determined to be, respectively, 76.6±4.6 and 23.3±2.0 μmol/mL/hr ($P<0.001$). These results clearly demonstrate the enhanced toxicity of cocaine following blockade of cholinesterase activity. The involvement of cholinesterase in the metabolism of cocaine as well as the similarity in chemical structure between this drug and many established cholinergic agents further suggests a possible direct or indirect effect of cocaine upon the cholinergic system. This possibility was examined by determining the choline and acetylcholine levels in seven mouse brain regions following treatment with 50 mg/kg cocaine, i.p., followed by sacrifice with microwave irradiation 5 min thereafter. The cocaine treatment was preceded by treatment with isotonic saline or DFP, as above, to determine the effect of cholinesterase blockade upon the levels.

104.20

CALCIUM CHANNEL ANTAGONIST ALTER SELF-ADMINISTRATION OF COCAINE IN RATS. L.G. Sharpe, N.L. Goodman* and J.H. Jaffe*. NIDA Addiction Research Center, Baltimore, MD. 21224

Two general classes of calcium channels have been identified (type I and type II) based on several years of research. One factor that distinguishes the two classes is that the dihydropyridines (nifedipine, nimodipine) interact with the type II calcium channels whereas other calcium channel antagonist (verapamil and diltiazem) interact with the type I calcium channel. Results from animal studies indicate that the dihydropyridine antagonist reduce dopaminergic transmission, a brain mechanism intimately involved in the reinforcing properties of cocaine and other psychoactive drugs. Our purpose was to assess the effects of both types of calcium channel antagonists in rats self-administering cocaine (0.5 and 1 mg/kg/inf, IV) on an FR 10 schedule of reinforcement. On day 1 and 2, the animals were treated with the antagonist vehicle before each 2-hr session to establish baseline responding. On days 3 and 4, the calcium antagonist were administered followed on day 5 with another vehicle pretreatment. The number of cocaine self-injections increased significantly after 0.5 and 1 mg/kg (IP) of nifedipine or nimodipine. Verapamil depressed responding at 20 mg/kg dose, whereas diltiazem (20 to 60 mg/kg, IP) was without effect. We conclude that the two types of calcium channel antagonist influence cocaine self-administration by separate mechanisms.

104.21

COCAINE BINDING SITES IN FETAL RAT BRAIN. J.S. Meyer and L. Collins*. Dept. of Psychology, Neuroscience and Behavior Program, Univ. of Massachusetts, Amherst, MA 01003

Although both human and animal studies have shown that cocaine exposure in utero can influence subsequent behavior in offspring, the mechanism of action of the drug prenatally is still unknown. As cocaine binding sites have been identified in the striatum, cortex, and other brain regions of adult animals, an important first step in elucidating prenatal cocaine effects is to determine whether similar sites are present during this stage of development. Fetuses were obtained from timed pregnant albino rats on gestational day (GD) 20, followed by preparation of standard P2 (synaptosomal) membrane fractions from whole brains. Saturation analyses were performed using a low-sodium medium (10 mM sodium phosphate buffer) and a range of ^{3}H-cocaine concentrations from 0.3 to 300 nM. EDBA/LIGAND analysis with a 2-site model yielded a high-affinity cocaine binding site with a K_D of 20-30 nM and a low-affinity site in the uM range. These affinities are commensurate with those reported for adult striatum under similar tissue preparation and incubation conditions (e.g., Calligaro and Eldefrawi, Membrane Biochem. 7: 87, 1988), although the B_{Max} values we obtained are understandably lower. The high-affinity binding site, which is already present even before GD 20, is an excellent candidate for mediating the effects of cocaine on the developing brain. (Supported by NSF BNS-8704702 and BRSG RR07048 from NIH)

105.1

THREE DIMENSIONAL STRUCTURE OF INTRACELLULARLY STAINED NEURONS AND THEIR PROCESSES REVEALED BY HVEM AND AXIAL TOMOGRAPHY D. N. Mastronarde, C.J. Wilson and B. McEwen*, HVEM Laboratory, Boulder, Colorado, Department of Anatomy and Neurobiology, Univ. of Tennessee, Memphis TN, and Wadsworth Ctr. for Labs and Res., NY State Dept. of Health, Albany NY.

The high voltage electron microscope can be used with very thick sections (i.e. 1-5 μm), and so can provide the images required for tomographic reconstruction of large pieces of dendrites, axons, or cell bodies of neurons injected with of electron dense markers. We have tested this approach with a portion of the dendritic tree of a spiny neostriatal projection neuron stained by intracellular HRP injection, postfixed with OsO_4, embedded in plastic, and cut into 3 μm thick sections. The sections were examined at 1000 kV with no further processing. Series of electron micrographs of a single section were taken at specimen tilts varying from -60 to +60° in 2° steps. These images were then digitized, converted to optical density, adjusted to remove differences in negative density, and aligned. A three dimensional reconstruction of the original dendrite was generated using the r-weighted back projection method. The accuracy of the reconstruction was evaluated by direct comparison with the original data set. Degradation of the reconstruction due to missing information was not severe, due to the unambiguous nature of the boundaries of intracellularly stained cell processes.

Tomographic reconstructions offer advantages for quantitative studies. Interactive computer programs can provide rapid and accurate linear measurements, such as dendritic or axonal diameter, spine lengths, etc., and can also extract measurements of membrane surface area or cytoplasmic volume. The resolution of the reconstruction is much higher than that available using any light microscopic methods, and is nearly as great as the resolution of conventional TEM.

105.2

SERIAL ELECTRON MICROSCOPY OF CA3 DENDRITIC SPINES SYNAPSING WITH MOSSY FIBERS OF RAT HIPPOCAMPUS. M.E. Chicurel and K.M. Harris (SPON: R. Tuttle). Dept. Neur. Children's Hosp. & Harvard Med. Sch. Boston, MA 02115

In their classic study, Blackstad and Kjaerheim (1961, JCN 11:133-146) obtained partial reconstructions of these CA3 dendritic spines from 10-12 serial thin sections and began to illustrate their complexity. We are obtaining more complete reconstructions of these spines so that modeling of their biophysical properties can be achieved. Preliminary results from a reconstruction through 42 serial sections revealed 2 spines synapsing with a single varicosity of the mossy fiber. One of these spines had 11 separate necks and heads (branches) emerging from a single primary neck at the dendrite. Seven branches were complete within the series, and 10 had synapses; the 11th was complete, contained 2 mitochondria, but had no synapse. The second spine had 4 branches; 2 were complete, and all had synapses. Of the 14 branches total, 6 had spinules, 5 had spine apparatuses, 10 had ribosomes, 10 had multivesicular bodies, and 8 had smooth endoplasmic reticulum. These results indicate: 1) that a single mossy fiber bouton can form synapses with more than 1 spine, and 2) that the complexity of the spine with multiple constrictions will require separate biophysical analysis for each branch. Supported by #NS21184, #P30-HD18655.

105.3

DETAILED COMPARTMENTAL MODEL OF AN EM RECONSTRUCTED SPINY STELLATE CELL IN THE MOUSE NEOCORTEX. I. Segev*, E.L. White and M.J. Gutnick, Dept of Neurobiology, Hebrew University, Jerusalem, and Depts of Morphology & Physiology, Faculty of Health Sciences, Ben Gurion University, Beersheva, Israel.

Integrative capabilities of neurons are determined by their morphology, synaptic architecture and electrical properties. At present there is only one neocortical neuron, a spiny stellate cell, that has be thoroughly reconstructed from serial thin sections (White,E.L and Rock,M.P.,*J. Neurocytol.*9:615,1980). We have used SPICE to construct a detailed compartmental model of this cell, based on the dimensions of its 5 dendrites and 310 spines, precise information on the sites and morphological types of all its synapses, and identification of each of its 48 thalamocortical synapses. Initial exploration revealed that a small R_m of 2000 ohmxcm^2 (R_i=70 ohmxcm) produced a large input resistance (R_N) of 140 Mohm; the presence of spines served to reduce R_N by about 20%. If, as physiological experiments indicate, GABAa-type IPSPs generated locally in a dendrite are depolarizing, they may serve to veto all excitation coming from that dendrite, while at the same time facilitating excitatory influences on other dendrites. Our observations demonstrate that neocortical neurons can have much greater computational power than the simple "integrate-and-fire" units that are often used in "neural network" models.

105.4

VESTIBULAR SYNAPTIC TRANSMISSION AT DEVELOPING CHICK TANGENTIAL NEURONS. K.D. Peusner and E. F. Stanley. LB, NINDS, NIH, Bethesda, MD 20892

The two main neuron classes in the tangential vestibular nucleus (TN) differ in membrane properties and synaptic properties following vestibular nerve stimulation. Elongate cells are activated by chemical transmission, whereas there is morphological evidence for mixed chemical and electrical transmission at the principal cells. In this study we have tested the mode of transmission by using low Ca solutions.

Field potentials were recorded from the TN in chick embryo (15-16 days) brain slices while stimulating the vestibular ganglion. An afferent volley and a biphasic response were recorded which were not abolished in low calcium solution or after addition of cobalt. The Ca-insensitive biphasic response cannot be accounted for by the elongate cells since intracellular recording confirmed that synaptic transmission to these cells was Ca sensitive. Supported in part by NIH grant R01 NS 18108.

105.5

MEMBRANE DENSITY HYPERTROPHY IN THE END BULB OF HELD IN CONGENITALLY DEAF CATS. S.A. Larsen and T.M. Kirchhoff*. Dept. of Anat. Sci. & Neurobiol., Univ. of Louisville Med. Sch., Louisville, Ky 40292.

Impulses conveyed from hair cells of the cochlea are received by neurons in the cochlear nucleus (CN). Bock et al (Brain Res, 239:608, 1982) concluded that synapses can be functional in the auditory pathway in the absence of peripheral stimulation and function may be restored by direct nerve stimulation contrary to Gulley's theory that stimulation is necessary to maintain synapses (Brain Res, 158:279, 1978). We studied synaptic terminations of the end bulb of Held (EB) on large spherical cells (LSC) of normal-hearing (NH) and white-deaf cats (WD). WD cats are prenatally deaf because of genetically determined cochlear degeneration.

We observed degeneration of cochlear hair cells, atrophy of the LSC and degeneration of the EB terminal in WD cats. Most of the remaining EB terminals in WD cats have few membrane densities, few synaptic vesicles and prominent presynaptic densities while in NH cats synaptic zones are asymmetrical and presynaptic densities are rarely observed. Cells were also observed in WD cats which exhibited hypertrophy of membrane densities by as much as 50%. While there is an increase of membrane densities in these cells, there is not an increase in the number of synaptic vesicles. Some of the membrane densities are synaptic zones as it is known that some neurons do respond to electrical stimulation.

Supported by Deafness Res. Found. & Univ. of Louisville Graduate Research Grant.

105.6

Pertussis toxin prevents long term potentiation (LTP) of intracellularly recorded hippocampal excitatory postsynaptic potentials (EPSP's). J.W. GOH AND P.S. PENNEFATHER. Faculty of Pharmacy, University of Toronto, Toronto, Ontario, CANADA M5S 2S2.

A brief, high frequency (400 Hz, 0.5s) stimulation of fiber tracts in brain slices from the rat hippocampus results in a long lasting potentiation of synaptic events mediated by those axons. Last year we reported that inactivation of hippocampal G-proteins with pertussis toxin (PT) prevented the induction of LTP measured in terms of the synaptically evoked population spike (see SFN Abst. 14, 12.8). It was of interest to confirm that LTP of EPSP's was also blocked by PT. We have recorded intracellularly from CA_1 pyramidal cells of transversely sectioned rat hippocampal slices and evoked EPSP's by stimulating the stratum radiatum. PT (2-5 μg total) was injected stereotaxically above the right hippocampus and slices were obtained 3-4 days post-injection. The effectiveness of PT pretreatment was assessed by monitoring the ability of adenosine (100 μM) to cause a membrane potential hyperpolarization of the CA_1 neuron. That action is mediated by a PT sensitive G-protein. In 6 out of 6 cells PT abolished the adenosine induced hyperpolarization and prevented the induction of LTP of the EPSP's. The presynaptic action of adenosine to reduce the EPSP amplitude was not significantly affected by PT pretreatment.

105.7

SYNAPTIC TRANSMISSION BETWEEN INDIVIDUAL NEURONS IN RAT VISUAL CORTEX. A.J.R. Mason*, A. Nicoll*, K.J. Stratford* & C. Blakemore. Univ.Lab. of Physiol., Oxford OX1 3PT, UK.

Using simultaneous intracellular recordings, we have investigated the properties of synaptic connections between pairs of neurons in slices of rat visual cortex. Impalements were made less than 250 μm apart in layer 2/3. Depolarizing current pulses, adjusted in strength and duration to evoke single spikes, were injected into one cell of each pair at 2 Hz. Spike-triggered averaging revealed synaptic connections between 8 of the 70 cell pairs tested. In 2 of these pairs, action potentials evoked in either cell elicited synaptic potentials in the other cell. All post-synaptic potentials (PSPs) were depolarizing at resting potential (67-83 mV). Their mean peak amplitudes ranged from 0.14 to 1.54 mV. Latencies were less than 2 msec, 10-90% rise-times were between 1.0 and 2.6 msec, and widths at half-amplitude ranged from 9.0 to 19.1 msec. All cells had electrophysiological characteristics typical of pyramidal neurons. The pyramidal nature of one cell of a synaptically coupled pair was confirmed by intracellular staining using biocytin. Amplitude fluctuation analysis was applied to 3 connections for which we had recorded at least 3000 sweeps. Under the assumption that the noise and PSP amplitude distributions could each be modelled by a single Gaussian distribution, the synaptic potentials fluctuated more than could be accounted for by the noise. Thus, for local synaptic connections between pyramidal cells in rat visual cortex, it appears that single pre-synaptic action potentials do not generate PSPs of constant amplitude.

105.8

COMPARISON OF FLUCTUATIONS IN ELECTRICAL AND CHEMICAL TRANSMISSION EVOKED BY STIMULI IN THE SAME SYNAPSE. Ch. Stricker*, H.-R. Lüscher and H.P. Clamann.. Dept. of Physiol., Univ. of Bern, Switzerland.

Synaptic transmission from spindle afferents to motoneurons in the frog is both electrical and chemical. By comparing fluctuations of the electrically and chemically evoked components, it may be possible to determine whether fluctuations are a result of uncertainty of potential propagation in afferent branches or of failure of transmitter release. The components of the EPSP can be identified by shape and latency. After isolation from anesthetized frogs, whole spinal cords were placed into a lucite chamber maintained at 10±1°C and superfused with oxygenated frog Ringer's solution. Motoneurons of the 9th or 10th spinal segment were impaled with electrodes filled with 3 M KCl and having 15-30 MΩ resistance. Cell input impedance was measured. 1024 composite EPSP's evoked by stimulating the corresponding dorsal root at ≥ 5 X threshold at 1/sec were recorded. The mean time course of the EPSP, of its variance and of a 10 msec segment of the baseline variance preceding the stimulus artifact were computed. The variance time course of the EPSP was corrected for baseline variance. In 7 of 14 cells, the variance of the monosynaptic chemical component did not differ significantly from that of the electrical component. Both corrected variances were nearly zero. In 3 cells, a slight monotone variance increase from the onset of the EPSP through the monosynaptic chemical component was seen. In 4 cells, the variance of the monosynaptic chemical component of the EPSP was less than the baseline variance. We conclude that in the isolated frog spinal cord at 10°C, neither electrical nor chemical transmission show much fluctuation. Thus electrical invasion of boutons and transmitter release are both reliable in this system. A decrease in the variance, and hence in the noise during an EPSP below baseline values suggests that noise signals may not always add linearly.

105.9

A POSSIBLE SOURCE OF ATTENUATION OF QUANTAL CURRENTS AT THE CRAYFISH NEUROMUSCULAR SYNAPSE.
J.M. Wojtowicz, K. Miller*, J. Winslow*, H.L. Atwood, Dept. of Physiology, University of Toronto, Toronto, Ontario. M5S 1A8.

Experiments were performed on the opener muscle of the crayfish in vitro. In each experiment the excitatory axon was stimulated at the frequencies of 1-5 Hz, and quantal release of transmitter was followed simultaneously with both intracellular and macropatch electrodes. The intracellular electrode, impaling a muscle fiber can detect individual quantal EPSPs; however, their site of release on the fiber is not known. The macropatch pipette (typically 20 microns diameter) detects quantal currents generated by transmitter released from a circumscribed area of the axonal terminal. Combined recordings showed that certain quanta are strongly attenuated or eliminated during transmission between the release site and the core of the postsynaptic muscle fiber. We propose that the attenuation of these quanta is caused by decremental conduction along the subsynaptic cytoplasmic folds. These filamentous projections appear to be analogous to dendrites or dentritic spines of neurones. Their structure has been investigated using serial sectioning and reconstruction from electron micrographs. Subsynaptic structures may regulate the transfer of quantal information. Supported by MRC and NSERC, Canada

105.10

KINETICS AT THE NEUROMUSCULAR JUNCTION: A MONTE-CARLO SIMULATION. T.M. Bartol Jr.*, B.R. Land[1]*, E.E. Salpeter[2]*, M.M. Salpeter (SPON: A. Cohen). Section of Neurobiology and Behavior, [1]Cornell Theory Center/Cornell National Supercomputer Facility, and [2]Laboratory of Nuclear Studies Cornell University, Ithaca, NY 14853.

This study models events produced by the release of an acetylcholine (ACh) quantal packet at the vertebrate neuromuscular junction. Such modeling must include the chemical kinetics for two ACh binding and unbinding steps, an isomerization step for the acetylcholine receptor (AChR) as well as for ACh diffusion along a primary and secondary cleft. In the past we have done this using numerical methods for solving differential equations, but only for simplified versions of the cleft geometry (e.g. Land et al PNAS 1984 Vol. 81, pp.1594-1598). We have now implemented an alternative modeling method using a three dimensional Monte-Carlo computer simulation which follows individual released ACh molecules in space and time. The Monte-Carlo model presented here has considerable advantages over previous methods by allowing the inclusion of more realistic conditions. In particular we can 1) specify arbitrary three dimensional synaptic cleft geometry and 2) study more complex assumptions on the chemical kinetics, such as two non-equivalent binding sites on the AChR complex.

This simulation has been used to explore the effect of different initial geometric conditions on the spacial distribution of bound and unbound ACh and AChR, and on the shapes of the resultant miniature endplate currents (MEPC's). We report 1) that the presence of a secondary synaptic cleft can affect the MEPC shape, depending on the distance between the presynaptic ACh release site and the secondary fold and 2) that the extent of potentiation of MEPC amplitude and decay time depends on the distance between two ACh release sites. Comparisons with experimentally obtained MEPC shapes as a result of varying AChR and acetylcholine esterase (AChE) concentrations now permits the determination of rate constants for ACh diffusion and binding and for AChR gating which apply to the complex geometry of the vertebrate neuromuscular junction. This research was conducted using the Cornell National Supercomputer Facility, a resource of the Center for Theory and Simulation in Science and Engineering.

105.11

AN EVALUATION OF MEDIA FOR MAINTENANCE OF MOUSE DIAPHRAGM MUSCLE IN LONG TERM ORGAN CULTURE.
D. M. Wetzel & M. M. Salpeter. Section of Neurobiology, Cornell University, Mudd Hall, Ithaca, NY. 14853.

Denervation of vertebrate skeletal muscle results in many physiological changes such as the development of spontaneous fibrillations, increased levels of extrajunctional acetylcholine receptors (AChR), and accelerated turnover rates of junctional AChR. Studies of the molecular mechanisms by which the nerve might control these muscle properties would be greatly aided by the availability of a good *in vitro* system which would allow long term investigation and manipulation of denervated adult muscle. Yet to date no organ culture system has been able to maintain these denervation induced muscle responses for prolonged periods, especially if muscles have not been pre-denervated several days prior to being placed in organ culture. In this study we describe organ culture conditions and media which allow us to maintain both pre-denervated and non-predenervated muscle (i.e. muscles denervated at time of transfer to organ culture) for prolonged periods (up to 20 days) comparable in response and fine structure to that of denervated muscle *in vivo*.

A variety of culture media formulations were examined for their ability to support mouse diaphragm muscle in long-term organ culture. These included Trowell's 8, DMEM/F12, DMEM/F10, and M199. Medium 199 (with 5% fetal bovine serum, 1.17 iu/ml insulin, 1.6 mM L-glutamine, 2 mM β-hydroxybutryic acid, 10 μM ascorbate, 1nM dihydrotestosterone, and other supplements) was identified as best for maintaining diaphragm muscle for > 2 weeks as judged by the development of the above listed denervation induced changes as well as the maintenance of high quality muscle fine structure. Although muscles denervated *in vivo* and transferred to organ culture 6-8 days later (pre-denervated) are easier to maintain *in vitro* than muscles denervated at time of transfer to the organ culture (non-pre-denervated), this medium maintained even non-pre-denervated muscle for up to 16-20 days in culture.

105.12

NERVE TERMINALS AT SNAKE TWITCH AND SLOW ENDPLATES RESPOND DIFFERENTLY TO ISOTONIC POTASSIUM PROPIONATE. L.M. Coniglio*, G.M. Hendricks* and R.L. Parsons. Dept. of Anat. & Neurobiol., Univ. of Vt, Burlington, VT 05405.

We observed that during exposure to isotonic potassium propionate (KP) solution, MEPC frequency at twitch fiber endplates was maintained at high levels for up to 3 hrs., whereas at slow fiber endplates, examined after 10 min. to 3 hrs. in KP, the MEPC frequency was not elevated. Ultrastructural studies were undertaken to test whether morphological differences were correlated with the different MEPC responses to isotonic KP. Control preparations and preparations maintained in isotonic KP for 15-90 min. were examined by electron microscopy. We found that nerve terminals at slow fiber endplates were depleted of synaptic vesicles within 15 min., whereas even after 90 min., the nerve terminals at twitch fiber endplates contained numerous vesicles. These results indicate that the difference seen in MEPC frequency between fast and slow twitch fibers in isotonic KP is correlated with a morphological difference at the nerve terminals of fast and slow twitch fibers. Also, our results show that nerve terminals at snake twitch fiber endplates respond differently to isotonic K than nerve terminals at frog twitch fiber endplates, which are depleted of synaptic vesicles after a few minutes in isotonic K (Gennaro et al., J. Physiol. 280:237, 1978). Supported in part by a Grant from the MDA.

105.13

PRESYNAPTIC ACTIVE ZONE INTEGRITY AND ACH RELEASE FROM FROG MOTOR NERVE TERMINALS. S.D. Meriney, P.A. Pawson and A.D. Grinnell. JLNRC, UCLA Sch. of Med., Los Angeles, CA 90024.

At the frog motor nerve terminal, the active zone (AZ) is oriented linearly in regularly spaced double row segments directly opposite ACh receptor-dense postsynaptic folds. Patients with Lambert-Eaton myasthenic syndrome exhibit neuromuscular weakness, and make IgG that disrupts this tight organizational alignment (Fukunaga et al., 1982). Recently, Nystrom and Ko (1988) have shown that proteolytic enzymes also disrupt AZ structure. We have examined AZ structure and nerve terminal function following a 2 hour incubation in purified collagenase, and show that AZ segments average only about 50% of control length, frequently lose their orientation, and often break up into single rows or dispersed particles. Despite this change in structure, ACh release is not grossly affected. Subtle changes, such as a decrease in the average MEPP amplitude, probably due to a misalignment of pre- and postsynaptic elements, do occur and may decrease neuromuscular efficacy. However, disrupted AZ pieces appear to maintain function as they "float" in the presynaptic membrane. Supported by grants from the MDA and NIH.

105.14

AN INCREASE IN OSMOTIC PRESSURE INCREASES THE PERCENTAGE OF GIANT AND SKEW-MEPPs WHICH ARE COMPOSED OF SUB-UNITS. M.E. Kriebel, J. Vautrin and F. Llados. Dept. Physiol., SUNY Health Sci. Ctr., Syracuse, NY 13210.

Bell- and skew-MEPPs are composed of similarly sized sub-units (Erxleben and Kriebel, J. Physiol. 400:659, 1988) and the number is readily changed (Kriebel et al., J. Physiol. 262:553, 1982). Van der Kloot and Van der Kloot (Expt. 41:47, 1985) report an increase in MEPP size after hypertonic saline treatment whereas Kriebel and Pappas (Neurosci. 23:745, 1987) found more skew-MEPPs. BWSV, Ca^{++}, La^{+++}, ionophore, heat, K^{+} (Kriebel, Handbook Exp. Pharm. 86:537, 1988) reduce the size of the bell-MEPP and increase the percentage of skew-MEPPs with no change in sub-MEPP size. We have measured MEPC amplitudes and fiber input impedance during and after soaking frog and mouse muscle in up to 1M NaCl saline (gradual and step changes). Input resistance increased up to 50 times and stayed higher (20%) than normal after returning to normal saline. These results explain the increased bell-MEPP size. There was also an increase in skew-MEPPs, giant and atypically shaped MEPPs which had the same initial rising phase, and rising phases deviated at similar times. Thus it is meaningless to refer to the "average MEPP size." Skew-, giant and atypical MEPPs are explained with a sub-unit composition and with synchronizing and timing mechanisms that can be altered in number and time. NIH - NS 25683.

105.15

MORPHOMETRIC AND ACH ANALYSIS OF ISOLATED SYNAPTIC VESICLES AFTER LONG TERM, LOW FREQUENCY STIMULATION. G.Q.Fox,G.H.C.Dowe*and D.Kötting*. AbG.161, Max-Planck-Institut für biophysikalische Chemie, 3400 Göttingen, FRG.

Cholinergic(ACh) containing synaptic vesicles from Torpedo electric organ were isolated by sucrose density centrifugation and analysed morphometrically and biochemically after 1800 pulses of 0.1 Hz stimulation. Two subclasses of vesicles were identified based on previously established ACh criteria. Stimulation produced a 99% loss of estimated terminal vesicle numbers. These values did not recover after 2 h of rest indicating an absence of local recycling. Only a single 68 nm diameter size class of ACh vesicle was found. ACh levels were depleted by 66% and similarly failed to recover following rest. Gradient location of (^{3}H)choline uptake was influenced by stimulation but uptake efficiency amounted to only 1 label per 10^{7} vesicles or 10^{4} terminals. ACh content/vesicle covered a range of $1\text{-}3\times10^{5}$ molecules for both subclasses exceeding estimated quantal content by a factor of 15.

These findings contradict numerous claims pertaining to stimulation-induced heterogeneity of this vesicle population.

105.16

ULTRASTRUCTURE OF MUSCLES AND NEUROMUSCULAR JUNCTIONS IN THE LARYNX OF MALE AND FEMALE *XENOPUS LAEVIS*. Darcy Kelley, Martha Tobias and Mark Ellisman, Dept. Biol. Sci., Columbia Univ., New York, NY 10027 and Dept. of Neurosci., UCSD, La Jolla, CA 92093

Sex differences in the electrophysiological properties of the larynx contribute to the distinctive male and female vocalizations of *Xenopus laevis* (Tobias and Kelley, J. Neurosci.,1987, 1988). In response to repetitive nerve stimulation, female muscle fibers immediately produce action potentials while male fibers first produce sub-threshold potentials. Muscle fibers of adult females but not males are dye-coupled. Freeze fracture replicas as well as thick and thin sections were examined for ultrastructural clues into sex differences in synaptic efficacy and dye-coupling.

Male laryngeal muscles contain unusual multilamellar structures that surround lipidic inclusions,"whorls" not seen in females. In both male and female freeze fracture material, square arrays of intramembranous particles were apparent. No gap junctions have yet been observed between muscle fibers in either females or males. Areas of close membrane apposition that include apparent cytoplasmic continuity were seen in freeze fractured and sectioned muscles. To date, these specializations have been observed only in females and could account for female-specific dye-coupling of muscle fibers. In many female specimens, multiple small axon terminals occupy the primary synaptic cleft. Most male neuromuscular junctions appear to contain a single axon terminal. The number of synaptic vesicles and the dimensions of active zones appear similar in the sexes. These results suggest that differences in synaptic efficacy in males and females are not due to vesicle availability. Supported by NS 23684; NS 14718; NSF and RR00592.

105.17

FOCAL ADHESIONS OF NERVE TERMINAL TO SYNAPTIC MATRIX AND SCHWANN CELL AT MOUSE NEUROMUSCULAR JUNCTIONS. N. Robbins and J. Polak. Center for Neurosciences, Case Western Res. Schl. of Medicine, Cleveland, Ohio 44106.

The neuromuscular synaptic matrix contains information for localizing and aligning presynaptic and postsynaptic specializations, but it is not clear whether the matrix is uniformly adhesive or if there are unique and specific sites of nerve terminal adhesion. To address this question we utilized hypertonic fixatives to induce shrinkage at the mouse soleus NMJ.

In electron micrographs of such preparations, the synaptic matrix adhered to postsynaptic components, while the presynaptic terminal frequently retracted from the matrix except for discrete adhesive regions invariably overlying secondary folds. Some but not all of these adhesive sites were active zones. The adhesion of nerve terminal membrane was so strong that extended tabs of the nerve terminal membrane remained attached to the apices of junctional folds while the rest of the terminal retracted. Similar tabs were occasionally found at the apices of junctional folds in normally-fixed NMJ's. Also, phosphotungstic acid-stained material showed fibrillar elements running from active zones to the synaptic matrix. Hypertonic-fixed preparations also revealed adhesive focal attachments of the nerve terminal to the lateral borders of the Schwann cell cap. Finally, in tissue processed for immunoelectron microscopy, nerve terminal-matrix and nerve terminal-Schwann loci were sites of sub-membranous actin accumulation.

The density and strength of focal adhesive sites to matrix or Schwann cell may be critical in determining the stability or retraction of mature motor nerve terminals. (Work supported by NIH AG00795)

106.1

TIMING OF PREHENSILE COMPONENTS IN THE ELDERLY. V. Diggles and T. Iberall, Andrus Gerontology Center and Department of Computer Science, University of Southern California, Los Angeles, Calif 90089.

The disruption of movement automaticity into less fluid, modular motion in the elderly may be due to age-related changes in the CNS that affect the constraints that simplify motor control by reducing controllable degrees of freedom. The purpose of this study was to assess the integrity of these constraints in the elderly by examining the timing and kinematics of prehensile movement components. The transport and grasping components are believed to have independent control, but their timing appears synchronized such that the occurrence of specific kinematic landmarks are highly correlated (r=.76 to .89) in young adults (Jeannerod, 1984).

In this study, a WATSMART system was used to analyze the kinematics of female subjects, aged from 57-67, while reaching and grasping a cylinder. Movement amplitude, cylinder size and speed instructions were varied. There is a main effect of movement amplitude on movement time, as predicted by Fitts' law. Results indicate that the onset of the slow velocity phase of transport and the time of peak finger separation were not highly correlated (r=.10 to .47). While it is likely that a temporal correlation between the transport and grasping components reduces the control burden by constraining degrees of freedom temporally, the disruption of movement in the elderly may be due to the disintegration of this constraint.

106.2

DIFFERENTIAL EFFECTS OF AGING ON FORELIMB AND HINDLIMB MOTONEURONS IN THE RAT. K. Kanda and K. Hashizume*. Dept. of Physiol. Tokyo Metropol. Inst. of Gerontol., Itabashi-ku Tokyo 173, Japan.

We studied the number and the sizes of forelimb, ulnar motoneurons (Ul-MNs), which send their axons into the Ul nerve, of young (9 months of age) and old (27 months) rats. The data were compared with the data on hindlimb, MG-MNs. The MNs were labeled by retrograde transport of horseradish peroxidase (40% solution) injected bilaterally into both the Ul and MG nerves. Individual labeled cells were identified and counted. The cross-sectional area (CSA) of their somata was also measured with an image analysis system. The mean number of MG-MNs was significantly less in the old rats compared with that in the young rats, confirming our previous findings (Hashizume et al., J. Comp. Neurol., 269: 425, 1988). In constrast, no significant difference was found in Ul-MNs between young and old rats (mean $\pm$ SD: 285 $\pm$ 17 for the young and 290 $\pm$ 11 for the old rats). The mean cross-sectional area of Ul-MNs, however, was a little (7%) smaller in the old rats as was found in MG-MNs. The mean wet weight of the flexor carpi ulnaris muscle, which were innervated by the Ul nerve, was not significantly different for the two age groups, whereas that of the MG muscle in old rats was about 25% lighter than that in young rats. The results suggest that the effects of aging are weaker for forelimb MNs than for hindlimb MNs.

106.3

NEURONAL CHANGES IN AREA 4 DURING THE LIFE SPAN OF THE RHESUS MONKEY. J. Tigges, J. Herndon and A. Peters. Yerkes Reg. Primate Res. Ctr., Emory University, Atlanta, GA 30322, and Dept. of Anatomy, Boston Univ. Sch. Med., Boston, MA 02118.

One right or left area 4 of each of 19 rhesus monkeys, ranging in age from 1 day to 35 years, was processed (frozen sectioned at 30 or 40 μm) for light microscopic analysis in order to assess age-related changes. All neurons were considered regardless of their size. In addition, Betz cells were analyzed separately; to be regarded as Betz cells, pyramidal somata had to display a minimum height of 38 μm. — No age-related change in thickness of area 4 was found. A significant decrease of approximately one third was observed in the number of neurons in maturing monkeys (< 5.5 years). In contrast, in maturing rhesus monkeys significant increases with age were observed in the mean number of Betz cells, and in the means of Betz cell area, height, width, perimeter, and estimated volume. In adult monkeys (> 4.5 years), no age-associated loss of neurons was observed. Also, no loss of Betz cells occurred, although the perimeter, area, and estimated volume of Betz cells decreased slightly, but significantly, with increasing age in adult monkeys. Lipofuscin granules were discernable in Betz cells beginning at the age of 5 years and grew in number with increasing age. In the older rhesus monkeys, the granules were so large and numerous that in some Betz cell somata they displaced the nucleus from its usual location in the center of the cell. (NIH grants RR-00165 and 2PO1-AG00001).

106.4

GLIAL FIBRILLARY ACIDIC PROTEIN mRNA INCREASES WITH AGE IN THE MOUSE HIPPOCAMPUS AND CEREBELLUM. J.R.Goss, Kay-Min Chan*, and D.G.Morgan. Andrus Gerontology Center & Dept. of Biological Sciences, University of Southern California, Los Angeles, CA 90089-0191.

Previously, we reported an 80% increase in GFAP RNA in mouse cortex using a solution hybridization (RNase protection) analysis. The same study demonstrated no change in three other message levels: Thy-1 antigen, glutamine synthetase, and beta-tubulin. We report here that GFAP RNA increases 50% in mouse cerebellum and 70% in mouse hippocampus with aging.

Total RNA was prepared from cerebellum and hippocampus of female C57BL/6J mice of four different age groups: 4 months, 9-10 months, 17 months, and 26 months. Four samples for each age group were analyzed by solution hybridization for GFAP and Thy-1 antigen message levels. Solution hybridization was performed in triplicate using six concentrations of total RNA per sample. The mass of each specific message per mass of total RNA was calculated (pg/ug).

A 50% increase in GFAP RNA (4.2 ± 0.74 to 5.95 ± 1.1) in the cerebellum and the 70% increase in GFAP RNA (1.68 ± 0.17 to 3.0 ± 0.6) in the hippocampus were the only significant changes. We feel that this increase in GFAP RNA represents a change in the fibrous character of the astrocytes in these regions, perhaps similar to reactive gliosis. Supported by NIA Training Grant AG00093 (JRG), The Anna Greenwall Award and The American Federation for Aging Research (DGM).

106.5

TEMPORAL RELATED CHANGES IN THE AGING RAT LATERAL ENTORHINAL CORTEX (LEC): A GOLGI-EM STUDY. A.A. Carboni and W.G. Lavelle. Dept of Surgery, Div. of Otolaryngology/Head and Neck Surgery, UMASS. Med. Center, Worcester, MA. 01655.

Focal neuropil lesions and neuronal changes in olfactory cortical structure (Hooper and Vogel, '76; Bondareff et al, '82) and function (Hyman et al, '86; Doty et al, '87) have been implicated in the earliest changes of Alzheimer's disease. Similar changes in the rat (Vaughan and Peters, '81), however, may be found as a result of normal aging.

Correlated light and electron microscopic observations were made of tissue impregnated by the Golgi-Colonnier-Gold method (Braitenberg, '85). Layers III and V of the LEC, in a series of 22 day and 22 mo. old rats, were analyzed and a significant thickening of the layers and basal laminae of the blood vessels was found with an increase in the primary dendritic lengths of the pyramidal neurons. There appeared to be no change in the terminal dendritic segment lengths, branching or synaptic density of these pyramidal neurons. There was, however, a significant decrease in the synaptic sizes of these neurons determined by ultrastructural serial sections. Supported by BRSG #6-32724.

106.6

CHRONIC EXPOSURE TO ELEVATED CORTICOSTERONE ALTERS SPATIAL MEMORY AND HIPPOCAMPAL MORPHOLOGY IN YOUNG, ADULT RATS. S.R. Bodnoff, M. M. Miller, R.M. Sapolsky, & M.J. Meaney. Douglas Hospital Research Ctr., Depts. of Psychiatry and Obstetrics & Gynecology, McGill Univ., Montreal H4H 1R3, Canada and Dept. Biological Sci., Stanford Univ., Stanford, CA 94305.

Chronic exposure to elevated, stress-like levels of corticosterone results in the loss of neurons in the hippocampus (Sapolsky et. al, J. Neursci., 1985), a brain region of considerable importance for learning and memory. We have implanted 4 month-old, Long Evans rats with fused pellets of corticosterone that produce plasma corticosterone levels in the upper physiological range (20-25 µg/dl), comparable to peak basal levels produced by aged animals. Spatial memory was assessed using the Morris water maze, a test that is extremely sensitive to hippocampal dysfunction. Following 3 months of treatment, the corticosterone-treated animals showed significant spatial memory impairments compared with cholesterol-treated controls. The impairments were robust and highly significant for the first 6 trials, but eventually the corticosterone-treated animals reached the same level of performance as controls. Treatment for 1-month has no effect whatsoever on spatial memory.

Corticosterone treatment resulted in the loss of hippocampal neurons in selected regions. These data will be discussed in the context of the corticosterone-induced morphological changes and their relevance for the study of aging.

106.7

AGING, BLOOD VESSELS AND NEUROVASCULAR APPOSITIONS IN THE RAT DENTATE FASCIA. A. Topple, E. Fifkova and K. Cullen-Dockstader*. Dept. of Psychology, Univ. of Colorado, Boulder, Co 80309.

Aspects of mural ultrastructure of capillaries and larger vessels as well as frequency of different types of appositions made by neuronal processes onto the vessel wall were studied. A report on the preliminary findings of this study has appeared (Topple, et al., Soc. Neurosci. Abstr., 1987). Tissue was taken from the left dentate gyrus of 3-, 9-, 24- and 30-month old male Fischer 344 rats (n=18). 719 vessels were analyzed for 15 variables. We report that with increasing age there is thickening of the basal lamina and an increase in mitochondrial presence in capillaries but not in large vessel populations. These age-related changes may be considered as possible vascular markers for the aging brain. A comparison of capillaries and large vessels irrespective of age shows differences in wall size, mitochondrial area and the fraction of vessel wall occupied by mitochondria. In addition, there are more axon terminals, axons and dendrites on capillaries than on large vessels. If these appositions are functional, this suggests a more significant role for them in capillary function than for the larger vessels. Since age did not alter this arrangement, it also suggests that age-related compromises in vessel function may not be related to neuronal regulation. The unchanged frequency of appositions with age is in contrast to the finding in the preliminary study (355 vessels).

Supported by #AG 04804.

106.8

DENDRITIC EXTENT OF GRANULE CELLS IN AGING F344 RAT DENTATE GYRUS. D. G. Flood and R. Moromisato*. Depts. of Neurology and Neurobiology and Anatomy, Univ. of Rochester Sch. of Med. & Dent., Rochester, NY 14642.

Aging human dentate gyrus granule cells have shown dendritic growth between middle age and old age, followed by regression between old age and very old age. The growth is thought to be compensatory for age-related loss of neighbor neurons. Because rat granule cells also die in aging (Curcio and Hinds, Neurobiol. Aging, 4, 77, 1983) and because rat dentate gyrus displays a remarkable degree of lesion-induced plasticity in both adult and aged subjects, we suspected that rat dentate gyrus granule cell dendrites might also be plastic in normal aging. We measured dendritic extent in 9 male rats aged 12, 20, or 30 months (3 per group). Rats aged 3, 27, and 37 months are currently being analyzed. Twelve neurons per rat were randomly selected from the lateral blade, point, or medial blade of the dentate gyrus in coded 240 µm thick, Golgi-Cox stained, horizontal sections. Camera lucida drawings were made at 1000X and digitized using a graphics tablet linked to an Apple II Plus microcomputer. Dendritic measures were made as a function of the entire dendritic tree and of the segments ordered centrifugally or centripetally with respect to the cell body. Although there was a slight increase at 30 months in total dendritic length and average segment length for both the entire dendritic tree and the terminal segments, this increase was not statistically significant. The addition of new subjects may help to clarify whether rats do mimic humans in their ability to demonstrate dendritic plasticity in aging or whether dendritic extent of rat granule cells does not change in age. Supported by NIH grant AG 03644 and ADRDA grant IIRG-87-116.

106.9

ADRENALECTOMY DECREASES CALCIUM SPIKE DURATION IN RAT HIPPOCAMPAL NEURONS. D.S. Kerr*, L.W. Campbell*, S-Y Hao*, and P.W. Landfield (SPON: Dr. J. Ryu). Dept's. Anatomy and Physiol. & Pharmacol., Bowman Gray Sch. Med., Wake Forest Univ., Winston-Salem, NC 27103.

Recently, we reported that adrenalectomy (ADX) reduces and corticosteroid (CORT) administration increases the duration of the Ca-dependent afterhypolarization in rat hippocampal neurons. Moreover, the effect of ADX was more pronounced in neurons from aged rats in which the AHP normally is prolonged (Kerr, Landfield, Soc. Neurosci. Abstr., 1988; Kerr et al, under review).

If this CORT effect is mediated by Ca conductance, it could link together the glucocorticoid hypothesis of brain aging (cf. Landfield et al, 1981, Science; Sapolsky et al, 1984, J. Neurosci.) with the altered Ca hypothesis of brain aging (cf. Khachaturian, 1984, Handbook of Studies on Psychiatry and Old Age, Elsevier; Landfield, Pitler, 1984, Science; Gibson, Peterson, Neurobiol. Aging, 1987). Moreover, it could have important implications for the normal functions of glucocorticoids in the hippocampus (cf. McEwen, 1982, Adrenal Actions on Brain, Springer-Verlag). However, the AHP provides only an indirect measure of Ca influx. In the present studies, therefore, we quantified the effects of ADX (31 cells, 24 intact and ADX rats) on relatively "pure" calcium spikes which are prominent in cesium-loaded hippocampal pyramidal cells treated with tetrodotoxin. In this preparation, the Ca spike elicited by an intracellular depolarizing current pulse is characterized by a fast, large spike, followed by a longer, lower amplitude plateau phase. (ADX rats were maintained on 1% NaCl drinking saline, and all were healthy at study).

Significant decreases (ANOVA) in Ca fast spike width, plateau amplitude and spike duration were found in cells from ADX animals, although no clear differences in fast spike amplitude or spike inactivation during a 2-Hz train were found. Although additional studies are needed, these data suggest that adrenal steroids modulate brain Ca conductance. This possible relationship may have implications for our understanding of both the normal and the aging-like effects of adrenal steroids in the hippocampus. (Supported by AG04542, NIH).

106.10

AGING-RELATED INCREASES IN L-LIKE CALCIUM CURRENTS IN RAT HIPPOCAMPAL SLICES. L.W. Campbell*, S-Y Hao*, and P.W. Landfield (SPON: C.E. Dunlap). Dept. Physiol. & Pharmacol., Bowman Gray Sch. Med., Wake Forest Univ., Winston-Salem, NC 27103

Recent studies have indicated that Ca-dependent afterhyperpolarizations and Ca spikes are prolonged in CA1 hippocampal slice neurons from aged rats (cf. Landfield, Pitler, Science, 1984; Soc. Neurosci. Abstr, 1987; Pitler, Landfield, under review), which could be a factor in the altered Ca homeostasis that occurs in nerve cells during aging (cf. Khachaturian, 1984, Handbook of Studies on Psychiatry and Old Age, Elsevier; Gibson, Peterson, Neurobiol. Aging, 1987).

However, it is not clear which components of voltage-sensitive Ca currents may be affected most by aging. In the present studies, therefore, we used single-electrode voltage-clamp methods to conduct quantitative analyses of Ca currents in cesium-loaded, tetrodotoxin- and TEA-treated CA1 neurons in slices from 15 young-adult (3-5 mo. old) and 10 aged (24-27 mo. old) specific-pathogen-free Fischer rats.

Studies triggering large spike currents from a holding potential of -70 mV did not show aging differences. However, at holding potentials of -40 mV, large regenerative currents were partially inactivated (possibly T- or N-like currents) and good voltage control could be achieved over remaining L-like currents. Under those conditions, significant aging-related increases in Ca current were found.

When nimodipine, a DHP-class of Ca channel antagonist that blocks L channels, was applied, Ca current was reduced. Moreover, this effect of nimodipine was stronger in cells from aged rats. The sensitivity to nimodipine and the prominence of the aging effect at a -40 mV holding potential, suggest that aging is associated with increases in L-like Ca currents in hippocampal neurons, which could have implications for our understanding of aging-related neuronal degeneration. However, these data do not exclude the possibility of aging changes in other Ca channels or Ca homeostatic mechanisms as well (supported in part by AG 04542 and Miles, Inc.)

106.11

CHANGES IN CHOLINERGIC AND ADRENERGIC RECEPTORS IN PREFRONTAL CORTEX OF AGED RHESUS MONKEY: CORRELATION WITH PERFORMANCE ON DELAYED-RESPONSE TASK. M.V. Wagster*, L.C. Cork*, L.C. Walker and D.L. Price. Neuropathology Lab., The Johns Hopkins Univ. Sch. Med., Baltimore, MD 21205.

Neurotransmitter markers for the cholinergic and adrenergic systems were examined in the prefrontal cortices of 13 rhesus monkeys (*Macaca mulatta*) ranging from 2-34 years of age. Three years prior to sacrifice, the four oldest animals were tested on a spatial delayed-response task (DRT). In all subjects, *in vitro* receptor autoradiography was used to measure the binding concentrations of: [^{3}H] pirenzepine (PZ) for M1 cholinergic sites; [^{125}I] iodocyanopindolol (ICYP) for β1 and β2 adrenergic sites; and β-([^{125}I]iodo-4-hydroxyphenyl)-ethyl-aminomethyl-tetralone (HEAT) for α1 sites. Total ICYP binding concentration correlated negatively with age and DRT performance. A trend for a decrease with age in ICYP binding for β1 sites was detected but did not correlate with DRT performance. ICYP binding to β2 sites did not change appreciably with age but correlated negatively with DRT performance. HEAT binding decreased with age but did not correlate with performance on DRT. No correlation between PZ binding and age was detected, but PZ binding correlated positively with DRT performance.

106.12

DISCRIMINATION AND REVERSAL LEARNING IN THE AGED MONKEY. P.R. Rapp. The Salk Institute, La Jolla, CA 92037

Previous studies have demonstrated that behavioral deficits in the aged nonhuman primate are task-dependent. Since different brain regions make relatively specific contributions to learning and memory, the pattern of deficits aged monkeys exhibit across a battery of memory tasks provides a basis for predicting which neural structures mediate age-dependent cognitive dysfunction. In an ongoing series of studies, 4 young adult and 5 aged rhesus monkeys were tested in a variety of discrimination and reversal tasks. These monkeys were previously trained in 3 memory tasks, and 2 tests of response preference. Testing was conducted in a WGTA. Subjects were trained to a 90% correct level of performance in a pattern discrimination task in which one of two concurrently presented stimuli was always associated with reward. During subsequent reversal training, subjects were rewarded only when they chose the previously nonreinforced pattern. Although the average performance of the groups did not differ during pattern discrimination acquisition, some aged subjects required 2.5 to 5 times more training than young animals to reach criterion levels of performance. Since this form of learning is not dependent on intact medial temporal lobe function, these findings point to the involvement of other neural structures in mediating age-related cognitive decline. Most aged monkeys, however, performed as well as younger subjects during pattern discrimination reversal training. The normal discrimination and reversal learning observed in many aged monkeys therefore suggests that the acquisition of simple stimulus/reward associations remains largely intact during normal aging.

106.13

REACTION TIME CHANGES WITH AGING IN THE MONKEY. J. Bice*, H. Edwards*, J.W. Ashford. Southern Illinois Univ. Sch. of Med., Springfield, IL 62794.

Reaction time (RT) slows with age and Alzheimer disease (AD), particularly choice RT. We assessed simple (alerting function) and choice RT in young and old rhesus monkeys. In 3 old monkeys (23 years old), simple and choice RT measures declined over a 2 year period (average 3 sessions per week, 240 trials per 1 hour session). Simple RT was measured following a white flash, a delay interval (0-3 sec) and a blue light (down response required). Choice RT was measured to red (down) vs. green (up) light. The simple RT improved over this time from an average of 970 ms (880,780,1250) to 630 ms (600,490,800). The choice RT-red improved from 790 ms (730,470,1170) to 487 ms (550,400,510). Younger monkeys (6 years old) showed little improvement of RT over 4 months of training, average simple RT=675 ms (623,717,685) and average choice RT-red=497 ms (524,448,517). These data confirm studies in the aged human showing that extensive practice can overcome the RT disadvantage associated with age. Further analysis of the alerting function showed similar peak performances between 400 and 600 ms for both groups, similar to humans. However, the choice RT-red remained faster than the minimum simple RT, suggesting a confounding design factor. Manipulation of these variables by drugs and comparing them to human data will assist in the development of a monkey model of AD.

106.14

EVALUATION OF L-ACETYL CARNITINE (LAC) TREATMENT ON BRAIN AND BEHAVIORAL MEASURES IN AGED RATS. R.L. Spencer, J. Ganem*, S. Chapman*, W.K. O'Steen[1], B.S. McEwen. Rockefeller Univ., New York, NY 10021, and [1]Bowman Gray Sch. Med., Winston-Salem, NC 27103.

LAC has been shown to attenuate some of the age-related decline in physiological and behavioral function of rats. We studied the effect of LAC treatment on some biochemical and anatomical measures of septal-hippocampal circuitry and on behavioral measures of spatial learning. Old male and female Sprague-Dawley rats (21 mos at onset of treatment) were given LAC in their drinking water (75mg/kg) for 4 months. Choline acetlytransferase (ChAT) was significantly lower in old rats at 21 and 25 mos of age compared to young rats (2-4 mos). Age differences in spatial learning as measured in the Morris water maze were, however, confounded by visual ability. Based on retinal morphometry, there was 50-100% retinal degeneration in old rats by 21 mos of age. Of 37 old rats with the least retinal degeneration, 36 were able to learn the location of the hidden platform. Thus, when factoring in visual ability, spatial ability was retained in rats as old as 25 mos, even though some decline in ChAT activity was evident. Further evaluation of the utility of LAC treatment of aged rats may require more sensitive measures of behavior and perhaps an earlier onset of treatment.
(Supported by Sigma Tau)

106.15

DIETARY NIMODIPINE IMPROVES ASSOCIATIVE LEARNING AND OPEN FIELD BEHAVIORS IN AGING RABBITS. K.T. Straube, R.A. Deyo*, J.R. Moyer, Jr. and J.F. Disterhoft. Dept. of Cell Biology and Anatomy, Northwestern Univ. Med. Sch., Chicago, IL 60611.

Recently, the Ca^{++} channel blocker nimodipine (NIM) administered intravenously was shown to facilitate learning in aging rabbits (Deyo, et al. *Science, 243:* 809-811, 1989). In the present experiment we evaluate the effects of NIM given orally on associative learning and open field behaviors.

Sixteen aging rabbits (32-50 mo) were assigned to 1 of 2 groups (n=8) and fed 860 ppm NIM or the same diet without NIM (aging-controls). An additional 8 (3 mo) rabbits were maintained on the control diet (young controls). Animals were fed these diets for 28 days prior to and during testing. Rabbits were trained a maximum of 25 days in a trace conditioning task in which the CS was a 100 ms, 85 db, 6 KHz tone followed by a 500 ms trace interval followed by a 150 ms 2.5 psi corneal airpuff (UCS). A conditioned response (CR) was any response occurring after the CS onset but prior to the UCS onset. Immediately after training, rabbits were tested on a 4' x 4' open-field. The number of crossings, rearing and grooming behaviors were recorded for 10 minutes each day for 5 consecutive days.

NIM accelerated acquisition (4 CRS in any block of 5 trials) in aging rabbits relative to aging controls ($p < .05$). The oral dose of 860 ppm NIM used here was not as effective as i.v. administration at 1.0 µg/kg/min as was previously reported (Deyo, et al., 1989). Aging rabbits showed significant learning impairments when compared to young animals ($p< .05$). In addition, the increased open field activity observed in aging rabbits was reduced by NIM ($p < .01$), while rearing behavior increased ($p < .05$). These behavioral rates in aging NIM rabbits were similar to the young controls. These data provide further evidence of NIM facilitation of learning and open field behavior in aging subjects.

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106.16

EFFECTS OF NIMODIPINE AND OTHER CA^{++} CHANNEL AGENTS ON THE ACTIVITY OF SINGLE HIPPOCAMPAL PYRAMIDAL CELLS AND CLOSELY ASSOCIATED INTERNEURONS. L.T. Thompson, R.A. Deyo*, & J.F. Disterhoft. Dept. Cell Biology & Anatomy, Northwestern Univ. Med. Sch., Chicago, IL 60611.

Studies of eye-blink conditioning show that the calcium-dependent afterhyperpolarization following firing is reduced in hippocampal pyramidal cells in slices taken from conditioned rabbits (Disterhoft *et al., PNAS 83:* 2733,1986). The firing of these neurons is enhanced during conditioned eye-blink responses (Berger *et al., J. Neurophysiol. 50:* 1197, 1983). Nimodipine, a dihydropyridine calcium antagonist, facilitates learning of the eye-blink response in aging rabbits (Deyo *et al., Science 243*, 809, 1989). Since pyramidal cell activity is regulated by calcium -dependent currents, we examined the effects of nimodipine and other antagonists and agonists on hippocampal neurons to begin describing the neural mechanisms underlying nimodipine's behavioral facilitation in aging.

Anesthetized young or aging adult albino rabbits were chronically implanted with driveable bundles of ten 32 µm nichrome microwire electrodes over the dorsal hippocampus and with jugular catheters. After one week of recovery, extracellular unit activity was analyzed using a BrainWave™ workstation, allowing simultaneous discrimination of up to 32 single-units. The characteristic waveforms and firing frequencies of pyramidal cells and interneurons permitted separation of these neuronal populations for study. Unit activity was continuously recorded prior to, during, and following infusion of calcium channel agents. Nimodipine enhanced firing of pyramidal cells in aging animals and suppressed activity of interneurons at the same time. This effect on activity was reversed by removal of the drug. Other agents, including BAY-K-8644 and flunarizine, are also being tested.

Nimodipine enhanced pyramidal cell firing in the aging hippocampus. These cells were previously shown to have enhanced activity during associative responses in young animals. The simultaneous reduction in interneuron firing rates is also consistent with nimodipine facilitation of hippocampal function in aging.

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106.17

NEUROCHEMICAL, ENDOCRINE AND IMMUNOLOGICAL RESPONSES TO STRESS IN YOUNG AND OLD F344 MALE RATS. S. Lorens, N. Hata*, R. J. Handa*, L. Van de Kar, M. Guschwan* and J. Clancy*. Dept. Pharmacology and Anatomy, Loyola University Medical Center, Maywood, IL 60153.

Forebrain dopamine (DA) but not norepinephrine (NE) or serotonin (5-HT) metabolism is significantly reduced in old non-stressed rats. Conditioned fear increased medial frontal cortex, nucleus accumbens and amygdaloid DA turnover in both young (7 mo) and old (22 mo) rats; decreased medial frontal NE content only in young rats; and, increased medial frontal cortical and hypothalamic 5-HT turnover only in old animals. These observations suggest age-related differences in the response of central NE and 5-HT systems to stress. No age-related differences were observed in the basal (morning) plasma corticosterone levels. However, the corticosterone response to ether stress and to conditioned fear was significantly higher in old rats. Hippocampal corticosterone type I but not type II receptors were decreased by 47% in 17.5 mo old rats. Thus, age-related changes in hippocampal corticosterone receptor types do not occur in unison, and the exacerbated corticosterone response to stress precedes the reported down-regulation of hippocampal type II receptors in aged rats. Immune function was impaired in the old non-stressed rats, and further compromised by exposure to conditioned fear. The old non-stressed rats showed an increased percentage of splenic large granular lymphocytes, reduced natural killer cytotoxicity, and impaired Con-A stimulated T lymphocyte proliferation. Compromised immune function also was observed in the young rats subjected to fear conditioning, but not to the same extent as in the old rats. Thus, aging male F344 rats evidence major alterations in central monoamine, endocrine and immune functions, and an increased sensitivity of these systems to stress.

106.18

STABILITY OF POSTSYNAPTIC DENSITY DIMENSIONS IN HIPPOCAMPAL SYNAPSES OF AGED RATS WITH POOR AND GOOD SPATIAL MEMORY. L. deToledo-Morrell, Y. Geinisman and F. Morrell. Depts. of Neurol. Sci. and Psychol., Rush Med. Coll. and Dept. of Cell Biol. & Anat., Northwestern Univ. Med. Sch., Chicago, IL 60612.

Spatial memory (SM), which crucially depends on the integrity of the hippocampal formation, is impaired in the majority, but not all, aged rats (deToledo-Morrell et al., Behav. Neurosci., 1984, *98:* 902). Structural substrates of this memory dysfunction may include decreases in the number and size of hippocampal synapses. We have reported earlier that the extent of loss of perforated axospinous synapses in the hippocampal dentate gyrus of aged rats correlates with the degree of SM deficit (Geinisman et al., Brain Res., 1986, *398:* 266). The present study was designed to verify whether the size of the postsynaptic density (PSD), the area of greatest concentration of postsynaptic neurotransmitter receptors and ion channels, is diminished in hippocampal synapses as a function of the age-related decline in SM. Young adult rats (5 mo. old) with good SM (as assessed in an 8-arm maze), aged rats (27 mo. old) with impaired SM and equally aged rats with intact SM were compared. Axodendritic, axospinous perforated (with discontinuous PSDs) and axospinous nonperforated (with continuous PSDs) synapses were differentially analyzed in the middle molecular layer of the dentate gyrus. Using serial sections, the maximal profile length was determined for each PSD sampled. Differences on this measure among the groups of rats under comparison were found to be small and statistically not significant. There was no significant correlation between the PSD maximal profile length and the extent of SM loss. Thus, PSD dimensions are unaffected by advancing chronological age and by the age-related memory dysfunction. It appears that a specific loss of certain hippocampal synapses, rather than a reduction in synaptic size, is a major morphological substrate of the age-related decline in SM. Supported by Grants AG 03410 from NIA and BNS 87-107 from NSF.

106.19

LONG-TERM BEHAVIORAL AND NEUROCHEMICAL EFFECTS OF INTRADENTATE ADMINISTRATION OF COLCHICINE IN RATS. H.A. Tilson, K. Nanry, M. Bonner, S. Barone Jr., E.G. Drust, and P. Tandon. LMIN, NIEHS/NIH, RTP, NC 27709.

Deficits in learning and memory and alterations in the signal transduction process for the cholinergic muscarinic receptor have been observed 12 wks after colchicine (COL) treatment. To study the long-term effects of COL administration on cognitive function and the cholinergic system, 6 mo-old male, Fischer-344 rats were injected with 2.5 ug of COL bilaterally in the dorsal and ventral hippocampus. A significant deficit in acquisition in the water maze was observed in animals 1 yr after COL administration. After [^{3}H]-inositol was incorporated into hippocampal slices, neurochemical studies showed an increase in the carbachol-induced PI metabolism in the rat hippocampus 1 yr post-lesion with COL. A significant hyperstimulation of ibotenic acid-induced PI turnover was also observed 1 yr after COL treatment. However, in contrast to results obtained 12 wks after lesioning, no significant changes were observed in norepinephrine or serotonin-induced PI metabolism 1 yr post-lesion. Pirenzepine, an M_1 receptor antagonist, produced a greater degree of inhibition (60%) in lesioned animals as compared to the age matched controls (20%). Increased staining for AChE was found in the hippocampus of treated rats, similar to that observed 12 wks post-lesion.

106.20

MONOAMINERGIC & CHOLINERGIC CORRELATES OF IMPAIRED SPATIAL MEMORY IN AGED RATS. V. Luine, D. Bowling*, M. Hearns* & C. Milio* Psychology Dept., Hunter College, N.Y., N.Y. 10021

Spatial memory performance in aged (25 months) and young (3 mo.) Fisher 344 rats was assessed on the 8-arm radial maze, and levels of NE, DA, 5HT and metabolites, and ChAT activity were measured in micropunched brain samples. Analyzed were cholinergic and monoaminergic cell body areas, frontal cortex (FCx), hippocampal regions, and other areas. Monoamines were measured by HPLC with EC, and ChAT by radiochemistry. Aged rats showed significant decrements in choice accuracy and trials to criterion. Numerous age related differences, some of which correlated with performance, were found. ChAT activity decreased by 17-25% in aged rats in the vertical diagonal bands (vDB), striatum and dentate gyrus; only vDB changes correlated with performance. Also correlated with performance were: 50% declines of NE in locus coeruleus and n. basalis (nBM) of aged rats and 30-66% declines of DA, DOPAC or HVA in entorhinal Cx, FCx, nBM and substantia nigra. DA and metabolites showed the greatest number and magnitude of age related changes and correlations with choice accuracy. The relatively few age related differences in the hippocampus were noteworthy. Results suggest that attenuated catecholaminergic activity, especially DA, makes a substantial contribution to the impaired performance of aged rats on a memory task. Cholinergic contributions, as indexed by ChAT activity, were less evident. (Supported by AG06384).

106.21

BIOGENIC AMINES IN THE AGED RAT BRAIN: RELATION TO BEHAVIOR. P.R. Miller*, M.H. Kodsi*, S. Southerland*, R.D. Burwell*, M. H. Lewis and M. Gallagher (SPON: R.J. Fanelli). Departments of Psychology and Psychiatry, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599.

Young (5 mo, N=29) and aged (25 mo, N=31) male Long-Evans rats were tested on a number of behavioral tasks providing measures of learning, diurnal behavior and sensorimotor function. A subpopulation of the aged rats (N=17) had a spatial learning deficit that was of comparable severity at both the beginning and end of the 13 wk testing protocol. The spatial learning capacity of the remaining aged rats (N-14) was within the range of the young group's performance. The "impaired" aged subgroup was also distinctly impaired on some, but not all, other behavioral measures that declined with age. Markers for biogenic amines (NE, DA, DOPAC, HVA, 5HT, 5HIAA, AND ChAT) were measured using HPLC and radioenzymmatic assays. For the measures that showed a significant change with age, some were equally altered in both subgroups of aged animals, i.e. 5HIAA elevation in all forebrain regions and a decline in striatal DA, DOPAC and HVA. In contrast, other neurochemical parameters were significantly altered only in the "impaired" subgroup of aged rats, i.e. ChAT reduction in basal forebrain and frontal cortex, DA and DOPAC reduction in basal forebrain, and NE elevation in both frontal and parietal cortices. The relation between age-related neurochemical changes and other behavioral indices of aging in these animals will be discussed.Supported by a NSF Predoctoral Fellowship to RB, a NIMH Research Scientist Development Award to MG (K02-MH00406) and grants NIMH MH39180 and NSF BNS 87-19881.

106.22

CHRONIC DEPRENYL TREATMENT AND PSYCHOMOTOR FUNCTION IN AGED MICE. *M. E. Chachich[1,4], M. Gupta[2], H. L. Wiener[3]*, J.A. Joseph[4], and D.K. Ingram[4]*.* [1]Dept. Psychol., Towson St. U.; [2]Dept. Anat., U. Louisville Sch. Med., Louisville, KY 40292; [3]N.S. Kline Inst. for Psychiat. Res., Ward's Island, NY 10035; [4]Geront. Res. Ctr., NIA, NIH, Baltimore, MD 21224.

MPTP cytotoxicity can be blocked by inhibiting monoamine oxidase-B (MAO-B). This observation has suggested a hypothesis that aging of the nigrostriatal system might result from the generation of oxy-radicals through normal action of MAO-B metabolism of dopamine (Cohen, G., *Adv. Neurol.*, 45:119, 1986). To examine this hypothesis, 18-mo old male C57BL/6J mice were tested in a psychomotor battery that included locomotor activity in an open field (10-min), a runwheel cage (48-hr), and on an inclined screen (10-min) and for their ability to perform on a rotorod (3 rpm), an accelerated rotodrum (up to 48 cm/sec), and a tightrope (three 60-sec trials). Mice were then provided *l*-deprenyl (a MAO-B inhibitor) in distilled water (0, 0.5 or 1.0 mg/kg per day) for 6-9 mo. When mice were retested at 24 and 27 mo of age, longitudinal declines in control groups were observed in tests of open field, runwheel, inclined screen, and rotodrum performance. No deprenyl effects were observed in any parameter except the rotodrum test, *i.e.* mice treated with 1 mg/kg maintained their maximum running speed over 9 mo. Compared to values observed among controls, HPLC analysis of catecholamines and enzymatic analysis revealed significant reduction in striatal MAO-B activity induced by deprenyl as early as 3 weeks after treatment and sustained over 6 months. Analyses of age-related loss of striatal dopamine receptors as well as changes in dopaminergic neurons in substantia nigra using fluorescence histochemistry are being conducted. However, present results indicate that chronic MAO-B inhibition during normal aging does little to retard decline in psychomotor performance in mice as assessed in this battery.

106.23

AGING AND SERIAL LIST MEMORY. A.S. Gilinsky and M.Korsnes Department of Psychology, University of Bridgeport,Bridgeport, CT 06601.

The easiest item to remember in a series is either the first or the last depending on the length of the interval between exposure to the series and the recognition test. A short interval favors the recent item; a long interval shifts superiority from recency to primacy; both primacy and recency effects operate at intermediate intervals. Wright, Santiago, Sands et al, Science,229:287,1985, found the list memory of pigeons, monkeys, and humans for visual recognition items showed the same modification of the memory process in the the three species with increasing retention intervals but the time course of these changes was fastest for pigeons and slowest for humans. Our experiment investigated human age differences in the serial position modification effect using the Wright et al paradigm in young (25-35 year-old) and old (65-75 year-old) college graduates. Increasing delay intervals gave a consistent modification of the recency effect in both groups. Short intervals gave strong recency effects;long intervals reduced the recency effects to chance levels. Older subjects were significantly more accurate than the young at brief delays and crossed over to primacy effects earlier in the time course than younger persons, not unlike Wright et al's monkeys compared to humans.

107.1

CNS CELL GROUPS INNERVATING THE PARASYMPATHETIC PREGANGLIONIC NEURONS REGULATING THE PTERYGOPALATINE GANGLION AS REVEALED WITH THE RETROGRADE TRANSNEURONAL VIRAL LABELING METHOD

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The pterygopalatine ganglion, also called the sphenopalatine ganglion, provides parasympathetic innervation to the lacrimal gland, the nasal and palatal mucosa, and the cerebral vasculature. The central innervation of these preganglionic neurons is unknown. We have used the retrograde transneuronal viral technique to identify central nuclei that innervate the preganglionic neurons regulating the pterygopalatine ganglion.

Pseudorabies virus injections were made into the pterygopalatine ganglion of rats. Four days later, the rats were perfused with 4% paraformaldehyde. The brains were processed for immunohistochemical detection of viral infections. Labeling of the ipsilateral parasympathetic preganglionic neurons was seen in the ventrolateral medullary reticular formation. In addition, viral infections of second order neurons in the brain that project to the preganglionic neurons were identified at several CNS levels. These were labeled as a result of retrograde transneuronal viral transfer.

Bilateral labeling was seen in the ventrolateral medullary reticular formation, A5 cell group, parabrachial nuclei, raphe magnus and pallidus nuclei, central gray matter, lateral and paraventricular hypothalamic nuclei, zona incerta, and bed nucleus of the stria terminalis. Ipsilateral cell body labeling was seen in the nucleus tractus solitarius, parapyramidal nucleus, and amygdala. Contralateral labeling was seen in the tuberomammillary nucleus of the hypothalamus.

107.2

ALTERED BONE REMODELING IN SYMPATHECTOMIZED AND SENSORY DENERVATED RATS.

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Bone metabolism may be influenced by the nerves in bone tissues. Neuropeptides such as vasoactive intestinal peptide (VIP, from sympathetic nerves) and calcitonin gene-related peptide (CGRP, from sensory nerves) have been implicated as local modulators of bone metabolism. The effect of guanethidine sympathectomy or capsaicin sensory denervation in rats on the following was studied : 1) the presence of VIP-, CGRP-, substance P (SP)-, and dopamine-ß-hydroxylase (DßH)-immunoreactive nerve fibers in periosteum; 2) the radial growth and apposition rate in tibiae (normal growth and modeling); 3) the percentage of periosteal surface of the mandible occupied by osteoclasts during induced remodeling. Neonatal rats were treated with guanethidine, capsaicin, or appropriate vehicle. At 7 weeks, maxillary molars were removed to induce remodeling on the buccal surface of the mandible. Animals were perfused 4 days after surgery. Whole mounts of periosteum were stained for immunofluorescence using antisera to VIP, CGRP, SP, and DßH. Tibial cross-sectional cortical area, medullary area, and periosteal apposition rate were measured by histomorphometry in ground sections. The percentage of periosteal surface at the remodeling site occupied by osteoclasts (stained for acid phosphatase) was measured in frozen, undecalcified sections. CGRP-and SP-immunoreactive nerves fibers were dramatically reduced in periosteum of capsaicin-treated animals as compared to controls; VIP- and DßH-immunoreactivity (IR) appeared unchanged. VIP- and DßH-IR were dramatically reduced in guanethidine-treated animals; CGRP- and SP-IR appeared unchanged. There was no significant difference in cortical or medullary area or periosteal apposition rate between each drug treatment and its control. Sympathectomy resulted in a 45.5% ($p \leq .005$) increase over controls in the mandibular bone surface occupied by osteoclasts. Sensory denervation resulted in a 21.2% ($p \leq .04$) decrease from controls. These data suggest that selective denervations did not affect normal bone growth and modeling. The alteration of at least one parameter (osteoclast surface) of bone remodeling by both treatments indicates that sensory and sympathetic nerves play a role in focal bone metabolism.

107.3

A GENERAL PATTERN OF CNS INNERVATION OF THE SYMPATHETIC OUTFLOW DEMONSTRATED BY TRANSNEURONAL PSEUDORABIES VIRAL INFECTIONS.

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Pseudorabies virus (PRV) injections of various sympathetic ganglia (viz., superior cervical, stellate, celiac and lumbar paravertebral ganglia and the adrenal gland) were made in rats. Two days later, colchicine (100 µg/10 µl saline) was injected into the lateral ventricle. After two days, the rats were perfused with 4% paraformaldehyde. Immunohistochemically detectable retrograde viral infections of ipsilateral sympathetic preganglionic neurons (SPNs) and transneuronal infections of the specific sets of second order neurons in the spinal cord and brain that innervate the infected SPNs were seen.

Five cell groups in the brain appear to regulate the entire sympathetic outflow: the paraventricular hypothalamic nucleus (PVH), A5 noradrenergic cell group, caudal raphe region, rostral ventrolateral medulla, and ventromedial medulla. Other CNS areas also became transneuronally labeled after infections of certain sympathetic ganglia, most notably the superior cervical and stellate ganglia. These areas include the central gray matter and lateral hypothalamic area. The zona incerta was uniquely labeled after stellate ganglion infections. The cell body labeling was specific. This specificity was demonstrated in the PVH where the neurons of the parvocellular PVH that form the descending sympathetic pathway were labeled in a topographic fashion.

107.4

ULTRASTRUCTURAL ANALYSIS OF AUTONOMIC PREGANGLIONIC NEURONS IDENTIFIED WITH A MONOCLONAL ANTIBODY TO CHOLINE ACETYLTRANSFERASE.

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A monoclonal antibody to the acetylcholine synthesizing enzyme choline acetyltransferase (ChAT) was used to identify autonomic neurons in the intermediolateral nucleus (ILp) of the adult rat thoracic spinal cord. Labeled cells were identified in the electron microscope, and an ultrastructural analysis was made of their dendritic processes and afferent input. Synaptic terminals on identified ChAT(+) ILp neurons consisted of three types: (1) terminals containing round, clear vesicles with or without dense-cored vesicles (DCVs); (2) terminals containing clear pleomorphic or flattened vesicles; and (3) terminals containing a large proportion of DCVs. The first group was the predominant terminal type, and serial section analysis indicated that the third group may be a subset of the first, with the predominance of DCVs being a consequence of the section plane. Dendrites of labeled ILp cells formed extensive transverse bundles. Longitudinal bundles of labeled dendrites were also found between ILp cell clusters located rostrocaudally along the spinal cord. Such dendritic bundles were closely associated with fascicles of thin, unmyelinated axons. Both dendritic and axonal groups were surrounded by astroglial sheaths. Axons contained within fascicles occasionally formed varicosities that synapsed upon labeled ILp dendrites. The results suggest that dendritic bundling may maximize the convergence of afferent systems upon groups of autonomic neurons, and that astroglial processes may isolate these units from the surrounding neuropil. *Supported by NIH grant NS25784.*

107.5

DIFFERENTIAL COEXISTENCE OF SUBSTANCE P (SP)-IMMUNOREACTIVITY (IR) WITH OTHER NEUROCHEMICALS IN INTERMEDIOLATERAL CELL COLUMN (IML)-PROJECTING NEURONS IN THE VENTRAL MEDULLA OBLONGATA (VM)

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SP-IR coexists with serotonin (5HT)- and thyrotropin releasing hormone (TRH)-IR in VM neurons that project to the IML. Enkephalin (ENK)-IR neurons are similarly distributed and also project to the IML. Neuropeptide Y (NPY)-IR neurons in the VM have a small overlap in distribution with SP-IR neurons. The overlapping distribution of ENK- and NPY- with SP-IR suggests that these neurochemicals may coexist with SP-IR in IML-projecting neurons. The present study investigated the possible coexistence of SP- with NPY- or ENK-IR in IML-projecting neurons.

Rats received 40nl injections of rhodamine beads into the T3 IML. Colchicine was given ICV 4d later, and 24h later animals were perfusion fixed. Tissue was sectioned at 4µm and stained using dual color immunofluorescence.

Despite their similar distributions ENK- and SP-IR coexisted only rarely in IML-projecting neurons. NPY-IR was identified in IML-projecting neurons but coexistence of NPY- and SP-IR was also rare, although it was somewhat greater than that of ENK- and SP-IR. The results of these studies indicate that there are several neurochemically coded subsets of IML-projecting neurons in the VM. (NS24876-CH; HL07565-CS)

107.6

SPATIAL RELATIONSHIP OF NERVE TERMINALS CONTAINING OPIOID PEPTIDES TO THE DENDRITIC TREE AND CELL BODY OF SACRAL PREGANGLIONIC PARASYMPATHETIC NEURONS.

J. Wang, W. Wu and R. Elde (SPON: V. Seybold). Dept. Cell Biology and Neuroanatomy, Univ. Minnesota, Minneapolis MN 55455.

Although much is known about the regional distribution of opioid peptides in the CNS, less is known concerning the cellular arrangements which enable opioids to be delivered to receptors on target neurons.

In order to address this, preganglionic parasympathetic neurons were retrogradely labeled by exposing the proximal end of the severed pelvic nerve to 1% FluoroGold. After perfusion fixation, one or two such labeled neurons in 200µm vibratome sections were intracellularlly filled by iontophoresis with 3% Lucifer Yellow. Sections were immunostained for enkephalin and dynorphin using rhodamine-labeled second antibodies. Sections were examined using laser scanning confocal microscopy.

Neurons filled with Lucifer Yellow were often found to have two aspiny, primary dendrites, one of which extended laterally into the white matter. The other dendrite often extended medially toward the central canal. Branching of dendrites was sparse. Nerve terminals containing enkephalin and dynorphin immunoreactivity were not restricted to the vicinity of the cell body, but were found to appose even the most distal portions of the dendrite. Supported by DA 02148

107.7

SACRAL SPINAL REFLEX PATHWAYS IN THE CLAWED FROG, **XENOPUS LAEVIS**. J.C. Bresnahan, H.L. Campbell, and M.S. Beattie. Depts. of Anat., Surg., and Neurosci. Prog., Ohio State U., Cols., OH 43210.

HRP was applied to pelvic visceral structures, or to the 10th spinal nerves, in juvenile **Xenopus laevis** frogs in order to determine the location and morphology of neurons involved in sacral spinal reflexes.

Labelling of the 10th nerve showed, in addition to lateral motor column (LMC) neurons, a group of small neurons located in the medial and lateral intermediate zones. These cells were similar in location and morphology to mammalian sacral parasympathetic nucleus (SPN) preganglionic neurons (PGNs). Labelled dorsal root afferents made apparent direct contacts with their somata and proximal dendrites. HRP applied to peripheral nerves innervating the cloaca, cloacal sphincter, and bladder labelled this same population of cells, and also revealed a group of lamina IX neurons located in a medial column extending from the caudal cord through the 10th spinal segment. These putative motoneurons were smaller than LMC motoneurons, and their location and morphology was reminiscent of neurons in Onuf's nucleus in mammals. The organization of sacral reflex systems in **Xenopus** is thus similar to that in mammals. (NS-10165)

107.8

DISTRIBUTION, CONNECTIVITY, AND ORIGIN OF TRH-IMMUNOREACTIVE (TRH-I) FIBERS IN THE DORSAL MOTOR NUCLEUS OF THE VAGUS AND NUCLEUS OF THE SOLITARY TRACT (DMV/NST) IN RAT. R.B. Lynn*, L. Rinaman, and R.R. Miselis (SPON: G.R. Christoph). Animal Biology & Institute of Neurological Sciences, University of Pennsylvania, Philadelphia, Pennsylvania 19104-6045.

Central TRH stimulates gastric activity, perhaps by direct effects on gastric neurons in the DMV/NST. This anatomical study examined that possibility in rats. The stomach wall was injected with 25 µl of the β-subunit of unconjugated cholera toxin (CTB; Sigma; 0.25%) to label gastric vagal motoneurons and dendrites. Rats were perfused 72 h later, and brain sections processed for localization of transported CTB and/or TRH-I using goat anti-CTB (Calbiochem) and rabbit anti-TRH (gift of M.S. Kreider, Univ. Penn.). A two-color peroxidase reaction showed TRH-I fibers associated with CTB-labeled dendrites in the DMV/NST. Ultrastructural analysis showed TRH-I terminals forming asymmetric synaptic contacts with CTB-labeled gastric vagal motoneuronal dendrites. In addition, TRH-I terminals formed asymmetric and symmetric synapses with unlabeled dendrites, and non-synaptic TRH-I varicosities were present throughout these nuclei. In a second group of rats, the DMV/NST was injected with 50 nl of Fluorogold to retrogradely label the source of these afferents. After 3-8 days, rats received colchicine ICV, and were perfused 48 h later. Brain sections containing retrogradely-labeled neurons were processed for TRH-I using FITC immunofluorescence. Double-labeled neurons were found only in the nuclei raphe obscurus and pallidus. This work demonstrates that 1) TRH-I terminals in the DMV/NST synapse on the dendrites of gastric vagal motoneurons and non-motoneurons; 2) TRH may also be released non-synaptically in the DMV/NST; and 3) the raphe nuclei provide at least part of this TRH-I innervation. This work was supported by GM27739 and E.I. duPont de Nemours & Co.

107.9

REPRESENTATION OF CECUM IN LATERAL DORSAL MOTOR NUCLEUS AND COMMISSURAL SUBNUCLEUS OF THE NUCLEUS TRACTUS SOLITARIUS IN RAT. D. A. Ferenci*, S. M. Altschuler*, R. R. Miselis (SPON: J. Metzler). Depts Pedia., An. Biol., and Inst. Neurol. Sci., Univ. of Penn., Phila., PA 19104.

This study demonstrates vagal innervation of the cecum using a sensitive retrograde neural tracer, cholera toxin-horseradish peroxidase(CT-HRP). CT-HRP was injected into exteriorized cecum(n=22). In separate rats stomach, ileum, or ascending colon were injected. After 72-120 hour survival periods brainstems were processed. With all cecal injections, strong labeling of the lateral 1/3 of the dorsal motor nucleus(DMN) occurred bilaterally above, at and below the level of the area postrema. Dendrites of laterally positioned neurons projected medially and rostrocaudally within the DMN and dorsally into the medial and commissural subnuclei(sn) of the nucleus tractus solitarius(NTS). Afferent labeling occurred in the anterolateral and medial area of the commissural sn of the NTS. Stomach injections labeled the medial column of the entire rostrocaudal extent of the DMN and the gelatinosus sn of the NTS. Ileal and ascending colonic injections labeled neurons very sparsely in the lateral columns. The vagal complex has a prominent viscerotopography: DMN has a mediolateral organization corresponding to end organ innervation; NTS has a rostrocaudal axis of visceral representation corresponding to rostrocaudal positioning along the alimentary canal. Supported by NIH grants GM27739, DK01747, and DK07066.

107.10

VISCEROTOPIC ORGANIZATION OF ABDOMINAL VAGAL BRANCHES AS DETERMINED BY INDUCED MOTILITY RESPONSES. N. Carlson*, H.-R. Berthoud, and T.L. Powley (SPON: F.R. Brush). Lab. of Regulatory Psychobiology, Purdue Univ., W. Lafayette, IN 47907.

In order to examine the functional implications of the longitudinal columnar organization of the dorsal motor nucleus of the vagus (dmnX), the innervation field for each column and associated branch needs to be identified. Cervical vagal stimulation-induced motility responses were monitored all along the GI tract, using miniature strain gauges, before and after selective abdominal vagal branch cuts in anesthetized rats. Because motility fronts can propagate "enterically" along the gut without vagal input, only short latency (<30sec), stimulus-locked responses were considered. The branches were found to mediate such responses at the following sites:

	STOMACH	DUODENUM prox.	DUODENUM mid.	DUODENUM dist.	JEJUNUM & ILEUM	CECUM & COLON
gastrics	X	X				
hepatic		X	X			
celiacs		X	X	X	X	X

Therefore, because the gastric columns are medial in the dmnX and the celiac columns are lateral, there is a viscerotopic organization of vagal control over gastrointestinal motility. The oral-aboral axis of the gut is represented on a medial-lateral axis within the dmnX. Furthermore the duodenum is a major site of overlapping innervation of all vagal branches/columns. Finally, this is the first functional demonstration of vagal innervation of the cecum and supports the results of anatomical studies (Berthoud, H.-R., et al., this meeting; and Miselis, R.R., personal communication). (DK27627 and NS26632)

107.11

SIMULTANEOUS IDENTIFICATION OF AFFERENT INPUTS AND EFFERENT PROJECTIONS OF THE DORSAL MOTOR NUCLEUS OF THE VAGUS WITH DiI. H.-R. Berthoud, A. Jedrzejewska* T.L. Powley. Lab. of Regulatory Psychobiology, Purdue U., W. Lafayette, IN 47907.

To develop a protocol that would concurrently label CNS inputs to vagal preganglionics as well as the terminals of these motor neurons in the periphery, we made bilateral DiI injections into the dmnX (0.2-5%, in EtOH, 2-4 per side, 50nL per injection) in SD rats. At sacrifice (15-30days), animals were perfused with, and the tissue samples stored in, 10% formalin at 4°C. Brains were cut with a cryostat; gut tissues were prepared as peels of particular wall layers. Both were processed in PBS, counterstained with Bisbenzimide, cleared and mounted in Glycerol, and viewed with epifluorescence.

The extensive injections labelled most, if not all, dmnX cells and typically encroached on neighboring structures. Afferent projections to the dmnX, including those from the parabrachial n., the PVN, and the central n. of the amygdala, were brightly and selectively labeled. Corroborating and extending previous anterograde tracer demonstrations of vagal preganglionics (e.g Kirchgessner & Gershon, Neurosci. Abs., 14(1988) 1317), labelled vagal profiles (single and multiple axons, fine and large varicose endings) were found exclusively in the myenteric plexus throughout the GI tract, including the most distal small bowel as well as the cecum and proximal colon. The stomach (all parts) showed by far the densest innervation, with labelled vagal profiles present in virtually every myenteric ganglion and connective. The cecum evidenced extensive vagal innervation as well. In the intestine, single fibers could be observed coursing through a number of ganglia, giving off collaterals and fine endings in each of them. (DK27627 and NS26632)

107.12

3-D DIGITAL ATLAS OF THE DORSAL MOTOR NUCLEUS OF THE VAGUS. T.L. Powley, F.B.Wang and E. Baronowsky.* Lab. of Regulatory Psychobiology, Purdue Univ., W. Lafayette, IN 47907.

To facilitate high resolution analyses of vagal motor function (e.g. electrophysiology, picospritzing, microsurgery, dendroarchitectonic reconstruction, etc.) and to make it feasible to map these functions onto the defined columns or subnuclei of the dorsal motor nucleus (dmnX), we have developed a 3-D database and atlas of the different subpopulations of dmnX preganglionics.

In separate groups of rats (10 or more/group), each of the primary branches of the abdominal vagus was independently soaked with HRP to retrogradely label the corresponding dmnX cell column. Serial coronal sections (56µm) of the medulla oblongata were saved and processed with TMB. Additional groups representing the full ventral and dorsal trunks as well as secondary gastric branches were similarly prepared. Using standard medullary surface landmarks, the x-, y-, and z-coordinates of all labeled cells were then digitized (Bioquant System IV). For each defined pool of preganglionics as well as for the entire dmnX, exact dimensions (confidence limits) in all three planes were then calculated and expressed in stereotaxic coordinates. The results confirm our earlier descriptions (e.g. Fox & Powley, Br.Res., 1985; Powley et al., AJP, 1987) and graphically illustrate the fine-grained pattern of the columnar organization of the dmnX. For each identified pool of motorneurons, numbers of cells, packing densities, and relative concentrations in different regions of the dmnX were determined. These results provide a 3-dimensional database for experimental manipulations of the vagal preganglionic neurons controlling the different viscera. (DK27627 & NS26632)

107.13

DIFFERENTIAL PROJECTIONS TO THE NUCLEUS OF THE SOLITARY TRACT FROM UTERUS. R. Guevara-Aguilar, M. García-Bazán and M. Ortega-Villalobos. Depto. de Fisiol. UNAM. México, D.F.

Previously, we reported labelled cells in the nucleus of the solitary tract (N.T.S.), after the application of HRP in the body of the uterus of the rat. The aim of this paper was to learn more about the distribution of the afferent fibers from the different parts of the uterus to the different nuclei which compound the NTS complex. WGA-HRP 40% solution was injected at different levels of the uterus body. The first group was injected in the anterior third; the second group was administered in the half third, including both ductus; in the third group the HRP was injected in the cervix and the fourth one, was injected in all parts mentioned above. The percentage of labelled cell bodies in the medial, lateral and commissural nucleus of the NTS complex was different for each group. The first group has the highest percentage of labelled cell bodies localized in the lateral nucleus; the medial nucleus was weak labelled and no labelled cells were found in the commissural nucleus. The second group had similar label cells in the lateral as well as in the medial nucleus. The commissural nucleus presented more label cells. In the third group, the highest percentage of label cell bodies was observed in the commissural and in the lateral with the lowest percentage of labelled cells in the medial nucleus. The fourth one has similar percentage of cell bodies label in the three nucleus.

107.14

EFFERENT PROJECTIONS FROM THE BED NUCLEUS OF THE STRIA TERMINALIS TO THE NUCLEUS OF THE SOLITARY TRACT AND VENTROLATERAL MEDULLA IN THE RAT. A.M. Zardetto-Smith, D.J. Magnuson and T.S. Gray. Dept. of Anatomy, Loyola University Stritch School of Med., Maywood, IL 60153.

The lateral portion of the bed nucleus of the stria terminalis (BST) is closely related to the central nucleus of the amygdala (CeA) in terms of connectivity to central autonomic nuclei, such as the nucleus of the solitary tract (NTS) and ventrolateral medulla (VLM). The CeA has previously been shown to selectively innervate subpopulations of catecholaminergic cells within the NTS and VLM (Neurosci. Lett. 97:252, 1989). To examine in detail the pattern of termination of BST efferents within the NTS and VLM, small unilateral deposits of the anterograde tracer Phaseolus vulgaris leucoagglutinin (PHA-L) were iontophoretically placed within lateral and ventral subnuclei of the BST of male Long-Evans rats. PHA-L fibers and terminals were visualized using the avidin-biotin immunoperoxidase technique. Catecholaminergic cell bodies were visualized via glucose-oxidase immunocytochemistry to catecholamine markers (TH, PNMT, DBH). The densest projection to the NTS and VLM arose from posterior and lateral parts of the BST. PHA-L fibers and terminals were found in close proximity to both the A2 noradrenergic and C2 adrenergic catecholaminergic groups. In the VLM, PHA-L fibers terminated in and around both the A1 and C1 catecholaminergic groups. Amygdaloid axon contacts on PNMT-immunoreactive dendrites within the ventrolateral reticular formation were frequently observed. The results demonstrate that the lateral BST, like the CeA, may directly influence both subpopulations of catecholaminergic neurons within the NTS and VLM. (Supported by NS 20041)

107.15

NEUROPEPTIDE IMMUNOREACTIVITY OF LATERAL PARABRACHIAL NEURONS THAT PROJECT TO THE MEDIAN PREOPTIC NUCLEUS IN THE RAT. N.L. Chamberlin and C.B. Saper. Depts. of Pharm. & Physiol. Sci. and Neurology, University of Chicago, Chicago, IL 60637.

The median preoptic nucleus (MnPO), in the center of the anteroventral third ventricular region, receives a major projection from the parabrachial nucleus (PB), mainly originating in the dorsal, central and external lateral subnuclei. A small percentage of these neurons are immunoreactive for corticotropin releasing factor (CRF) or enkephalin (ENK) (Lind and Swanson, Brain Res. 321:217, 1984). However, the neuropeptide content of most of the PB neurons that project to the MnPO is unknown. In a further attempt to identify the neurotransmitters in this pathway, we injected the MnPO in a series of rats with 5-20 nl of 3% Fluorogold. After 12 to 15 days, the animals were treated with intraventricular colchicine, perfused and the brains were cut at 30-50 μm. Sections through the parabrachial nucleus were immunohistochemically stained with antisera raised against galanin (GAL), somatostatin (SOM) or porcine brain natriuretic peptide (BNP). Small numbers of retrogradely-labeled neurons, mainly in the dorsal lateral subnucleus but occasionally in the central lateral nucleus as well, were immunoreactive with ezch of the antisera. Our data indicate that GAL, SOM and BNP, in addition to CRF and ENK, contribute to the projection from the PB to the MnPO. However, a predominant neurotransmitter for this pathway has yet to be identified.

107.16

A POTENTIAL ROLE FOR RECEPTOR CELLS IN A BRAINSTEM MIDDLE EAR AERATION REFLEX. P.J. Gannon, J.T. Laitman*, K.J. Chandross* and A.R. Eden. Departments of Otolaryngology and Anatomy, Mount Sinai Medical Center, New York, NY 10029.

The dynamics of air pressure maintenance in the mammalian middle ear (ME) are poorly understood. Whether physiologic levels of ME gas partial pressures (critical for efficient audition) are passively or neurally maintained remains controversial. Our previous anatomic and physiologic studies have demonstrated a polysynaptic brainstem reflex which may subserve control of ME aeration. The means by which this reflex may monitor ME pressure is currently unknown. This study advances our understanding of the hypothesized reflex by examining potential chemo/baro-receptor cells located within the tympanic plexus (TP) of the ME. These cells are intimately associated both with autonomic (caroticotympanic and tympanic) and sensory (branches of CN VII, IX, & X) nerves.

The anatomy of the TP in an age graded (newborn, 3mo, 6mo, adult, n=10) series of Macaca fascicularis was studied. In 3 adults, the superior cervical ganglion (SSG) was removed unilaterally. After degeneration of postganglionic fibers, glomus/ganglion cell clusters (GGC) in the TP were processed for EM analysis. In normals, when present, the GGC were processed for EM. Results indicate a maturational sequence in which the GGC first appear at adult levels in 6mo animals. The 2 newborns did not have GGC. Axosomatic contacts with glomus/ganglion cells were numerous at all other ages. Similar, but degenerating, synapses, were seen bilaterally after gangliectomy. The bilateral distribution of sympathetic postganglionics identified here supports similar findings of physiological studies in the parotid gland. Our studies also demonstrate the delayed/later postnatal development of a system which may represent the receptor cell end of the hypothesized ME aeration mechanism. Such a maturational sequence, if also present in humans, could explain the prevalence of middle ear disease in human infants as being due to an immaturity of the neural substrate.

TRANSPLANTATION: CORTEX AND BRAINSTEM

108.1

FETAL RAT FRONTAL CORTICAL TISSUE TRANSPLANTATION INTO THE LESIONED CEREBRAL CORTEX OF ADULT RATS. B.W. Chopko, T.J. Voneida, and R.E. Stoll. Neurobiology Dept., NE Ohio Univs College of Medicine, Rootstown, OH 44272.

Blocks of presumptive frontal cortical tissue (initial total volume ranging from 10 to 100 microliters/host) obtained from fifteen day fetal rats were transplanted into adult rats immediately after the production of a transcortical lesion cavity. Two transplants were placed into cavities in the rostral sensorimotor cortex, and one was placed into a cavity which included the olfactory bulb, prefrontal cortex, and rostral frontal cortex. After a three to five month survival period, the rats were perfused, and the brains sectioned and stained for cells (cresyl violet) and myelin (Weil). Estimated total graft volumes ranged from 11 to 17 mm^3. All transplants contained a heterogeneous population of cells exhibiting morphologies consistent with both neuronal and glial elements. Although an obvious laminar pattern was not present, transplants contained foci demonstrating columnar packing, cell nests, and rosette formation. All transplants demonstrated widespread evidence of myelin production. The myelin was arranged in compact, whorled bands and was typically confined entirely to the transplant. In a few instances, wisps of myelinated fibers extended between the host and transplant.

We conclude that fetal frontal cortical transplants survive and proliferate after transplantation into cerebral cortical lesion cavities in adult rats. The transplants produce myelin and exhibit subregions suggestive of cellular organization.

108.2

THE LONG-TERM FUNCTIONAL AND MORPHOLOGICAL EFFECT OF THE CORTICAL TRANSPLANT DEPENDS ON THE DELAY BETWEEN BRAIN DAMAGE AND GRAFTING. V. Valousková*, R. Macías-Gonzales*(SPONS: K. Anderson), Institute of Physiology, Czechoslovak Academy of Sciences, 142 20 Prague 4-Krc.

The degree of functional integration of cortical grafts and their capability to substitute for the function of the sensory-motor cortex removed by suction was studied in adult Long Evans rats by correlating the behavioral effects with the character of the host-transplant integration.

Embryonic neocortex (ED 14) was transplanted into the cavity in the left hemisphere either 14 days (group TR_{14}, n=11) or immediately (group TR_0, n=12) after the lesion. Food deprived animals were trained 4 months later to reach into a horizontal tubular feeder for food pellets. This task, specifically subserved by the ablated cortex, was mastered to the level comparable with intact rats by 38% of transplanted rats with adequately connected graft (n=13), but none of the rats with lesions only (group C, n=9) or the rats with poorly connected graft (n=9). Seven months after the surgery rats were trained in the Morris navigation task, and found to be impaired by the lesions in a non-specific way. No statistically significant differences were found between TR_0 and intact rats. TR_{14} and C had longer escape latencies than the former groups. There was an inverse relationship between the area and type of the host-transplant contact and the size of the opposite hemicortex cavity.

The data indicate that cortical grafts are capable of substituting for the specific function of damaged sensory-motor host cortex and prevent long-term deterioration of non-specific function, probably by the reduction of secondary degenerative changes. These effects depend on the delay between brain damage and grafting and on the corresponding characteristics of the host-transplant integration.

108.3

FUNCTIONAL MOTOR CORTEX TRANSPLANTS. Rick Sandor*, Manuel F. Gonzalez, Frank R. Sharp (SPON: J. Mueller). Depts. of Neurology and Physiology, University of California and VA Med. Ctr., San Francisco, CA 94121.

Fetal frontal cortex was transplanted into cavities formed in the motor cortex of neonatal rats. When host subjects reached adulthood, they were trained to use one arm to press two levers in succession as rapidly as possible. Attempted removal of the transplant in adult rats significantly increased the time for three subjects to accomplish the task. This occurred in 2/4 attempted surgical removals and 1/6 attempted immunological removals. Since the motor deficits produced were similar to those produced by removal of normal motor cortex, it is proposed that these fetal motor cortex transplants were functional.

No histological evidence of host brain injury was present in any subject. Therefore, host brain damage is unlikely to explain the behavioral deterioration following transplant removals. The failure to produce behavioral deterioration in 2/4 attempted surgical removals and 5/6 attempted immunological removals may be due to significant residual transplant tissue present in the host brain of all of these subjects, or to the fact that some transplants are non-functional.

108.4

KAINIC ACID METABOLICALLY ACTIVATES TRANSPLANTS AND ACTIVATES FUNCTIONAL CONNECTIONS BETWEEN FETAL CORTICAL TRANSPLANTS AND HOST THALAMUS AND STRIATUM. S.F. Ciricillo*, M.F. Gonzalez, and F.R. Sharp (SPON: P. Weinstein). Depts. of Neurology, Neurosurgery, and Physiology, UCSF and VA Med Ctr, SF, CA 94121.

Since little is known concerning the epileptic potential of fetal cortical transplants, we have examined the effects of kainic acid injections in rats with surviving grafts. Fetal cortical transplants were transplanted into cavities formed in sensory or motor cortex of adult rats. Following 1-2 month survivals, kainic acid (10mg/kg) was injected subcutaneously into the host subjects. Following induction of limbic seizures, (14C) 2-deoxyglucose was injected. Local cerebral glucose utilization (LCGU) increased markedly in all fetal cortical transplants compared to adjacent host neocortex. Marked, symmetrical increases of host LCGU occurred in the cingulate cortex, hippocampus, subiculum, and other limbic structures. Moreover, focal increases of LCGU occurred in the host thalamus and host caudate-putamen of some subjects ipsilateral to the transplant. These data suggest (a) fetal cortical transplants are particularly susceptible to systemic kainate which induces seizures and increases LCGU in transplants and (b) kainic acid activated neurons in transplants which project to host brain and increased LCGU in host thalamus and striatum.

108.5

SPROUTING OF TRANSECTED RAT MEDIAN FOREBRAIN BUNDLE CATECHOLAMINE FIBERS INTO FETAL NEOCORTICAL GRAFTS. A.A. Dunn-Meynell and B.E. Levin, Neurology Service, VA Med. Cent., E. Orange, NJ 07019

Catecholamine (CA) fibers in the median forebrain bundle (mfb) fail to regenerate after 6-hydroxydopamine (6OHDA) lesions made at the level of the anterior commissure. To examine the effect on sprouting, E15-16 rat fetal neocortical grafts were placed into lesion sites 1 or 14d following mfb 6OHDA injections into adult SD rats. Animals were sacrificed at 12 weeks. Fluorescent histochemistry showed little or no sprouting from transected mfb fibers in control brains (without grafts) with a 79.3±1.9% depletion of frontal cortex norepinephrine (NE) content measured by HPLC. However, grafts became innervated by fluorescent sprouts from both the mfb and striatum, and frontal cortex NE depletion was less (69.3±4.8%; $P<0.05$) than controls. Both NE and dopamine fibers appeared to innervate the graft as shown by autoradiographic demonstration of high affinity uptake sites on graft CA terminals by binding of ^{3}H desmethylimipramine and mazindol. Thus neocortical grafts appear to stimulate collateral sprouting from intact CA fibers and regenerative sprouting from otherwise non-regenerative severed CA fibers. This work was supported by the VA Medical Research Svc.

108.6

IMMUNOHISTOCHEMICAL CHARACTERIZATION OF THE EARLY DEVELOPMENT OF AND DIVERSE NEUROTRANSMITTER PHENOTYPE DISPLAYED BY EMBRYONAL CARCINOMA-DERIVED NEURONS Wm.A.Staines, D.Morassutti and M. McBurney* Depts. of Anatomy and Medicine, University of Ottawa Medical School, Ottawa CANADA, K1H 1M5

Cultures of a mouse embryonal carcinoma cell line, P19, were induced with retinoic acid to form a mixed population of neurons, glial cells and fibroblasts. This cell population was subsequently characterized up to 32 days post-induction using a wide variety of immunohistochemical and histochemical techniques.

Subpopulations of neurons were found to display immunoreactivity for a large number of neuropeptides (most notably somatostatin and neuropeptide Y), glutamic acid decarboxylase and GABA, tyrosine hydroxylase and other catecholaminergic marker enzymes. Individual cultures displayed a great deal of mophological diversity within the neuronal population. The immunochemical characterization also indicated a marked heterogeniety within each culture but an equally marked consistency between cultures. In terms of morphological diversity, neurotransmitters expressed, patterns of coexistence, and relative representation of transmitter phenotypes, these cultures were very reminiscent of natural mammalian forebrain neurons. As such, embryonal carcinoma-derived neurons may prove a useful source of tissue for transplantation experiments.

108.7

TRANSPLANT SURVIVAL IN AN IN VIVO WINDOW CHAMBER. W.J. Levy, J. Mora, M.F. Humphrey, V. Holets. Dept. of Neurological Surgery, Univ. of Miami Sch. of Med., Miami, Florida 33136.

We have previously described an in vivo chamber which is implanted in the hindlimb of the rat and allows repeated observation of neural elements. The chamber consists of two cover slips, 150-200 microns apart, separated by a silicone elastomer spacer. The chamber is sealed circumferentially except for two ports at opposite sides. The chamber is implanted under surgical conditions and the sciatic nerve severed, and each end placed in one of the ports, then secured with a suture. The nerves will regrow through the chamber an innervate the distal stump. The contents can be reexamined by opening the chamber and visualizing the contents with an operating microscope or a video enhancing system. In six animal transplants were done of E 16 fetal motor cortex into the chamber, through a removable top. The transplants were placed at the tip of the growing fan of regenerating nerve when it was about one third of the way into the chamber. We observed that the transplants grew and attracted a blood supply. The transplant merged into the distal nerve stump, but the proximal nerve stump did not integrate into it. Sacrifice of the chamber and histological examination revealed neuron specific enolase positive cells, and GFAP positive cells, and neurofilaments supporting the survival of neural and astrocytic elements.

108.8

OLFACTORY BULB REPLACEMENT IN RODENTS. T. Zigova,* P. P. C. Graziadei and G. A. Monti Graziadei (SPON: P. F. Cancalon). Dept. of Biological Science, Florida State University, Tallahassee, FL 32306.

The results from a previous study in rats seem to suggest that new central connections may be established after transplantation of an embryonic olfactory bulb in place of a neonatal bulb. In order to explore this eventuality, before transplantation we autoradiographically labeled the embryos' (E13-E14) olfactory bulbs by injecting the mother with 3H-thymidine. The hosts were sacrificed at survival times from 1 to 3 months. Forty-eight hours before sacrifice, they received several WGA:HRP injections in the pyriform cortex. Sections from the pyriform cortex and the olfactory bulb were processed for HRP histochemistry. Furthermore, the sections from the olfactory bulb were processed for autoradiography. Several autoradiographically labeled neurons were also HRP-positive. This finding provides evidence that output neurons from the transplanted olfactory bulb can send their axons to the appropriate target location in the host brain. (NIH Grant NS 20699.)

108.9

TRANSPLANTATION OF FETAL NEURONS INTO THE THALAMUS INDUCES A TRANSITORY PERIOD OF ACTIVE SYNAPTOGENESIS IN ADULT SOMATOSENSORY FIBERS M. Peschanski and F. Nothias* INSERM U 161 rue d'Alésia 75014 Paris. France.

Interactions between adult axons of host origin and fetal neurons transplanted into an excitotoxically lesioned CNS area allow reconstruction of specific circuitry. In the somatosensory thalamus, synaptic contacts between host specific afferents and fetal neurons transplanted as a cell suspension permit somesthetic activation of these neurons. In the present study, time-course of establishment of these synaptic contacts was studied using anterograde axonal labeling with WGA-HRP from the dorsal column and trigeminal nuclei. Rats were perfused with aldehydes 7 to 30 days post-grafting (dpg).

Transplanted neurons first occupied only the mechanical lesion produced during implantation then progressively spread into the neuron-depleted area (between 10 and 20 dpg). There was no ingrowth of somatosensory fibers into the transplant, and therefore no synaptogenesis in the original transplantation site. In contrast, when fetal neurons were displaced into the lesioned host (10 to 20 dpg), intermingling with WGA-HRP labeled fibers, synaptic contacts were formed. Morphology of synaptic contacts progressively acquired mature characteristics of large terminals with round vesicles, asymmetric contacts and adhesion plaques.

This study demonstrates that adult sensory fibers previously deprived of their normal target-neurons are induced -by hitherto unknown mechanisms- to perform active synaptogenesis when, but only when, neurons enter their topographically determined terminal region.

108.10

SEROTONERGIC AND HISTAMINERGIC NEURONS IN DOUBLE GRAFTS IN OCULO: ELECTROPHYSIOLOGICAL AND STRUCTURAL CORRELATES. A.-C. Granholm and A. Blomqvist. Department of Cell Biology University of Linköping, S-58185 Linköping, Sweden.

The monoaminergic transmitter systems have long been postulated to play a role in diseases like Alzheimer, schizophrenia and affective disorders. With this focus of attention on transmitter-defined systems, the need for a simplified brain model has emerged. By grafting monoamine neurons with appropriate target areas each system may be studied separately during normal or pathologic conditions. This study was focussed on histamine neurons in tuberomamillary nucleus and serotonin neurons in raphe dorsalis. Brain stem and hypothalamic tissues were dissected from fetal rat(E17) and injected into the anterior eye chamber of adult hosts. Fetal hippocampal (E18) and cerebellar (E15) tissues were co-grafted 4 weeks after initial grafting. Double grafts were studied with electrophysiological and morphological techniques after cessation of growth of the second graft. Serotonin and histamine nerve cell bodies were identified in corresponding grafts by immunohistochemistry. Monoamine-containing neurites innervated cerebellar and hippocampal grafts with functionally appropriate connections. The receptor specificity of this innervation in double grafts was evaluated using extracellular recordings and local application of monoaminergic agents. In conclusion, the present study suggests that histamine and serotonin physiology and structure can be studied during isolated conditions in a controlled environment using intraocular double grafts.

108.11

HABENULA CELL TRANSPLANTS AMELIORATE SLEEP CYCLE DYSFUNCTION AND INCREASE LEVELS OF SUBSTANCE P IN THE INTERPEDUNCULAR NUCLEUS OF RATS WITH FASCICULUS RETROFLEXUS LESIONS. T. Eckenrode, F. Haun, P. Onda, and M. Murray, Dept. of Anatomy, Medical College of Pennsylvania, Phila., PA 19129.

Bilateral lesions of the fasciculus retroflexus (FR) result in diminished Substance P (SP) and Choline acetyltransferase (ChAT) staining in FR target areas in the interpeduncular nucleus (IPN). We tested whether these transmitter levels would be altered by transplants of SP- and ChAT-containing medial habenula cells into the FR lesion site, and whether these transplants also produced a functional effect. Medial habenula cells from E15 Sprague-Dawley rats were "pre-cultured" then transplanted into adult rats with bilateral FR lesions sustained at either postnatal day 3 (P3) or as adults. Three months later the transplant (TP) animals, lesion-only (LO) controls, and normals were tested for normalcy of their sleep cycles. Animals were placed on a small-base inverted flower pot in a tank of water during their sleep period; the normal decrease in muscle tone associated with stage IV (paradoxical) sleep results in the animal falling toward the water. Normals have 14.5 sleep periods/hr, contacting the water .81 times/period; both P3 and adult LO's have <1 sleep period/hr and rarely contact the water. TP animals are intermediate, with 7.9 sleep episodes/hr overall; those with P3 lesions contact the water 1.14 times/sleep period, those with adult lesions .76 times/period. Compared to LO animals, TP's have enhanced intrinsic SP staining in the IPN, plus the presence of SP staining in the denervated lateral subnuclei, normal target of the medial habenula SP projection. Supported by NIH Grant NS16556.

108.12

IS OUTGROWTH FROM THIRD VENTRICULAR PREOPTIC AREA (POA) GRAFTS OF THE HYPOGONADAL (HPG) MOUSE LIMITED TO GNRH FIBERS? R.C. Silverman, A.J. Silverman, and M.J. Gibson. Dept. Anat. and Cell Biology, Columbia Univ., N.Y., 10032 and Dept. Medicine, Mount Sinai School of Medicine, N.Y. 10029

Hpg mice are infertile as a consequence of a deletion in the gene which encodes gonadotropin hormone releasing hormone (GnRH). Some reproductive function is restored in hpg mutants when GnRH rich POA grafts from normal mice are placed into the third ventricle. GnRH neurons from the graft extend fiber projections to the median eminence (ME), one of the normal targets of these neurons.

Although the outgrowth of GnRH axons has been documented, it is unclear whether other neurons project out of the graft. In order to discover if there is non-ME outgrowth, we employed the carbocyanine dye, DiI. Five or 30 days after surgery, hpg mice were sacrificed by perfusion and brain tissue cut into 1mm slices. A DiI crystal was applied to the graft tissue with a micropipette. After storage in buffer at 37 degrees for two weeks, 40u sections were cut and examined with rhodamine optics. Fluorescent axons appeared along a trajectory similar to that of GnRH fibers. Axons generally exited at the level of the arcuate nucleus or directly into the ME. Fibers were rarely found exiting at other levels into the hypothalamus. A few axons left the ME into the hypothalamus. A few axons left the ME and coursed laterally along the ventral surface of the brain. In addition to anterogradely labeled axons, retrogradely filled neurons in the graft and host arcuate nucleus were identified. These observations suggest that the majority of graft outgrowth is to the ME. These studies also support the idea that host neurons can send axons into the graft tissue. Supported by NS 20335.

108.13

RECOVERY OF SEXUAL BEHAVIOR BY HYPOTHALAMIC FETAL BRAIN TRANSPLANTS IN MEDIAL PREOPTIC AREA LESIONED RATS. R. Paredes*, A.L. Piña*, J. Fernández* and F. Bermúdez-Rattoni. Instituto de Fisiología Celular, UNAM. Apdo. Postal 70-600, 04510. Escuela de Psicología, Universidad Anáhuac, 11000 México, D.F.

The medial preoptic area (MPOA), plays a crucial role in the expression of sexual behavior in different species. Bilateral lesions within this area completely abolish sexual behavior in male rats. In the present study, sexually experienced rats were lesioned in the MPOA. Those rats who did not show sexual behavior within 15 days after the lesion, received fetal hypothalamic grafts. After one week of recovery the animals were tested once a week with a receptive female, until they reached 15 weeks. From 18 animals that received bilateral grafts, the histological (Nissl) and immunocytochemical analysis showed that in 8 animals the grafts were healthy and well integrated. No signs of transplanted tissue was observed in 10 animals. All grafted animals recovered sexual behavior by the fifth week, while the lesioned subjects without graft did not show any parameter of sexual behavior even 15 weeks after the transplant. The grafts contained tyrosine hydroxylase but not methionine-enkephaline immunoreactive neurons. These results showed that fetal brain transplants can restore an innate complex behavior in MPOA lesioned rats.

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108.14

EFFECT OF TARGET TISSUES ON THE EXPRESSION OF PNMT AND TH IN GRAFTS OF FETAL RAT MEDULLA OBLONGATA GROWN IN OCULO A. Seiger[1], V.R. Holets[2] and M.C. Bohn[3]. [1]Dept. of Geriatric Medicine, Karolinska Institute, Stockholm, Sweden; [2]Dept. of Neurological Surgery, Univ. Miami, Miami, FL 33136; [3]Dept. of Neurobiology & Anatomy, Univ. Rochester, Rochester, NY 14642.

The expression of PNMT and TH was studied in fetal rat medulla oblongata (MO) neurons grown in the anterior eye chamber of adult rats. The effect of age (E12.5 and E18.5), and the presence of a co-graft (thoracic or cervical spinal cord, hypothalamus) on neuron survival, PNMT and TH expression, and PNMT activity were analyzed.

Nearly twice the number of PNMT- and TH-immunoreactive neurons were observed in single MO grafts obtained from E12.5 rats compared to E18.5 grafts. In co-grafts of E18.5 MO with hypothalamus, thoracic or cervical spinal cord, a significant increase in the number of PNMT- and TH-immunoreactive cells was observed, as well as an increase in the PNMT activity. No effect of the co-grafts on the number of PNMT-immunoreactive cells was observed in the E18.5 group, although there was a four-fold increase in the number of TH-immunoreactive neurons in the MO-hypothalamus co-grafts. These data suggest that central targets promote the survival and differentiation of central catecholaminergic neurons. Funded by The Miami Project, The Daniel Heumann Foundation for Spinal Cord Research (VRH); NS20832, Dysautonomia Foundation, PSW Charitable Trust (MCB).

109.1

EFFECTS OF PIMOZIDE IN A REACQUISITION PARADIGM. J.L. Wiley, J.H. Porter, and D.I. Schiff*. Dept. of Psychology, Virginia Commonwealth Univ., Richmond, VA 23284.

Neuroleptic drugs have been shown to produce both motor and anhedonic effects in animals. Previous studies (Horvitz & Ettenberg, 1989; Wiley, Porter, & Faw, in press) have shown that haloperidol blocks the reacquisition of operant running during extinction following a single priming trial with food reward. This reacquisition paradigm, in which drug injection and behavioral testing occur on different days, allows the effects of neuroleptics on reward processes to be examined without the confound of motor effects. The present study tested pimozide (.25, .50, and 1 0 mg/kg) in this reacquisition paradigm. Fifty-one rats (80% BW) were run (single daily trials) in a straight-arm alleyway reacquisition procedure for four successive phases: acquisition, extinction, injection day, and test day. Vehicle control animals that received food reward on injection day ran significantly faster on test day than animals that did not receive food reward. The rats that received pimozide and food reward on injection day also ran faster on test day. Thus, pimozide at the doses tested did not produce the anhedonic effects in this reacquisition procedure that have previously been shown with haloperidol.

109.2

DIFFERENTIAL EFFECTS OF CLOZAPINE AND PIMOZIDE ON FIXED-RATIO RESPONDING DURING CHRONIC DOSING. J.H. Porter, J.L. Wiley, and A.D. Compton. Dept. of Psychology, Virginia Commonwealth Univ., Richmond, VA 23284.

With a chronic dosing regimen, tolerance to the rate suppressing effects of clozapine (CLZ) on fixed-interval responding develops within 6 to 10 days, whereas pimozide (PMZ) continues to suppress responding (Kaempf & Porter, JPET, 243: 437-445, 1987). Rats tested on a multiple fixed-interval (FI) fixed-ratio (FR) reinforcement schedule develop complete tolerance to CLZ's disruption of FI responding, but only partial tolerance to CLZ' disruption of FR responding (Villanueva et al, NEUROSCI. ABST., 1989). The partial tolerance to CLZ during the FR schedule was unexpected, and the present study was conducted to determine if an interaction between FI and FR responding on the multiple schedule was responsible for this effect.

Twelve rats were tested on a FR 30 food reinforcement schedule. Chronic dosing with 10 mg/kg CLZ produced an initial disruption of responding, and over 10 days responding recovered to 50% of baseline levels. Subsequent dosing with 1 mg/kg PMZ produced an initial disruption of responding that was maintained over 10 days of dosing. These results confirm that only partial tolerance to CLZ's disruption of operant behavior occurs for FR responding, and no tolerance occurs to PMZ's rate disrupting effects.

109.3

MECHANISMS OF TOLERANCE TO THE EFFECTS OF CLOZAPINE AND PIMOZIDE ON A MULTIPLE FI 60-SEC FR-30 OPERANT SCHEDULE IN RATS. H.F.Villanueva, J.H.Porter, N.Narasimhachari, J.K.Stewart, B.Landa* & J.L.Wiley. Depts. of Pharmacology Psychology & Biology, VCU/MCV, Richmond, VA 23298.

10 mg/kg clozapine (CLZ) and 1 mg/kg pimozide (PMZ) were administered to male Sprague-Dawley albino rats in order to assess the mechanisms of tolerance to these compounds. Behavioral effects were measured on a multiple FI 60-sec FR-30 food reinforcement schedule; the biochemical effects of CLZ and PMZ were measured in the plasma, frontal cortex and striatum via HPLC.

Presession administration of both drugs produced initial disruptions of response rate, response duration and reinforcement rate. Complete tolerance developed to CLZ's disruption of FI responding, but only partial tolerance developed for FR responding over the 10 day chronic dosing regimen. The disruption of FI and FR responding by PMZ increased over the 10 days. Post-session administration of CLZ and PMZ failed to disrupt operant responding and did not affect subsequent presession drug administration, suggesting that both the tolerance to CLZ and the sensitivity to PMZ are mediated by functional mechanisms (i.e. behavioral tolerance).

Biochemical assays revealed no significant differences between acute and chronic drug administration, suggesting that dispositional mechanisms (i.e., changes in metabolism) were not responsible for the behavioral changes.

109.4

DIFFERENCES IN REGULATION OF D1, D2, AND 5-HT2 RECEPTORS AFTER CHRONIC TREATMENT WITH CLOZAPINE OR HALOPERIDOL. S.J. O'Dell, G.J. LaHoste, C. Widmark*, R. Shapiro*, J.F. Marshall, and S.G. Potkin* Depts. of Psychobiology & Psychiatry; University of California, Irvine, CA. 92717

The atypical neuroleptic clozapine (CLZ) is often effective in diminishing psychosis in schizophrenics who respond poorly to typical antipsychotics such as haloperidol (HAL). In addition, CLZ is relatively free of the extrapyramidal side effects (EPS) associated with typical neuroleptics. To investigate the neural mechanism by which CLZ can attenuate psychosis without inducing EPS, we used quantitative autoradiography to measure changes in dopamine and serotonin receptors in rats after injection with CLZ or HAL for 28 days at clinically relevant doses. Levels of D1, D2, and 5-HT2 receptors were determined in frontal cortex, caudate-putamen, nucleus accumbens, and substantia nigra. Rats that received CLZ chronically showed CNS receptor changes markedly different from those in chronic HAL-treated animals. Rats treated chronically with HAL showed enhanced D2 binding, while those treated with CLZ did not. In contrast, chronic CLZ induced enhanced D1 binding, whereas these sites were unchanged in HAL-treated rats. Finally, CLZ treatment decreased 5-HT2 receptor binding while HAL had no significant effect. The differential effects of CLZ and HAL on D1, D2 and 5-HT2 receptors may be relevant to their antipsychotic potential and their propensity to induce EPS.

109.5

IN VIVO DIALYSIS STUDY OF THE CHRONIC ADMINISTRATION OF CLOZAPINE (CL) OR HALOPERIDOL (H) ON DOPAMINE RELEASE AND METABOLISM IN RAT STRIATUM (S) AND NUCLEUS ACCUMBENS (NA). J. Ichikawa* and H.Y. Meltzer. Department of Psychiatry, Case Western Reserve University, Cleveland, Ohio 44106.

CL has been suggested to produce less impairment of dopaminergic neurotransmission in the S than the NA compared to H. Male Sprague-Dawley rats were given CL (20 mg/kg/day) or H (2 mg/kg/day) in their drinking water. After 21 days, dialysis probes of differing sizes were implanted into the S and NA. After perfusion with Ringer's for 24 hours, (one day drug withdrawal), CL (40 mg/kg, S; 20, NA) or H (2 mg/day, S and NA) were administered after obtaining 3-5 basal samples. Chronic administration of H decreased but CL had no effect on basal DA efflux in the S and NA. In the 3 hr period after H administration, DA efflux in the S or NA in the rats receiving chronic H was less than that in vehicle-treated rats; DA efflux following CL was decreased only in the S. These results indicate that H produces a greater decrease in DA release in the S than CL which could account for the greater ability of H to produce EPS.

109.6

S-ADENOSYL-L-METHIONINE REVERSES TOLERANCE PRODUCED BY CHRONIC HALOPERIDOL TREATMENT IN RATS. A.M. KASK*, C. MARIN, T.N. CHASE, (Spon: R.-T. Wang) ETB,NINDS, Bethesda, MD 20892.

Haloperidol (HAL) induced catalepsy has been reported to be mediated by dopamine (DA) D2 receptors. Chronic treatment with HAL results in an increase in D2 receptor number and tolerance to its cataleptogenic action. Some neuroleptic drugs, including HAL, have been reported to decrease membrane fluidity after long term use. Such physical/chemical transformations in the cell membane may compromise receptor integrity and thus responsivity. S-adenosyl-L-methionine (SAMe) is a methyl donor to phosphotidylcholine, a major component of cell membranes, and has been shown to increase membrane fluidity. The present study was designed to determine whether SAMe's effect on membrane fluidity could affect DA receptor activity, and whether supersensitivity and tolerance induced by chronic HAL could be manipulated by changing the physical/chemical properties of the membrane. Catalepsy ratings were performed on individual animals; receptor content was measured in pooled groups. Chronic HAL treatment (5 weeks, 1mg/kg s.c.) increased D2 receptor content from 0.26 to 2.3 pmoles/mg protein and decreased catalepsy ratings from 2.7±0.15 (Scale 0-3.0) to 1.1±0.37. Adding SAMe (50 mg/kg i.p.) to the treatment schedule for the last two weeks reduced D2 content to 1.5 pmoles/mg protein and increased catalepsy ratings to 2.5±0.12. The data suggest that it may be possible to modify or prevent onset of tolerance to certain neuroleptic drugs by the addition of S-adenosyl-L-methionine to the treatment regimen.

109.7

THE ELECTROANALYTICAL DETERMINATION OF HALOPERIDOL IN VIVO WITH CARBON BASED ELECTRODES. M.T. Morocco*, T.A. Patterson, and J.O. Schenk. Department of Chemistry and Prgms. of Biochemistry and Pharmacology/Toxicology, Washington State University, Pullman, WA 99164-4630.

An electroanalytical probe for the determination of Haloperidol (HAL) in vitro and in vivo was developed. Adsorption of HAL to surfaces of these electrodes enables HAL to be detected electroanalytically. The sensitivity of the measurement is enhanced by the use of carbon paste electrodes, presumably by adsorption/extraction mechanism.

Experiments were conducted using conventional as well as 300 um diameter electrodes. Carbon paste electrodes were constructed by mixing ultra-carbon with nujol oil or silicon grease. Calibration curves were obtained by adding incremental amounts of HAL stock solution to 150 mM NaCl/20 mM $NaHCO_3$ buffer (pH=8.1). For the carbon paste electrodes, the linear range was 0.1-1 uM, while for the solid carbon electrodes, no signal was obtained below 3 uM. Therefore, the solid carbon electrodes were judged inadequate for in vivo measurements of HAL.

To illustrate the usefulness of this method for HAL detection, the determination of some in vivo parameters of the pharmacodynamics of HAL were investigated. The striatal extracellular diffusion coefficient was determined to be 0.36 ± 0.15 SEM x 10^{-6} cm^2/sec, N=4, and the uptake rate of HAL into striatal tissue was 200 um/g/sec. Supported by MH42759 (J.S.) and the State of Washington.

109.8

ACUTE HALOPERIDOL INHIBITS THE EFFLUX OF CALCIUM FOLLOWING DEPOLARIZATION IN THE STRIATUM IN VIVO. T.A. Patterson and J.O. Schenk. Dept. of Chemistry and Programs of Biochemistry and Pharmacology/Toxicology, Washington State University, Pullman, WA 99164-4630.

Recent studies of the ability of haloperidol (HAL) to act as a Ca^{++} channel blocker resulted in mixed results. Gould, et al. (PNAS 80:5122 (1983)) observed that HAL did not antagonize the voltage dependent Ca^{++} channel, while Flaim, et al (PNAS 82:1237 (1985)) found that HAL does inhibit the influx of Ca^{++} through the voltage sensitive Ca^{++} channel. We have studied the effect of acute HAL on Ca^{++} influx and efflux during depolarization in vivo and have found that HAL inhibits the efflux of Ca^{++}.

Experiments were conducted using small (< 10μm) Ca^{++} selective electrodes (Fluka) with a Ag/AgCl reference electrode < 5μm away. An injection syringe was placed 1 mm from the sensing electrode and the electrode assembly placed in the striatum of a chloral hydrate anaesthetized rat. At baseline, $[Ca^{++}]$ was measured, a control stimulation of 200 nl of 300 mM KCl given, and then either 1 mg/kg HAL or saline was injected ip and a second stimulation was performed 1 hr later. The results of these experiments showed a significant increase ($p < .10$) in the $t_{1/2}$ of the efflux of Ca^{++}. Studies on the effect of HAL on the influx of Ca^{++} as well as the effect of chronic administration of HAL on Ca^{++} influx and efflux are currently underway. Supported by MH42759 and the State of Washington.

109.9

METABOLITES OF HALOPERIDOL EXIBIT HIGH AFFINITY AND BIOLOGICAL ACTIVITY AT SIGMA RECEPTORS. E.L. Moses[1]*, P. Varghese[1]*, J.M. Walker[2], and W.D. Bowen[1] (SPON: J. Daniels). [1]Sect. Biochem., Div. Biol. & Med. and [2]Dept. Psychol., Brown University, Providence, RI 02912.

We examined the relative binding affinities of haloperidol and its metabolites for sigma and dopamine-D_2 receptors in rat brain homogenates. Haloperidol bound with K_i = 2.8 nM to both receptors. Compared to haloperidol, reduced haloperidol (RH) bound to sigma receptors with equal affinity, but to dopamine receptors with 85-fold lower affinity (K_i = 239 nM). The piperidine metabolite (PM) lacked affinity for dopamine receptors, but bound to sigma sites with K_i = 326 nM. Sigma-related biological activity of metabolites was determined in vitro by assessing ability to attenuate carbachol-stimulated phosphoinositide (PPI) turnover (Eur. J. Pharmacol. 149:399), and in vivo by assessing ability to produce postural changes upon microinjection into the rat red nucleus (Neurology 38:961). Preliminary results indicate that RH exibits potency in both assays comparable to that of haloperidol. PM was devoid of activity in the PPI assay, suggesting possible antagonist properties. These results suggest that haloperidol treatment may result in formation of sigma-active metabolites which can produce sigma receptor-mediated effects, long after dopaminergic activity is terminated.

109.10

EFFECTS OF CHRONIC ADMINISTRATION OF NEUROLEPTICS ON SYNAPSES OF LAYER VI MEDIAL PREFRONTAL CORTEX. S.L. Vincent, J. McSparren*, R.Y. Wang and F.M. Benes. Department of Psychiatry, Harvard Medical School and Mailman Research Center, McLean Hospital, Belmont, MA 02178, and SUNY, Stony Brook, NY 11794.

It was previously shown that high dose treatment with haloperidol (HAL) for 4 months induces synaptic rearrangements in layer VI of rat medial prefrontal cortex (MPF-VI), where the cortical dopamine projection terminates. In the present study a quantitative electron microscopic analysis of MPF-VI was carried out to determine the effects of low dose, long-term administration (1 yr) of HAL and clozapine (CLOZ) on the ultrastructure and arrangement of synapses. No change was found in the size of axon terminals, dendritic profiles or synaptic vesicle density. On both shafts and spines of CLOZ-treated animals, there was a decreased number of asymmetric synapses, while those with symmetric thickenings or no membrane specializations are increased. HAL-treated animals showed similar changes only on shafts. These various alterations occurred most frequently on larger caliber shafts and spines. These data suggest that low dose, long-term neuroleptic treatment may induce shifts among excitatory and inhibitory synaptic elements, and such shifts may provide important information about the interaction between neuroleptics and intrinsic components of MPF-VI. Supported by MH00423, MH31154, MH41440 & MH00378.

109.11

LATENT INHIBITION AS AN ANIMAL MODEL OF ANTIPSYCHOTIC DRUG ACTION L.A. Dunn*, G.E. Atwater*, G.W. Christison* C.D. Kilts. Dept. of Psychiatry, Duke Univ. Med. Ctr., Durham, NC 27710.

Latent Inhibition of a conditioned emotional response (CER) is a quantitative measure of selective attention. LI has been shown to be deficient in schizophrenic patients and to improve with antipsychotic medication (Baruch et al, J. Ment. Dis., 176:598-606, 1988). We have assessed the pharmacologic isomorphism of LI in rats with the clinical pharmacology of schizophrenia. Unless otherwise stated, all drugs were administered i.p. daily for 7 days. Drug and vehicle treated rats were placed individually into darkened test cages. Half were preexposed 20 times to cage illumination as a CS, and half were not preexposed. All groups were conditioned with 2 CS-US pairings. The US consisted of a 1 sec., 0.75ma. scrambled footshock. The following day CER, as measured by interruption of drinking in response to CS presentation, was recorded. LI was enhanced by haloperidol 0.3 mg/kg after 7 or 14 daily treatments, but not after a single acute dose. Haloperidol doses of 0.3 and 0.03 mg/kg enhanced LI, while doses of 0.003 and 3.0 mg/kg had no effect. Haloperidol enhancement of LI was unaffected by co-administration of the anticholinergic agent trihexyphenidyl. Enhancement of LI is exhibited by a broad range of antipsychotic drugs including fluphenazine, chlorpromazine, thiothixene, thioridazine, mesoridazine, and metoclopramide. The effect is specific to antipsychotic drugs as pentobarbital, imipramine, chlordiazepoxide, tihexyphenidyl, and promethazine all failed to enhance LI. LI exhibits striking parallels to the clinical pharmacology of schizophrenia in terms of dose response, latency to onset of effect, effect of anticholinergics, and drug sensitivity and specificity. (Supported by the Scottish Rite Foundation).

109.12

INTERACTION OF HALOPERIDOL AND (+) SKF 10,047 DETECTED BY THE BRAIN WAVE FACTOR SCAN. K.L. Marquis, R.P. Gussio*, M.G. Webb* and J.E. Moreton. Dept. of Pharmacol. & Toxicol. Univ. of Maryland School of Pharmacy, 20 North Pine St., Baltimore, MD 21201.

The (+) isomer of SKF 10,047 (SKF), the prototypic sigma agonist, alters rat cortical EEG in a fashion which is distinct from that of the PCP selective ligand MK-801. Haloperidol (HAL), also a potent sigma ligand, blocks SKF discriminative stimulus effects (Steinfels et al., 1987), and thus may alter the EEG response to SKF. Female Sprague-Dawley rats, prepared with frontal-parietal cortical recording electrodes and intravenous cannulae, were treated with haloperidol (0.1 mg/kg i.v.) or saline 15 min prior to SKF (10 mg/kg i.v.). Cortical EEG responses were analyzed for 10 minutes following the second injection by transforming analog EEG to power density spectra. Spectral quantities estimating frequency and energy responses in specific frequency bands were subjected to univariate statistical and factor analyses. No demonstrable change was observed in the average power spectra and no significant differences were detected by t tests. Alternatively, several factors emerged that described the patterned variation of the signal induced by SKF following HAL as distinctly different from that of SKF alone. These data indicate the need for more sensitive quantitative methods of analysis for cortical EEG. The implications to the pharmacology of the sigma receptor are a subject for further study. (Supported by grant DA03173)

109.13

CONTINUOUS HALOPERIDOL INDUCES ORAL MOVEMENTS IN RATS WHICH HAVE A TARDIVE DYSKINESIA-LIKE FORM, WHEREAS WEEKLY HALOPERIDOL INJECTIONS INDUCE A PRIMED DYSTONIA-LIKE SYNDROME. G. D. Ellison and R. E. See. Department of Psychology, UCLA, 405 Hilgard Ave., Los Angeles, CA 90024

Oral movements (OMs) in rats administered chronic neuroleptics were repeatedly observed and also measured by a computerized video system. If "typical neuroleptics" such as haloperidol (HAL) are continuously administered, rats gradually show an increase in smooth, relatively small OMs; upon drug withdrawal, persisting increases in larger amplitude OMs are seen. FFT analysis in these animals indicates a shift in peak energy to 1-3 HZ (the same frequency shown by humans with tardive dyskinesia). A different type of OM pattern is observed when HAL is administered in weekly, large injections, similar to the "priming" studies used to induce dystonic reactions in monkeys. In this case OMs develop during chronic administration which are large in amplitude, rapid in slope of onset, and have a peak energy at 4-6 HZ. Atypical neuroleptics such as clozapine or raclopride do not induce these syndromes, but with each of these drugs distinctively different effects on OMs gradually develop. Drug regimen thus appears to play an important role in producing acute dystonic- versus tardive dyskinesia-like syndromes in rats.

109.14

IN VIVO RECEPTOR BINDING IN RAT BRAIN OF THE POTENTIAL ANTIPSYCHOTIC ORG 5222. J.A.D.M. Tonnaer, W.M.J.B. van Gemert*, Th. de Boer* and L.P.C. Delbressine* Scientific Development Group, Organon International B.V., 5340 BH Oss, The Netherlands.

ORG 5222 is a tetracyclic compound with an antidopaminergic potency comparable to haloperidol but superior to chlorpromazine and clozapine. Behavioral and neurochemical testing further indicated profound antiserotonergic properties and revealed potential antipsychotic activity of ORG 5222 with a reduced risk of extrapyramidal side effects. We studied the in vivo binding of [^{3}H]ORG 5222 (50 μCi; 4 μg/kg i.v.) to synaptic membranes in rats. Displaceable binding reached equilibrium within 1 hour and was stable for at least 3 hours. HPLC analysis indicated that more than 80% of radioactivity bound to the membranes consisted of intact [^{3}H]ORG 5222. The topography of [^{3}H]ORG 5222 binding sites was assessed by quantitative autoradiography 1 hour after i.v. administration. Displaceable binding was prominent in the choroid plexus (300 fmol/mg t.e.), high in the claustrum and layer IV of the cortical fields (60-120 fmol/mg), intermediate in dopaminergic projection areas (45-70 fmol/mg) and low in the hippocampal formation, thalamus and brainstem areas (<45 fmol/mg). This regional distribution of ORG 5222 binding in vivo shows a conspicuous overlap with the localization of 5-HT$_{1c}$, 5-HT$_2$ and, to a lesser extent, of DA receptors. The serotonergic interactions may play a role in the antipsychotic action of ORG 5222.

109.15

EFFECTS OF THE NOVEL DOPAMINE AUTORECEPTOR AGONIST PD 118717 ON THE ACTIVITY OF SUBSTANTIA NIGRA DOPAMINE NEURONS. C.L. Christoffersen and L.T. Meltzer. Depart. of Pharmacology, Parke-Davis Pharmaceutical Research Division, Warner-Lambert Co., Ann Arbor, MI 48105.

PD 118717 (7-[3-[4-(2-Pyrimidinyl)-1-piperazinyl]-propoxy]-2H-1-benzopyran-2-one sulfate) binds to dopamine (DA) D-2 receptors and is active in biochemical tests of DA autoreceptor agonist activity (Pugsley et al. this meeting) and in behavioral studies shows selectivity for the DA autoreceptors versus postsynaptic DA receptors (Williams et al., this meeting). In the present experiments we compared the effects of PD 118717 with those of apomorphine and (-)-3-PPP on the firing activity of substantia nigra DA neurons recorded extracellularly in chloral hydrate anesthetized male S-D rats. IP administration of PD 118717 produced a dose-related inhibition of DA neuron firing. The maximal inhibition at 20 mg/kg IP was approximately 80% of the baseline firing rate. In contrast, apomorphine (1 mg/kg IP) and (-)-3-PPP (20 mg/kg IP) produced maximal inhibitions of 100% and 50%, respectively. PD 118717 inhibited DA neuron activity in rats depleted of DA by treatment with reserpine plus alpha-methyl-paratyrosine, indicating direct DA agonist actions. These data indicate that PD 118717 is a partial DA agonist, with greater intrinsic activity than (-)-3-PPP but less intrinsic activity than apomorphine.

109.16

IN VITRO AND IN VIVO NEUROCHEMICAL EFFECTS OF A DOPAMINE (DA) AUTORECEPTOR AGONIST PD 118717 IN RAT BRAIN. T.A. Pugsley, S.L. Myers*, L.C. Coughenour*, Y.H. Shih*, S.Z. Whetzel*. Dept. Pharmacol., Parke-Davis Pharmaceutical Res. Warner-Lambert Co., Ann Arbor, MI 48105.

We have previously reported that PD 116795 exhibits a selective DA action on presynaptic DA autoreceptors (Wise, L. et al. Soc. Neurosci. 13:359, 1987). In a continuation of these studies we have examined the effects of PD 118717 (7-[3- [4-(2-pyridimidinyl)-1-piperazinyl]propoxy]-2H-1-benzopyran-2-one sulfate), an analog of PD 116795, on DA autoreceptors regulating synthesis and release of DA. PD 118717 bound selectively to rat striatal DA-2 receptors in vitro and dose-dependently (1-30 mg/kg i.p. and p.o.) inhibited the gamma-butyrolactone (GBL)-enhanced rat striatal DOPA synthesis. These results together with behavioral data (Heffner, T., et al this meeting) showing a lack of effect of PD 118717 on normosensitive postsynaptic DA receptors indicates a selective action of this agent on DA autoreceptors. CI-954 decreased striatal DA synthesis in non-GBL treated rats, slowed the rate of utilization of brain DA, decreased striatal DA metabolism and increased rat serum corticostersone levels, findings consistent with DA autoreceptor agonist action. No effect on the synthesis and turnover of norepinephrine was found up to 20 mg/kg i.p. indicating a selective action of PD 118717 on DA neurons. These results indicate that PD 118717 is a orally active DA autoreceptor agonist.

109.17

BEHAVIORAL EFFECTS OF THE NOVEL DOPAMINE AUTORECEPTOR AGONIST PD 118717. **A.E. Williams*, J.N. Wiley* and T.G. Heffner. (SPON: D.A. Downs). Dept. of Pharmacology, Parke-Davis Pharm. Res. Div., Warner-Lambert Co., Ann Arbor, MI 48105.**

PD 118717 (7-[3-[4-(2-pyrimidinyl)-1-piperazinyl]-propoxy]-2H-1-benzopyran-2-one sulfate) is a dopamine autoreceptor agonist as determined in neurophysiological and neurochemical tests. PD 118717 inhibits spontaneous locomotion in mice and rats, consistent with activation of presynaptic brain dopamine receptors. Relative selectivity for pre-vs postsynaptic dopamine receptors is demonstrated by the lack of locomotor stimulation at large multiples of behaviorally-active doses in normal and reserpine-treated rats and by a 20-fold separation between doses that cause locomotor inhibition and those that cause stereotypy. In line with presynaptic dopamine agonist effects, PD 118717 reduces the locomotor stimulation caused by amphetamine, a drug that releases dopamine from central neurons. Absence of postsynaptic dopamine antagonist effects is suggested by the failure of PD 118717 to block climbing induced by the direct-acting dopamine agonist apomorphine. Like other dopamine agonists, PD 118717 increases locomotion in rats with supersensitive dopamine receptors following depletion of brain dopamine by 6-hydroxydopamine. PD 118717 is active after oral dosing and its effects persist for 4 hours. These results indicate that PD 118717 is an orally-active brain dopamine autoreceptor agonist.

109.18

U-68553B, A NOVEL DOPAMINE AUTORECEPTOR AGONIST WITH CHRONIC ACTIVITY. R.A.Lahti, M.F.Piercey, D.L.Evans*, K.J.Carrigan*, G.L.Neff*, C.Barsuhn*, G.Vogelsang*. CNS Diseases Research, The Upjohn Company, Kalamazoo, MI 49001.

Schizophrenia may be due to an overactive dopamine system and since autoreceptors regulate the activity of these neurons a selective autoreceptor agonist may reduce the symptoms of schizophrenia. A clinical study using NPA demonstrated the utility of this approach, but tolerance developed to its clinical effect.

U-68553B, a dihydrophenalene, is a potent autoreceptor agonist in: the GBL model, reducing HVA and plasma prolactin, and in reducing the firing rate of VTA dopaminergic neurons. In receptor binding studies, U-68553B had an IC50 of 11.0 nM at the D2 receptor, 270 nM at the 5-HT2 receptor, 7.0 nM at the 5-HT1A receptor, and was relatively weak at various adrenergic receptors. Following 2 weeks of administration, U-68553B was not found to develop tolerance in the GBL model, or HVA suppression, whereas apmorphine did develop some degree of tolerance to HVA. U-68553B was active in both the limbic and striatal areas.

These data suggests that U-68553B is a potent dopamine autoreceptor agonist in both the limbic and striatal areas, and does not appear to develop tolerance upon chronic administration.

109.19

NEUROLEPTICS: PREDICTION OF ANTIPSYCHOTIC RESPONSE. F.P. Zemlan, D.L. Garver and O.J. Thienhaus. Dept. of Psychiatry, Univ. Cincinnati Sch. Med., Cincinnati, OH 45267-0559

The purpose of the present study was to identify clinical, diagnostic or drug response variables which predict antipsychotic response to neuroleptic treatment in schizophrenic patients. Consenting inpatients (N=40) received a 14 day fixed dose neuroleptic trial with patient symptoms quantified three times per week employing the modified NHSI. Responders (N=25) were discharged after 15 ± 2 days of hospitalization, for nonresponders (N=15) neuroleptic was altered as clinically indicated. Stepwise regression analysis indicated that 84% of the variation in length of hospitalization was accounted for by the number of pharmacotherapy alterations required for symptom remission ($F=207.76;df=3,36;p<0.0001$). Bayesian analysis indicated that clinical response could be predicted for 65% of the patients after 3 days of neuroleptic treatment from combined thought disorder and auditory hallucinations scores, while by Day 7, response could be accurately predicted for 80% of the patients employing the same clinical symptoms.

Most predictive tests employed in medicine demonstrate a prediction accuracy of about 70% to 90%, similar to that presently reported for a 7 day neuroleptic trial to predict clinical response. It is suggested that the length of hospitalization for schizoprenic patients may be decreased by decreasing the length of time a clinician prescribes pharmacotherapy that subsequently proves not effective.

109.20

ADDITION OF DOPAMINE ANTAGONISTS IN FLUVOXAMINE-REFRACTORY OBSESSIVE COMPULSIVE DISORDER. C.J. McDougle, M.D.*, W. K. Goodman, M.D., L.H. Price, M.D., P.L. Delgado, M.D., J.H. Krystal, M.D., D.S. Charney, M.D., G.R. Heninger, M.D., Yale Univ. Dept. of Psychiatry, 34 Park St., New Haven, CT 06508.

The efficacy of serotonin (5-HT) reuptake inhibitors, e.g. clomipramine and fluvoxamine (FVX), in Obsessive Compulsive Disorder (OCD) suggests that 5-HT dysregulation may be involved in the treatment and pathophysiology of OCD. However, as many as 50% of OCD patients do not improve with 5-HT reuptake inhibitors. OCD in this subgroup of unresponsive patients may involve neurochemical abnormalities different from or in addition to 5-HT dysfunction. In this study, a dopamine (DA) antagonist was added to ongoing treatment in OCD patients refractory to FVX ± lithium. Methods: 17 patients with OCD (DSM-III-R) unresponsive to FVX ± lithium (mean ± S.D. duration = 10.9 ± 3 weeks) had DA antagonist (pimozide equivalents=6.5 ± 5.4 mg/day) added to their regimens for 2-8 weeks (mean, 4.7 ± 1.9 weeks). Yale-Brown Obsessive Compulsive Scale (Y-BOCS) scores before and after addition of DA antagonist were used to assess outcome. Clinical Global Improvement (CGI) ratings were also made. All cases were blindly reviewed to determine whether comorbid tic-spectrum disorders or schizotypal personality disorder (SPD) predicted a response to DA antagonist addition. Results: Nine of 17 (56%) patients were judged responders (CGI = "much" or "very much improved") after combined DA antagonist - FVX ± lithium treatment. Addition of DA antagonist was associated with a highly significant decrease (36%) in Y-BOCS scores (mean ± S.D. = 9.1 ± 8.9; p<.001). Seven of 8 (88%) patients with comorbid tic-spectrum disorders or SPD were responders, whereas only 2 of 9 patients without these comorbid diagnoses were responders (p=.02). Conclusion: This study documents the efficacy of DA antagonist addition in OCD patients refractory to treatment with FVX ± lithium. Comorbid diagnoses of tic-spectrum disorders or SPD, in which DA dysregulation has been postulated, were more frequently associated with a positive DA antagonist response. These data suggest that both 5-HT and DA systems may contribute to the pathophysiology of obsessive-compulsive symptoms in some patients.

109.21

ACUTE EFFECTS OF WY-47,846 AND RELATED COMPOUNDS ON SEROTONERGIC AND DOPAMINERGIC NEURONS. **C.W. UZZLE*, D.E. JONES* AND J.T. HASKINS.** Wyeth-Ayerst Research Princeton, N.J. 08543-8000

Wy-47,846 (3a,4,4a,6a,7,7a-hexahydro-2-[4-[4-(2-pyrimidinyl)-1-piperazinyl]-butyl]-4,7 ethenocyclobut [f] isoindole-1,3,(2H)-dione), a compound with preclinical anxiolytic activity, was examined to determine its effects on the neuronal activity of both 5-HT and DA neurons. The effects of Wy-47,846 on 5-HT neurons of the dorsal raphe were compared to those of 8-OH-DPAT and gepirone while the effects of Wy-47,846 on A9 and A10 DA neurons were compared to those of buspirone. Standard neurophysiological methods were used for recording. The rank order of potency of these compounds for inhibiting 5-HT neurons was 8-OH-DPAT > gepirone = Wy-47,846 (ID50's = 1.9, 28.9 and 31 µg/kg, i.v., respectively). The activation of A9 and A10 DA neurons by Wy-47,846 and buspirone was similar although buspirone was slightly more potent in both areas. These findings indicate that the putative nonbenzodiazepine anxiolytic, Wy-47,846, has effects on 5-HT and DA neurons similar to those of other nonbenzodiazepine anxiolytics.

109.22

SEROTONERGIC AND DOPAMINERGIC INVOLVEMENT IN THE FEEDING INDUCED BY BUSPIRONE, GEPIRONE AND IPSAPIRONE. P.J. Fletcher* and M. Davies* (SPON: T.B. Wishart). Neuropsychiatric Research Unit, MR Bldg., University of Saskatchewan, Saskatoon, Sask., Canada, S7N 0W0.

The possible roles of 5-hydroxytryptamine (5-HT) and dopamine (DA) systems in mediating the increased feeding induced by buspirone, gepirone and ipsapirone were investigated. Following peripheral injection each compound dose-dependently increased 1h food intake in free-feeding rats. Depletion of brain 5-HT with PCPA prevented eating induced by gepirone (2.5mg/kg) and ipsapirone (2.5mg/kg), but not by buspirone (1mg/kg). Microinjection of gepirone (0.2µg) and ipsapirone (0.04 and 0.2µg), but not buspirone (0.04-5µg), into the dorsal raphé nucleus (DRN) significantly increased food intake, presumably by inhibiting the activity of DRN neurons, thereby reducing forebrain 5-HT neurotransmission. Pretreatment with haloperidol (0.1mg/kg, 30 min) attenuated eating induced by the peripheral administration of all three compounds, indicating that DA is involved in mediating the increased feeding responses. Increased striatal DA activity results in general behavioural activation, which under certain conditions is observed as enhanced feeding. This mechanism may account for the feeding induced by buspirone, gepirone and ipsapirone, since these drugs have been shown previously to increase striatal DA turnover. In the cases of gepirone and ipsapirone induced feeding an indirect action involving inhibition of serotonergic DRN afferents may be involved. However a direct interaction with DA receptors may underlie the effect of buspirone.

109.23

REGIONAL CEREBRAL GLUCOSE METABOLISM IN THE RAT AS A CORRELATE OF THE ONSET AND BLOCKADE OF RAT VCMs

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The evalution of regional cerebral glucose metabolism (RCGM) in the haloperidol-treated rat may suggest the brain areas which mediate development and blockade of vacuous chewing movements (VCMs). Sprague Dawley rats were treated for 1,3,6, and 12 months with water (W), haloperidol (H), haloperidol plus progabide (H+P), and progabide (P), with H at 1.5 mg/kg/day and P at 100 mg/kg/day. Quantitative glucose metabolism using 14C-2-deoxyglucose and autoradiography were carried out in rats from each group; multiple brain areas were examined for glucose utilization using the Sokoloff method. After 6 months, H increased RCGM in the caudate (H:96.6 (10.6) mg Glu/100 gm tiss/min; W:86.5 (3.47), p<.05; mean (SD). P increased RCGM in medial prefrontal cortex (P:96.5 (7.4); W:80.9 (7.8); p < .005); P also increased RCGM in hippocampus (P:62.8 (3.6); W:54.1 (5.2), p < .005). This effect in caudate is similar to the chronic neuroleptic effect on glucose metabolism in man (PET/FDG technique) but different than the acute H effect in previous rat studies. Areas whose RCGM correlate with the VCMs after 12 months of treatment will be reported.

109.24

DISSOCIATION OF ALTERED STRIATAL GAD ACTIVITY FROM VACUOUS CHEWING MOVEMENTS. S.E. Bachus, L. Goodman*, J.Dale*, H.Kaneda & C.A.Tamminga. Maryland Psychiatric Res. Ctr., Baltimore, Maryland, 21228.

The use of vacuous chewing movements (VCMs), which develop after chronic neuroleptic exposure, as an animal model for tardive dyskinesia[1] allows regional biochemical analysis to generate hypotheses for testing in the human condition. Both reduced nigral GAD activity[1] and increased striatal GAD activity with unchanged nigral GAD activity[2] have been reported to accompany VCMs in rats after chronic haloperidol (H) exposure. Production of VCMs by H is blocked by concurrent administration of the GABA agonist progabide (P)[3]. We have examined whether biochemical alterations occur in basal ganglia GABA pathways which correlate with onset of, or are reversed by blockade of VCMs.

Male Sprague-Dawley rats were treated for 6 mo. with water (W), H (1.5 mg/kg/day po), P (100 mg/kg/day po), or H+P. GAD activity was measured radioenzymatically[4] after specific brain regions were dissected.

Nigral GAD activity was not changed after 6 mo. of H, despite the development of VCMs. While striatal GAD activity was not increased by H alone, it was elevated by H+P treatment (.24 ± .01 µmol/mg protein/hr) relative to W (.20 ± .01, p<.025) or P (.20 ± .01, p<.05). The ratio of striatal/nigral GAD activity was also increased by H+P (.23 ± .02) relative to W (.17 ± .01, p<.005) and P (.17 ± .01, p <.005). Neither nigral GAD activity (r=-.36) nor striatal GAD activity (r= .25) was significantly correlated with individual variability in VCMs in H-treated rats. Thus, elevated striatal GAD activity can persist despite the blockade of enhanced VCMs after chronic H, and this change does not correlate with VCMs, suggesting that it is not directly causally related to VCMs.

[1]Gunne & Häggström, Psychopharm 81:191,1983. [2]Mithani et al., Psychopharm 93:94, 1987. [3]Shirakawa et al. Soc Neurosci Abst. 1989. [4]Sims & Pitts, J Neurochem 17:1607, 1970.

109.25

GABA AGONIST ACTION ON HALOPERIDOL-INDUCED VACUOUS CHEWING MOVEMENTS

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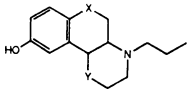
Vacuous Chewing Movements (VCMs) are an animal analogue of the human motor disorder tardive dyskinesia (TD). VCMs are slow in onset, nonsuppressible by atropine, continue after neuroleptic cessation, and have a prevalence in rats of 63% after 3-6 months of treatment. The pathophysiology of TD, and perhaps these VCMs, may include both dopamine (DA) system changes, and GABA system hypofunction. In an attempt to demonsrate a prophylactic effect of GABA agonists in VCMs, we have treated rats with water alone (W), haloperidol alone (H), progabide alone (P), and haloperidol plus progabide (HP). Sprague Dawley rats were fed H (1.5 mg/kg/day) in their drinking water and P (100 mg/kg/day) in their food for 1,3,6, and 12 months. VCMs/2 minutes were analyzed regularly by blind raters. After 6 months of treatment the 4 groups show significantly different rates of VCMs/2 minutes: 1.54 ± 0.43 for W; 9.81 ± 1.5 for H; 4.12 ± .93 for H+P; 0.97 ± .37 for P. Progabide to inhibited the onset of haloperidol-induced VCMs. Moreover, the P-treated animals do not merely show "dyskinesia" suppression but actual lack of onset as we have determined from an additional progabide-withdrawal experiment. These data would suggest that progabide and perhaps other GABAmimetic compounds can prevent the development of TD.

109.26

DOPAMINE AUTORECEPTOR AGONIST PROPERTIES OF (+)-trans-3,4,4a,10b-TETRAHYDRO-4-PROPYL-2H,5H-[1]-BENZOPYRANO-[4,3-b]-1,4-OXAZIN-9-OL AND ENANTIOMERS.

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PHNO and CGS 15855A possess potent DA autoreceptor agonist activity. We have now synthesized and evaluated the dopaminergic properties of the trans-3,4,4a,10b-tetrahydro-4-propyl-2H,5H-[1]-benzopyrano-[4,3-b]-1,4-oxazin-9-ol (PD 125530) and its enantiomers, compounds which combine the structural features of the above compounds.



PHNO • X = CH_2, Y = O
CGS 15855A X = O, Y = CH_2
PD 125530 X = O, Y = O

Both PD 125530 and its (+)-enantiomer, PD 128907, bound to DA D_2 receptors, and in rats attenuated gamma-butyrolactone-stimulated brain DA synthesis, decreased firing of substantia nigra DA neurons, and selectively inhibited exploratory locomotor activity, a profile consistent with a DA autoreceptor agonist mechanism of action. However, the (-)-enantiomer, PD 128908, lacked activity. Thus, like PHNO and CGS 15855A, PD 125530 is a potent DA autoreceptor agonist, and this activity resides in one enantiomer.

109.27

INTERACTION BETWEEN THE POTENTIAL ANTIPSYCHOTIC CI-943 AND AMPHETAMINE.

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CI-943 (8-ethyl-7,8-dihydro-1,3,5-trimethyl-1H-imidazo [1,2-c]pyrazolo[3,4-e]pyrimidine) is a novel antipsychotic candidate that is not a dopamine receptor antagonist (Soc. Neurosci. Abst. 13:459-460,1987). In addition to reducing conditioned behavior and apomorphine-induced climbing, we reported previously that CI-943 does not block stereotyped behavior caused by apomorphine or amphetamine but can augment the locomotor stimulant effect of amphetamine in rats (Soc. Neurosci. Abst. 14:370,1988). Further studies indicate that CI-943 does not augment locomotor stimulation caused by amphetamine in rats habituated to the test environment, a paradigm that enhances the ability to detect stimulation. High doses of CI-943 reduce amphetamine locomotor stimulation in habituated rats. Moreover, CI-943 does not augment the increase in hypothalamic self-stimulation responding or Sidman avoidance responding caused by amphetamine, but instead attenuates the stimulant effects of amphetamine in these tests. CI-943 also does not enhance amphetamine-induced stereotyped behavior in rats. These results indicate that while CI-943 does not cause dopamine antagonist-like blockade of amphetamine-induced stereotyped behavior, neither does it enhance the psychostimulant effects of amphetamine in animals.

STRESS, HORMONES AND THE AUTONOMIC NERVOUS SYSTEM

110.1

HUMAN VASOPRESSIN (AVP) AND ADRENOCORTICOTROPIN (ACTH) RESPONSES TO STRESSFUL SENSORY INPUT: INDIVIDUAL DIFFERENCES AND ADAPTIVE RESPONSES.

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Plasma levels of AVP and ACTH were determined in human subjects before and after exposure to stressful Coriolis stimulation on a rotating chair assembly (Kohl, R.L., Aviat. Space Environ. Med., 58:125, 1987). Subjects made head movements out of the plane of rotation until stimulation of vestibular systems induced advanced nausea and autonomic system dysfunction (i.e., motion sickness). Pre- and posttest levels of AVP were linearly correlated ($r = 0.561$, $p<0.05$). The amount of stimulation tolerated before nausea occurred was significantly correlated with the subjects' ability to mobilize AVP (Kruskal-Wallis 1-way ANOVA, $p<0.04$), but not with resting levels of AVP or ACTH. The coefficient of variation determined from six measurements of resting levels of AVP was inversely correlated with susceptibility to stressful motion ($p<0.006$). After five consecutive daily exposures to nausea, a significant diminution of postnausea AVP and ACTH occurred. Corelease of AVP and ACTH in response to nausea followed a linear relationship ($p<0.05$). These findings suggest the participation of the paraventricular hypothalamic nucleus in endocrine and autonomic responses to stressful sensory input and indicate that individual differences and adaptive responses involving these endocrine systems may offer predictive and etiologic insights into the autonomic dysfunctions observed.

110.2

STRESSFUL INTERVIEW ELICITS RISE IN PLASMA VASOPRESSIN AND RENIN ACTIVITY.

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Release of hormones from the anterior pituitary might be affected by several neurohumors known to increase in the circulation in response to stress. A stressful 30 min interview increased plasma levels of vasopressin (VP), epinephrine (EPI), norepinephrine (NE), and plasma renin activity (PRA), as well as cyclic AMP, ACTH, β-endorphin (β-EP), β-lipotropin (β-LPH) and prolactin (PRL). These increases occurred within 7 min of beginning the interview and were quite robust: VP (40%), EPI (200 %), NE (170%), PRA (123%), cyclic AMP (57%), ACTH (59%), β-EP (79%), β-LPH (42%) and PRL (46%). Plasma levels of atrial naturetic factor were unaffected by the stressful experience. Two of the stress-responsive circulating substances have been reported to affect the release of ACTH: VP and EPI. VP stimulates ACTH release from pituitary cells in vitro, and is believed to be released from the median eminence into the portal blood to stimulate corticotrophs in vivo. Our finding of stress-induced increases in circulating VP suggests that VP is also released from posterior pituitary during stress and may augment stress-induced CRF-mediated release of ACTH. Since angiotensin II (AGII) is reported to be a secretogogue for ACTH, and PRA is involved in the synthesis of the precursor for AGII, PRA might indirectly contribute to ACTH release.

110.3

CHANGES IN THE ELECTROGASTROGRAM AND THE ONSET OF EXCESSIVE WHEEL RUNNING IN RATS DURING ACTIVITY-STRESS. N.S. Morrow and S.W. Kiefer. Dept. of Psychology, Kansas State University, Manhattan, KS 66506.

To examine the relationship between changes in gastric function and the elevation of wheel running during activity-stress, surface recordings from the stomach (the electrogastrogram) were recorded. Initially, the stomachs of 14 rats were translocated so that the stomach was located just beneath the abdominal skin. Rats were then divided into an activity group (n = 7) and a home cage group (n = 7). Following baseline measurements, all rats were fed for 1 h each day. The electrogastrogram (EGG) was recorded daily. Results indicated that changes in the frequency and amplitude of EGG potentials were both predictive and correlated with increases in activity and ulceration. Home cage control rats did not exhibit changes in the EGG and did not become ulcerated. It is suggested that changes in gastric factors may influence the development of excessive wheel running.

110.4

ONTOGENY OF BEHAVIORAL AND HORMONAL RESPONSES TO STRESS IN PRENATALLY STRESSED MALE RAT PUPS. L.K. Takahashi, E.W. Baker*, and N.H. Kalin. Dept of Psychiat., Univ. of Wisc. Med. Sch., and VA Med. Cntr., Madison, WI 53705.

We reported that prenatally stressed 14-day-old rat pups (PS) showed marginal increases in stress-induced analgesia but elevated plasma ACTH (Physiol. Behav. 1988, 42:325). This study examines, in both 14- and 21-day-old male pups, effects of prenatal stress on occurrence of ultrasonic distress calls, freezing behavior, analgesic reactions, and plasma ACTH. Sprague-Dawley rats were shocked every other day during pregnancy. PS pups (n=12-15) were compared to home cage controls (HC, n=11-15) in 10-min tests that assessed responses to: 1) separation from the litter; and 2) foot shock. At 14 days, PS pups emitted fewer ultrasonic calls, showed lower stress-induced analgesia, and had higher plasma ACTH than HC pups ($p<.05$ or less). At 21 days, number of ultrasonic calls, duration of freezing, and level of analgesia were similar between groups. However, plasma ACTH again differed between PS and HC pups, with the latter exhibiting a significant elevation after shock exposure ($p<.05$). Differences in hormonal responses to stress, shown in early life of PS pups, reflect altered hypothalamic-pituitary-adrenal regulation which may be linked to the expression of stress-induced behavior.

Supported by NIMH grant MH-43986.

110.5

ONTOGENY OF DEFENSIVE BEHAVIORS AND HORMONAL ACTIVATION IN INFANT RHESUS MONKEYS. N. H. Kalin, S. E. Shelton*, and J. Turner*. Psychiatry Lab., VA Hospital; and Dept. of Psychiatry, Univ. of Wis., Madison, WI 53705

Infant rhesus monkeys (20- to 52-wk-old) emit frequent coo vocalizations when separated from their mothers. If a human enters the room and does not engage the infant in eye contact (NEC), it freezes. However, staring (ST) at the infant induces barking and hostility (Science 243:1718, 1989). This study examined the ontogeny of these responses in 0-2, 2-4, 5-7, and 9- to 12-wk-old infants (8 per group). With separation, 0-2 wk-old infants displayed distress, but failed to modulate their responses as the parameters of the threat changed. Two- to 4-wk-old infants froze during NEC, but during ST did not selectively respond with increased barking. Five- to 7-wk-old infants froze very little and did not increase their barking during ST. The 9- to 12-wk-old infants responded much like the older infants in our previous study, *i.e.*, NEC selectively induced freezing and ST selectively induced barking. ACTH levels were higher in the 9-12 wk-old group, whereas GH levels were elevated in the 0- to 2-wk-old infants. Separation increased levels of both hormones, but significant increases in ACTH did not occur before 5 wk of age. Thus, different developmental time courses exist in the rhesus monkey for defensive behaviors and stress-induced ACTH and GH secretion.

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110.6

THE EFFECT OF PRENATAL STRESS ON THE HIPPOCAMPUS IN RATS. H. Uno, B. Schroeder*, P. Alsum*, L. Takahashi and N. Kalin. Wisconsin Regional Primate Research Ctr. and Dept. of Psychiatry, UW-Madison.

Our previous studies have revealed that prenatal stress in rats induced in offspring increased stress-induced secretion of ACTH and corticosterone. The hippocampal neurons are known to regulate the hypothalamic-pituitary-adrenal axis and the pyramidal neurons in the CA3 and 2 contain a high concentration of glucocorticoid receptors. In the present study, 28-day-old rats from 24 litters (16 stressed and 8 control, males and females) were perfused through the heart with 10% neutral buffered formalin. The hippocampal sections were embedded with glycol methacrylate resin and cut 3 microns thick with a glass knife, then stained with cresyl violet. The most pronounced change was found in the CA3 region; approximately 75% of prenatally stressed pups showed a 10-20% reduction of pyramidal neurons and the dendritic processes were poorly developed compared to those in control brains. In the other regions of the hippocampus, the pyramidal neurons in the CA1 and the dentate granular neurons showed no striking difference between stressed and control brains. These results suggest that the CA3 pyramidal neurons are most vulnerable to the effect of prenatal stress. Perhaps an increased level of glucocorticoids induced by stress during pregnancy led to developmental changes in the hippocampus of offspring. Our earlier work showed that prenatal administration of dexamethasone induced neuronal damage in the CA3 neurons of rhesus fetal brains.

110.7

α- AND β- ADRENERGIC RECEPTORS AND TELEMETERED AUTONOMIC RESPONSES TO SOCIAL STRESS. W. Tornatzky*, K. A. Miczek (SPON: J. Ellingboe) Psychology Department, Tufts University, Medford, MA 02155

Animals react to social stress with defensive and submissive behavior as well as with increased cardiovascular activity and core temperature. To evaluate the involvement of adrenergic receptor systems and anxiolytics in the heart-rate (HR), core temperature (TC) and behavioral reaction of a rat that is exposed for 1 hour to the threats by an opponent, clonidine (0.01 to 0.1 mg/kg i.p.), a centrally acting α2-receptor agonist, β-blockers and benzodiazepines were administered. The acute social stress situation consisted of brief physical agonistic interactions until the experimental rat was forced into a prolonged submissive supine posture, emitted ultrasounds and, subsequently, exposure to the opponent's threats, while being shielded from physical contact by a wire mesh. HR and TC of these individually housed Long-Evans rats (300g) were monitored by a PC-based telemetry system (Mini-Mitter Dataquest III) throughout the light/dark cycle. A stable circadian pattern developed within 7 days after implant of the telemetry sender with mean values of 350 beats/min and 37.6oC during the dark period; decreasing by 30-60 b/min and 0.4-1oC respectively during the light period. Physical interaction with the resident induced large elevations above dark cycle mean values (+150 b/min for HR and +1.8oC for TC). During the following threat period TC remained elevated. The HR declined to a level which was similar to the elevated HR (+50 b/min) measured during activity bouts. Clonidine (0.01-0.1 mg/kg) prevented the increase in HR and TC during the 1 h agonistic interaction dose-dependently. The 0.1 mg/kg dose restored the baseline levels, and did not prevent defensive postures and ultrasounds. Diazepam (3, 6, 10 mg/kg) attenuated the increase in TC, and also decreased defensive upright postures. Threat during social conflict results in massive autonomic reactions that appear to depend on central adrenergic receptors.

110.8

BLOOD PRESSURE VIA TELEMETRY DURING SOCIAL STRESS IN FREELY MOVING RATS. W.P.Meehan*, W.Tornatzky* and K.A.Miczek (SPON: D.Lindsley). Dept.Psych., Tufts Univ., Medford, MA 02155.

Daily variations in blood pressure (BP) and changes in BP due to social stress were recorded from freely moving rats. Implantable transmitters (Mini-mitter, OR) with a catheter inserted into the aorta allowed the remote recording of systolic and diastolic BP, heart rate (HR) and coarse motor activity. Rats were housed individually for baseline measures and, for social stress, they were placed as intruders into the cage of a rat selected for its aggressive behavior ("resident"). The 1 h test began with unrestricted physical interaction with the resident until the intruder displayed a submissive supine posture (30 to 180 s). Then, a wire cage was placed over the intruder to prevent futher physical contact by the resident. Baseline BP was 125/80 (HR=350) at rest and increased to 135/90 (HR=400) during activity. These periods of activity occurred at regular intervals and no differences in BP or HR were noted between lights on and off. BP during physical interactions with the aggressive rat was 165/115. In the protective wire cage, BP remained elevated at ca. 145/95 mm Hg and returned to baseline in the home cage. Clonidine (0.06 mg/kg) attenuated the stress-induced increase in BP. In summary, BP and HR were found to increase in response to non-stressful activity within the home cage of the rat and more so in response to aggression and threat of attack by another rat.

110.9

DIFFERENTIAL CARDIOVASCULAR EFFECTS OF FOOTSHOCK AND AIRPUFF STRESSORS IN WISTAR-KYOTO AND SPONTANEOUSLY HYPERTENSIVE RATS. R.F. Kirby, C.H. Woodworth, G.G. Woodworth* and A.K. Johnson. Dept. of Psychology and The Cardiovascular Center, Univ. of Iowa, Iowa City, IA 52242.

Cardiovascular responses to noxious and non-noxious stressors were compared in the present study with and without peripheral beta-adrenoceptor mediated-vasodilatory mechanisms blocked. Adult male WKY and SHR were implanted with carotid artery catheters for recording of arterial pressure and heart rate and jugular vein catheters for administration of vehicle or the beta-2 adrenoceptor antagonist. After recovery, animals were exposed to their first stress episode of 5 acute trials of either footshock (0.5 mA, 0.5 sec) or airpuff (15 psi, 100 ms) with each trial separated by 1 min. Animals were tested to the other stressor two days later with the drug treatment they had not previously been exposed to. Order of the stressors and drug treatment were counterbalanced between days. Both stressors led to immediate pressor responses that decreased across trials, but the responses were greater to airpuff than to footshock. However, blood pressure and heart rate rose gradually across footshock trials but remained stable between each airpuff trial. The effect of beta-2 adrenoceptor blockade was to increase the duration of the pressor response to both stressors. These results indicate that unique cardiovascular response patterns occur to airpuff and footshock stress but both involve activation of beta-2 adrenoceptors.

110.10

SPONTANEOUSLY HYPERTENSIVE AND WISTAR-KYOTO RATS SHOW BEHAVIORAL DIFFERENCES BUT CARDIOVASCULAR SIMILARITIES IN TACTILE STARTLE. C.H. Woodworth, R.F. Kirby, G.G. Woodworth* and A.K. Johnson. Depts. of Psychology and Statistics, and The Cardiovascular Center, Univ. of Iowa, Iowa City, IA 52242.

Concurrent behavioral and cardiovascular responses to tactile startle were investigated in SHR and WKY with and without blockade of peripheral vasodilatory mechanisms with a beta-2 adrenoceptor antagonist. These data were collected as part of a study comparing cardiovascular responses to noxious and non-noxious stressors (see Kirby et al., Soc Neurosci Abstr, 1989). Although SHR showed significantly greater motor responses to 100- msec, 15-psi airpuffs than WKY, there were no strain differences in pressor responses or tachycardia measured immediately or 30 sec following airpuff stimuli. Both strains showed significant habituation of motor and pressor responses and increasing tachycardia over 5 consecutive startle trials (60- sec ITI). Motor and HR responses changed more rapidly in WKY than SHR. Prior treatment with the beta-2 adrenoceptor antagonist ICI 118,551 (0.5 mg/kg, iv) had no effect on either behavioral or cardiovascular responses to airpuff stimuli in either strain, although motor responses habituated more slowly in ICI-treated rats than in vehicle controls.

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110.11

DIABETIC RATS DISPLAY A HEIGHTENED STRESS AND ANXIETY RESPONSE TO THE OPEN FIELD AND ELEVATED PLUS MAZE. Q. Ahmad[1], S. Farag[1] and Z. Merali[1,2]. [1]Psychology and [2]Pharmacology, University of Ottawa. Ottawa, Ontario, Canada, K1N 9A9.

This study assessed the anxiety and stress responses of the insulin treated male Spontaneously Diabetic Rat (SDR) (4-6 months diabetic), matched non-diabetic and genetically distinct controls to: 1) the open field (OPFD); 2) the elevated plus maze (EPM) following either vehicle or the anxiolytic, Chlordiazepoxide (CHLOR 2.0, 4.0, 6.0 mg/kg; i.p.). 30 min after treatment, the rats were placed in the OPFD for 10 min, followed by 10 min in the EPM. In the OPFD, CHLOR induced a dose-dependent increase in locomotion, of similar magnitude, in all groups. In all groups, CHLOR dose-dependently increased the number of entries into the center (anxiogenic) area of the OPFD. This response however, was significantly attenuated in the SDR. In the EPM, CHLOR dose-dependently increased the time spent in the open (anxiogenic) arms. Again, this response was significantly attenuated in the SDR. In conclusion, the SDR show a heightened stress response and blunted sensitivity to the anti-anxiety effects of CHLOR, across 2 different stress and anxiety assessment paradigms.

110.12

MEDIAL FRONTAL CORTEX LESIONS ELIMINATE ULTRASONIC VOCALIZATIONS DURING STRESS IN THE RAT

E.J. Neafsey and R.J. Frysztak, Department of Anatomy, Loyola University Stritch School of Medicine, Maywood, IL 60153

During stress rats emit ultrasonic vocalizations (USV). Since in primates the anterior cingulate gyrus has been linked to production of the distress call (MacLean and Newman, BR 450,111, 1988), we examined the effect of lesions of the rat medial frontal anterior cingulate cortex (MFC) on USV emitted during tone-footshock classical conditioning and during presentation of the tone stimulus alone on extinction trials following conditioning. Bilateral lesions of the prelimbic and infralimbic regions of the medial frontal cortex of 13 male Sprague-Dawley rats were made by stereotaxic injection of 0.4 ul of N-methyl-D-aspartate; sham lesions were made in 13 other animals. After several weeks to several months of recovery, all SHAM animals emitted USV during tone-footshock conditioning and during subsequent tone presentation alone, while only 1 of 13 MFC-lesioned rats emitted any USV during these procedures. These results replicate those seen in primates following similar lesions (MacLean and Newman, ibid.). Supported by Loyola BRSG funds.

110.13

STRESS PRODUCES AN ACUTE AND LONG-TERM ENHANCEMENT OF THE ACOUSTIC STARTLE RESPONSE: A ROLE FOR ENDOGENOUS OPIOIDS. Collins, D.L.*, Mickley, G.A. and Cohen, S.J.* (SPON: J.L. Ferguson). BHS, AFRRI, Bethesda, MD. 20814-5145.

Intermittent (but, not continuous) footshock causes an analgesia mediated by endogenous opioids. Since opiates can alter acoustic startle responding, this experiment measured the startle amplitude of Sprague Dawley rats following either intermittent or continuous footshock. Rats in the former group, but not the latter group, exhibited significantly enhanced startle response amplitudes compared with control (i.e., non-shocked) rats. The startle amplitude of rats receiving intermittent footshock (but, not continuous footshock) was significantly reduced by naloxone (1 mg/kg, i.p.). In a follow-up test (conducted 27 days after the initial shock) rats that had been exposed to either intermittent or continuous footshock stressors demonstrated greater startle response amplitudes than control rats, and reduced their initial spontaneous activity in a novel environment. These data suggest that, following certain stressful conditions startle hypersensitivity may be long lasting and mediated by endogenous opioids.

110.14

STRESS INDUCED CHANGES OF ALPHA-2-RECEPTORS IN THE LOCUS COERULEUS. G.K. Weiss*, A. Voltura*, D. Savage, C. Lucero*, T. Hoffman*, & D. Karnaze* A. Ratner. Univ. of New Mexico Sch. of Med., Albuquerque, NM 87131,

Stress is known to activate the central noradrenergic (NA) system. Presynaptic alpha-2-adrenergic receptors act to suppress NA activity. Thus, adaptation to stress may involve changes in these alpha-2-receptors. This study examines the effect of various periods of mild stress on alpha-2-receptors in the Locus Coeruleus (LC). Adult male rats were placed in a cold room (4 C) and switched to a new novel environment every 30min. Control rats were kept in their home cage (24 C). Autoradiographic measurements of alpha-2-receptors within the LC were made by incubating tissue sections with the specific alpha-2-antagonist, H^3-Idazoxan. Computer assisted microdensitometry was used to determine specific binding. A significant increase in alpha-2-receptor binding was seen 1hr after stress. After 4hrs of stress, binding was significantly decreased. Exposure to the same stress procedure for 4hrs per day over a 4 day or 10 day period showed no change in binding. These results indicate that a mild short term stress can alter the binding of Idazoxan to alpha-2-receptors in the LC. The increase at 1hr may act to down regulate LC activity, while the decrease at 4hrs could lead to enhanced LC activity. The lack of change, after long term stress may be due to adaptation to the stressor, since corticosterone levels in these animals were not found to be increased.(Supported by NIH NS23262 and MBRS RR08139).

110.15

SYMPATHO-ADRENAL EFFECTS ON INHIBITORY RESPONSES TO STRESS IN RATS. P.Terry* and P.Salmon* (SPON: J.D.Feigenbaum). Dept. of Psychology, Univ. Coll. London, Gower St., London WC1E 6BT, U.K.

These experiments investigate the ways in which disruptions of the sympatho-adrenal system influence the growth and amelioration of inhibitory responses to stress. Sympatho-adrenal function was disrupted by beta-blocking drugs, by neurotoxic sympathectomy, and/or by adrenal demedullation. Behavioral inhibition by frustrative nonreward (extinction of rewarded running) was retarded by acute i.p. injection of the beta-blocker propranolol. Adrenal demedullation reproduced this effect of beta blockade, but neurotoxic sympathectomy (by 6-hydroxydopamine) facilitated inhibition. These two interventions in combination tend to cancel out. The results suggest a functional dissociation between the two components of the sympatho-adrenal system with regard to the inhibitory effects of stress.

110.16

CONTINGENCY, EMOTIONALITY AND NALOXONE-INDUCED ANALGESIA (NIA). C.X. Poulos*, A.D. Le*, D. Knoke* and H. Cappell* (SPON: Y. Israel) Dept. of Psychology, Univ. of Toronto and Addiction Res. Foundation of Ontario, Toronto, Canada.

Recently, we and other investigators have independently reported that repeated administration of naloxone (N) can produce analgesia. The present experiment was designed to examine the possibility that NIA might be a form of stress-induced analgesia. During acquisition, 2 groups of rats received N (5 mg/kg) and 2 groups received saline (S). One drug group and one S group were given a hot-plate test 20 min after injection (N-contingent and S-contingent). The other 2 groups received hot-plate tests 24 hrs following injection (N-noncontingent and S-noncontingent). Pawlick latency was used as an index of pain sensitivity. After 23 acquisition trials, all rats were injected with N and tested on the hot-plate. Only the N-contingent showed significant NIA. On the next test day, rats were given their usual injection substance and 15 min later they were placed in an open field box. The N-contingent group had the highest level of freezing as well as defecation. These findings showed that NIA per se is contingent upon painful stimulation during opiate blockade and that NIA might be mediated by heightened emotionality, which is similarly contingent.

Supported in part by a grant from NSERC of Canada.

110.17

PLASMA AND TISSUE CONCENTRATIONS OF CATECHOLAMINES AFTER DIFFERENT TYPES OF STRESSES IN RATS: NALOXONE EFFECTS. H.M.Rhee. Dept. of Pharmacology, Oral Roberts University School of Medicine, Tulsa, OK 74137.

Stresses are known to be responsible for sympathetic overdrive with an elevated level of plasma catecholamines. To investigate the role of opioid receptors in immobilization, heat, and exercise stresses, young adult male Sprague-Dawley rats were surgically implanted with arterial and venous catheters two days before the stress. Immobilization stress was applied in a rat restrainer. Heat stress was given under an infrared lamp (39°c) for 5 minutes. Other rats were forced to run on a treadmill for 3 weeks with a gradual increase in the speed as well as in the duration of running time. Blood pressure and heart rate were continuously measured before, during, and after the stress. Plasma and tissue catecholamines were analyzed using HPLC with EC detector. All stresses increased blood pressure and heart rate significantly. Naloxone did not prevent the rise of blood pressure and heart rate after the immobilization. Naloxone had little effect on plasma catecholamines after the immobilization, but it increased them after the heat stress. Naloxone had no effect on either total cholesterol or high density lipoprotein. The data suggest that different types of stresses selectively release catecholamines, which is regulated by opioid receptors, particularly under stressful conditions.

110.18

NEUROENDOCRINE AND BEHAVIORAL CORRELATES OF ACTIVITY-INDUCED ANOREXIA IN THE RAT L. Shih*, J. R. Glowa and A. Riley Clinical Neuroendocrinology Branch, NIMH, Bethesda, MD 20892 and Psychopharmacology Laboratoory, Depart. of Psychology, The American University, Washington DC 20016.

Rats were individually housed and allowed to acclimate to home cages, with free access to food and water and daily intake monitored. Based on wgts over the last three of five acclimatization days, rats were divided into two groups with similar wgts, and put into a home cage with (running group) an attached running wheel which was locked or without (control group) a running wheel. Both groups were then subjected to a nine-day regimen of limited access (1-hr) to food with wgt and consumption monitored. After that period, (running) rats were allowed to run 22.5 hrs/day; both groups were weighed daily at 4:00 p.m. and fed 25-30 g at 4:30 and allowed to consume as much as possible for 1 hr. This procedure was followed for 30 days. The animals were then weighed, sacrificed (with blood taken for ACTH and corticosterone analysis) and the brain quickly removed for molecular probe analysis. Significant differences in ACTH and corticosterone occurred between the two groups, and there was a profound, but not life-threatening, weight loss in the running group. These data are consistent with the notion that there is a positive relationship between activity and stress hormone levels, and a negative relationship between these variables and the ability to maintain weight. As similar effects occur with human anorexia patients, behavioral procedures related to adaptation should be examined clinically.

110.19

STRESS-INDUCED INCREASES IN FOREBRAIN CHOLINE AND GLUTAMATE SYNAPTOSOMAL UIPTAKE IN AGED RAT STRAINS. G.M. Gilad, V.H. Gilad and Y. Tizabi[1]. Neuropsychiatry Branch, NIMH Neurosciences Center at St. Elizabeths, Washington, D.C. 20032; [1]Dept. of Pharmacology, Col. Med., Howard Univ., Washington, D.C. 20059.

The activity of septohippocampal cholinergic neurons undergoes adaptive changes in response to stressful stimuli. These changes are more pronounced in inbred Wistar Kyoto (WKY) rats that are behaviorally more reactive to stressors and have a shorter life span than Brown Norway (BN) rats. Moreover, degeneration of these neurons which may be accompanied by reduced hippocampal choline uptake, occurs earlier in the WKY strain. Here we report the effects of 2 hr restraint stress on choline and glutamate uptake in aged (24 mo.) rats of these strains. After stress, choline uptake was increased slightly in the hippocampus (~15%) and by as much as 80% in the frontal cortex. With one exception—unchanged choline uptake in aged WKY hippocampus—no strain or age differences were noted in this stress-response. Glutamate uptake, measured in the hippocampus and septum, increased ~3 folds in both regions independent of strain or age. We conclude: 1) an initial increase in choline uptake after acute stressful stimuli is a hallmark of the stress-response of basal forebrain cholinergic neurons, and 2) a large increase in glutamate uptake (re-uptake) occurs after 2 hr of restraint stress and its characteristics should be subject to further studies.

110.20

EFFECTS OF IONIZING RADIATION ON ISOLATED AND GROUP-HOUSED MICE. M.R. Landauer*, H.D. Davis*, D.M. Maier*, T.B. Elliot* & G.D. Ledney*. (SPON: D.R. Livengood) Behavioral Sciences & Experimental Hematology Depts., Armed Forces Radiobiology Research Institute, Bethesda, MD. 20814-5145.

The effects of group housing on radiation-induced lethality were examined in male Swiss-Webster mice exposed to 9-12 Gy gamma photons. Isolated mice survived significantly longer (1.5 days) than animals housed in groups of 10, although there was no difference in the LD50s. Haloperidol, which antagonizes amphetamine-induced aggregate toxicity, failed to attenuate radiation-induced lethality. The radiation-induced aggregate toxicity effect was maintained in grouped animals regardless of cage size (39 vs 98 sq cm/mouse) or age (3-9 months). Pre-radiation (isolated, grouped) and post-radiation housing conditions (isolated, grouped & returned to same group [stable], or grouped & introduced to new group [unstable]), were examined. Animals grouped pre-radiation (10.5 Gy) survived longer than mice isolated pre-radiation. Mice housed in stable groups post-radiation survived longer than those in unstable groups. Oral administration of the antibiotic, Pefloxacin, increased survival time in both isolated and grouped (unstable) animals. Isolated mice in both control and antibiotic conditions survived longer than grouped animals. It was concluded that the radiation-induced aggregate toxicity effect may be mediated by the transmission of pathogenic organisms and/or the stress associated with post-radiation grouping.

111.1

DEVELOPMENT OF THE HUMAN THALAMUS: GOLGI ANALYSIS OF PULVINAR NEURONS. N.Zečević and J.Mojsilović. Dept.Neurophysiology, Institute for biological research, University of Belgrade, 11 000 Belgrade, Yugoslavia.

Development of the human pulvinar was analysed on Golgi impregnated brains from 7 fetuses, 12 to 34 gestational weeks (gw). Brain tissue was obtained after legal abortions The posterior and lateral area of the thalamus was considered as the place where prospective pulvinar neurons could be found at early ages. Neurons were bipolar and branched bipolar at 12gw, while at 14gw first multipolar neurons appeared. At 16gw number of multipolar neurons increased and two subtypes could be observed: bush cells and sparsely branched multipolar cells. In later ages (34gw) both subtypes had either small (<20μm) or large (often 30-40μm)maximal diameter (Dmax) of the soma. Parameters measured: total dendritic length (TDL), area and Dmax of the soma, number of dendritic segments, average segmental length- increased several times in the period examined. The largest increase was observed from 24-34gw, when TDL increased 3 times. In conclusion, major pulvinar neuronal types are well differentiated by 24gw. Acceleration of maturation between 24 and 34 weeks coincides with development of pulvinar projections to peristratal cortex.

111.2

GOLGI STAINING METHOD REVEALS THAT LESIONS TO CHOLINERGIC BASAL FOREBRAIN NUCLEI IN NEWBORN MICE RESULT IN ABNORMAL DIFFERENTIATION OF NEOCORTICAL PYRAMIDAL CELLS. C.F. Hohmann, K. Kwiterovich*, M.L. Oster-Granite and J.T. Coyle. Depts. of Psychiatry, Physiology and Neuroscience, The Johns Hopkins School of Medicine., Baltimore, MD 21205.

We have previously reported abnormal cortical morphogenesis following lesions to the nucleus basalis magnocellularis (nBM) area in newborn mice. Such lesions resulted in a transient (>85% at PND4/5; 35% at PND14; 20% at PND30), ipsilateral depletion of cholinergic markers in neocortex paralleled by an apparent retardation of cortical cell differentiation, as revealed by Nissl stain. Most affected were the pyramidal neurons of layer V which undergo differentiation during the first postnatal week, the time of maximal cholinergic depletion in cortex. We are now using the Rapid Golgi method in an effort to define and quantify the abnormal morphogenesis of layer V pyramidal neurons ipsilateral to the nBM lesion.

BALB/CByJ pups received unilateral nBM lesions at the day of birth and were sacrificed on PND7 and PND14, respectively, and processed for Rapid Golgi staining. Hemispheres ipsi- and contralateral to the lesion were sectioned coronally on a vibratome, slide mounted separately and analyzed blindly using a Glaser/Van der Loos Image Combining Computer Microscope [ICCM]. Our results show that the average perikaryal size of layer V pyramidals is significantly reduced ipsilateral to the lesion. The size differential between ipsi- and contralateral cells is larger at PND7 than at PND14, confirming previous impressions that the severity of morphological disturbances attenuates with age and recovery of cholinergic innervation. However, ipsilateral PND14 pyramidal neurons continue to display qualitatively and quantitatively abnormal dendritic morphology, including increased apical dendritic branching, paucity of spines and other morphological features otherwise found in more immature or genetically abnormal mice. Abnormalities of cellular morphology, similar to those reported here, have also been observed in autopsy samples from Down Syndrome and other mental retardation syndromes.

111.3

EXTRATHALAMIC AFFERENT INNERVATION OF THE CEREBRAL CORTEX IN THE NEONATAL RAT. H.C. Fibiger and K. Semba. Div. of Neurological Sciences, Dept. of Psychiatry, Univ. of British Columbia, Vancouver, B.C., Canada V6T 1W5

In addition to thalamocortical projections, the cerebral cortex receives inputs from a variety of subcortical structures including the basal forebrain, hypothalamus, and brainstem. There is some evidence that during development these cortical afferent fibers grow into the cortex at different times. Furthermore, some afferents appear to "wait" in the subplate, while others do not. However, currently available data on cortical ingrowth are limited to selective populations of afferents. In the present study, we investigated the status of cortical innervation by individual extrathalamic afferents in the neonatal (postnatal day 1) rat using retrograde transport of WGA-HRP. Three representative cortical regions were selected for detailed studies: the primary somatosensory (vibrissal region), visual, and medial prefrontal cortices.

Following WGA-HRP applications to the somatosensory or the visual area, retrogradely labelled neurons were seen in the respective specific thalamic nuclei, as well as in topographically appropriate regions of the magnocellular basal forebrain, and the locus ceruleus. However, no or very few cells were labelled in the raphe nuclei or hypothalamus, both of which are closer to the cortex than is the locus ceruleus. These results indicate that not all extrathalamic afferent fibers have grown into all cortical regions in the neonatal rat, and that the differential timetable cannot simply be explained by different times of origin of the afferent source neurons as previously shown in our laboratory, or their distances from the cortex.

111.4

FATE MAP OF FUTURE ENTORHINAL AND PERIRHINAL CORTEX IN THE EMBRYONIC MOUSE. C.D. Claypool*, B.W. Coltman*, C.F. Ide. Biology Department, Tulane University, New Orleans, LA 70118.

Our long term goal in this project is the prenatal removal of cortical afferents which might influence the development of target cells found in the hippocampus and dentate gyrus. Our first experiment involved determining the location of the entorhinal primordium in the embryonic day 15.16 (E15.16) cortex. Using exo utero surgical methods, we placed small DiI crystals in different cortical regions of the E15.16 embryo. At post-natal day 1 (P1), brains were fixed in 4% paraformaldehyde, cut via vibratome into 200 um sections, and examined with fluorescent microscopy (n=10). The entorhinal cortex was mapped to the far postero-lateral region of the E15.16 cortex. Clear projections to the hippocampus and the alveus were present by P1, as well as evidence of communication with the dentate gyrus. The perirhinal cortex was mapped to the region just anterior to the entorhinal region. Fibers from this region were shown to project into the alveus and through the anterior commissure. Axons from cells in the olfactory bulb were also stained and present in the perirhinal region. We are now removing these regions in E15.16 embryos to determine trophic effects cortical afferents have on development of target areas. Supported by NIH 1 F33 NS08594-01.

111.5

MORPHOLOGICAL IDENTIFICATION OF TACHYKININ-CONTAINING NEURONS IN THE DEVELOPING NEOCORTEX OF THE CAT. M.K. Boylan*, and R.S. Fisher. (SPON: C.L. Wakefield). MRRC and Dept. of Anatomy and Cell Biology, UCLA, Los Angeles, CA 90024.

Tachykinins (Tks, including substance P, substance K and neuromedin K) are produced by neocortical (Cx) neurons during fetal development in the cat and are frequent postnatally. Morphological identification of Tk-producing neurons has not yet been performed. In order to demonstrate the cellular origin of Tks in the developing Cx, immunohistochemistry was combined with a single slice Golgi/gold-toning procedure in the Cx of fetal (F56) and postnatal (P1, P30 and P90) cats. Golgi/gold-toning revealed the somatodendritic morphology of Tk-positive neurons; axons were not apparent. At F56 and birth (P1), immature pyramidal-like and nonpyramidal neurons contained Tk immunoreactivity. In the P30 and P90 Cx, spiny and sparsely spiny nonpyramidal (stellate) neurons were double labeled. These were present in layers III-VI. Pyramidal neurons were occasionally double labeled and were distributed in layers II-III and V-VI. These developmental results are in contrast to previous reports of Tks in the adult Cx indicating that Tk-producing neurons were strictly nonpyramidal and sparsely spiny or aspiny. The results of the present study suggest that pyramidal neurons transiently express Tk immunoreactivity during development. Also, spiny nonpyramidal cells are prevalent postnatally but may represent neurons that subsequently lose dendritic spines. Supported by USPHS Grants NS 24596 and HD 05958.

111.6

DEVELOPMENT OF A CHEMICALLY DEFINED MEDIUM FOR ORGANOTYPIC TISSUE SLICE CULTURES. J. Edmond*, C.M. Annis, R.T. Robertson and J. Yu. Department of Biochemistry, Mental Retardation Research Center, University of California, Los Angeles, CA 90024, and Departments of Anatomy and Neurobiology and Physical Medicine and Rehabilition, University of California, Irvine, CA 92717.

The organotypic tissue slice culture technique (Gahwiler, *TINS*, 1988) provide an excellent system for the analysis of axonal connectivity and neural development. However, the study of molecular mechanisms of axonal and neural development necessitates the use of a chemically defined culture medium. We report here the development of a medium, EOL 1 defined medium, which meets these needs. The medium is based on a deficient DMEM/ Ham's F-12, which is supplemented with several stable and unstable additives necessary for proper neural function and metabolism. Several of the more important medium additives are creatine, fumarate and D(-)-hydroxybutyric acid. Organotypic slice cultures from several brain regions, including cerebral cortex, hippocampus and basal forebrain, grow well in this defined medium and can be maintained for periods of time in excess of four weeks. Studies of normal morphology along with histochemical and immunocytochemical characterization of these cultures demonstrate that this defined medium allows for a high degree of cytoarchitectural maintenance while preserving many normal neurotransmitter and neuropeptide distributions.

Supported by NIH grants HD 06576 (JE) and NS 25674 (RTR) and NSF grant 87-08515 (RTR).

112.1

A PARTIALLY PURIFIED MUSCLE FACTOR(S) INDUCES TYROSINE HYDROXYLASE EXPRESSION IN CULTURED NEURONS FROM VARIOUS BRAIN REGIONS IN RAT.

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We have previously demonstrated that a soluble muscle-derived factor(s) (MDF) induces novel expression of the catecholamine (CA) enzyme tyrosine hydroxylase (TH) in cultures of embryonic rat cerebral cortex and greatly amplifies CA traits in cultures of CA neurons from substantia nigra. In this study, we examined whether MDF similarly affects 1) other brain neurons, 2) peripheral neurons and 3) neurons of differing age. Brain cells from striatum, cerebellum, collicular plate, and spinal cord of embryonic day (E) 14-17 rats and peripheral neurons from dorsal root (E14 DRG) and superior cervical (E21 SCG) ganglia were dissociated and maintained in culture either on control media or media supplemented with partially purified MDF. As in cortex, control cultures of other non-CA brain neurons contained a low percentage of TH neurons which was dramatically increased by addition of MDF. Induction was greatest in striatal cultures (Control: 431 $\pm$ 78 vs. MDF: 22,542 $\pm$ 769 TH neurons) with up to 40% of total neurons expressing TH. Although striatal neurons were MDF-responsive at all ages tested, cerebellar and collicular neurons could not be induced after E17. In ^{3}H-thymidine labeling studies, in all regions, only postmitotic neurons expressed TH. In contrast to brain neurons, peripheral neurons were not induced by MDF; DRG cultures did not contain more TH neurons nor did MDF increase TH activity levels in SCG cultures. We conclude that MDF promotes TH expression in certain classes of brain but not peripheral neurons. These studies suggest that the transmitter phenotype of neurons throughout brain is highly plastic during the critical time just following withdrawal from the cell cycle.

112.2

DIFFERENTIAL EFFECTS BETWEEN SERUM AND MUSCLE EXTRACT ON MOTONEURON SURVIVAL AND NEURITE EXTENSION.

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Motoneurons were isolated according to the procedure of Dohrmann et al., 1986 [Develop. Biol. 118: 209-21] and grown on polyornithine coated glass coverslips [~60 cells/mm^2] in a medium consisting of DMEM/F12 containing the N1 supplements minus putrescine [CDM]. Horse serum [5% v/v] and/or muscle extract [50µg/ml] were added to the CDM for various experiments. The muscle extracts were prepared from E8, E11, E18 and P3 chick legs.

When grown in CDM alone no neurons survived longer than two days. The addition of only serum to CDM allowed the survival of 12% of neurons at 4 days and 4% at 8 days; however, neurite formation did not occur. Addition of muscle extract alone to CDM also allowed a similar number of neurons to survive but in addition promoted neurite formation. Neurons surviving longer than 4 days exhibited extensively developed neurites. The combination of adding both muscle extract and serum to CDM resulted in the survival of a much larger fraction of neurons [30% at 4 days and 18% at 8 days]. Furthermore, all neurons that survived 4 days or longer had very extensively developed neurites. There were no differences amongst the various muscle extracts tested in their ability to allow the survival of motoneurons and no marked differences in their ability to promote neurite formation. (Supported by the ALS of Canada)

112.3

THE FATE OF FGF IN THE OPTIC TRACT.

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^{125}I-FGF was injected intraocularly into adult rats and found to be internalized and anterogradely transported from the eye, through the optic nerve, to the contralateral lateral geniculate body and contralateral superior colliculus. ^{125}I-FGF was not retrogradely transported from superior colliculus to retina. Anterograde transport was blocked by excess unlabelled FGF and dependent on intact tertiary structure: Denatured, biologically inactive FGF was not transported. ^{125}I-NGF was not transported. Transport was not heparinase sensitive but was blocked by wheat germ agglutinin, arguing that high-affinity FGF receptors mediate transport. Time course studies showed the fastest transport rate of ^{125}I-FGF to be 3 mm/hour. In pulse label studies, radioactivity was lost from the superior colliculus with a half-life of 22 hours. Autoradiography of brain sections demonstrated that radioactivity was confined to retinal ganglion cell projections suggesting that it was not released from axons. Autoradiograms of optic tract tissues separated on SDS PAGE showed that ocular FGF is proteolytically degraded primarily after being exported from the eye.

Supported by NINDS grants: NS 25122 and NS 01230.

112.4

GINSENG SAPONINS : INFLUENCE ON GROWTH CONE OF FETAL MOUSE SPINAL CORD IN VITRO.

X.Y. Shen, S.M. Kuo*, S.C. Chang* and Z.L. Hao*, Dept. of Anatomy, Shanghai Medical University, Shanghai 200032, People's Republic of China.

Ginseng, the roots of Panax ginseng C.A. Meyer, has been used in traditional Chinese medicine for thousands of years. Much evidence has suggested that ginseng saponins may play a role in the regulation of neurotransmission. The authentic sample of ginsenosides is derived from Shanghai Institute of Pharmaceutical Industry. Primary organotypical cultures of 17 D fetal mouse spinal cord were done as usual (Shen,1985,1988). Each experiment was performed with five groups, one of which was a control group while the others contained 25, 50, 100 and 200 µg in 1 ml of the regular medium. The proliferative effect of neurites was consecutively observed at 2, 3 and 4 days in vitro under phase-contrast microscopy and some ultrastructural studies were also used. The criteria used for the evaluation of this activity is based on the neurite length as well as the total number of neurites produced by the explant. We found that the growth cones in 50-100µg/ml added groups were obviously enhanced when compared with other groups. The results suggest that ginsenoside of ginseng root has a promoting effect on neurite extension.

112.5

TROPHIC EFFECT OF OLFACTORY PROTEINS DEPENDS ON DNA SYNTHESIS.

M.P. Lambert, S.Hua*, and W.L. Klein. Dept. of Neurobiology and Physiology, Northwestern University, Evanston, IL 60208.

Previous work has shown that rat basal forebrain cultures respond to soluble olfactory proteins with increased survival and differentiation. Confirming this trophic effect using the MTT assay, we found that cells grown with olfactory bulb extract(OLBX) showed 100% survival for at least 48 hrs. By contrast, cells in control cultures showed 50% mortality after 6 hrs. In testing possible mechanisms for the response, we found that OLBX promoted DNA synthesis, with an initial (first 23 hr) 3H-thymidine incorporation rate that was double control values. Fetal calf serum(FCS) also promoted thymidine uptake but, as the effect was additive with that of OLBX, it appeared to act on different cells . To ascertain if DNA synthesis was necessary for the trophic effect of OLBX, aphidicolin was used to inhibit DNA polymerase I. During the first 1-5 culture days, aphidicolin not only inhibited thymidine uptake but completely inhibited the ability of OLBX to promote survival. In contrast, addition of aphidicolin after 7 or 9 culture days caused little morphological change in cultures, although it halted thymidine incorporation. These data support the hypothesis that olfactory bulb proteins increase survival of cultured neural cells by maintaining mitosis through a critical window of development. (Supported by NIH grant NS23348 to WLK)

112.6

THE DEVELOPMENT OF MESENCEPHALIC DOPAMINERGIC NEURONS *IN VITRO* IS AFFECTED BY NEUROTROPHIC ACTIVITIES DERIVED FROM NEURAL CELL LINES.

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Neurotrophic factors play a crucial role in neuronal differentiation and regeneration. To identify growth factors which affect the development of dopaminergic neurons, conditioned media (CM) were prepared as possible sources of neurotrophic activities from various cell lines, including a neuronal cell line B103, glial lines B49 and C6, PC12 pheochromocytoma and a microglia-like line R33 derived from a nickel sulphate induced eye tumor. CM were assayed for effects on high-affinity dopamine uptake and survival of dopaminergic neurons grown in low density cultures (80,000 cells/cm^2) of dissociated embryonic day 14.5 rat mesencephalon. In both the absence and presence of 0.5% horse serum, B49- and JScl1-CM produced a dose-dependent increase of ~250% in the number of tyrosine hydroxylase-immunoreactive (TH-IR) neurons after 8 days in vitro. In cultures treated with CM from the other cell lines, smaller, but significant, increases were observed. Moreover, the R33- and PC12-CM, but not the other media, also elicited a significant increase in (^{3}H) dopamine uptake per TH-IR neuron. These differential effects suggest the presence of at least two neurotrophic activities capable of influencing the maturation of central dopaminergic neurons.

Supported by: NIH grants NS 20832, NS 25778, the PEW Charitable Trust and DFG (En 187/1-1).

112.7

SOLUBILIZATION OF A SPINAL CORD MEMBRANE MOLECULE THAT STIMULATES TYROSINE HYDROXYLASE ACTIVITY IN CULTURED SYMPATHETIC NEURONS. V. Wong & J.A. Kessler, Depts. Neurology & Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461.

Neuronal contact with other cell membranes and with extracellular matrix (ECM) plays an important role in regulating neuronal growth and development. Effects of cell contact on neurotransmitter expression (e.g. substance P, acetylcholine, catecholamine) have been demonstrated in various cell types including sympathetic, spinal cord, striatal neurons, and PC12 and chromaffin cells. We found that treatment of cultured sympathetic neurons with membranes prepared from adult rat spinal cord stimulated levels of tyrosine hydroxylase (TH) enzymes activity and TH mRNA. The TH-stimulating molecule(s) is differed from the previously characterized cholinergic stimulating molecule (MANS; Wong & Kessler, PNAS 84:8726, 1987), also isolated from spinal cord membranes, in that it could not be extracted from membranes by high salt treatment. Solubilization of spinal cord membranes required 0.1% Triton X-100, a non-ionic detergent, suggesting that the TH-stimulating molecule(s) contains an integral membrane moiety. When cultured sympathetic neurons were treated with the Triton extract, TH activity was stimulated 2-fold, while choline acetyltransferase (CAT) activity was unaltered. The remaining membrane pellet also stimulated TH levels, suggesting incomplete solubilization by Triton. Further studies are underway to identify a more effective detergent for solubilizing spinal cord membranes, and to characterize this TH-stimulating molecule.

112.8

SAFETY OF INTRATHECAL (i.t.) INSULIN IN RABBITS. C.K. Haun, G. Goubran*, W.K. Engel & I. Tan*. Depts. of Anatomy & Cell Biology and Neurology, University of Southern California School of Medicine, Los Angeles, CA 90033.

In pre-clinical safety-testing prior to its direct i.t. application to the abnormal human CNS, insulin was infused continuously into the lumbosacral CSF space, permitting high concentrations of insulin to bathe the CNS. Doses of up to 6 U/kg body wt./day were well tolerated for up to 4 wks. In 29 male NZ White rabbits (3-3.5 kg) under general anesthesia, a PE-10 catheter was inserted into the sub-arachnoid space at vertebrae L7-S1; its tip lying at the S2-3 vertebral level. The catheter was connected to an Alzet 2ML2 minipump (5ul/hr.) via a PE-60 extender. The pump was implanted in a s.c. pocket, and changed every 2 wks. It contained crystalline porcine or human-recombinant insulin, up to 122.5 U/ml in artificial CSF, with glutamic acid, 5-7 mg/ml, to prevent insulin aggregation. The animals survived well, exhibited normal behavior, and gained weight, providing they ate normally; their blood glucose did not change significantly from pre-operative levels (90-170 mg/dl). Rabbits that failed to eat suffered hypoglycemic convulsions and died despite post factum i.v. or s.c. glucose injections. (Doses of 10, 25 or 50 U/kg produced lethal hypoglycemia despite eating.) This study predicts CNS safety of dose-controlled i.t.-administered insulin in patients, but indicates the need to maintain blood glucose adequate to prevent hypoglycemia.

112.9

ENKEPHALIN IMMUNOREACTIVITY IN ORGANOTYPIC CULTURES OF DEVELOPING RAT CEREBELLUM: EVIDENCE FOR TRANSIENT OPIOID EXPRESSION IN VITRO. J.G. Osborne* and K.F. Hauser (SPON: L. Yurkewicz). Dept. of Anat. and Neurobiol., Univ. of Kentucky Sch. of Med., Lexington, KY 40536-0084.

Enkephalins are reported to transiently appear within the neuronal progenitor cells of the cerebellar external granule layer (EGL), and are hypothesized to modulate EGL cell growth *in vivo* (Zagon et al., *Science*, 227: 1049, 1985). Enkephalin immunoreactivity (IR) was examined within cerebellar explant cultures to better understand the mechanisms governing opioid expression during development. Cerebella from male, neonatal Sprague-Dawley rats were isolated from the brainstem, dissected into 8 explants per cerebellum, and maintained *in vitro* for 1, 4, 7, 14, or 28 days as organotypic explants in 22 mm wells or in Maximow chambers. The distribution of enkephalin IR (antisera from Dr. B.E. Maley) was explored in 4% paraformaldehyde fixed, whole-mount explants. Controls, using antisera preabsorbed against both met- and leu-enkephalin, lacked staining. Based on morphology and distribution within the explant, enkephalin IR was present in Golgi cells and smaller immature EGL cells. With progressive development, detectable enkephalin IR was not seen in mature granule, stellate or basket neurons, which are derived from EGL progenitors, indicating that enkephalin IR present in EGL cells is lost during maturation. However, some EGL cells were not enkephalin immunoreactive, suggesting that other local factors also affect enkephalin IR in EGL cells. The loss of enkephalin IR by neurons derived from the EGL suggests that enkephalin expression by EGL cells is developmentally regulated, and that the decline in expression is normally mediated by local mechanisms present within the maturing cerebellum. Supported by NIH RR05374, and PSP and UKMC Res. Funds (Univ. of KY).

112.10

OPIOIDS DIRECTLY MODULATE THE GROWTH OF MIXED-GLIAL CULTURES: SUPPRESSION OF ASTROCYTE PROLIFERATION BY MET-ENKEPHALIN. A. Stiene-Martin* and K.F. Hauser, Dept. of Anat. and Neurobiol., Univ. of Kentucky Sch. of Med., Lexington, KY 40536-0084.

Opioid peptides have been reported to inhibit the proliferation and differentiation of neural cells. This study examined the action of methionine-enkephalin (ME) on the growth of astrocytes in mixed-glial cultures. Primary cultures of mixed-glial cells were isolated from the cerebral hemispheres of 1-day-old mice. After 24 h *in vitro*, at least 6 wells/group were continuously treated with basal growth media alone (controls), ME (1 μM), ME (1 μM) plus naloxone (3 μM), or naloxone (3 μM) alone. Cultures were examined at regular intervals, and counts were made of absolute numbers of cells in unstained preparations. To determine whether ME selectively influences astrocyte DNA synthesis, mixed-glial cultures were treated with 0.24 μCi [^{3}H]-thymidine for 16 h. Combined [^{3}H]-thymidine autoradiography and GFAP immunocytochemistry were performed. When compared to controls and naloxone-treated groups, ME caused a statistically significant decrease in both total cell numbers and in the mitotic index of type I astrocytes (GFAP+ flat cells) after 4 days *in vitro*. However, type II astrocytes (GFAP+ process-bearing cells) were not affected. The data indicate that ME selectively suppresses the growth of a morphologically distinct subpopulation of astrocytes in mixed-glial cultures, and that the action of ME is opioid receptor specific-since its action was reversed by naloxone and naloxone alone had no affect. This supports the general hypothesis that opioids influence brain maturation by modulating neural cell proliferation, and suggests for the first time that glia may be a direct target for opioid action during neural development.

Supported by NIH RR05374, and PSP and UKMC Research Funds from the Univ. of Kentucky.

112.11

ANALYSIS OF PREPROENKEPHALIN mRNA EXPRESSION IN THE DEVELOPING RAT CEREBELLUM BY *IN SITU* HYBRIDIZATION. K.F. Hauser, J.G. Osborne*, S.L. Sanders*, T.E. Curry*, and M.H. Melner*[1] (SPON: A.G. Gona). Depts. of Anat. and Neurobiol., and of Ob./Gyn., Univ. of Kentucky Sch. of Med., Lexington, KY 40536 and [1]Oregon Reg. Primate Res. Ctr., Beaverton, OR 97006.

Enkephalin immunoreactivity (IR) is reported to be transiently present within germinative cells of the developing rat cerebellar external granular layer (EGL), and is hypothesized to modulate EGL cell proliferation (Zagon et al., *Science*, 227: 1049, 1985). To determine whether preproenkephalin mRNA expression is responsible for the transient appearance of enkephalin IR, as well as to identify the possible cellular sites of opioid gene expression in the developing cerebellum, preproenkephalin mRNA expression was examined by *in situ* hybridization in the developing cerebellum. Adult and postnatal day 1, 3, 6, 11, 12, 16, 21, 28 and 35 male Sprague-Dawley rats were anesthetized and sacrificed by intracardiac perfusion with 4% paraformaldehyde in phosphate buffer. Frozen sections were hybridized with an [^{35}S]-labeled cRNA probe specific for preproenkephalin mRNA. Preproenkephalin mRNA was not detected in cerebellar cortical neurons from postnatal days 1-6; although, occasional heavily-labeled cells were observed in the choroid plexus of the IVth ventricle, in the pia after day 3, and in nuclei in the brainstem. On day 11, Golgi cells were moderately labeled and this pattern remained consistent in older rats and adults. However, under the present hybridization conditions, preproenkephalin mRNA was not detected in the germinative cells of the EGL. Thus, the transient appearance of enkephalin IR in EGL cells appears to be unrelated to increased levels of preproenkephalin mRNA expression by these cells. Supported by NIH BRSG RR05374, and PSP and UKMC Research Funds (Univ. of KY).

112.12

LAMININ EXPRESSION IN DEVELOPMENTAL RAT BRAIN: A QUANTITATIVE IN SITU HYBRYDIZATION STUDY USING IMAGE ANALYZER. C. T. Cheng, F. C. Zhou, and C. H. Lee Depts. Anatomy and Pathology, Indiana Univ. Sch. Med. Indianapolis, IN46223

Laminin promotes neurite outgrowth of cultured neurons (Davis et al., TINS 8:528, 1985) and guides fiber growth of grafted neurons in the rat brain (Zhou and Azmitia, J. Chem. Neuroanat. 1:133, 1988). Recently, we have studied the distribution of the laminin mRNA in the rat brain at different developmental stages by in situ hybridization (Cheng et al., Abst. Soc. Neurosci. 14, 73, 1988). Currently, we performe a quantitative study of the laminin mRNA in rat brains by computerized autoradiographic image analyzer.

Brains from Sprague-Dawley rats of 18 days embryo (E18), postnatal day 7 and 29 (P7 and P29) were used for the study of the distribution of laminin mRNA by in situ hybridization. A 50-base oligonucleotide which hybridizes to the B2 chain of laminin mRNA was labeled with alpha-^{35}S (or ^{32}P)-dATP at 3' end and used to detect laminin mRNA. The density of radioreactive silver granules were analyzed by image analyzer and showed quantitative changes in cells of various brain regions during different stages of development .

At the same stage (P7) of development, a regional difference in the density of silver granules was observed: meningeal cells> cortical neurons> cerebellum/purkinje cells > CA3/pyramidal cells. As compared with ages, the densities of silver granules in the meningeal cells are low in E18 and reached their highest level at P7 and P29, whereas the densities of silver granules in the cortical neurons were increased from E18 to P7 and decreased by P29. These results indicate that the laminin expression in different rat brain areas have changed quantitatively during the development, which is in consistent with our previous immunocytochemistry study. Sponsored by NIH grant 23027.

112.13

INTRANEURONAL LAMININ(LM)-LIKE IMMUNOREACTIVITY IN HUMAN CENTRAL NERVOUS SYSTEM. H.Suzuki*, T. Yamamoto#*, H. Konno*, Y. Iwasaki*, Y. Ohara*, H. Yamamoto**, (SPON: K. Kurata##). *Dept. Neurological Sciences, ##Dept. Physiology, Tohoku Univ. Sch. of Med., Sendai, 980 Japan. #Dept. Neurology, Fukushima Medical College, Fukushima, Japan. **Dept. Cell Biology & Anatomy, North Western Univ. Sch. of Med., Chicago, IL.

Several recent studies have revealed the existence of laminin-like immunoreactivity(LLI) and their mRNA signals in CNS neurons of the rodent(T. Yamamoto, J. Neurol. Sci., 1988;84:1, T. Manthorpe, Abstr. ,18th Annual Meeting, S.N., 1988;149.13, C.T. Hu, Abstr.,18th Annual Meeting, S.N., 1988;149.14). We Report here the presence of intraneuronal LLI in the human brain and their distributions. Laminin affinity column-purified rabbit antibodies raised against human placental LM were applied to the sections from the formalin-fixed human brains. In addition to the expected staining of basal lamina of the capillaries and pia matter, distinct LLI was observed in neurons of the hippocampal pyramidal and dentate granular layers, amygdala, septal nuclei, basal forebrain, striatum, neocortical layers(I-III and VI), anterior and dorsomedial nuclei of the thalamus, and supraoptic nucleus. A small number of the neurons in the ventrolateral and reticular nuclei of the thalamus were also immunoreactive. The cortical layers(IV and V), cerebellum, red nucleus, subthalmic nucleus, pallidum, and motor neurons in the brainstem and spinal cord were devoid of immunoreactions. The distributions are in essence similar to those in rodents. Although the signigicance of LLI in neurons remains to be clarified, the differential distribution of the LLI suggests the neuronal system-specific roles imposed on the intraneuronal laminin-like molecule(s).

112.14

DOES THE DISTRIBUTION OF GANGLIOSIDES IN NEURONAL MEMBRANES DEPEND ON UNDERLYING CYTOSKELETAL COMPONENTS? I.H. Fentie*, S.P. Mahadik[1] and F.J. Roisen (SPON: C. Shields). Dept. of Anat. Sci. & Neurobiol., Univ. of Louisville, Sch. of Med., Louisville, KY 40292 and [1]NY State Psychiatric Inst. NY, NY 10032.

Compelling evidence has been accumulating which suggests that gangliosides play a regulatory role in both in vitro and in vivo neuronal development. Recently, we employed immunocytochemistry to examine the topographical distribution of the ganglioside GM1 on murine Neuro-2a cells using high resolution SEM and STEM. Frequently, the colloidal gold markers were seen distinctly aligned along the perikaryal and neuritic surfaces. This linearity implied that endogenous molecules of GM1 were bound to an underlying cytoskeletal component. To examine this possibility and to determine the nature of the underlying cytoskeletal anchorages, we applied cytoskeletal disruptive and stabilizing agents to Neuro-2a cells prior to immunolocalization of GM1 with mAb to GM1 labeled with colloidal gold. Preliminary studies suggest that conditions which limit microfilament formation (cytochalasin D) seem to reduce the linear arrays on the perikaryal surfaces. The relationship of these alignments to microtubules is being probed with colchicine and taxol. Attachment of surface molecules to underlying cytoskeletal anchorages could provide a means of regulating membrane fluidity and receptor efficacy. USPHS grant NS24524.

112.15

MORPHOLOGICAL EFFECTS OF EXOGENOUS GM1 GANGLIOSIDE ADMINISTRATION IN INTACT ANIMALS. L. Lescaudron* and D.G. Stein. Brain Research Laboratory, Rutgers Univ., Newark, NJ 07102.

We previously demonstrated that chronic GM1 ganglioside injections (17 days) do not affect the behavior of intact (sham-operated) rats studied on open-field, Morris water-maze and passive avoidance tasks (Bitran et al.,Soc.Neurosci.Abstr.,494.6, 1988). However, a significant decrease in ventricle size (-35%) and number of Ach-E-positive neurons (-11%) in the nucleus basalis magnocellularis (NBM) was observed in GM1 as compared to saline-injected animals. This suggests that GM1 gangliosides may have a toxic effect on cholinergic neurons. To examine this, we counted Ach-E- positive neurons in the lateral (LS) and medial (MS) septal nuclei in the same animals. No difference in the number of Ach-E positive neurons was observed in LS and MS between the two groups of animals. Next, in the NBM, we observed decreases in cell body size of Ach-E stained neurons ($p<0.007$). There was also a reduction in the number of Ach-E-positive neural processes (probably dendrites)($p<0.02$). However, on Nissl-stained sections, no difference was observed in cell packing density and in size of the cells within the NBM. Taken together, these data suggest that GM1 ganglioside injections reduce the number and/or activity of the Ach-E in some neurons within the NBM. A densitometric analysis performed in the frontal (FC) and fronto-parietal cortex (FPC) and the hippocampus (HP) showed a reduction of Ach-E staining by -7.7% in the FC and -15% in the FPC and no difference in the HP (relative to white matter) in GM1 treated rats as compared to saline controls. In conclusion, chronic injection of GM1 gangliosides produces a decrease in Ach-E labeling both in the NBM and cerebral cortex suggesting a modification of the cholinergic function in these structures.

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112.16

GM 1 MODIFIES ADRENAL CHROMAFFIN CELLS "IN VITRO". G.Perez*,A.Posse*,V.Lauria* and J.A.Colombo. (SPON:E. Adler-Graschinsky). Unidad de Neurobiologia Aplicada (CEMIC-CONICET), Buenos Aires. Argentina.

The possibility of modifying viability rates and growth of neural cells prior to transplantation may represent a valuable tool for the improvement of their efficiency in attempting neural cell transfer.Among other trophic factors gangliosides affect neural growth. Under "in vitro" conditions we tested the effects of ganglioside GM1 (FIDIA) on cell morphology and adhesivity to a polylysine substrate. Adrenalchromaffin cells were enzymatically and mechanically dissociated from immature (PN 10) or adult rats, and seeded on polylysine-coated coverslips or 35 mm plastic dishes in DMEM-F 12. Incubation was carried out at 37°C and a mixture of 95% air/CO2. Twenty four hours after seeding, GM1 was added to each dish, in triplicates, to a final concentration of 10 -3, -5 and -7 M. Cell changes were registered daily. Addition of GM1 10 -3 to either immature or adult chromaffin cells resulted in a dramatic increase in cell size within 24 hs. after initiation of treatment, with a concomitant decrease in cell numbers adherent to substrate. Such changes were not apparent at 10 -7 M. Interaction between GM1 and NGF will be reported.-

This work was performed under grant support from the National Research Council of ARgentina (CONICET).

112.17

GM1 GANGLIOSIDE-INDUCED RECOVERY OF STRIATAL DOPAMINE LEVELS IN MPTP-TREATED ANIMALS IS INFLUENCED BY AGE AND EXTENT OF INITIAL INJURY. J.S. Schneider and C.F. Gross*. Dept. of Neurology, Hahnemann Univ. School of Medicine, Philadelphia, PA. 19102.

The present studies were performed to examine the effects of 2 variables-age and extent of injury-on the ability of GM1 ganglioside to stimulate recovery of striatal dopamine (DPM) levels following MPTP-induced damage to the DPM system. The effects of age were examined in C57 Bl mice. Mice aged 8 wks. to 12 months were given MPTP (18-30 mg/kg s.c., 2 times daily for 5 days). Some mice were then given 4 or 8 wks. of GM1 (30 mg/kg/day i.p.). Mice older than 6 mos. did not show any significant increase in striatal DPM levels with GM1 treatment in contrast to highly significant increases observed in 8-10 wk. old mice. Tyrosine hydroxylase immunohistochemistry suggested that older mice exposed to MPTP might have had more extensive substantia nigra cell loss than did younger mice. We have also observed that cats exposed to relatively low amounts of MPTP (and with mild to moderate motor deficits) have a larger magnitude increase in striatal DPM levels after GM1 treatment than cats given larger MPTP doses (and with initially severe motor deficits and extensive DPM depletions). These results suggest that increased age and extensive nigral cell loss are factors which decrease the restorative effects of GM1 ganglioside on striatal DPM levels in this model. (Supported by the American Federation For Aging Research, Inc. and the American Parkinson's Disease Assoc.).

112.18

POSSIBLE ROLE OF A PUTATIVE RPE-CELL TROPHIC FACTOR(S) IN PHOTORECEPTOR CELL RESCUE IN RCS DYSTROPHIC RATS. H.J. Sheedlo, L. Li and J.E. Turner. Department of Anatomy, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27103.

Normal retinal pigment epithelial (RPE) cell transplants have been shown to prevent photoreceptor cell (PRC) degeneration in retinas of 26 day-old Royal College of Surgeons (RCS) dystrophic rats (Li and Turner, 1988). This study revealed PRC rescue in areas distant from the RPE-cell graft site and under membrane debris, suggesting effects by putative trophic factors. The following studies were undertaken to determine if RPE cells release factors which affect PRC survival in dystrophic retinas. In one study medium conditioned by RPE cells (RPE-CM) from Long Evans rat pups was injected into the subretinal space of 26 and 27 day-old RCS dystrophic rats. Examination of the injected retinas 10 and 15 days later revealed a outer nuclear layer (ONL) thickness significantly greater than in noninjected retinas. Also, no outer segments (OS) were observed in retinas of 36 day and older RCS dystrophic rats; however, areas beneath the RPE-CM injection site showed OS as well as longer inner segments (IS). To further support the existence of trophic factors, normal RPE cells were transplanted into the vitreous and choroid of 26 day-old RCS dystrophic rats. At 60 days in grafted retinas, the ONL thickness was significantly increased when compared to 60 day control retinas. Although the PRC rescue effects were not as dramatic as shown by subretinal RPE-cell transplantation, IS and OS were detected. It is possible that RPE cell factors are able to affect the ONL by passing through the inner retina and Bruch's membrane. Biochemical analyses of RPE-CM and other injection studies are currently being performed. Supported by NEI EY-04337.

112.19

THE POLYPEPTIDE THYMOPOIETIN INDUCES PROCESS FORMATION IN PC12 CELLS. R. Cohen*, S. Geertsen*, T. Audhya*, G. Goldstein*, and M. Quik (SPON: B. Esplin) Dept. Pharmacol., McGill Univ., Montreal, Canada & Immunobiol. Res. Inst., Annandale, NJ.

Thymopoietin (Tpo), a peptide isolated from thymus, induces thymocyte differentiation. Tpo also binds to the nicotinic α-bungarotoxin (α-BGT) receptor in electroplax and brain. The present work shows that Tpo interacts with the α-BGT site in PC12 cells; Tpo resulted in a dose dependent inhibition of binding (IC50 = 3 nM). The effect of Tpo was also determined on cellular morphology in PC12 cells in culture as evidence exists that the α-BGT site may have a role in growth functions. Addition of Tpo to the cells in culture resulted in an enhanced formation of processes (≥ 25 um in length), which was dose and time dependent. Tpo-induced process extension occurred as early as 1 day after exposure to the peptide and increased with continued time in culture; for instance, after 5 days exposure, an increase (2 fold) in process extension was observed with 1 nM Tpo, which was maximal (100 fold) with 100 nM Tpo. These effects were not observed with splenin, a peptide identical to Tpo except for 1 amino acid substitution. The effects of Tpo appeared distinct from those of NGF. Thus Tpo, a peptide which interacts at the α-BGT site, can induce process formation in PC12 cells. The relationship between these events remains to be determined.

112.20

PROPERTIES AND BIOLOGICAL ACTIVITY OF AN *APLYSIA* LECTIN. M.P. Wilson*, G.M. Carrow, and I.B. Levitan, Graduate Dept. of Biochem., Brandeis Univ., Waltham, MA 02254.

The plant lectin concanavalin A (Con A) has a variety of effects on *Aplysia* neurons, including enhancement of neurite outgrowth in primary cell culture (Lin and Levitan, Science, 237:648, 1987). It is possible that there are endogenous lectins in *Aplysia* with similar biological activities. The gonads from *Aplysia depilans* have been reported to contain a lectin (*Aplysia* gonad lectin, AGL) which has been purified and partially characterized (Gilboa-Garber et al, FEBS Letters, 181:267, 1985). We have purified a galactose-inhibitable AGL from *Aplysia californica* and found it to be a disulfide linked dimer with a native molecular weight of 75 KD. It is found exclusively in gonads and eggs and has N-terminal amino acid homology with the plant lectin phytohemagglutinin-E, another galactose-inhibitable lectin. In preliminary experiments, AGL appears to enhance neurite outgrowth from *Aplysia* neurons in primary culture. Using ligand blotting, we have found that ^{125}I-AGL binds to three low molecular weight proteins from nervous system membranes. These proteins, which comprise a subset of those to which ^{125}I-Con A binds, have been purified. The amino acid sequences of these putative AGL receptors may provide clues about the mechanism of action of AGL on *Aplysia* neurons.

CORTEX I

113.1

DOPAMINE RELEASE IN THE FRONTOPARIETAL CORTEX: EFFECTS OF NIGRAL NEUROKININ STIMULATION. M.S.Reid* M.Herrera-Marschitz*, M.Goiny*, U.Ungerstedt* (SPON: R.A.Yokel) Dept. of Pharmacology, Karolinska Institute, Stockholm, Sweden.

Using an in vivo microdialysis technique, we have found that dopamine is detected in perfusates collected from the frontoparietal (sensorimotor) cortex and striatum of rats (dopamine: 0.7 ± 0.2 nM, n=10; 11 ± 2 nM, n=10, respectively). The cortical and striatal dopamine levels were strongly reduced (0.07 ± 0.06 nM, n=4; 0.7 ± 0.4 nM, n=4, respectively) following deafferentation induced by mesencephalic 6-hydroxydopamine injection (Herrera-Marschitz M. et al. Neuro. Lett., 97:266-270, 1989). Presently, we have found that dopamine release in the cortex and striatum could be stimulated by an injection of d-Amphetamine (2.0 mg/kg s.c.) (466 ± 174%, n=4; 1168 ± 161%, n=4, respectively). Furthermore, intranigral injections of substance P (0.07 nmol/0.2 µL) could also stimulate dopamine release in the cortex and striatum (106 ± 41%, n=4; 32 ± 13%, n=4, respectively).

113.2

INFRARED DIC-VIDEO MICROSCOPY OF LIVING BRAIN SLICES
H.U. Dodt*, H. Pawelzik* and W. Zieglgänsberger (Spon. R. Gerstberger). Clinical Neuropharmacology, Max-Planck-Institute of Psychiatry, Munich, FRG

To analyse complex neuronal networks such as in the neocortex, a correlation of neuroanatomical and neurophysiological data is necessary. In the present study we tried to visualize single neurons in an in vitro slice preparation. We exploited a nearly neglected property of the brain slices, namely their translucence. For this purpose a newly developed brainslice chamber with a coverslip bottom was mounted on an inverted microscope (Zeiss Axiovert) equipped with differential interference contrast (DIC) optics. The slice could thus be inspected from below. The image was projected on the target of a video camera and displayed on a TV monitor. The Hamamatsu videosystem used allows analog and digital contrast enhancement and background subtraction. As the DIC optics provide optical sectioning, cells in brain slices of 200-250 µm could be visualized to a depth of 50-100 µm if illuminated with light of λ>700 nm. The contrast enhancement provided by the video system proved to be crucial for visualization of unstained single cells in slices of this thickness. The method was applied to hippocampus and neocortex of the rat. Intracellular and field potentials elicited by orthodromic stimulation were recorded with standard electrodes to show the viability of the preparation.

In the neocortex pyramidal and nonpyramidal neurons could be differentiated. Dendritic bundling of assemblies of pyramidal cells was clearly visible with medium magnification (20X-40X objectives). The use of a high power objective (63X, N.A. 1.4) allowed the visualization of varicosities and of nuclear heterochromatin. With appropriate use of a micromanipulator a multibarrel electrode could be positioned close to neurons of interest.

In combination with extra- and intracellular recordings the optical technique described here should allow the investigation of functional connections between morphologically identified cell types in the neocortex.

Supported by a grant from the BMFT to W.Z.

113.3

INDUCTION OF LTP BY ACTIVATION OF CORTICOCORTICAL INPUTS TO THE MOTOR CORTEX: INTRACELLULAR RECORDINGS AND LABELING.
A. Keller, A. Iriki* and H. Asanuma.
The Rockefeller University, New York, NY 10021

In an attempt to identify cortical circuits related to motor learning and memory, motor cortical neurons in which LTP is induced were studied intracellularly. Twelve young adult cats were anesthetized with Nembutal, and several microelectrodes implanted in the somatosensory cortex (area 2) and the association cortex (areas 5a & 5b) for intracortical microstimulation. Intracellular recordings were obtained from neurons in the motor cortex using a glass pipette filled with 5% biocytin. Stable recordings of short-latency EPSPs in response to stimulation through one of the electrodes were obtained from 19 cells; 10 cells received short-latency input from area 5, and 9 from area 2. Tetanic stimulation (200Hz; 20s; 30µA) was then delivered through the responsible electrode, and amplitude of EPSPs to test stimulation before, and after tetanic stimulation were compared. Following tetanic stimulation, mean EPSP amplitudes were significantly increased in 17 cells. The cells were then labeled by intracellular injection of biocytin; most of the labeled cells were pyramidal neurons, whereas only 3 neurons were sparsely-spiny, nonpyramidal cells. All labeled cells were in layers II or III of the motor cortex. The results suggest that LTP can be induced in the motor cortex by activation of various CC pathways, and in both pyramidal and nonpyramidal cells. Supported by NIH #NS-10705 & F32NS08626

113.4

LTP IN CAT MOTOR CORTEX CAN BE INDUCED BY ACTIVATION OF THALAMOCORTICAL AFFERENTS ONLY WHEN COACTIVATED WITH CORTICOCORTICAL INPUTS.
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The Rockefeller University, New York, NY 10021

We have previously shown that tetanic stimulation of the sensory cortex (SI) produces LTP in motor cortical neurons, whereas tetanization of the ventrolateral (VL) nucleus of the thalamus does not (Brain Res. 1987, 413:360). In the present study we investigated LTP in the cat motor cortex using intracellular recording techniques, under nembutal anesthesia. Motor cortical neurons in which short latency EPSPs were produced by stimulation of both SI and VL were first identified. Then, simultaneous tetanization was delivered to SI and VL. This procedure resulted in an increase in amplitudes of EPSPs produced by test VL stimulation. This associative LTP was produced only when the rise time of EPSPs evoked by stimulation of SI and VL was similar, suggesting that both afferents synapsed on motor cortical neurons in close proximity to each other. All of these neurons were located in layer III of the motor cortex. The results suggest that this associative LTP in the motor cortex constitutes a basis for the acquisition and retention of motor skills. Supported by NIH Grant NS 10705.

113.5

ORIGIN AND FUNCTION OF HORIZONTAL LAYER I AFFERENTS TO RAT SI NEOCORTEX.

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Horseradish peroxidase (HRP) applied topically to the pia of anesthetized rats penetrated layer I and was transported retrogradely to the posterior thalamus and 2 ipsilateral cortical areas: the frontal agranular motor area, and the second somatosensory area. Thus, principal targets of SI outputs are also the source of its layer I inputs. This resembles the laminar pattern of forward and backward projections in the sensory cortical systems of monkeys and cats (Pandya and Yeterian, 1985). Transport of the fluorescent tracer DiI from a site in layer I in fixed hemishpheres revealed, after 4 weeks, a dense plexus of layer I fibers radiating 2-4mm in all directions.

A vertical cut was made through slices *in vitro*, sparing a bridge containing only the horizontal pathway in layer I (HLI). HRP injected into layer I on one side of the bridge showed that the HLI pathway does not originate from the collaterals of local neurons. Stimulation of layer I on one side evoked an early current sink restricted to layer I on the other side, at distances > 1mm horizontally. These HLI-evoked responses were reversibly blocked by the glutaminergic antagonist kynurenic acid (2mM). HLI-evoked EPSPs of up to 50ms duration were recorded intracellularly from pyramidal neurons in layers III and V; cells were identified with biocytin injections. While some layer V pyramidal cells within 1mm did not respond, others with profuse distal tufts of apical dendrites were excited up to 4mm away.

Supported by NIH NS01271 and NS25983.

113.6

CORTICOMOTONEURONAL POSTSPIKE EFFECTS IN AVERAGES OF UNRECTIFIED EMG ACTIVITY. G.W. Botteron* and P.D. Cheney. Dept.. of Physiology, Univ. of Kansas Med. Ctr., Kansas City, KS 66103.

To examine the extent to which clear postspike effects (PSEs) can be detected in spike triggered averages of unrectified EMG activity and to test the possible utility of this method, we compared postspike effects in rectified and unrectified EMGs from 44 corticomotoneuroal (CM) cells yielding 293 cortical cell - target muscles pairs (CMPs). Of the 293 CMPs tested, 110 showed clear PSF in averages of rectified EMG activity. Of these 110, 49 also showed clear postspike effects in simultaneously computed averages of unrectified EMGs. Twenty four CMPs showed clear postspike suppression (PSS). Remarkably, twelve of these 24 CMPs yielding suppression also showed clear PSEs in averages of unrectified EMGs. Thirteen CMPs showed clear PSEs in averages of unrectified EMGs but failed to show clear effects in corresponding averges of rectified EMGs. Simulations were used to confirm these findings and to investigate various interpretations. In conclusion, we have shown that clear PSEs associated not only with facilitation but also suppression can be detected in averages unrectified EMG activity. (Supported by NSF grant BSN-8216608 and NIH grant NS25646.)

113.7

SYNAPTIC INTERACTIONS BETWEEN NEIGHBORING NEURONS IN THE PRIMATE MOTOR CORTEX M. Matsumura*, D.-F. Chen* and E.E. Fetz, Regional Primate Research Center and Dept. of Physiology and Biophysics, University of Washington, Seattle, WA, 98195 USA.

To analyze synaptic interactions between primate motor cortex neurons we calculated spike-triggered averages (STA) of intracellular membrane potentials in a monkey anesthetized with halothane. Intracellular recordings were made with a glass micropipette while extracellular spikes were recorded from neighboring cells with a carbon-fiber electrode attached to a pipette for ionophoretic application of glutamate. Neurons could be identified by antidromic responses to pyramidal tract stimulation, response to natural stimulation and relative cortical location. To date we obtained simultaneous intra- and extracellular recordings from 118 pairs of neurons separated by less than 0.5 mm. 83 of the STAs showed broad depolarizing potentials straddling the trigger, indicating that both neurons received common synaptic input (mean amplitude ± SD = 800 ± 570 μv). 34 pairs showed postsynaptic potentials with onsets after the trigger, indicating the presence of a serial connection (often in combination with common input); these PSPs had onsets of 1.3 ± 1.0 ms, and amplitudes of 260 ± 200 μv. The types of synaptic interactions and their magnitude could be modulated by different forms of stimulation. Activating the cells by application of glutamate and by stimulation of receptive fields both reduced the relative amplitude of common input potentials, compared to those obtained with spontaneous activity.

113.8

INTRACORTICAL CONNECTIVITY OF CAT MOTOR CORTEX EVALUATED BY SPIKE-TRIGGERED AVERAGING AND CROSS-CORRELATION. P. Zarzecki, D.C. Gordon and E.E. Fetz Dept of Physiol, Queen's Univ, Kingston, Ontario, Canada; Dept of Physiol and Biophys, and Regnl Primate Res Ctr, Univ Wash, Seattle, WA, USA.

We studied the synaptic connections between neighboring motor cortex neurons in anesthetized cats by recording simultaneously with two electrodes. Intracellular (IC) recordings were made from 31 neurons. Extracellular (EC) spikes recorded by the second electrode were used to trigger averages of membrane potentials of IC neurons. For most of the more than 60 pairs of neurons analyzed so far, spike-triggered averages showed broad psps straddling the EC trigger spike. This is evidence of common synaptic input to the EC and IC neurons. Psps delayed after the trigger were interpreted as serial synaptic connections. There were eight serial epsps, ranging from 30 to 300 μV in amplitude, and two ipsps.

To assess the efficacy of the epsps, the IC neurons were made to discharge by current injection, and the EC and IC discharges were cross-correlated. Only one of the epsps produced a clear correlogram peak, confirming that cross-correlation underestimates the extent of intracortical connectivity.

Supported by the MRC of Canada and the US NIH.

113.9

RELATIONSHIPS BETWEEN SIMULTANEOUSLY RECORDED MOTOR CORTEX NEURONS IN PRIMATES. E.M. Schmidt and J.S. McIntosh. NIH, NINDS, Lab. of Neural Control, Bethesda, MD 20892.

One method of studying the organization of the motor cortex is to examine the firing patterns of closely spaced neurons during a controlled task and also during small perturbations that reveal sensory input to the neurons under study.

A Rhesus monkey was trained to flex and extend the wrist in response to movement of a visual target on a video monitor. The monkey's hand was held in a molded form coupled to a torque motor which produced a simulated spring load. The monkey was required to match a wrist-movement coupled cursor to a target for a period of at least one second to receive a reward. Halfway through the random duration hold period, a 50 ms torque pulse was applied to perturb the wrist in either a flexion or extension direction. After training, under pentobarbital anesthesia and sterile operating conditions, a recording chamber was implanted over the arm area of the contralateral precentral cortex, along with a head restraint device.

Thus far, the firing patterns of 42 pairs of simultaneously recorded neurons (each pair recorded with the same electrode) have been examined. At least one neuron of the pair was related in some fashion to the task. In half of the pairs the relationship was either similar (S) (29%), or reciprocal (R) (21%). In the remaining half, the relationship was complex (36%) or not related (14%). The sensory input was examined in all of the cells and only 25 pairs responded. The responses were similar in 2 pairs of S-cells and 1 pair of R-cells. The responses were reciprocal in 3 pairs of S-cells and 2 pairs of R-cells. In the remaining 17 pairs the responses were complex.

From this preliminary sample of closely spaced cortical neurons, 86% were task related. However, less than 1/3 have similar task related activity and only 17% have similar sensory input.

113.10

NEURAL ENCODING OF MOVEMENT PARAMETERS IN AREA 5 IN THE MONKEY. P. Burbaud*, C. Doëgle*, Ch. Gross*, and B. Bioulac. Lab. de Neurophysiologie, CNRS UA 1200, Université de Bordeaux II, 146 rue Léo Saignat, 33076 Bordeaux (France).

Electrophysiological and clinical studies have shown that area 5 of the parietal cortex is implicated both in the integration of complex proprioceptive inputs from area 2 and in the control of arm movement (Hyvarinen J. Springer Verlag (Eds) NY, 1982). Using unit recording techniques in the monkey, two populations of neurons have been described: somaesthetic-like neurons and neurons which exibit early movement-related changes in spike frequency (Seal et al., Brain Res. 250, 229-243, 1982). In this work, we studied the neuronal encoding of cinematic parameters of the movement (onset, duration, velocity) by area 5 neurons. For neurons showing changes in neuronal activity prior to the movement, the **stimulus or movement**-related activity has been investigated using two different statistical methods. It is concluded that these populations of neurons with different functional properties might represent the cellular substract of the somatognosic and the somatopraxic function ascribed to area 5 of the associative parietal cortex.

114.1

INTRINSIC CONNECTIVITY OF MOTOR CORTEX IN OLD WORLD MONKEYS. George W. Huntley and Edward G. Jones. Department of Anatomy and Neurobiology, University of California, Irvine, CA. 92717.

The intrinsic connectivity of the forearm and digit representations in the precentral cortex (areas 4 and 6) of macaque monkeys was examined using intracortical microstimulation (ICMS) followed by small extracellular HRP injections at defined sites. Multiple representations of movement about a single joint were found in both areas. Representations involving distal musculature were located nearest the central and arcuate sulci; more proximal representations were located between the two sulci. Small HRP injections were then made into one of several sites in area 4 from which the same movement could be elicited by low-threshold ICMS. The depth of the injection site varied. Axonal trajectories were reconstructed with a camera lucida; sites of termination within the precentral gyrus were then correlated with cortical locations physiologically identified using ICMS. From all injection sites, long processes were traced which extended both anterior and posterior to the injection site, and gave rise to foci of terminations which in certain instances joined sites with similar representations. The dimensions of the foci and their distance from the injection site varied both with the size of the injection and with the layer in which the injection site was located.

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114.2

ORGANIZATION OF PRIMARY MOTOR CORTEX OF ADULT MOUSE AS STUDIED BY MICROSTIMULATION AND RETROGRADE TRACING TECHNIQUES. C.X. Li* and R.S. Waters (SPON: R.P. White). Dept. of Anatomy & Neurobiol., Univ. of Tenn., Memphis, Col. of Medicine, Memphis, TN 38163.

Our understanding of motor organization of rodent motor cortex has derived, in large part, from previous investigations in rat. In this report, we describe the organization of primary motor cortex in mouse and compare our findings with those previously reported in rat.

Experiments were carried out on 27 mice. Animals were anesthetized with Ketamine and placed in a modified stereotaxic apparatus. In 21 animals, the primary motor and somatosensory cortices were exposed and covered with warmed silicon oil. Microstimulation consisting of trains of cathodal pulses (0.2 ms duration; 300 Hz; 100-mA maximum intensity) was delivered to the cortex through tungsten-in-glass microelectrodes. Movement and threshold for activation were recorded. All electrode penetrations were marked on a high quality photograph of the cortical surface. Following mapping, the brain was removed, frozen, sectioned at 60 mm, and stained with cresyl violet. In 6 animals, HRP was injected into the cervical enlargement of the spinal cord. Animals were sacrificed 72 h after injection, brains removed, and tissue processed according to the method of Graham and Karnovsky (1966).

Microstimulation: The map of primary motor cortex consisted of a single representation of the body. Motor responses were activated from both agranular and granular cortex. The forelimb representation consisted of a flexor, extensor, and overlapping hindlimb zone.

HRP: Following injection of HRP, labeled neurons were observed in three discrete cortical regions. The most extensive labeling was found in frontal and parietal cortices where two foci of different density were interconnected by a more sparsely labeled area. Two smaller regions of labeling were also identified in lateral cortex.

The motor organization in mouse is similar to that described in rat and suggests a common plan of rodent motor organization. (Supported by NSF Grant BNS 85-16076)

114.3

TOPOGRAPHY OF CONNECTIONS TO PRIMARY MOTOR CORTEX(M-I) OF MACAQUES. M.F. Huerta and T.P. Pons. BioStructure and Function, Univ. Conn. Hlth. Ctr., Farmington, CT 06032; Lab. Neuropsych., NIMH, Bethesda, MD 20982.

The use of multiple tracers within a single animal is a powerful way to demonstrate topography of neural connections. In this study the topography of sources of inputs to physiologically defined regions of M-I were determined. In three monkeys (M. mulatta) intracortical microstimulation was used to define topographic sectors of M-I. Single injections of different tracers (fast blue, diamidino yellow, fluorogold, latex microspheres and/or tritiated amino acids) were then placed into separate movement representations of M-I. Thalamic connections include the oral ventral posterior lateral nucleus (VPLo) and the oral ventral lateral nucleus (VLo), with the facial and forelimb sector of M-I being connected with medial and lateral parts of both VPLo & VLo, respectively. The forelimb sector of M-I is connected with lateral postarcuate cortex, whereas the facial sector is connected with adjacent, more lateral, parts of this cortex; the facial sector of M-I is bilaterally connected with the rostral supplementary motor area, while the forelimb zone of M-I is bilaterally connected with immediately caudal parts of the same area. Connections with other structures were also observed. Our findings indicate that a topograghical pattern of connections with M-I is more obvious in some areas and nuclei than in others, which confirms and extends previous anatomical and physiological results.
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114.4

PREMOTOR AREAS ON THE MEDIAL WALL OF THE HEMISPHERE: CORTICOSPINAL PROJECTIONS TO CERVICAL AND LUMBOSACRAL CORD. S.Q. HE*, R.P. DUM and P.L. STRICK. VA Med. Ctr. and Depts. of Neurosurg. & Physiol., SUNY-HSC @ Syracuse, Syracuse NY, 13210.

We have recently presented evidence that the medial wall of the hemisphere in macaques contains 3 premotor areas which are somatotopically organized and project directly to the spinal cord. One of these regions is the supplementary motor area (SMA) in a medial portion of area 6. The 2 other regions lie in the cingulate sulcus- one in area 23c, termed the caudal cingulate motor area (CMAc) and the other in area 24c, termed the rostral cingulate motor area (CMAr).

We examined the somatotopic organization of these areas more directly by injecting different fluorescent tracers into lower cervical and lower lumbar segments of the spinal cord in macaques. We found that the regions in each of the premotor areas which project to lower cervical segments did not overlap substantially with the regions that project to lower lumbar segments. In fact, the overlap observed in the premotor areas was comparable to what is seen in the primary motor cortex of the same animals. Few double labeled neurons were observed and these were found in regions of overlap. Our results support the concept that the premotor areas on the medial wall of the hemisphere contain arm and leg representations which are as distinct as those in the primary motor cortex.
Support: VA Med. Res. Serv. and Rehab. R&D; USPHS 24328, 2957.

114.5

PREMOTOR AREAS ON THE MEDIAL WALL OF THE HEMISPHERE: INPUT FROM VENTROLATERAL THALAMUS. J.W. HOLSAPPLE* and P.L. STRICK (SPON: D.S. Hoffman). VA Med. Ctr. and Depts. of Neurosurg. & Physiol., SUNY-HSC @ Syracuse, Syracuse, NY 13210.

We have examined the inputs from the ventrolateral thalamus to the caudal cingulate motor area (CMAc) using retrograde transport of wheat germ agglutinin conjugated to horseradish peroxidase (WGA-HRP). Small injections of tracer were placed into portions of the CMAc lying on either the dorsal or ventral banks of the cingulate sulcus in 4 monkeys (macaca nemestrina). We have focused our analysis on regions of the CMAc which are reciprocally connected with the 'arm area' of the primary motor cortex.

Following tracer injections into the dorsal bank of the CMAc, rostral portions of VLo contained the largest number of labeled neurons in the ventrolateral thalamus. In contrast, following an injection into the ventral bank of the CMAc, the largest number of labeled neurons was found in portions of VLc. None of our injections resulted in substantial labeling of thalamic neurons in the anterior nuclear group. Therefore, it is unlikely that the CMAc is a part of 'limbic-related' cortex. On the other hand, VLo and VLc are major sites of termination for pallidal output in the ventrolateral thalamus. Thus, our results suggest that the CMAc is a cortical target for the 'motor' component of basal ganglia output.
Support: VA Med. Res. Service and USPHS 24328, 8439, 2957.

114.6

COMPARISON OF OUTPUT EFFECTS FROM SINGLE PULSE AND REPETITIVE INTRACORTICAL MICROSTIMULATION APPLIED TO MOTOR CORTEX IN PRIMATES. G.L. Widener and P.D. Cheney. University of Kansas Medical Center, Kansas City, KS 66103.

Although electrical stimulation has been widely used to identify the sign and strength of motor output from various brain areas, there has been no systematic comparison of different stimulation methods in terms of the motor output effects they yield. Therefore, we compared the effects of single pulse and repetitive intracortical microstimulation (ICMS) on muscle activity at 20 corticomotoneuronal (CM) cell sites in monkeys performing an alternating wrist movement task. CM cells were identified using spike triggered averaging (STA) of rectified emg activity (Fetz and Cheney J. Neurophysiol. 44: 751, 1980). Single pulse ICMS (S-ICMS) consisted of a series of biphasic pulses at a frequency of 10 to 20 Hz whereas repetitive ICMS (R-ICMS) consisted of a train of 10 biphasic pulses at a frequency of 330 Hz. At 10 μA, peak magnitues expressed as a percent of baseline for S-ICMS and R-ICMS were 56% and 265% respectively. Muscle field and muscles showing the greatest effect were used to characterize the pattern of output distribution. At 95% of sites, the muscles with the greatest effect from S-ICMS matched those with the greatest postspike effects (STA). For R-ICMS, the match was poorer (65%). Muscle field overlap between STA and S-ICMS was 70% compared to 53% for STA and R-ICMS. S-ICMS yielded lower thresholds for effects than R-ICMS at 7 sites, 5 sites had equal thresholds and at 2 sites thresholds were lowest for R-ICMS. We conclude that output effects obtained with either S-ICMS or R-ICMS are similar to those of the corresponding postspike effects but that S-ICMS is clearly superior in revealing basic features of underlying ouput organization. (Supported by NSF Grant BSN-8216608 and NIH Grant NS 25646)

114.7

AREAL DISTRIBUTION OF CORTICAL NEURONS PROJECTING TO THE BRAINSTEM AND SPINAL CORD IN RATS. X.-G. Li, S.L. Florence and J.H. Kaas. Vanderbilt Univ., Nashville, TN 37240.

The present experiments were designed to determine the locations of corticospinal and corticotrigeminal neurons relative to the pattern of cytochrome oxidase dense patches that reveal the somatotopic organization of S-I in rats. Unilateral injections of 1% WGA-HRP were made in the ventral horn of the cervical or lumbar enlargement or into one of the trigeminal nuclei of anesthetized adult rats. We conclude: (1) Corticofugal projections originate from neurons throughout granular S-I. Injections in the lumbar enlargement labeled neurons throughout the lower limb and caudal trunk representations, injections at the cervical cord labeled the upper limb and adjacent trunk representations, and medullary injections labeled representations of the head and face. (2) Other projection neurons formed a somatotopic pattern in the dysgranular zones inserted in and around granular S-I. (3) Large numbers of projection neurons in a somatotopic pattern in presumptive M-I were found following injections at all sites including the lumbar enlargment. (4) Projection neurons were found lateral to S-I in S-II and/or the parietal ventral area (PV) and in perirhinal cortex. (5) Other projection neurons were found in the frontal cortex rostral to M-I. The results indicate that at least six cortical regions contribute to the corticospinal-trigeminal systems in rats. (Supported by NS16446.)

114.8

THE EFFECT OF LIMB POSITION ON ELECTROMYOGRAPHIC ACTIVITY EVOKED BY ELECTRICAL STIMULATION IN RAT MOTOR CORTEX

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We have found that motor cortex (MI) representation patterns are modified rapidly following nerve transection (Sanes et al., '88). Alterations in afferent input to MI may serve as a cue for MI reorganization in these cases. To begin to investigate the role of afferent feedback on MI organization, we examined the effect of different elbow positions on the amount of muscle activity (EMG) evoked in the biceps and triceps muscles following electrical stimulation in MI. In ketamine anesthetized rats biceps and triceps EMG evoked by intracortical electrical stimulation (30 msec pulse trains, 333 Hz, 10-60 μA) was recorded after the elbow was fixed in an extended or flexed position. The amount of EMG (recorded through percutaneously inserted intramuscular wires) was measured as the integral of the average of 10 cortical stimuli at 1 and 2x threshold, and at 45 μA. A total of 64 sites yielding either forelimb or whisker movements was sequentially tested with the elbow placed in extended, flexed and again in extended position in 4 rats. The biceps EMG evoked from MI showed a consistent relationship to elbow position. In the extended position, the amount of biceps activity recorded at each site was, on average, more than 3X larger than that recorded in flexion. The average threshold to evoke biceps EMG in the extended position was about 40% (16.5±1.2 μA) lower than in the flexed position. The amount of EMG and EMG thresholds were near original levels when the limb was again returned to the extended position. The generally absent or weak triceps response made it impossible to evaluate position effects in this muscle. Stimulation in the MI vibrissa representation showed no significant effect of elbow position on whisker movement thresholds. At some sites the biceps threshold was lower than the whisker threshold when the elbow was at extension, but not when flexed. These results show that limb position can affect the amount of muscle activity evoked by electrical stimulation and suggest that afferent feedback of muscle length may modify the effect of MI output. They further demonstrate that limb position must be rigorously controlled when constructing maps of MI based on electrical stimulation techniques. (Supported by NS 25074).

114.9

ANATOMICAL SUBSTRATES FOR SHIFTING SOMATOTOPY IN RAT MOTOR CORTEX. S.A. Lee*, S.J. Bonasera*, D.L. O'Donoghue and D.R. Humphrey. Laboratory of Neurophysiology, Emory University, Atlanta, Ga., 30322.

Studies have shown that amputation of the forelimb (FL) in the neonatal rat produces an expansion of vibrissae (VB) motor areas into what would have been FL motor cortex (Donoghue and Sanes, PNAS, 84: 1123). One factor that might contribute to this finding is an overlap in the distributions of cortical cells projecting to bulbar VB and spinal FL motor control nuclei, an overlap which might not be revealed by microstimulation maps that emphasize lowest threshold responses. To study this possibility, we first mapped the FL and VB control areas in adult rats. Fluorogold dye was then injected into the reticular formation where the corticobulbar fibers that control the VB terminate, and Fast Blue dye was placed into the grey matter of the cervical (C5) spinal cord. Marking lesions allowed us to correlate the cortical stimulation map with the distributions of labeled neurons observed histologically in the same animals. Both types of labeled cells overlapped across the stimulation defined boundary between the VB and FL motor areas. Thus, an anatomical substrate exists even in normal animals that might account for some of the motor cortex 'plasticity' observed after neonatal manipulation of the controlled motor periphery. (Supported by NIH Grant NS 20146).

114.10

SIMILARITIES AND DIFFERENCES IN THE LAMINAR ORGANIZATION OF CORTICOCORTICAL CONNECTIONS IN RAT AS COMPARED TO MONKEY. T. W. Deacon, D.-W. Wang* and A. Carpenter*. Biological Anthropology, Harvard University, Cambridge, MA 02138.

In macaque and squirrel monkey neocortex there are systematic laminar patterns distinguishing different classes of corticocortical connections. Reciprocal connections between many cortical areas exhibit an asymmetric pattern of termination in which one projection's terminations predominate in middle cortical layers iiic-iv originating from cells that predominate in layer iii whereas terminations of its reciprocal counterpart predominate in layers i and vi originating from cells predominating in upper layer v. We have investigated corresponding reciprocal patterns in rat neocortex using the anterograde-retrograde transport of WGA-HRP and the anterograde transport of tritiated amino acids. We find similarities and striking differences with respect to typical monkey patterns. For example, similarities include layer vi and layer i termination patterns of frontal motor projections to parietal areas and from secondary to primary areas in all modalities. However, in contrast to monkey findings, projections from parietal to frontal areas terminate predominantly in layer i and to a lesser extent in ii and upper layer iii but not middle layers, and primary sensory areas project to adjacent secondary areas into upper layer v and layer iv but also apparently to layer i. Retrogradely labeled cells of origin for most corticocortical projections nearly always predominate in upper layer v and to a lesser extent layer iii, however, labeled cells are also observed in layer ii in most areas. Layer ii cells are not thought to have interareal connections in monkey brains. These data indicate that corticocortical laminar connection patterns are not simply generalizable across mammalian orders, but that some features may be common and others derived independently.

114.11

Pelvic Floor Muscles. Definition of Cortical Output by Intracortical Microstimulation. D. Filipini* and B. Dubrovsky. McGill University Montreal, Quebec H3A1A1.

We investigated the output organization of pelvic floor muscles control in the primary motor cortex of cats under nembuthal anesthetia (30 mg/kg i.p.). Emg activity of the external anal sphincter (EAS) and levator ani (LA) muscles was recorded and taken as the index of response to cortical stimulation.

Initially anodal surface stimulation with a silver ball was given to determine the locus of interest (tail and lower axial muscles). Secondly focal depth stimulation by means of low current intracortical microstimulation (ICMS) was used to activate individual muscular groups. Glass-coated tungsten microelectrodes with exposed tips of 10-20 μm were used to deliver trains of cathodal current. Trains of 40 ms duration at 400 Hz with pulses of 0.2 ms and 10-50 μA intensities were used to elicit muscular responses. Activation of LA and EAS was obtained when electrodes were positioned with a posterior angulation of 30° in the depth of an area extending over the middle part of the posterior sigmoid gyrus 4.9 mm posterior to the cruciate dimple (fig.). Mirror area in the other hemisphere elicited same responses. Latencies varied between 12 and 25 ms following the train. Lowest thresholds for LA (7±3 μA) and EAS (9±2 μA) were obtained at 5mm deep from the site of penetration. Exploration of areas in the vicinity produced contraction of tail and hip muscles.

Histological reconstruction of lesions delivered at different sites of activation corresponded to the middle part of the dorsal bank of the cruciate sulcus (fig.) which is hidden from the surface. Our results show that:

1)LA and EAS have bilateral representation in primary motor cortex of the cat

2)neuronal populations controlling both muscles are specifically placed and partly enclosed by zones controlling tail and proximal hindlimb musculature.





*Supported by CONICET-Agentina

114.12

PATTERNS OF CONNECTIVITY IN RAT ORBITAL CORTEX. R.L. Reep, A. Wallach*, V. King* and J.V. Corwin. Depts. of Neuroscience and Physiological Sciences, J-144, Univ. Florida, Gainesville, FL 32610; Dept. of Psychology, Univ. New Orleans-Lakefront, New Orleans, LA 70148.

Previously we have demonstrated bilateral reciprocal connections between ventrolateral orbital cortex (VLO) and medial agranular cortex (AGm). Unilateral lesions of these cortical areas produce multimodal neglect. In order to further elucidate the neuronal circuitry pertinent to neglect, we have studied the connections of VLO, as well as the adjacent medial (MO) and lateral (LO) orbital areas, using retrograde and anterograde tracers.

Most of MO has cortical connections with anterior cingulate cortex bilaterally, insular cortex, and the ventral subiculum. Ventral MO and VLO have reciprocal connections with AGm bilaterally, posterior parietal cortex, and area Oc2MM of visual association cortex. Cortical connections of LO are limited to insular cortex. All three orbital fields have commissural connections with the homotopical area contralaterally.

Thalamic afferents to orbital cortex involve primarily the gelatinosus, ventromedial, and mediodorsal (MD) nuclei. Nucleus gelatinosus neurons projecting to VLO are located in the central portion of the nucleus, while those to MO and LO are arranged around the periphery. A heavy input to MO and LO arises from the ventromedial nucleus, but VLO receives none. All three orbital fields receive mediodorsal nucleus afferents, from partially overlapping portions of central, dorsolateral and posterior MD.

Amygdala connections are extensive with MO, and involve the basolateral, basomedial, cortical and amygdalo-hippocampal areas. VLO has moderate connections with the basolateral nucleus; LO with the anterior basolateral and lateral nuclei. Caudate projections are topographically arranged.

Supported by UF College of Veterinary Medicine, NIH-BRSG program.

114.13

FORELIMB FLEXION EVOKED BY MOTOR CORTEX OR PYRAMID STIMULATION IN RATS IS DUE TO THE LARGEST PYRAMIDAL TRACT AXONS. C.A.Chapman* and J.S.Yeomans (SPON:P.Li). Dept. Psychology, Univ. Toronto, Canada, M5S 1A1.

Double pulses were delivered to the motor cortex forelimb area, or ipsilateral pyramid, and the maximum flexion of the contralateral forelimb measured at the wrist. The movements increased as the C-T intervals increased from 0.4-1.0 ms in pyramid sites, and increased at C-T intervals from 0.6-2.0 ms in cortical sites, suggesting longer refractory periods for cortical substrates. In cortical sites, as the C-T interval was increased from 4-20 ms, the movements decreased gradually to the single-pulse level, due to decreasing temporal summation. When C pulses were delivered to the cortex and T pulses to the pyramid, the movements increased at C-T intervals from 0.8-2.5 ms. When the C pulses were delivered to the pyramid and the T pulses to the cortex, the movements increased at slightly longer C-T intervals, due to the longer refractory periods in cortex, but estimated conduction times (0.5-1.0 ms) between the two sites were similar. This suggests that collision occurred between orthodromic and antidromic action potentials in the pyramidal tract axons responsible for the limb flexion. The large collision-like effect suggests that the largest pyramidal tract axons (conduction velocities of roughly 13-30 m/s) are responsible for most of the forelimb flexion in pentobarbital anesthetized rats.

114.14

UNILATERAL AND BILATERAL EFFECTS OF UNILATERAL FRONTAL AGRANULAR CORTEX LESIONS ON VISUAL REACTION TIME PERFORMANCE, PAW USE AND SOMATO-SENSORY NEGLECT IN THE RAT. V.J.Brown* and T.W.Robbins, Dept. of Exp. Psychology, Cambridge University, Downing Street, Cambridge, CB2 3EB, U.K.

The effects of unilateral aspirative lesions of medial agranular frontal cortex (AGm) were compared in three separate tests of sensorimotor integration. In an automated visual reaction time task with low stimulus response compatibility rats were trained to respond to brief visual stimuli presented unpredictably to either side of the head by making a nose poke response on the side opposite to that on which the stimulus occurred. Spatial response bias and reaction time to the visual stimuli were recorded (Carli et al., *Nature*, 313:679, 1985). The same rats were also trained to reach for food pellets (Whishaw et al., *Brain*, 109:805, 1986) and were tested for their removal of sticky patches applied bilaterally to the radial aspect of their forepaws (Schallert et al., *Pharmacol. Biochem. Behav.*, 18:753, 1983).

The animals showed a postoperative bias to the ipsilateral side on all tests. This had stabilized by 1 month, leaving deficits apparent in paw use and on the reaction time task. This latter task was found to be acutely sensitive to deficits following the lesion, with long lasting effects (3+ mths) on spatial response bias. In contrast to the ipsilateral side bias, the lesioned rats showed lengthened reaction times bilaterally, unlike previously reported effects of either unilateral striatal or unilateral AGm lesions in rats.

As the AGm lesion includes the region thought to be homologous to the supplementary motor area (SMA) in man, this bilateral effect on reaction time may parallel similar bilateral effects on motor performance of unilateral lesions of the SMA (Goldberg, *Behav. Brain Sci.*, 8:567, 1985). The effects on spatial response bias resemble those seen following unilateral striatal lesions and are consistent with the concept that cortico-striatal loops mediate behavioral functions of these related areas.

BASAL GANGLIA AND THALAMUS I

115.1

DYNAMIC NETWORKS DERIVED FROM SINGLE NEURONAL RECORDINGS IN PERFORMING MONKEYS. Erwin B. Montgomery, Jr., Department of Neurology and Neurological Surgery (Neurology), Washington University School of Medicine, St. Louis, MO. 63110.

A method described previously derives network models of neuronal interactions (patterns of connection strengths) from single neuron recordings in performing monkeys (Montgomery, Abs. of the 11th meeting of the Euro. Neurosci. Assn., 1988). A series of simultaneous equations are constructed relating the discharge frequencies of a target neuron (F_t) and driver neurons (f_1 through f_n) of the form

$$F_t = a_1 * f_1 + \ldots + a_n * f_n + c$$

where a_1 through a_n reflect connection strengths and c, the target neuron excitability. Since the discharge frequencies of the target and driver neurons are experimentally determined, the target neuron excitability and connection strengths can be mathematically determined.

One implication is the "connect period" where one neuron may influence another for a limited time period. Furthermore, multiple "connect periods" may be strung together to produce a dynamic network which determines behavior. This study determined connection strengths between putamen neurons in overlapping segments through the course of a behavior in performing monkeys.

Results show connection strengths vary through the time course of behavior suggesting a dynamic network. Thus, the driver neuron contribution to a target neuron is the product of discharge frequency and connection strength which may vary with connections to different target neurons and within the time course of the behavior. A dynamic network may provide a syntactical structure which some have argued to be missing in current concepts of network function (Fodor and Physlysn, Cognition 28:2849-2857, 1988).

115.2

THE ACTIVITY OF STRIATAL NEURONS CHANGES DURING THE INITIATION OF HAND MOVEMENTS MADE IN RESPONSE TO VISUAL AND VIBRATORY CUES. T.W. Gardiner and R. J. Nelson, Dept. of Anatomy and Neurobiology, College of Medicine, Univ. of Tennessee, Memphis, 875 Monroe Ave., Memphis, TN 38163.

The activity of striatal neurons was recorded while Rhesus monkeys made identical wrist flexion and extension movements in response to either vibratory or visual cues. Animals were rewarded with fruit juice for correct performance during these self-paced tasks. Vibratory cues consisted of palmar vibration (low-amplitude sine wave at 27, 57 or 127Hz) delivered to the control handle of the apparatus. Visual cues consisted of changes in the lamp display that also signaled the current wrist position. Visual and vibratory-cued trials were presented randomly in blocks of ten trials with the same movement being required in response to each of the sensory cues. Of the 108 presumptive striatal neurons recorded, 83 were task-related. Of these, 19 (23%) responded to the onset of sensory stimuli: 10 to vibratory stimuli, 3 to visual stimuli and 6 to both. Seven of 15 vibratory responsive neurons were tested and did not respond to the stimuli when the monkeys were instructed not to move after stimulus onset. Sixty-four neurons showed pre-movement activity changes. Whereas 12/64 showed late changes in activity, 52/64 (~80%) exhibited early activity changes (>80msec) before movement onset. Of the movement-related neurons, 24/64 (~37%) showed directional bias and responded differentially depending upon the direction of the subsequent movement.

These findings suggest that some vibratory responsive striatal neurons are conditionally responsive to these stimuli when they trigger movement. In addition, the significant percentage of movement-related cells showing activity changes that preceded movement suggests that the striatum may play a role in the initiation of planned motor behaviors as well as its more accepted role in the execution of the movements themselves. This study met NIH guidelines for animal utilization.

Support by NIH Program Project Grant NS26473.

115.3

A COMPARISON BETWEEN PRIMATE CAUDATE NUCLEUS AND PUTAMEN SINGLE UNIT ACTIVITY IN A PRECUED REACHING TASK. D.Jaeger, S.Gilman and J.W. Aldridge, Dept. of Neurology, Univ. of Michigan, Ann Arbor, MI 48104

There is growing evidence that the caudate nucleus (Cd) is concerned with the cognitive aspects of a motor task whereas the Putamen (Pu) is concerned with movement execution. To test this hypothesis, single unit activity was recorded from the Cd and Pu of the left hemisphere of a monkey during a task which required contralateral or ipsilateral reaching movements to touch one of a set of 4 target knobs on each side. Stimulus lights, mounted on each target knob, cued the target for each trial. In addition, a subset of stimulus lights was flashed as a precue for 2 s before the go-cue. The location of the flashing lights precued partial to complete information about the target position of the next movement. Of 149 Pu and 100 Cd units analyzed with respect to contralateral target reaching movements, 58% of Pu and 19% of Cd units were activated during the movement. The average spike rate increase in Pu responses was twice that of Cd and better aligned to movement onset. Pu responses were typically dependent on movement amplitude and direction. The onset of 46% of these responses was simultaneous with movement onset while 44% of the responses had an early component preceding movement onset by as much as 1 second. Of 39 Pu and 22 Cd units analyzed with respect to ipsilateral target reaching movements 38% of Pu and 14% of Cd units were activated during movement execution. Unit responses in Cd were typically related to the presentation of sensory cues and the response onset preceded the cue onset in most cases. For 24% of Cd and 7% of Pu units such an anticipatory cue response was found with respect to the precue, which was a cue without an immediate motor demand to the animal. The results of this study support the hypothesis that Pu activity is tightly linked to limb movement and that movement control is organized bilaterally to some extent. Early Pu responses indicate that this structure is involved in movement planning as well as execution. Cd unit activity on the other hand is rarely linked to limb movement and the predominance of anticipatory cue responses indicates that it may be related to attentional switching in predictable sequential tasks and to the processing of cognitive task requirements. NIH grants NS19613, NS07222.

115.4

THE TIMING OF CHANGES IN PALLIDAL DISCHARGE IS CORRELATED WITH MOVEMENT TIME. M. Anderson and R.S. Turner*, Depts. of Physiol. and Biophys. and Rehab. Med. and Reg. Primate Res. Center, Univ. of Washington, Seattle, WA. 98195

The discharge of many neurons in the globus pallidus of monkeys changes during an arm-reaching movement, and when the normal pallidal activity is interrupted, the movement time (MT) is prolonged. Does the discharge of these neurons at particular times predict or reflect movement time?

This question was addressed by a trial-by-trial analysis of the discharge of 40 pallidal neurons whose discharge was task-related and could also be modified by "passive" movement around one or more joints of the arm. The linear correlation between the number of spikes and the movement time was evaluated for each 50 ms bin during before and after the movement. For 35 of the 40 cells, there was a correlation at the $p<.05$ level between the number of spikes and the MT for one or more 50 ms bins, and for 19 of these, the correlation was significant at the .001 level. The pattern of correlations across time showed that for only 3 cells was the correlation significant at the time of the peak change in firing. Instead, significant correlations were usually positive during the onset of a period of decreased firing or on the falling phase of a burst and negative at the end of a decrease in firing. Significant changes usually occurred near the end of the movement.

These data indicate that it is the timing of changes in pallidal activity that is most strongly related to the duration of the movement.

NS 15017, GM 07108, and RR 00166 and by Dept. of Ed. grant H133B80081.

115.5

ACTIVITY OF PALLIDAL NEURONS DURING REACHING MOVEMENTS UNDER THREE CONDITIONS OF SPATIAL AND TEMPORAL CUING. R.S. TURNER* and M.E. ANDERSON (SPON: A. RUSSELL)

Depts. of Physiol. and Biophys., Rehab. Med., and Reg. Primate Res. Center, Univ. of Washington, Seattle, WA 98195

Clinical evidence and the study of SNr cells during eye movements suggest that the BG are especially involved in movements made in the absence of immediate sensory cues, e.g. visually precued or self triggered movements. We would expect to find larger changes in cell activity, and/or more GP cells related to a particular movement when it is performed under precued or self triggered conditions. We have addressed this hypothesis by recording GP cell activity in monkeys performing reaching movements under 3 task conditions. The monkey moves its hand across a slightly inclined work surface from a central start position to one target LED in an array offering 8 possible target directions and 3 possible distances from center. Sensory targeted and triggered: A target LED is illuminated and trigger tone sounds simultaneously. The monkey is rewarded to move to the target within 0.8 s. Visually precued/Sensory triggered: A target lights briefly, up to 1.5 s. before the trigger tone sounds. The monkey must wait until the trigger tone sounds before moving to the then invisible target position. Visually targeted/Self triggered: One target LED is continuously lit during a block of trials, but no trigger tone sounds. The monkey must hold at the start position for at least 1.5 s. before moving to the target position. Among the 203 GP cells studied under more than one condition, 57 could be driven by manipulation of the contralateral arm. Preliminary analysis shows that of these 'arm' cells, 11 had larger responses during precued trials, and 11 had larger responses during sensory targeted and triggered trials. There is thus no initial support for the simple interpretation of the above stated hypothesis.

PHS grants NS15017, GM07108 & RR00166

115.6

PATTERNS OF SENSORIMOTOR INTEGRATION IN THE PRIMATE NEOSTRIATUM: PRIMARY SOMATOSENSORY CORTEX (SC) AND MOTOR CORTEX (MC) PROJECT TO COEXTENSIVE TERRITORIES IN THE PUTAMEN. M. Fotuhi, V.E. Koliatsos, G.E. Alexander, and M.R. DeLong. Departments of Neuroscience and Neurology, The Johns Hopkins University, School of Medicine, Baltimore, MD, 21205.

Previous findings in our laboratory (Alexander et al., Soc. Neurosci. Abstr. 14:720, 1988) have indicated that the arm areas of the supplementary motor, arcuate premotor, and motor cortex project to adjacent but non-overlapping regions of the putamen. In combination, these three motor and premotor areas appear to include most of the arm territories of the putamen.

To determine the relationship between the terminal fields of SC and MC projections to the putamen, we carried out a double anterograde tracing study in two monkeys. The arm regions of SC and MC of one hemisphere received multiple small injections of wheat germ agglutinin-horseradish peroxidase and tritiated amino acids, respectively. Terminal fields labelled from both sets of injections extended over long rostrocaudal domains of the putamen in a discontinuous distribution. Patches of the MC terminals overlapped extensively with patches containing terminals from the sensory SC. In contrast to the segregation of terminal fields from the precentral motor and premotor areas, the present data indicate that selected pre-and post-rolandic areas converge upon the same region of the neostriatum.

115.7

STIMULUS-LOCKED CHANGES IN NEURONAL ACTIVITY IN THE SUPPLEMENTARY MOTOR AREA (SMA), MOTOR CORTEX (MC), AND PUTAMEN OF THE MONKEY. M.D. Crutcher and G.E. Alexander. Dept. of Neurology, Johns Hopkins Univ. Sch. of Med., Baltimore, MD. 21205.

We have previously described neuronal activity in the SMA, MC, and putamen related to 'high level' aspects of the preparation and execution of visually guided limb movements. These studies used rhesus monkeys trained to perform a set of visually guided, delayed step-tracking tasks. During the course of those experiments, we noticed cells whose changes in activity were more closely related to the time of occurrence of the visual stimulus that triggered the movement than to the time of onset of the movement. To examine this more closely, a correlational analysis was used to determine whether changes in single-cell activity in the SMA, MC and putamen were stimulus- or movement-locked. Approximately one-third of the onsets of movement-related activity were stimulus-locked in all three motor areas (SMA, 38% (N=123); MC, 28% (N=166); Putamen, 31% (N=231)). Moreover, in each of these areas approximately one-half of the cells with preparatory activity showed stimulus-locked offsets of activity (SMA, 57% (N=67); MC, 43% (N=40); Putamen, 51% (N=53)). During the preparation and execution of visually guided limb movements the brain must translate information concerning the location of the target into appropriate patterns of muscle activation. These stimulus-locked changes in neuronal activity may represent the earliest stages of this stimulus/response transformation. Alternatively, they can be viewed as neural correlates of the initiation or 'triggering' of these highly overlearned limb movements. In either case, the fact that stimulus-locked changes in neuronal activity were seen often, and with comparable frequency, in all three structures suggests that relatively 'high' levels of motor processing are distributed across multiple components of the motor system. (Supported by NIH grant NS - 17678).

115.8

SERIAL VS. PARALLEL PROCESSING WITHIN THE PRIMATE BASAL GANGLIA-THALAMOCORTICAL 'MOTOR CIRCUIT'. G.E. Alexander and M.D. Crutcher. Dept. of Neurology, Johns Hopkins Univ. Sch. Med., Baltimore, MD 21205.

The problem of controlling visually guided limb movements can be divided analytically into sequential levels of processing that successively determine: a) target location; b) limb trajectory; c) joint kinematics; d) joint torques; and e) muscle activations. Whether the motor system employs such a serial approach, rather than parallel processing, is not known. This issue was addressed in the present study. Monkeys were trained to perform a set of motor tasks that dissociated behavioral variables related to three of the analytically defined motor processing levels (a,c,e). We recorded task-related neuronal activity in the supplementary motor area (SMA, 222 cells), motor cortex (MC, 202 cells) and putamen (317 cells). All are components of the basal ganglia-thalamocortical 'motor circuit'. We found that multiple levels of processing ('target', 'joint' and 'muscle') were represented by neuronal activity within the 'motor circuit', which argues against a direct mapping of target coordinates onto muscle activation patterns. Target-level representations were more prevalent than joint-level representations during the preparation for movement, while the pattern was reversed during movement execution. This suggests that 'higher' levels of processing are emphasized during movement *preparation*, with a shift toward 'lower' levels during *execution*. That multiple levels of processing were represented in all three structures indicates that motor processing is spatially distributed and not strictly localized or hierarchically segregated. The relative timing of onsets and offsets of task-related activity suggested a cascade of influences leading from SMA and MC to putamen. Nevertheless, both within and across structures, there was strong evidence that multiple levels of motor processing proceed concurrently, that is, *in parallel*, during both the preparation and execution of visually guided arm movements. (Supported by NIH grant NS 17678.)

115.9

SOMATOTOPY IN RAT STRIATUM MAPPED WITH ^{14}C DEOXYGLUCOSE AUTORADIOGRAPHY. L.L. Brown, S. Salinas and S.M. Feldman. Dept. of Neurology, Albert Einstein Coll. of Med. Bronx, NY 10461.

Primary sensory (SI) corticostriate projection patterns in cat show a patchy somatotopic organization in the dorsolateral striatum (Malach & Graybiel, 1986). Is somatotopic information also segregated in the rat striatum and can it be mapped functionally? Twelve adult rats were prepared for quantitative ^{14}C deoxyglucose studies and stroked with a nylon bristle which exerted a 2.5 gm force on either the forelimb, trunk or hindlimb. Serial sections were collected throughout the striatum. Autoradiograms were analyzed with an image analysis system which produced isodensity maps of the ten highest grey levels above threshold. Distinct patches were observed in the dorsolateral striatum at several anterior-posterior levels, unlike controls and the contralateral striatum. In one area, patches were observed regardless of body region stimulated. In other regions, forelimb patches were ventral and lateral to trunk and hindlimb patches. The data suggest that somatotopic information is both segregated and integrated by the striatum in the rat.

115.10

FORELIMB-CORRELATED AND VIBRISSAE-CORRELATED SINGLE UNIT ACTIVITY IN THE DORSOLATERAL STRIATUM OF THE AWAKE, FREELY MOVING RAT. R.M. Carelli, J. Crescitelli*, P. Schriever* and M.O. West. Dept. Psychology, Rutgers Univ., New Brunswick, NJ 08903.

We have previously demonstrated somatic representations in the dorsolateral striatum of the rat (Neurosci.Abstr. 14: 76, 1989). The objective of the present study was to further evaluate sensory characteristics of two types of striatal units: forelimb-correlated and vibrissae-correlated neurons. Long-Evans male rats (300g) were chronically implanted with a detachable microdrive for recording in the lateral striatum (+2.0 to -2.0 mm A-P, 3.5 to 4.5 mm M-L, and 3.0 to 6.0 mm D-V from Bregma). The first study examined the response latency of forelimb-correlated units. Electrical pulses (0.1-1.0 mA, 0.2 msec) were delivered randomly (1-10 sec intervals) through a bipolar stainless steel wire routed subcutaneously to the contralateral forelimb. Preliminary results showed a mean latency to striatal discharge of 10.5 msec (n=6). Forelimb-correlated SI cortical units in the same recording track exhibited a mean latency of 6.0 msec (n=3). In the second study, sensorimotor aspects of vibrissae-correlated units were examined. Water-deprived rats were trained to obtain water reinforcement by making a nose-poke through a notch containing a photocell beam, which involved vibrissae contact with the notch. Computer-synchronized videotape recordings (60 frames/sec) allowed the construction of peri-event histograms showing correlations between neural and behavioral events. Preliminary results suggest that vibrissae-correlated units fire rhythmically, in phase with vibrissae beating during voluntary movement (4-6 cycles/sec). Ongoing studies are examining the relationship between these rhythmic discharges and sensory discharges related to notch contact. Supported by NSF BNS-8708523, DA 04551 and PHS RR 07058-21.

115.11

PUTAMEN NEURONAL ACTIVITY ASSOCIATED WITH WRIST MOVEMENT UNDER DIFFERENT BEHAVIORAL CONDITIONS. Samuel L. Liles, Dept. of Physiology, Louisiana State Univ. Med. Ctr., New Orleans, LA 70119.

Two monkeys were trained to extend or flex the wrist in a horizontal arc in response to visual cues. A panel containing two rows of light-emitting diodes (LEDs), 32 LEDs in each row, was located in front of the monkey. The lower row provided a visual display related to wrist position, while visuo-spatial targets were presented in the upper row. Three different behavioral conditions were tested: (i) simultaneous presentation of the "go" stimulus and the visuo-spatial target; (ii) presentation of the visuo-spatial target as a prestimulus cue which remained lighted after the "go" stimulus to provide a visible target for movement; and (iii) presentation of the prestimulus cue briefly (extinguished 1-3 sec prior to the "go" stimulus), so that subsequent wrist movement was made to a "remembered" target position. Electromyographic recordings were made from 14 different muscles to verify that the patterns of muscle activity were comparable during wrist movement under these three different behavioral conditions.

The major finding is that 31 neurons in a sample of 72 exhibited enhanced tonic or phasic discharge rates during trials involving movement to a remembered target position compared to discharge rates in which the target was visible. (Supported by NIH Grant NS-22275).

115.12

TRAINED CIRCLING BEHAVIOR INDUCES FIRING RATE CHANGES IN STRIATAL AND VENTROMEDIAL THALAMIC NEURONS IN FREELY MOVING RATS. P. Patino *, M. Garcia-Munoz and C. R. Freed. SPON: (Evelyn Kriek). Depts. of Med. and Pharm., Univ. of Colorado Health Sci. Ctr., Denver, Co, 80262.

The unilateral lesion of the dopaminergic nigrostriatal pathway causes postural asymmetry and dopamine agonist induced circling behavior. Our laboratory has reported that forced locomotion increases the firing rate of nigral dopamine cells. We have now studied firing changes in the medial lateral or ventral lateral striatum (ST) and ventromedial thalamus (VMT) during trained circular locomotion. VMT receives afferents from substantia nigra pars reticulata and constitutes one of the main output pathways for this circling behavior. Rats were trained to run on a circular disk treadmill for water reward and then implanted with Parylene C coated stainless steel wires for single unit chronic recording in ST or VMT. Some units were identified antidromically in ST from SNR. Basal firing rates in ST ranged from 0.2 to 2.5 hz and respond to changes in motor activity. Many (50%) cells in ST increased their firing rates to contralateral locomotion, a few ipsilateral (2%) and a few to bilateral (25%) while others did not change firing with movement (23%). In comparison to the ST, changes in firing rate in VMT are smaller, bilateral in more cases (30%), and there are more non movement related units (26%). We have seen movement related changes in firing in ST and VMT some of which are lateralized.

115.13

ROLE OF VISUAL INFORMATION IN MONKEY PUTAMEN DURING OPERANT FEEDING MOVEMENT. M.Fukuda, T.Ono, H.Nishijo* and E.Tabuchi*, Dept. Physiol., Fac. Med., Toyama Med. and Pharmaceu. Univ., Sugitani, Toyama 930-01, Japan

We recorded single unit activity from the monkey putamen to study processing of visual information during operant feeding behavior. We divided the behavior sequence into four phases; visual, bar press, procurement, and ingestion. Of 489 neurons recorded, 119 (24.3%) responded in at least one of the four phases. Of these, 51 (10.4%) responded during the visual phase, 62 (12.7%) during bar press, 26 (5.3%) during procurement and 27 (5.5%) during ingestion. Most of the neurons that responded during bar press and procurement were probably related to movement, extension and flexion of the hand. Some neurons responded to visual stimuli, food and/or nonfood objects. For example, some neurons responded strongly at the sight of preferred food, but weakly at the sight of non-preferred food, and did not respond to nonfood. These responses depended on special situations of motor demands such as waiting, or preparing for movement, but not on reflex movement or automatic movement. These results suggest that differential responses in the putamen to visual stimuli might be related to some cognitive functions such as visual discrimination, habit, or motor control required for task execution.

115.14

CUE & MOVEMENT RELATED RESPONSES OF PALLIDAL AND NEOSTRIATAL NEURONS IN THE RAT DURING A TARGETED HEAD MOVEMENT. A.C. Moretta*, T.W. Gardiner and S.T. Kitai (SPON: L. Johannsen). Dept. Anatomy & Neurobiol., College of Medicine, Univ. Tenn. Memphis, Memphis, TN 38163.

Neuronal responses related to limb movement and to sensory cues that trigger locomotion have been described previously in the neostriatum (STR) of the rat. We have examined neuronal activity in both the STR and globus pallidus (GP) of rats performing a head movement task. Rats were trained to hold their heads centered and then to move to a left or right target in response to a sensory cue. Single-unit recordings obtained from 14 GP units and 11 STR units showed alterations in their firing patterns during this task. Six GP units and 5 STR units altered their activitiy in association with the cue for movement. Changes in activity associated with the subsequent movement were observed for 24 of the 25 units. Most movement-related responses (10 in GP, 5 in STR) were directionally specific. These results suggest that responses associated with the head movement are prevalent in both GP and STR and that neuronal activity in these structures is at least qualitatively similar to that observed in other species. [Supported by NIH NS 20702 and NS 23886 to STK]

115.15

EFFECT OF ETHANOL ON MANY-NEURON ACTIVITY IN RAT NEOSTRIATUM DURING LOCOMOTOR BEHAVIOR. R.-S. Lee, N. Shimizu*, and D.J. Woodward. *Dept. of Cell Biol. and Anat., The Univ. of Texas. Southwestern Med. Ctr. at Dallas, TX 75235.*

The objective of the present study was to clarify the following: (1) how ethanol affects the general activity of neostriatal neuronal circuitry in rats, (2) how ethanol affects context dependent behavior of neostriatal neurons at different stages of the experience of treadmill (TM)-induced locomotion. Long-Evans rats (n=21) were prepared for chronic recording with 20 microwires (25 or 45 μ), which were implanted into neostriatum. Rats were trained to walk on a treadmill (30 sec on / 30 sec off). A tone served as a cue for the onset of TM. The spontaneous activity of multi-channel single units (2-6) in neostriatum was recorded extracellularly. Some neostriatal neurons were recorded daily for 2-7 weeks. The firing rate of many neostriatal neurons elevated during TM-on phase. The results demonstrated that: (1) The normally elevated unit activity during TM was suppressed by ethanol (0.8-1.4 g/kg, i.p.) on most neostriatal neurons recorded with microwires chronically, consistent with previous study that used tungsten electrodes (Soc. Neurosci. Abst. Vol. 14, 718, 1988). (2) Heterogeneous responses to ethanol were demonstrated in simultaneously recorded neostriatal neurons. The responses of some cells were unaffected or even increased, while those of the other neurons decreased. (3) Furthermore, the firing rate or pattern of neuronal activity of TM trained rats changed remarkably over 30 sec on-off intervals in which several trials expressed high activity with occasional shifts to very low or no neuronal activity after ethanol. Overall, ethanol suppressed the response of increased activity on most neostriatal neurons during TM exercise. We postulate that in the TM trained rat after ethanol there emerges a labile context "mode" switch which yields changes similar to those produced during transitions found between activity patterns in mode of " familiar" versus "novel" TM cycle intervals. *(Supported by AA-3901, DA-02838, Biol. Humanics Found.)*

115.16

REACTION TIME STUDIES IN MONKEYS WITH DAMAGE TO THE NIGROSTRIATAL DOPAMINERGIC SYSTEM. E. Trouche and P. Apicella. LNF 3, CNRS, 13402 Marseille Cedex 9, France.

In this study, we investigated the effects of striatal dopamine (DA) depletion on monkeys' reaction time (RT) performances. For this purpose, Papio papio baboons with 6-hydroxydopamine (6-OHDA)-induced lesion of the nigrostriatal DA system were tested with a variety of motor tasks involving either rapid accurate forelimb reaching towards a visual target or a similar movement with no target. In some tasks, the subjects had to choose the appropriate movement when the signal to move was given (choice RT) by a visual starting signal, the spatial location of which was randomly varied on the test panel. The results show that injections of 6-OHDA into the substantia nigra, even those resulting in small (40 to 60 %) to moderate (60 to 80 %) striatal DA depletion, disrupted the RT performances whatever the testing procedure used. The increase in RT does not seem to have been modified by the complexity of the programmed motor response or the amount of preparation for movement. This may indicate that premovement central neural processing was not impaired in the monkeys studied here. On the other hand, the extent of the lengthening of the RTs depended on the position of the trigger signal in the visual field, which shows that the severity of the movement initiation impairment can depend on the sensory context.

115.17

STRIATAL EXCITOTOXIC LESIONS CAUSE DYSKINESIAS AND CHOREA-LIKE MOVEMENTS FOLLOWING APOMORPHINE ADMINISTRATION IN NON-HUMAN PRIMATE (Papio papio). O. Isacson, P. Hantraye, D. Riche and M. Maziere. Dept. Neurology, Harvard Med. Sch., McLean Hospital, Belmont, MA 02178, CNRS, and C.E.A. Dept. de Biologie, F-91906 Orsay, France.

We have categorized the dyskinesias induced by 0.5-2.0 mg/kg apomorphine 1-82 weeks following subtotal excitotoxic lesions of the caudate-putamen in baboons (Papio papio). In parallel with behavioral studies, in vivo positron-emission tomography (PET) using labelled bromolisuride was used to assess striatal degeneration. Post-mortem histological results were compared with the dyskinetic profile in each animal. The dyskinesias appeared within 7 days after the unilateral ibotenic-acid lesion and only after pharmacological activation. The motor symptoms included dystonia, chorea-like movements, head-dyskinesia, oro-facial dyskinesia and rotation. PET-scans revealed a 30-60% reduction of the dopamine receptor ligand binding in the caudate-putamen, depending on extent of neuronal loss in this area. Post-mortem morphological analysis indicated a striking similarity with the regional neuronal and axon-sparing lesions seen in Huntington's disease, although interneurons were not spared. Our results show that a unilateral excitotoxic caudate-putamen lesion in non-human primates can produce dyskinesias, PET and post-mortem pathology not unlike that seen in Huntington's disease.

115.18

KINEMATIC DEFICITS IN PARKINSON'S DISEASE VARY AS A FUNCTION OF MOTOR TASK Nadine P. Connor, James H. Abbs and Greg S. Turner, Speech & Motor Control Lab., Waisman Center, Univ. of Wisconsin, Madison, WI 53706.

During rapid goal-directed arm movements, Parkinson's Disease (PD) subjects typically show a disproportionate reduction in peak velocity for a given movement amplitude and an increased movement duration. However, these manifestations of bradykinesia may be confounded by task variables, such as novelty or use of visual guidance. Consistent with this hypothesis, recent research suggests that these movement disturbances may not generalize to well-learned, natural movements performed without visual guidance.

This study compared jaw opening movements in six male PD subjects with those of age/sex matched controls under two conditions: 1) rapid visually-guided jaw movements toward discrete targets and 2) non-visually guided movements for speech. During visually-guided jaw movements, PD subjects manifested the same deficits previously reported for the proximal arm; namely, reduced peak-velocity/amplitude ratios relative to normal and increased duration. In contrast, these measures were not different from normal during speech movements of comparable amplitude. These results suggest that the PD motor impairment must be viewed as a complex, task-dependent disorder influenced by a variety of factors, including the degree to which sensory information is available and utilized. Supported by NIH Grants NS-13274, HD-03352, and NS-16373.

115.19

TURNING EVOKED BY STRIATAL OR CORTICAL STIMULATION: LONG "REFRACTORY PERIODS" DUE TO TRANSYNAPTIC COLLISIONS. J.S.Yeomans and K.E.Buckenham*, Dept. Psychology, University Toronto, Canada, M5S 1A1.

Frequency thresholds for turning were reduced at C-T intervals of 0.6-4.0 ms. in both cortical and striatal sites. In several sites, thresholds were also reduced at C-T intervals of 10-50 ms, which is too long to be attributed to axonal refractoriness. This late recovery was associated with large electrode tips (r=+.67) and high currents (r=+.38). When C pulses were presented via the cortical electrode and T pulses via the striatal electrode, frequency thresholds for turning decreased at short C-T intervals (0.6-4 ms). When C pulses were presented via the striatal electrode and T pulses via the cortical electrode, frequency thresholds for turning decreased at long C-T intervals (10-50 ms). This asymmetric collision suggests that one-directional synapses are interposed on the descending pathway between cortex and striatum. The size (10-40%) and C-T intervals of the late asymmetric recovery in the collision experiment were almost the same as the size and C-T intervals of the late recovery in the striatal refractory period data. The striatal electrodes showing late recovery are proposed to activate both presynaptic and postsynaptic axons. The late recovery is attributed to recovery from transynaptic action potentials occurring 10-50 ms after the C pulses.

BASAL GANGLIA AND THALAMUS II

116.1

ORGANIZATION OF PRIMATE VENTROLATERAL THALAMUS: MICROEXCITABILITY AND SENSORIMOTOR PROPERTIES. J. Ashe', J.L. Vitek', M.R. DeLong and G.E. Alexander. (SPON: R.T. Richardson). Johns Hopkins Univ., Baltimore, MD 21205.

Anatomic and physiologic studies have demonstrated that the cerebellum and basal ganglia project to different subnuclei within the ventrolateral thalamus, and these in turn project to separate cortical areas. To further characterize this region of thalamus, and to establish whether ventrolateral thalamic subnuclei have distinctive physiological properties, we carried out the following study. African Green monkeys were conditioned to permit a detailed sensorimotor exam that included passive joint rotation, muscle palpation, tactile stimulation and the elicitation of active movements of the limbs and orofacial structures. Electrode penetrations were made in parasagittal planes by an anterior oblique approach. Neuronal activity was sampled throughout each penetration and microstimulation (maximum current: 45 μA) was carried out at 250 μ intervals.

Discrete movements of the contralateral face, arm and leg, were evoked by microstimulation over a region of the thalamus, that was largely coextensive with VPLo. The pattern of microstimulation effects, and the distribution of neuronal sensorimotor response properties, revealed clear evidence of somatotopic organization.

Areas of the ventrolateral thalamus corresponding to regions that receive basal ganglia input showed little microexcitability. In contrast, areas in which microstimulation effects were prominent, appeared to correspond to regions receiving cerebellar input. The microexcitability seen in VPLo may be related to its strong functional connection to motor cortex.

116.2

ORGANIZATION OF PRIMATE VENTROLATERAL THALAMUS: NEURONAL RELATIONS TO ACTIVE AND PASSIVE LIMB MOVEMENTS. J.L. Vitek', J. Ashe', M.R. DeLong, G.E. Alexander. (SPON: D. Hanley). Dept. of Neurol., Johns Hopkins Univ., Baltimore, MD 21205.

This study was undertaken to examine the functional properties of neurons in ventrolateral thalamus in order to: 1) test the hypothesis of segregated basal ganglia and cerebellar thalamocortical circuits and 2) clarify the differential role of ventrolateral thalamic subnuclei in motor performance.

African Green monkeys were trained to perform a visuomotor step-tracking task requiring elbow flexion and extension. Torque perturbations sufficient to displace the forearm were randomly interspersed with the movement trials and monkeys were trained to permit passive somatosensory exams.

Approximately one-half of the movement-related neurons (48/95) showed unidirectional responses. Examination of the sensorimotor response properties of these unidirectional cells revealed 25 whose response properties were restricted to active and/or passive movement about the elbow or shoulder.

Elbow or shoulder specific cells located in more antero-medial penetrations (8/25) discharged only during active arm movements and all but two gave no response to torque perturbations. In contrast, shoulder (2/25) or elbow (15/25) specific cells located in more postero-lateral penetrations were responsive to both passive sensory examination and torque perturbations. The relative number of torque-responsive cells increased and the torque response latency decreased in more lateral penetrations. In the most lateral sites many of the torque response latencies were in the 7-15 ms range. Cells in this region may act to relay short latency proprioceptive input to the motor cortex. The functional differences observed between antero-medial and postero-lateral regions of ventrolateral thalamus may be explained by differential inputs to these areas from the basal ganglia and cerebellum, respectively.

116.3

CHRONIC STIMULATION OF THE VIM THALAMIC NUCLEUS SELECTIVELY INHIBITS THE PARKINSONIAN AND ESSENTIAL TREMORS IN HUMAN PATIENTS. A.L. Benabid, P. Pollak*, D.M. Gao*, L. Jeaugey*, D. Hoffmann*, C. Feuerstein. Dept. of Clin. and Biol. Neurosciences, INSERM U-318, Joseph Fourier Univ.of Grenoble, 38700 La Tronche, FRANCE.

During stereotactic procedures in non anesthetized patients suffering from Parkinson disease or essential tremor, suppression of the tremor was obtained by acute test stimulation of the nucleus ventrointermedius of the Thalamus (VIM) at high (130 Hz) frequency. This was then used as a treatment in 20 patients: VIM was chronically implanted with DBS electrodes connected to an Itrel Medtronic stimulator. Stimulation at 65 Hz was not efficient. In all patients, the tremor was consistently suppressed and the effect was maintained for as long as 2 years although the intensity of the stimulation had to be increased within acceptable limits. This increase actually reached a stable level after three months. Additionnal L-DOPA, when needed for akinesia, enhanced the effect of thalamic stimulation. Side effects were not observed at the intensities necessary for tremor suppression but when the voltage was increased, paresthesias were induced and, for even higher intensities, cerebellar dysmetria. Bilateral implantation was performed in 5 patients and in 3 the implantation was performed on the contralateral side of a previous thalamotomy. No neuropsychological deficit was observed following these bilateral procedures and old patients were successfully submitted to this procedures without complications. The mechanism of this effect is not fully understood but could be due to an inhibition of a retroactive loop in the same manner thalamotomy does. The reversibility of the effect, its possible adaptation to the particular circumstances of each patient makes this procedure very flexible and this should replace the thalamotomy in the regular surgical treament of the parkinsonian tremor. However, this effect is selective and does not work as spectacularly in other types of tremor, for which new targets, if any, should be found. Experimental approaches (including micro and semi-microelectrodes electrophysiological recordings in Macacus Monkeys and in human patients) are in progress to understand the mechanism and the characteristics of this highly efficient therapeutic effect.

116.4

DiI AS AN *IN VIVO* AND *IN VITRO* TRACER IN BASAL GANGLIA AND OTHER SYSTEMS. S. Milošević*, A.F. Sadikot, M.-C. Bélanger* and A. Parent. (SPON: L.J. Poirier) Lab. of Neurobiol, Fac. of Med., Laval Univ., Québec.

The fluorescent carbocyanine dye 1',1-dioctadecyl-3,3,3',3', tetramethylindocarbocyanine perchlorate (DiI) was used for a variety of *in vivo* and *in vitro* applications in rat CNS. Brains were cut on cryostat, freezing microtome, or vibrotome, and kept for up to 2 months in phosphate buffer saline (PBS) or PBS-30% sucrose solution, with no apparent differences in quality of fluorescent labelling. The striatonigral system was used to study applicability to anterograde and retrograde axonal transport *in vivo*. Effective injection sites limited to 50-150 microns were possible using micropipettes filled with DMSO solution of DiI either implanted into brain to allow passive diffusion, or using iontophoresis. Following injections into striatum, axon terminals and cell bodies were noted in substantia nigra at as early as 20 hours. Optimum transport of dye occurs within 5-10 days, although survival times up to 4 months also resulted in excellent labelling. Combination with tyrosine hydroxylase immuno-histochemistry gave good results in the substantia nigra. *In vitro* experiments included application of crystals into embryonic brain tissue and blood vessels of adult cerebral cortex. Application of DiI to cerebral vessels results in profuse axonal labelling but no cell labelling was seen with diffusion times as long as 4 months. However, following application of DiI to adult cortical surfaces labelling of cell bodies was observed due to uptake of dye by thick apical dendrites. In embryonic tissue, excellent axonal labelling was obtained following application of DiI, with retrograde labelling of cell bodies in visual and olfactory systems.

116.5

DIFFERENTIAL PROJECTIONS OF CENTRE MEDIAN AND PARAFASCICULAR NUCLEI IN SQUIRREL MONKEY. A.F. Sadikot and A. Parent, Lab. of Neurobiol., Fac. of Med., Laval Univ., Québec, Canada.

Efferent projections of the centre median (CM) and parafascicular (PF) thalamic nuclei were examined in 3 squirrel monkeys (*Saimiri sciureus*), using anterograde transport of lectin-conjugated horseradish peroxidase(WGA-HRP) in one case and *Phaseolus vulgaris*-leucoagglutinin (PHA-L) in two cases. Striatal distribution of calbindin-28K, prosomatostatin, tyrosine hydroxylase and acetylcholinesterase was examined on a few sections from each case to delineate patch-matrix compartments. Efferents to the striatum are principally distributed to the matrix compartment. The putamen receives a dense projection from the CM, which is organized in the form of bands in the dorsolateral putamen, becoming patchy at more caudal levels. Striatal inputs from the Pf are found more rostrally, supplying principally the ventromedial putamen, ventrolateral caudate and tail of the caudate, lateral nucleus accumbens, and olfactory tubercle. Both pallidal segments receive inputs from CM and Pf nuclei; CM efferents terminate preferentially in the external segment, whereas Pf efferents preferentially supply the internal segment. Other basal forebrain areas receiving parafascicular inputs include the septum, islands of Calleja, ventral pallidum, nucleus basalis of Meynert and amygdala. Projections from the CM or Pf nuclei are also seen in the hypothalamus, substantia nigra, ventral tegmental area, retrorubral field, deep layers of the superior colliculus, and peribrachial tegmentum. The results suggest that in the primate, the CM nucleus projects principally to motor areas of the basal forebrain, whereas the Pf nucleus projects principally to limbic-associative areas.

116.6

EVIDENCE FOR COLLATERALIZATION OF THE PROJECTION FROM PARAFASCICULAR NUCLEUS TO GLOBUS PALLIDUS AND CAUDATE-PUTAMEN IN THE RAT. A.E.Kincaid, S.W.Newman, A.B.Young and J.B. Penney, Jr. Dept. of Anat. and Cell Bio. and Dept. of Neuro. Univ. of Michigan Medical School, Ann Arbor, MI 48109.

The parafascicular nucleus (PF) of the thalamus, one of the intralaminar nuclei, is known to project to the caudate-putamen (CPu), subthalamic nucleus (STN) and cortex (Cx), but has not been shown to project to the globus pallidus (GP) of the rat. We have presented evidence for a projection from the PF to the GP of the rat. To test the hypothesis that the axons from PF to GP are collaterals from the PF to CPu projection, we injected fluorescent retrograde tracers into the GP and CPu. Adult, male Sprague-Dawley rats received iontophoretic injections of Fluoro-gold (FG) into GP and pressure injections of rhodamine-filled microspheres in CPu. Four days later, animals were perfused and their brains processed for microscopy. Slides were viewed on a fluorescence microscope and double-labeled neurons were identified on the same section; FG-labeled cells under UV light excitation, and rhodamine-labeled cells under green light excitation. A substantial population of double-labeled neurons were noted in the PF following these injections. Not all neurons that project to the GP and the CPu from the PF, as determined by our injections, were double-labeled. Supported by NIH NS20629 to SWN and NIH NS19613 to ABY and JBP.

116.7

BASAL GANGLIA $GABA_A$ AND BENZODIAZEPINE RECEPTORS AFTER IBOTENIC ACID LESIONS OF INTRALAMINAR THALAMUS. K. O'Mara*, A.B. Young and J.B. Penney (SPON: M.B. Bromberg). Dept. of Neurology, University of Michigan, Ann Arbor, MI 48109.

The rat parafascicular thalamic nucleus provides a major input to striatum. We have found compensatory $GABA_A$ and benzodiazepine receptor changes in basal ganglia after lesions of striatum (Pan et al., J. Neurosci. 3:1189, 1983), substantia nigra (Pan et al., J. Neurochem. 45:1396, 1985) and cortex. To study the effects of parafascicular lesions, 10 μg in 1 μl of ibotenic acid was infused over 8 min 20 sec at 2.9 mm caudal, 6.0 mm ventral and 1.0 mm lateral to bregma into the parafascicular nucleus. Eight weeks later autoradiographic saturation assays of $GABA_A$ (15 - 90 nmol [^{3}H]muscimol) and benzodiazepine (2.5 - 25 nmol [^{3}H]flunitrazepam) receptors were performed on striatum, globus pallidus, entopeduncular nucleus, substantia nigra and ventromedial/ventrolateral thalamus. No changes were found in B_{Max} or K_d of either ligand in any region. These findings suggest that parafascicular input does not greatly influence striatal GABAergic output.

Supported by NS 19613.

116.8

DIRECT STRIATO-THALAMIC PROJECTIONS IN NEONATAL RATS. M.S. Nishihama*, M. Takada, T. Moriizumi* and T. Hattori.Dept. of Anatomy, Univ. of Toronto, Toronto, Ontario M5S 1A8, Canada.

In the course of investigation on the development of the basal ganglia-related neuronal connections, we have found direct projections from the striatum to the thalamus in neonatal rats. Following WGA-HRP injections into the core of the striatum at postnatal day 0 (P0), anterogradely labeled axon terminals were observed ipsilaterally in the ventrolateral (VL), ventrobasal (VB), centrolateral (CL) and parafascicular (Pf) thalamic nuclei at P1. By contrast, WGA-HRP deposits in the overlying cortex as control labeled terminals only in the VB. Moreover, thalamic injections of a fluorescent retrograde tracer Fluoro-gold (FG) at P0 resulted in perikaryal labeling in the ipsilateral striatum at P1. These FG-positive cells were aggregated in a mosaic fashion similar to the patchy distribution characteristic of perinatal striato-nigral projection cells (J. Neurosci., 7:1969, 1987). Thus, the present data provide evidence that the neonatal rat striatum has efferents projecting outside the basal ganglia (at least to the VL, CL and Pf of the thalamus). Since no available studies have previously suggested the existence of direct striato-thalamic projections in the adult, this novel pathway might transiently appear during development. Supported by the MRC of Canada.

116.9

THALAMIC AFFERENTS FROM THE ENTOPEDUNCULAR NUCLEUS IN THE DOG. B.A. Hannah* and S.T. Sakai (SPON: J. Krier) Department of Anatomy, Michigan State University, East Lansing, MI 48824.

As part of our ongoing studies on the organization of the motor control pathways, the present study investigated the distribution of the entopeduncular (EP) efferents to the canine thalamus using the autoradiographic (ARG) tracing technique. A series of tritiated amino acid injections were made into the EP in anesthetized dogs. Labeled fibers were observed emanating from the EP toward the thalamus within the ansa lenticularis and the fasciculus lenticularis. Silver grains were observed throughout a wide extent in the ipsilateral thalamus including the ventral anterior nucleus (VA) and the ventromedial nucleus (VM). Heavy ARG label was observed in the lateral habenular nucleus (HL) and the centromedian (CM) and parafascicular (Pf) nuclei. In addition, labeled fibers were observed in the massa intermedia distributing to the contralateral VM, CM and HL.

It is of interest that the pallidothalamic regions of VA, VM and the caudal intralaminar nuclei labeled in the present study appear to coincide in location with the thalamic regions known to project to area 6 in the dog (Stanton et. al. 1986). Moreover, comparison of the present results with our data on the canine nigrothalamocortical projections suggest that area 6 may receive ascending inputs from two basal ganglia sources: the entopeduncular nucleus and the substantia nigra.

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116.10

THE DISTRIBUTION OF THE NIGROTHALAMOCORTICAL PROJECTIONS IN THE DOG: A COMBINED HORSERADISH PEROXIDASE AND AUTORADIOGRAPHIC STUDY. S.T. Sakai Department of Anatomy, Michigan State University, East Lansing, MI 48824.

The purpose of the present study was to determine the extent to which the canine area 6 thalamocortical projections receive inputs originating from the substantia nigra (SN). In the anesthetized dog, a series of tritiated amino acid injections were made into the SN . At the same time, a series of horseradish peroxidase (HRP) injections were made into area 6. The HRP labeled area 6 thalamocortical neurons were found to be coextensive with nigrothalamic autoradiographic (ARG) label in the ventral anterior nucleus (VA), ventromedial nucleus (VM), mediodorsal nucleus (MD) and parafascicular nucleus (Pf). However, HRP labeled cells were also observed in the ventral lateral nucleus (VL) in a region where no ARG label was observed. In addition, both VA and VM contained areas of dense ARG label where no HRP filled cells were observed. While these results suggest that area 6 receives indirect input from the SN via the thalamus, comparison of these results with our previous data obtained from the cerebellothalamic and pallidothalamic projections suggest that area 6 may also receive inputs originating from these additional sources. Finally, the possibility remains that an additional cortical target of the nigrothalamic projections exists. This region may include the prefrontal cortex. In the monkey, Ilinsky et. al. (1985) demonstrated nigrothalamic afferents to the prefrontal cortex as well as the supplementary motor area and the frontal eye fields.

(Supported by NIH grant NS 18551 and B.R.S.G. funds to the College of Human Medicine).

116.11

NEURONAL AND SYNAPTIC ORGANIZATION OF THE VENTRAL ANTERIOR NUCLEUS PARS MAGNOCELLULARIS (VAmc) OF THE MACACA MULATTA THALAMUS. I.A. Ilinsky and K. Kultas-Ilinsky, Dept. of Anatomy, Univ. of Iowa Coll. Med., Iowa City, IA 52242.

The magnocellular subdivision of the ventral anterior nucleus is recognized only in primates. It receives nigrothalamic projections throughout its extent and is reciprocally connected with the prefrontal cortex (Ilinsky et al., JCN 236:315-330, 1985). No data are available on physiological characteristics or ultrastructural organization of this nucleus and its functional significance remains obscure. The goal of this study was to provide neuroanatomical data on organization of neuronal circuitry in the VAmc. A variety of techniques including antero- and retrograde HRP labeling, EM-autoradiography and GAD-immunocytochemistry were used. Two major cell types were identified: thalamocortical projection neurons (PN) and small GAD-positive cells, apparently local circuit neurons (LCN). The major input to PN was from nigral afferents distributed to the soma and all levels of dendritic arbor with the heaviest concentration on secondary branches and bifurcation sites. These boutons formed symmetrical synapses and were all GAD positive. PN somata received also sparse indirect cortical input mediated by GABAergic dendrosomatic synapses and symmetrical synapses from boutons of unknown origin on axon hillocks and initial axonal segments. Corticothalamic terminals provided major input to PN tertiary dendrites with only some boutons contacting secondary dendrites directly and indirectly through LCN dendro-dendritic synapses. Two additional very sparse populations of synaptic boutons of unknown origin were observed on distal PN dendrites. LCN received mainly cortical and very sparse nigral input on their distal dendrites plus a symmetrical synapse from a large bouton of unidentified origin on soma. The results suggest the prevalence of inhibitory input from various sources on PN without obvious strategically placed excitatory input which could drive these cells. Supported by NS RO1 24188.

116.12

AUTORADIOGRAPHY OF GABA AND BENZODIAZEPINE RECEPTORS IN THE MONKEY MOTOR THALAMUS. K. Kultas-Ilinsky, J. Kallsen*, S. Tewfik*, L. Freedman and I.A. Ilinsky. Dept. of Anat., Univ. of Iowa, Iowa City, IA 52242.

Quantitative receptor binding autoradiography technique was used to study subtypes of GABA and benzodiazepine receptors in projection zones of basal ganglia and cerebellar afferents to the thalamus in two species of subhuman primates: Macaca Rhesus and Erythrocebus pates. The binding assays were carried out as described by Kultas-Ilinsky et al. (1987, Brain Res., 459:1-16) using [^{3}H]-muscimol ([^{3}H]MUS), [^{3}H]-flunitrazepam ([^{3}H]FLU)], [^{3}H]-baclofen and [^{3}H]-bicuculline. Consistently saturable specific binding was obtained only with [^{3}H]FLU and [^{3}H]MUS. Bicuculline (10 μM) inhibited [^{3}H]MUS binding by 30-50% in different nuclei but enhanced [^{3}H]FLU binding (17-30%). Enhancement of [^{3}H]FLU binding by 10 μM GABA was more pronounced (20-40%). No substantial differences between the two monkey species were detected in the distribution and binding parameters of receptors. In both, the highest number of [^{3}H]FLU and [^{3}H]MUS binding sites was found in the nigrothalamic projection zone (VAmc). In the nuclei receiving pallidal (VAdc and VApc) and cerebellar (VL) input the Bmax values for both ligands were lower. Binding affinities were comparable in all nuclei analyzed (KD = 2-3 nM for [^{3}H]FLU and KD = 36.9 nM for [^{3}H]MUS). When compared to our previous data on the GABA receptor binding in the cat motor thalamus several differences could be noted. The most significant: the higher concentration of both binding sites in the nigral projection zone compared to pallidal, little difference between thalamic nuclei with respect to [^{3}H]MUS/[^{3}H]FLU binding site ratio, and overall lower [^{3}H]MUS binding affinity. The differences in GABA receptor distribution between the feline and primate thalami may be related to differences in topography of subcortical afferents and neuronal organization of the nuclei in these species. Supported by NSRO119280.

116.13

THERMAL DEPENDENCE OF INDUCTION AND EXPRESSION OF LONG-TERM POTENTIATION IN THE HAMSTER HIPPOCAMPAL SLICE. M.P. Thomas*, M.S. Krelstein*, and J.M. Horowitz, Animal Physiol., Univ. Calif. Davis, CA 95616. (SPON: P.Pappone)

The mechanisms involved in the induction of long-term potentiation (LTP) differ from those involved in its maintenance. We investigated the thermal sensitivity of the induction process and the initial expression of LTP. We prepared hippocampal slices from hamsters as described previously (Thomas et al. J. therm. Biol. 11:213, 1986) and measured field PSP initial slopes in response to Schaffer collateral/commissural fiber stimulation. Bath temperature was varied via an external water bath. LTP was reliably evoked above 25°C by high-frequency pulse train stimulation, and could not be evoked below 20°C. However, when a high-frequency train was delivered to slices at a bath temperature of 20°C and the slice subsequently warmed to 25°C, LTP was observed relative to control responses recorded previously at 25°C. Furthermore, when LTP was elicited at a bath temperature of 25°C, it persisted when the slices were cooled to 20°C (determined by comparing post-tetanic responses with control responses recorded previously at 20°C). In summary, the first experiments show that when the temperature is held at 20°C the process is initiated but then arrested. The second set of experiments show that once expressed at a higher temperature, the synaptic enhancement persists when the tissue is cooled. [NSF grant BNS-88-19973.]

116.14

ULTRASTRUCTURAL AND IMMUNOHISTOCHEMICAL OBSERVATIONS ON A PROJECTION FROM THE MAGNOCELLULAR BASAL NUCLEUS TO THE THALAMIC RETICULAR NUCLEUS IN RATS. by L.L. Porter and C. Asanuma. Department of Anatomy, USUHS, Bethesda, MD, 20814 and Laboratory of Neurophysiology, NIMH, Poolesville, MD, 20837.

We have examined some details of the projection from the caudal magnocellular basal nucleus (cMBN) to the thalamic reticular nucleus (TRN) in rats. Axons of this pathway are known to give rise to clusters of boutons apposing TRN somata and 'en-passant' beads upon TRN dendrites.

The terminal boutons of the cMBN ⇒ TRN pathway were examined at the EM level following PHA-L injections into the cMBN. Following the immunohistochemical processing, many PHA-L positive terminal profiles are apparent within the TRN. Labeled axonal terminals frequently synapse upon both somata and proximal dendrites of TRN neurons, with symmetric membrane specializations. These may, therefore, differ from the ChAT positive synapses within the TRN which are asymmetric and terminate upon distal dendritic processes.

Following WGA-HRP injections into the TRN, neurons were identified within the cMBN that are both retrogradely labeled and positive for GABA. Such doubly labeled neurons occur in the same general cMBN region as ChAT positive neurons that project to the TRN.

Thus, a portion of the cMBN ⇒ TRN projection synapses directly upon the somata and proximal dendrites of TRN neurons with symmetric membrane specializations, and some of the cells giving rise to this projection are GABAergic. We infer from these observations that at least a portion of the cMBN ⇒ TRN projection is GABAergic. Since GABA iontophoresis inhibits TRN spike activity presumably by increasing chloride ion permeability (McCormick, D.A., & Prince, D.A., *Nature* 319: 402, 1986), this pathway may, in part, serve to directly 'shunt' the excitability of TRN neurons at their somata and proximal dendrites. Further work is needed to understand the physiology of this projection and its relation to thalamic gating functions.

116.15

NEURAL CONNECTIONS OF THE SUPRACHIASMATIC NUCLEUS WITH MIDLINE THALAMIC NUCLEI B.G.Orpen, Z.Woskowska*, M.O.Miceli and M.Steiner. St.Joseph's Hospital Research Institute, and Dept.of Psychiatry and Biomedical Sciences, McMaster University, Hamilton, Ontario, Canada.

The suprachiasmatic (SCN) and adjacent hypothalamic nuclei project to several midline thalamic nuclei. The thalamic nuclei project in turn to the limbic system and striatum, and are thus in a position to modulate circadian time-keeping information. We used the neural tracer, horseradish peroxidase-wheat germ agglutinin (HRP-WGA), to study in more detail the afferent and efferent neural connections of the midline thalamic nuclei in the golden hamster, Mesocricetus auratus, with particular emphasis on connections with the SCN and adjacent hypothalamic nuclei.

Hamsters injected with HRP-WGA were sacrificed after 24-48 hours. The brains were then processed using tetramethyl benzidine histochemistry. Five subjects sustained small, confined injections in the paratenial (PT) and paraventricular (PVT) thalamic nuclei. The remaining five animals sustained larger thalamic injections, which incorporated much of the anterior-posterior and lateral extent of the PT and PVT, and, in some instances, the paracentral and reuniens thalamic nuclei. Control injections included the immediately dorsal cortex, septum-fornix and the adjacent bed nucleus of the stria terminalis.

After confined injections, only a few labelled cells and terminals were observed in the SCN; after larger injections, many labelled SCN cells, but only a few terminals were observed. This pattern suggests that each SCN cell projects to only a small area in the PVT and/or PT. Labelling was uniformly distributed in the anterior SCN, but was confined to the ventral and lateral portions of the mid- to posterior SCN. These findings will be discussed in relation to labelling of other potential components of the circadian rhythm system.

(Supported by the St.Joseph's Hospital Foundation. B.G.O. and M.O.M. are Ontario Mental Health Foundation fellows.)

AUDITORY, OLFACTORY AND OTHER SENSORY SYSTEMS

117.1

ADAPTIVE CHANGES IN BINAURAL TUNING OF NEURONS IN THE OPTIC TECTUM OF BARN OWLS RAISED WITH ALTERED EXTERNAL EARS. J.F. Olsen* and E.I. Knudsen (SPON: T. Iismaa). Dept. Neurobiol., Stanford Univ., Stanford, CA 94305

Experiments in which barn owls were raised with monaural earplugs have shown that the auditory space map in the optic tectum can be modified in response to altered cues for sound localization (Knudsen, 1983). Here we show that the tuning of tectal neurons to interaural differences in time (ITD) and intensity (IID), measured directly with dichotic stimuli, is altered adaptively when owls are raised with abnormal external ears. The direction-dependent sensitivities of the ears were changed radically by removing the ruff feathers and preaural flaps before they grew in. After the owls reached adult size, ITDs and IIDs were measured with microphones in the ear canals. Although ITDs varied with azimuth, as found in normal owls, the change in ITD with azimuth was faster than normal for frontal source locations (out to 40° azimuth), and was slower than normal peripherally. This abnormal pattern of ITD variation correlated with an adaptive change in ITD tuning of tectal neurons: For bimodal units with visual receptive fields within the frontal 40°,best ITDs changed faster with visual best azimuth in ruff-cut owls than in normal owls. In normal owls, IID cues vary primarily with source elevation. In ruff-cut owls, IID cues varied primarily with azimuth, and spanned an abnormally small range. Unit tuning to IID was altered in ruff-cut owls: In general, best IIDs varied with best azimuth but not with best elevation, a pattern opposite to normal. However, for units that had best azimuths within 15° of the vertical meridian, best IID varied with best elevation. Thus, despite severely altered cues, units that represent the vertical meridian developed a nearly normal pattern of IID tuning. NIH grant R01 NS 16099-09.

117.2

CALLOSAL AND THALAMOCORTICAL TERMINAL FIELDS IN PRIMARY AUDITORY CORTEX OF DEVELOPING RATS. R. T. Robertson, K. A. Gallardo* and J. Yu. Depts. of Anatomy and Neurobiology and Physical Medicine and Rehabilitation, University of California, Irvine, CA 92717.

We have examined the areal and laminar distributions of callosal and thalamocortical terminal fields in primary auditory cortex of developing rat pups. Infant Sprague-Dawley rat pups, 6-11 days of age, were anesthetized and injections of 10% HRP were made unilaterally in auditory cortex. Following survival periods of 1-2 days, animals were perfused and frozen sections were processed for HRP or AChE histochemistry. Callosal terminal fields were identified by anterograde transport of HRP; thalamocortical terminal fields were identified by endogenous AChE activity.

Injection sites within primary auditory cortex (cortical area 41) were identified by strong retrograde labeling of neurons in the ipsilateral ventral medial geniculate nucleus. In animals sacrificed before postnatal day 10, contralateral auditory cortex showed widespread retrograde and anterograde HRP labeling; the areal pattern overlapped extensively with AChE staining. However, HRP labeling was most pronounced in cortical layers II/III and V while AChE labeling was most pronounced in layer IV. In animals sacrificed after day 12, anterogradely transported HRP also occurred in layer II/III and V, but the areal pattern appeared patchy, occuring mainly in regions other than those displaying prominant AChE staining. These data indicate that after an initital "exuberant" phase, callosal terminal fields reduce to complement the geniculocortical terminal fields in auditory cortex.

Supported by NIH grant NS 25674 and NSF grant 87-08515.

117.3

RAPID MITOCHONDRIAL RESPONSE TO COCHLEA REMOVAL IN CHICKEN BRAIN STEM AUDITORY NEURONS. G.E. Hyde* and D. Durham. Hearing Development Labs, RL-30, U. of W., Seattle, WA 98195

Removal of excitatory input to second order auditory neurons (n. magnocellularis, NM) in the chicken brain stem results in rapid *increases* in the activity of mitochondrial enzymes as early as 6 hours after cochlea removal. Light microscopic results of histochemical staining for cytochrome oxidase (CO) suggest that mitochondria may also be redistributed within the neuronal soma as a result of cochlea removal. An ultrastructural study was done on chicks surviving 6 hours or 14 days after unilateral cochlea removal at 10 days of age. Vibratome sections of the brain stem were incubated for CO histochemistry followed by preparation of thin sections. Because the cochlear ganglion cells project to the ipsilateral NM, the contralateral NM was used as a within-animal control.

Six hours following cochlea removal, neurons in the ipsilateral NM show *proliferating* mitochondria. Stereologic measurements show at least a 53% (±4% S.E.M.) increase in mitochondrial surface density in ipsilateral NM neurons compared to contralateral neurons. In addition, a greater percentage of cristae within the proliferating mitochondria contain CO reaction product. A small sub-population of NM neurons ipsilateral to cochlea removal shows neither mitochondrial proliferation nor increased CO staining; these neurons also exhibit polyribosome dissociation as described by Rubel et al. (Soc. Neurosci. Abstr. 14:1117, 1988). At 14 days survival, there is a *decrease* in the number and CO staining of mitochondria in NM neurons ipsilateral to cochlea removal. At this time, the number of lipid-containing vacuoles, which may represent products of mitochondrial degradation, is increased within the ipsilateral NM neurons. (Supported by PHS grants NS26521, NS07246, NS24518)

117.4

DEVELOPMENT OF ACTION POTENTIALS OF EMBRYONIC CHICK COCHLEAR GANGLION CELLS *IN VITRO*. R. Batra and K. Book*. Dept. of Anatomy, Univ. of Conn. Health Cntr., Farmington, CT 06032.

Earlier studies have shown that embryonic neurons from a sensory ganglion can produce action potentials mediated by a combination of sodium and calcium ions, or by sodium alone. In some cases, a transition from sodium-calcium to sodium action potentials occurs during development. The present *in vitro* study of the cochlear ganglion of the chick provides evidence that ganglion cells with sodium-calcium action potentials and those with sodium action potentials coexist at intermediate stages of development. Intracellular recordings were made from cochlear ganglion cells of white leghorn embryos explanted during the period when cochlear nerve fibers begin to innervate hair cells (embryonic day 9-15, Hamburger-Hamilton St. 35-41). The neurons were grown for various lengths of time *in vitro* prior to recording.

In response to a positive step of current (50-300 pA), an explanted ganglion cell exhibited its characteristic action potential, which could be of one of two types. The first type of action potential had a rapid rise followed by a broad plateau that could last tens of milliseconds. It resembled action potentials produced by a combination of sodium and calcium currents. The second type consisted of a rapidly rising spike that was brief (~1-3 ms) and resembled action potentials produced by sodium currents alone. Greater steps of current produced a burst of action potentials in some cells, but others always fired only one action potential at the onset of the step. Ganglion cells did not fire action potentials spontaneously.

The two types of action potentials may represent two different populations of neurons or the same population at different stages of development. To distinguish these possibilities, we are presently recording from cochlear ganglion cells explanted later in development and from the cells of the cochleo-vestibular ganglion at the earliest stages of differentiation.

This research was supported by NINCDS grants NS-14354 to D.K. Morest and NS-18027 to S. Kuwada.

117.5

VISUAL OR AUDITORY DEPRIVATION PREVENTS THE EMERGENCE OF AN ELECTROPHYSIOLOGICAL MAP OF AUDITORY SPACE IN THE GUINEA PIG.
D.J. Withington-Wray* & M.J. Keating* (SPON: Brain Research Association). National Institute for Medical Research, London NW7 1AA, UK.

Multi-unit electrophysiological recording from the superior colliculus of the guinea pig shows that the emergence of an auditory space map follows a protracted postnatal course. Spatially diffuse auditory responses are present on the first day after birth (DAB). Localized response areas and a topographic map do not appear until 32 DAB. Animals were reared from birth in either total darkness or in a chamber providing continuous omnidirectional white noise and mapped between 34-67 DAB. They showed no evidence of an auditory space map and their responses were similar to those of normal animals shortly after birth. The results of experiments in which the sensory deprivation was limited to particular developmental periods indicate that, for both visual and auditory peturbation, the period from 26-30 DAB represents a critical susceptible period. These data are compatible with the view that the normal elaboration of the auditory space map requires coincident visual and auditory sensory input.

117.6

DEVELOPMENTAL SHIFT OF THE TONOTOPIC ORGANIZATION OF THE CHICK AUDITORY CORTEX ANALOGUE.
P.Heil and H. Scheich. Inst. Zoology, Techn. Univ., 6100 Darmstadt, F.R.G.

A developmental shift of the place coding of sound frequencies along the basilar papilla has been proposed as one solution of the discrepancy between the morphological maturation of the cochlea, which starts at the base and proceeds to the apex, and the physiological and behavioral ontogeny of hearing, initiating at low and progressing to higher frequencies (Rubel, E.W., in Handbook of Sensory Physiology IX:135, 1978). If with age no rewiring of the frequency-specific projections occurs along the auditory system, concomitant developmental shifts of tonotopic organizations should be demonstrable in higher auditory centers. We therefore mapped the tonotopic organizaion of field L, the chick auditory cortex analogue, using the (14C)-2-deoxyglucose (2DG) technique. Methods of stimulation and quantitative densimetric analysis were described elsewhere (Heil, P. and Scheich, H., Exp. Brain Res. 58:532, 1985). In each of the three postnatal ages examined (P0, P7, P27) there was a clear tonotopic gradient. But during this first postnatal month basically all 2DG-isofrequency contours shifted continuously and significantly (up to 8%) to relative positions, where formerly lower frequencies were represented. These results are likely to indicate changes in best frequencies of individual neurons. Furthermore, this first demonstration of a developmental change in the place coding of sound frequencies at the forebrain level raises the question of constancy of perceptual correlates with age.

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117.7

A PROPOSED MECHANISM FOR HYPERPLASTIC GROWTH OF THE OLFACTORY RECEPTOR SHEET IN GROWING RATS.
E. Meisami, M.A. Paternostro and M.J. Sichlau*. Dept. of Physiology, Univ. Illinois, Urbana, IL 61801.

Rat olfactory epithelium (OE) grows continuously by hyperplastic expansion and thickening (Meisami, Dev. Brain Research, 46: 9-19, '89). From birth to day 90, OE surface area increases 23x, total number and surface density of olfactory receptor neurons [ORN] increase 26x and 1.8x; OE thickness 1.5x. Total numbers of basal and supporting cells increase as well but their density per unit OE surface area remains unchanged. We propose that OE hyperplastic growth occurs by two mechanisms: first, a horizontal growth accompanied by a proliferative division of basal cells; this is the major source of expansion and hyperplasia. Second, a vertical growth, caused by a differentiative division of basal cells, resulting in ORN accretion and OE thickening. Morphometric and topological analyses reveal that OE growth between days 1 to 90 occurs across both septal and turbinal surfaces (10x and 30x, respectively). Relative contribution of turbinate OE to total OE increases from 69% at birth to 82% at day 90. At all ages, about 90% of total OE is found in the heavily turbinated middle and posterior olfactory regions, even though absolute amount of growth in these areas is very marked, compared to anterior areas. The results indicate a "ballooning" pattern of growth in all OE areas.

117.8

IDENTIFIED MAMMALIAN OLFACTORY RECEPTOR NEURONS IN CELL CULTURE.
P.Q. Trombley† and G.L. Westbrook. †Dept. Biology, Univ. of Oregon, & Vollum Institute, Oregon Health Sciences Univ., Portland, OR.

Recent studies in vivo suggest that the glomerular layer of the olfactory bulb, the site of olfactory receptor neuron (OR) input to Mitral cells, shows significant alterations during olfactory learning. Thus detailed physiological studies of this excitatory synapse could be of importance to an understanding of synaptic plasticity in this brain region. In order to examine this synapse, we have first established primary cultures of OR cells and examined their voltage-dependent conductances. OR cells were identified by retrograde transport of rhodamine labelled latex beads. Beads were injected into the olfactory bulbs of 2-5 day postnatal rats; 48 hours after injection, the olfactory epithelia were dissociated and plated on a confluent layer of olfactory bulb astrocytes.

ORs could be identified morphologically as small bipolar cells with a fine axon on one end and a thick dendritic process on the other, some with cilia. These cells were stained by antibodies against neuron specific enolase, while a smaller proportion were stained by antibodies against olfactory marker protein (OMP); most OMP(+) cells also contained rhodamine beads suggesting that these were mature OR cells. Whole-cell recordings from both fluorescent-labelled and unlabelled OR cells after 3-25 days in culture were similar. In physiological saline, the apparent input resistance was 1-10 GΩ and the OR cells fired a single action potential in response to depolarizing current injection. The spike was abolished by 100 nM TTX. Under voltage clamp, depolarizing steps from -80mV revealed: 1) a fast inward current with steady-state inactivation which was TTX-sensitive and Cd-insensitive 2) a slow persistent inward current in 10 mM Ba which was TTX-insensitive and washed out over 1-2 minutes (with 1 mM ATP /0.1 mM GTP in the pipette); 3) a persistent outward current activated at steps positive to -10mV which was blocked by 25mM TEA or intracellular Cs, but unaffected by 100 µM Cd. No transient outward current or Ca-K current(s) were seen under these conditions. Supported by NIH and the McKnight Foundation.

117.9

INFLUENCE OF OLFACTORY PLACODE TRANSPLANTS ON THE DEVELOPMENT OF THE OLFACTORY BULB IN *XENOPUS LAEVIS*.
C.A. Byrd and G.D. Burd. Dept. of Molecular and Cellular Biology, University of Arizona, Tucson, AZ 85721.

Olfactory receptor cell axons may play an inductive role in the development of the olfactory bulb (OB). When an extra olfactory placode is added to *Xenopus* embryos, the OB that develops appears to be larger than normal (Stout and Graziadei, 1980). To determine how the primary afferents affect development in this system, we quantified afferent innervation to and neuron number in the OB. Olfactory placodes were transplanted into *Xenopus* embryos (stages 28-32) so that the resulting epithelium would innervate the OB. Animals with successful transplants (i.e. those with a nerve innervating the OB) were sacrificed just before metamorphosis. The transplanted tissue developed into a closed epithelium that contained the same cell types found in normal olfactory epithelium--basal cells, receptor cells, and two types of supporting cells. Axon counts were performed to determine the number of axons innervating the OB in normal and transplant animals. We found that animals with a transplant did not have more afferents innervating the OB, even though there was an extra nerve. The possibility that there are more afferents in transplant animals earlier in development is being explored. We also counted the cells in the mitral cell/external plexiform layer (MCL/EPL) in normal and transplant animals in an effort to determine whether the output neurons (mitral/tufted cells) are influenced by the transplant. Preliminary results suggest that the transplant can cause an increase in neuron number in the MCL/EPL, but it does not have an effect in all cases.
Supported by NIH-NINCDS #NS25596.

117.10

THE DEVELOPMENT OF ELECTRO- AND MECHANORECEPTORS IN EIGENMANNIA IS INDUCED BY INNERVATION OF EPIDERMAL CELLS.
H.A.Vischer and W.Heiligenberg. SIO, Neurobiol. Unit, UCSD, La Jolla, CA 92093.

Gymnotiform fish of the genus Eigenmannia were induced to spawn under laboratory conditions. In order to visualize outgrowing nerves of the anterior lateral line ganglion (ALLG), primordial cells of the ALLG were labeled with the fluorescent dye DiI in embryos (2-3 days after spawning, 55-75 somites). Labeled rami of the anterior lateral line nerve (ALLN) were ablated 12-48 hours later with a short (t<500 ms), high-intensity (U>1500V) electric current pulse applied through an Indium-filled electrode. In a significant number of cases (90%; n=15), no lateral line receptors developed in those areas which are normally innervated by the lesioned rami. In another set of experiments, more than 90 cells were labeled iontophoretically with Lysinated Rhodamine Dextran in the vicinity of the ALLG primordium in a total of 30 embryos, 2-3 days old. 4-8 days later, traces of the marker could only be found in neural crest-derived cells, such as melanophores, but not in any lateral line receptor cells. We conclude: 1) Cephalic mechano-and electroreceptors have no placodal origin and 2) innervation is essential for the formation of anterior lateral line receptors.

117.11

CRUSTACEAN PROPRIOCEPTOR ORGAN RETAINS NORMAL SENSORY RESPONSES IN CULTURE. H. B. Hartman, S. N. Wright* and R. L. Cooper. Department of Biology, Duquesne Univ., Pittsburgh, PA 15282 and Biocenter, Univ. of Basel, CH 4056 Basel, Switzerland.

The crustacean proprioceptor, the propus-dactylus organ (PD), can be maintained in culture in either crab serum with gentamicin added or in defined medium at 11°C for at least 10 days without loss of patterned sensory activity. Blood from Dungeness crabs Cancer magister was pooled, then chilled to form a clot which was discarded. Serum was sterilized by filtration. The defined medium consists of crab saline, L-15 medium Leibovitz, BSA, gentamicin, and HEPES buffer adjusted to 950 mOsm/l at pH 7.3 which was sterilized by filtration.

The PD organ distal cuticular protuberance and proximal tendon were pinned firmly to the bottom of a Sylgard-lined glass Petri dish with the elastic strand stretched between them to approximate its normal rest length. Survival of the sensory neurons in the two media was assayed in the following manner. Action potentials were recorded daily from selected identified movement sensitive neurons (both ESC and RSC), the entire position nerve, and a nerve containing a subset of movement sensitive neurons over a period of 10 days. The responses quantified by the statistical index eta^2 remained normal for the test period. PD organs from early juvenile crabs where sensory hyperplasia is known to occur (Hartman and Cooper, in preparation) are being cultured and examined for growth cone formation. Supported by NSF Grant BNS-8700506 to H.B.H.

117.12

MORPHOLOGIC AND ELECTROPHYSIOLOGIC CHARACTERISTICS OF RAT NODOSE GANGLION NEURONS CO-CULTURED WITH CAROTID BODY CELLS J. Alcayaga* and C. Eyzaguirre (SPON: P. Zapata). Dept. Physiol., Univ. Utah Sch. Med., Salt Lake City, UT 84108

Nodose ganglion (NG) neurons and carotid body (CB) cells obtained by enzymatic dissociation were plated on poly-lysine, collagen, or fibronectin-coated dishes and cultured in Medium 199, MEM, or F-12 supplemented with 10% FBS, 10% HS, 20 mM HEPES and NGF (15 ng/ml). Co-cultures were made either in one step or CB cells were added to NG neurons previously treated with cytosine arabinoside (3.7 μM) for 3 days. Cultures were maintained for up to 26 days at 37°C in a 5% CO_2-air environment. Conventional intracellular recordings were made during superfusion with 5% CO_2 in O_2-equilibrated Earle's saline.

NG neurons maintained in Medium 199 or MEM were mainly round; only few cells presented short neurites (<50 μm). However, 2% of neurons grown in F-12 had longer neurites (50-1500 μm). In co-culture, many NG neurons showed neurites (>70 μm) regardless of the media. The soma diameter was 20-40 μm in all cases. Neurons cultured alone had passive electric properties similar to those previously reported but no spontaneous activity. In co-culture their passive properties remained similar but spontaneous action potentials, sometimes preceded by slow depolarizing potentials, occurred in 14% (5/36) of them. Thus, CB cells seem to induce morphological and physiological changes in cultured NG neurons although the nature of this influence needs to be elucidated. Supported by grant NS05666.

117.13

A POST-NATAL INCREASE IN AMPLITUDE OF THE T-TYPE Ca^{2+} CURRENT AND THE APPEARANCE OF BURST FIRING IN FRESHLY ISOLATED RAT SENSORY NEURONS G. White,* D.M. Lovinger,* and F.F. Weight (SPON: K.L. Zbicz), Section of Electrophysiology, NIAAA, Rockville, MD 20852.

An afterdepolarizing potential (ADP) generated by a T-type Ca^{2+} current produces burst firing in neurons isolated from adult rat dorsal root ganglia (DRG) [White et al., Soc. Neurosci, Abst: 14, 297,1988]. The amplitude of the T-current in adult neurons is much larger than reported for cultured embryonic or neonatal DRG neurons. To determine if this difference in current amplitude is present prior to culturing, we recorded electrophysiological responses in DRG neurons freshly isolated from 1 day-old, 12 day-old and adult rats using the whole-cell patch-clamp technique. The frequency of occurance of an ADP was the same across all age groups (≈55% of the neurons); however the amplitude of the ADP increased with age as did the frequency of observations of ADP-associated burst-firing (>80% of neurons from adult rats[n>50], 43% from 12 day-old rats [n=18], and 0% from 1 day-old rats [n=22]). Voltage-clamp experiments revealed that the amplitude of the T-current (corrected for cell capacitance) was also greatest in neurons from adult rats (29.8 pA/pF, n=22), smaller in neurons from 12 day-olds (8.7 pA/pF, n=18) and smallest in neurons from 1 day-olds (4.4 pA/pF, n=18, $p<.05$, Kruskal-Wallis). These observations suggest that the post-natal increase in T-current density contributes to the development of burst-firing.

BIOCHEMICAL AND PHARMACOLOGICAL CORRELATES OF DEVELOPMENT I

118.1

DEVELOPMENT OF CENTRAL α_2-ADRENORECEPTORS IN THE SHEEP: A PHARMACODYNAMIC APPROACH. RK Zoltoski, JC Rose*, and CE Dunlap, III. Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC 27103.

Guanabenz (GB), a centrally acting α_2-adrenergic agonist, lowers blood pressure in hypertensive rats and human patients and normotensive dogs. The effects of this drug on fetal cardiovascular function are not known. Therefore, we undertook experiments to test the hypothesis that GB produces similar cardiovascular effects in the fetal lamb compared with the maternal ewe.

Changes in fetal and maternal mean arterial pressure (MAP), heart rate (HR), amniotic fluid pressure (AFP), and GB plasma levels were determined in chronically catherized pregnant ewes and their fetal lambs of known gestational ages. Fetal lambs were divided into two groups, 100-115 and 120-135 days of gestation.

Following maternal administration of 2 mg GB iv, a biphasic response of maternal MAP was observed; a large increase in MAP (10±2 torr at 0.5 min), due to stimulation of peripheral α_2-adrenoreceptors, followed by a sustained decrease (-12±4 torr at 5 min) lasting 40 mins. A decrease in HR was noted at 1 min (-16±6 bpm) and lasted 75 mins (-15±2 bpm). GB also increased AFP which artifactually raised MAP in both groups of fetal lambs; however, following correction of MAP for the increase in AFP, no change was detected. A reflex-like decrease in fetal HR occurred (-39 to -53 bpm at 3 mins), but this response was not sustained. Plasma level measurements indicated that there was a rapid decline in GB from the maternal compartment ($t_{1\backslash 2\alpha}$=3 min, $t_{1\backslash 2\beta}$=58 min) and equilibration into the fetal compartment did not appear to occur. When 0.1mg/kg GB was administered to the fetal lamb, an increase in fetal MAP (12-14 torr at 1 min) occurred, but no decrease in pressure, up to 2 hours, was noted. HR in these lambs appeared to be reflexly decreased due to the increase in pressure (-31 to -32 bpm at 1 min). No changes in maternal MAP, HR, or AFP were seen.

We conclude that GB diffuses poorly across the placental barrier into the fetal lamb from maternal circulation, and that the central α_2-adrenergic hypotensive actions are not expressed in fetal lambs, as they are in adult sheep.
(Supported by NIH grant HL34460 and BGSM-WFU graduate school)

118.2

N-METHYL-D-ASPARATATE RECEPTOR ACTIVATION IS REQUIRED FOR DEVELOPMENTAL EXPRESSION OF THE CAT-301 ANTIGEN. Robert G. Kalb and Susan Hockfield, Section of Neuroanatomy, Yale University, New Haven, CT 06510

Neuronal activity in early postnatal life can have a profound influence on the development of neuronal properties. The N-methyl-D-aspartate (NMDA) class of glutamate receptor has been implicated in this process, but the molecular events which follow receptor activation and lead to alterations in neuronal characteristics are largely unknown. We have used monoclonal antibody Cat-301 to identify a chondriotin sulfate proteoglycan whose expression requires NMDA receptor activation during early postnatal life. The Cat-301 antigen is a good candidate for NMDA-regulated expression because previously we have shown that its expression on motor neurons in the hamster spinal cord depends on neuronal activity during a circumscribed period in postnatal life.

Neonatal hamsters were administered a range of doses of the NMDA-receptor antagonist MK-801 from postnatal day 7 (P7) to P21. Normally all sciatic motor neurons are Cat-301 positive by P14. In MK-801-treated animals, Cat-301 expression on motor neurons was inhibited in a dose-dependent manner. Two other motor neuron antigens were unaffected by MK-801 treatment. Adult animals treated with MK-801 for two weeks continued to express Cat-301 on all motor neurons. These results demonstrate a temporally-restricted period in motor neuron development during which the expression of the Cat-301 antigen is sensitive to NMDA receptor blockade. The Cat-301 antigen provides a positive molecular marker for NMDA-mediated development of motor neurons.
Supported by EY06511 (S.H.) and NS01247 (R.G.K.)

118.3

DEVELOPMENT OF CHOLINERGIC TERMINALS IN RAT STRIATUM AS VISUALIZED BY [^{3}H]HEMICHOLINIUM-3 AUTORADIOGRAPHY. H.K. Happe and L.C. Murrin. Dept. of Pharmacology, Univ. of Nebraska Med. Ctr., Omaha, NE 68105.

Cholinergic terminals can be quantitatively analyzed at the light microscopic level by use of [^{3}H]hemicholinium-3 (HC-3) autoradiography. HC-3 binds with high affinity and specificity to the high affinity choline transport site, a specific marker for cholinergic neurons. We characterized HC-3 binding (Sandberg & Coyle, Brain Res. 348:321, 1985) to slide mounted tissue sections from 5, 10, 15 and 21 day old rats. Binding is saturable (K_D = 10 nM), reachs equilibrium by 20 min at RT, is sodium-dependent and is inhibited by choline. Specific HC-3 labelling of tissue sections increases 3-fold between 5 and 21 days of age. Quantitative autoradiographic analysis finds a 6-fold increase in HC-3 binding in certain regions of striatum over this same time period. There is a 4-fold lateral to medial gradient in the body of the striatum in all animals 5 to 21 days of age. There is also a large rostral to caudal gradient in receptor density. The head of the striatum is relatively homogeneous in labelling. The shell of the accumbens and the olfactory tubercle are also heavily labelled. A striosomal pattern is apparent in caudal portions of the striatum at 5 days of age, coincident with patterns for mu opiate receptors and acetylcholinesterase. By 21 days of age this striosomal pattern of HC-3 labelling is no longer apparent. Supported by NS23975 and a McDonald Fellowship.

118.4

EFFECTS OF NEONATAL 6-OHDA LESIONS ON DOPAMINE RECEPTORS, DOPAMINE AND DOPAC IN STRIATUM. L. Arqueros* and L.C. Murrin. Dept. of Pharmacology, Univ. Nebraska Md. Ctr., Omaha, NE 68105.

As a basis for studies on the importance of dopaminergic input to the development of other neuronal systems in the striatum, we carried out a detailed study on the effects of 6-hydroxydopamine (6-OHDA) lesions on the development of markers for the dopamine system. Two-day old rat pups were administered desmethylimipramine (20 mg/kg i.p.) and 60 min later 50 μg of 6-OHDA was injected into each lateral ventricle in a volume of 5 μl over 5 min followed by a 5 min wait. From 4 to 30 days of age dopamine (DA) and 3,4-dihydroxyphenylalanine (DOPAC) were undetectable in striatum of most pups and means were <1% of controls, indicating no recovery of DA. Norepinephrine levels in hippocampus were unaffected and were reduced 30-40% in striatum. D1 receptor number is unchanged at 4 days of age but is reduced by 30% in 15 and 30 day animals. D2 receptor number remains statistically unchanged from 4 to 30 days of age. These data suggest that D1 and D2 receptors are affected differently in development by DA terminals and presumably DA release. This further supports the idea that the development of these two receptors is regulated by different mechanisms. Supported by NS23975 and the Burroughs Wellcome Fund.

118.5

DIVERSE DEVELOPMENTAL PATTERNS OF RECEPTOR BINDING IN RAT NEOCORTEX. J.L. Fuchs. Dept. of Biol. Sciences, Univ. of North Texas, Denton, TX 76203.

This study investigated the ontogeny of receptor binding in relation to underlying features of the rat neocortex. Autoradiographs of in vitro labeled sections were prepared from Long-Evans rats ages P0 through adult. While all of the ligands examined showed developmental changes in laminar distribution, some also showed radial transitory patterns associated with identified cortical areas. The only radial pattern extending through all laminae was that of [^{125}I]α-bungarotoxin binding, which labeled all primary sensory cortical areas as early as P2-4. Radial patterns for other ligands emerged 1-4 days later, but were confined to laminae IV and the V-VI border. [^{3}H]Muscimol was dense in area 17, SI and areas anterior to SI, [^{3}H]imipramine marked SI, area 17 and TeI, and [^{3}H]nicotine labeling was heaviest in area 17. Other ligands, including [^{3}H]QNB, [^{3}H]flunitrazepam and [^{3}H]phorbol 12,13-dibutyrate, did not reveal obvious distinctions between cortical areas. The observed radial differences were maximal around P8-12 and then "faded" during the 3rd week so that little if any contrast remained between adjacent areas in adults. The present survey is compatible with a heterogeneous, dynamic picture of developing neocortex in which various neurotransmitter systems gain and recede in prominence as they vie with each other for cortical territory.

118.6

DOPAMINE RECEPTOR ONTOGENY IN MOUSE CNS TISSUES. B.D. Perry, M. Wainwright*, L. Won, A. Heller and P.C. Hoffmann. Depts. Psychiatry, Pharmacol/Physiol Univ Chicago, Chicago, IL 60637

As part of studying determinants of D-1 and D-2 dopamine receptor expression we have examined 125I-SCH 23982 (SCH:D1) and 125I-Spiperone (SPIP:D2) binding sites in developing mouse brain regions (caudate: CS; cortex: CX ros. mesenceph. teg.: RMT). At embryonic day 16 (ED 16) specific binding sites for both ligands are detectable (for each, Bmax approx. 0.05 fmol/mg prot.). The ligands have lower affinities for these sites than in mature CNS (e.g. SCH Kd for ED 16 CS 4.0 nM and 0.4 nM in 28 day postnatal). The density of sites increases steadily through 28 day postnatal. This is the first report that we are aware of that examines prenatal ontogeny of D1-receptor binding sites. These studies will facilitate examination of the developmental determinants of phenotypic expression of dopamine receptors.

Support: MH-28942 and Brain Research Foundation

118.7

FUNCTIONAL EFFECTS OF PRENATAL COCAINE EXPOSURE. L.A. Freed, T.A. Fico, H.E. Hughes and D.L. Dow-Edwards. Lab of Cerebral Metabolism, Dept of Neurosurgery, SUNY-Health Science Center, Brooklyn, NY 11203 and Department of Developmental Psychobiology, NY State Psychiatric Institute, NY, NY 10032.

Infants prenatally exposed to cocaine are reported to have neurobehavioral deficits. Recent animal studies have suggested direct effects of cocaine on brain development. Following exposure to cocaine during critical periods of postnatal development in the rat (days 1-10 or days 11-20), we found long-lasting changes in functional activity in several brain regions (Dow-Edwards, et al., Dev. Brain Res. 42:137, 1988) and (Soc Neurosci abstr, 290.15, 1988). We have now quantified (^{14}C) 2-deoxyglucose patterns in rat brain following prenatal cocaine exposure and correlated these findings with D_1 and D_2 receptor binding.

Wistar rats were mated and housed under standard laboratory conditions. Dams received either 0 or 60 mg/kg cocaine-HCL po, from gestional day 8 through 22. Pair-feeding and surrogate fostering techniques were used. At 58-64 days of age, local rates of glucose utilization were determined in only the male offspring (1/litter) using the deoxyglucose method of Sokoloff et al. (J. Neurochem. 28:987, 1977). Selective tritiated D_1 and D_2 receptor ligands were used to correlate altered dopamine receptor concentrations autoradiographically in discrete brain regions in these same animals. Compared to metabolic responses in females receiving cocaine during the neonatal period, prenatal cocaine appears to produce minimal alterations in patterns of brain metabolic activity in male offspring.

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118.8

REGIONAL DEVELOPMENT OF DOPAMINE AND MUSCARINIC RECEPTORS IN RAT BRAIN. J. K. Rowlett*[1], M. A. Rice*[2], S. A. McDougall*[1], M. T. Bardo[1] and N. W. Pedigo.[2] (SPON: J. F. Zolman[1]). [1]Depts. of Psychology, [2]Pharmacology and Anesthesiology, Univ. Kentucky, Med. Ctr., Lexington, KY 40536.

The purpose of the present study was to characterize muscarinic and dopamine-1 (D-1) binding sites in the developing rat anterior striatum, medial prefrontal cortex and hippocampus-entorhinal cortex. Ages were based on those commonly used in behavioral ontogeny studies: 11, 17, 28, and 35 days after birth. ^{3}H-SCH 23390 (0.2-2.0 nM) in the presence or absence of 10 or 100 uM (+)-butaclamol, was used to define D-1 binding, with mianserin (100 nM) added to mask serotonin-1C sites. ^{3}H(-)quinuclidinyl benzilate (^{3}H-QNB; 20-500 pM) in the presence or absence of 10 uM atropine was used to define muscarinic binding. Saturation studies were done at equilibrium for each age and region. D-1 receptors reached adult levels between 17 and 35 days after birth, whereas muscarinic receptor density generally increased throughout development. For example, adult levels of D-1 binding in striatum were 2393 ± 45 fmol/mg protein, but binding in 11, 17, 28 and 35 day old rats was 68, 69, 138 and 130% of adult, respectively. Striatal levels of muscarinic binding were 5203 ± 763 fmol/mg protein in adults and 45, 55, 81 and 66 % in the neonatal rats. Significant differences were due to increased receptor concentration without age-related changes in receptor affinity. The differential development of muscarinic and D-1 receptors may account for behavioral differences between neonatal and adult rats.

118.9

IMMUNOSUPPRESSION BY PRENATAL DIAZEPAM.
M. Schlumpf, *H.R. Ramseier, W. Lichtensteiger, *G. Seiler and **J.B. Baumann. Institute of Pharmacol. and *Inst. of Immunol. and Virol. Univ. Zürich and **Kantonsspital Basel, Switzerl.

When administered between gestational day (GD) 14 and 20 diazepam (1.25 mg/kg) as well as clonazepam (0.25 mg/kg, s.c.), a selective ligand for the central benzodiazepine (BDZ) receptor, suppressed cellular immune responses in male and female Long Evans rat offspring, i.e. the lymphocyte proliferative response to allogeneic and polyclonal stimuli. Treatment of the pregnant animal with the peripheral type agonist Ro 5-4864 did not consistently affect immune responses in offspring, although peripheral BDZ receptors are present in fetal brain and periphery. The involvement of the central type benzodiazepine receptor suggests a neuroendocrine link. However corticosterone plasma levels in fetuses at GD 20, 1 hour after the last drug injection, were significantly decreased only in females, whereas the immune depression was seen in both sexes. At PN 4 female pups but not male had significantly increased plasma hormone levels. The postnatal rebound of plasma corticosterone might have contributed to the immunosuppressive effects but seemingly represents not a main factor in suppression of T cell responsiveness.

118.10

DEVELOPMENTAL EXPRESSION OF ENKEPHALIN CONTAINING SYSTEMS IN THE RAT BRAIN.
T.T. Quach, C. Llorens-Cortes*, J.C. Schwartz*, R.J. Wyatt, A.M. Duchemin INSERM U. 109, 2ter rue d'Alésia, 75014 Paris (France), NIMH St Elizabeths Hospital, Washington D.C. and School of Medicine, Ohio State University.

The expression of the gene encoding for enkephalin (Enk) was studied pre- and post-natally in rat brain with a pre-pro-Enk specific cDNA probe to quantitate mRNA. In addition, specific RIAs were used to measure the levels of met-enkephalin and of Tyr-Gly-Gly (YGG), its principal metabolite. Poly A^+ enriched RNA from rat brain was analyzed for the presence of Enk-mRNA by agarose gel electrophoresis, followed by transfer to mitrocellulose and hybridization to a ^{32}P-labeled Enk-cDNA probe. Brain pre-pro-Enk mRNA detectable by embryonic day 14 represented more than 30 % of adult levels. It increased continuously until post-natal day 28 when it reached maximal concentrations. In young adult rats, level was decreased. Enk and YGG immunoreactive peptides detected in the samples from embryonic day 12, represented respectively 0.05 % and 0.78 % of the adult level. Marked development of peptides was seen only post-natally with a rapid increase occurring within the first 2 weeks after birth. The highest contents of the peptides were present in young adult rats.

118.11

THE DEVELOPMENT OF THE OXYTOCIN RECEPTOR IN RAT FOREBRAIN: CHEMICAL NEUROANATOMY AND BEHAVIORAL CORRELATIONS.
T.R. Insel, L. E. Shapiro*, and D. Dang*. Section on Comparative Studies of Brain and Behavior, Laboratory of Clinical Science, NIMH, Poolesville, MD 20837.

The ontogeny of oxytocin receptors in rat forebrain was studied using the selective oxytocin receptor antagonist, ^{125}I-d(CH_2)$_5$[Tyr(Me)^2,Thr^4,Tyr-NH_2^9]OVT ([^{125}I]-OTA). With *in vitro* receptor autoradiography, binding was noted on the first postnatal day in dorsal subiculum and thalamus. From postnatal days 5-18, intense labeling was evident in posterior cingulate cortex, dorsal subiculum, lateral septum, and the CA1 subfield of hippocampus. Of these regions only the lateral septum expressed oxytocin receptors in adult brain. Competition studies on coronal sections through posterior cingulate, septum, and dorsal subiculum at P10 demonstrated that transient binding sites in these areas were indeed oxytocin selective (OXY>AVP>V_1>V_2). Results from saturation studies on cingulate membranes from 10-day-old pups agreed favorably with previous reports of the kinetics of [^{125}I]-OTA binding to adult oxytocin receptors, K_d = 0.099 nM in P10 cingulate cortex vs. 0.073 nM for adult ventral subiculum (Elands et al., 1987). In contrast to these evanescent developmental sites, oxytocin receptors in the bed nucleus of the stria terminalis and the ventromedial nucleus of the hypothalamus only appeared in adulthood, presumably in response to the surge of gonadal steroids at puberty.

To determine if oxytocin receptors during development were functionally responsive to exogenous peptide, oxytocin was administered intracerebroventricularly to pups at ages 5-7 days. Injection of oxytocin (0.1 µg) resulted in an 80% decrease in the rate of ultrasonic calls following social separation without altering locomotor activity. This effect was blocked by co-administration of the selective oxytocin antagonist, [d(CH_2)$_5$Tyr(Me)^2, Orn^8]-vasotocin, at a dose which lacked intrinsic effects.

These results demonstrating a marked, transient increase in the expression of oxytocin receptors during development and a decrease in the response to social separation following oxytocin administration, suggest a unique role for this peptide during development.

118.12

ONTOGENY OF α_2 RECEPTOR SUBTYPES IN RAT BRAIN.
H.K. Raymon and F.M. Leslie, University of California, Irvine, CA. 92717.

Monoamines may play a neurotrophic role in early ontogenesis by regulating cell differentiation in monoamine receptive cells. To investigate possible sites of noradrenergic action in development, the ontogeny of α_2 adrenoceptor subtypes and norepinephrine (NE) uptake sites were examined in rat brain using *in vitro* autoradiography. Brain sections from developing rats were incubated with [^{3}H]desmethylimipramine (DMI), which labels NE uptake sites, in the absence and presence of 10 µM nortriptyline to define nonspecific binding. α_2 binding sites were defined with either [^{3}H]idazoxan (which labels the total population of α_2 binding sites) or [^{3}H]rauwolscine (which labels only a subpopulation of α_2 binding sites). Specific binding was defined as the difference in binding in the absence and presence of 10 µM phentolamine. [^{3}H]DMI uptake sites are present at postnatal day (PND) 1 in the septum and the anterior olfactory nucleus. By PND 4, binding sites are visible in several areas, including the bed nucleus of the stria terminalis, septum, the vertical limb of the diagonal band and anterior cingulate cortex. The α_2 receptor subtypes show a differential distribution during development. [^{3}H]Idazoxan binding sites are visible at PND 1 in the anterior olfactory nucleus, the frontal cortex, the caudate-putamen, several thalamic nuclei and the hippocampus. In contrast, [^{3}H]rauwolscine binding sites are not seen in the thalamus on PND 1, indicating a differential distribution of sites. They are, however, seen in other areas labeled by [^{3}H]idazoxan. Preliminary data indicate that there is a transient, laminar distribution of rauwolscine binding sites in cerebellum by PND 14, which disappears by PND 21. This suggests that the rauwolscine binding sites may play a role in cerebellar development. Developmental processes in regions which exhibit [^{3}H]DMI uptake sites and α_2 receptor binding may be under the influence of endogenous NE systems. In addition, the differential appearance of the α_2 binding sites is consistent with the existence of α_2 adrenoceptor heterogeneity. *Supported by NIH NS 19319 and NIH 1F31MH09737.*

118.13

OPEN FIELD ACTIVITY IN OFFSPRING OF DIAZEPAM-TREATED MALE MICE.
A. Márquez-Orozco*, M. C. Márquez-Orozco*, M. Ramos-Avila* and R. A. Prado-Alcalá (SPON: B. Ortega). Embryol. Dept. and Physiol. Dept., Med. Sch, Natl. Univ. of México, México, D.F., México 04510.

To assess if diazepam (DZP) administered to males produces motor impairments to their offspring, male CD-1 mice were given DZP (2.7 mg/kg, i.p.) daily during six weeks. Immediately afterwards they were mated with females. Five months after birth, the litters (males and females) were tested in an open field (100 x 100 x 20 cm, divided into 9 squares of equal area). Litters of saline treated males were also studied. They were habituated for 5 min, and the number of crossings between squares was measured during the next 5 min. All mice derived from DZP-treated males displayed a significantly higher exploratory behavior than the control mice. In other groups of the same characteristics as the ones described, scopolamine (SCOP, 6 mg/kg) or saline solution was injected (I.P.) 5 min before habituation: in all cases SCOP induced an increase in motor activity. These results suggest that DZP administered to males before mating, may produce behavioral alterations in their offspring.

118.14

ULTRASTRUCTURAL ALTERATIONS IN THE RETINA OF MICE FETUSES PRENATALLY EXPOSED TO DIAZEPAM.
M.C. Márquez-Orozco, A. Márquez-Orozco and V. Gazca-Ramírez. (Spon: H. Brust-Carmona). Embriol. Dept. Sch. of Med. Natl. Univ. of México, México 04510, D.F, México.

Diazepam accumulation during gestation in the retinas of mice fetuses delays cellular differentiation. We investigated if diazepam causes ultrastructural changes that could account for this delay. Three groups of gestating mice of the CD-1 strain were injected, i.p., between the 6th and 15th day of gestation, either with single daily doses (2.7 mg/kg b.w.) of diazepam (D), the vehicle (V), or 0.9% saline solution (S). On the 16th day, the mice were sacrificed by beheading, the fetuses removed, and perfused intracardially with 1% paraform-aldehyde and 2.5% glutaraldehyde, the eye globes were postfixed in OsO_4 and embedded in epoxic resin. Histological sections were contrasted with uranyl acetate and lead citrate and observed under a transmission microscope Zeiss EM-10. Nuclear density per area was greater in the D fetuses than in the S and V fetuses ($p < 0.05$). Chromatin was atipically distributed in the nuclei, the rough endoplasmic reticulum showed distended cisternae; the Golgi Complex, the mitochondrias, and the polyribosomes were more abundant; the photoreceptors' microvilli were disorganized. These ultrastructural changes were not observed in groups V and S. The greater nuclear density and the alterations of the cytoplasmic organelles could reveal disruptions in cell multiplication.

118.15

A FLOW CYTOMETRIC ANALYSIS OF DEVELOPMENTAL CHANGES IN $GABA_A$ RECEPTOR FUNCTION IN EMBRYONIC CHICK SPINAL CORD CELLS. A. Prasad*, S.V.Smith*, G.D. Lange and J.L. Barker, (SPON: H. Lansdell). Laboratory of Neurophysiology, NINDS, NIH, Bethesda, MD 20892.

Suspensions of acutely dissociated spinal cord cells from chick embryos were incubated with a voltage-sensitive oxonol dye and analyzed using flow cytometry. In a series of experiments, the Na^+/K^+ ionophore gramicidin was added to oxonol-stained cell suspensions, while varying the ionic conditions. The analysis of these results provided a basis for correlating changes in oxonol fluorescence, as measured by flow cytometry, with changes in cellular membrane potential.

Membrane potential responses to GABA and muscimol, a $GABA_A$ agonist, were probed in cells from animals ranging in age from embryonic day 3 (E3) to E13. There was a major subpopulation of cells scattering a significant amount of light, which, beginning at E4, depolarized in response to muscimol and GABA. By E12 however, much higher doses of agonist were routinely required to produce any shifts in oxonol fluorescence.

Both types of agonist-induced shifts were blocked by bicuculline, a competitive antagonist at $GABA_A$ receptors and picrotoxin which blocks a $GABA_A$ receptor coupled chloride conductance at the channel rather than the receptor level. We are investigating the possibility that some of the properties of $GABA_A$ receptor-coupled choride channels which emerge early in embryogenesis on spinal cord cells are transformed during development.

118.16

$GABA_A$ RECEPTOR-MEDIATED RESPONSES IN RAT HIPPOCAMPAL CELLS CHANGE THEIR PROPERTIES DURING DEVELOPMENT. J.L.Barker, M.L.Fiszman*, E.A.Novotny* and G.D.Lange Laboratory of Neurophysiology, NINDS, NIH, Bethesda, MD 20892.

Embryonic and early postnatal rat hippocampi were papain digested in order to obtain cell suspensions suitable for analysis in a fluorescence-activated cell sorter (FACS). Oxonol (DiBa-C4(3)), a voltage-sensitive indicator dye, was used to detect membrane polarization changes.

In embryonic hippocampal cell suspensions nanomolar concentrations of GABA and muscimol depolarized all cells scattering significant levels of light in a dose-dependent manner with a threshold concentration of 10nM. This effect was prevented by preincubation with bicuculline or picrotoxin. In hippocampal cell suspensions obtained from 5-7 day old pups $GABA_A$ agonists depolarized one subpopulation and hyperpolarized another. The threshold concentration to induce this effect was ten times higher than in prenatal preparations. At all the ages tested (-)pentobarbital failed to modulate GABA-induced responses. However, it did induce a GABA like effect with concentrations ranging from 50 to 200μM. This effect was blocked by bicuculline or picrotoxin.

Our results indicate that there is a change in the responses to $GABA_A$ agonists as well as in their affinity during development. The low effective concentrations of the agonists suggest that a high affinity state of the receptor is functionally operative in early stages of development.

118.17

THE DEVELOPMENTAL EXPRESSION OF GABA PRECEDES GAD IN THE RAT SPINAL CORD. T.N. Behar*, A.E. Schaffner, S. Nadi and J.L. Barker, (SPON: M. Freed) LNP, LN, NINDS, NIH, Bethesda, MD 20892.

Developing rat spinal cord was analyzed for the presence of GABA and GAD by immunocytochemistry and HPLC. Levels of GABA determined biochemically paralleled the development of a GABAergic phenotype in acutely dissociated cells. GABA was first detected by HPLC and indirect immunofluorescence at embryonic day (E)13. The concentration of GABA and the number of GABA+ cells began to increase at E15. By E19 the level of GABA reached 200 nmoles/mg protein and remained at this level through postnatal day (PN) 5. During the same period the number of GABA+ cells increased to 15-30% of the total cell population. Double labeling with anti-neurofilament (NF) antibodies revealed that the GABA+ cells were also NF+. Over the period E15 through PN7, the number of NF+ cells expressing GABA increased dramatically such that by PN7 >50% of the NF+ cells were GABA+. The pattern of GABA staining also changed during development. Initially, GABA was diffusely distributed throughout the cytoplasm and processes of the cells. A more punctate pattern was evident in some cells at PN7 and by PN21 virtually all GABA+ cells displayed the punctate pattern of GABA staining. GAD was first detected by immunofluorescence at E21 in 1% of the cells and initially appeared in a perinuclear patchy pattern. GAD expression increased such that by PN 14, 50% of the GABA+ cells were also GAD+. At PN21 all GABAergic cells were GAD+ and comprised 5% of the total population. When various regions of the cord at E17 were dissociated and stained for GABA, there was a distinct rostral to caudal and ventral to dorsal gradient of GABA expression. Detection of GABA before GAD suggests that GABA is synthesized through an alternative pathway, such as polyamines, and that GABA may play a role in differentiation or morphogenesis of the spinal cord.

118.18

METABOLISM OF DOCOSAHEXAENOIC ACID (DHA) IN BRAIN AND RETINA IN NEONATAL MOUSE. **F. Cai* and N.G. Bazan (SPON: K. Kratz). LSU Eye Center and Neuroscience Center, New Orleans, LA 70112.**

DHA is a fatty acid that is uniquely and rapidly accumulated during synaptogenesis and photoreceptor membrane biogenesis. Its physiological role and metabolism are still unclear. The synthesis of DHA, and its supply to brain and retina, were studied using ^{14}C-linolenic acid (18:3), given intraperitoneally (IP) or orally to 3-day-old mice. Mice were killed from 10 min to 4 h later. Liver (L), serum (S), brain (B), retina (R), and gastrointestinal (GI) tissue were removed for lipid analysis, and analyzed by TLC into different lipid classes and by HPLC for conversion of 18:3 to DHA. ^{14}C in lipids appeared in S and L within 10 min after IP injection, peaked at 30 min, and then declined. Oral ^{14}C peaked at 60 min in GI. Thirty min after oral administration, 67% of the ^{14}C was in phospholipids (PL) in B, and 62% in R; in L most of the ^{14}C in triacylglycerols (TG) remained as 18:3, but in PL at 2 h 40% of the 18:3 in TG had been converted to DHA and 10% to 22:5, an intermediate in DHA metabolism. In B only 10% of the ^{14}C was 18:3 at 1 h, while 20% was 20:5 and 6% was 22:5. These results suggest that DHA is synthesized in liver (as 22:6-PL) and supplied to neuronal membranes via the bloodstream. (Supported by NIH EY04428)

NEURAL-IMMUNE INTERACTIONS: STRESS

119.1

GLUCOCORTICOID BINDING IN IMMUNE TISSUES IS LESS AFFECTED BY STRESS LEVELS OF CORTICOSTERONE COMPARED TO BRAIN. A.H. Miller*, R.L. Spencer, M. Stein, B. McEwen (SPON:S. Barclay). Mt. Sinai Med. Cntr., NY,NY 10029 and Rockefeller Univ., NY,NY 10021.

Glucocorticoids may be important in neural-immune interactions. Therefore, Type I and II glucocorticoid receptor (GR) binding was measured concurrently in thymus,spleen,hippocampus (HC) and pituitary (PIT) after various neuroendocrine manipulations. Sprague-Dawley rats were divided into 3 treatment groups (N=3 per group): 1) 16 hr adrenalectomy (ADX), 2) intact/no stress and 3) intact + 1 hr restraint stress. For ADX rats, Type I binding was absent in thymus and low in spleen relative to HC and PIT. Type II binding in ADX rats was highest in thymus, intermediate in spleen and lowest in HC and PIT. After restraint, Type I binding was absent in spleen and HC and very low in PIT. Compared to intact rats, Type II receptor level of stressed rats was significantly reduced in HC but unchanged in thymus, spleen and PIT. Likewise, the Type II Kd in HC after stress was greatly increased compared to nonstressed rats. A much smaller elevation in Kd was noted in thymus,spleen and PIT after stress. In sum, stress levels of corticosterone appear to have a lesser effect on GR binding in thymus, spleen and PIT than in HC.

119.2

EFFECTS OF HANDLING ON IMMUNE REACTIVITIES OF BALB/C MICE

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We have studied the specific and non-specific immunological consequences of psychosocial manipulation in an animal model. BALB/c.ByJ mice were picked up and held for 2 minutes/day for 2 weeks prior to being: injected intravenously with $1x10^5$ syngeneic Line 1 lung carcinoma cells; injected with intraperitoneally with 100 μg of the protein antigen keyhole limpet hemocyanin (KLH); or evaluated for splenic NK cell cytotoxicity and T cell proliferation. This handling protocol was consistently associated with the appearance of a significantly increased number of Line 1 metastases in the lungs of the handled animals relative to home caged unhandled controls (no differences were noted when animals were handled after tumor cell injection). This handling protocol was also associated with decreased IgG anti-KLH antibody responses. Glucocorticoid levels were higher 30 minutes after KLH injection than they were immediately before injection in the unhandled animals, but not in the handled mice. Finally, 2 weeks of daily handling has been consistently associated with decreased Con A-induced T cell proliferation, but not with increased in vitro NK cell cytoxicity.

119.3

STRESS MODULATES SENSORY EVOKED POTENTIALS FROM THE HYPOTHALAMUS, HIPPOCAMPUS AND SUPERIOR COLLICULUS. J. Casada*, R. Perry*, and N. Dafny. (SPON: R. Guynn) Dept. Neurobiol. and Anat., The University of Texas Medical School at Houston, 77225.

Stress has been shown to modulate immunity in experimental animals and in human subjects. The present experiment was designed to investigate whether single or repeated exposure to an acute stressor elicits changes in sensory evoked potentials recorded from brain sites implicated in neuroimmune modulation using freely behaving rats. Thirty male Sprague Dawley rats were permanently implanted with 120 μm stainless steel semimicroelectrodes in the dorsal hippocampus (DH), medial basal hypothalamus (MBH), and superior colliculus (SC). Animals were restrained for four hours per day on each of four consecutive days. Four sets of 32 averaged visual evoked responses were recorded on the day prior to the first restraint (control) and on the first and fourth day of restraint. Restraint generally elicited an increase in the amplitude of sensory evoked potentials recorded from the MBH, DH, and SC without changing the neuronal recovery function. A persistent increase in the averaged evoked potential amplitudes seen on both the first and fourth daily presentation of the stressor indicates that the animals did not adapt to the stressor as measured electrophysiologically.

119.4

CONDITIONED STRESSOR INDUCED ALTERATIONS OF IMMUNE FUNCTION: EFFECTS OF CONDITIONED EXCITATORY AND INHIBITORY STIMULI. DT Lysle, JE Cunnick, BJ Kucinski*, H Fowler* & BS Rabin. Depts. of Pathology and Psychology, Univ. of Pittsburgh, Pittsburgh, PA 15213.

Our research program is designed to investigate the effect of psychological stress, in the form of a conditioned aversive stimulus (CS) based on electric shock, on different compartments of the immune system. The present research used Lewis rats to evaluate the effect of a CS on the mitogenic response of lymphocytes from the blood, spleen, and lymph nodes. The blood and spleen lymphocytes showed suppression of mitogenic responsiveness to Con-A and PHA. In contrast, lymphocytes derived from the lymph nodes were unaffected by exposure to the conditioned stimulus. There was also a reduction of natural killer cell activity for spleen lymphocytes. The reduction in immunoresponsiveness of spleen and blood lymphocytes was not the result of a decrease in interleukin-2 (IL-2) production, as those cells produced normal levels of IL-2. We also have extended our assessments to include other forms of conditioning such as conditioned inhibition (i.e. a signal which predicts the absence of shock and thus inhibits fear by inducing relief or relaxation). We assessed whether a conditioned inhibitor would attenuate the suppression of lymphocyte responsiveness produced by a signal for the shock, or on its own enhance immune function.

119.5

THE EFFECT OF DIFFERENTIAL HOUSING ON ANALGESIA, TASTE AVERSION, AND NATURAL KILLER CELL CYTOTOXICITY IN TWO MOUSE STRAINS

K.S.Kelly, L.J. Grota, and R.Ader*. Dept. of Psychiatry, University of Rochester Medical Center, Rochester, NY, 14642.

Female Swiss Webster (SW) and C57Bl/6J mice were shipped at weaning, housed singly or in groups of five, and remained undisturbed for 10 weeks. The mice then received access to chocolate milk or water for 1 hr and were injected s.c. with morphine sulfate (150 mg/kg) or saline. Analgesia testing on the conditioning day yielded a significant morphine effect in both strains. SW mice exhibited a differential housing effect with group housed animals showing shorter response latencies. One week later, all mice were tested for taste aversion. Only the SW mice exhibited a conditoned aversion with no differential housing effect observed. A second conditioning trial was given the following week. Prior to the morphine injection, a main effect of housing was observed; individually housed mice consumed more chocolate milk. The taste aversion seen in SW mice had extinguished. A final test trial showed the conditioned taste aversion was not reinstated in SW mice; C57B1/6 mice still failed to show an aversion to the chocolate milk. Thus, this dose of morphine had a strain dependent effect on taste aversion. The weak aversion shown by SW mice extinguished in one trial and was not reinstated with a second conditioning trial. Finally, mice were sacrificed and spleens assayed for NK cell cytotoxic activity. Differential housing had a significant effect on NK cell cytotoxicity, with individually housed mice showing enhanced responses relative to group housed animals.

119.6

ROLE OF GLUCOCORTICOIDS AND CATECHOLAMINES IN THE MEDIATION OF SHOCK-INDUCED SUPPRESSION OF T-LYMPHOCYTE MITOGENIC RESPONSIVENESS. JE Cunnick, DT Lysle, H Fowler, and BS Rabin. Div. of Clinical Immunopathology, Dept. of Pathology, Univ. of Pittsburgh, Pittsburgh, PA 15213.

We have previously shown that presentations of mild-footshock to Lewis rats can suppress splenic and peripheral blood responses to non-specific T-cell mitogens. Although the mechanism(s) mediating the in-vivo suppression have not been identified, glucocorticoids and catecholamines are immunosuppressive in-vitro. Testing of adrenalectomized, sham operated and normal, unoperated rats demonstrated that adrenalectomy prevented the suppression of the mitogenic response of peripheral blood T-cells but only slightly attenuated the suppression of splenic T-cells. The beta-antagonists, propranolol and nadolol were both capable of attenuating the shock-induced immune suppression of splenic cells in a dose dependent manner. Furthermore, neither drug was able to attenuate suppression of the blood mitogen response.

These data indicate that, in-vivo, there are two distinct mechanisms mediating the suppression of T-cell responsiveness to mitogens in the spleen and the peripheral blood. As nadolol does not cross the blood/brain barrier, it suggests that the peripheral release of catecholamines is responsible for splenic immune suppression.

119.7

STRESS-INDUCED IMMUNOMODULATION: I. BASIC PARAMETERS. LR Watkins, M Fleshner*, DR Rager, DA Warren*, C Broussard*, D Bellgrau, SF Maier & M Laudenslager. Psych. Dept., U CO Boulder, CO 80309 HSC U CO Denver, CO 80204 & 80262.

This lab has previously reported that inescapable tail-shock can suppress in vivo IgG production against s.c. lyophilized keyhole limpet hemocyanin (KLH). In order to (a) study splenic rather than lymph node response & (b) attain temporally discrete in vitro spleen placque and in vivo IgM & IgG responses, we are now examining the antigenicity of i.p. and i.v. soluble KLH in rats. Plasma samples were obtained by tail bleed on days 3, 5, 7, 9, & 14 following KLH injection. Dose dependent elevations of anti-KLH IgM & IgG were found (ELISA) following 100, 500, & 1000 ug KLH. I.V. KLH produced larger increases in IgM & IgG than did equal doses of i.p. KLH. The IgM response was also far more temporally discrete following i.v. than i.p. KLH, with a sharp peak in IgM response occurring 5 days after i.v. KLH. Preliminary spleen placque assay results support these findings. Confirming and extending this lab's previous results, 100 inescapable tail shocks delivered immediately after 100 ug i.p. KLH led to significant decreases in anti-KLH IgM & IgG. Ongoing studies with i.p. and i.v. KLH are aimed at identifying the critical temporal relationship(s) between shock delivery and KLH administration required for immune alterations to occur. Once the critical period(s) is/are identified, the effect of escapable vs. inescapable shock will be examined and the potential role of pituitary-adrenal hormones assessed (see companion abstract). Supported by ONR00014-85K0211 and NSF BNS-8808840.

119.8

STRESS INDUCED IMMUNOMODULATION: II. PITUITARY-ADRENAL INFLUENCES.

M. Fleshner*, L. R. Watkins, M. L. Laudenslager & S. F. Maier (*upon D. M. Diamond*) Psych Dept. U CO Boulder, CO 80309 U CO HSC Denver, CO 80204.

Rats exposed to stressors show changes in immune function. One possible mechanism underlying these changes involves activation of the pituitary-adrenal axis. These studies investigate the role of ACTH and corticosterone (CORT) in stress-induced modulation of IgM and IgG production (described in Part II companion abstract). Because adrenalectomy induces widespread changes in physiological and neural function, we have avoided the potential confounds posed by this technique. Instead, three alternative methods for investigating pituitary-adrenal involvement are currently being pursued and each will be used to examine tail-shock induced immunosuppression. The first approach involves i.p. administration of dexamethasone (DEX) prior to shock. Using this method, we determined a dose of DEX that reduces stress levels of CORT as measured by RIA without significantly affecting the antibody response to s.c. KLH. The second method is intracerebroventricular (ICV) injections of DEX which, importantly, avoids directly exposing immune cells to this steroid. With multiple ICV injections prior to shock we found that CORT remained at basal levels during shock. A third method being developed involves surgical isolation of the adrenal glands followed by revascularization. This should allow the animal to release basal but not stress levels of steroids. An important advantage of these methods is that animals should be physiologically normal and have normal levels of circulating CORT at the time of clonal proliferation and antibody production when steroids seem important for immune function regulation. These techniques should allow us to clearly define the potential role of pituitary-adrenal factors in tail-shock induced immunomodulation. (N0014-85-K-0411 ONR, NSF BNS 8808840).

119.9

EVIDENCE FOR A CAUSAL RELATIONSHIP BETWEEN THE SUPPRESSIVE EFFECT OF STRESS ON NATURAL KILLER CELL ACTIVITY AND ENHANCED METASTATIC TUMOR GROWTH IN RATS S. Ben-Eliyahu, R. Yirmiya, R. P. Gale, and J. C. Liebeskind Dept. of Psychology, UCLA, Los Angeles, CA 90024

Acute stress can facilitate malignant tumor growth and can suppress the cytotoxic activity of natural killer (NK) cells. However, because stress can affect tumor growth in ways other than via the immune system, the causal relationships among stress, immune deficiency, and enhanced tumor growth are only suggestive. In this study, we used a malignant cell line, MABD106, which is syngeneic to Fischer 344 rats and was shown to be highly controlled by NK cell activity in vivo. Using a 2x2 design, Fischer 344 rats were injected with either naltrexone (s.c. 10mg/kg) or saline and were either subjected to intermittent forced swimming stress or were not stressed. In the first experiment, one hour after the end of the stress session rats were sacrificed, spleens were taken, and a 16 hour chromium release assay was used to measure splenic NK cytotoxic activity. In the second experiment, one hour after the end of the stress session rats were intravenously injected with 10^5 MADB106 cells in .5 ml PBS under halothane anesthesia. Twelve days later, lung surface metastases were counted. Stress significantly suppressed splenic NK cytotoxic activity against MADB106 and enhanced MADB106 metastatic growth in vivo. Naltrexone had no effect on either end point. Taken together with the previously established role of NK cells in limiting MABD106 lung metastases, this evidence supports causal relationships among stress, suppressed NK activity, and tumor development. (NIH grant NS07628)

119.10

RESTRAINT STRESS-INDUCED IMMUNOSUPPRESSION OF A LYMPHOKINE RESPONSE TO INFLUENZA VIRUS. J.F. Sheridan , N. Feng and B.S. Huneycutt (SPON: S. Travers). Depts. of Oral Biology, Medical Microbiology and Immunology, Ohio State Univ. Colleges of Dentistry and Medicine, Columbus, Oh. 43210.

Stress has been shown to inhibit a variety of immune responses in both experimental animals and humans. However, the health consequences of stress are still unclear. The purpose of this experimental study was to assess the effect of restraint-induced stress on the immune response to viral infection in the mouse. Mice (4 to 6 week old C57/B6, males) were infected intranasally with influenza A/PR8 virus. One group was stressed 16 hours (5:00 pm - 9:00 am) each day, for one day prior to infection and 14 days post infection. A second group was stressed for 6 hours (10 am - 4 pm) one week prior to infection and 14 days post infection. Control groups consisted of stressed, not infected and non-stressed, infected mice. Cells from lymph nodes and spleen were stimulated in vitro with influenza virus and the production of lymphokines, IL-2/IL-4, was measured. IL-2/IL-4 secretion in the stressed group was significantly reduced for both lymph node and spleen cells. The virus-specific response by mediastinal lymphocytes and splenocytes from the 16 hour stressed group was suppressed by 95.7% and 59%, respectively, when compared to the non-stressed group. Immunosuppression persisted at 25 days after termination of stress. Suppression of IL-2/IL-4 activity by 6 hour stress was not significant (20.6% for splenocytes) at 14 days after initiation of stress, while significant suppression was present two weeks after termination of stress.

119.11

DEVELOPMENT OF PRIMATE PSYCHOSOCIAL MODELS FOR NEUROIMMUNOMODULATION. W.B. Iturrian, J.L. Weed*, M.A. Hebert* and B.N. Bunnell. Pharmacology and Psychology Depts., Univ. Ga., Athens, GA 30602.

The objective was to develop nonhuman primate stress models correlating social, immune and neuroendocrine modulation strategies. We monitored cortisol; ACTH; PRL; PBL number; viability; and mitogen (PHA, Con A) responses as well as various aggressive, submissive and affiliative social behaviors. Social stress produced by adding a stranger into a group (N=42) depressed SIs acutely, with overshoot on day 4. The precise pattern varies with social rank and individual behavioral type. Increased aggressive and affiliative behaviors accompanied by reversals in rank increased the winner's Con A SI while the loser's decreased. Physical and social stressors caused complex biphasic cycles of immune status lasting weeks. Con A mitogen responses were related to social manipulation and behavior.

119.12

SOME LONG-TERM BEHAVIORAL AND IMMUNOLOGICAL EFFECTS OF BRIEF, EARLY MATERNAL SEPARATION IN NONHUMAN PRIMATES: PRELIMINARY OBSERVATIONS D.R. Rager*, M.L. Laudenslager, P.E. Held*, and M.L. Boccia*. Univ. of Colorado Health Sciences Center, Denver, CO 80204.

A number of well-documented behavioral and physiological changes occur acutely in response to maternal separation in nonhuman primate infants. These include behavioral agitation (increased activity and vocalization) followed by depression (decreased activity and social withdrawal). Physiological changes include alterations in heart rate, body temperature, EEG activity, and several measures of immune function. Preliminary data from our lab suggest that behavioral and immunological effects of early maternal separation may be observed up to 30 months later. The nature of these effects is dependent upon the particular macaque species studied. Additional pilot data regarding the ability of benzodiazepines (e.g., aprazolam) to modulate these long-term immunological changes will be discussed. (Supported in part by NIMH research grant MH37373 and a generous gift from the Upjohn Company).

119.13

EARLY SOCIAL ISOLATION IN RHESUS MONKEYS: LONG-TERM EFFECTS ON SURVIVAL AND CELL MEDIATED IMMUNITY. J.P. Gluck*, H. Ozer*, L.L. Hensley*, A. Beauchamp*, R.B. Mailman, and M.H. Lewis. (SPON: K. Suzuki) Biological Sciences Research Center and Departments of Psychiatry and Medicine, University of North Carolina, Chapel Hill, NC 27514, and Department of Psychology, University of New Mexico, Albuquerque, NM 87131

Considerable attention has been given recently to the effects of early life events on vulnerability to disease and psychiatric disorder. The behavioral effects of early social isolation in some non-human primates has been well documented. We have examined the survival rate and cell mediated immunity in a group of 18-23 year old rhesus monkeys, half of whom were socially isolated during their first 9 mos. of life. A comparison of survival rates indicated that the socially deprived monkeys had a significantly decreased survival rate, males being particularly vulnerable. Comparisons of the immunological phenotype between socially isolated and control animals were conducted on three separate occasions (the last following administration of the antigen tetanus toxoid) using monoclonal antibodies directed at human T-cell, B-cell, NK, LAK and macrophage phenotypes. A significant decrease in the ratio of helper to suppressor T cells was associated with early social isolation. A second effect of early social deprivation was a significantly increased percentage of natural killer (NK) cells. These findings, combined with preliminary data from functional studies, including mitogen stimulation and NK and LAK cell activity, provide important evidence to support the relationship between early psychosocial insult and life-long alterations in immune function. (Supported by PHS grant MH42938 and Center grants HD03110 and MH33127.)

119.14

EFFECTS OF SOCIAL SEPARATION ON T-CELL SUBSETS AND FECAL CORTISOL IN CEBUS MONKEY: RELATION TO NEUROTRANSMITTER ACTIVITY IN AMYGDALA. A. Kling, R. Lloyd, K. Tachiki, B. Ring*, and H. Prince*. Psychiatry Service, UCLA/VA Med. Center, Sepulveda, CA 91343, and American Red Cross.

We suggest that the influence of separation on the immune system may be mediated by the amygdaloid nuclei. Four Cebus monkeys (2 ♂ C. albifrons and 2 ♀ C. apella) were group-housed in an outdoor enclosure. We measured the T-cell subsets using flow cytometry during the social vs. separated condition. Samples obtained after two 3-5 day separations were compared with 5 samples during group housing. Blood and fecal samples for concentration of cortisol were obtained after each treatment condition. The major effects of social separation were an increase in the percentage of lymphocytes expressing a T-suppressor marker in all subjects. Further, the females showed an increase in T-helper percentages as well. Fecal cortisol concentrations were higher during separation compared to group housing and chair restraint. Subsequent analysis of brain neurotransmitters and metabolites obtained by microdialysis of the amygdala in the same subjects showed a robust and consistent increase in 5-HIAA and NE during separation. These findings suggest that social separation results in alterations of lymphocyte subsets along with an increase in brain serotonin and NE. This study was supported by the Joan B. Kroc Foundation and the Veterans Administration.

119.15

STRESSOR PREDICTABILITY AND IMMUNE FUNCTION. J. Irwin and N. Custeau*. Psychology Dept., Queen's University, Kingston, Ont. K7L 3N6, Canada.

The predictability of an aversive stimulus may be an important determinant of physiological or behavioral responses to stress. That is, when a light or tone signal precedes stressor onset, an organism may be able to adopt preparatory responses which buffer the aversive consequences of the stressor. Recently it was reported that this variable also influences stress-induced changes in immune activity, including mitogen-induced lymphocyte proliferation (Mormede et al, 1988). In the present study, the role of predictability in the mediation of stress-induced changes in Natural Killer (NK) cell activity was investigated.

NK activity was assessed in male C57BL/6J mice following exposure to 1 hr of intermittent inescapable footshock. For some animals shock onset was preceded by a 10 sec light CS (signalled shock), whereas for others, the light was not contiguous with shock onset (unsignalled). In addition, shock was presented either at regular intervals or on a variable interval schedule. Twenty-four hr later, splenic NK activity was assessed with a ^{51}Cr release assay. Compared to control animals, mice exposed to signalled shock at regular intervals exhibited a significant reduction of NK activity. Surprisingly, those in the unsignalled groups showed less NK suppression than those in the light-signalled shock group. It may be the case that the light was not sufficiently salient to predict shock onset. Alternatively, the warning signal and the temporal regularity of the shock may have added an increment of stress which is absent when stress is unpredictable. Nonetheless, these data do suggest that predictability may influence stress-induced alterations in immunity. (Supported by NSERC Grant U0569).

119.16

EFFECT OF BRIEF STRESSORS ON LYMPHOCYTE FUNCTION IN RATS: MECHANISTIC IMPLICATIONS J.P.Halper*, A.H.Miller*, C.Lackner*, B.Trestman*, F.Berkenbosh*, A.Santucci, V.Haroutunian, M.Stein. Mt. Sinai Med. Cntr., NY, NY 10029

Many studies have implicated that stressors lead to diminished lymphocyte function. However, little is known regarding the mechanism. The studies reported herein indicate that short periods of stress (2 minutes of intermittent footshock or 5 minutes of restraint) lead to diminished lymphocyte proliferation in response to mitogenic lectins. This defect in mitogenesis appears to occur at or beyond the level of Protein Kinase C (PKC), since mitogenesis in response to phorbol ester and ionomycin (which directly activate PKC) was also diminished. Brief stressors have the advantage of minimizing nonspecific effects associated with more prolonged or intense stress. Brief stressor effects on lymphokine production and lymphocyte subset distribution will also be presented.

119.17

INESCAPABLE FOOTSHOCK STRESS PROLONGS SURVIVAL IN MRL-LPR/LPR AUTOIMMUNE MICE

L.J. Grota, J.A. Moynihan*, S.G. Schmidt,* T.R. Schachtman*, N. Cohen* and R. Ader*. Depts. of Psychiatry and Microbiology and Immunology, University of Rochester, Rochester, NY, USA 14642

MRL-lpr/lpr mice develop autoimmunity that is characterized by production of autoantibodies to a wide spectrum of self-antigens; arthritis; and glomerulonephritis caused by both immune complex deposition in the kidneys and a T cell inflammatory response. They die beginning at approximately 20 weeks of age. Additionally, the MRL-lpr/lpr mice display massive lymphadenopathy due to the proliferation of a novel subset of T cells that are dull Thy 1^+ and Lyt 1^+.

We have demonstrated that footshock stress delivered 5 days/week beginning at 19 weeks of age consistently and significantly prolongs survival of MRL-lpr/lpr mice compared to both apparatus control and home cage control animals. A sigificant difference was found at 26 weeks of age, shocked animals having fewer deaths than controls (Chi-square analysis, $p<0.01$). In one such study, after 7 weeks of shock, 1/16 (6.25%) footshocked animals, 6/16 (37.5%) apparatus control, and 8/12 home caged (66.7%) had died

After 3.5 weeks of "therapy", animals were bled to measure corticosterone levels 15 minutes following the end of the shock session. Preliminary data indicate similar steroid levels in shock and apparatus control animals, suggesting that the difference in survival may not be mediated by the anti-inflammatory effects of steroids.

119.18

PSYCHOLOGICAL STRESS DOWN-REGULATES IL-1 PRODUCTION IN HUMAN MACROPHAGE/MONOCYTES. R. Glaser, A. Griffin, D. Bucci, J. Hillhouse, S. Kennedy, M. Kotur and J. Kiecolt-Glaser. The Ohio State University Medical Center, Columbus, Ohio 43210.

Work from our laboratory has focused on the interaction between the CNS and the immune system, and how psychological stress in humans can modulate this interaction. Since cytokines are important in cell-to-cell interactions, we have examined the synthesis of IL-1 by macrophage/monocytes in blood samples obtained from medical students taking examinations or from cell obtained approximately one month prior to the examination block (baseline). Supernatants from adherent cell cultures were assayed for IL-1 synthesis using the mouse thymocyte proliferation assay. We found that there was a significant decrease in IL-1 synthesis by cell cultures obtained at the time of examinations, as compared to the levels of IL-1 synthesized in cells obtained at baseline. We also found that this change was not related to the total number of macrophage/ monocytes in each sample. The results are consistent with previously published data from our laboratory and others, that psychological stress can down-regulate cellular immunity at several different levels.

NEURAL PLASTICITY IN ADULT ANIMALS: MOTOR SYSTEMS

120.1

SYNAPTIC REORGANIZATION IN THE MOTOR CORTEX INDUCED BY LESIONS OF THE DEEP CEREBELLAR NUCLEI. H. Asanuma, K. Arissian*and A. Keller. (SPON: E. L. White) The Rockefeller University. New York, NY 10021

We have previously reported that elimination of the thalamic input to the motor cortex induces proliferation of the corticocortical (CC) afferents in the motor cortex, at sites of synaptic contacts previously occupied by the thalamocortical terminals (Ichikawa et al., Brain Res., 437:131 1987). To determine whether similar plastic processes occur without synaptic vacancies, the deep cerebellar nuclei, which project to the motor cortex via the thalamus, were lesioned unilaterally in 7 adult cats. Operations were performed under Nembutal, and sterile conditions. Cerebellar lesions did not produce degenerative changes in the motor cortex. One month later, CC afferents from area 2 to the motor cortex were labeled by lesion-induced degeneration. Quantitative electron-microscopy revealed that sections of motor cortex contralateral to the cerebellar lesions contained significantly higher numbers of labeled CC terminals, and higher proportions of axospinous synapses, compared to control hemispheres. In addition, electrophysiological examination revealed that the polarity of evoked potentials induced by area 2 stimulation reversed in the depth of the motor cortex, whereas in control hemispheres there was no reversal. The results demonstrate that a functional, but not anatomical, elimination of input to the motor cortex can induce a reorganization of synaptic connections. Supported by NIH #NS-10705 (H.A.) & NRSA #1 F32 NS08626 (AK)

120.2

DENERVATION IN VITRO: CHANGES IN FUNCTIONAL PROPERTIES OF PERIPHERAL NICOTINIC ACETYLCHOLINE RECEPTOR (AChR). R. Rozental*, W. Randall* and E.X. Albuquerque. (Spon: S.R. Max) Dept. of Pharmacol., Univ. of Md. Sch. of Med., Baltimore, MD 21201.

Although the AChR is the best studied agonist-gated ion channel, some of its properties are still controversial. One question is whether the altered kinetic properties of the AChR observed after denervation are due to post-translational modifications or to expression of new transcription. Single fibers were dissociated from frog interosseal muscle, kept at 4°C, for a period of up to 14 days, and cell attached patch-clamp recordings were made at 10°C. After acute dissociation, ACh (0.4 μM) and (+)anatoxin (0.2 μM) induced channel openings with 30 pS conductance and voltage-dependent lifetimes. On the 2nd and 7th days after fiber dissociation, 2nd (18 pS) and 3rd (smaller than the previous) conductance states were observed. The channel lifetimes of the 2nd and 3rd conductance states were shorter than the 1st one described, and by 12–14 days, each one accounted for 33% of the total observed openings. Chronic treatment of the fibers kept at 4°C with cycloheximide (10 μg/ml) did not prevent the appearance of the two lower conductance states, yet inhibited all incorporation of ^3H-leucine into TCA precipitable protein. α-Bungarotoxin (5 μg/ml) blocked all currents. Thus, we conclude that: 1–protein synthesis may not be required for the expression of the above two lower conductance states. 2–these above channels are different from the "embryonic" type observed in the denervated muscle. 3–our results may represent the expression of different subconductance states of the same AChR. (Support: US Army Med. Res. & Dev. Comm. Contract DAMD17-88-C-8119 and NIH Grants NS25296 & NS26885).

120.3

MORPHOLOGICAL AND PHYSIOLOGICAL CORRELATES OF SYNAPTIC COMPETITION AT FROG NEUROMUSCULAR JUNCTIONS. R. Dunia and A.A. Herrera. Dept. of Biological Sciences, Univ. of Southern California, Los Angeles, CA 90089.

In the pectoral muscle of adult Xenopus (10-44g), half the fibers have two junctions. By recording curare-blocked EPPs from identified sites on these dually-innervated fibers, we found the following 3 innervation patterns: 1) those with the same axonal input innervating each of two distant sites (a/a), 2) those with different inputs innervating distant sites (a/b), and 3) those similar to type a/a, but with one of the two sites innervated by a second axon (a/ab). EPP amplitudes (corrected for input resistance and resting potential) measured at each site confirm previous reports that type a/a terminals produce larger EPPs than either type a/b or a/ab terminals. It has been hypothesized that these differences in synaptic efficacy are due to competitive interactions between axons of different motoneurons.

We are currently examining other aspects of synaptic transmission such as facilitation and depression at junctions of each type to more fully characterize the physiological consequences of synaptic competition. In addition, we are correlating the histological appearance of the identified junctions with innervation pattern to test whether competition induces enhanced structural remodelling. Supported by NIH.

120.4

CHANGES IN THE SIZE OF LUMBAR MOTONEURONES AFTER ROSTRAL SPINAL CORD TRANSECTION. E. Eidelberg, L. H. Nguyen*, and M. Gutierrez*. Division of Neurosurgery, U. of Texas Health Science Center, San Antonio, Texas 78284

Rats were prepared by complete transection of the spinal cord at T_8-T_{10}, followed by retrograde labeling of soleus (SOL) and extensor digitorum longus (EDL) motoneurones by injection of WGA-HRP into each muscle (one muscle/side). We measured the crossectional area of the soma of densely labeled cells. There were no significant differences in the mean size of SOL vs. EDL cells in intact rats. The cells in both groups became significantly larger, even a few hours after the transection, but EDL cells returned to baseline size sooner than SOL cells. Pre-treatment with intraventicular puromycin, a blocker of protein synthesis, prevented the size increases. It seems possible that a rapid increase in protein synthesis may underlie the morphological changes.

Supported by a Merit Review grant from the V.A. and a grant from NIH NS-14546.

120.5

MONOCLONAL ANTIBODIES TO NEONATAL HUMAN AND MOUSE CEREBELLUM. J. Raisanen,* L.B. Pickford,* D.N. Mayer* and R.V. Rouse. (SPON: M.E.Smith) Dept. of Pathology, Stanford Univ. School of Medicine, Stanford, CA.

The mammalian cerebellum has a highly ordered morphology which is composed of several distinct types of neurons and glia. Morphogenesis depends on the successive proliferation, migration and differentiation of primitive neuroectodermal cells of the metencephalon. Each of these stages is likely defined by a regulated expression of certain neural, glial and interstitial molecules. Identification of these molecules would, therefore, help to characterize these developmental processes. As an initial step, monoclonal antibodies to neonatal human (35 weeks gestational age) and Balb/c mouse (P10) cerebellum were generated using standard fusion methods. Generated antibodies were screened for binding specificity on acetone fixed, frozen sections of developing cerebellum.

In the human cerebellum, mouse monoclonal antibodies defined antigens specific to the molecular layer, to small blood vessels, Purkinje cells and cells of the dentate nucleus. Similar recognition patterns were observed in both neonatal and adult cerebellum and cerebrum. In the mouse, rat monoclonal antibodies included ones recognizing Bergmann and radial glia and glial endfeet on blood vessels. Others recognized nuclear membranes of many neural cell types and blood vessels throughout the brain. Further immunohistochemical and biochemical characterization of the molecules recognized by these antibodies is in progress. These molecules may be important in the anatomical or physiological development of the mammalian cerebellum.

120.6

MEMORY TRACES IN PRIMATE SPINAL CORD: CORRELATION WITH BEHAVIOR. J.R. Wolpaw, C.L. Lee, and J.S. Carp. Wadsworth Laboratories, NYS Dept Health, Albany, NY 12201.

Operant conditioning of the H-reflex, the electrical analog of the monosynaptic stretch reflex, changes the spinal cord itself (J Neurophysiol 61:563-572, 1989). Conditioned side-to-side reflex asymmetry is still present in the anesthetized animal three days after transection removes supraspinal influence. We compared the asymmetry in the awake behaving animal to the asymmetry that persists in the transected spinal cord.

Nineteen Macaca nemestrina underwent conditioning to increase or decrease triceps surae H-reflex in one leg. Then, each animal was deeply anesthetized, reflexes were measured in the transected spinal cord, and animals were sacrificed by overdose and perfused for anatomic study.

The conditioned reflex asymmetries present in awake behaving animals remained after transection isolated the spinal cord from supraspinal influence. Larger asymmetry in the behaving animal was associated with larger asymmetry in the transected spinal cord ($r=.75$, slope=.70, $p<.001$). This relationship suggests that the memory trace in the spinal cord accounted for most of the asymmetry seen in the awake behaving animal. Physiologic and anatomic studies (Carp et al and Lee et al, this volume) are evaluating the most likely site of this memory trace, the Ia synapse on the alpha motoneuron.
(Supported by NIH NS22189 & Paralyzed Veterans of America)

120.7

CHOLINE-ACETYL-TRANSFERASE (CHAT) DISTRIBUTION IN THE LUMBOSACRAL SPINAL CORD CHANGES FOLLOWING CHRONIC SACRAL ROOT RESECTION. A.M. Booth, S. Erdman* V. Erickson* M. Kawatani, J.R. Roppolo, S. Smerin and W.C. de Groat. Department of Pharmacology and Center for Neuroscience, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

In the spinal cord CHAT, a marker for cholinergic neurons, is located in preganglionic cells in the intermediolateral nucleus, in motoneurons, and in some interneurons in the medial dorsal horn and the intermediomedial nucleus. These experiments examined the change in CHAT distribution following long term (more than one year) unilateral transection of the sacral dorsal and ventral roots in adult cats. Following fixation by intracardiac perfusion the tissue was frozen and cut at 42 μm. Localization of CHAT-like immunoreactivity used a rat monoclonal antibody and a double bridge, nickel intensified PAP process on free floating tissue. CHAT-like immunoreactivity in lesioned tissue was different in several regions of the lumbosacral cord. Most notable was a tangle of CHAT positive processes projecting into lamina VII and into the ventral white matter where they extended both rostrally and caudally. Quite unexpected were intensely labeled processes coursing from the S_1 intermediolateral and lateral ventral horn (lam VII & IX) toward the commissural region and into the dorsal columns and a group of processes extending along the lateral margin of the dorsal horn and into Lissauer's tract. These bundles of CHAT positive projections traveled both rostrally and caudally from S_3 to L_7. There was no change in size, number or intensity of labeling of CHAT containing neurons. These findings raise the possibility that axotomy of efferent cells increases the intensity of CHAT staining in the processes of these cells and/or that long term rhizotomy causes an expansion of the processes.

120.8

CHANGES IN PEPTIDE-IMMUNOREACTIVITY IN RAT DORSAL HORN FOLLOWING ELECTRICAL STIMULATION OF THE SCIATIC NERVE. C.M. Klein*, L.S. Sorkin, S.M. Carlton, K.Westlund and R.E. Coggeshall. (SPON: H.W. Burden). Dept. of Anat., MBI, Univ. of Texas Med. Br., Galv., TX 77550.

The purpose of this study was to examine changes in peptide-immunoreactivity in the L4-L5 dorsal horn following acute sciatic nerve stimulation. Three peptides known to be contained in primary afferent fibers were targeted:substance P (SP), calcitonin gene-related peptide (CGRP), and cholecystokinin-8 (CCK). Adult Sprague-Dawley rats were anesthetized (Nembutal), paralyzed (Gallamine) and the sciatic and tibial nerves on one side were exposed. The sciatic nerve was then electrically stimulated (1 Hz, 0.2 ms) for 20 mins.; the compound action potential was monitored from the tibial nerve. The C wave was present and did not diminish for the entire stimulation period. Immediately after stimulation, the rats were perfused with 3% paraformaldehyde, 3% glutaraldehyde, 0.1% picric acid in cacodylate buffer. The L4-L5 spinal cord segment was removed and processed for immunocytochemistry by the peroxidase anti-peroxidase method. Results show that immunoreactive staining for SP, CGRP and CCK in the medial half of the superficial dorsal horn was significantly decreased following acute stimulation of the sciatic nerve. This data suggests that electrical activity in primary afferent fibers causes depletion of peptides in their terminal distribution in the dorsal horn.

120.9

CHANGES IN PEPTIDE-IMMUNOREACTIVITY IN RAT DORSAL HORN FOLLOWING PLACEMENT OF SCIATIC NERVE STUMPS INTO SILICONE TUBES. R.E. Coggeshall, C.M. Klein, T.E. Sherburn AND S.M. Carlton. The Marine Biomedical Institute, Dept. of Anatomy and Neurosciences and Dept. of Phys. and Biophy., The University of Texas Medcial Branch, Galveston, Texas 77550.

Transection of large peripheral nerves is known to cause loss of dorsal root ganglion cells and of peptides in the dorsal horn. Recently we found that putting the stumps of a transected nerve in an impermeable tube avoided the cell loss. To test whether the tube also influenced the immunocytochemical staining, rat sciatic nerves were transected and the animals were placed into two groups; 1) simple nerve transections and 2) nerve transections treated by tubes. The data indicate that there is loss of immunocytochemical staining in the dorsal horn in the tubed animals but not as much as with the simple transections. If this is confirmed quantitatively and if cell numbers do not change (to be determined), the conclusion will be that transection of a peripheral sensory axon causes depletion of peptides in the central processes of dorsal root ganglion cells which is not correlated with the death of the cells. Supported by NIH grants NS10161, NS 11255 and NS 07185.

120.10

SYNAPTIC REPRESSION IN THE CRAYFISH AFTER PARTIAL DENERVATION. L.L. Gould* and S.J. Velez (SPON: F. McCann). Department of Biological Sciences, Dartmouth College, Hanover, New Hampshire 03755

The superficial flexor muscle system of the crayfish Procambarus clarkii was used to study the effect of partial denervation on established synaptic contacts. The six motoneurons that innervate the 40 muscle fibers have their cell somas at precise locations within the abdominal ganglia. A portion of the third segment ganglia was cut and removed from cold-anesthetized animals at a location that removed the cell soma of three of the six neurons going into the muscle. The remaining cell somas (neurons 1, 3 and 5) were left in place, along with the nerve and the muscle fibers. At regular intervals after the surgery, the connectivity patterns generated by neuron 3 over the muscle surface were analyzed by recording jp's in phase with axonal spikes. In control animals, jp's ranging from 0.1 to 5 mV (generated by the spontaneous firing of the neuron) are easily recorded all over the muscle surface. In experimental animals, no jp's could be recorded at the 0.1 mV limit of resolution of our system even after eight weeks after surgery, yet they could be seen after stimulating the axon at 10 Hz or after cold treatment. Therefore, synaptic contacts are present but are not generating the normal postsynaptic jp sizes under physiological conditions. This suggests that the synaptic contacts from axon 3 are being repressed when some of the neurons that innervate the muscle are missing.

120.11

THE EFFECT OF PARTIAL DENERVATION ON ULTRATERMINAL SPROUTING IN THE EDL MUSCLE OF ADULT AND AGED RATS. J.L. Rosenheimer, Y.P. Xu*, and L.A. Trotter* Dept. of Physiol., Univ. of Wis., Madison, WI 53706.

In rat extensor digitorum longus (EDL) muscle end plates, there is an age-related decrease in nerve terminal number but an increase in ultraterminal sprouting, characteristic of muscle denervation. To see if the sprouting response following denervation would be affected by age, EDL muscles from 10- and 25-mos animals were partially denervated and end plates were examined 4 to 14 days after denervation.

There was a significant increase in ultraterminal sprouting after 4 days denervation in the 25-mos rats, but not until 7 days after denervation in the 10-mos rats. Sprouting frequency in 25-mos muscle end plates was 2x greater than in 10-mos muscle. However, the number of sprouts in 25-mos denervated muscles was only 1.3x greater than in 25-mos innervated muscles; in 10-mos denervated rats the number was 2.5x greater. Average ultraterminal sprout length was 2x greater in 25- compared to 10-mos muscles. In general, innervated end plates with sprouts and all denervated end plates had larger areas and fewer nerve terminals than innervated end plates without sprouts. The results suggest that aging at the neuromuscular junction is accompanied by changes reminiscent of functional denervation. Supported by NIH grant AG01572.

120.12

PROTEIN SYNTHESIS IS REQUIRED FOR LONG-TERM ADAPTATION OF TRANSMISSION AT NEUROMUSCULAR SYNAPSES. P.V. Nguyen* and H.L. Atwood. Dept. of Physiol., Univ. of Toronto, Toronto, M5S 1A8 Canada.

Previous work has established that chronic stimulation of the crayfish claw closer muscle's phasic motoneuron can induce a long-term adaptation (LTA) of its neuromuscular synaptic responses (J.Neurosci. 5:459-67). LTA is characterized by reductions in both initial EPSP amplitudes and synaptic depression. In the present study, these changes were evident 2 days after a 3-day conditioning regimen (2h/day,5 Hz). To determine if protein synthesis was required for LTA, we injected cycloheximide (CHX; 5ug/g), a protein synthesis inhibitor, into intact crayfish at various times relative to each conditioning period. CHX reversibly and maximally blocked protein synthesis for 2 hours following a single injection. Injection of CHX before each conditioning period significantly attenuated LTA, while injection during or after conditioning had no effect. Chronic CHX injection had no adverse effect on neuromuscular transmission in control animals. Thus, short-lived, pre-existing protein(s) may be required for neuromuscular LTA in the claw's phasic motoneuron.

Funded by the MRC (Canada) and NSERC (Canada).

120.13

DISTRIBUTION OF MYOSIN ISOENZYMES IN AGING SKELETAL MUSCLE OF MICE. K. Y. Saiki* and M. A. Fahim. Andrus Gerontology Center, University of Southern California, Los Angeles, CA 90089-0191.

To delineate morphological differences in aging skeletal muscle, the proportion of alkali-1 and -2 myosin light chains (MLC) in extensor digitorum longus (EDL) in young and old mice (C57BL/6NNia) were determined by immunocytochemical techniques. The EDL of 6 month old and 24 month old mice was either partially ablated (ABL) or the nerve innervating the muscle crushed (CN) to invoke partial denervation. Five weeks post-surgery fluoroscein-labeled antibody, specific for alkali-1 and -2 MLC showed a redistribution towards a greater proportion of alkali-1/alkali-2 MLC in both young and old CN-EDL with young muscle showing a higher ratio. ABL-EDL also demonstrated similar changes with old muscle, however, showing a higher ratio. Twitch tensions/unit mass of young CN-EDL decreased when compared with young controls while old ABL-EDL attenuated compared to old controls. This suggests that strength decrement may be associated along with other age related changes with the redistribution of alkali-1 and -2 MLC. Supported by NIH grant AG-04755.

120.14

DISSOCIATED CELL CULTURE OF ADULT RAT PELVIC GANGLION. W.D. Steers and J.B. Tuttle Urology and Neuroscience Univ of Virginia Hlth Sci Cntr, Charlottesville, VA 22908

Parasympathetic decentralization fails to block neuronal hypertrophy in rats with partial urethral obstruction. Thus, the alterations in function, morphology and electrophysiology found in this animal model for outlet obstruction appear to depend upon events in the target organ: the bladder. Methods were developed to test the hypothesis of target-derived trophic factor involvement in obstructive pathophysiology by examining growth factor action in cell culture. Dissociation protocol and medium formulation were optimized to culture neurons from pelvic ganglia 6 weeks following ligation or sham surgery. The adult rat neurons adapted to culture with only a 10-30% initial yield. Neuronal counts were confirmed by immunostaining for neuron-specific beta-tubulin (TuJ1). NGF (0.1 ug/ml) doubled initial survival. The retrograde fluorescent tracer diO, injected into the bladder 36 hrs prior to dissociation, labeled some cultured neurons indicating survival of the subpopulation innervating bladder. Neurons were immunoreactive for TH and NPY, indicating survival of a mixed cell population. Cultured neurons retained excitability, with voltage-sensitive Na^+ and K^+ currents serving action potentials. Thus, the adult rat pelvic ganglion is suited to studies of trophic interaction in vitro, and may be useful in providing further insight into plasticity of the adult peripheral nervous system.

120.15

GROWTH ASSOCIATED PROTEIN, B-50 (GAP43) IS PRESENT IN THE MAMMALIAN ENTERIC NERVOUS SYSTEM. K.A. Sharkey*, W. Tetzlaff*, P.J. Coggins*, H. Zwiers*, M. Bisby and J.S. Davison*. (SPON: G.E. Lucier) Dept. of Medical Physiology, University of Calgary, Calgary, Alberta, Canada.

B-50 (also known as GAP43) is a neuron-specific, membrane bound phosphoprotein whose synthesis is selectively enhanced during neural development, growth and conditions of neuronal plasticity. In the adult peripheral nervous system B-50-like immunoreactivity (B-50-LI) is normally present at low levels. However, there is an increase in B-50-LI after injury. The presence of B-50 has not been investigated in the mammalian enteric nervous system. Experiments were performed on adult rats. B-50-LI was localized in nerves throughout the wall of the intestine using affinity purified rabbit B-50 antibodies. Most striking was the dense immunoreactivity in the myenteric plexus. A few scattered endocrine cells were stained in the ileum. B-50-LI nerves were found in the longitudinal and circular muscle layers, both myenteric and submucous plexuses and in the peri-and sub-glandular and villus portions of the lamina propria. Numerous nerve cell bodies in both plexuses were lightly stained. In-situ hybridization with a cDNA probe to GAP43 revealed a positive signal in enteric neurons. In extracts of ileum a band of B-50-LI material was observed on Western blots. The role of B-50 in the gut has yet to be determined, but it is tempting to speculate that the adaptive ability of the gut after injury might be related to the presence of B-50 in enteric nerves.

120.16

POTENTIAL ROLE FOR NGF IN NEURONAL PLASTICITY AFTER BLADDER OBSTRUCTION. D.J. Creedon, W.D. Steers, and J.B. Tuttle.Depts Urology, Neuroscience, Physiology, Univ of Virginia Hlth Sci Cntr, Charlottesville, VA 22908

Urethral obstruction causes bladder enlargement associated with neuronal hypertrophy and functional changes in the reflex pathways controlling micturition. Since smooth muscle cells can secrete trophic substances that influence peripheral neuronal growth including nerve growth factor (NGF), neurotrophic factors may be involved in the pathophysiological sequence following obstruction. NGF (0.1 ug/ml) enhanced the survival of adult rat major pelvic ganglion (MPG) neurons in culture. This suggests at least a subset of MPG neurons remain responsive to NGF in the adult. Using a two-site ELISA to measure NGF, obstructed bladders produced 2X more NGF (ng per mg protein) than controls. In one experiment, the amount of NGF per mg protein was similar to controls, but the increased size of the obstructed bladder (wet wgt 684 vs. 75mg) resulted in 10X more total NGF in the enlarged organ. Since the number of neurons innervating the bladder following obstruction is unchanged, MPG neurons may access 10-20X more NGF following bladder enlargement. This change in NGF supply could mediate the neuronal hypertrophy and some aspects of adult reflex plasticity. The results suggest that regulation of target organ size and factor synthesis in response to functional demands may alter the supply of trophic material available to innervating neurons in the adult.

120.17

PRESYNAPTIC INFLUENCE ON POSTSYNAPTIC CHANNEL KINETICS IS LOCALIZED TO INDIVIDUAL SYNAPSES. L.M. Marshall. Dept. of Physiology, Univ. of North Carolina, Chapel Hill, NC 27599.

In the two types of principal neurons in frog sympathetic ganglia, B- and C-cells, a twofold difference in the mean open time of nicotinic synaptic channels accounts for the twofold difference in the decay rate of their fast excitatory postsynaptic currents (EPSCs). When B-cells are denervated and incorrectly innervated by preganglionic C fibers, B-cells acquire the slowly decaying EPSCs and long channel open times normally seen in C-cells only (Nature 317: 621, 1985). This study examined the EPSCs from B-cells that were denervated and reinnervated by both B and C preganglionic axons.

Dually innervated B-cells in the 9th and 10th paravertebral ganglia of adult Rana catesbeiana were examined by whole voltage clamp. In individual B-cells, stimulation of B preganglionic fibers evoked EPSCs with a fast decay, while C preganglionic nerves evoked EPSCs with a slow decay. These results suggest that presynaptic influence on synaptic channel kinetics is localized to the region of each synaptic contact (Supported by grant NS14899)

120.18

NEURON NUMBER INCREASES IN POSTMETAMORPHIC BULLFROGS: SYMPATHETIC GANGLIA. P.B. Farel and J.K. Baek*. Dept. of Physiol., Univ. N. Carolina, Chapel Hill, NC 27599.

In most species, body size continues to increase after the period of nervous system development is over. **How does the nervous system accomodate to the expanded target size?** We retrogradely labeled sympathetic neurons by applying HRP to the nerve trunk supplying the posterior thigh in bullfrogs ranging from 3.3-11.5 cm in length (n = 12). Labeled and unlabeled sympathetic neurons were counted in the three sympathetic ganglia that supply the leg. Serial reconstruction of labeled neurons demonstrated that no correction factor is needed for nucleolar counts.

The total number of neurons was strongly correlated with size (r = 0.75). Large frogs (10.5-11.5 cm, n = 4) had 2-4 times as many total neurons as small frogs (3.3-3.5 cm, n = 4) and 4-8 times as many HRP-labeled neurons.

Neurons are added to the sympathetic ganglia in adulthood and these added neurons send axons to the periphery. Experiments in progress are directed toward estabishing the generality, kinetics, and mechanism of the increase in neuron number.

120.19

NEURON NUMBER INCREASES IN POSTMETAMORPHIC BULLFROGS: DORSAL ROOT GANGLIA. P.G.R. St.Wecker* and P.B. Farel (SPON: D.L. McIlwain). Dept. of Physiol., Univ. N. Car., Chapel Hill, NC 27599.

To further our study of neuronal increase in the adult bullfrog, we retrogradely labeled lumbar DRG neurons by applying HRP to the posterior profundus nerve in bullfrogs ranging from 3.3-11.5 cm in length. Labeled and unlabeled neurons were counted in the three DRGs that supply the hindlimb. Serial reconstruction of labeled neurons demonstrated that split nucleoli occur in approximately 5% of counted neurons.

The total number of neurons was strongly correlated with body length (r = 0.88). Large frogs (10.5-11.5 cm, n = 3) had a mean (±SD) of 12,368±1,580 total neurons, versus a mean of 6,943±739 in small frogs (3.3-3.4 cm, n = 2). Counts of HRP-labeled neurons showed a similar ratio, with a mean count of 384±84 for large frogs and 226±40 for small frogs.

These data indicate that peripherally projecting neurons are added to the DRG in adulthood. These results are consistent with previous data on sympathetic ganglia, spinal motoneurons, and brainstem projection neurons in adult bullfrog.

121.1

BRAIN-BEHAVIOR RELATIONSHIPS IN AGING OBSERVED THROUGH QUANTITATIVE MAGNETIC RESONANCE (MR) IMAGING AND MEMORY MEASURES. J. Rawles*, E. V. Sullivan, K. O. Lim*, R. B. Zipursky*, and A. Pfefferbaum*. Stanford University School of Medicine, Stanford, CA and Veterans Administration Medical Center (116A3), Palo Alto, CA 94304.

Although declines in memory function and changes in brain morphology occur with normal aging, little direct evidence exits for a link between these two processes. This linkage was assessed by comparing quantitative measures of medial temporal-lobe integrity from MR scans with performance on memory tests in 8 young men (mean=24yrs) and 7 older men (mean=73yrs). We expected that scores of long-term memory (LTM) tests, subserved by medial temporal-lobe structures, would be negatively correlated with measures of temporal horn cerebrospinal fluid (CSF) in the older group.

MR quantification for this analysis comprised three specific measures (left, right, left+right temporal horn CSF) and one global measure (ventricular volume excluding the temporal horns)(Lim, et al., 1989, *Soc. Neurosci. Abstr.*). Memory assessment included tests of short-term memory (STM)(Wechsler Memory Scale [WMS] and block span) and LTM (delayed recall of WMS subtests).

The older group had significantly greater CSF volumes for all MR measures and scored significantly lower than the young group on four STM and two LTM tests. Scores of two LTM tests (stories and drawings), but of no STM test, correlated significantly with specific brain measures and with the global MR measure in the older group; no correlation was significant in the young group.

Thus, coupling structural imaging measures of circumscribed brain regions with tests of component processes of memory is a viable method for observing specific brain-behavior relationships in normal aging that transcend nonspecific deterioration commonly associated with the aging process.

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121.2

EVIDENCE FOR ATYPICAL NEURAL ARCHITECTURES IN NEUROPSYCHOLOGICALLY NORMAL ADULTS. J.P. Brandt*, D. Tranel, H. Damasio, A.P. Tranel* and R.D. Jones. Div. of Behav. Neurol. & Cognitive Neuroscience, Dept. of Neurology, Univ. of Iowa Col. of Medicine, Iowa City, Iowa, 52242.

The development of aphasia following a right hemisphere lesion in dextral adults is extrememly rare. We conducted neuroanatomical and neuropsychological studies in 2 such adult males. Both had handedness quotients of +100 (complete right-handedness), and normal cognitive development and intellectual abilities. Also, both had typical anatomical asymmetries of auditory cortex, i.e., favoring the left. In the acute epoch after a lesion involving right primary and association auditory cortices, the subjects had (a) fluent, paraphasic speech, with profound disturbances of comprehension, repetition, reading, and writing, AND (b) anosognosia, left neglect, and visuoperceptive defects.

Handedness is generally used as a predictor of cortical functional dominance and has even been thought to be a factor in its development (indirectly via an asymmetry for motor control). The evidence here contradicts that notion and reveals that atypical neural architectures can occur in cognitively normal adults with typical anatomical asymmetries, even when characteristic indexes, e.g., sinistrality, ambidexterity, or developmental learning disorder, are entirely absent.

121.3

HUMAN EXTRACELLULAR STEREOTRODE ANALYSIS OF NEURONAL ENSEMBLE DYNAMICS DURING LANGUAGE TASKS. D.F. Cawthon*, G.A. Ojemann, E. Lettich*, D.F. Kalk*, and D.B. Percival* (SPON: A.B. Harris). Dept. Neurosurg.and Appl.Physics Lab., Univ. of Washington, Seattle, WA 98195.

With informed consent and under institutional rules, we have found modulation of normal human temporal lobe neuronal activity by language and memory tasks at nonessential sites during epilepsy surgery. Separation of action potentials of different neurons is critical for frequency analysis if one is interested in the dynamics of firing in an ensemble of cells, but could not be reliably accomplished by time-window discriminators from multiunit activity obtained by standard tungsten microelectrodes. We therefore adapted a "stereotrode" (McNaughton, B.L. et al. *J Neurosci Methods* 8: 391-397, 1983) which in our version has two stainless steel microwires (60μ diameter, 70μ apart) threaded through a 30G hypodermic shaft, used as reference. After recording, the location of the tips is marked thermally and alternating 20 μ sections are stained by Nissl or Fink-Heimer methods. Recordings were played back from FM tapes via National Instruments' A/D and DMA boards and software drivers into a Macintosh II computer, with additional software written in Pascal and then analyzed using PITTSA, a time series analysis package written in Lisp. Each cell's action potential appeared on both microelectrode channels but with different amplitudes due to different distances from the two electrode tips. Cells of nearly identical waveform were often indistinguishable on one channel, only to be revealed as separate by the other channel. A dual amplitude scatter plot yields 3 to 6 well-defined clusters, each corresponding to a different cell, as corroborated by differences between cell firing patterns with different linguistic tasks and by statistical cluster analysis techniques. Action potential duration can be examined as a third clustering parameter, in a 3D scatter plot. (Supported by NIH Grants NS21724, NS20482, NS17111 and NS07289 and by the Epilepsy Foundation of America.)

121.4

STIMULUS-SPECIFIC MEMORY DEFICITS AFTER THALAMIC LESIONS IN MAN. J.Ilmberger,W.Fries*,A.Danek*, Inst.Med.Psychology,Univ. of Munich, Munich,FRG.

It is well known that circumscribed lesions in the left or right temporal lobe lead to stimulus-specific verbal or nonverbal memory deficits. It is unclear whether this specificity also holds true for memory disorders due to thalamic lesions. One problem in testing is finding nonverbal stimulus material resistant to verbal encoding. Milner's (1973) recency test seems to offer an adequate solution to this problem.

We used a similar test consisting of serially presented stimulus cards; at regular intervals a question card was interposed. The subject had to indicate whether he had seen the two items on that card before and, if yes, in which order. Both a verbal (nouns) and a nonverbal (partial reproductions of abstract paintings) form of the test was administered to patients with uni- and bilateral thalamic lesions and a healthy control group. Bilateral lesions led to a high error score in both forms of the test; a left-sided lesion decreased verbal, a right-sided lesion nonverbal performance, indicating that the verbal -nonverbal distinction also is valid at the thalamic level.

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121.5

EFFECTS OF AUDITORY ASSOCIATION CORTEX LESIONS ON THE PROCESSING AND RETENTION OF ACOUSTIC STIMULI IN MONKEYS. M. Colombo*, M. R. D'Amato*, H. R. Rodman, and C. G. Gross. Dept of Psychology, Rutgers University, New Brunswick, NJ, 08903 and Dept. of Psychology, Princeton University, Princeton, NJ, 08544.

Unlike previous studies that used auditory-visual or auditory-spatial delayed matching-to-sample (DMS) designs, we employed an auditory-auditory DMS task to examine the effects of auditory association cortex lesions on the short-term memory capacity of monkeys.

Three *Cebus apella* monkeys were preoperatively overtrained on closely similar visual and auditory DMS tasks with delays of .5, 4, and 32 seconds. Each then received a two-stage bilateral lesion, with testing after each lesion. The lesions included most of the superior temporal gyrus and upper bank of the superior temporal sulcus, and the anterior part of the lower bank of the lateral fissure.

Although visual DMS performance was completely unaffected by the auditory cortical lesions, auditory DMS performance suffered, particularly after the second operation. One monkey was able to match the acoustic stimuli but displayed a severe retention loss at all delays. The other two monkeys were unable to match the acoustic stimuli, even at the shortest delay. The fact that these subjects could discriminate tones less separated than the tones used in the matching task indicates that the matching deficit was not due solely to sensory impairment; rather, it reflects a deficit in higher-order processing of auditory information.

121.6

BEHAVIORAL DISTURBANCES IN THE DEVELOPING RHESUS MONKEY FOLLOWING NEONATAL LESIONS OF INFERIOR TEMPORAL CORTEX (AREA TE) RESEMBLE THOSE IN ATTENTION-DEFICIT HYPERACTIVITY DISORDER. P. M. Merjanian, J. Bachevalier, K. D. Pettigrew*, and M. Mishkin. Laboratory of Neuropsychology, NIMH, Bethesda, MD 20892.

Early damage to the limbic system in monkeys yields severe socio-emotional disturbances resembling those seen in autistic children (Merjanian et al., Soc. Neurosci. Abstr., 12:23, 1986 and 14:2, 1988). Although the 6 operated controls that had received early damage to area TE did not show these disturbances, they did display other behavioral abnormalities. Six monkeys had received area TE lesions (TE) neonatally, 6 were normals (N/TE) reared with monkeys from Group TE and 6 were normals reared with other normals (N/N). At 2 and 6 months of age, each animal from Group TE was paired in a play cage with its age-matched control (N/TE), and their behavior was observed for 12, 5-minute intervals on 6 days. Similarly, each N/N was paired with another N/N. At 2 months, the monkeys in Group TE were hyperactive, shifted more from one behavior to another, and showed more stereotyped behaviors than monkeys in either Group N/TE or Group N/N. In addition, animals in Group N/TE showed more aggressive behavior towards animals with TE lesions and had more temper tantrums than both other groups. Although these behavioral disturbances in both the operated animals and their unoperated controls were less severe at 6 months of age, they strongly resemble those of people with attention-deficit hyperactivity disorder and the responses of their peers.

121.7

RETROSPLENIAL CORTEX: POSSIBLE ROLE IN HABITUATION OF THE ORIENTING RESPONSE. S.E. Kwon*, S.E. Nadeau*, and K.M. Heilman, Dept. Neurol., Univ. FL, Gainesville, FL 32610.

A patient following a retrosplenial(RS) area lesion developed a propensity to attend contralaterally (Neurol., 38(suppl.1)1988). To learn if a RS regional loss was inducing this defect, unilateral RS and cingulum(CG) lesions in rats were reproduced by aspiration. Subjects were assessed preop and postop for orientation and habituation to bilateral simultaneous stimulation in 3 modalities; visual, tactile and auditory. At each session, independently tested, the orientation test was terminated upon completion of 5 trials per modality and the habituation test ended at habituation (4 consecutive response failures) or upon completion of 15 trials per modality.

Contralesional vs ipsilesional orientation was not significantly different suggesting no evidence of neglect. A significant delay in habituation to contralesional stimulation in postop weeks 2 and 3-5 was revealed by two-way ANOVA for its interactions ($p<0.01$) and its post-hoc comparison ($p<0.02$).

Our results suggest that RS-CG lesions are associated with a failure to habituate to contralesional stimuli. While the mechanism underlying this defect is unknown, this limbic region has close anatomic connections with areas such as the frontal lobe that are known to play a role in directional attention. Perhaps RS lesions release attentional networks from limbic control.

121.8

ACQUISITION OF SIGN LANGUAGE FOLLOWING LEFT HEMISPHERE DAMAGE AND APHASIA. S.W. Anderson, H. Damasio, A.R. Damasio, U. Bellugi, A.P. Tranel*, J.P. Brandt*, and L. O'Grady*. Div. Behav. Neurology & Cognitive Neuroscience, U. Iowa College of Medicine, Iowa City, IA 52242.

Because American Sign Language (ASL) shares principles of auditory-based languages but is based on visual-motor signs and has linguistic mechanisms unique to this mode of transmission, studying its processing after brain damage offers a new window into the neural basis of cognition. We describe a case-control experiment of the acquisition of ASL following a left temporo-parietal infarct which caused severe aphasia for English. This 28 year old man with no prior knowledge of sign language was able to acquire ASL lexicon at a rate equal to an age matched normal control. Specific signs were produced with great accuracy (words - 97%; letters - 96%; numbers - 100%), in striking contrast to a near complete inability to speak their word counterparts (less than 10% correct). These findings were replicated in another English-speaking man with left temporo-parietal damage and severe aphasia. The finding that specific semantic knowledge may be readily expressed by a visual-gestural symbolic system after damage to posterior language-related cortices and aphasia for English, indicates that such knowledge is represented independently of a verbal lexicon and does not depend on the posterior cortices necessary for normal verbal-auditory language.

121.9

KNOWING THAT "COLORADO" GOES WITH "DENVER" DOES NOT IMPLY KNOWLEDGE THAT "DENVER" IS IN "COLORADO." A.R. Damasio and D. Tranel. Div. of Behav. Neurol. & Cognitive Neuroscience, Dept. of Neurol., Univ. of Iowa Col. of Medicine, Iowa City, IA, 52242.

Patient Boswell, who is severely amnesic in both the retrograde and anterograde compartments, can provide the name of a state that a particular city is in, given the name of the city, e.g., given "Denver," he will respond "Colorado." By contrast, he is entirely unable to answer the request to name cities in Colorado, and is unable to provide even superficial information about the unique characteristics of the city of Denver. To investigate this phenomemon, we conducted two experiments. In the first (State Completion), Boswell was given 77 names of cities in the USA, and asked to provide the associated state name; the second (City Generation) required Boswell to provide names of cities, given the name of 27 states. For State Completion, Boswell's performance was quite accurate: he correctly supplied 41/77 (53%) state names and improved to 95% correct with forced choice. For City Generation, Boswell produced only 4 city names (on average, 0.15 cities per state). The dissociation indicates that the ability to complete a well learned verbal set (e.g., DENVER-COLORADO) given its first component, implies nothing about (1) the underlying verbal or nonverbal knowledge associated with either item in the set, or (2) their relationship to one another.

121.10

DEVELOPMENTAL PROSOPAGNOSIA: A NEW FORM OF LEARNING AND RECOGNITION DEFECT. D. Tranel and A.R. Damasio. Div. of Behav. Neurol. & Cognitive Neuroscience, Dept. of Neurol., Univ. of Iowa Col. of Medicine, Iowa City, IA, 52242.

In the course of our studies on learning and recognition, we have learned about individuals who complain of never having developed a normal ability to recognize the identity of human faces. The problem is first noted at school age and is severe, i.e., it is not a mere "difficulty" with face learning, and it is not a problem of "putting names to faces." It can often be circumvented by adaptive compensatory strategies, is relatively isolated, and is compatible with normal intelligence. We have termed the defect developmental prosopagnosia. Based on preliminary observations, we have identified several salient characteristics: (1) Male preponderance; (2) Preponderance of left-handedness; (3) Association with at least one other pattern recognition defect; (4) Intelligence at a level compatible with major professional achievement; (5) Presence of artistic abilities. There is no description of this defect in the neurological and neuropsychological literatures, the reason probably being that affected persons successfully disguise the defect, are considerably embarrassed by it, and cope with it effectively. In its general profile, developmental prosopagnosia has several characteristics of the acquired form of the disorder, i.e., the form of prosopagnosia that occurs with sudden onset following focal brain damage.

121.11

VISUAL EVOKED POTENTIALS (VEP) AND EVENT-RELATED POTENTIALS (ERP) IN TRAINED MONKEYS. A.Glover*, M.F.Ghilardi*, M.Basciani*, and I.Bodis-Wollner. VEP Laboratory, The Mount Sinai Sch. Med., New York, NY 10029 Using an "odd-ball" paradigm, we studied the VEPs and ERPs in 2 cynomolgus monkeys. Electrodes were at Fz, Cz and Oz, O'1 and O'2 (3 cm left and right of Oz, respectively) and in a primate chair and positioned 27 cm from a Tektronix 608 monitor. The size of the visual display was 17.3 deg., the stimuli were 2.5 cpd vertical ("target") or oblique ("non target") sinusoidal gratings. The mean luminance and contrast were 85 cd/m2 and 40%, respectively. Stimulus duration was 742 msec. Target stimulus probabilities of 0.5, 0.3 and 0.1 trials were studied. Trials were initiated by the monkeys by pressing and holding a lever. Lever release for the target resulted in fruit juice reward. In the fully-trained monkeys, discriminating accurately 95% of the time, the VEPs to either stimuli consisted of an initial negative potential at 53 msec followed by a positive peak (56 msec) and a succeeding negative-positive deflection (68 and 73 msec). The mean peak-peak amplitudes were: 14.0, 13.2, 11.1 and 10.2 uV, respectively. We obtained P300-like signals at the centro-parietal derivations only to the target stimulus. At 0.5 target presentation, the mean latency and peak-to-peak amplitude of P300 at Cz are 408 msec, and 25 uV,respectively. Eye movements recorded during the study had no relationship to P300-like potentials. Supported by N.I.H. grants NS 11631;EY01708;EY01867.

121.12

MEMORIZED OBJECTS MUTUALLY INTERFERE ON DELAY DISCHARGES FOR PICTORIAL SHORT-TERM MEMORY IN NEURONS OF PRIMATE TEMPORAL CORTEX. MIYASHITA,Y., SAKAI,K.* and HIGUCHI,S.* Dept. Physiol., University of Tokyo, School of Medicine, Tokyo 113, Japan.

Human short-term memory is limited in its capacity. No neural basis for this cognitive limitation has so far been discovered. We previously reported that, in the anterior ventral temporal cortex of monkeys, individual neurons exhibited sustained activity highly selective to a few of 100 colored fractal patterns during a visual working-memory task (Miyashita et al., Nature 331, 68-70, 1988). Now we have developed a listed delayed matching-to-sample task, in which several sample stimuli are successively presented and a match stimulus is given after a 16sec delay interval. Two monkeys (Macaca fuscata) were trained to memorize the sample stimuli and to decide whether the match stimulus was included in the samples. In all of the 20 tested neurons, the optimum discharge rate during the delay period was weaker when the monkeys memorized several stimuli at a time than it was when they memorized only one optimal stimulus. This result suggests that the capacity limitation of short-term memory originates from a limited memory representation rather than a bottleneck in a decoding process.

121.13

MACAQUES ARE AS SEVERELY IMPAIRED IN OBJECT-REWARD ASSOCIATION AFTER COMBINED ABLATION OF AREA TE AND THE SUPERIOR TEMPORAL SULCUS AS AFTER AMYGDALO-HIPPOCAMPAL ABLATION. J.A. Weinstein*, R.C. Saunders, and M. Mishkin. Laboratory of Neuropsychology, NIMH, Bethesda, MD 20892.

Following amygdalo-hippocampal (AH) removals, macaques are severely impaired on a variety of trial-unique object-reward association tasks (Phillips and Mishkin, Soc. Neurosci. Abstr. 10: 136, 1984.) Attempts to fully reproduce this impairment by disconnecting the limbic system from its visual inputs by means of inferior temporal lesions (of either area TE, area TEO, or both combined) consistently failed (Spiegler and Mishkin, Soc. Neurosci. Abstr. 5: 323, 1979). Here we investigated the possibility that the preserved visuo-limbic interaction observed in that study reflected preserved transmission of information through the depths and upper bank of the superior temporal sulcus (STS). Monkeys (Macaca mulatta) with either TE, TE+STS, or AH lesions, and their unoperated controls (N), were retrained on a trial-unique object-reward association task (Gaffan, Learn. and Motiv. 10: 419-444, 1979). At all list lengths (1, 2, 3, 5, and 10) Groups TE+STS and AH, but not TE, were severely and equivalently impaired relative to Group N. The results suggest that, for purposes of object-reward association, visual information can be transmitted to the limbic system via either area TE or STS.

121.14

RETROGRADE AMNESIA AFTER NEOSTRIATAL LESIONS IN MONKEYS: PRESERVED NON-MATCHING RULE AND IMPAIRED OBJECT MEMORY. E.C. Gower and S. Jacobson. VAMC, Boston MA 02130.

The design of this experiment separated a test of procedural memory from a test of preoperatively acquired object information using the non-matching-to-sample (NMTS) recognition memory task. Cynomolgus monkeys learned NMTS with one set of objects, and relearned it using a different set of objects. Three monkeys with ibotenic acid lesions of the caudate nucleus were only mildly retarded in reacquisition compared with 4 normal monkeys. A subsequent test combined long-term memory trials for preoperatively encountered objects with short-term memory trials for a list of recently presented objects. Here the experimental group demonstrated normal immediate memory for the first list sample, a significant decline in performance for the 2nd and 3rd items in the list, and a dramatic deficit on trials requiring the recognition of familiar objects last seen 3-6 months previously. For these trials, the range of normal performance was 76-83% correct choice of the novel object, in contrast to 53-66% correct for the experimental monkeys. The latter still represented above-chance performance however ($X^2_3 = 15.85$, $p<.01$). This pattern of results parallels the dissociation of preserved task-related procedural information from a significant loss of memory for events observed in human retrograde amnesia. (Supported by VA Medical Research funds.)

121.15

A BEHAVIORAL COMPARISON OF TWO MODELS OF DEMENTIA-AMNESIA IN THE RAT. C. T. Dickson* and C. H. Vanderwolf. Dept. of Med. Physiology, Univ. of Calgary, Calgary, Alberta T2N 4N1, and Dept. of Psychology, Univ. of Western Ontario, London, Canada N6A 5C2.

Two animal models of human dementia and/or amnesia have been proposed recently: 1) combined lesions of the hippocampal formation and amygdala; and 2) joint blockade of ascending serotonergic and cholinergic projections to the cerebral cortex. To compare these models, groups of rats prepared with 1) stereotaxic lesions of the ventral hippocampus and the amygdala; 2) intra-brain stem injections of 5,7-dihydroxytryptamine (DHT, 25 ug/ul, 0.5 ul in each of 4 sites); 3) p-chlorophenylalanine (PCPA, 500 mg/kg/day, i.p. X 3) plus scopolamine (5 mg/kg, s.c.); and 4) control treatments, were tested on: a) an open field; b) a swim-to-platform test; and c) a Lashley III maze. DHT impaired maze performance, especially after scopolamine, and PCPA plus scopolamine virtually abolished swim-to-platform performance, but combined hippocampal-amygdala lesions had little effect on either of these tests. All 3 treatments increased open field activity to varying degrees. From this it can be concluded that blockade of central serotonergic activity, especially when combined with scopolamine treatment, results in a more severe impairment of behavior than do lesions of the hippocampus and amygdala. (Supported by funds from NSERC)

121.16

IMPAIRMENT ON A PRETRAINED SPATIAL NONMATCH-TO-SAMPLE TASK IN AN ANIMAL MODEL OF HUMAN DIENCEPHALIC AMNESIA. R. L. Knoth, Department of Psychology, Chapman College, R. G. Mair, Department of Psychology, University of New Hampshire, Durham, NH 03824, S. A. Rabchenuk, Department of Psychology, Dartmouth College, P.J. Langlais, San Diego, VAMC.

The post-thiamine deficient (PTD) rat is an animal model for human Wernicke-Korsakoff's disease. The primary symptoms of human Korsakoff's disease are retrograde and anterograde amnesia. Behavioral testing of PTD animals has demonstrated global anterograde learning and performance deficits in a variety of discrimination and memory tasks (Mair et al., Behav Brain Res., 27, 223-239; Knoth et al., Neurosci Abstr., 14, 1230). No experiment however, has adequately examined retrograde deficits in these animals.

The purpose of this experiment was to examine retrograde memory deficits using a pretrained spatial NMTS task. A total of 24 animals were trained to 90% correct responding on NMTS in daily sessions of 25 trials. Following pretraining, 16 animals underwent thiamine deficiency. Following recovery, 11 experimental and 8 control animals were retested on NMTS. PTD animals performed significantly worse than controls on Day 1 of behavioral retesting. Both groups, however, showed improved performance over the next 15 training sessions.

These results support the assumption of retrograde memory deficits in PTD animals.

121.17

EFFECTS OF IBOTENIC ACID LESIONS OF THE HIPPOCAMPAL CA1 FIELD ON A DELAYED NON MATCHING TO PLACE TASK IN MICE. BERACOCHEA, D.,CHO, Y. and JAFFARD, R., Lab. Psychophysiologie, CNRS URA 339,Univ. Bordeaux I,33405 TALENCE FRANCE.

We have investigated the role of the CA1 hippocampal area on a delayed-non-matching to place task (DNMTP; 8-arm radial maze) and determined whether preoperative training might influence post-operative performance.Mice were first trained on the DNMTP rule to a criterion of 85% correct responses then submitted to memory testing. The memory test was divided into a study phase and a test (choice) phase. The time-interval between the visit to a target arm and subsequent recognition was either free (0 sec or 30 sec) or occupied by forced visits to other arms (1, 3 or 5 arms) according to a mixed design. Results showed that animals trained before CA1 lesions required twice as many trials as controls to master the DNMTP rule. They subsequently exhibited only a slight transient impairment of memory performance in the more difficult condition (5 interposed arms). Performances were not impaired by delays. Animals trained post-operatively were as rapid as controls in reaching the DNMTP rule criterion, but were unable to perform correctly in the mixed design procedure whatever the conditions. Our results weigh in favor of a differential effect of CA1 lesions on post-operative memory performance as a function of preoperative training.
* This research was supported by the C.N.R.S. URA 339.

121.18

A RAT MODEL OF MEDIAL TEMPORAL LOBE AMNESIA: NONRECURRING-ITEM DELAYED NONMATCHING-TO-SAMPLE. D.G. Mumby*, J.P.J. Pinel, and E.R. Wood*. Dept. of Psychology, Univ. of British Columbia, Vancouver, B.C., V6T 1Y7.

The study of the neural bases of memory and amnesia has been greatly facilitated by the development of a monkey model of medial temporal lobe amnesia: the nonrecurring-item delayed nonmatching-to-sample paradigm. We have adapted this paradigm for use with rats. Performance of intact rats (n=14) at retention intervals of up to 120 s was comparable to that commonly reported for monkeys; the rats scored approximately 93%, 90%, 84%, and 82%, at retention intervals of 3-s, 15-s, 60-s, and 120-s, respectively. Aspiration lesions of the hippocampus had only slight effects on performance. When amygdalar lesions were subsequently added, performance was dramatically reduced at retention intervals longer than 15 s, but was unaffected at shorter intervals. These results suggest that there is considerable continuity among humans, monkeys, and rats in the neural basis of recognition memory, and in so doing, they illustrate the potential of the rat model in its investigation.

121.19

ISCHEMIA-INDUCED DAMAGE TO THE RAT HIPPOCAMPUS PRODUCES DEFICITS IN NONRECURRING-ITEM DELAYED NONMATCHING-TO SAMPLE. E.R. Wood*, D.G. Mumby*, J.P.J. Pinel, and A.G. Phillips. Dept. of Psychology, Univ. of British Columbia, Vancouver, B.C., V6T 1Y7.

Rats subjected to transient forebrain ischemia induced by bilateral carotid occlusion combined with hemorrhagic hypotension develop irreversible neuronal loss limited to the CA1 region of the hippocampus (Mudrick, L.A., et al., Prog. Brain Res., 78:87-93, 1988). Evidence suggests that selective damage to this brain area produces memory deficits both in humans (Zola-Morgan, S., et al., Neurosci., 6:2950-2967, 1986) and in monkeys (Bachevalier, J., and Mishkin, M., Neuropsychologia, 27:83-105, 1989). Ischemic rats and sham-surgery controls were tested on a nonrecurring-items delayed nonmatching-to-sample task (Mumby, D.G., Pinel, J.P.J., & Wood E.R., this meeting). This test is analogous to tests that are sensitive to medial temporal lobe damage in humans and other primates. Ischemic rats took longer to reach criterion (at least 21/25 correct on two consecutive sessions) than controls (725 trials vs 350). Once criterion had been achieved the interposition of 15-, 30-, and 60-s delays between the sample and the test produced significant performance deficits. These findings suggest that the performance deficits produced in humans and monkeys by selective CA1 cell loss can be modelled in the rat.

121.20

SEVERE IMPAIRMENT ON CONCURRENT OBJECT DISCRIMINATION FOLLOWS CEREBRAL ISCHEMIA IN RATS. L.A. Rothblat, J.R. Graham* and T. Zheng*, Dept. of Psychology, The George Washington University, Washington, D.C., 20052.

It is now well established that restricted areas of the hippocampus, most notably the CA1 subfield, are especially susceptible to ischemic injury. Recent clinical findings suggest that such limited damage can result in severe and enduring amnesia. To further explore the relationship between ischemia, the hippocampus and memory, we trained rats which had been subjected 3 months earlier to an ischemic episode on a 4-pair visual concurrent object discrimination (COD).

Rats subjected to cerebral ischemia were severely impaired on COD. Ischemic animals required significantly more days to learn the discriminations (X=22.8, S.E.=2.5) than did normal (X=13, S.E.=1.1) or sham-operated controls (X=10.0, S.E.=1.7). In fact, 4 of 10 ischemic animals failed to reach criterion within 30 days. Further analysis indicated a significant correlation between degree of damage in the CA1 subfield and discrimination performance.

The present findings indicate that COD may be a particularly sensitive assay of hippocampal dysfunction. They also suggest that temporal lobe structures in rats may serve mnemonic functions which are qualitatively similar to those of human and nonhuman primates. Supported by ONR N00014-88-K-0227.

121.21

MK-801 REDUCES MORPHOLOGIC AND BEHAVIORAL SEQUELAE OF KAINIC ACID-INDUCED STATUS EPILEPTICUS. B.A. Stein, R.M. Sapolsky, J. Nagode *, H.P. Davis, & B.T. Volpe. Dept Biol Sci, Stanford Univ., Stanford CA 94305; Dept Psychol, Univ Colorado at Col Springs, 80933; Dept Neurol, Cornell Univ Med Ctr, White Plains, NY 10605.

The hippocampus is vital to learning and memory, and neuropathologic insults to the structure impair cognition. Various insults, including hypoxia-ischemia, hypoglycemia and seizure, appear to damage the hippocampus via excessive stimulation of the NMDA receptor by amino acid neurotransmitters. Thus, the degenerative consequences of these insults are diminished with NMDA receptor antagonists. It is not clear if only partial hippocampal protection (with receptor blockade) is sufficient to spare cognitive function. We test this possibility.

Rats were injected ip with either saline, or the excitotoxin kainic acid (KA; 10mg/kg) with or without treatment 20 minutes prior with the NMDA antagonist MK-801 (10mg/kg). Rats received 5 seizures in 15 days, recovered for 30 days, and were tested for 60 trials on a 12-arm radial maze with 7 arms baited. Rats were perfused and volumes of hippocampal damage. KA-alone rats sustained considerable damage, with $24 +/- 12 \times 10^5$ cubic microns of damage in their hippocampi. KA + MK-801 rats had 35% less damage than did KA-alone rats. The groups did not differ in seizure intensity or mortality rate.

The reduction in damage with MK-801 also spared cognition; saline and KA + MK-801 rats did not differ in working errors (reentry of a baited arm), reference errors (entry of an unbaited arm), or working-reference errors (reentry of an unbaited arm). KA-alone rats were impaired in all three categories ($p < .01$ in each case). Thus, even partial attenuation of hippocampal damage can protect cognitive function.

121.22

LESIONS OF THE PERIRHINAL CORTEX BLOCK FEAR-POTENTIATED STARTLE. J.B. Rosen, J.M. Hitchcock, M.J.D. Miserendino* and M. Davis. Dept. of Psychiatry, Yale Univ. Sch. of Med., 34 Park St., New Haven, CT 06508.

The acoustic startle reflex can be potentiated by presentation of a stimulus previously paired with a shock (i.e., fear-potentiated startle). Neuroanatomical, electrical stimulation and lesion studies have demonstrated that this potentiation involves a direct projection from the central nucleus of the amygdala to the acoustic startle pathway. However, little is known about brain areas which may activate the amygdala during fear-potentiated startle. The present study sought to determine cortical areas which may be critical for fear-potentiated startle using a light as a conditioned stimulus.

Rats were trained to be fearful of a light by pairing it with shock. The following day, five groups of 7-10 rats were lesioned in one of the following areas: the visual (including areas 17, 18a, 18b, and retrosplenial cortex), frontal (including parietal and cingulate cortices), medial prefrontal, insular, or perirhinal cortices. Six days later, rats were tested for fear-potentiated startle by presentation of an acoustic startle stimulus alone or in the presence of the light conditioned stimulus. Fear-potentiated startle was markedly attenuated only in rats with perirhinal cortex lesions. Subsequent experiments determined that lesions of only the rostral part of the perirhinal cortex (as defined by Paxinos and Watson, 1986) were necessary to block fear-potentiated startle.

These results indicate that the perirhinal cortex (a multisensory association cortex that has direct projections to the amygdala), but not many other cortical areas, plays an important role in fear-potentiated startle using a visual conditioned stimulus. Neuroanatomical tracing will be performed to elucidate the perirhinal afferents and efferents that may be important for fear-potentiated startle.

121.23

IBOTENIC ACID LESIONS OF RETROHIPPOCAMPAL AREA: EFFECTS ON BEHAVIOUR SLEEP/WAKING PATTERNS AND EEG. Hagan J.J., Verheijck E., Spigt M.H. and Ruigt G.S.F. (SPON F. JENCK). CNS Pharmacology, ORGANON B.V. OSS, 5340 BH - The Netherlands.

The effects of bilateral ibotenic acid injections in the entorhinal cortex of rats were studied. Following histology rats were divided into two groups: (RH) had lesions in subiculum, medial and lateral entorhinal cortex and posteroventral hippocampus: EC/SUB had a similar pattern but with no hippocampal lesion. Ache staining was increased in the molecular layer of the dentate gyrus. Place navigation learning was unaffected but EC/SUB animals were impaired when retrained to a new location. For a second set of experiments lesioned rats were implanted with cortical electrodes. Light period deep sleep was increased, REM sleep decreased and spindles increased in the EC/SUB group. REM sleep EEG peak frequency was reduced in RH rats and both groups showed reduced amplitude. During active waking both lesions reduced peak frequency and peak amplitude. No changes were found in deep sleep EEG. Both groups were nocturnally hyperactive but open field behaviour was unchanged. SHAM and RH rats, but not the EC/SUB group, reacted to a novel object with an increase in contact time. Partial excitotoxic lesions of the entorhinal cortex and subiculum cause subtle and selective impairments in behaviour and cortical EEG.

122.1

CIRCUITRY OF THE EXTENDED AMYGDALA: INTRODUCING THE USE OF FLUORESCENT DEXTRANS FOR RAPID, ANTEROGRADE AND RETROGRADE TRACT TRACING. L.C. Schmued* and L. Heimer. Dept. of Otol., Univ. of VA, Charlottesville, VA 22908.

This study introduces the use of two rhodamine conjugated dextran derivatives, RBD70K, and TMRD10K. Like the PHA-L technique, TMRD10K allows for a detailed visualization of anterogradely labeled fibers and terminals, is compatible with numerous other histochemical procedures, but does not require any histochemical processing. The ECAC (extended central amygdaloid continuum) is named for its best characterized component, the central amygdaloid nucleus. It also includes the lateral bed nucleus of stria terminalis, and portions of sublenticular substantia innominata. Injection of three different retrograde fluorescent tracers including RBD70K into the vagal complex, mesopontine tegmentum, and substantia nigra verified that all ECAC components project to these brainstem areas. It was also found that 2-5% of ECAC neurons with brainstem projections send axon collaterals to one or both of the other injected brainstem nuclei. Injection of retrograde fluorescent tracers into the vagal nuclei and substantia nigra, and the anterogradely transported fluorescent dextran TMRD10K into the mesopontine tegmentum, revealed ECAC cells with identified brainstem efferents relative to the location of the reciprocal afferents from the mesopontine tegmentum. Supported by NIH Grant NS17743.

122.2

UPTAKE, REPLICATION & TRANS-SYNAPTIC PASSAGE OF PSEUDORABIES VIRUS IN BRAINSTEM CIRCUITS. L. Rinaman[1], A. Robbins*, M. Whealy*, J. P. Card and L. Enquist*. The DuPont Co., Wilmington, Delaware, 19880 and [1]Institute of Neurological Sciences, University of Pennsylvania, Philadelphia, PA 19104.

Previously, we established that pseudorabies virus may be effectively used to define neural circuits in the rat brainstem which influence gastric function. Injection of virus into the stomach wall results in uptake, retrograde transport and replication of virions within motoneurons of the dorsal motor vagal nucleus (DMV). In addition, the virus passes trans-synaptically to infect second-order neurons which synapse upon the peripherally projecting motoneurons. In the present ultrastructural investigation, we have used two polyvalent antisera generated against either the entire virus or gIII, the major envelope glycoprotein, to gain further insight into the specificity of the trans-synaptic transport and to examine the replication and assembly of the virus within neurons. Both antisera identify virus within a di-synaptic brainstem circuit consisting of DMV motoneurons and interneurons in the adjacent nucleus of the solitary tract (NST). The most intense and complete labeling of this circuit is achieved with the whole virus antiserum, which identifies major forms of the virus. Dense immunoreactivity is characteristically associated with replicating capsids within both the cell nucleus and cytoplasm. All other forms of the virus in the cell cytoplasm and dendrites also exhibit dense immunoreactivity. Particular associations with the nuclear membrane, Golgi cisternae and smooth endoplasmic reticulum (SER) are apparent. Antiserum to gIII identifies only enveloped forms of the virus, and results in dense immunoreactivity related to the Golgi cisernae and SER. Immunoreactivity from both antisera is also densely concentrated at postsynaptic densities of motoneuron perikarya and dendrites. It is striking that only the whole virus antiserum detects virus within presynaptic afferents at these sites, suggesting that the virus loses its envelope as it passes through the synapse. These data provide considerable insight into the replication, assembly and trans-synaptic transport of pseudorabies virus in the nervous system and reiterate the usefulness of this neurotropic virus for studying neuronal circuits.

122.3

REVEALING DISYNAPTIC PATHWAYS BY COMBINING ANTEROGRADE DEGENERATION, RETROGRADE TRACING, INTRACELLULAR STAINING AND ELECTRON MICROSCOPY. E.H. Buhl#+*, W.K. Schwerdtfeger#* P. Germroth#* and W. Singer# (SPON: A. MacKay-Sim). #Max-Planck-Institute for Brain Research, 6000 Frankfurt 71, FRG; +Vision, Touch and Hearing Research Centre, University of Queensland, St. Lucia, Qld 4067, Australia.

Synaptic circuitry was investigated by combining retrograde tracing, intracellular staining, anterograde degeneration and electron microscopy in the same piece of tissue. This methodological procedure was successfully applied to disentangle a disynaptic neuronal chain, which originated in the olfactory bulb, was synaptically relayed in the entorhinal cortex and terminated in the ipsilateral hippocampus. Presumed entorhinal relay cells were retrogradely labelled from their hippocampal termination site by means of the fluorescent tracer Fast Blue. Subsequently, the marked projection neurones were intracellularly injected with Lucifer Yellow in fixed slice preparations. Following a simple photo-conversion procedure, dye filled cells were flat-embedded and processed for electron microscopy. The origin of presynaptic afferents to identified relay cells was revealed ultrastructurally after lesion induced anterograde degeneration of olfactory mitral cell axons. Due to its reliability, technical simplicity and a high degree of selectivity the new approach is considered an appropriate tool for unravelling neuronal networks.

122.4

LABELLING OF AXONS OF PASSAGE BY *PHASEOLUS VULGARIS* LEUCOAGGLUTININ (PHA-L) B. R. Schofield. Dept. of Neurobiology, Duke Univ. Med. Cntr., Durham, NC 27710.

Phaseolus vulgaris leucoagglutinin has emerged as an important tool for tracing neuroanatomical pathways. Its reported advantages include little or no uptake by axons of passage. I report here that such uptake can occur and can lead to extensive labelling in both anterograde and retrograde directions.

In guinea pigs, 2.5% biotinylated PHA-L was injected by iontophoresis (+5uA for 15 - 60 min) through glass micropipettes (12-25 um tip dia.) into the trapezoid body, the spinal trigeminal tract, or the inferior cerebellar peduncle. Five days later, the brain was fixed by perfusion and PHA-L was localized with avidin-biotin-peroxidase immunohistochemistry.

Injections in the spinal trigeminal tract labelled axons in the trigeminal nerve and axons and terminals in the principle and spinal trigeminal nuclei. After trapezoid body injections, labelled axons extended more than 13 mm to reach the contralateral inferior colliculus. Injections into the inferior cerebellar peduncle labelled fibers entering the cerebellum, as well as trigeminocerebellar cells in the spinal trigeminal nucleus.

These results suggest that the possibility of labelling axons of passage should be considered whenever PHA-L is used. Supported by NIH grants NS 08177 and NS 14655.

122.5

THE ASSOCIATION OF FRAGMENT C OF TETANUS TOXIN WITH THE NEURONAL MEMBRANE. E.A. Neale, L.M. Bowers*, H. I. Trenchard, and W.H. Habig*. Laboratory of Developmental Neurobiology, NICHD, NIH and Laboratory of Bacterial Toxins, FDA, Bethesda, MD 20892

Fragment C, the binding portion of the tetanus toxin molecule, can be used with a specific, non-neutralizing monoclonal antibody (18.2.12.6) as a neuronal label for fetal mouse neurons in cell culture (Neale et al., Soc. Neurosci. Abst. 14:547,1988). Dissociated neurons treated with the Fragment C/18.2.12.6 complex shortly after plating, and with fluorescent secondary antibody 24 hours later, show fluorescence extending to the tips of the growing neurites. Such staining could result from lateral diffusion of the complex along the membrane during growth, or from dissociation of the complex and reassociation with newly formed receptors. When neurons are plated into a culture already containing neurons pre-labeled with complex, and the culture is treated at a later time with secondary antibody, both populations of neurons appear fluorescent. Binding and staining data indicate that the presence of a neutralizing monoclonal antibody (18.1.7) in the medium during the interval between exposure to complex and to secondary antibody reduces the amount of cell-associated Fragment C/18.2.12.6. These experiments suggest that Fragment C/18.2.12.6 can dissociate from and subsequently rebind to the neuronal membrane. In contrast, a complex of tetanus toxin/18.2.12.6 cannot be detected on the neuronal surface after 2 hr and is visualized only inside the cell after detergent treatment. These results suggest that the initial processing of Fragment C by the neuron is different from tetanus toxin.

122.6

NEURONAL PROCESSING OF TETANUS TOXIN FRAGMENT C DIFFERS FROM PROCESSING OF THE HOLOTOXIN. H.I. Trenchard, S.C. Fitzgerald*, W.H. Habig*, and E.A. Neale. Lab. of Develop. Neurobiol., NICHD, NIH and Lab. of Bacterial Toxins, FDA, Bethesda, MD 20892

The use of Fragment C, the binding portion of the tetanus toxin molecule, as a neuronal marker raises questions about the processing of this ligand by the cell. We have studied the binding of ^{125}I-labeled tetanus toxin and Fragment C (FrC) in 3 wk old dissociated cultures of fetal mouse spinal cord. For all experiments, toxin or FrC was applied to cultures in a physiologic salts solution for two hours at room temperature, after which cultures were rinsed and returned, in growth medium, to the incubator (37°C, 10% CO_2). Culture fluid was removed at various times and the cells dissolved in 1% SDS for assay of cell-associated radioactivity and gel analysis of toxin degradation.

Bound FrC is released rapidly into the culture medium during the first 24 hr, while tetanus toxin dissociates more gradually. FrC which is released from cells during 2 or 16 hr following initial incubation will rebind to new cultures. Tetanus toxin, that has been bound and released, loses some ability to rebind. A non-neutralizing monoclonal antibody against FrC (18.2.12.6), when present during binding, increases the amount of FrC and tetanus toxin bound to cells, and appears to stabilize the association of FrC with the membrane. A neutralizing antibody (18.1.7), when present in the culture medium during the interval following the initial incubation, decreases the amount of cell-associated FrC. Neither antibody has much effect on bound tetanus toxin, suggesting a more loose association of FrC with the membrane. SDS gels reveal that toxin breakdown products are found in the culture medium and not in cells. However, toxin breakdown occurs only in the presence of cells and not in conditioned medium. This suggests that cells have degraded the toxin and excreted the products.

122.7

EVALUATION OF C-FRAGMENT OF TETANUS TOXIN TO TRACE GUINEA PIG CENTRAL SYMPATHETIC PATHWAYS. R.L. Meckler, R. Baron*, & E.M. McLachlan*. School of Physiol. & Pharmacol., New South Wales Uni., Kensington, NSW 2033, AUSTRALIA.

The C- fragment of tetanus toxin (TTC) has been evaluated as a tool for retrograde transsynaptic identification of pathways in the sympathetic nervous system using immunohistochemical methods. TTC injection into medial gastrocnemius (MG) muscle, in addition to its retrograde transport within somatomotor, sympathetic and sensory axons, was taken up and concentrated by terminal varicosities within pre- and paravertebral ganglia at all thoracolumbar levels, after post-injection times of 6 to 55 h. Staining was absent in chronically denervated sympathetic ganglia, demonstrating the specific association of the antigen with preganglionic varicosities. Preganglionic varicosities at all thoracolumbar levels were also labelled after TTC injection into the peritoneal cavity or into denervated MG, either of which failed to label somatic motor or sensory neurones. The results indicate that this tracer cannot be used to demonstrate sympathetic pathways within the central nervous system. The data may help explain the widespread involvement of the sympathetic nervous system in tetanus.

122.8

A MODIFIED TMB HRP REACTION FOR LM AND EM PATHWAY TRACING. R.J. Weinberg, S.L.Van Eyck, and A. Rustioni. Depts. of Cell Biology & Anatomy, and of Physiology, U of North Carolina, Chapel Hill, NC 27599.

Compromises must be made in HRP histochemistry among many factors, including sensitivity, specificity, tissue preservation, and identifiability of label. Reactions at low pH with TMB chromogen and nitroferricyanide stabilizer provide unexcelled sensitivity, but give high background and poor preservation. Stabilization with molybdate reduces background and improves preservation, but is less sensitive. Tungstate stabilization of TMB by the following protocol is particularly useful for anterograde tracing.

Tissue sections in glass vials are incubated at room temperature on a shaker for 10' in 1 ml of 0.1M phosphate buffer (pH 6.0) with 50 µl of 1% ammonium paratungstate (K&K) in H_2O and 25 µl of TMB (Sigma) 0.2% in EtOH. Add 1 µl of H_2O_2 and incubate 30'-1 hr. Rinse twice in buffer and dry on subbed slides. Pass through alcohols to xylene, mount with DPX. The reaction produces small blue-green crystals; under crossed polarizers, anterograde label is bright pink, and labeled cells are green. Thionin gives an excellent stain with little loss of label; counterstaining enhances visibility of the retrograde labeling, although anterograde labeling becomes less visible in darkfield. The tungstate-TMB method is also suitable for EM. Optimal results are achieved when grain size is reduced (by shortening incubation to ~15') and label is stabilized with DAB: Rinse sections well in pH 6.0 cacodylate buffer (add 1 ml 1M HAc to 13 ml .05M sodium cacodylate); incubate 8' in 1 ml buffer with 0.5 mg DAB, 20 µl 1% $CoCl_2$, and 2 µl 3% H_2O_2. Rinse well, osmicate, dehydrate, and embed in Epon-Spurr; thin sections are post-stained with uranyl acetate and lead citrate. Small flattened crystals of reaction product are clearly visible in dendrites, somata, and axon terminals. The protocol has been combined with post-embedding immunocytochemistry to demonstrate glutamate in primary afferent terminals in rat caudal trigeminal nucleus.

This work was supported by NIH grants NS 12440 and NS23804.

122.9

NEW APPLICATIONS FOR HORSERADISH PEROXIDASE-REACTIVE CHROMOGENS IN IMMUNOCYTOCHEMISTRY AND TRACT TRACING AT THE LIGHT AND ELECTRON MICROSCOPIC LEVELS. M.N. Lehman and R.B. Norgren, Jr. (SPON: P. Sanberg) Anat. and Cell Biol., Univ. of Cincinnati Med. Coll., Cincinnati, OH 456267

We have found that chromogens developed for HRP-tract tracing or peroxidase based immunocytochemistry (ICC) can be profitably utilized in other applications and in combination with other chromogens (Norgren & Lehman, J. Histochem. Cytochem, 1989). Diaminobenzidine (DAB) has been used for both tract tracing and ICC experiments. Other chromogens have been utilized primarily for either tract tracing or ICC. Recently, we have been investigating the properties of Indophane Blue (Viomedics). We have found that this chromogen can be used in tract tracing as well as avidin-biotin-peroxidase immunocytochemistry. Indophane Blue is sensitive enough to visualize anterogradely transported cholera-toxin-HRP in the visual system and appears to be more sensitive than DAB in this application, though not as sensitive as tetramethylbenzidine (TMB). An important advantage of this chromogen over TMB is that tissue sections can be processed at pH 6.0 rather than pH 3.3. The blue color of this chromogen is easily distinguishable from the brown DAB reaction product making it an excellent second label for immunocytochemistry. Electron microscopic observation suggest that Indophane Blue is electron dense. [Supported by NIH grants HD21968 and NS24292 (MNL)].

122.10

REACHING THE UNREACHABLE: USING THE CARBOCYANINE DYE DiI TO TRACE FIBRES IN THE RAT FETUS AND HUMAN BRAIN. Stuart Bunt, Husni Al-Goshae* and Greg Irwin*. Dept. of Anat. and Physiol., The University, Dundee, DD1 4HN, U.K.

The stable, highly fluorescent DiI embeds in lipid membranes and diffuses slowly to cover the cell envelope. As this diffusion can occur even in fixed material it enables visualization of fibre morphology and pathways in previously difficult or inaccessible sites. We place crystals of DiI (1, 1'-dioctadecyl, -3, 3, 3', 3'-tetramethylindocarbocyanine perchlorate) directly on the fibres to be traced and maintain the tissue in fixative at 37° or room temperature for up to six weeks. The dye has enabled us to observe ganglion cell axons in the human retina and we have successful examples from whole and 50-250um vibraslice sections of hippocampus, thalamus, neocortex, cerebellum, lateral geniculate body, superior colliculus and corpus callosum of adult human brains; a) from donated specimens held in 4% formalin for up to six months, b) postmortem specimens fixed in formalin within 12 hours of death and c) unfixed postmortem tissue obtained within 12 hours of death.

In E12-E15 rat embryos we have labelled growth cones of the first spinal axons. In E14 and E15 embryos most of these are club like endings between 3 X 8 um and 7 X 35 um. At earlier stages and in ventral tracts larger filopodial structures between 5 X 30 and 7 X 30, resembling the wider growth cones seen in vitro, are more common.

122.11

WITHDRAWN

122.12

Preliminary Attempts at Retrograde Labelling and Culturing of Spinothalamic (ST) Neurons in Fetal and Postnatal Rats K. Thor, C. Pechura, V. Smallwood*, N. Silva, J. Barker NIH, Bethesda, MD 20892

We are attempting to establish long-term cultures of ST neurons that are retrogradely labelled with rhodamine beads (RB) by refining techniques for 1) culturing postnatal ST neurons and 2) *in utero* injections for labelling fetal ST neurons. Postnatal (PN) day 4-5 pups were injected with 200 nl RBs into the thalamus while under halothane anesthesia. 5 days later, the spinal cords were dissociated with various proteases and cultured in various media. In optimal cases, obtained using papain dissociation and culturing in media with 5% newborn calf serum, 3-5 RB-labelled neurons/plate survived for at least 9 days in culture. Postnatal neurons were more susceptible to dissociation damage than were embryonic cells, and recording was difficult. RB-labelled neurons did not develop to the same extent as unlabelled neurons. *In utero* RB injections into the brains of E17-E19 fetal rats were made in chloral hydrate-anesthetized dams, whose uteri were placed into a specially-designed stereotaxic holder. A needle (30g), inserted through the uterine wall and fetal skull, delivered 200 nl of RBs into the fetal brain. The uterus was returned to the peritoneal cavity and the abdomen sutured. At various times post-injection (1-3 days) the fetuses were removed, and their spinal cords were frozen for sectioning or dissociated for culturing. All of the dams survived with no complications (n=4), and 25% of the injected fetuses (n=20) developed normally. Numerous RB-labelled neurons were seen in the fetal brains and/or spinal cords, but these preliminary injections were not accurate. Refinement of coordinates should allow placement of the needle tip into the fetal thalamus *in utero* and eventual culturing of ST neurons. We thank C. Asanuma for advice on *in utero* injections.

122.13

IDENTIFICATION OF ISOLATED MAGNOCELLULAR NEUROENDOCRINE CELLS (MNCs) FROM THE HYPOTHALAMUS. M.L. Weiss and P. Cobbett Pharmacology & Neurosci. Prog., Michigan State Univ. E. Lansing, MI 48824

We wanted to stain selectively neuroendocrine cells from young adult rats following intravenous (IV) injection as others have done with HRP. In order to visualize dispersed, living cells, however, we needed to substitute HRP with a retrograde fluorescent tracer. Evans Blue (EB; 10%) was found to produce specific hypothalamic labeling similar to that of HRP, after injection into either subclavian or tail veins and a 1-2 day survival. Following EB treatment, small blocks of tissue containing the supraoptic nucleus were enzymically and mechanically dissociated and plated onto Con-A coated coverslips. Apparently healthy dispersed neurons were located using phase contrast microscopy; the majority of these cells contain EB. Visualization of the dye using epi-fluorescence did not appear to affect the phase brightness of neurons, even after long exposures to incident beam. We plan to confirm that the EB labeled MNCs contain neurophysin with immunocytochemistry. These results show that MNCs can be recovered and identified following dispersion, and suggest that it may be possible to electrophysiologically record from isolated, identified MNCs. Supported by NS 08125 and the Pharmaceutical Manufacturers Association Foundation.

122.14

IN VITRO LABELLING TO DEMONSTRATE CONNECTION BETWEEN HOST RETINA AND FETAL TECTUM OR CEREBELLUM GRAFTS IN ADULT RATS. R.V. Stirling, R.Aramant, M.Seiler and A.R. Adolph. Eye Research Institute, Boston, MA 02114

A previous study has shown that embryonic brain tissue can be grafted to adult rat retina (Aramant et al., Soc. Neurosci. Abstr. 14:1276). We have developed a technique to investigate how these grafts connect with the host retina. Pieces of E15 tectum or cerebellum were placed through an incision through sclera, choroid and retina into a retinal lesion site (posterior approach). Such grafts survive, develop and integrate with the host retina.

In vitro labelling with horseradish peroxidase allows the label to be placed selectively either to where extensions from the graft join the host retina or into the graft itself. Following a warm saline perfusion, the retina is removed from the sclera and flattened down onto a nylon mesh in a dish containing cold modified Ames medium (Langdon, pers. comm.), perfused with 95% 0_2 5% $C0_2$. HRP is applied using fine microelectrodes tipped with concentrated HRP. The retina is pinned down with a fine veil and left in the oxygenated solution at 28°C for 5-6 hrs. The retina is fixed between two coverslips for 1-2 hrs. at 4°C; it is processed affixed, vitreous up, to a glass slide with albumin/gelatin embedding medium using the ion intensified DAB reaction (Adams 1971). The retina can easily be removed for resectioning.

Using this technique, we have labelled cells in tectal grafts by HRP application to the host retina, and have seen fine fibers leaving the graft. These usually do not progress very far, often making hairpin turns back to the graft. Occasionally, fibers from the graft do enter the host where they make extended randomly oriented arbors in the outer part of the inner plexiform layer, with swellings which may indicate synaptic contact.

This whole-mount in vitro method is simple to use and yields complete filling of RGC's, and their axons, and has demonstrated connection between tectal grafts and host retina.

122.15

DISTINCT IDENTIFICATION IN VIVO OF TWO LABELED POPULATIONS OF GRAFTED MOUSE NEUROBLASTOMA CELLS USING RHODAMINE AND FLUORESCEIN LATEX NANOSPHERES. **B.A. Bonsack,* J.D. Macklis* and R. Madison,+*Dept. of Neurology and Program in Neuroscience, Harvard Medical School, Children's Hospital, Boston, MA and +Division of Neurosurgery, Duke Univ. Durham, NC**

Latex nanospheres with a wide range of incorporated chromophores were developed for selective labeling of neuronal subpopulations in vitro and in vivo. In vitro studies show some neuronal populations accumulate and store these nanospheres more avidly than non-neuronal populations. Neurons are retrogradely labeled by these nanospheres in vivo(1) Mouse neuroblastoma cells were chosen as a model system to show that separate populations of neural cells can be distinctly labeled, grafted in vivo, and distinguished from host cells and from each other.

Mouse neuroblastoma cultures were grown using standard methods, labeled in vitro with either rhodamine or fluorescein nanospheres by direct application of concentrated nanospheres, and washed. After 24 hours, cells were examined for label intensity, labeling efficiency, and viability. Rhodamine and fluorescein labeled cells were combined in single cultures and followed for one week. Cells from each initial culture remained healthy and uniquely labeled with eccentric granular fluorescence after coculturing; no double labeling was seen. In vivo grafts of two distinctly labeled cultures into overlapping regions of mouse neocortex demonstrate unique identification of cells from each graft. Preliminary studies suggest that mouse neocortical neurons can be similarly labeled, transplanted, and distinctly identified from multiple donor sources. Supported by NS26311, HDOO859, Alzheimer's Assoc., & Whitaker Fdn.

1) Macklis JD., Madison R., SOC Neurosci Abst 1988,14;219.12

122.16

ULTRASTRUCTURAL EVIDENCE FOR GABA-ERGIC BRAINSTEM PROJECTIONS TO SPINAL MOTONEURONS. J.C. Holstege*(SPON: M. Godschalk) Anatomy Dept. Erasmus Univ. Rotterdam, P.O.Box 1738, 3000 DR Rotterdam, The Netherlands.

Several studies have shown the existence of serotonergic and non-serotonergic brainstem projections to spinal motoneurons. In the present study in rat it was investigated whether the non-serotonergic projections contained GABA. WGA-HRP was injected in the ventro-medial part of the lower brainstem. After perfusion, the L5 and L6 spinal segments were dissected, treated for TMB histochemistry, osmicated and plastic embedded. Immunogold postembedding immunocytochemistry was performed with a GABA antibody (a gift from Dr. R. Buys, Amsterdam). In the lateral motoneuronal cell groups 505 WGA-HRP labelled terminals were analysed at the ultrastructural level. Nearly 40% of these terminals were also labelled for GABA. 81% of the double labelled terminals were of the F-type, 12% were G-type (indicating possible co-existence of serotonin and GABA) and 7% were S-type. The results show the existence of a substantial GABA-ergic (inhibitory) brainstem projection to lumbar motoneurons. These projections may act as a gain setting system, thereby counteracting the serotonergic fibers in the control of the excitability of spinal motoneurons.

122.17

IMMUNOCYTOCHEMISTRY OF AFFERENTS TO HRP-LABELLED MOTONEURONS (MNs): SEQUENTIAL LIGHT AND EM ANALYSIS USING ACROLEIN FIXATION. M.S. Beattie, M.G. Leedy, and J.C. Bresnahan. Depts. of Surgery and Anatomy and Neurosci. Prog., Ohio State Univ., Columbus, OH 43210.

Acrolein (CH_2=CH-CHO) has been reported to be a superior fixative for EM immunocytochemistry (King, J. et al., J.Histochem.Cytochem.,31:62, 1983). We have employed this technique in combination with retrograde HRP.

HRP was applied to rat sciatic nerve; after 2 days, rats were perfused with 5% acrolein in PBS. Tissue was vibratomed and processed simultaneously for HRP and immuno-staining using antibodies to 5-HT, L-enkephalin (ENK), and dopamine-beta-hydroxylase (DBH), the ABC technique, and glucose oxidase. Plastic-embedded 60 um sections were used for LM analysis, then thin-sectioned for EM. Densely-stained DBH and L-ENK terminals in apposition to HRP-labeled MNs were evident through the depth of 60 um plastic sections. 5-HT label was inconsistent. EM showed stained synaptic appositions to HRP labelled MNs. The procedure should be useful for quantitative analyses of afferents to identified MNs, and provides excellent morphology in thick plastic sections. (NS10165)

122.18

DOUBLE LABELING OF CYTOCHROME OXIDASE AND GAMMA AMINOBUTYRIC ACID IN CENTRAL NERVOUS SYSTEM NEURONS OF ADULT CATS. X.G. Luo*, R.F. Hevner and M.T.T. Wong-Riley. Dept. of Anatomy & Cellular Biology, Med. College of Wis., Milwaukee, WI 53226.

The relationship between the levels of cytochrome oxidase (C.O.) and gamma aminobutyric acid (GABA) was investigated within single neurons by double labeling the two markers in the same section. Seven adult cats were used. Frozen brain sections were cut at 15 um and divided into two groups. One group was incubated with GABA immunogold-silver staining (modified from Holgate et al., J. Histochem. Cytochem., 31:938-944, '83) followed by indirect immunoperoxidase for C.O. (Hevner & Wong-Riley, J. Neurosci., in press). The second group was incubated for C.O. histochemistry (Wong-Riley, Brain Res., 171:11-28, '79) followed by immunogold-silver staining of GABA. Double staining was equally effective for both methods. The discrete black silver grains were clearly distinguishable from the brown enzyme reaction product within the neuronal cytoplasm. There was no evidence of cross reaction between the two primary antibodies in our experiments. These combinations enabled the demonstration of four different types of labeling within neurons in the same sections: C.O.+/GABA+, C.O.+/GABA-, C.O.-/GABA+, and C.O.-/GABA-. Neurons in the perigeniculate nucleus and basket cell terminals in the cerebellum were GABA positive and rich in C.O. Interneurons of the lateral geniculate nucleus as well as stellate and Golgi cells of the cerebellum were GABA-rich but poor in C.O. These results demonstrate that GABAergic neurons in the CNS can have either high or low levels of C.O. (Supported by NIH NS18122 and EY 05439 to MWR and MCW MSTP Fellowship to RFH).

122.19

NOVEL, LAMINAR- SPECIFIC IMMUNOSTAINING OF NEURONS IN LAYERS 1,2,3 AND 5 OF CAT VISUAL CORTEX. Mala Glenwright,* Maynard Morrison,*+ David Dobbie*+ and J.A. Matsubara (SPON: D.P. Phillips). Depts. of Ophthalmology, Anatomy and the Neurosciences, Univ. of Brit. Columbia, V5Z 3N9. Canada and + Dominion Biologicals, Dartmouth, Nova Scotia B3B1M1. Canada.

Recent advances in hybridoma technology have provided us with new markers for specific cell types and cell layers in the central nervous system. We report a new monoclonal antibody which recognizes neuronal populations in layers 1,2,3 and 5 in cat visual cortex.

Monoclonal antibody #73 7A4-2 (Dominion Biologicals, Limited) was raised against an antigen isolated from human blood cells and derived from overgrown tissue culture supernatant. While not yet fully characterized, it is specific for human blood group A. We screened this antibody, and others, on fixed tissue from cat and rat.

Animals were deeply anaesthetized and perfused transcardially using a series of standard fixatives. Sections were first incubated overnight in primary antibody (or in phosphate buffer for control sections), followed by standard procedures using the avidin-biotin method (Vector Labs). Vibratome sections yielded better immunostaining than frozen sections.

Monoclonal antibody #73 7A4-2 labeled neurons in supragranular layers 1,2 and 3 and in layer 5 in areas 17 and 18 of cat visual cortex. The cytoplasmic, but not the nuclear, compartment of both pyramidal and non-pyramidal cells in these layers were labeled. Among these cells, the larger pyramidal cells in layers 3 and 5 were significantly darker and more intensely stained than the other neurons. Furthermore, the apical dendrites of these cells exhibited exceptionally intense immunostaining. Diffuse labeling was present in adjacent cortical areas (cingulate cortex and area 19), but the strong, laminar specificity was confined to areas 17 and 18. No additional organizational features were observed in sections cut in the horizontal plane. (This work funded by MRC MA-9150 to J.M.).

122.20

WITHDRAWN

122.21

"FLUORO NISSL GREEN": PURIFICATION AND FUNCTIONAL CHARACTERIZATION OF A NOVEL FLUORESCENT NISSL STAIN FOR NEUROANATOMIC TECHNIQUES. B. Quinn, Dept. of Brain & Cognitive Sciences, MIT, Cambridge, MA 02139.

Fluorescent neuroanatomical techniques, such as immunofluorescence and retrograde tracing with novel markers like Di-I, derive great utility from their specificity. However, this specificity can be a drawback as well, in that it may be difficult to achieve a detailed assessment of labeled neurons or axons in their cytoarchitectonic context. We have reported a protocol which led to a green fluorescent counterstain for neuronal perikarya (Quinn & Weber, Soc Neurosci 14:219.6, 1988). An enhanced synthesis and purification of this fluorochrome is now described, along with functional characterization. 200-400 mg of m-phenylene diamine (Sigma; Aldrich), 400 ul glycerol, and 200 ul acetic acid in 10 ml H2O are heated to boiling in a lab microwave, developing a lemon yellow color. The solution is diluted 20-fold with H2O and applied to a SepPak C18 disposable cartridge (Waters, Milford MA, No.51910). The cartridge is washed with 20 ml H2O and the fluorochrome eluted with 2 ml aliquots of 10-20-30-40-50% MeOH or acetonitrile. The most active fluorescent fraction, confirmed by spot illumination, is dried in a Speedvac vacuum centrifuge or by an N2 stream. We further purified the compound by C8-RP-HPLC, but it can be used brought up directly in buffered glycerol pH 7.5-8.0 (e.g., 100 mM Tris:Glycerol 1:1).

The SepPak-purified compound has a maximum absorbance of 434 nm and maximum emission at 517 nm, with respective half-intensity bandwidths of 54 and 46 nm (C. Earley, Dept. of Chemistry, MIT). Thus, it is excited by most fluorescein, and certain UV, filters; the emission peak is very close to FITC. It provides an excellent green counterstain to rhodamine, and to Di-I (S. McConnell et al., this meeting). Preferential perikarya staining is obtained above pH 7, and nuclear staining at acid pH. In a model on-slide system, staining was preferential for rRNA over protein; and like a classical Nissl stain, cresyl violet, staining of perikarya and nucleolus is attenuated by RNAse treatment. Although the earlier 1988 protocol was suggestive of the Skraup reaction, the reaction is active in the presence of polyalcohols from ethylene glycol to dextrose. The rapid, but low, yield is consistent with a contaminating reactant, although one candidate, m-nitroaniline, which would lead to chrysoidin-like azo fluorochromes, was inactive. Full structural characterization of this compound is in progress.

122.22

TRAUMA INDUCED GOLGI-LIKE STAINING OF DENDRITIC TREES. A.N.van den Pol and F.Gallyas*, Sect.Neurosurgery, Yale Univ.Sch.Med.,New Haven,CT. 06510.

A critical problem in neuroscience has been that of identifying damaged neurons immediately after moderate brain injury. We report here a new approach which allows the silver staining of the soma and dendritic tree, including spines, of traumatized neurons throughout the CNS. Unilateral brain injury in heavily anesthetized rats resulted in labeling only in the vicinity of the trauma, while non-traumatized contralateral loci showed no labeled neurons. The stain worked equally well on pig, mouse, and human brain, and labeled neurons injured by micropipette, pressure, toxic chemicals, and other means. Even with fixation starting as soon as 1 min after injury, neurons were labeled. For experimental neurocytology, selected neurons can be traumatized in vitro under microscopic control, allowing the stain to be used as a method for selectively labeling dendritic trees with a Golgi-like appearance as demonstrated in the hypothalamic paraventricular and suprachiasmatic nucleus. Unlike Golgi impregnations which generally start as an expansion from a single crystal in a given cell, many independent regions of metallic precipitation appear in traumatized dendritic trees. Subsets of neurons in regions of the brain such as the hypothalamus or hippocampus appear differentially sensitive to different types of insult or injury, allowing characterization of neurons based on dendritic tree and response to injury.

122.23

POSTNATAL MAMMALIAN MOTONEURONS IN CULTURE. C. Krieger* and S.U. Kim. Division of Neurology, Dept. of Medicine, Univ. of British Columbia, Vancouver, BC, V6T 1W5.

Investigations of cultured mammalian motoneurons have generally used embryonic material which has been retrograde labelled using fluorescent dyes. Embryonic neurons differ, however, from those in the adult animal in that they undergo a period of naturally occurring cell death. Since there is no significant postnatal cell death in neurons of the rat or mouse, investigations on neonatal motoneurons may be more relevant to understanding the pathophysiology of disorders affecting the adult mammalian motoneuron.

Under cold anaesthesia, neonatal rats or mice (1-6 days of age) were injected with Rhodamine isothiocyanate into the proximal muscle masses of the extremities. After 24 to 48 hours, spinal cords were removed, dissociated and the labelled cell fraction was enriched using fluorescence-activated cell sorting. Cells were plated onto poly-L-lysine coated coverslips or feeder layers of rat brain astrocytes. Some of these labelled motoneurons were capable of survival for more than 5 days in vitro and demonstrated neurofilament and neuron-specific enolase immunoreactivity.

Supported by the Canadian MRC and the ALS Society of Canada.

122.24

IMMUNOGOLD SILVER STAINING OF INTRACELLULARLY INJECTED SUPRAOPTIC NEURONS. LL. Smithson*, K.G. Smithson and G.I. Hatton. Neurosci. Prog., Michigan State Univ., E. Lansing, MI 48824.

Our studies have sought to integrate functional and structural properties of neuroendocrine cells by combining intracellular electrophysiology and injection of Lucifer Yellow CH (LY) with immunocytochemical (ICC) analysis (J. Neurosci. Meth., 10:59,1984). This procedure used an avidin biotin-HRP complex and 3,3'-diaminobenzidine (DAB) as a marker and while generally successful, one limitation has been the opacity of the ICC marker. Incompletely filled cells, such as those dye coupled to an injected cell, may be dim and sometimes rendered unidentifiable. To alleviate this problem we have evaluated another ICC marker, 5-15 nm colloidal gold. After dye injection, tissue is fixed, dehydrated, and embedded in polyethylene glycol. Serial sections (3-5 μm) are cut, and ICC is done on those sections containing portions of cytoplasm and/or the nucleus of dye filled cells following which the gold-labeled sections are silver intensified. This silver-intensified colloidal gold marker produces a punctate reaction product within the cytoplasm of an immunopositive cell. Unlike DAB, this reaction product results in little or no attenuation of the injected cell's LY fluorescence. The use of a silver-intensified colloidal gold marker should improve the reliability of this ICC procedure, especially for dimly fluorescent cells and allows positive identification of sections that contain only a small portion of the cell. Supported by NS 16942 and a fellowship from the Medical Scientist Training Program to KGS.

122.25

A TRIPLE-LABELING TECHNIQUE TO IDENTIFY AND REVEAL THE DETAILED MORPHOLOGY OF MOTONEURONS RECORDED IN VITRO. F. Viana*, L. Gibbs* and A.J. Berger (SPON: W. Spain). Dept. Physiol. & Biophys., Univ. of Wash., Seattle, WA 98195.

Techniques such as antidromic activation commonly used in vivo to characterize cells in a motor pool (motoneurons vs. interneurons) may not be possible in vitro. We devised a triple-labeling protocol to circumvent this problem in guinea pig hypoglossal nucleus (HN) studies. The retrogradely transported marker, rhodamine-dextran (RhD), was applied to the cut central end of the 12th nerve in the neck. After 2 days, brainstem slices (500 μm) were prepared and intracellular recordings obtained from HN neurons. After characterizing the cell physiologically, a mixture of 1.5% Lucifer Yellow (LY) and 1% biocytin (dissolved in 1M LiCl) was injected via iontophoresis into the cell. Slices were then fixed, frozen and sectioned at 80 μm and sections were examined using a fluorescence microscope; 91% (n=21) of LY-injected cells were recovered. Nineteen of these cells were double-labeled (fluorescence for both Rh and LY), thereby positively identifying them as HN motoneurons. After photomicrography, the sections were rehydrated and the biocytin visualized with streptavidin-biotin and diaminobenzidine as the chromagen. All LY-labeled cells then exhibited permanent HRP-like staining quality, allowing detailed reconstruction of somal, dendritic and axonal morphology. We conclude that: 1) RhD can be used as a retrograde marker for central neurons projecting to the periphery; 2) Biocytin injected with LY can provide permanent and detailed morphology of Rh-labeled central neurons. (NS 14857)

122.26

QUANTITATIVE MORPHOLOGICAL COMPARISON OF RAT DENTATE GRANULE CELLS INJECTED IN FIXED SLICES AND THOSE FILLED IN VITRO. **A.M. Felthauser* and B.J. Claiborne. Division of Life Sciences, University of Texas, San Antonio, TX 78285.**

Previous studies have shown that intracellular injections can be made in fixed cortical tissue (Neurosci. 28:3, 1989), but it is not yet known if neurons labeled using this method are adequately stained for quantitative analyses. Here we compare the dendritic structure of granule neurons injected with Lucifer Yellow in fixed hippocampal slices to the structure of granule cells filled with HRP in vitro and analyzed in our earlier work (Claiborne et al., in press). Hippocampi were removed from 7 Sprague-Dawley rats between the ages of 37 and 41 days. Transverse slices 400 um thick were cut on a tissue chopper, fixed for 45 min. in 4% para-formaldehyde, rinsed in buffer and mounted on gelatin-coated slides. Granule neurons in the dorsal blade were filled with Lucifer Yellow (2.5% in 0.5 M LiCl). Slices were re-fixed overnight, dehydrated in EtOH and cleared in methyl salicylate. Results showed that almost all dendrites extended to the pia, as is seen with in vitro injections, and that there were 29.6 ± .9 segments per cell (mean ± s.e.m.; n=16). This was not significantly different (t-test) from the 31.5 ± 1.0 segments seen in neurons (n=26) filled with HRP in vitro. These results indicate that neurons filled in fixed slices are adequately labeled for quantitative studies. (Supported by grants BNS 8709366 and AG 07141.)

122.27

A VERSATILE, HIGH-RESOLUTION, MULTI-USER IMAGE ENHANCEMENT AND ANALYSIS WORKSTATION FOR NEUROBIOLOGY. R.S. Nowakowski, M.D. Egger, H.M. Geller, J.P. Grierson, T. Liang,* and †J.A. Blumert*. Image Enhancement and Analysis Facility, Departments of Anatomy and Pharmacology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854, and †Silicon Graphics, Inc., East Hanover, NJ 07936.

In order to exploit recent advances in computer imaging technology, we have assembled a versatile, general-purpose, high-resolution imaging workstation for use by a diverse group of neurobiologists. We use a Silicon Graphics IRIS (Model 4D/70GT) Unix-based graphics workstation equipped with a digitizing and image analysis board (Androx ICS 400), a high-resolution (1024 x 1024 pixels) video camera (Dage 81), a color screen-printer (Seiko CH-5103) and software packages (Androx ICL and Synoptics Semper 6). Images can be displayed and analyzed in gray-scale (up to 1024 gray values) or in 24-bit color (over 16 million shades) at resolutions up to 1024 x 1024 pixels. An ethernet port allows rapid transfer of images to and from remote sites, including PC's.

Capabilities of our system include: 1) 3-dimensional display of neuron trees from Golgi-stained or HRP-filled neurons, 2) enhanced (digitized, averaged and histogram-stretched), high-resolution images of immunohistochemically-stained neurons in culture, 3) image-ratioing and cross correlations to demonstrate co-localization of multiple fluorophores, 4) automated counting of BUdR-labeled proliferating cells in the developing brain, and 5) 3-dimensional graphs of flow cytometry data.

Supported in part by NIH grant 1S10RR04019.

122.28

THE STEREOLOGY OF AUTORADIOGRAPHY: MEASURING THE SIZES AND DENSITIES OF TRITIUM LABELED CELL NUCLEI. S.J. Clark* J. Cynx. (SPON: D.R. Griffin). The Rockefeller University, Field Research Center, Millbrook, NY 12545

True cell size distributions and densities cannot be measured directly from tissue sections. Stereological models have been developed to correct this problem. Unfortunately, these models are not applicable to autoradiographically labeled nuclei.

Here we present equations which can be used in conjunction with stereological models of the "unfolding" type (e.g. Wicksell, S.D., Biometrica 17:84, 1925) to reconstruct the sizes and densities of autoradiographically labeled nuclei.

We show how these equations were developed, how they can be modified so as to be applicable to different experimental parameters, and the assumptions and limitations of the models. Finally, we illustrate the use of the model on data from a study of neurogenesis in the avian telencephalon (Nottebohm et al. in prep.).

122.29

A TRUE QUANTITATIVE FLUORESCENT MICROSCOPY SYSTEM FOR VOLTAGE SENSITIVE DYE. R. M. Dasheiff and D. S. Sacks* Veterans Admin. and Univ. of Pittsburgh Epilepsy Center, Pgh. Pa. 15213.

Fluorescent microscopy is a powerful imaging technique. There has been an increasing interest and need to measure actual dye concentration in a sample. Recently, fluorescent voltage sensitive dyes (VSD) have been used as a histological marker for the membrane potential in neural tissue. However, to maximize the utility of this technique, the absolute dye concentration needs to be calculated. The dye can both record and *save* the information about the state of depolarization or hyperpolarization in the tissue for latter analysis. The actual voltages can be calculated if the dye concentration can be accurately determined. In our experiments, diO-C_2-(5) is injected through the carotid artery and is accumulated into neural tissues. The brain is immediately removed, frozen, sectioned on a cryostat, and thaw-mounted onto microscope slides to produce a permanent and stable record. An epifluorescence microscope with a computer controlled scanning stage provides the image to a SITS camera. The entire cross section of the brain can be scanned and is analysed by creating a montage. The gain and high voltage of the camera is kept constant, and only the gating is used to compensate for high or low light levels. A uniformly fluorescent standard is used to subtract out inhomogeneities in the camera image caused by uneven lighting from the lamp, and shading effects in the camera. A set of VSD standards, of known concentration and thickness, is used to construct a calibration curve. By using this curve and the gate number, the "relative" intensity of fluorescence in the raw image is transformed into an actual dye concentration in the tissue. The voltage difference produced between the control and experimental conditions can then be calculated from the dye concentrations.

123.1

THE RELATIONSHIP BETWEEN STIMULUS-EVOKED METABOLIC ACTIVITY AND WGA-HRP LABEL IN THE VENTROBASAL THALAMUS OF MONKEYS. W. Ma[1] and S.L. Juliano[1,2]. [1] Dept. of Anatomy, USUHS, Bethesda, MD 20814; [2] Lab. of Neuropsychology, NIMH, Bethesda, MD 20892.

Earlier studies investigating the coincidence between cortico-cortical connections and stimulus-evoked 2-deoxyglucose (2DG) activity in the somatosensory cortex of monkeys indicated that the location of metabolic patches could be predicted by the presence of transported HRP patches, after injections into different somatosensory fields. This observation was more clearly true following injections into area 3b than following injections into area 1. In the present study, electrophysiological recordings were conducted to identify small fields of skin (RFs) that activated neurons at the recording site, when stimulated with a mechanical stimulator. These RFs were small and on glabrous skin. An iontophoretic injection of WGA-HRP was then made into the recording site. The injection sites were approximately 500-750 um in diameter. Two days later, each monkey (*Macaca fasicularis* or *Saimiri sciureus*) received an injection of 2DG while the stimulus identified in the recording experiment was delivered. Evaluation of the transported WGA-HRP and the stimulus-evoked 2DG activity in the VPL nucleus of the thalamus, revealed that the relationship between the 2 forms of label was similar to that found in the somatosensory cortex. That is, following WGA-HRP injections into cytoarchitectonic area 3b, the 2 forms of label were co-localized in VPL. Following injections into area 1, the 2 forms of label were not coincident, although both *patterns* of label were remarkably similar. This finding suggests that the information conveyed to the somatosensory cortex arises from separate populations of VPL neurons and that information projected to area 3b more clearly predicts the loci of evoked metabolic activity. Supported by NS-24014.

123.2

MODULAR ORGANIZATION OF THE THALAMIC VPM NUCLEUS IN MONKEYS. E. Rausell* and E.G. Jones (SPON: C. Avendaño). Dpt of Anatomy and Neurobiology, College of Medicine, University of California, Irvine, CA 92717.

The ventralis posterior medialis (VPM) nucleus of the monkey thalamus, which forms the relay for trigeminal inputs to the first somatic sensory area (SI) of the cerebral cortex, is divided into a series of elongated rod-like configurations of relay neurons. The rods are cytochrome oxidase (CO) positive and physiologically form the basis for the projection of modality and place specific information to column-like arrays of SI neurons (Jones et al. *Exp. Brain Res* 62: 438, 1986). The present study, in *M. Fascicularis* further characterizes the population of neurons in the rods and defines a second set of SI projecting neurons situated in different compartments of VPM. After defining the SI face areas of both sides by multiunit mapping, Fast Blue was either injected into the middle layers or applied directly to the cortical surface. The deeper injections led to retrograde labeling of cells in the CO positive rods, while superficial deposits affecting only layer I led to retrograde labeling of cells in medium and small celled zones of VPM that stain less strongly for CO. These zones are situated adjacent to the borders with the centre median and ventralis posterior inferior nuclei.

In parallel immunocytochemical experiments, the rod cells projecting to middle layers of SI were shown to stain positively for parvalbumin and with the monoclonal antibody CAT 301. The cells outside the rods and projecting to layer I were shown to stain positively for the 28 kd calcium binding protein, calbindin. Gaba immunoreactive interneurons are situated among both sets of cells and do not stain for parvalbumin or calbindin.

These results show that thalamic relay neurons project differentially upon layers of the SI cortex, as in the cat (Rausell and Avendaño, *Brain Res.* 347:165, 1985). In the monkey, these cells can be further characterized by the differential expression of calcium binding proteins.

Supported by NIH Grant Number NS 22317, and by a Fulbright/MEC Fellowship to E.R.

123.3

Classes of thalamocortical relay neurons defined by differential immunoreactivity for calcium binding proteins in monkeys. E.G. Jones and S.H.C. Hendry Department of Anatomy and Neurobiology, University of California, Irvine, California 92717.

The distributions of neurons displaying immunoreactivity for parvalbumin and 28Kd calbindin were studied in the thalamus of Macaca fascicularis. Relay cells were identified by retrograde transport of fast blue from cortical injections and intrinsic neurons by GABA immunoreactivity in the same sections. Anterograde transport and degeneration studies also revealed parvalbumin or calbindin immunoreactivity in thalamocortical axons.

Thalamic nuclei are distinguished by different patterns of parvalbumin and calbindin immunoreactivity. In certain nuclei, e.g. in the anterior pulvinar nucleus, relay neurons stain for one only. In other nuclei that stain for both, the two calcium binding proteins can be found in separate relay neurons, e.g. in the intralaminar nuclei, or colocalized in some of the relay neurons, e.g. in some members of the ventral group. GABA is colocalized with parvalbumin in cells of the reticular nucleus but only in a few cells in the medial and lateral geniculate nuclei and in no cells in other nuclei.

Supported by NIH grants numbers NS21377, 22317, EY07193, EY06432.

123.4

COLLATERAL THALAMOCORTICAL PROJECTIONS TO AREAS 3b AND 1 OF SOMATOSENSORY CORTEX IN SQUIRREL MONKEYS: A FLUORESCENT DOUBLE LABELING STUDY. C.G. Cusick, S.L. Florence, P.E. Garraghty, and J.H. Kaas. Anatomy Dept., Tulane Univ. Med. School, New Orleans, LA 70112, and Psychology Dept., Vanderbilt Univ., Nashville, TN 37240.

Injections of the retrograde anatomical tracers WGA-HRP and ^{3}H-WGA into separate representations of the finger tips in somatosensory cortex of squirrel monkeys produce overlapping zones of label and double labeled cells in the thalamus (Cusick, et al., Somatosensory Res., 3:1, 1985). The present study was undertaken to determine if thalamic neurons project to both areas 3b and 1, and if branched projections include representations of the proximal fingers and palm as well as finger tips. Injections of retrograde fluorescent tracers fast blue and diamidino yellow, or fluorogold and rhodamine labeled microspheres, were placed in electrophysiologically identified representations of the proximal phalanges and digital pads in areas 3b and 1 in anesthetized squirrel monkeys (Saimiri sciureus). Overlapping and non-overlapping zones containing retrogradely labeled cells were revealed in the ventroposterior lateral, ventroposterior superior, ventropsterior inferior, and anterior pulvinar nuclei. Double labeled cells were identified in all four nuclei. The results suggest that branched projections terminate in areas 3b and 1 and include all parts of the hand representation.

123.5

ORIGINS OF THALAMIC PROJECTIONS TO LAYER I OF THE CEREBRAL CORTEX OF THE MONKEY. D.P. Friedman[1,2], Lin Li[1], and L.G. Ungerleider[1]. Lab Neuropsychology[1], NIMH Bethesda, MD, 20892 and Div Preclinical Res[2], NIDA, Rockville, MD, 20857.

We have previously described widespread projections from the region of the medial thalamus to layer I of the macaque cortex (Friedman et al., Soc Neurosci Abstr 13:251, 1987). To determine the nuclei of origin of these projections, we have made injections of retrogradely transported markers into large cortical regions in 8 rhesus and cynomolgus monkeys. All injections were performed under deep ketamine and barbiturate anesthesia while aseptic precautions were observed. After survival times of 2 days, the animals were again deeply anesthetized and perfused with 3% paraformaldehyde.

Injections into prefrontal, cingulate, temporal, parietal, and occipital regions all produced labeled cells in the midline nuclei and in the adjacent portions of the paracentral nucleus (PCN) and the magnocellular division of the ventral anterior nucleus (VA). However, both the density and distribution of this label differed depending on the site of the injection. Thus, whereas injections into prefrontal, cingulate, or parietal cortex led to labeling primarily in the midline nuclei, injections into the inferior temporal or prelunate gyrus led to sparse labeling on the midline, but impressive numbers of cells in VA and PCN.

The present results supply additional evidence that the midline nuclei have widepsread projections to cortex, but indicate that these nuclei may not supply the entire thalamic projection to layer I. Thalamic input to layer I of cortical areas in receipt of only sparse midline projections may arise instead in VA and/or PCN.

123.6

THALAMOCORTICAL RELATIONSHIPS IN THE VIBRISSA/ BARREL SYSTEM. P.W. Land and J.D. Yoskosky*, Dept. of Neurobiol., Anat. and Cell Sci., Univ. of Pittsburgh Sch. Med., Pittsburgh, PA 15261.

We investigated the topographic relationship between somatosensory cortical barrel columns and corresponding thalamic barreloids in rats using orthograde and retrograde transport of horseradish peroxidase (HRP). Electrophysiological recordings were used to target iontophoretic injections of HRP in lamina IV or VI of the posteromedial barrel subfield. Patterns of labeling in the medial division of the thalamic ventrobasal complex (VBm) were examined in sections cut parallel or orthogonal to the long axis of the barreloids. Injections into lamina VI or into the region of the V/VI border beneath a barrel labeled corticofugal axons that arborized throughout the corresponding thalamic barreloid. Divergent projections were more common "within a row" than "within an arc".

Both lamina IV and lamina VI injections led to labeling of neurons in the thalamus. Deep injections yielded labeled neurons throughout the extent of the barreloid corresponding to the injected barrel column. By contrast, lamina IV injections tended to produce labeled neurons primarily in the ventral lateral portion of VBm. These results indicate that each barrel column in rat provides cortical feedback to the barreloid providing the primary thalamic input, and perhaps to barreloids representing vibrissae in the same row. The data also suggest that, despite the morphological homogeneity of VBm neurons, cells in different parts of the nucleus may have distinct patterns of axonal termination in the somatosensory cortex. (supported by NIH grant NS 23047).

123.7

INTRINSIC CONNECTIONS OF RAT SmI BARREL CORTEX. N. D. Akhtar and P. W. Land, Dept. of Neurobiology, Anatomy and Cell Science, Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA 15261.

Small iontophoretic injections of HRP placed into individual laminae of the rat somatosensory cortex were used to trace the inter- and intralaminar distribution of neurons projectiong to the target site. The overall distribution of retrogradely labeled neurons is similar whether injections are within a barrel column or between adjacent barrel columns. Following injections into lamina II/III, the majority of labeled neurons appear in lamina V. A few labeled cells also are present in lamina IV below the injection site. Injections in lamina V always produce labeled neurons in lamina VI, and in lamina II/III. In addition, larger infragranular injections that either extend to the region of the IV/V border, or include lamina VI result in some labeled neurons within lamina IV above the labeling site. In all cases, there is a column of labeled cells extending through the cortex. Injections in lamina IV, in particular, label a narrow column of cells primarily in the infragranular laminae. Finally, within each lamina a target site tends to receive more convergent projections from cells within the same barrel row than from adjacent rows. Our results demonstrate specific patterns of connectivity for individual laminae in rat SmI as has been reported for other sensory cortical regions. The tendency for connections between neurons representing vibrissae in the same row may contribute to a bias in the processing of sensory information in the barrel cortex. (Supported by grant MH09773)

123.8

CHARACTERIZATION OF ENSEMBLE PROPERTIES OF SIMULTANEOUSLY RECORDED NEURONS IN SOMATOSENSORY (SI) CORTEX. J. Chapin, M. Nicolelis*, C.-H. Yu*, and S. Sollot*, Hahnemann Univ., Phila, PA. 19102

The CNS accomplishes its actions through the joint activity of large numbers of neurons, yet standard neurophysiological analysis involves recording neurons only one at a time. To define network mechanisms of cortical processing, we have quantitatively characterized patterns of discharge among neurons simultaneously recorded through arrays of 25μ microwire electrodes in the SI cortex of awake, behaving rats. All neurons were initially classified according to classical criteria, such as cutaneous or proprioceptive receptive fields, motor-behavioral correlates, and functional connections defined by spike-triggered cross-correlation. Next, multivariate statistical techniques were used to characterize the "ensemble properties" of the neurons. Cluster analysis was used to define the "state-space" of the ensemble. The joint activity of the N simultaneously recorded neurons over certain time intervals (10-100 msec) were represented as points in N-space. Clusters of these points defined activity states corresponding to particular behavioral conditions, such as sensory stimuli, or to phases of limb movement. These multi-neuronal states yielded more accurate predictions of the behavioral condition than did any individual neuron. Factor analysis was used to identify the elemental factors underlying these activity states. Some factors were identified as representing particular types of sensory inputs, but others were correlated specifically with movement, or were "unexplained". Principal components analysis was used to define component factors which could account for most of the variance in the network over several behavioral conditions. As composites of the elemental factors these components represented emergent properties of the ensemble. They were also used as a reduced criterion set for subclassification of the recorded neurons. This was useful, for example, for subclassifying sensory neurons which possessed similar receptive fields, but which discharged differently during various behaviors. In summary, these approaches not only provided measurement and visualization tools appropriate for multi-neuronal studies, but also allowed a conceptualization of cortical physiology in terms of distributed activity across neurons. *Supported by PHS grants NS26722, AA06965, and AA00089.*

123.9

AXONAL PROJECTIONS IN MOUSE BARREL CORTEX. K.L. Bernardo, J.S. McCasland and T.A. Woolsey, Dept. Neurosurg., Div. Exp. Neurol. & Neurosurg. and McDonnell Center for Studies of Higher Brain Function, Washington University School of Medicine, St. Louis, MO 63110.

The distribution of retrogradely labeled **cell bodies** in barrel cortex following focal extracellular HRP injections directly into the barrel field is in columns of the same barrel row as that which was injected (Bernardo et al, '88). Quantitative conclusions were drawn from automated analyses of histological slides using a Grinell image processor, video camera and a microscope with a computer-contolled motorized stage.

We now describe the distribution of labeled **axons** in these materials, using the same automated image analysis system with new software designed to extract data about axonal trajectories instead of cell somata.

Results have been analyzed so far for injections confined to the supragranular layers within barrel cortex. In layer IV and in the infragranular layers of each cortex, axons project primarily ***laterally***, down the barrel row which was injected (e.g. C3 -> C4), as well as into the ***anterior*** adjacent barrel row (e.g. B -> C, C -> D, etc.). Presumably these projections serve to convey information to specific adjacent barrel columns. The different cell and axonal labeling patterns identify distinct **populations** of cortical neurons subserving different functions for integration of inputs from the whiskers.

123.10

LAMINAR INTERACTIONS IN RAT VIBRISSA/BARREL CORTEX SHI DI[1,2,3], CHRISTOPH BAUMGARTNER[1,4]* and DANIEL S. BARTH[1,2], *Departments of [1]Neurology and [2]Psychology, Univ.of California, Los Angeles, (U.S.A), [3]Mental Health Institute, Beijing Medical University (China) and [4]Neurological University Clinic, Vienna (Austria)*

The rat vibrissa/barrel cortex was studied as a model of sensory information processing. Previous receptive field and latency studies have suggested sequential processing among cortical laminae in barrel cortex. The present study applied the current source-density (CSD) analysis combined with principle component analysis (PCA) to analyze field potentials in rat vibrissa/barrel cortex evoked by mechanical whisker displacements. The potential complex consisted of biphasic fast components followed by long lasting slow waves. It began with activity in supragranular cells consisting of a source in layers I-II and a sink in layers IV-V, this was followed by activation of the infragranular cells with a paired sink and source in layers I-IV and V-VI, respectively. The slow wave sequences also began in the supragranular cells followed by infragranular neurons. We propose that the fast components reflect the sequential interlaminar depolarization processes, and the slow waves, the hyper- or repolarization processes. These results suggest that a basic neuronal circuit, consisting of sequential activation of the supragranular then infragranular pyramidal cells, gives rise to the field potentials evoked by physiological stimulation. This is consistent with our previous studies of direct cortical responses (DCR) and pathological discharges of penicillin focus.

123.11

REGULAR-SPIKING CELLS BUT NOT BURSTING CELLS IN THE DEEP LAYERS OF MOUSE BARREL CORTEX RECEIVE MONOSYNAPTIC THALAMIC INPUT. A.Agmon and B.W.Connors, Dept of Neurology, Stanford Univ., Stanford, CA 94305 and Brown Univ., Providence, RI 02912.

The deep layers of rodent neocortex contain two physiologically-distinct types of pyramidal cells (Connors, Gutnick and Prince, J. Neurophysiol. 48, 1302-1320 (1982); Agmon and Connors, Neurosc. Lett., in press). Whereas the majority of cells ("regular-spiking") respond to an intracellular suprathreshold current step by firing trains of individual action potentials, about one third of the cells in the same layers generate single or repetitive bursts of spikes. Using the thalamocortical slice preparation (Agmon, Ph.D. Dissertation, 1989) we characterized the thalamocortical synaptic responses of both types of cells and used their response latencies to determine their synaptic order. Half of the regular-spiking cells (7/14) were found to receive thalamus-evoked EPSP's at a monosynaptic latency. In contrast, only 1 of 10 bursters was activated monosynaptically, the rest being polysynaptic or non-responsive. We conclude that the intrinsic physiological properties of cortical cells are correlated with their thalamocortical inputs, and that bursting and regular-spiking cells occupy different positions in the synaptic circuit of the barrel cortex. Regular-spiking and bursting cells, though both pyramidal, belong to distinct morphological subtypes (Chagnac, Luhmann and Prince, submitted). It remains to be seen whether these two classes of cells also differ in their axonal targets. Supported by MH17047 and NS12151.

123.12

ORIGIN OF OPTICAL SIGNALS RECORDED WITH VOLTAGE SENSITIVE DYE IN THE RAT SOMATOSENSORY CORTEX. S.Ito*(SPON:S.Kawamura).Dept. Physiol., Kumamoto Univ. Med. Sch., Kumamoto, 860, Japan.

To estimate what aspects of neuronal activity are monitored by optical recording with voltage sensitive dye, fluorescence signals of the dye RH414 recorded with a single-element photodiode were compared with field potentials recorded with a metal microelectrode in the rat somatosensory cortex. Rats were anesthetized with sodium amobarbital, ventilated artificially, and mounted on a stereotaxic apparatus. Responses of the hindlimb area evoked with contralateral sciatic nerve stimulation were monitored. The optical signals had the very same latency and shape as those of the initial negative component of the field potential recorded at several hundred micrometers below the surface. On this component the stimulus intensity-response curve was identical. When double shocks were used, the relationship between the amplitude of the response to the second stimulus and the interstimulus interval was also identical. Thus, the optical signal was exactly alike the field potential recorded at a certain depth, suggesting that these two recordings represent the same process in the cortex, that is, the summated PSPs in layers I-IV. Supported in part by a grant of the NISSAN SCIENCE FOUNDATION.

123.13

COMPUTER SIMULATIONS OF INFORMATION PROCESSING IN A BARREL OF RAT SOMATOSENSORY CORTEX. H. T. Kyriazi* and D. J. Simons (SPON: N. Schor) Dept. of Physiology, Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA 15261.

A computer model has been developed that incorporates known anatomical and physiological features of whisker-related barrels in cortical layer IV. The model consists of 98 interconnected stellate cells (84 excitatory, spiny and 14 inhibitory, smooth) each of which receives monosynaptic, excitatory inputs from up to 10 cells in the corresponding thalamic barreloid. These numbers correspond roughly to 5% of the estimated populations. Adjustable parameters include cell connectivity, synaptic strength, refractory period, synaptic delay, EPSP and IPSP decay rates, and shape of the function relating membrane potential to probability of spike discharge. Parameter values, set at the beginning of a simulation, are adjusted separately for spiny and smooth cells. Individual cell connectivities and synaptic strengths are assigned randomly according to a Gaussian distribution, with only the mean and range values being prespecified. The purpose of this randomization is to simulate natural variability. The network is activated by actual pre-recorded spike trains from barreloid cells. Circuit dynamics involve both temporal and spatial integration, and the activity of each cell is updated every msec.

Output consists of simulated spike trains that can be compared quantitatively to those of real barrel neurons. We have focused on the spontaneous and stimulus-evoked activities of spiny and smooth cells in response to single- and multi-whisker deflections. Specific response components include magnitudes of ON and OFF responses, temporal patterns of excitation/inhibition following single-whisker stimuli, and cross-whisker inhibition following sequential deflections of adjacent whiskers. Sets of parameter values have been identified that enable the model network to generate spike trains that reasonably simulate our neurophysiological data. Such computational studies may yield quantitative estimates of the relative importance of neuronal parameters which form the basis of cortical information processing. Supported by NS-19950.

SOMATOSENSORY CORTEX AND THALAMOCORTICAL RELATIONSHIPS II

124.1

DISSOCIATION OF THE EFFECTS OF INSULAR AND INFERIOR TEMPORAL (AREA TE) LESIONS ON TACTUAL AND VISUAL MEMORY IN MACAQUES. T.P. Pons, E.A. Murray and M. Mishkin. (SPON: M. Haenlein) Lab Neuropsychology, NIMH, Bethesda, MD 20892.

A severe deficit for both visual and tactual recognition memory in monkeys follows combined bilateral removal of the amygdala and hippocampus (Murray and Mishkin, 1984, J. Neurosci.). Bilateral removal of cortical area TE also produces a severe impairment in visual recognition memory (Mishkin, 1982, Phil. Trans. R. Soc. Lond. B.). In the present study we asked whether bilateral removal of the insula, which appears to be the somatosensory analogue of visual area TE by several connectional and physiological criteria, would produce a tactile memory deficit. To answer this question 6 cynomolgus monkeys were trained preoperatively on a delayed nonmatching-to-sample (DNMS) task in both the visual and tactual modalities. After achieving a criterion of 90% correct responses in 100 trials in both modalities, with 10 s delays between sample and test presentations, the animals' tactual performance was measured in blocks of 100 trials each with delays of 30, 60, and 120 s. Postoperatively animals were retrained on the tactual task to criterion or for 1500 trials with 10 s delays, and then given the tactual, followed by the visual, performance test. Four monkeys with insular lesions were severely impaired on tactual (59%) but not visual (>90%) DNMS. Conversely, 2 animals with TE lesions were impaired on visual (76%) but not tactual (>90%) DNMS. These findings provide evidence that the insula is a critical link in a cortico-limbic pathway for tactual memory, just as area TE is for visual memory.

124.2

NEURAL CODING OF TACTILE ROUGHNESS: CORRELATION BETWEEN HUMAN PSYCHOPHYSICAL ESTIMATES AND MONKEY PERIPHERAL AFFERENT RESPONSES. C.E. Connor*, S.S. Hsiao*, and K.O. Johnson (SPON: G.F. Poggio). Bard Laboratories, Dept. Neuroscience, The Johns Hopkins University Sch. Medicine, Baltimore, MD. 21205.

Previous results suggested that subjective roughness of embossed dot patterns is correlated with two measures of firing rate variation in macaque slowly-adapting (SA) Merkel's and rapidly-adapting (RA) Meissner's afferents (K.H. Fasman et al., Neurosci. Abstr., 11:906, 1985). One measure is based on temporal changes in firing rate within fibers due to intermittent stimulation as the dot pattern moves across the skin surface. The other is based on differences in firing rate between fibers due to the spatial distribution of dots across the skin surface. The present study was designed to distinguish between the temporal and spatial measures as neural codes for tactile roughness. The stimuli were 16 plastic surfaces with arrays of embossed 0.5 mm diameter dots. Dot spacing was separately varied in two directions, one parallel to the direction of scanning motion and one orthogonal, in an attempt to independently alter temporal and spatial stimulation. In psychophysical experiments, subjects were asked to scan the distal pad of their index finger sideways across the surfaces and report their estimates of roughness magnitude using a numerical scale. In neurophysiological experiments, the same surfaces were swept repeatedly across the receptive fields of macaque SA and RA afferents innervating the distal pads of the fingers.

Psychophysical results from 35 subjects showed that subjective roughness magnitude declines rapidly as dot spacing in the scanning direction decreases from 4.0 to 1.5 mm., and declines somewhat less rapidly as dot spacing decreases over the same range in the orthogonal direction. Temporal firing rate fluctuations in both SA's and RA's increase as orthogonal spacing decreases, and thus cannot explain the psychophysical results. Spatial (between-fiber) firing rate differences, however, provide a close match to the human roughness estimates. Supported by NIH grant NS18787.

124.3

FEATURE ANALYSIS OF SPATIAL PATTERNS IN THE MONKEY SOMATOSENSORY SYSTEM. S.S. Hsiao*, K.O. Johnson, J.R. Phillips. Bard Laboratories of Neurophysiology, Johns Hopkins University Sch. of Med. Baltimore, MD 21205.

Embossed letters of the alphabet were used as stimuli in an investigation of the representation and transformation of spatial patterns in the somatosensory system. Single unit recordings were made from peripheral afferents in the anesthetized monkey and from neurons in SI cortex in the awake monkey to generate a two-dimensional spatial event plot (SEP).

In order to quantify the responses, an analysis was developed to assess the degree of isomorphism and to see if neurons had any selective preference to particular features in the letters. The degree of isomorphism was assessed by investigating the distribution of distances of the neural impulses from a stick representation of the stimulus letters. Neurons with low distance distributions (i.e. most of the impulses near the letters) are more isomorphic than neurons in which the impulses are distant from the letters. Impulses from the SA afferents had mean rms distances from the stick letters of less than 1mm. The RA afferents had mean rms distances of slightly greater than 1mm. The PC afferents had much larger rms distances (>3.5mm), indicating their spatial structure was unrelated to the letters. The cortical responses had rms distances ranging from less than 1mm to very large values, with the SA neurons in area 3b having the lowest values.

The SEPs were also investigated for their sensitivity to letter features. The density of impulses were quantified relative to their spatial location around the letters. These distributions were then mapped into a feature map. Peripheral SA and QA neurons were isomorphic and showed leading edge enhancement with a loss of internal detail. The cortical responses were much more varied than the peripheral responses, ranging from being highly structured and isomorphic, to neurons with high feature sensitivity, to neurons with weakly structured responses. Supported By NIH grant NS18787.

124.4

ACTIVE VS PASSIVE TOUCH IN A LETTER RECOGNITION TASK: HUMAN PERFORMANCE AND VELOCITY EFFECTS. F. Vega-Bermudez*, K.O. Johnson, K.H. Fasman, S.S. Hsiao* (SPON:L. Haynes). Bard Laboratories of Neurophysiology Dept. of Neuroscience, Johns Hopkins Univ. Sch. of Med., Baltimore MD 21205.

The performance of human subjects in a letter recognition task was measured under two conditions: active touch where subjects scanned letters, 6 mm in height, with the tip of their right index finger and passive touch where the same letters were applied to the constrained fingertip by means of a rotating drum. Twenty five subjects were tested in the active task and 15 subjects in the passive task. Overall performance was nearly identical in the two tasks as measured by the distributions of individual percent correct identifications (Kolmogrov-Smirnov D=0.146, p 0.10), by the grand means (49.0% active, 50.7% passive) and by the correlation of confusion matrices from the two tasks (R=0.956, p 0.001). Finger scanning velocities in the active task varied from 10 to 60 mm/sec. For the purposes of comparison, the passive task was run at 20, 40 and 80 mm/sec. These observations suggest that self initiated movements do not contribute relevant information for fine form discrimination at the fingertips.

124.5

MODULATION OF CUTANEOUS INPUT FROM THE HAND TO SI CORTEX PRODUCED BY ICMS OF MOTOR CORTEX IN THE MONKEY. W. Jiang, Y. Lamarre and C.E. Chapman, Centre de recherche en sciences neurologiques, Université de Montréal, Canada H3C 3J7

We have previously shown that cutaneous inputs from the hairy skin of the arm to primary somatosensory cortex (SI) are decreased topographically by intracortical microstimulation (ICMS) of motor cortex. The present study tested the effects of ICMS on inputs from the glabrous and hairy skin of the hand.

ICMS (train of 11 pulses at 330 Hz, 0.2 ms pulse width) was applied to low threshold (<14 μA) sites in motor cortex and airpuff-evoked potentials (20 ms) were recorded in the adjacent SI areas. Data were pooled from 141 pairs of SI recording/ICMS (1-1.5 x threshold) sites in 3 monkeys. Evoked potentials elicited from the glabrous skin of the hand were not modulated (n=15 pairs). Hairy input from the hand was less often modulated (8/21) than input from the arm,' e.g. the forearm (43/72). Activation of hand and/or digit muscles (including intrinsic muscles of the hand) was less likely to modulate hairy input from the hand (3/13) than was the activation of elbow muscles (5/8). The results suggest that motor cortex modulates hairy, but not glabrous, inputs from the hand. This is consistent with the importance of glabrous inputs in active touch. Supported by the MRC of Canada and the FRSQ.

124.6

Gating of Cutaneous Inputs During an Active Tactile Discrimination Task versus Passive Receptive Field Testing in the Monkey. S.A. Ageranioti and C.E. Chapman, Université de Montréal, Montréal, CANADA

It is known that somatosensory transmission is reduced prior to, and during, voluntary movement, but this may vary as a function of the context in which the movement is made. The present study compared the discharge properties of neurones in the cutaneous hand area of SI cortex (n=150) in 2 monkeys during active movement and passive receptive field (RF) testing. The monkeys were trained to actively scan and to discriminate between a rough and a smooth surface.

Units were classified according to the location of their RF: those with a RF on the tip(s) of the digit(s) in contact with the surfaces (single digit, sd (n=18); multiple digit, md (n=95)); those with a RF elsewhere on the digit(s) (n=24); glabrous palm (n=9); or hairy dorsum (n=7). The majority of units with cutaneous RFs on the tips of the digits in contact with the surfaces showed as much (49-59%) or more (15-29%) modulation during the active task than during passive RF testing. The greatest modulation was seen in md units with large RFs including the glabrous palm. Only a small proportion (12- 36%) was more responsive to passive, than active, testing. In contrast, two-thirds of the remaining units with cutaneous RFs (other digits, palm or hairy dorsum) were more responsive to passive RF testing than to active movement. While the latter were also less frequently modulated in the task (43%), they showed no evidence for increased inhibitory influences during the active task (10% vs 13% for the units with RFs on the digit tips). These findings suggest that in a task in which the sensory inputs are behaviourally significant to the animal, the inhibitory controls over sensory transmission are suppressed. Supported by the MRC of Canada and the FRSQ.

124.7

NORMAL TACTILE FUNCTION PERSISTS DESPITE SENSORY DEPRIVATION IN INFANT MACACA M.Carlson & M. Pearce* Depts. Psychiatry, Anatomy & Neurobiology, Washington University School of Medicine, St. Louis, MO 63124

Infant macaques as young as 2 months of age, and infants with restricted tactile experience from birth to 4 months, can discriminate between textures as well as adults (Carlson,'84a). Although tactile function appears to be mature soon after birth, other features of the somatic system change with age. Infants with unilateral or bilateral removal of primary (SI) (Carlson,'84b,c) or secondary (SII) (Carlson and Burton,'88) somatic sensory cortex can recover normal tactile function whereas juveniles and adults have persistent deficits after the same damage. To explore the possible role of sensory input in tactile function early in life, we produced total sensory deprivation of the glabrous hand by crush of the medial and ulnar nerves (70mm from the digit tips) in two 5 week-old infants. When tested on texture discrimination tasks at 20 weeks, moderate retardation in tactile learning was seen. However, discrimination thresholds for sandpaper and nyloprint textured surfaces in both the normal and early-deprived hands were found equal (or superior) to that of adult macaques and humans. Currently we are testing an infant in which these nerves were crushed 120mm from the tips (extending the deprivation period to 17 weeks) and we will examine input to the SI and SII areas contralateral to the deprived hand in all animals. Given the mature level of function in normal infants and the failure to induce deficits by sensory deprivation, we suspect that tactile input is not needed for normal development of this system or that the critical period may occur in the late prenatal rather than the early postnatal period.

124.8

INTEGRATION OF INPUTS FROM MULTIPLE SKIN SITES BY CORTICAL TOPOGRAPHIC UNITS. M. E. Diamond and O. V. Favorov, Dept. of Physiology, Univ. of North Carolina, Chapel Hill, NC 27599.

The minimal RF ("minRF") method, which maps the dominant skin input to small neural populations, reveals that the somatic cortex of cat is a mosaic of discrete, columnar topographic units (Proc. Natl. Acad. Sci.(1988) 84, 6606-10). All recording sites within a topographic unit yield the same minRF; each unit is 250-400 um in diameter and is surrounded by six others. Single neuron maximal RFs (maxRFs) are larger and more variable than minRFs, but the site of overlap of all the maxRFs in a topographic unit matches the minRF of the unit. Experiments were carried out to determine whether the skin site of maxRF overlap, in comparison to sites in the RF periphery, is relayed to a topographic unit along a physiologically distinguishable pathway. Tactile stimuli were delivered to site 1 (corresponding to the minRF; the point of maxRF overlap) and to sites 2-4 (5, 10, and 15 mm from site 1) and single neuron response was recorded. On average, stimulation of site 1 led to shortest response latency and highest response probability; site 4 led to the opposite. When sites 1-3 were stimulated, middle layer neurons responded earliest; when site 4 was stimulated superficial neurons respond earliest.

A model is proposed whereby the site of maxRF overlap (the minRF) of a given cortical topographic unit is relayed to that unit directly through thalamus and site 4 is relayed primarily through neighboring cortical units; the model is compared to proposed pathways to the barrel field cortex in rodent. The role of intracortical inhibition is discussed. (Supported by NIH Grant DE07509)

124.9

VIBRISSAL ROUGHNESS DISCRIMINATION IS BARREL CORTEX DEPENDENT. E. Guic-Robles*, H. Bravo*, and W.M. Jenkins. Dpto. Fisiologia y Biofisica, U. Chile, Santiago 7, Chile.

Recently we have developed a behavioral discrimination procedure to study the rat's vibrissal sensory capacities. This procedure has been used to demonstrate that rats can learn a roughness discrimination using only their vibrissal system (Guic-Robles et al, Arch. Biol. Med. Exp., 20(2) R209, 1987; Behav. Br. Res., 39, 285-289, 1989).

In the present experiment we have investigated the contribution of the neocortical barrel field devoted to vibrissal representation (PMBSF) to the high performance levels obtained by rats in this complex tactile discrimination. Eight binocularly occluded rats were trained to make vibrissal system based roughness discriminations. All trained rats obtained the 85% correct discrimination criterion. The behavioral procedures, apparatus and test stimuli were identical to those previously reported. The PMBSF was subsequently bilaterally ablated after localization of this cortical area by electrophysiological recordings. The locus and extent of the cortical lesions were confirmed by histological analysis. There was no evidence of task retention after cortical lesions and barreless rats were not able to obtain prelesion discriminative performance levels when restricted to using vibrissal cues. After extensive post lesion training and testing, four of these rats were allowed to palpate the discriminanda with their forepaws. These rats rapidly reached the 85% correct criterion once again.

The present results indicate that the PMBSF cortex is essential for complex tactile discriminations when sensory information is obtained through the vibrissae. In contrast, the PMBSF is not essential for complex tactile discriminations when the source of the somatosensory information is provided by other non-vibrissal sensory receptors. Supported by NSF -INT8713322 and FONDECYT 5057 and 0133.

124.10

FUNCTIONAL DIFFERENCES OF HUMAN SOMATOSENSORY SI AND SII CORTICES: NEUROMAGNETIC RECORDINGS. H.Hämäläinen, R.Hari*, J.Kekoni*, and J.Tiihonen*. Dept. Psychol., Univ. Helsinki, and low Temp. Lab., Univ. Technol., Espoo, Finland.

Magnetic fields were measured with a 7-channel 1st-order SQUID-array to trains (ITI=10s; ISI=1s) of electrical stimuli applied to the left middle finger, or to stimuli presented to either the 1st or 3rd finger in an oddball paradigm (standards, p=0.9; deviants, p=0.1).

The responses showed clear deflections at 45-50 ms and 100-110 ms. According to field maps the earlier deflection (corresponding to P50 wave in electric scalp recordings) can be explained by an equivalent dipole located in the SI hand area, whereas the later one (corresponding to P100 wave in electric measurements) fits the anatomical location of SII area.

Both deflections decreased during the train of stimuli, and the decrement was largest between the 1st and 2nd stimuli. thereafter the responses tended to increase again suggesting that this phenomenon is based on active inhibitory processes.

The 100-ms magnetic deflection was significantly larger for infrequent deviants than for standards, suggesting that SII area codes even small changes in stimulus location.

124.11

CONTRALATERAL PARESTHESIA CAN UNUSUALLY BE ELICITED BY MAGNETIC COIL STIMULATION NEAR HUMAN CENTRAL SULCUS.
M. Somasundaram*, V.E. Amassian, R.Q. Cracco and P.J. Maccabee*. (SPON: Dr. Sloane Wolfson)
Depts. of Neurology and Physiology, SUNY Health Sci. Ctr. at Brooklyn, New York 11203.

Paresthesias are elicited by tetanic, but not by individual electrical stimuli to exposed, human postcentral gyrus (Libet et al. J. Neurophysiol. (1964) 27, 546-578). Although individual transcranial magnetic coil (MC) stimuli to motor cortex could elicit a sense of movement in a paralyzed limb (Amassian et al. Brain Research (1989) 479, 355-360), we also failed usually to elicit paresthesias by cortical stimulation. However, exceptional individuals describe reproducible, localized paresthesias following individual MC stimuli. For focal stimulation, we used a Cadwell MC composed of two coils, each 5.0 by 4.8 cm, optimal stimulation occurring under their junction. The 'best' subject is a physician, 56 years old and in good health. Using near threshold MC stimuli near central sulcus, the paresthesias (tingling) were projected to the palmar surface of fingers IV and V; no movements were elicited in the relaxed hand. To determine whether the paresthesias resulted from stimulation of pre- or postcentral cortex, the MC pulse was delivered during hand muscle contraction. Optimal sites for paresthesias correspond to those for muscle activation, implying a precentral origin. Stronger MC stimuli added other digits to the projected experience.

The paresthesias might result from an increased tendency to repetitive discharge by precentral neurons or an increased response by neurons more immediately related to perception. The latter is more likely because muscle responses elicited by the MC were unremarkable.

124.12

RESPONSE SUSCEPTIBILITY OF HUMAN BRAIN STRUCTURES TO EXTERNAL RHYTHMIC SENSORY STIMULATION.
L. Narici°, G. Iori°*, V. Pizzella*, G.L. Romani&, P.M. Rossini#*, G. Torrioli*, R. Traversa#*Istituto di Elettronica dello Stato Solido, CNR Roma, Italy.

We show results of a study on the relationship between brain spontaneous activities and inputs from visual and somatosensory systems as measured with neuromagnetic techniques.

It is often assumed that the response susceptibility of brain structures upon external rhythmic sensory stimulation features peaks in the same frequency channels where spontaneous activity can be detected. Using sensory inputs, a suitable stimulation paradigm, and the powerful localizing capability proper of the neuromagnetic method we are able to identify the source location and frequency characteristic of three underlying oscillating circuitry. Upon visual rhythmic stimulation we studied a 10 Hz rhythm, linked to the alpha rhythm generators. Using somatosensory stimulation we identified an oscillating 12 Hz activity linked to the mu-rhythm, and possibly to the generators of the short latency evoked fields, and another rhytmic activity, at 6 Hz, probably genarated by two widely separated sources (parietal and frontal) driven by the same, deep clock (possibly thalamic).

The method proposed is able to investigate non-invasively rise and time evolution of synchronization processes in the human brain and, therefore, can be of interest in the study of all those pathologies describing disruption or enhancement of the brain rhythms.

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REGENERATION: GENERAL

125.1

ESTABLISHMENT OF SCHWANN CELL CULTURES FROM ADULT PERIPHERAL NERVE.
G. Ferrari*, M. Fabris*, L. Maffei* and M.G. Fiori. Fidia Research Laboratories, Abano Terme (PD) and Ist. Neurofisiol. CNR, Pisa - Italy.

Schwann cells are known to play a crucial role in promoting adult peripheral nerve regeneration following damage. Elucidation of underlying molecular event will be important to understand Schwann cell functions. For this purpose, availability of large numbers of relatively pure and well-characterized Schwann cells, in particular of primate origin, would be an important asset. While many studies have derived from neonatal tissue, the yield of Schwann cells from adult nerves is currently scarce due to the presence of abundant myelin and connective tissue. In this study we present a method for obtaining Schwann cells from adult sciatic nerve that circumvents these problems. To evaluate the percentage of Schwann cells in culture a time-course analysis of Schwann cell-associated markers was performed employing immunofluorescence and immunoperoxidase. At 1 day in culture 90% of the cells were bipolar and S100, NGF-R, 217C-immunopositive. Two weeks later 70-80% of the cells still expressed the above markers. Thus, this method provides proliferating Schwann cells from adult nerves in large numbers and in a reasonable time both for morphological and biochemical analysis, and for studying the effects of mitogenic agents.

125.2

EXPRESSION OF CYTOTACTIN IN SCHWANN CELLS DURING DEVELOPMENT AND REGENERATION OF NERVES.
J.K. Daniloff, K. Crossin*[1], N. Satterlee*, A. Smith*, and G.M.Edelman[1]. Dept. of Anatomy, LA. St. Univ., Sch. of Vet. Med., Baton Rouge, LA 70803 and [1]Lab. of Dev. & Mol. Biol., Rockefeller Univ. N.Y.,N.Y.10021

Cytotactin is an extracellular glycoprotein involved in neuron-glia adhesion in the CNS. We have described its action in the normal and regenerating neuromuscular system and at nodes of Ranvier (J. Cell Biol. 103:379, 1986 and 108:625, 1989). To extend these studies, we examined the localization and synthesis of cytotactin by embryonic, reactive, and transformed Schwann cells of the PNS of rats.

Embryonic sciatic nerves (E18) were double-labeled with antibodies to cytotactin and S100 protein. All Schwann cells were labeled at the light and electron microscopic level. Cultured sciatic Schwann cells treated briefly with antimitotics to remove fibroblasts synthesized high levels of cytotactin, as did transformed cells from the C-6 Schwannoma cell line.

Cytotactin is present only in small amounts in normal Schwann cells of adult sciatic nerves. When the nerve was transected, reactive Schwann cells were intensely labeled by antibodies to cytotactin. Synthesis returned to normal low levels upon reinnervation.

These data support our hypothesis that cytotactin is involved in development and regeneration of the PNS. Cytotactin expression by Schwann cells may mediate certain identified effects of Schwann cells in these processes.

125.3

REACTIVE ASTROCYTES AND NEONATAL ASTROCYTES DIFFER IN THEIR ABILITIES TO SUPPORT NEURITE EXTENSION IN VITRO.
A.M. Stewart* and E.E. Geisert, Jr. (SPON: J.W. Brown). Department of Cell Biology and Anatomy, Neurobiology Research Center, University of Alabama at Birmingham Medical Center, Birmingham, AL 35294.

To test the hypothesis that reactive astrocytes are involved in the lack of axonal regeneration following CNS injury, we compared the growth of rat E18 cortical neurons on reactive astrocytes, cultured from injured corpus callosum, to the growth of neurons on neonatal astrocytes. Following two days in culture, cells were fixed and neurons were stained selectively with a monoclonal antibody directed against class III ß-tubulin. Neurons contacting cultured astrocytes were digitized for a quantitative analysis of neurite growth. Neurons contacting neonatal astrocytes had a mean of 163 µm of neurite/neuron; while, neurons contacting reactive astrocytes had on average 84 µm of neurite/neuron. This difference was more pronounced when neurons were contacting process-bearing astrocytes (neonatal astrocytes 221 µm of neurite/neuron, and reactive astrocytes 81 µm of neurite/neuron). In control experiments neurons were grown on PLL coated coverslips suspended over the cultured astrocytes. No significant difference in neurite length between reactive astrocytes (88 µm of neurite/neurons) and neonatal astrocytes (81 µm of neurite/neuron) was seen, demonstrating that differences in soluble factors produced by the two populations of astrocytes cannot account for the ability of the cells to support neurite extension. These data demonstrate that neonatal astrocytes actively facilitate neurite growth; while, reactive astrocytes at best are a neutral substrate. These effects appear to be due to physical contact between the cultured astrocytes and the growing neurites. Supported by Whitehall Foundation, Inc. and PHS grant NS23613.

125.4

THE FATE OF THE SCHWANN CELL BASEMENT MEMBRANE IN PERMANENT NERVE TRANSECTION.
C. Giannini,* P. J. Dyck. Peripheral Nerve Center, Mayo Foundation, Rochester, MN 55905

Following nerve degeneration, Schwann cell basement membranes (SCBM) maintain Schwann cells in linear rows and serve as conduits for fiber regeneration to target tissue. Quantitative data on SCBM number, size, and integrity after denervation without reinnervation is lacking. SCBM (continuous or discontinous) were evaluated in transverse electronmicrographs of groups of permanently transected peroneal nerves of C57BL6J mice at various times after transection. A definite trend was already established by 1 month.

Groups	No.	No.SCBM/ nerve mean±SD	Continous profiles (%)mean±SD	Mean of mean length(um)	Σ SCBM length/ nerve(um)
Control	6	1234±109	100	14.5±.8	17,905±1138
1 wk	8	1538±220	93±3	13.5±1.9	19,934±3371
4 wk	7	2179±444	51±8	6.7±.9	14,137±3178
Cont. vs 1 wk		p>.05	p<.001	p>.05	p>.05
Cont. vs 4 wk		p<.001	p<.001	p<.001	p=.02

These results indicate that even within 1 month of permanent transection many SCBM tubes become discontinous in the transverse plane.The average length of continuous and discontinous SCBM becomes shorter. In addition, summated length of all SCBM becomes less, possibly suggesting degradation of nerve SCBM. Discontinuity and decrease in amount of SCBM may have implications for nerve regeneration.

125.5

ASTROCYTES AND THEIR RESPONSE TO SPINAL CORD LESIONS IN THE DEVELOPING OPOSSUM. Ganesh Ghooray, R.H. Ho, and G.F. Martin. Department of Anatomy and Neuroscience Program, The Ohio State University, Columbus, OH 43210.

The aim of this study was to observe the normal development of astrocytes in the spinal cord of the opossum and to see if the development of a glial scar correlates temporally with the loss of rubrospinal plasticity. We examined astrocyte development by immunostaining for vimentin and glial fibrillary acidic protein (GFAP) using the ABC technique. The results indicate that vimentin-like immunoreactivity is well developed at birth (12 days after conception) whereas very little GFAP-like immuno-reactivity is present. The subsequent development of GFAP follows ventral to dorsal and rostral to caudal gradients. GFAP and vimentin are first expressed in radial glia. In the lesion experiments, pouch-young animals were subjected to spinal hemisections at low to mid-thoracic levels and allowed to survive for two weeks. The tissue was then immunostained for GFAP. The earliest indication of a glial scar was seen when the lesion was made at estimated postnatal day (EPD) 26 (50 mm snout-rump length (SRL)); scar density increased gradually to reach adult levels by EPD 73 (125 mm SRL). Since rubrospinal axons can still grow around a lesion of their pathway at EPD 26 (Xu and Martin), the development of an astrocytic scar may not be the only factor which impedes developmental plasticity. (Supported by NS-25095).

125.6

CULTURED SCHWANN CELLS, BUT NOT ASTROCYTES, SUPPORT AXONAL GROWTH IN THE CENTRAL NERVOUS SYSTEM OF ADULT RATS. C.T. Montgomery* and J.A. Robson. Dept. of Anatomy and Cell Biology, SUNY Health Science Center, Syracuse, NY 13210.

Growing axons require an appropriate substrate to elongate. In the peripheral nervous system this substrate is provided by Schwann cells and in the developing central nervous system (CNS) astrocytes appear to be involved. We are comparing the abilities of Schwann cells and astrocytes to support axonal growth in the adult CNS by transplanting purified glial cells into the brain. Schwann cells are collected from sciatic nerves of newborn rat pups and astrocytes are taken from embryonic rat forebrain. Purified cultures are made according to standard techniques. For transplantation, cells are transferred into polycarbonate tubes. One end of each tube is inserted into the thalamus of an adult rat and the other end is secured subcutaneously. Five to eight weeks later, horseradish peroxidase (HRP) is placed on the external end of the tube. The next day the animal is perfused and the brain is sectioned and processed for HRP histochemistry.

Results show that implanted Schwann cells support axon growth. The tubes become vascularized and contain bundles of axons, many of which appear myelinated. Following HRP application, many retrogradely labeled cells are found in the CNS. Most are in the diencephalon although some are in the telencephalon. Tubes containing astrocytes do not appear to support axonal growth. They are sparsely filled with tissue and are not well vascularized. No retrogradely labeled cells have been found in the CNS. These results indicate that transplanted Schwann cells are able to support axonal growth in the adult brain in the absence of a preformed basal lamina. In comparison, cultured embryonic astrocytes, transplanted in the same fashion, do not support such growth. (Supported by NIH Grant EY03940 and NATO Grant 0235/87)

125.7

AXON GROWTH IN THREE DIMENSIONAL ASTROCYTE CULTURES. J.W. Fawcett*, L. Housden*, L. Smith-Thomas and R.L. Meyer. Physiological Laboratory, Downing St., Cambridge CB2 3EG, England, and Developmental Biology Center, U.C. Irvine, CA 92717.

A tissue culture model of axon regeneration in the CNS should contain neurons, astrocytes and oligodendrocytes. When we examine the growth of axons on astrocyte-oligodendrocyte combinations in flat cultures, we find that axons grow readily, between the astrocytes and oligodendrocytes. We have therefore been unable to reproduce the inhibition of axon growth by the mammalian CNS in two dimensional cultures.

We have designed a technique in which three dimensional tissues of selected cell types can be created and cultured in 0.5mm diameter cellulose ester tubes. Rat glia are cultured in flasks, then trypsinized off, pelleted, resuspended and packed into tubes attached to pipette tips, using a combination of air pressure and centrifugation. A source of axons is then inserted into one end of the tube. Later the tube is sectioned, and immuostained for neurofilament and GFAP.

Axons from newborn rat dorsal root ganglia (DRG's) will not penetrate astrocytes which have been cultured 3 weeks or more, but will grow sparsely in cultures less than 10 days old. However, axons from E15 embryonic DRG's will penetrate aged astrocyte cultures, even when they contain oligodendrocytes. Similarly, axons from adult retina do not grow into older astrocyte cultures, while axons from embryonic retina grow profusely, even when oligodendrocytes are present. Axons from all these types of neuron will grow for considerable distances on flat astrocyte cultures. Supported by MRC, ISRT and grant #EY06746

125.8

NEURITE OUTGROWTH ON CNS TISSUE SECTIONS: DIFFERENCE IN AGE, ANIMAL SPECIES AND EFFECT OF BRAIN LESION. E.Watanabe* and F.Murakami. Dept. of Biophysical Eng.,Fac.of Eng.Sci., Osaka Univ., Toyonaka 560, Japan (SPON: T. Bando)

Neurite-elongation inhibiting proteins in mammalian CNS myelin (Caroni and Schwab'88) might be responsible for infrequent occurrence of regeneration in CNS. Cell culture on cryostat tissue sections provides useful information on the role of such proteins in vivo (Watanabe and Murakami'89). We cultured E6 chick neocortical and E9-11 chick DRG neurons on cryostat tissue sections of nerve tissues. While cell attachment and neurite outgrowth were inhibited on the white matter of adult rat CNS, no inhibition was observed on frog and neonatal rat spinal cord, and adult rat sciatic nerve, where regeneration takes place in vivo. Inhibition of cell attachment was abolished in rat near the lesion site after transection of superior-cerebellar-peduncle decussation, in consistent with reported success of regeneration (Kawaguchi et al.'86). Correspondence of the presently obtained results to the phenomena occurring in vivo provides further evidence suggesting possible involvement of the inhibitory proteins in regulation of axonal regeneration.

125.9

PATTERN OF LECTIN BINDING TO NORMAL PERIPHERAL NERVE AND PERIPHERAL NEUROMAS. S. Kumar*, P.L. Wade*, G.P. Cole*, and A.K. Gulati* (SPON: S.D. Stoney,Jr.). Department of Anatomy, Medical College of Georgia, Augusta, GA. 30912.

In the present study we report binding of 10 different lectins to normal rabbit nerve and neuromas. These neuromas resulted from epineurial sutures used to repair gaps. Neuromas were obtained at 2 and 12 weeks after transplantation. The following lectins, Maclura pomifera agglutinin (MPA), Triticum vulgaris (WGA), Canavalia ensiformis (Con A), Griffonia simplicifolia-I (GS-I) and GS-II, Glycine max (SBA), Ulex europaeus (UEA), Dolichos biflorus (DBA), Arachis hypogaea (PNA), Bachinia purpurea (BPA) bound to the perineurium, with intense binding for MPA, WGA, GS-I and Con A. Endoneurium and blood vessels also bound by MPA and WGA. Morphological analysis of peripheral neuromas revealed an increase in connective tissue with increased duration after transplantation, along with axon regeneration. Lectin binding increased with the increase of connective tissue in neuroma. Some lectins (MPA, WGA, Con A) presented a uniform increase in binding, whereas others (GS-I) exhibited a network like binding. These observations describe selective changes in glycoconjugates in experimentally induced peripheral neuromas. Supported by NIH grant NS24834.

125.10

THE EFFECT OF HeNe LASER IRRADIATION ON THE REGENERATION OF THE RAT FACIAL NERVE. J.J. Anders, R.Borke,S.Woolery* and W.P.Vandemerwe*. Dept. of Anatomy and Laser Biophysics Center, USUHS, Bethesda, MD 20814

The use of laser radiation as a photobiological stimulator of nerve regeneration was examined. The facial nerve was exposed unilaterally in anesthetized rats and crushed for 90 secs. The wound was sutured and transcutaneously irradiated daily with a HeNe continuous wave laser (632nm, 8.5mW, for 60min) for 7, 8, or 9 days. Rats were injected subcutaneously, on the side of the face supplied by the injured nerve, with 20 μl of HRP (20%) in 5 areas, 24 to 30 hrs before aldehyde perfusion. The number of labeled neurons in the facial nucleus was counted as an assay of the degree of regeneration. Control, uninjured rats injected with HRP, had 1600 to 2000 facial motor neurons labeled. Rats in which the facial nerve was crushed but not irradiated had an average of 8 neurons labeled on day 7 post crush, 18 on day 8, 89 on day 9 and 1140 on day ten. After laser irradiation, 15 neurons were labeled on day 7, 1055 on day 8, 1365 on day 9. These results show that laser irradiation caused an increase in the rate of facial nerve regeneration.

125.11

CORTICO-SPINAL TRACT REGENERATION OCCURS IN X-IRRADIATED, MYELIN-FREE RAT SPINAL CORD, OR BY APPLICATION OF AN ANTIBODY AGAINST MYELIN-ASSOCIATED NEURITE GROWTH INHIBITORS. T. Savio, L. Schnell and M.E. Schwab. Brain Research Institute, University of Zurich, August-Forel-Str. 1, CH-8029 Zurich/Switzerland

Regeneration of nerve fibers in the adult CNS does not occur, although many central neurons have the capability to regenerate their lesioned axons inside PNS tissue transplants. Recently, inhibitory substrate components which could be involved in lacking CNS regeneration have been discovered in central myelin and oligodendrocytes (Schwab and Caroni, J. Neurosci. 8: 2381-2993, 1988). They consist of two main proteins of 35 kD and 250 kD which are responsible for the inhibition of the neurite extension observed in vitro on CNS myelin or on white matter as a substrate. Their effects can be neutralized by a specific monoclonal antibody (IN-1) produced against these proteins (Caroni and Schwab, Neuron 1: 85-96, 1988). In order to study regeneration in the spinal cord of lesioned rats, we have eliminated the inhibitory substrate components either by neutralizing them with IN-1 mAB through implantation of hybridoma cells, or by preventing myelin formation through X-irradiation of the thoracic and lumbar spinal cord of newborn rats. At 15 days of age, a time when regeneration of the cortico-spinal tract (CST) in normal rats is known to be totally absent, the dorsal spinal cord was cut, and 2-3 weeks later, the lesioned CST was traced by injection of WGA-HRP into the frontal cortex. Results showed clear fiber regeneration, which could be followed for up to 11 mm caudal to the lesion area. In non-irradiated controls or in rats implanted with hybridoma cells producing anti-HRP mAB no fibers were detected at > 1 mm caudal to the lesion. Therefore, our results show that lesioned central fibers can regenerate in the spinal cord after a treatment which either eliminates oligodendrocytes and myelin or neutralizes some of their specific proteins, confirming the hypothesis that inhibitory CNS myelin components are crucially involved in the lack of CNS regeneration.

125.12

RELATION OF MACROPHAGE ACTIVITY TO AXONAL REGENERATION IN THE INJURED SPINAL CORD. C.P. Barrett*, L. Guth* and R.P.Rees*, (SPON. T.H. OH) Dept. of Anat., U. MD. Sch. Med., Balto, MD 21201

The macrophages that accumulate at sites of tissue injuries secrete superoxides and other cytotoxic agents. In spinal cord trauma, they promote a progressive necrosis resulting in an environment hostile to axonal ingrowth. Conversely, other macrophage secretions promote mitogenesis, angiogenesis and neuritogenesis, which are favorable to the repair of injured neural tissues. Thus selective regulation of macrophage activity might enhance axonal regeneration. After crushing the spinal cord of adult rats, we treated them with intravenous E.coli lipopolysaccharide (LPS) , 0.4 mg/kg b.i.d., a dose known to prime monocytes and macrophages for secretion of growth factors. After 2, 4, or 8 weeks, the spinal cords were examined by LM histology and immunocytochemistry. In comparison with saline-treated controls, the lesion site of the LPS-treated rats exhibited (a) reduced tissue necrosis; (b) enhanced ingrowth of glial and ependymal cells which formed cellular bridges across the lesion; (c) accumulation of macrophages whose unusually basophilic cytoplasm was indicative of protein synthetic activity; (d) considerable ingrowth of regenerated axons; and (e) reduced density of the glial scar at the severed ends of the spinal cord. These observations corroborate the early findings of Windle who used Piromen, a bacterial LPS, to stimulate axonal regeneration in the cat spinal cord. We suggest that the favorable effect of LPS results from altered macrophage activity. Supported by NIH grant NS-21460.

125.13

EFFECT OF VARIOUS STRENGTH ELECTRIC FIELDS ON PERIPHERAL NERVE REGENERATION. M. F. Zanakis and M. J. Politis. American BioInterface Corp., New York, NY 10276 and University of Saskatchewan, Saskatoon, Canada S7N 0W0

Cathodally directed DC electric fields enhance the regenerative capabilities of the damaged mammalian nervous system. Several studies have demonstrated this effect, but few have focused on the critical parameters required. In this experiment, adult rat sciatic nerves were transected at mid-thigh and the distal stump was frozen with dry ice. DC stimulating devices (Traxon®) incorporated into a nerve cuff were applied over the lesion site, with the cathode located 10mm distal to the transection. The current delivered to the nerve was 0, 0.6, 1.4, 6.0, 19.0, or 38.0μA. After 14 days the distal stump was removed and processed with toluidine blue, and with a fluorescent probe for neurofilament protein. Results indicate a clear dose response to the treatment, with 1.4μA of current resulting in a greater number of axons distally as shown using both histological techniques. This enhanced regenerative effect is currently being investigated using other parameters of efficacy.

125.14

THE LACK OF AN EFFECT OF APPLIED DC ELECTRIC FIELDS ON THE RATE OR QUALITY OF PERIPHERAL NERVE REGENERATION IN ADULT GUINEA PIGS. M. E. McGinnis* (SPON: S. Ostroy). Ctr. for Paralysis Research, Sch. of Veterinary Medicine, Purdue Univ., W. Lafayette, IN 47907.

This study was undertaken to provide evidence of enhanced regeneration of mammalian peripheral nerves in response to applied electric fields. The peroneal nerves of adult guinea pigs were either transected or crushed under appropriate anesthetic and surgical conditions. Constant current DC stimulators (20 μA) were implanted subdermally in the flank and Pt electrodes routed to each ankle. One electrode served as a cathode or as an anode (the return electrode was at the stimulator implant site), and the other as a sham electrode delivering no current. All surgery and analyses were done without knowledge of electrode identities. Animals with crush lesions were tested for toe spreading ability from the 14th to the 23rd day. Animals with transection lesions were allowed to recover for 40 days before the sciatic nerve was exposed for supramaximal square pulse stimulation. The foot was clamped by two isometric force transducers to measure toe abduction and foot flexion. After evaluation, all animals were euthanized and the nerves removed for histology. Counts of myelinated fibers were made from toluidine blue stained thick sections and counts of unmyelinated fibers from electron micrographs.

There was no difference between legs with an anode, a cathode, or a sham electrode as evaluated by: the time to return of the toe spreading reflex, the isometric force of either twitches or tonic contractions, the latency between stimulation and contraction, or the number or density of either myelinated or unmyelinated fibers. These negative results are at variance with our previous work showing effects on the CNS of guinea pigs (Borgens et al, Science, 238:366-369, 1987) and those of others showing positive effects on the PNS of rats (Politis et al, J of Trauma, 28:1375-1381, 1988).

125.15

EFFECT OF GUIDANCE CHANNEL SURFACE MICROGEOMETRY ON PERIPHERAL NERVE REGENERATION. V. Guénard, P. Aebischer. Artificial Organ Laboratory, Brown University, Providence, RI 02912.

Various parameters such as permeability, electrical activity or ability to release growth or trophic molecules influence the outcome of peripheral nerve regeneration through synthetic guidance channels. In the present study, we focused on the surface microgeometry of the entubulating membrane. Non-porous and expanded polytetrafluoroethylene (PTFE) tubes with internodal distances of 1, 5, and 10 μm were used to bridge a 4 mm nerve gap in a transected mouse sciatic nerve model. Increase in surface roughness correlated with increased internodal distance. Asymmetric semipermeable acrylic copolymer (AC) tubes with smooth inner skins and rough microfibrillar outer surfaces were also compared to AC channels featuring rough microfibrillar inner surfaces and smooth outer skins. Cohorts of 5 animals were implanted for 4 and 12 weeks for each channel type. In all animals, regenerated neural tissue bridged the nerve ends. Non-porous PTFE and AC channels with smooth inner skins contained a well-defined centrally located nerve cable surrounded by an acellular gel whereas porous PTFE and AC channels with a rough inner skin contained nerve fascicles scattered in a loose connective tissue entirely filling the tubes' lumen. The greater the porosity of the PTFE tubes, the more randomly spread were the fascicles. Sleeving the 10 μm PTFE channels with silicone tubes so as to render them impermeable resulted in the same scattering pattern of nerve fascicles. These results suggest that the surface microgeometry of guidance channels, not their permeability characteristics, influences the spatial arrangement of the regenerated nerve elements within synthetic nerve guidance channels. Supported by NIH grant NS 26159.

125.16

CNS AXONAL REGENERATION IS INFLUENCED BY TRANSPLANTS OF MATRIX FILLED POLYMER TUBES. F. Massoudi*, J. J. Collins* and L. F. Kromer, Dept. of Anatomy & Cell Biology, Georgetown University, Washington, DC 20007

Since extracellular matrix (ECM) molecules can directly promote neurite outgrowth in vitro, the present study was undertaken to evaluate whether ECM substrates also could exert a direct effect on CNS axonal regeneration in vivo. For these experiments adult female Sprague-Dawley rats received bilateral aspiration lesions of the fornix-fimbria to transect the septal cholinergic projections to the dorsal hippocampus (HPC). Each animal then received bilateral transplants of tumor-derived ECM or pure collagen matrix cables located within selectively permeable whole or hemi-polymer tubes which bridged the lesion gap between the septum and HPC. The following observations were noted at 1, 2, and 4 weeks after transplantation. 1) Directed axonal growth from the septum to HPC only occured through a matrix cable contained within a whole polymer tube. 2) ECM regeneration substrates were no more effective in promoting axonal regeneration than simple collagen matrices. 3) Migration of immature astrocytes and fibroblasts into the collagen or ECM cables always preceded the initial wave of axonal growth onto the cables. These observations suggest that host-derived astrocytes and possibly fibroblasts play an important role in fostering post-trauma axonal regeneration in the adult CNS under the experimental conditions employed in this study. Our data also indicate that laminin containing ECM does not appear to be advantageous over a pure collagen matrix in enhancing axonal growth from injured CNS pathways.

125.17

COLLAGEN NERVE GUIDE TUBES SUPPORT AXONAL ELONGATION IN THE RAT CENTRAL NERVOUS SYSTEM. SM Weil[1*], R Madison[2], K A Crutcher[1] (Spon: J Tew, Jr.). [1]Dept. of Neurosurgery, Univ. of Cincinnati, Cincinnati, OH 45267 and Div. of Neurosurgery, [2]Duke Univ., Durham, NC 27710

Collagen nerve guide tubes support peripheral nerve regeneration (Madison, Soc. Neurosci. Abst. 13:1042, 1987; Archibald and Madison, 14:499, 1988). In the present study, 26 female rats (180-210 gm) received bilateral (n=20) or unilateral (n=6) suction lesions of the fimbria. Collagen nerve guides (1.0mm inner diameter, 3.0mm in length) were then oriented longitudinally to bridge the septum and hippocampal formation. Animals were killed at 2 weeks (n=12), 4 weeks (n=11), 5 weeks (n=2) and 8 weeks (n=1). The tube was consisently present at 4 weeks, but at longer periods only remnants of the tube remained. There was no evidence of inflammatory reaction. Consecutive coronal sections (16 μ thick) were obtained for histological evaluation using Nissl, acetylcholinesterase (AChE), catecholamine histofluorescence, collagen and silver staining methods. Monoclonal antibody to 68kd polypeptide neurofilament (Boehringer Mannheim Biochemica) was used to detect intraluminal axons. All 12 animals killed at 2 weeks exhibited a cellular tissue core in the proximal lumen of the tubes. Eleven of the animals also showed evidence of AChE staining that decreased toward the hippocampal end of the tube. All 11 animals killed at four weeks also contained a cellular core that spanned the length of the tube. Catecholamine fluorescent positive fibers were present in 2 week animals (17/22 tubes) and 4 week animals (17/21 tubes). Neurofilament-positive fibers were present in 2 week animals (6/10 tubes) and 4 week animals (10/16 tubes). In preliminary experiments, 8 tubes were pretreated with nerve growth factor (.2ug/ml). Animals killed at 2 weeks (n=2) and at 4 weeks (n=2) exhibited a marked increase in intraluminal cellular density and AChE staining. Moreover, the cellular response formed a concentric ring intimately related to the inner wall of the tube. These results suggest that synthetic collagen tubes persist for at least 4 weeks in the CNS, during which time they are colonized by various cellular elements including axons. Further studies, including ultrastructural analysis, are needed to unequivocally identify the contents of the tube and the origin of the axons. Tubes supplied by Colla-tec, Inc., Plainsboro, NJ.

125.18

SEMI-PERMEABLE COLLAGEN-BASED NERVE GUIDE TUBES ARE AS EFFECTIVE AS STANDARD NERVE GRAFTS TO REPAIR TRANSECTED PERIPHERAL NERVES: AN ELECTROPHYSIOLOGICAL STUDY IN THE NON-HUMAN PRIMATE. S.J. Archibald[1], C. Krarup*[2], J. Shefner*[2], B. Bonsack[3], and R. Madison[1] (SPON: E. Gregory). [1]Division of Neurosurgery, Duke University, N.C. 27710, [2]Laboratory of Neurophysiology, Harvard Medical School, Brigham and Womens Hospital, Boston, 11 MA., and [3]Division of Neuroscience Research, Childrens Hospital, Boston, MA.

Collagen nerve guide tubes permeable to macro-molecules up to 68kd have been shown to support sciatic nerve regeneration in rats that is equal to that achieved with a nerve autograft (Arichibald and Madison, Soc. Neurosci. Abst., 14:499, 1988). In the present study, five adult male Macaca Fasicularis monkeys received bilateral median nerve transection at the wrist, removal of a 4mm segment of nerve, and the deficit repaired with either a collagen nerve guide tube or a nerve autograft. Serial evoked EMGs of the APB muscle and sensory nerve conduction studies (SAP) were performed approximately biweekly up to 250 days following initial surgery. EMG activity was first detected between 48-61 days, and SAPs evoked by stimulating the index finger, after 54-77 days. EMG amplitudes recovered to 6.5-52% (conduction vel. 68% normal) and 45-101% (conduction vel. 74% normal) of baseline values for the autograft and tube repair groups respectively. SAPs recovered to 3.5-18% (velocity 73% normal) and 3.5-19% (velocity 64% normal) of baseline values for the autograft and tube repair respectively. Supported by Colla-Tec, Inc.

125.19

ELECTRICALLY CHARGED POLYMER GUIDANCE CHANNELS ENHANCE PERIPHERAL NERVE REGENERATION. R.F. Valentini, A. Sabatini *, P. Dario *, P. Aebischer. Artificial Organ Laboratory, Brown University, Providence, RI 02912 and Scuola Sup., Pisa, Italy.

Electrically charged guidance channels may provide a means of studying the effects of electrical phenomena on nerve regeneration. Polymer electrets are a unique class of dielectric materials which develop either transient (ie piezoelectric materials such as polyvinylidene fluoride [PVDF]) or static (ie true electrets such as polytetrafluroethylene [PTFE]) surface charges due to their molecular structure and mode of preparation. Thus, they do not require an external power source. In the present study, we compared the ability of various tubular electrets to support PNS regeneration using a transected mouse sciatic nerve model with a 4 mm gap. PVDF and PTFE tubes with an 0.9 mm ID were submitted to a high intensity corona poling procedure in order to render them electrically active. Positive or negative surface poling was achieved by connecting a custom-designed electrode array to a high voltage DC power supply. PVDF tubes were poled at 20°C for 12 hr at 21 kV and PTFE tubes were poled at 150°C for 20 min at 14 kV. Cohorts of 5 animals received (+), (-), or unpoled PVDF and PTFE 6 mm long tubes for 4 and 12 wks. At explantation all animals contained regenerated nerve cables bridging the nerve gap and the tissue reaction to the polymer was minimal. At 4 and 12 weeks the number of myelinated axons (MA) was significantly greater in the (+) and (-) PVDF and PTFE tubes than in the unpoled tubes. (+) poled tubes showed greater numbers of MA than (-) poled tubes although the difference was not statistically significant. These results suggest that the nerve regeneration process is affected by electrically active tubes.

Supported by a Whitaker Foundation grant and NIH NS 26159

125.20

DELAYED NERVE REPAIR STIMULATES NERVE REGENERATION IN A SILICONE CHAMBER MODEL. N. Danielsen*, L. B. Dahlin* and L. R. Williams (SPON: H. Müller). Dept. of Anatomy, Univ. of Gothenburg, Gothenburg, Sweden and CNS Diseases Research, The Upjohn Co., Kalamazoo, MI 49001.

Several studies have demonstrated that a previous injury to a nerve (preconditioning) increases the regeneration rate. In this study we used the silicone chamber model to analyze the effects of delayed nerve repair on nerve regeneration (a combination of preconditioning of the proximal nerve stump and predegeneration of the distal stump). The rat sciatic nerves on both sides were transected and repaired after a delay of 3-14 days with a silicone chamber, leaving a 10 mm gap between the two nerve stumps. Light microscopy demonstrated that chambers implanted after a delay of 3-7 days had a significant more advanced axonal migration into the chamber matrix than that of control chambers implanted immediately. The effect was most pronounced in chambers implanted after a delay of 7 days. Chambers implanted after 10-14 days did not differ from controls. Chambers implanted after a delay also showed an increased vascularization as compared to controls. We conclude that delayed nerve repair stimulates nerve regeneration in the silicone chamber model.

REGENERATION: GAP-43

126.1

DIFFERENTIAL POST-LESION EXPRESSION OF B-50/GAP43 AND OLFACTORY MARKER PROTEIN IN THE OLFACTORY SYSTEM. J. Verhaagen*, A.B. Oestreicher*, M. Grillo*, Y.-S. Khew-Goodall*, W.H. Gispen* and F.L. Margolis

Roche Inst. Molec. Biol., Nutley, N.J. 07110, U.S.A. and Rudolf Magnus Institute for Pharmacology, Utrecht, The Netherlands

The olfactory neuroepithelium contains a population of stem cells that gives rise to new neurons throughout the life of the animal. Following lesioning the degenerated olfactory epithelium is able to reconstitute itself by an activation of stem cell division resulting in the formation of new neurons. The regeneration of the olfactory neuroepithelium was studied with mRNA probes and antibodies for B-50/GAP43 and olfactory marker protein (OMP). The well-documented growth related expression of B-50/GAP43 makes this protein a reliable marker for immature neurons. In contrast, OMP is specifically expressed in olfactory neurons that have achieved a relatively advanced state of maturation. Thus, using these two proteins as markers, we were able to discern two stages in the regeneration of the lesioned olfactory pathway. The first stage occurs following either peripheral lesioning with Triton X-100 (TX) or after olfactory bulbectomy (BX) and is characterized by the formation of a large number of new olfactory receptor neurons. These newly formed neurons can be identified by virtue of their expression of B-50/GAP43. The second stage of the regeneration process differs between TX and BX animals. Thus, the formation of a full complement of OMP-expressing neurons is dependent on the presence of the olfactory bulb since this occurs only following TX-lesioning and not after BX. Apparently the stem cells in the olfactory epithelium are autonomous in their ability to undergo mitosis and initial maturation. However, in the absence of the olfactory bulb the complete post-lesion recovery of the olfactory nerve is clearly disturbed. This indicates that the olfactory bulb provides the primary olfactory neurons with as yet unidentified signals (e.g. trophic factors, electrical activity) that are necessary for their maturation and maintenance.

126.2

CHANGES IN THE SYNTHESIS OF CHROMATIN PROTEINS DURING GOLDFISH OPTIC NERVE REGENERATION. J.M. Gossels, S.E. Lewis*, N.I. Perrone-Bizzozero, and L.I. Benowitz. Harvard Medical School; McLean Hospital, Belmont, MA 02178.

Regeneration of the goldfish optic nerve is associated with the expression of specific proteins including β-tubulin, GAP-43, the ON complex and others. Similar changes have also been observed in mammalian peripheral nerve regeneration and during neuronal development, suggesting the existence of a common mechanism for nerve growth and regeneration involving a specific program of gene expression. To begin to elucidate this mechanism, we explored changes in levels of synthesis and phosphorylation of goldfish retinal chromatin proteins in response to optic nerve crush since non-histone DNA-binding proteins are major regulators of gene expression. Control retinas or those undergoing axonal regeneration for 5 or 14 days were dissected, incubated in the presence of radioactive amino acids or phosphate, and chromatin was isolated. The two-dimensional gel pattern of non-histone (acidic) chromatin proteins was entirely distinct from those of the nonnuclear particulate and soluble and the nuclear soluble fractions. In addition to an overall increase in synthesis of chromatin proteins during regeneration, specific increases were observed in the synthesis of 3 proteins, two at 58 kD and one at 51 kD. The specific changes were particularly evident at 14 days of regeneration. The 3 proteins were all phosphoproteins and had pI's at approximately 5.5. Further characterization of these proteins may lead to the identification of DNA-binding proteins which act as trans-acting elements to regulate gene expression during nerve regeneration. Supported by NEI EY 05690 and EY 06152.

126.3

REGULATION AND SEQUENCE OF THE GOLDFISH GAP-43 PROTEIN N.I.Perrone-Bizzozero, R.L.Neve, E.Franck*, J.Gossels, and L.I.Benowitz. Harvard Medical School; McLean Hospital, Belmont, MA, 02178, and Children's Hospital, Boston, MA, 02135.

GAP-43 is a protein that is involved in the development and plasticity of synaptic connections . It is expressed at high levels in all developing and regenerating systems, and is particularly evident in the goldfish optic pathway during axonal regeneration. To gain insight into the mechanisms that regulate the expression of this protein, we have studied the regulation of GAP-43 mRNA levels during regeneration of the goldfish optic nerve. A goldfish GAP-43 cDNA clone was isolated from a cDNA library prepared from regenerating goldfish retinal mRNA. Sequencing revealed that the size of the goldfish protein is similar to its mammalian counterparts, with an extensive conservation of the first 58 amino acids. Beyond this region the goldfish protein differs considerably, with a higher content of acidic residues. The N-terminal conserved region includes the PKC phosphorylation site and the putative calmodulin-binding domain . By Northern blot analysis, levels of GAP-43 mRNA were found to increase within 2-3 days after axotomy, peak at 5-14 days to 20 times the baseline value, and return to control values within 4-8 weeks. Nuclear run-off studies showed that the accumulation of GAP-43 mRNA can not simply be accounted for by an increase in transcription, since transcriptional levels increased only 1.5-fold during regeneration. By immunocytochemistry, GAP-43 could be detected in regenerating optic nerve terminals 17 days post-crush, but was not seen in the contralateral (intact) optic tectum. These results are consistent with the idea that GAP-43 is a specific marker of axonal growth .

Supported by NEI EY 05690.

126.4

GAP-43 IS A MARKER FOR GROWING FIBERS IN THE STRIATUM OF MPTP-TREATED MONKEYS S.K. DOSTER, K. BANKIEWICZ, I.J. KOPIN and M.A. PALMATIER. Clinical Neuroscience Branch, NINDS, NIH, Bethesda, MD 20892

GAP-43 is a neuronal protein found in developing or regenerating neurons which is enriched in growth cones. MPTP-parkinsonian monkeys which receive cavities in the caudate nucleus show some reversal of motor abnormalities. The behavioral improvements are accompanied by evidence of growth into the denervated striatum of dopaminergic nerve fibers from the ventral tegmental area. Striatal sections from MPTP-hemiparkinsonian monkeys, with or without cavities, were examined for GAP-43 immunoreactivity. GAP-43 immunoreactive fibers are present around the cavities and in areas of the striatum that coincide with the immunoreactive dopaminergic fibers. GAP-43 immunoreactivity is present in the striatum several months after cavitation. No GAP-43 immunoreactivity is seen in the striatum of a non-cavitated monkey 2 months after MPTP treatment. GAP-43 can be used as a marker for growing nerve fibers in the central nervous system of non-human primates. All monkeys were treated according to NIH Animal Care guidelines.

126.5

INCREASED EXPRESSION OF THE GROWTH-ASSOCIATED PROTEIN B-50 (GAP-43) IN UNILATERAL FIMBRIA-FORNIX LESIONED RATS. L.H. Schrama, B.J.C. Eggen*, H.B. Nielander*, P. Schotman*, A.B. Oestreicher*, B.M. Spruijt* and W.H. Gispen*. Div. Mol. Neurobiol., Rudolf Magnus Inst., Lab. Physiol. Chem., and Inst. Mol. Biol. & Med. Biotechnol., Univ. of Utrecht, Padualaan 8, 3584 CH Utrecht, NL.

The neuron-specific phosphoprotein B-50 (GAP-43) has been shown to be associated with neurite extension in the nervous system. The expression of B-50 increases after crush lesioning of the sciatic nerve (Van der Zee et al., J. Neurosci., 1989, in press). It has also been reported that lesioning of central nerves is accompanied by an increase in expression of B-50. This study investigates if the expression of B-50 is also affected by transection of the fimbria-fornix. Male Wistar rats (220-240 g b.w.) were subjected to left unilateral fimbria-fornix transection or sham operation. B-50 expression was studied by means of *in situ* hybridization with random-priming labeled [^{35}S]-B-50 cDNA at 4, 24, 48 and 96 h after transection. Control hybridizations were performed with a random-primed [^{35}S]-labeled linearized pBR328. Quantification of the autoradiogram by digital video-imaging showed that there were no major changes in any of the regions studied 4 h after transection. There was, however, a 5-fold increase in the expression of B-50 in the CA3/CA4 region of the hippocampus 24 h after transection in the ipsilateral as compared to the contralateral side. This increase persisted but was reduced to 2-fold at 48 and 96 h after transection. In the medial and lateral septum a bilateral increase was observed at 24, 48 and 96 h after transection. In the cingulate cortex, an ipsilateral increase was seen at the earlier time points, whereas a contralateral effect was only observed at 96 h. The enhanced expression of B-50 mRNA illustrates the cellular regenerative response following mechanical brain damage.

126.6

A CORRELATION BETWEEN THE RATE OF AXONAL REGENERATION AND THE AMOUNT OF GAP-43 AND ACTIN mRNA IN CULTURED RAT SYMPATHETIC NEURONS. M.I. Johnson[1] and M. Willard. Dept. Anat. & Neurobiology ([1]also Pediatrics, Neurology) Washington Univ. Sch. Med., St. Louis, MO.

Embryonic rat superior cervical ganglion neurons elongate axons approximately three times more rapidly when they are cultured on a laminin substrate than when they are cultured on collagen. We have performed in situ hybridization experiments to determine whether the amount of messenger RNA for GAP-43 (a protein associated with axonal growth) and for actin is correlated with the rate of axonal extension. Superior cervical ganglion neurons from embryonic day 21 rat embryos were dissociated and cultured on either laminin or collagen. After one to five days in culture, the cells were fixed and hybridized with ^{35}S-labeled cRNA probes for either GAP-43 or actin. Following in situ hybridization with GAP-43 cRNA, the average number of silver grains over neurons grown on laminin was approximately 2.5 times higher than the average number over neurons extending neurites more slowly on collagen. When the actin cRNA was used for in situ hybridization, we observed five times as many grains over neurons extending axons rapidly on a laminin substrate as compared to those extending axons on a collagen substrate. A control sense cRNA for GAP-43 hybridized equally to the neurons in the two types of cultures. We conclude that the nature of the substrate, which can influence the rate of axonal extension, also influences the accumulation of both GAP-43 and actin mRNA in superior cervical ganglion neurons. [Supported by NIH NS21771, (MIJ), NIH EYO2682 (MW)]

REGENERATION: CNS I

127.1

PLASTICITY OF A SPINAL MOTONEURON NUCLEUS AFTER PERIPHERAL NERVE GRAFT REPAIR D.M. Weinert*, H. Jaksche*, H. Ludt*, A.C. Nacimiento, Neurosurgical Research Laboratory, Saarland University Medical School, 6650 Homburg/Saar, F.R.G.

In adult Sprague-Dawley male rats under pentobarbital anesthesia, the left peroneal nerve was transected and the gap bridged by microsurgical autologous transplantation of a 1 cm long radial nerve segment. After 2,4,8 and 10 weeks, HRP was injected bilaterally into the anterior tibialis (AT) muscle, followed by perfusion 46 - 59 h later. Serial 40 m cross-sections of spinal cord L2-L6 segments were reacted with tetra-methyl-benzidine. Measurements included number, diameter, cell density and 3-D reconstruction of AT motor column. Position of labeled motoneurones in lamina IX was dorsolateral. Their number, relative to the intact side, increased over time from 10% (2 weeks) to 65% (8 weeks). Diameter range bilaterally was 18-66 m, unimodally distributed. Reconstruction revealed on the grafted side caudal shifts a) of peak cell density, which returned to its normal level in the rostral part of the column 8 weeks p.o. and b) of motor column as a whole.

127.2

CHANGES IN SOMA SIZE OF TROCHLEAR MOTOR NEURONS FOLLOWING AXOTOMY. P. G.Iannuzzelli*, J.Brown*, E. H. Murphy and R. Baker. (SPON: H. Warren Goldman). Depts. Anatomy (EHM,JB) and Pharmacology (PI), Medical College of PA, Philadelphia, PA 19129, and Dept. Physiol. & Biophys. New York Med Ctr., New York, NY 10016 (RB).

When the trochlear nerve is cut unilaterally, on average 50% of the cells eventually die and the remainder regenerate to innervate the superior oblique (SO) muscle. We measured the mean soma size of TMNs in Nissl material during the early postoperative period and in TMNs retrogradely labelled with HRP after the regenerating axons had reached the SO muscle. Soma sizes of neurons in the control and axotomised nucleus were compared within each animal (n = 15).Soma size changes were not observed until 7 days postaxotomy. At 2 and 3 weeks, axotomised cells were significantly smaller than controls. At 4 weeks, when axons first reach the SO muscle, soma size returned to normal. At 8 - 25 weeks mean soma size of axotomised TMNs was significantly larger than controls. However the average percent soma size increase was correlated with the extent of cell death. The data indicate that when the population of neurons innervating a target is reduced, the soma undergoes hypertrophy, and the extent of the hypertrophy varies with the extent of cell death and the change in the cell/target ratio. The time course of the changes indicate that this hypertrophy is not related to the process of regeneration (weeks 2-4) but must involve trophic signals transported retrogradely from the target during the restorative process of functional reinnervation.

Supported by EY20007 and NS24707.

127.3

TESTOSTERONE-INDUCED ACCELERATION OF RECOVERY FROM FACIAL PARALYSIS IN MALE HAMSTERS: TEMPORAL REQUIREMENTS OF HORMONE EXPOSURE. K.A. Kujawa * and K.J. Jones. The Chicago Medical School, North Chicago, IL 60064.

We have previously demonstrated that exposure to subcutaneous implants of testosterone propionate (TP) following crush axotomy of the facial nerve in adult male hamsters results in acceleration of recovery from facial paralysis (Kujawa et al., Exp Neurol., 1989). Recovery was defined behaviorally in the form of return of semi blink, blink reflex, full vibrissae movement, and complete recovery (=normal orientation of vibrissae). In this study, we examined the temporal requirements of TP exposure necessary for acceleration of recovery from facial paralysis to occur following facial nerve crush. For each of 2 experiments, castrated male hamsters were anesthetized via intraperitoneal injection of Nembutal, subjected to right facial nerve crush, and divided into 2 groups. In experiment #1, one group received TP implants on day 6 postoperatively (PO), and the other group was sham-implanted. In experiment #2, one group of animals with facial nerve crushes received TP implants on day 0 PO, with implant removal on day 7 PO. A control group of crush-axotomized animals was sham-implanted under the same time schedule. Signs of facial nerve recovery were monitored daily for 4-5 weeks PO in both experiments. The results of the first experiment indicated that, without exposure to TP during the first week PO, the accelerative effects of the hormone were essentially abolished. The results of the second experiment indicated that, while an initial acceleration of semi blink return was noted before capsule removal, no acceleration of return of the blink reflex and full vibrissae movement was observed after capsule removal. Also, an effect of TP, at 3 weeks PO, on acceleration of complete recovery was found. Thus, it appears that there is a priming effect of TP that 1) is exerted at the level of the neuron, 2) temporally precedes behavioral recovery by a week or more and 3) is critical to subsequent acceleration of recovery from facial paralysis. Supported by a grant from the Spinal Cord Research Foundation (KJJ).

127.4

PROGRESSIVE MORPHOLOGICAL CHANGES IN RAT SPINAL MOTOR NEURONS WITH LONG-TERM REGENERATED AXONS AFTER SCIATIC NERVE CRUSH INJURY. C.M.Bowe, V.Vlache*, N.H.Evans*. Section of Neurobiology, Brown University, Providence, RI 02912

Perikaryal enlargement and dendritic abnormalities are observed in spinal motor neurons (SMNs) with long-term regenerated axons after peripheral nerve crush (Bowe et al. Brain Res.(1988) 463:69-77). Since cord examinations in that study were performed only after the first year post-crush, progressive vs. chronic morphological changes could not be distinguished. The present study examined SMNs and dorsal root ganglion (DRG) cells with regenerated axons (REG) at 5 and 10 months following sciatic crush in adult rats. REG neurons were identified by retrograde labelling with HRP applied to the sciatic nerve distal to the point of the earlier crush. Control data were derived from labelled SMNs and DRG cells contralateral (CL) to the side of crush and from sham-operated (SO) rats in which HRP was applied to the sciatic nerve at a site comparable to that used for application on the crushed side.

Statistically significant differences in cell area were not observed between the REG and either control group of SMNs or DRG cells examined at 5 or at 10 months following nerve injury. However, morphological changes could be appreciated in REG SMNs examined at both post-crush intervals. HRP labelling was consistently more dense in REG SMNs compared to CL SMNs or to labelled cells in the SO rats. REG SMNs were more densely clustered than were CL or SO control SMNs. Many, but not all, of the REG SMNs had multiple, thickened dendritic processes. Abnormal morphology was noted in labelled SMNs with both large and small cell areas and was observed with increased frequency in cord sections at 10 months following crush injury compared to those at 5 months after the lesion. Morphological changes were not appreciated in REG DRG cells examined at either post-crush interval. These findings indicate that SMNs with long-term regenerated axons exhibit progressive morphological changes.

127.5

REGENERATIVE AXONAL SPROUTS IN THE TROCHLEAR NERVE FOLLOWING AXOTOMY. E. H. Murphy,J. Brown*, P. G. Iannuzzelli* and R. Baker. Depts. Anatomy (EHM, JB) and Pharmacology (PI), Medical College of PA, Philadelphia, PA 19129, and Dept. Physiol. & Biophys., New York Med. Ctr., New York, NY 10016 (RB).

In the previous study we showed that soma size of trochlear motor neurons (TMNs) which have regenerated is significantly correlated with cell death. In this study we investigated the mechanisms underlying this hypertrophy of the soma. The trochlear nerve was cut unilaterally in cats. Following a survival of 3-9 months, we studied soma size, dendritic arborization, axon diameter and the number of axonal sprouts in those cells which survived the nerve cut and regenerated to reinnervate the superior oblique muscle. Results show that (i) dendritic diameter and extent of arborization do not increase with increased soma size; (ii) axonal diameter increases between the third and sixth postoperative month and then reaches a plateau; (iii) axonal diameter does not increase with increased soma size; (iv) the number of axonal sprouts in the regenerated nerve is approximately 1000, the same as the number of axons in the normal nerve, regardless of the number of surviving cells; (v) the average number of axonal sprouts per surviving cell correlates with soma size. Thus, the results suggest that when the neuronal population is decreased, the surviving cells produce a sufficient number of axonal sprouts to maintain the original number. Hypertrophy of the soma of these surviving cells appears to be associated with the production and maintenance of additional axonal sprouts to compensate for altered cell\target ratio.

Supported by EY20007 and NS24707.

127.6

INTRAMEDULLARY REGENERATION BY "DENDRAXONS" IN CAT SPINAL MOTONEURONS AFTER VENTRAL RHIZOTOMY.
L. Havton* and J.-O. Kellerth* (SPON: H. Aldskogius). Dep. of Anatomy, Univ. of Umeå, S-901 87 Umeå, Sweden.

Peripheral nerve injury may lead to a series of retrograde effects including the formation of supernumerary axons (Havton and Kellerth, Nature 325:711, 1987), which originate from the cell body area and have variable but anomalous trajectories within the ipsilateral spinal cord. The aim of the present study was to investigate the regeneration in cat spinal motoneurons after ventral rhizotomy.

Three months after the injury, single axotomized motoneurons were labeled with horseradish peroxidase (HRP) through micropipettes. Fixation and histochemical processing of the spinal cord allowed morphological analyses.

In addition to the original stem motor axon, axon-like structures originating from the dendritic trees of the axotomized neurons were frequently encountered. The "dendraxons" (Lindå et al., Brain Res., 358:329, 1985) had variable projections and termination fields. In two cats the unilateral ventral rhizotomy was combined with bilateral implantation of peripheral nerve grafts into the dorsal region of the spinal cord. Three months later, a large number of myelinated axons originating from the axotomized motoneurons had regenerated into the ipsilateral peripheral nerve graft, whereas the contralateral control graft was almost devoid of axonal profiles.

127.7

CHANGES IN SYNAPTIC BOUTONS AFTER INJURY OF THE HYPOGLOSSAL NERVE IN THE ADULT HAMSTER. T.E. Durica and S.K. Jacob. Rush Medical College, Anatomy Department, Chicago, IL 60612.

This study examined changes in axosomatic synaptic bouton contacts on injured hypoglossal neurons. The right hypoglossal nerve was either cut or crushed and at 5 or 15 days postoperative (dpo) the brainstems were processed for EM examination. The Bioquant System IV was used for measurement and data analysis. A quantitative study assayed the percent coverage of somal profiles by synaptic boutons on normal and injured neurons. At 5dpo both types of injuries resulted in a decrease in the percent coverage: normal = 28%; cut = 17%; crush = 14%. At 15 dpo cut injury resulted in a continued decrease in percent coverage (9.3%) while crush injured neurons began to show some recovery (19%). Synaptic numbers also showed the same trend. There was a continued decrease after cut injury; crush injured neurons showed an initial decrease followed by a return to normal values at 15dpo. These results may reflect the fact that by 15dpo crush injured neurons have begun to reinnervate the tongue musculature while cut injury has prevented any reinnervation from occurring.

127.8

SYNAPTIC BOUTON CHANGES FOLLOWING INJURY TO THE FACIAL NERVE. S.K. Jacob and T.E. Durica. Rush Medical College, Anatomy Department, Chicago, IL 60612.

This study examined axosomatic synaptic bouton contacts on facial motoneurons following different injuries to the facial nerve. Two groups of adult hamsters underwent either 1) a crush of the right facial nerve or 2) a resection (cut and removal of a segment) of the right facial nerve. Brains were processed for routine ultrastructural examination at 5 or 15 days postoperative (dpo). A quantitative study assayed the percent coverage of somal profiles by synaptic boutons in normal and injured neurons. Both injuries result in significant decreases in bouton contacts at 5dpo (normal = 30%; crush = 16%; resection = 8%) and these values remain low at 15dpo (crush = 15%; resection = 12%). The mean number of synaptic boutons per neuronal profile was compared and the same trend was noted; decreased numbers at 5 and 15 dpo for both injuries. The initial response of the resected series is more severe but by 15 dpo the values are similar to the crush group.

127.9

REFLEX DEPRESSION RESULTS FROM ANAESTHESIA RATHER THAN SPINAL SHOCK FOLLOWING SPINAL TRANSECTION IN THE LAMPREY L. Margolin* and J. Ayers. Dept. of Biology and Marine Science Center, Northeastern University, East Point, Nahant, MA 01908

Lamprey exhibit a variable period of reflex depression following spinal transection. The present experiments were designed to determine whether this depression resulted from spinal shock or anaesthesia.

Tricaine anaesthesia elicited the following sequence of events: cessation of respiration, followed by the cessation of spontaneous movement, and finally the concurrent disappearance of the tail pinch and Mauthner reflexes. Recovery from anaesthesia was also stereotyped with respiration recovering first, followed by the recovery of the response to tactile stimulation, resumption of spontaneous movement and the return of the Mauthner reflex (J. Exp. Biol. **133**: 121-135) in response to electrical stimulation of the tail. These latencies are dose-dependent.

The effect of acute high spinal transection upon recovery from anaesthesia was determined in larvae and compared with that seen in normal ammocoetes and acutely transected adult specimens. Spinal transection had a profound effect upon latency to reflex recovery but the evoked behaviors remained the same even in chronic transectees. Recovery latencies in transected specimens ranged from two times normal (respiration) to a six-fold increase (Mauthner reflex). Recovery latencies of similar duration were seen in transected adult specimens, although the latency for recovery of the Mauthner reflex was significantly shorter in lesioned adults than in lesioned larvae. To control for the effects of anaesthesia 5 specimens were tested acute to unanaesthetized decapitation. All specimens exhibited caudal reflex responses within seconds of decapitation, eliminating the possiblity of spinal shock.

We conclude that the sea lamprey does not exhibit spinal shock, that reflex depression results from delayed effects of the anaesthetic and that spinal transection greatly increases the duration of anaesthetic-induced reflex depression.

127.10

RECOVERY OF FICTIVE LOCOMOTOR ACTIVITY FOLLOWING COMPLETE SPINAL CORD TRANSECTION IN BULLFROG LARVAE. J.K. Baek* and P.B. Farel (SPON: J. Capowski). Dept. of Physiol., Univ. N. Car., Chapel Hill, NC 27599.

Although anatomical continuity is restored across a complete thoracic spinal cord transection within 30 days, the pattern of neural connectivity across the site of injury is perturbed. Long ascending and descending projections between lumbar spinal cord and brainstem are replaced by axons that synapse within 1-2 mm of the injury site. We were interested in whether locomotor recovery could be mediated by this abnormal pattern of connections.

The isolated CNS of bullfrog larvae expresses ventral root activity that, in the intact animal, represents swimming. We found that spontaneous episodes of activity begun above the injury site elicited activity below the injury. Caudal activity was frequency-locked to rostral bursts. Phase relations between rostral and caudal bursts were fairly constant within an episode, although somewhat longer than normal.

These experiments show that aberrant connectivity, similar to that found following embryonic grafts into mammalian spinal cord, can mediate approximately normal locomotor activity.

127.11

EFFECTS OF SPINAL LESIONS ON EMBRYONIC MICE. R. Tompkins, M. Lail*, G. Romeo*, J. Gogola*. Biology Department, Tulane University, New Orleans, LA 70118

The ability of embryonic mice to recover from spinal transection was assessed behaviorally and histologically. At 14 days after mating, pregnant females were prepared for surgery. Full transects of the spinal cords, midway between the limbs, were made with scissors (n=6), forceps (n=4) or fine (10/0) sutures (n=10). Zero time controls showed complete section in nearly all cases. After lesion, exo utero development proceeded to term. Operated animals and their controls were stimulated by stroking and squeezing the face and anterior limbs to evoke posterior limb movement and stroking and squeezing the tail and hindlimbs to evoke head and forelimb movement. No such behaviors were seen in experimental animals cut with forceps or scissors. Such behavior was seen in all controls and those experimental animals cut with a suture. Histological analysis showed that few experimental spinal cords, regardless of how the cord was cut, showed any significant abnormalities. Silver stained sections clearly showed fibers traversing the level of the cut. Thus, embryonic spinal cords are capable of considerable restoration of structure and even function in some circumstances. The embryonic spinal cord of the mouse may provide a permissive environment for assessing the regeneration potential of appropriately marked transplanted cells from older animals.

127.12

ADULT DORSAL ROOT AXONS REGENERATE INTO INTRASPINAL TRANSPLANTS OF FETAL SPINAL CORD (FSC) MORE READILY THAN INTO FETAL BRAIN TRANSPLANTS. Y. Itoh, C. Rogahn*, A. Tessler. VA Medical Center and The Medical College of Pennsylvania, Philadelphia, PA 19129.

We have shown that the cut adult dorsal roots regenerate and form synapses within transplants of FSC. It is unknown whether the growth is specific to FSC, a normal target of DRG axons, or whether dorsal root axons will also grow into fetal brain transplants. In this study we used Nissl-Myelin staining to evaluate the growth and differentiation of spinal cord and brain transplants, calcitonin gene-related peptide (CGRP)-immunocytochemistry to label regenerated DRG axons in transplants, and video image analysis to measure the area occupied in transplants by regenerated CGRP-labeled axons.

Transplants of embryonic day (E)14 spinal cord, E14 or E18 cerebral cortex, E15 cerebellum, or E18 hippocampus were placed into a cavity in the L4 segment of adult rat spinal cord, dorsal roots L3 and L5 were transected, and the severed L4 dorsal root was juxtaposed to the graft. One month later sagittal cryostat sections were processed for Nissl-Myelin stain and CGRP immunocytochemistry. Fetal transplants of brain as well as spinal cord grew and differentiated in the damaged adult spinal cord. CGRP-labeled axons regenerated into transplants from all regions, but growth into FSC was the most robust. These results indicate that fetal CNS tissue whether an appropriate target or not provides an environment that supports or enhances dorsal root regeneration, but that the environment provided by FSC is the most favorable. Supported by the VA Medical Research Service, NIH grant NS 24707, and USAMRDC grant 51930002.

127.13

REGROWTH OF CORTICOSPINAL AXONS AFTER SPINAL INJURY IN THE NEONATAL RAT. C.A. Bates and D.J. Stelzner, Dept. Anatomy, SUNY-HSC at Syracuse, Syracuse, NY 13214.

In the rat, corticospinal (CS) axons grow around a neonatal spinal lesion which includes both CS tracts and the right hemicord. These axons are late growing, unlesioned axons or severed axons which regrow after the injury. Here, we distinguish between these possibilities by double labeling the CS axons before and after spinal injury.

Rat pups were anesthetized with cold on postnatal day (PND) 2,4 or 10. Fast blue (FB 4%) was injected into the CS tract at C4. Two days later, the FB was aspirated. This removes the FB and lesions the CS tract. Two months post-op, diamidino yellow (DY) was injected caudal to the lesion. Three days later the animals were sacrificed and the CNS was fixed, frozen sectioned and viewed under epifluorescence.

FB labeled cells are located in layer V of the dorsal cortex in each age group. These cells projected at least to C4 before the lesion. In the PND 2 and PND 4 groups, some of the FB cells are also labeled with DY, with more at PND 2 than PND 4. These cells have regrown an axon around the lesion in the intervening two months. About an equal number of cells in the PND 2 or PND 4 groups are seen with only DY labeling. These cells represent a small group of late growing axons which reach the C4 level after the lesion. No double or DY labeled cells are seen in the PND 10 group. Supported by NS 14096.

127.14

RESPONSES OF LIZARD EPENDYMA CELLS TO DENERVATION AND RENERVATION BY PERIPHERAL AXONS. D. R. Liebich*, V. M. Grant and S. B. Simpson, Jr. Dept. Biol. Sci./Comm. Neurosci., Univ. of Ill. at Chicago, Chicago, IL 60680.

As part of a continuing study of ependymal epithelium in spinal cord regenerates of Anolis c. we ask the following: 1) How do the ependyma cells respond to removal of the CNS axons that they fasciculate; 2) What is the fate of the scattered cerebrospinal fluid contacting neurons (CSFN) in the ependymal epithelium when CNS axons are absent; and 3) How do ependyma cells respond to peripheral nerves? Autografts of the regenerated cartilage tube and ependyma were made to the backs and hind limbs of the same lizard. In the latter the graft was aligned with the cut sciatic nerve. The grafts were fixed at various times and examined using light and transmission electron microscopy. We have observed that CSFN are absent in back grafts that are sparsely innervated by peripheral axons. Grafts to the limb which fasciculates larger numbers of peripheral axons exhibit larger than normal numbers of CSFN. These experiments also demonstrate that the ependymal epithelium can fasciculate peripheral axons, some of which become myelinated.

127.15

NEUROGENESIS IN NATURALLY OCCURRING SPINAL CORD REGENERATION. B.M. Davis, J. L. Carlson*, L.D. Goldstein*, M.C. Anderson* and S.B. Simpson, Jr. Dept. of Biological Science, Univ. of Illinois at Chicago, Chicago IL 60680.

It has been proposed that regeneration of CNS axons naturally occurs only in those systems with ongoing neurogenesis (Holder and Clarke, TINS, **11**:99, 1988). To test whether neurogenesis plays a role in regeneration in salamander CNS, unlesioned and spinally transected adult salamanders (*Notophthalmus viridescens*) were injected (for a 2wk period) with ^{3}H-thymidine. Few if any supraspinal neurons were thymidine labeled in unlesioned salamanders, indicating no ongoing neurogenesis in spinally projecting nuclei. However, a significant number of labeled brainstem cells (neurons and glia) resulted from injections given during the first month, but not the second month, post-transection.

To examine whether axotomized CNS neurons regrow their axons, thoracic spinal cords were transected (abolishing normal walking and swimming behaviors), and HRP was applied to the lesion site. Following behavioral recovery (60-90d post-transection), Fast Blue was applied to a second transection 1.0 cm caudal to the first. In these salamanders (n=4) 18-27% of the supraspinal neurons which contained Fast Blue also contained HRP, indicating that a significant number of the supraspinal axons had undergone frank regeneration.

Thus, although damaged axons can regrow, a burst of neurogenesis is observed during regeneration, suggesting that the environment present during neurogenesis may be important for successful frank regeneration of CNS axons. (Supported by NIH NS25617 to BMD).

127.16

IS NEUROGENESIS NECESSARY FOR CNS REGENERATION? M. T. Duffy*, B. M. Davis and S. B. Simpson, Jr. (SPON: E. Pollack). Dept. of Biological Science, University of Illinois at Chicago, Chicago, Il. 60680.

We have previously shown that regeneration in the lizard tail spinal cord is accompanied by supernormal descending projections from local intraspinal and supraspinal nuclei. We have proposed that this results from a combination of frank regeneration and sprouting of axons. However, it has recently been proposed (Holder & Clarke, TINS, 11:99, 1988) that natural regeneration occurs only in animals exhibiting ongoing neurogenesis.

To test this hypothesis, normal lizards were injected every other day for 30 days with ^{3}H-Thymidine. This resulted in labeling of the telencephalon (as seen by Lopez-Garcia et al., 1987), but not in neurons of supraspinal nuclei. In a separate experiment, lizards had their tails autotomized and were injected with ^{3}H-Thymidine until their tails had regenerated. This resulted in an increase in labeling throughout the CNS due to angiogenesis and gliogenesis. No labeled cells were identifiable as neurons. Further, the number and spatial pattern of the thymidine labeled cells was inconsistent with the previously identified origins of "regenerated" and "sprouted" fibers (as demonstrated with HRP pathway tracing). We conclude that the portions of normal lizard CNS involved in locomotion, somatosensory and proprioceptive functions exhibits no ongoing neurogenesis and that regeneration causes no measurable neurogenesis. (Supported by NS24162 to SBS).

127.17

TRANSFORMATION OF ESCAPE REFLEX FUNCTION FOLLOWING ASEXUAL REPRODUCTION IN AN AQUATIC OLIGOCHAETE. C.D. Drewes* and C.R. Fourtner. Dept. Zool., Iowa State Univ. Ames, IA 50011, Dept. Biol. SUNY Buffalo, NY 14260.

Lumbriculus variegatus reproduces asexually by fragmentation (formation of complete worm from body fragments). Since fragments from either anterior or posterior body regions always regenerate short heads (7-8 segments) and much longer tails, segments originating in posterior regions become transformed, acquiring a more anterior axial identity in the regenerating worm. In the giant axon mediated withdrawal reflex, there is a constant ratio of sensory field partitioning along the body axis regardless of body length (Medial Giant Fiber [MGF] = anterior 40% and Lateral Giant Fiber [LGF] = posterior 70% of body length). Using electrophysiological and anatomical correlates, we described the day-by-day transformation in the proportional partitioning of the sensory field to the giant fibers mediating escape. Our results reveal that: 1) posterior fragments, originally subserved solely by the LGF sensory field, gradually become subserved by the MGF sensory field, conforming to their new anterior position; 2) appropriate increases in the ratio of MGF:LGF caliber and conduction velocity accompany the LGF to MGF sensory field transformation; 3) sensory field transformation can be repeatedly reversed by additional amputations. These results demonstrate that neural organization of the reflex is highly plastic and that transformation may result from the counterbalance of morphogenic influences localized within the anterior and posterior ends of regeneration body fragments.

130

SYMPOSIUM. HORMONES, NEURAL CIRCUITS, AND COMMUNICATION. A. Arnold, UCLA (Co-chairperson); E. Brenowitz, Univ. of Washington (Co-chairperson); A. Bass, Cornell Univ.; D. Kelley, Columbia Univ.; J. Wingfield*, Univ. of Washington; H. Zakon, Univ. of Texas.

Steroid hormones have a great influence on the structural organization and function of sexually dimorphic neural circuits that underlie the production and detection of communicatory signals in diverse animal species. The speakers will identify common principles that emerge from comparative studies of this topic and propose directions for future research. Wingfield will consider the interactions of ontogenetic hormone cycles, social behavior, and environmental variables in the regulation of communication. Brenowitz will discuss hormone accumulation by song control brain nuclei in bird species that vary in the degree of sexual dimorphism in song behavior. Bass will discuss the influence of hormones upon acoustic communication in fish. Zakon will consider steroid effects upon the production and reception of electrocommunication signals in fish. Finally Kelley will discuss how gonadal steroids direct sexual differentiation of neuroeffectors for vocal communication in frogs, and will provide concluding remarks.

131

SYMPOSIUM. THE INITIAL EVENTS IN TASTE: CHEMOSENSORY TRANSDUCTION IN THE VERTEBRATE TASTE BUD. Stephen D. Roper, (*Chairperson*), Colorado State Univ; John H. Teeter, Monell Chemical Senses Cntr; Myles Akabas, Columbia Univ; Patrick Avenet*, Univ. Saarlandes; Sue C.Kinnamon, Colorado State Univ.

Until recently, little was understood about how taste cells function. Taste receptor cells were considered to be passive transducers of the external chemical milieu, converting sapid stimulation of the apical chemosensitive region into graded receptor potentials that controlled transmitter release from synapses in the basolateral region. In 1983, two laboratories independently reported that taste cells generate action potentials in response to stimulation, thereby establishing that cells in taste buds possess voltage-gated ion channels (Kashiwayanagi, et al., *Am. J. Physiol.* **244:** C82; Roper, *Science* **220:** 1311). Since then, there has been a rapid accumulation of data concerning ion channels on taste cells and their role in chemosensory transduction. Key findings have been made in the last few years utilizing intracellular and patch recording methods and Ca-sensitive dye techniques.To date, the data suggest that amiloride-sensitive Na^+ channels mediate NaCl taste; apical voltage-dependent K^+ channels mediate sour taste; a ligand-gated non-selective cation channel mediates amino acid responses; and a receptor-stimulated second messenger system mediates bitter taste.

The intent of this symposium is to synthesize findings from a number of laboratories and to arrive at a working hypothesis for how the different types of cells present in taste buds and how the various ion channels and second messengers present in taste cells are orchestrated to produce intracellular signals that indicate the presence of salty, sweet, sour or bitter compounds on the tongue.

132.1

TEMPORAL RESPONSE OF MACAQUE V1 NEURONS TO CHROMATIC FLICKER D. D. DePriest* and P. Lennie, Center for Visual Science, University of Rochester, Rochester, NY 14647.

We recently characterized the responses of parvocellular (P) lateral geniculate (LGN) neurons in Macaque to temporal modulation of chromatic and achromatic stimuli, and compared these results to human psychophysical profiles obtained under identical conditions (ARVO, 1988). When stimuli are modulated along a line of preferred chromaticity (either constant B cone excitation, or constant R,G cone excitation) P cells behave as low-pass filters, with a corner frequency of 20Hz in most cases. The temporal characteristics of P cells are quite uniform and do not account for the limitations of performance seen psychophysically. These limits must arise centrally.

We have extended our analysis to striate cortex (V1). We measured chromatic flicker sensitivity in 47 chromatically responsive V1 neurons using either a spatially uniform field or a moving sinusoidal grating. Thirty (61%) of these units had non-oriented receptive fields; twenty-three (50%) demonstrated frequency doubling to chromatic stimuli. Twenty (42%) of all chromatically responsive neurons yielded temporal sensitivity profiles like those seen in P cells: low-pass with a corner frequency near 20Hz. The remaining cells formed a heterogeneous group with band-pass temporal characteristics.

Envelopes of V1 response profiles show relatively much higher sensitivities to high temporal frequencies than is found in psychophysical measurements. Neurons in striate cortex are therefore unlikely to set the limits to chromatic flicker sensitivity.

Supported by EY04440 and EY01319.

132.2

A NEW TWO-DIMENSIONAL PSEUDORANDOM STIMULUS FOR THE STUDY OF RECEPTIVE FIELDS IN THE LGN AND STRIATE CORTEX R. Clay Reid*[123], Robert M. Shapley[13], and Jonathan D. Victor[23], New York University[1], Cornell University Medical College[2], and The Rockefeller University[3].

We have adapted a new method of visual stimulation to the study of receptive fields in the LGN and striate cortex of both cat and macaque. The method employs pseudo-random binary stimuli, known as m-sequences (first used in VEP's by Sutter, 1988), which modulate the luminance or chrominance of each region in an 8x8 or larger array of squares. M-sequences hold several advantages over other random stimuli: specifically the auto- and cross-correlations among the regions are perfectly flat, allowing for an efficient estimation of first and second-order responses of receptive fields. Further, several mathematical relations make the extraction of all first as well as second-order responses, normally a lengthy calculation, very rapid.

This approach has proven useful in a variety of experiments. First, we have used it to study the the 2-dimensional chromatic structure of neurons in the macaque, using isoluminant, red or green cone isolating, and luminance-modulating stimuli. Second, we have studied the first and second-order components of the spatio-temporal interactions leading to directional selectivity in the cortex. Finally, we have used it in multielectrode experiments along with the BrainWave Systems data acquisition software. Since the stimulus elevates the firing rate of cortical neurons in an 'unbiased' way— being spatially distributed, unoriented, and non-repetitive— it has helped in the study of cross-correlations among cortical and LGN neurons while simultaneously mapping their receptive fields. *Supported by EY1472 and EY7977.*

132.3

SPATIOTEMPORAL CONDITIONS FOR MOTION DETECTION IN CAT STRIATE CORTEX NEURONS. C.L. Baker (Jr.) Dept. of Psychology, McGill Univ., Montreal, PQ, Can., H3A 1B1

Responses of single neurons in striate cortex of the cat were measured in response to sinewave grating stimuli. Firstly, a neuron's spatial frequency tuning was determined, and subsequent stimuli were set at the optimal spatial frequency for that neuron. Then a "jumping grating" stimulus was used: a sinewave grating subjected to a series of abrupt spatial displacements, while remaining stationary for a fixed exposure time between displacements. The amount of direction selectivity elicited by this stimulus was measured as a function of the amount of spatial displacement. Striate cortex neurons generally showed an optimal spatial displacement, corresponding to somewhat less than one quarter of a spatial period of the neuron's optimum spatial frequency (close to "quadrature phase").

This optimum displacement was not affected by increasing the exposure time between displacements, indicating that the measurement is not a simple consequence of temporal frequency tuning.

These results closely parallel recent human psychophysical data (Baker, Baydala, and Zeitouni, 1989), obtained from measurements of the motion aftereffect elicited by jumping grating stimuli.

Supported by Canadian MRC grant (MA-9685).

132.4

DIRECTIONAL NEURONS IN AWAKE RHESUS MONKEYS: IMPLICATIONS FOR MOTION TRANSPARENCY. R.G.Erickson, R.J. Snowden, R.A. Andersen, and S. Treue MIT, Cambridge, MA.

The observation that motion cues alone are sufficient for perception of two transparent surfaces indicates that the brain can simultaneously resolve different motion vectors from an array of moving elements, a fact not well accounted for by current visual-motion algorithms. To examine how different cortical cells respond to one versus two transparent surfaces we compared the responses of 77 striate and prestriate neurons (32 directional) to the oppositely directed motion of two dot fields moved separately or simultaneously over their receptive field. Three results were obtained. 1) All nondirectional cells responded equally well to single and double surfaces. 2) There were two groups of directional cells within both V1 and V2. One had similar responses to a single surface moved in the preferred direction regardless of the presence or absence of a second moving surface. Thus, subgroups with different preferred directions can independently encode coherent motion occurring on separate transparent surfaces. Responses of the other group of V1 and V2 cells showed interactions (usually suppressive) between the two surfaces. 3) Development of suppressive interactions appeared to be hierarchical. Only weak interactions were observed in V1 while stronger suppression was found in V2. Complete suppression of excitatory responses by addition of a second surface moved in the null direction was observed only in V2 and other prestriate areas.

These results imply that subpopulations of V1 cells can encode the motions of individual, transparent surfaces.

132.5

ORIENTATION SELECTIVE RESPONSES TO MOTION CONTRAST BOUNDARIES IN MACAQUE V1. T.D. Albright and A. Chaudhuri*, The Salk Institute, La Jolla, CA 92037.

There are a variety of figural cues that our visual system can use to segregate objects (e.g., luminance, texture, relative motion, depth). We previously reported that many neurons in extrastriate visual area MT exhibit similar direction selectivity for stimuli defined by different figural cues (Albright, Soc. Neurosci. Abs., 13:1626, 1987). These neurons thus exhibit the same "form-cue invariance" that is manifest perceptually. We have now examined the possibility that such invariance may be present as early as V1. Single V1 neurons were isolated in alert fixating rhesus monkeys. Visual stimuli included drifting bars defined solely by either luminance contrast or motion contrast. The latter stimuli, possessing no coherent luminance boundary, have also been referred to as kinetic edges and non-fourier motion (Chubb and Sperling, JOSA, A5, 1988). Three-quarters of our sampled V1 neurons responded to the motion contrast stimuli. More specifically, of those V1 neurons exhibiting significant orientation selectivity for the luminance contrast stimuli, 50% responded selectively to the orientation of the motion contrast stimuli. In most cases the preferred orientation was similar for the two stimulus types but motion contrast stimuli typically elicited broader tuning and weaker responses. These results suggest that form-cue invariance seen in MT results from convergence of cue information as early as V1. (Supported by NIH EY07605)

132.6

SINGLE-UNIT RESPONSES TO TEXTURE PATTERNS IN AREA V1 OF THE ALERT MONKEY. J.J. Knierim and D.C. Van Essen. Div. of Biology 216-76, Caltech, Pasadena, CA 91125.

Human observers can effortlessly detect a target element in a field of distractors if the target and distractors differ in orientation. We recorded from single units in V1 of an alert macaque monkey and found possible neural correlates of this "pop-out" phenomenon.

Visual stimuli consisted of 1) a single line element, either at the cell's preferred orientation or orthogonal to it, and/or 2) concentric rings of elements, either of the same orientation as the central element (uniform orientation) or orthogonal to the central element (orientation contrast). The central element was within the classical receptive field, whereas the surrounding rings were entirely outside it. The response to the central element was significantly modulated by at least one type of surround pattern in 46/60 cells tested (77%). In about half of these cells (22/46, 48%), the response was significantly greater to the orientation contrast pattern than to the uniform texture field. Other cells were suppressed by all surround patterns (13/46, 28%). Cells responding better to the uniform texture field than to the orientation contrast pattern were rare. Taken together, these results show that, in many cells, static texture patterns placed outside the classical receptive field elicit strong orientation specific effects that correlate with the perceptual salience of a central target.

132.7

EVIDENCE FOR TEMPORAL FACILITATION ALONG THE LONG-AXIS OF VISUAL CORTICAL RECEPTIVE FIELDS. F.Wörgötter*† & U.T.Eysel (SPON: H. Orbach) Inst. f. Neurophysiologie Universität Bochum 4630 Bochum FRG.; †Present address: Div. of Biol., CALTECH, Pasadena, CA 91125.

Temporal mechanisms in visual cortical cells have been described mostly in terms of interactions between subfields. This study focusses on interactions along the long axis of the central excitatory zone. In Area 17 of anesthetized cats 81 cells were studied with moving light spots of 0.1-0.3° diameter. For most cells *the strongest response to a spot is elicited when the spot moves along the long axis of the receptive field.* Thus, this response component has a preferred direction (PD) which is orthogonal to PD for the response component elicited with a long bar. Applying short bar stimuli, a superposition of the two response components was also observed. This results in cortical tuning curves with four symmetrically arranged response peaks. We have reported that the first order fourier coefficients of cortical tuning curves can be regarded as directional component whereas the second order coefficients correspond to the orientation tuning (Wörgötter & Eysel Biol.Cybern. 57:349,1987). Consequentially the fourth order fourier coefficients can be used to quantify four-symmetrical tuning curves and it is statistically shown that pure axial response components predominate for spot stimuli, whereas they cannot be detected with long bars. Superposition between both components most often occurs at intermediate bar length. The importance of higher order symmetries in cortical tuning curves is further supported by tuning curves recorded in higher areas in monkey's visual cortex that already are four-symmetrical when stimulated with long stimuli (DeValois et al Vis. Res. 22:531,1982; Felleman & VanEssen J.Neurophysiol. 57:889, 1987).

Since the response to flashing spots is mostly much weaker than to moving spots, it is concluded that temporal facilitation can be elicited by motion along the receptive field long axis and that this response component is independent from response components that can be elicited with oriented long bars.

132.8

Bicuculline induced changes in excitability and orientation selectivity of striate cortical neurones. K.Albus and U.Baumfalk*. Dept. Neurob., MPI Biophys. Chem., 3400 Göttingen, FRG.

The effect of microiontophoretic application of bicuculline methiodide(BMI) on the orientation selectivity of single neurones was investigated in cats. BMI increased orientation tuning width (OTW) in 60% of the cells, decreased OTW, i.e. improved orientation selectivity(OS) in 10% and did not alter OTW in the remaining 30%. An increase in OTW was combined with either an enhancement(80%) or a suppression(20%) of the mean response to an optimally oriented light bar (ROPO). In most cells with an increase in OTW both ROPO and the response to a light bar oriented orthogonal to the optimal(RNOPO) were enhanced and the enhancement was stronger for ROPO. In a minority of cells with increases in OTW(20%) a strong enhancement of RNOPO was combined with either an increase or a decrease in ROPO. A substantial decrease in OS was usually present in neurones with increases in OTW of more than 20°; however, the general excitability of most of these neurones seemed to be altered as indicated by the appearance of spontaneously occuring and/or evoked bursting activity. Interestingly the glutamate or aspartate induced changes in OS were qualitatively and often also quantitatively similar to the BMI induced changes in OS in about 80% of the cells tested so far. We conclude that BMI causes only moderate changes in OS, in most cells; an actual loss of OS under BMI might be due to unspecific increases in excitability of cell(s) close to the application pipette.

132.9

ORIENTATION TUNING IN CAT STRIATE CORTEX INVOLVES INTRACORTICAL SUPPRESSION OF CROSS-ORIENTATION EXCITATION. U.Th. Eysel, J.M. Crook* and H.F. Machemer*. Dept. of Neurophysiology, Ruhr-Universität Bochum, D-4630 Bochum, F.R.G.

Orientation specificity in striate cortex of the lightly anesthetized cat was tested with flash presented and moving light bars. Based on the previous finding that orientation specificity in area 17 of the cat can be broadened most easily by local inactivation of cortical tissue in a distance of about 500µm (Wörgötter and Eysel, Soc.Neurosci.Abstr., 14:898,1988) we have constructed a circular array of four micropipettes glued externally to a guide tube with a radius of 500µm. The micropipettes were placed at a depth of 500-800µm and a tungsten in glass electrode was lowered through the guide tube so that single cells were recorded from the visual cortex in a lateral distance of 500µm from the surrounding pipettes. GABA (0.5M, pH=3.0) was ejected microiontophoretically from the micropipettes during continuous recording from single cells with the center electrode. During remote inactivation induced by GABA application, the responses to non-optimal orientations increased and orientation selectivity was reduced in 70% of the investigated S- and C-cells. In a number of cells the inactivation of surrounding tissue resulted in a broadening of orientation tuning at and close to the orientation perpendicular to the optimal orientation. A concomitant broadening of the excitatory receptive field and loss of directionality were often observed. The effects on orientation tuning were not dependent on stimulus motion; the orientation selectivity in response to flash presented bars was reduced in the same way. The results clearly demonstrate intracortical suppression of responses to orientations oblique and perpendicular to the optimal orientation. Cross-orientation inhibition seems the most probable underlying mechanism.

132.10

THE INFLUENCE OF CONTEXTUAL PATTERNS ON ORIENTATION SELECTIVITY IN V1 OF THE CAT. *C. D. Gilbert and T. N. Wiesel.* Laboratory of Neurobiology, The Rockefeller University, New York, NY 10021.

The extent of long range horizontal connections within the primary visual cortex suggests that a single cell can integrate information over a much larger part of the visual field than that covered by the cell's receptive field. In a similar vein, psychophysics has shown that perception of local features can be influenced by the visual context in which such features are presented. In the domain of orientation, psychophysical studies show that perception of an oriented line is influenced by nearby lines of a different orientation, the "tilt effect." To explore the neural mechanism underlying this illusion, we investigated contextual sensitivity in the domain of orientation at the level of single cells in the superficial layers of cat primary visual cortex. In these experiments we placed sets of lines outside the receptive field (the "surround lines"), in positions either along the orientation axis or orthogonal to it. The surround lines by themselves did not activate the cell, but when presented in conjunction with a line placed within the receptive field usually suppressed and on occasion facilitated its response, confirming earlier studies. An observation relevant to the tilt effect was a small shift in the optimal orientation of some cells (approx. 1/3, N=36) which was produced by the surround lines: when the surround lines differed in orientation from the preferred orientation of the cell by 20° to 30°, the peak in the orientation tuning curve shifted by 10°. For some cells the orientation shift showed hysteresis, such that when the surround lines were removed, the orientation tuning curve did not return to its original position and bandwidth, but had broadened. Presenting the cell with a succession of surround lines of different orientations caused the cell to "reset" its orientation tuning to its original width and position. These results suggest that neural processes allowing lateral interactions in the domain of orientation are seen even at an early level in the visual pathway, and that the tuning properties of cells are more dynamic than previously thought. (supported by NIH grant RO1EY05253 and the Rita Allen Foundation)

132.11

ORIENTATION TUNING CHARACTERISTICS OF V1 NEURONS MEASURED IN THE DISCRIMINATING MONKEY. G.A. ORBAN and R. VOGELS. Lab. Neuro- en Psychofysiologie, K.U.Leuven, Campus Gasthuisberg, B-3000 Leuven, Belgium.

In our search for neuronal mechanisms underlying simple discrimination tasks, single striate cell responses were measured while the monkey performed an orientation discrimination task. Task of the monkey was to fixate a LED and to maintain fixation when two square wave gratings (duration 350 msec, diameter 8°, 2 cycles/deg, 80% contrast and luminance 7 cd/m^2) presented in succession over the receptive field had an identical orientation, and to make a saccade to the grating when they differed in orientation. Data were obtained from 285 neurons recorded in two monkeys. Striate neurons displayed a wide range of responses to the homogeneous screen during fixation and to the grating pattern. The orientation tuning characteristics of the striate neurons were derived from the responses to the first grating. The median response latency was 70 msec (first and third quartiles : 40, 100 msec respectively), the median orientation tuning width at half-height was 41° (first and third quartiles 25° and 75°). The variance correlated strongly with the response strength and equalled on average 1.9 times the response strength. Neurons were orientation tuned from the start of the responses. While a number of neurons had orientation tunings which remained stable over 350 msec, many neurons showed a decrease in gain and a few a change in tuning width over time. These results show that tuning characteristics vary over a wide range in striate cortex of the monkey.

132.12

ORIENTATION DISCRIMINATION THRESHOLDS OF SINGLE STRIATE CELLS IN THE DISCRIMINATING MONKEY. R. VOGELS and G.A. ORBAN. Lab. Neuro- en Psychofysiologie, K.U.Leuven, Campus Gasthuisberg, B-3000 Leuven, Belgium.

In our search for neuronal mechanisms underlying single discrimination tasks, we measured just noticeable differences in orientation of the monkey with the same task as used to investigate the orientation tuning characteristics of striate neurons (Orban and Vogels, Soc. Neurosci. Abstr., 1989). Monkeys are able to discriminate orientation difference as small as 1.5°, a capacity similar to that of humans. We then estimated how well single striate neurons can discriminate these small orientation differences. Nonparametric ROC analysis was performed on the responses to the first grating used to probe orientation tuning. Since the monkey responded behaviorally with an average latency of 250 msec we compared estimates for two time intervals : 175 msec and 500 msec. While 15 out of 45 neurons discriminate differences smaller than 3.5 deg for 500 msec interval, only 6 out of 45 did so for 175 msec. In order to isolate the contribution of short term variability we performed a ROC analysis on the difference between the responses to the pair of orientations presented in a single trial. Three out of 21 striate neurons could discriminate differences as small as 2.5 degrees. These results show that single striate neurons can discriminate orientation differences nearly as small as the behaving animal, but only on a restricted part of their tuning curve.

132.13

RELATING DISCRIMINATION KINETICS TO THE RELIABILITY OF SINGLE NEURONS.

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The reliability of identification of visual targets in pop-out tasks increases with time available for stimulus inspection (i.e. the interval between stimulus and mask onsets, SOA). We have determined the experimental time course of this improvement and constructed a model to account for its kinetics. The model is based on the assumption that the reliability of the observer's response is limited by the variability of the responses of individual neurons. We assume that sensory information is coded in the neuron spike response integrated over the available time. The reliability of the discrimination at the neuronal level is then directly related to the ratio of the mean response to its standard deviation. This reliability increases with inspection time. Psychometric functions are derived from the firing rates of monkey V1 cortical neurons. Comparison with human psychometric functions suggests that very few cells may be needed to supply the psychophysical discriminating power.

132.14

COMPLEX CELLS ARE IDEAL BINOCULAR DISPARITY DETECTORS. I. Ohzawa*, G. DeAngelis* and R.D. Freeman. Neurobiology Group, School of Optometry, University of California, Berkeley, California 94720.

An ideal detector or sensor for any parameter **X** should have a high sensitivity to variations of **X**, but should be *insensitive* to all other parameters. Although both simple and complex cells of the visual cortex are sensitive to binocular disparity, it is not known if they differ with respect to sensitivity to other parameters of binocular stimuli. We have studied the sensitivities of striate neurons in anesthetized and paralyzed cats with respect to disparity, position, and contrast polarity.

After isolation of single cells, drifting sinusoidal gratings are used to determine the optimal orientation and spatial frequency for each eye. Sensitivity to binocular (phase) disparity is also evaluated by presenting optimal gratings dichoptically at a variety of relative phases. Then, using a binocular version of a receptive field mapping technique based on reverse correlation (Jones & Palmer, 1987), disparity selectivity is determined by brief (53msec) dichoptic flashes of bar stimuli at optimal orientations. For each eye, a stimulus is presented randomly with respect to position and polarity.

Results are as follows. For complex cells, disparity selectivity is much finer than the size of either monocular receptive field at all positions within the fields. In addition, both bright-bright and dark-dark pairs show identical disparity selectivity profiles. The optimal disparity for bright-dark pairs differs from that for the same polarity pair by 1/2 period of the cell's optimal spatial frequency. In contrast, simple cell responses depend critically on position of the bars. Bright-bright and dark-dark pairs with optimal disparity cause responses at mutually exclusive positions within the receptive fields.

In conclusion, complex cells are sensitive to disparity, but they are relatively insensitive to changes in stimulus position and contrast polarity. They are thus ideally suited as disparity detectors. Simple cells, however, do not exhibit these properties. (EY01175)

EXCITATORY AMINO ACIDS: RECEPTORS III

133.1

SIMILARITY BETWEEN QUISQUALATE RECEPTORS AND THE GLYCINE SITE ON NMDA RECEPTORS: EVIDENCE FROM ANTAGONIST PHARMACOLOGY. **N. W. Kleckner and R. Dingledine, Department of Pharmacology, University of North Carolina, Chapel Hill, NC 27599.**

Agonist-induced inward currents in Xenopus oocytes injected with rat brain mRNA were used to identify and characterize compounds that antagonize both the glycine site on NMDA receptors, and quisqualate/kainate receptors. Schild analysis of 7-chlorokynurenic acid (7-Cl-kyn) or 6,7-dichloro-3-hydroxy-2-quinoxaline carboxylic acid (6,7-diCl-HQC) vs glycine indicated competitive antagonism for both compounds with K_Bs of 350 and 300 nM, respectively. Both compounds also blocked the receptor mediating kainate-induced currents. Schild analysis of 6,7-diCl-HQC (K_B=3.0 μM) indicated competitive antagonism of kainate currents, but with a potency 10-fold lower than at the glycine site. 7-Cl-kyn antagonized kainate-evoked currents (K_B=14.1 μM), but the slope of the Schild regression was less than one. Thus 7-Cl-kyn was approximately 40-fold more potent at the glycine site than at the quisqualate/kainate receptor, but is probably not entirely competitive at the latter receptor. Omission of the Cl groups from 7-Cl-kyn or 6,7-diCl-HQC drastically reduced activity at both glycine and kainate sites. DNQX and CNQX were both more potent antagonists of kainate than glycine, but substitution of a Cl at the 6-position and especially the 6- and 7- positions increased potency at the glycine site. These results suggest that the glycine allosteric site of the NMDA receptor and the agonist binding site of the quisqualate/kainate receptor have some structural similarity. Halogenated derivatives of quinoxalines and kynurenines should be useful in evaluating the function of the glycine modulatory site in synaptic transmission mediated by NMDA receptors. In this regard we found that 7-Cl-kyn (5 and 15 μM) selectively attenuated NMDA receptor mediated epileptiform bursts in the CA1 region of hippocampal slices perfused with zero-Mg medium, without reducing the amplitude of the primary population spike. This block could be overcome by 300 μM D-serine. These results together indicate that the glycine site is functional on pyramidal neurons of the adult rat hippocampus.

133.2

GLYCINE REGULATES DESENSITIZATION OF ADULT RAT BRAIN NMDA RECEPTORS EXPRESSED IN XENOPUS OOCYTES. J. Lerma*, C. Samathanam*, S.G. Fan*, R.S. Zukin and M.V.L. Bennett, Dept. of Neuroscience, Albert Einstein Coll. of Med., Bronx, NY. (SPON: E.B. Masurovsky)

The N-methyl-D-aspartate (NMDA)-type glutamate receptor is a ligand-gated cation channel with several distinct regulatory sites. These include a positive allosteric site for glycine, an inhibitory site for Zn^{2+}, and a site or sites within the channel for phencyclidine (PCP) and Mg^{2+}. In Xenopus oocytes injected with adult rat brain mRNA, glycine potentiated NMDA-evoked inward current with no significant effect on NMDA affinity or Hill coefficient. The relationship between NMDA response and glycine concentration indicated a single component response with apparent affinities (K_D's) for glycine of 500 and 634 nM for the peak and steady state responses, respectively and Hill coefficients $n \approx 1$. In the absence of added glycine, a small transient was often observed at the onset of NMDA application. This response could be reduced by pre-washing with fresh Ringer solution and probably was due to glycine contamination. This response could be suppressed completely by pre-washing with the glycine antagonist 7-chloro-kynurenic acid. As glycine concentration was increased (30 nM-1μM), the NMDA response became bigger and desensitized less. The time course of desensitization at 100μM NMDA was fit by a single exponential with a time constant of 480 msec (n=15); this value was not affected significantly by altering glycine concentration. At higher NMDA concentration (300- 500μM), a second time constant of 1-2 sec became apparent. This component was enhanced by increasing extracellular Ca^{2+} and may have been due to Ca^{2+} influx. These results indicate that glycine has two actions on the NMDA channel; it enables channel opening and decreases one component of desensitization.

133.3

DESENSITIZATION OF NMDA RESPONSES IN OUTSIDE-OUT PATCHES PERSISTS IN LOW CALCIUM HIGH GLYCINE SOLUTIONS. W. Sather*, J.W. Johnson*, G. Henderson* and P. Ascher, Laboratoire de Neurobiologie, Ecole Normale Supérieure, 46, rue d'Ulm, 75230 Paris Cedex 05, France.

We have analyzed the influence of glycine and calcium on the desensitization of NMDA induced currents recorded in cultured mouse cortical neurons.

In outside-out patches, the response to NMDA (100 µM) always desensitized rapidly and profoundly. The decay of the response was well described by an exponential with a time constant of 100-400 ms. The amplitude of the steady-state response was always less than 20% of the peak response. Increasing the glycine concentration from 0 to 10 µM increased the absolute values of both the peak current and the steady-state current, but did neither alter the desensitization speed nor its amplitude. Similarly, reducing the extracellular Ca concentration from 1 to 0.01 mM did not alter markedly the desensitization.

The desensitization observed in the whole-cell recording mode differed from that observed in outside-out patches by its greater complexity (it often showed two components), by its smaller amplitude, by its variability from cell to cell and by its sensitivity to glycine and Ca. Increasing the glycine concentration and/or reducing the external Ca concentration often reduced the amount of desensitization, so that in certain cells desensitization was nearly absent in low Ca (0.1 mM) high glycine (10 µM) solutions. In other cells, however, desensitization was still observed in such conditions.

Our observations in the whole-cell mode do not contradict those recently reported by Mayer, Vyklicky and Clements (Nature, 1989, 338, 425) and by Clark, Clifford and Zorumski (Soc. Neurosci. Abstr., 1988, 14, 790). However the observations made in outside-out patches rule out the interpretation according to which the potentiation of NMDA by glycine results from a reduction of desensitization, since potentiation and desensitization are both present in outside-out patches.

133.4

USE- AND VOLTAGE-DEPENDENCE OF PCP BLOCK OF NMDA RECEPTORS EXPRESSED IN XENOPUS OOCYTES. M.V.L. Bennett, J. Lerma*, L. Kushner*, and R.S. Zukin, Dept. of Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461.

NMDA activated channels have several modulatory sites. In addition to the site of agonist action there is the glycine site which must also be occuped for the channel to open, a Zn^{2+} site which is inhibitory and a site or sites within the channel where PCP and Mg^{2+} block permeation. In oocytes injected with rat brain mRNA block of NMDA response by PCP is use dependent and very slow in the absence of NMDA. NMDA I_{max} is reduced without change in affinity or Hill coefficient. Rate of onset of block is increased at higher NMDA concentrations because of higher channel open probability, but the degree of block depends on PCP and not NMDA concentration. Also, recovery is very slow in the absence of NMDA and the rate increases with NMDA concentration. These data indicate that PCP enters and leaves the channels primarily when they are open, and that PCP can be trapped in the closed channel. Mg^{2+} decreases PCP's apparent affinity for the channel site; moreover Mg^{2+} slightly speeds recovery from PCP in the presence of NMDA, presumably by preventing PCP's rebinding. In contrast, Zn^{2+} slows both onset of and recovery from PCP block. In the absence of agonist, PCP produces a slowly developing block. PCP open channel block is greatly reduced by depolarization. The slow rate of recovery in the absence of NMDA is much less dependent on voltage than is the degree of open channel block. Although spontaneous openings of the channel cannot be ruled out, these findings suggest that PCP can enter and exit the NMDA channel via a lipophilic pathway. [Supported by NIH Grants NS20752 and NS07512 and Fogarty Fellowship TW 04040].

133.5

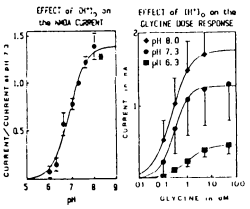
ZINC MODULATES NMDA CHANNELS AT VOLTAGE SENSITIVE AND VOLTAGE INSENSITIVE SITES. C.W. Christine and D.W. Choi. Dept. of Neurology, Stanford Univ. Med. Sch., Stanford CA 94305.

Recent data show that Zn^{++} can selectively block central neuronal excitation mediated by NMDA receptors. In outside-out patches removed from cultured murine cortical neurons, 3 μM glutamate in the presence of 5 μM glycine activated 50 pS channels that were blocked in a voltage-dependent manner by Mg^{++}. Zn^{++} appeared to have two effects on these NMDA channels. First, 1-10 μM Zn^{++} produced a concentration-dependent reduction in channel open probability, insensitive to membrane voltage between -60 and 40 mV; ED_{50} was about 3 μM. This reduction was mostly due to a decrease in opening frequency, and only weakly mimicked by Mg^{++}. Second, at higher concentrations (10-100 μM) and negative membrane voltages, Zn^{++} produced an apparent reduction in single channel amplitude associated with an increase in channel noise, suggestive of a channel block faster than that of Mg^{++} and hence only partially resolved with 2 kHz filtering. The amplitude reduction was voltage-dependent with a δ of 0.51 and K_d (0 mV) of 0.9 mM; amplitude distribution analysis suggested that this voltage-dependence may be primarily mediated by the "on" blocking rate. Zn^{++} may act at two sites: one outside the channel and affecting f_o, the other inside the channel and interfering with the passage of cations.

133.6

MECHANISM OF NMDA CHANNEL MODULATION BY H^+ AT NEAR PHYSIOLOGICAL pH. C.M. Tang*, M. Dichter, and M. Morad*. (SPON: E. Kicliter). Depts. of Physiology and Neurology. Univ. of Pa. and Graduate Hospital, Phila., PA 19104.

The NMDA-activated current is strongly modulated by $[H^+]_o$ at near physiological pH (Tang et al., S.N. Abst. p. 791, 1988). The lack of voltage sensitivity and the lack of sensitivity to changes in $[H^+]_i$ suggest that the protonation site is external to the voltage-sensing portion of the channel pore. Protonation at near physiological pH's primarily effects the probability of channel opening and not its unitary conductances. The major component of NMDA current modulation by H^+ cannot be explained by simple competition for glycine binding, since the glycine dose responses show prominent non-competitive behavior. However, a part of this modulation of NMDA channel gating by H^+ is due to shifts in the K_d of the glycine potentiation. (NMDA was constant at 100 uM.) K_d's are 900 nM at 6.3, 290 nM at pH 7.3, and 200 nM at 8.0. The sensitivity of the NMDA current to H^+ may play a protective role in hypoxic-ischemic conditions, and may, in part, contribute to the sensitivity of seizure threshold to pH.



133.7

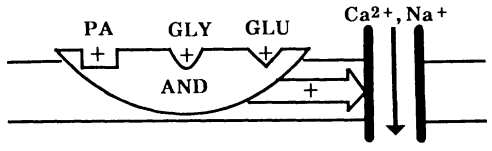
POLYAMINE ACTIVATION OF NMDA EFFECTOR REQUIRES OCCUPATION BY AGONISTS OF BOTH GLYCINE AND NMDA SITES. **H. Canton*, F. Colpaert and J. Lehmann, FONDAX-Groupe de Recherche SERVIER, 92800 Puteaux France**

Agonists activate the NMDA-receptor effector complex, by acting at the glutamate (glu), glycine (gly), or polyamine (PA) site, stimulating the binding of [3H]MK-801 to the open state of the NMDA-linked cation channel.

In well-washed membranes, the NMDA site antagonist 2-amino-5-phosphonopentanoate (AP5; 100 uM) or the gly site antagonist 7-chlorokynurenate (7ClKyn; 100 uM) inhibited [3H]MK-801 binding, presumably due to residual neurotransmitter. Glu, gly, or spermidine (spmdn; each at 10 uM) alone stimulated [3H]MK-801 binding.

NMDA receptor blockade by AP5 prevented gly and/or spmdn from stimulating [3H]MK-801 binding. Likewise, gly receptor blockade by 7ClKyn prevented glu and/or spmdn from stimulating [3H]MK-801 binding.

Therefore the action of spmdn requires both glu and gly site activation. It remains to be determined if all three sites must be occupied for effector activation.



133.8

IFENPRODIL AND SL 82.0715 ARE ANTAGONISTS AT THE POLYAMINE MODULATORY SITE OF THE NMDA RECEPTOR COMPLEX. C. Carter*, J. P. Rivy*, F. Thuret*, K. Lloyd and B. Scatton. Synthélabo Recherche (LERS), Biology Dept., 31 Ave P.V. Couturier, 92220 Bagneux, France.

In an attempt to characterise the mechanism of action of the non-competitive NMDA antagonists ifenprodil and SL 82.0715, we have examined their interactions with the polyamine modulatory site of the NMDA receptor (Ransom and Stec, J. Neurochem., 51:830, 1988). Spermine and spermidine (0.1-100 μM) increased the binding of the competitive NMDA receptor antagonist ^{3}H-CPP and of the NMDA channel blocker ^{3}H-TCP to rat brain membranes. These increases in binding were blocked by ifenprodil and SL 82.0715 (0.1-10 μM). The polyamine-induced increase in ^{3}H-CPP binding was insensitive to MK-801 or 7-chlorokynurenate. Spermine or spermidine did not increase ^{3}H-glycine binding (NMDA modulatory site) nor did ifenprodil or SL 82.0715 (100 μM) affect ^{3}H-glycine binding. Spermine or spermidine (10-1000 μM) had no effect per se on cGMP production in immature rat cerebellar slices, but both increased the maximal effects of NMDA (80 μM) on this parameter (by 400 and 160% respectively). Spermine (100 and 1000 μM) reversed the inhibitory effects of ifenprodil (but not those of MK-801 or kynurenate) on the NMDA-induced increase in cGMP levels. The data show that spermine and spermidine increase the effects of NMDA receptor stimulation via a distinct modulatory site and suggest that ifenprodil and SL 82.0715 are antagonists at this site.

133.9

GLUTAMATE-MEDIATED GENE EXPRESSION IN PRIMARY CULTURES OF CEREBELLAR NEURONS. A.M.Szekely*, D.Grayson* and E.Costa (SPON: M.Santi). FIDIA-Georgetown Inst. for Neurosciences, Georgetown University, Washington D.C.,20007

The activation of glutamate receptors sensitive to N-methyl-D-aspartate (NMDA) is critical in the development of transsynaptically induced neuronal plasticity. We reported that in neurons the stimulation of this glutamate receptor subclass induces the expression of c-fos proto-oncogene mRNA . C-fos protein together with jun protein participate in a DNA-binding protein complex (AP-1) and may control the expression of target genes involved in neuron-specific responses, effectively functioning as a nuclear third messenger. In primary cultures of cerebellar granule cells we show that NMDA-receptor activation increases the mRNA content of both c-jun and jun-B genes, and these changes are reflected in an increased formation of AP-1 complex as shown by gel-retardation assay. To identify potential target genes we constructed a cDNA library from mRNA isolated from glutamate-stimulated cerebellar granule cells. By differential screening a number of clones have been isolated and characterized in respect to their cross-reactivity, hybridization to known clones, Northern blot and sequence analysis. This study may provide insights how a neurotransmitter receptor activation may alter the transcriptional program of the neuron.

133.10

SIGMA RECEPTOR LIGANDS MODULATE THE EXCITATORY EFFECT OF NMDA: AN ELECTROPHYSIOLOGICAL STUDY IN THE RAT DORSAL HIPPOCAMPUS. F.P. Monnet*, G. Debonnel and C. de Montigny. McGill University, Montréal, Québec, Canada

The functional role of sigma (σ) receptors is unknown. We have shown that haloperidol, a high affinity ligand for σ receptors, potentiates the antagonism by MK-801 of NMDA-induced activation of hippocampal pyramidal neurons. In the present experiments, we studied the effects of the σ ligands haloperidol and 1,3-di(2-tolyl)guanidine (DTG) on the neuronal responsiveness to excitatory amino acids.

Male Sprague-Dawley rats were anesthetized with urethane (1.25 g/kg, i.p.). Five-barelled glass micropipettes were used for extracellular recording of CA_3 dorsal hippocampus pyramidal neurons. One side barrel was used for automatic current balancing and the others were filled with three of the following solutions: NMDA (10 mM in 200 mM NaCl, pH: 8), quisqualate (QUIS; 1.5 mM in 400 mM NaCl, pH: 8), kainate (KA; 1 mM in 400 mM NaCl, pH: 8), DTG (0.2 mM in 200 mM NaCl, pH: 4) and haloperidol (0.2 mM in 200 mM NaCl, pH: 4).

DTG (0.25 - 3 μg/kg, i.v.) increased in a dose-dependent manner, the excitatory effect of NMDA but not those of QUIS and KA; at the dose of 1 μg/kg, DTG increased by seven fold the excitatory effect of NMDA. Haloperidol (10 μg/kg, i.v.) had no effect on NMDA-induced activation but completely suppressed the effect of DTG. In contrast, spiperone (10 μg/kg, i.v.), a butyrophenone with low affinity for σ receptors, did not block the effect of DTG. Microiontophoretic application of DTG also potentiated the excitatory effect of NMDA; this effect of DTG was abolished by the concurrent application of haloperidol.

The marked and selective potentiation of NMDA-induced activation by DTG, and the blockade of this effect by haloperidol, but not by spiperone, suggest that DTG is an agonist and haloperidol an antagonist of σ receptors. The present data suggest that a function of σ receptors might be to modulate the sensitivity of NMDA receptors.

133.11

DEVELOPMENTAL CHANGE IN THE MAGNESIUM SENSITIVITY OF THE NMDA RESPONSE IN HIPPOCAMPAL CA3 PYRAMIDAL CELLS. R.J. Brady and J.W. Swann, Wadsworth Ctr. for Labs. & Res., NYS Dept. of Health, Albany, NY 12201.

In the presence of 2mM magnesium the NMDA-induced depolarization of a CA3 pyramidal cell in a TTX-treated, mature hippocampal slice is strongly voltage-dependent. The size of the response decreases as the neuron is hyperpolarized relative to its resting membrane potential. Intracellular recordings were obtained of the responses to iontophoretic application of the agonist into the proximal portion of the basilar dendrites. As expected, changing to a perfusate with no added magnesium potentiated the NMDA response and dramatically decreased its voltage-dependence. These results stand in contrast to those obtained from CA3 pyramidal cells in slices taken from rats 10-15 days of age. Under control conditions the NMDA response of these immature neurons was also strongly voltage-dependent. However, a change to nominally magnesium free perfusate, although potentiating the response, did not remove its voltage dependence. This further supports the conclusion of our previous work that there is a developmental change in the divalent cation sensitivity of the NMDA receptor-channel complex. In immature CA3 neurons an isomeric form is expressed that is more sensitive to calcium than to magnesium. Supported by grants NS-23071 to RJB and NS-18309 to JWS from DCDND-NIH.

133.12

NMDA-ANTAGONISTIC PROPERTIES OF THE ENANTIOMERS OF 3-(2-CARBOXYPIPERAZIN-4-YL)-PROPYL-1-PHOSPHONIC ACID (CPP) AND OF ITS UNSATURATED ANALOGUE 3-(2-CARBOXYPIPERAZIN-4-YL)-1-PROPENYL-1-PHOSPHONIC ACID (CPP-ENE). P.L.Herrling+, B.Aebischer*+, P.Frey*+, H.J.Olverman*# and J.C. Watkins*##. +Sandoz Res. Inst., CH-3001 Berne, Switzerland;# Dept. Pharm. Univ. Edinburgh, U.K.;## Dept. Pharm. Med. Sch. Bristol BS8 1TD U.K.

A [3H]-CPP binding assay in rat cortical membrane preparations yielded Ki values in nM of: 138 for D-CPP, 2332 for L-CPP, 44 for D-CPP-ene and 597 for L-CPP-ene. There is no comparable binding to other neurotransmitter binding sites:NE α1+2, 5HT 1+2, DA 1+2, opiate, benzodiazepine, ACh, QUIS or KA. In frog hemisected spinal cord inhibition of NMDA induced depolarizations yielded following pA2 values: 6.6 for D-CPP, 5.4 for L-CPP, 6.8 for D-CPP-ene and 5.7 for L-CPP-ene. Testing several antagonist concentrations suggested competitive antagonism. D-CPP-ene was active after p.o. application in the rat electroshock test: around 80% protection at 10mg/kg (6h value). These results indicate that the enantiomerically pure D-CPP-ene is a potent and systemically active competitive NMDA antagonist.

DIFFERENTIATION, MORPHOGENESIS AND DEVELOPMENT: CELLULAR AND MOLECULAR STUDIES I

134.1

EXPRESSION OF THE MATURE ARRAY OF NEUROFILAMENT PROTEIN ISOFORMS BY A CLONAL CELL LINE DERIVED FROM THE CNS. H.J. Lee, G.J. Elliot, D.N. Hammond, Y.M.-Y. Lee§, and B.H. Wainer. Committee on Neurobiology, The University of Chicago, Chicago, IL 60637 and §Department of Pathology, University of Pennsylvania, Philadelphia, PA.19104.

The intermediate filaments of mammalian neurons are composed of neurofilament triplet proteins. Each protein is expressed in a developmentally regulated manner, differentially phosphorylated, and preferentially distributed within a neuron. However, the functional significance of these properties is poorly understood. SN 48.B12.2.C6 is the first reported cell line that expresses the mature array of neurofilament (NF) proteins in a normal fashion. The cell line was derived by somatic cell fusion of postnatal day 21 murine septal cells to N18TG2 neuroblastoma cells. Using immunofluorescence, the low molecular weight NF protein (NF-L) and differentially phosphorylated isoforms of the middle (NF-L) and high molecular weight (NF-H) NF proteins were detected. Growth cones contained the highest concentration of each NF protein. Within the perikarya and processes, NF-L was observed mainly in the cell body; NF-M was equally distributed to the cell body and processes; and NF-H was observed slightly more in the processes than in the cell body. Weak staining for vimentin was also noted. N18TG2 cells exhibited no immunoreactivity for neurofilament proteins. Neither SN 48.B12.2.C6 or the N18TG2 neuroblastoma expressed glial fibrillary acidic protein or cytokeratins 8, 18, and 19. Intermediate filament protein expression by both lines was confirmed by Western blot analysis.

SN 48.B12.2.C6 is unique in that it i) expresses the highly phosphorylated isoform of NF-H, ii) expresses all three NF proteins, iii) does not express other intermediate filament proteins, and iv) does not contain perinuclear aggregations of NF proteins. SN 48.B12.2.C6 cells provide an *in vitro* model which may be usedful in the examination of neurofilament phosphorylation events and the elucidation of the role of differentially phosphorylated neurofilaments in neural function. Supported by NIH grants T32HD070009, NS 01244, and NS 25787; grants from the Alzheimer Disease and Related Disorders Association; and the Illinois Department of Public Health.

134.2

EXPRESSION OF CA^{2+} CHANNELS IN CULTURED NEURONAL PRECURSOR CELLS FROM THE RAT TECTUM PARALLELS THE EXPRESSION OF N-CAM AND TETANUS TOXIN RECEPTORS. R.Grantyn, L.Hofer* and M.Perouansky*. Max Planck Inst. Martinsried. F.R.G.

Neuronal precursor cells were dissociated from the tectal plate of rat embryos at day E12, seeded on collagen-coated glass coverslips and maintained in MEM with 10% horseserum and 10^{-5}M AraC. The latter suppressed further mitosis. Only 5% of the freshly plated cell population stained with antibodies to N-CAM or Tetanus toxin. These cells generated voltage activated Na^+ currents ($I_{Na(v)}$) as well as high voltage activated Ca^{2+} currents ($I_{Ca(HV)}$) and bound [^{125}I]Ω-conotoxin (Ω-CT), a blocker of $I_{Ca(HV)}$. However, within the following 10 hours all cells acquired not only $I_{Na(v)}$ and $I_{Ca(HV)}$ but also N-CAM- and Tetanus toxin immuno-reactivity. Initially, only the protruding parts of growing cells were labeled with Ω-CT. Later on the label was distributed over the whole cell surface, including the neurite. After 10 hours in vitro all cells were Ω-CT-positive. Three different markers indicate thus the rapid, probably substrate-stimulated acquisition of neuronal properties by undifferentiated cells from the mammalian brain.

134.3

The expression of calbindin D-28k or substance P by sensory neurons in dorsal root ganglion cell cultures is regulated by soluble factors present in peripheral or central target tissues. I. Barakat* and B. Droz. Institute of Histology and Embryology, Univ. of Lausanne, CH-1005 Lausanne, Switzerland.

Primary sensory neurons display various neuronal phenotypes which may be influenced by factors present in central or peripheral targets. Dorsal root ganglion (DRG) cells were cultured from chick embryos at E6 or E10 (before or after functional connections with targets). Calbindin D-28k (CaBP) or substance P (SP) were detected in culture either by immunocytochemical reaction or by in situ hybridization. At E6, DRG cells were all devoid of CaBP immunoreaction at any time of culture; in contrast at E10, 22% or 11% of neurons were CaBP immunoreactive in neuron-enriched or mixed DRG cell cultures. The addition of muscle or skin extracts to cultures at E6 induced the expression of CaBP by 7.5% or 2.5% of the neurons respectively. In muscle extract, the factor, which induced CaBP in responsive cells according to a dose-dependent manner, was a protein with a M_r greater than 30kD.

SP was expressed by 98% of the neurons grown at E6 but only by 60% at E10. The addition of muscle, skin or brain extract at E6 reduced the percentage of SP+ neurons.

In conclusion, the expression of CaBP and repression of SP by DRG cells in culture is controlled by factors present in peripheral or central target tissues.(SNF N°3.097-86)

134.4

HEPARIN-BINDING FACTORS IN CNS NEURONAL CELL LINE CONDITIONED MEDIUM STIMULATE PROLIFERATION OF O-2A GLIAL PROGENITORS. W. Thangnipon*, G. H. Frost* and J. E. Bottenstein. Marine Biomedical Institute and Dept. of Human Biological Chemistry and Genetics, Univ. of Texas Medical Branch, Galveston, Texas 77550.

Our previous studies have identified mitogenic activity in conditioned medium (CM) derived from the B104 CNS neuronal cell line on A2B5$^+$ O-2A glial progenitors in dissociated neonatal rat brain cultures (Bottenstein et al., J. Neurosci. Res. 20:291-303, 1988). The CM is generated by growing B104 cells on microcarrier beads in serum-free N4 medium. Its growth-promoting activity is evaluated by a serum-free bioassay using either A2B5 immunostaining of neonatal rat brain cells or a colorimetric proliferation assay on C62B rat glioma cells. Both O-2A progenitors and C62B cells exhibit a dose-dependent increase in cell number when treated with B104 CM. Maximal responses are obtained at 8-16 μg protein/ml for O-2A progenitors and 2-4 μg protein/ml for C62B cells. Concentrated and dialyzed CM was applied to a heparin-Sepharose column, and all activity appeared in the bound fractions. Gel filtration chromatography on a Sephacryl S-200 column generated the most active fractions with apparent molecular weights of 38-44K and 98-110K. Various criteria suggest that this trypsin- and heat-labile activity is not identical to that of PDGF, FGF, IGF 1, interleukin 2, GMF, EGF, GPF, GGF, or TGFα. Supported by NIH grant NS20375.

134.5

INSULIN GROWTH FACTORS REGULATE MITOSIS AND SURVIVAL IN CULTURED CEREBELLAR GRANULE CELLS AND PRECURSORS E. DiCicco-Bloom, R.C. Cohen *&I.B. Black Div. Developmental Neurology, Cornell Univ. Med. Coll., New York, NY 10021

Although neurogenesis has been characterized descriptively, regulatory mechanisms remain undefined. Based on our previous work, we have developed a new cell culture system to study central neuronal precursor or neuroblast mitosis.

A highly enriched population of dissociated granule cells and precursors was obtained from 7 day rat cerebella (Hatten,M.E.,J.Cell Biol., 100:384, 1985) and was cultured in fully-defined medium. Immunocytochemical analysis indicated that <2% of cells were stained by markers for glia, fibroblasts and Purkinje cells. Time-lapse phase photomicrography revealed ongoing division and neuritic outgrowth. Further, >50% of cells exhibited nuclear incorporation of [3H]thymidine, a marker for DNA synthesis.

To define effects of insulin on central precursors, cells were cultured in various hormone concentrations and assayed for survival and incorporation of [3H]thymidine (Inc.). Increasing concentrations of insulin produced a marked 10-20 fold rise in Inc. at 48hrs, with peak effect at 5-10ug/ml, indicating that the hormone stimulated neuroblast DNA synthesis. In contrast, maximal survival was achieved at low hormone concentrations (3-10ng/ml), suggesting that insulin mitogenic stimulation was not due to increased cell survival.

Insulin is but one of a family of factors, including insulin-like growth factor-I (IGF-I), that bind to homologous and heterologous receptors. To define IGF-I effects, cells were cultured in control medium or in the presence of insulin (10ng/ml), IGF-I (25ng/ml) or both factors. IGF-I treatment reproduced the marked 10-20 fold stimulation of Inc., while eliciting no further increase in survival over that produced by insulin. These observations suggest that during granule cell development, insulin plays a trophic role whereas IGF-I serves as a neuroblast mitogen. (Support:NIH grants HD23315, NS10259)

134.6

REGIONAL SPECIFICITY OF NEURONAL REGULATION OF ASTROGLIAL DIFFERENTIATION M.E. Hatten and U.E. Gasser. Dept. Pathology, College of Physicians and Surgeons of Columbia University, New York NY 10032

The control of astroglial cell growth is critical to brain development, tumorigenesis and repair of brain injury. Recently we have shown that neuron-glia cell contacts arrest astroglial DNA synthesis (Hatten, ME, J. Cell Biol. 104: 1353, 1987). In the present experiments, we analyzed whether neurons isolated from one brain region would regulate the proliferation of glia from a heterotypic region. Early postnatal cerebellar granule neurons rapidly arrested the growth of late embryonic or early postnatal hippocampal glial cells and induced astroglial differentiation into forms resembling those seen *in vivo*. Quantitation of glial process number and length in homotypic and heterotypic neuron-glial co-cultures revealed that glial form is intrinsic to the region of origin of the glial cell and is not specified by the region of origin of the neuron. The mechanism of granule cell regulation of hippocampal glial growth appeared to be membrane-mediated, since membrane material purified from cerebellar granule neurons arrested hippocampal glial growth in a dose-dependent manner. These results are consistent with previous results showing that granule cell membranes inhibit the growth of astrocytoma cells derived from different brain regions (Hatten, M.E. and M.L. Shelanski, J. Neurosci. 8:1447, 1988) and suggest that neuron-glia cell contacts provide a general mechanism for regulating glial proliferation in the brain.

134.7

REGION- AND GENDER-SPECIFIC MATURATION OF RAT ASTROGLIA IN VITRO. I. REISERT*, Co. BEYER* and Ch. PILGRIM. Abteilung Anatomie und Zellbiologie, Universität Ulm, D-7900 Ulm, FRG.

Previous studies using dissociated cell cultures of fetal rat brain have revealed considerable regional diversity as well as sex differences in developmental schedules of dopaminergic neurons. Because these phenomena might be related to glial heterogeneity, cultures of male and female diencephalon, mesencephalon, and rhombencephalon of gestational day 14 rats were investigated with respect to the development of astrocytic markers. Cultures were incubated for 3-8 days in serum-supplemented or serum-free medium. Vimentin and GFAP were quantified by counting of immunolabeled cells and immunoblotting. Vimentin content was highest in mesencephalon and lowest in rhombencephalon and was more or less stable over the period investigated. GFAP content was initially low and increased with time. It reached high levels in rhombencephalon but remained low in mesencephalon. Sex differences were observed in diencephalic cultures only. Transiently, female cultures contained more GFAP-immunoreactive cells than male cultures.

The results thus demonstrate considerable regional heterogeneity of astrocytic maturation. However, neither the regional nor the sex differences show a clear-cut correlation with previous data on regional development of monoaminergic neurons. It may be concluded that the dependence of neurons on glial environment for realization of an inherent developmental program may vary among neuronal cell types.

134.8

ASTROGLIAL TRANSFORMATIONS IN THE DEVELOPING MURINE CEREBRAL WALL. V.S. Caviness Jr., J-P. Misson*,T.Takahashi*, J. Crandall, Dept. Neurology, Mass Gen Hosp,Boston MA 02114,and Shriver Center,Waltham, MA 02254

Bipolar radial glia are present in the developing murine cerebral wall during the period of active neuronal migration. They disappear during the first postnatal week after migrations are terminated. Until P0 the classical bipolar form is the dominant RGC in the cerebral wall. However, during the final week in utero and during the first postnatal week the bipolar is succeeded by monopolar and multipolar astroglial forms. We describe here four stages of cellular change involved in the glial population change. The analysis is based upon immunohistochemical staining with RC2(Misson et Al., Dev. Br. Res ,1988,44,95). The bipolar cells, dominant before P0, are grouped in fascicles. Through E17, Stage 1, the nuclei of these cells are confined to the VZ and SVZ. Between E17 and P0, Stage 2, the nucleus appears to be translocated to the upper IZ in many of the classical bipolar cells. At the same time monopolar cells with somata in the upper IZ and SP become prominent. These glial cells have multiple processes ascending radially from the soma and have no descending process. In the interval P0-P3, Stage 3, there is an explosive emergence of multipolar astrocytes which stain strongly with RC2 and which progressively predominate over declining populations of bipolar and monopolar RG. Beyond P4, Stage 4, cells of the astroglial lineage cease to stain with RC2; concurrently these cells begin to stain well with AB to GFAP. The set of observations suggest that nuclear translocation, previously considered to be a mechanism involved in neuronal migration.occurs in the course of radial glia to astrocyte transformation. The antigens recognized by RC2 are developmentally regulated in the course of transformations occurring within the astroglial lineage.

134.9

GAP-43 mRNA IS DEVELOPMENTALLY REGULATED IN CULTURED RAT CORTICAL ASTROCYTES L. Vitković, A. daCunha, and V. J. Aloyo Lab of Immunoregulation, NIAID, NIH, Bethesda, MD 20892; Deptartment of Pharmacology, Medical College of Pennsylvania, Philadelphia, PA 19129

Growth-associated protein 43 (GAP-43 also termed B-50, F1, P57, or pp46) is an intensely investigated membrane-associated phosphoprotein whose expression is characteristically altered during normal CNS development and reinduced during successful nerve regeneration. It is currently believed that GAP-43 is exclusively a neuronal protein and thus, its function(s), still unknown, are explored in that context. However, we have recently demonstrated the presence of this protein in cultured rat cortical astrocytes (Vitkovic, L., et al. Proc. Natl. Acad. Sci. USA, 85: 8296, 1988). We show here that the mRNA to GAP-43 is also present in astrocytes. This suggests that the presence of GAP-43 in astrocytes is due to expression of its gene rather than uptake of a neuronally-synthesized protein. Astrocytes in culture develop with the same schedule as in cortex. We found that the developmental pattern of GAP-43 mRNA expression in astrocytes was similar to that in vivo as shown by others (Basi, G.S. et al. Cell, 49: 785, 1987). This suggests that (1) molecular mechanism(s) controlling the developmental regulation of GAP-43 operates in astrocytes; (2) astrocytes contribute significantly to the overall experession of GAP-43 in cortex.

134.10

NEURAL BC1 RNA: TOWARDS AN UNDERSTANDING OF ITS FUNCTIONAL SIGNIFICANCE. Henri Tiedge*, Robert T. Fremeau, Jr., Peter Weinstock*, James L. Roberts, and Jürgen Brosius. Fishberg Research Center for Neurobiology, Mount Sinai School of Medicine, New York, NY 10029

BC1 RNA is a 152 nucleotide long ribonucleic acid which is prevalent in rat nervous tissue. *In vivo*, this non-messenger type RNA is most likely complexed with proteins in a ribonucleic acid protein particle (RNP). In order to try to understand the functional significance of the RNP in the nervous system, we have used *in situ* hybridisation histochemistry to analyse the spatial and temporal expression pattern of BC1 RNA, both at a cellular and a subcellular level. BC1 RNA is expressed by most, but not all neurons, and high amounts of the RNA are found in neuronal cell bodies. Furthermore, in stratified regions such as the retina, the olfactory bulb, the hippocampus, and cerebral and cerebellar cortices, high levels of BC1 RNA are detectable in layers that contain little or no cell bodies but are rich in dendrites, axons, and synapses. The expression pattern in some of these layers seems to coincide with dendritic fields. We are currently trying to localise BC1 RNA in processes of cultured neurons. A dendritic and/or axonal location would seem to indicate an unorthodox functional role of this RNA.

135.1

IMMUNOCYTOCHEMICAL EVIDENCE FOR DISRUPTED THALAMIC PROCESSING IN ALZHEIMER'S DISEASE. W.G. Tourtellotte, G.W. Van Hoesen, B.T. Hyman, and A.R. Damasio. Depts. of Anatomy and Neurology, Univ.of Iowa College of Medicine, Iowa City, IA 52242.

The thalamic reticular nucleus (Rt) forms a narrow band of neurons interposed among external medullary axons of the lateral and rostral convexities of the thalamus. It receives collateral projections from virtually all thalamocortical and corticothalamic pathways *en route* to their respective targets. In turn, the inhibitory neurons within Rt send projections to the thalamus. The neuronal loss and neurofibrillary tangle (NFT) formation that occur in the basal forebrain in Alzheimer's disease (AD) are thought to contribute to dementia due to loss of cholinergic projections to the amygdala, hippocampal formation, and to the entirety of the cerebral cortex. However, recent evidence demonstrating a powerful basal forebrain projection to Rt suggests that an additional consequence of basal forebrain pathology would be partial deafferentation of Rt. Thus, destruction of basal forebrain afferents to Rt could lead to altered thalamic processing. These predictions are particularly relevant to AD considering experimental evidence that Rt gates thalamic transmission and plays a role in attention mechanisms. Rt was studied in 10 cases of AD and 4 non-demented controls using Alz-50 immunocytochemistry to recognize an AD-related protein, and thioflavin S to demonstrate neurofibrillary tangles (NFTs) and neuritic plaques (NPs). Alz-50 demonstrated a terminal-like pattern in Rt in all 10 AD cases but none of the 4 controls. However, neither NFTs, NPs nor immunoreactive neurons were present in Rt, and Nissl stain revealed a normal appearing cytoarchitecture in both AD and control cases. Projections from the basal forebrain were the likely source of the Alz-50 immunoreactivity in AD since other known afferents of Rt such as layer VI of cortex, principal thalamic nuclei, and the mesencephalic reticular formation lacked immunoreactive neurons. By contrast, the basal forebrain was invested with NFTs and immunoreactive neurons in AD but not in control brains. We interpret our results to suggest disruption of the basal forebrain-Rt pathway in AD and propose that such changes may lead to altered thalamic and cortical processing. (Support: NS 14944, PO 19632, 5T32 GM0733. We thank P. Davies for Alz-50.)

135.2

Neurofilaments and MAPs in Human Olfactory Epithelium in Alzheimer's Disease. B.R. Talamo, R. Rudel, V. Lee, K. Kosik and J.S. Kauer. Tufts U. Medical School, Boston, MA 02111.

Nasal epithelia obtained at autopsy from Alzheimer's (AD) patients contains pathological accumulations of neurites (Talamo et al, Nature **337**:736, 1989). This tissue and that obtained from non-demented patients have been further characterized immunocytochemically. In normal and AD tissue, olfactory neurons were immunoreactive (ir) with antibody to microtubule associated protein MAP1b(5), but not to MAP2 using 4 different antibodies. In AD, MAP1b(5)-ir was also present in diffuse material near the basal lamina. Olfactory neurons were not ir for *tau*, using 3 different monoclonal antibodies. *Tau*-ir was normally seen only in nerves in the vicinity of blood vessels, glands, and in occasional varicose fibers in non-sensory epithelium. However, abnormal neurites in AD tissue showed *tau*-ir with all 3 antibodies and also stained with ALZ50, as previously noted. Most neurofilament (NF) antibodies do not stain olfactory neurons, but the olfactory axons in control tissue were stained with antibodies to dephosphorylated NF medium and heavy subunits. AD epithelium was stained for both dephosphorylated and phosphorylated NF. It appears that normal olfactory neurons have a cytoskeletal profile similar to developing neurons in the CNS. The pathological neurites of AD tissue showed staining for proteins more characteristic of mature neurons and contained epitopes reported to be present in neurofibrillary tangles and dystrophic neurites in AD brain.

135.3

LAMINAR AND REGIONAL DISTRIBUTIONS OF PLAQUES AND TANGLES IN THE VISUAL CORTEX OF ALZHEIMER PATIENTS. M-C de Lacoste, L.W. McCallister, K.S. Pollan*, N.Hirstein*, G.A. Mihailoff, C.L. White III*. *Depts. Cell. Bio. and Pathol., U.T. Southwestern Medical Center, Dallas, TX 75235.*

This study was undertaken to determine if there is a relationship between patterns of intracortical connectivity and the laminar and regional distributions of neuritic plaques (NP) and neurofibrillary tangles (NFT) in striate (ST), parastriate (PARA) and peristriate (PERI) cortical areas. Formalin-fixed blocks of tissue from neuropathologically confirmed Alzheimer (AD) brains obtained at autopsy were coronally sectioned at 50μm using a large-stage freezing microtome. NFT and NP were labelled on mounted sections by the avidin-biotin immunoperoxidase method using a polyclonal antiserum to AD paired helical filament. A basic Nissl and the Gallyas silver stain were used for cyto- and myeloarchitectural differentiations. CARP software (Biographics, Inc.) was utilized for semiautomated counts of NP and NFT by lamina and region and to obtain precise three-dimensional reconstructions of the distribution of AD neuropathology.

Preliminary results (n=2) suggest that although there are some minor regional variations, NP are located predominantly in supragranular layers in the visual areas studied. The overall density of NP is also similar for ST, PARA and PERI cortex. In contrast, there are regional differences in the distribution of NFT: 1) Few NFT are located in ST cortex; and 2) PARA and PERI cortical areas exhibit differences in the relative proportion of supra- and infragranular NFT. Regional and laminar differences in the distribution of NFT point to a possible relationship between the spatial course of AD and patterns of intracortical connectivity. *Supported by NIH-AG-08013-01, HD21711 and the Biological Humanics Foundation.*

135.4

THY-1 DISTRIBUTION AND DENDRITIC GROWTH IN ALZHEIMER'S DISEASE HIPPOCAMPUS. D. Leifer, S. A. Lipton, and N.W. Kowall. (Sponsor: R. H. Ackerman). Department of Neurology and Neurobiology, Harvard Medical School.

The membrane glycoprotein Thy-1 belongs to the immunoglobulin superfamily and is present in high concentrations in the brain. Growing dendrites in rat Purkinje cells are intensely Thy-1 immunoreactive and antibodies against Thy-1 enhance neuritic regeneration in cell culture. We examined the distribution of Thy-1 immunoreactivity in normal elderly and Alzheimer's disease (AD) hippocampus using a well characterized monoclonal antibody and immunoperoxidase methods. In normal elderly hippocampus,Thy-1 was primarily located in irregular patches on the perikarya and dendrites of pyramidal neurons and their axons. Dentate granule cells were lightly stained. A blush of granular immunoreactivity often surrounded small blood vessels in grey and white matter. In AD brain, the pattern of immunoreactivity was often polarized on the base of the apical dendrite of pyramidal neurons and within the cytoplasm of neurofibrillary tangle bearing neurons. Dendritic spines and filopodial outgrowths were occasionally labelled with Thy-1 but dystrophic neuropil neurites were not identified. Occasional reactive neurites were seen in senile plaques and a blush of Thy-1 immunoreactivity often surrounded senile plaques cores. The persistent labelling of neuronal perikarya and blood vessels in the human hippocampus is similar to the transient labelling of developing Purkinje cells and blood vessels in the newborn rat. The enhanced pattern of Thy-1 staining in AD suggests that active dendritic remodelling is a feature of this disease.

135.5

MOLECULAR AND CELLULAR CHARACTERIZATION OF CYTOKINES IN ALZHEIMER'S DISEASE. S.A. Allen, S.D. Styren, and J. Rogers. Institute for Biogerontology Research, Sun City, AZ 85351.

As part of an ongoing study of immune system involvement in the pathogenesis of Alzheimer's disease (AD), we are characterizing cytokines present in brain using Western blot analysis, in situ hybridization, and immunohistochemistry at the light and electron microscopic levels. Cytokines examined include interleukin-1 (IL-1), IL-2, IL-2R, IL-3, IL-6, tumor necrosis factor (TNF), and interferon-gamma. Several of these intercellular mediators of immune function show differential CNS expression in AD compared to nondemented elderly patients. For example, IL-1 (which activates peripheral T and B cells) exhibits sparse immunoreactivity in nondemented elderly cortex. In AD cortex, however, there is clear IL-1 immunoreactivity on Western blots, with many immunopositive glia evident in light and EM sections. Gray matter staining of AD cortex frequently shows dense clusters of IL-1 immunoreactive glial profiles, which in our other studies of immune markers in AD have been found to correspond to senile plaques. TNF is a cytokine that is expressed by activated monocytes and, among numerous cellular actions relevant to immune function, activates or induces T helper cells. In brain, TNF immunoreactivity is sparse in control cortex, but strongly expressed in AD cortex. Astrocytes are the predominant brain cell type immunopositive for TNF, with labelled cells evident in both white and gray matter. The presence and, especially, the differential expression of cytokines in AD brain begins to suggest a functional role of immune mechanisms in the pathogenesis of AD. Supported by NIA AGO 7367-01A1 (JR).

135.6

NEURONAL MRNA LEVELS ARE MAINTAINED IN DOWN'S BRAINS WITH ALZHEIMER PATHOLOGY. K.L.Goodison*, A.W.Clark, I.M.Parhad Pathology Dept., U. of Calgary, Alta, T2N-1N4, Canada.

Down's syndrome (DS) subjects almost invariably develop Alzheimer type (AT) pathology in the 4th decade. We have previously shown marked decrements of specific neuronal mRNAs in the neocortex of individuals with senile dementia of Alzheimer's type (SDAT). More recently we have shown that in SDAT the neuronal mRNA levels correlate inversely with the number of neurofibrillary tanlges (NfT). In this study we asked whether the AT changes seen in adult DS are associated with similar decrements. We used frontal cortex from 6 adult DS (25-57 yrs) and 2 controls (29, 53 yrs); 2 fetal DS and 1 control. There were amyloid plaques (AP) and NfT in neocortex of DS cases > 30 yrs. The least severe had 2 NfT and 31 AP/mm^2, and the most severe had 73 NfT and 68 AP/mm^2. Total RNA was isolated and Northern analysis performed using cDNAs for amyloid precursor protein (APP) and neurofilament light subunit (Nf-L). APP mRNA levels in DS remained 1.5X the control value, and Nf-L levels were the same as in controls. No decrement was detected even in the presence of the most extensive AT degeneration. We conclude that cortical neurons in DS, unlike those in SDAT, maintain their mRNA levels as NfT appear in the cortex. These findings indicate a difference between AT degeneration in DS and in SDAT at the level of gene transcription or RNA processing.

135.7

TAU MODIFICATION AND ALZ 50 REACTIVITY

K.S. Kosik, S.K. Bakalis*, H. Scoble*. Harvard Medical School, Boston, MA 02115 and Massachusetts Institute of Technology, Cambridge, MA 02139

Convergent data has demonstrated that tau is the antigen for Alz 50. The utility of this antibody is its robust reactivity on Alzheimer brain tissue sections where it can detect not only those neurons with neurofibrillary lesions, but also populations of neurons vulnerable to the lesions. This observation has led to the idea that Alz 50 may recognize a modification of tau that occurs early in the process of neurofibrillary degeneration. Human tau cDNAs which expressed a carboxy terminal tau fusion protein were reactive with Alz 50 on immunoblots after SDS-PAGE. However, when the bacterial plaque isolates were lifted onto nitrocellulose filters and reacted with Alz 50, no reaction was observed. The carboxy terminal location of the epitope was confirmed by direct sequencing of an Alz 50 reactive proteolytic fragment from bovine tau. Fast atom bombardment mass spectroscopy of the fragment contained an ion peak consistent with the presence of a phosphate in the sample with the reactive peptide. This in vivo phosphate bears an unknown relationship to the epitope. We conclude that the Alz 50 epitope is revealed by an unfolding of the carboxy terminus of tau and can be induced in vitro by denaturation with SDS, a process that might be analogous to the early transformation of tau into paired helical filaments.

135.8

TAU IMMUNOREACTIVITY IN ALZHEIMER'S DISEASE, NEURAL EXPLANTS UNDER ADVERSE CONDITIONS, AND IN HEAT-SHOCKED RATS. S.Ch. Papasozomenos, Dept. of Path., Univ. of Texas Med. Sch., Houston, TX 77225.

We have previously shown that localization of excessive tau immunoreactivity (TI) with ribosomes might be the primary event and its localization with the Alzheimer's abnormal filaments might be an epiphenomenon (Papasozomenos, S.Ch., Lab. Invest. 60:123 and 375, 1989). To test the hypothesis that excessive TI in Alzheimer's and other neurodegenerative diseases might be caused by stressful stimuli, we have examined by immunohistochemistry and immunoblotting rat fetal brains, rat fetal cerebral and dorsal root ganglia (DRG) explants following treatment with 1 μM monensin for 24 hr or 0.005% SDS for 18 hr or heat shock at 45^{o}C for 15 min. In cerebral explants, monensin, SDS and heat shock treatment produced marked increase in TI. In DRG explants, monensin, which interrupts the vesicular traffic in the Golgi region, produced accentuation of staining in a juxtanuclear region. Increased TI was also present in heat-shocked rats. Immunoblot analysis showed that: a)reduced amounts of the fetal form at tau, a 52 kd polypeptide, were always present in adult tissue, b)peripheral axons contained only one$\sim$130-140 kd tau polypeptides, which was always present in the DRG explants together with a 62 kd polypeptide, and in the CNS only in regions containing peripheral axons, c)in cerebral explants, 4 tau polypeptides of 52,54, 59, and 60 kd were progressively revealed with longer duration in vitro following dephosphorylation, and d) in all regions of CNS, 3 tau polypeptides of 52, 60, and 66 kd and another of 68 kd revealed only after dephosphorylation were present. These findings suggest that tau may play a role in the stressful response and its precise molecular changes can be studied by correlative analysis of in vitro and in situ models.

135.9

A DEVELOPMENTALLY-REGULATED CHYMOTRYPSIN-LIKE SERINE PROTEASE IN RAT BRAIN. R.B. Nelson and R. Siman, Medical Products Dept., The DuPont Co., Wilmington, DE. 19880.

Using substrate-containing polyacrylamide gels, we have identified a M_r 25 kD protease in rat brain which is active at neutral pH. This activity was classified as a serine protease on the basis of its inhibition by DFP and PMSF. The protease, termed SP-25, was more specifically characterized as a chymotrypsin-like enzyme (C-terminal cleavage of aromatic amino acids) using peptide boronic acid synthetic inhibitors. SP-25 was distinct from plasminogen activator as its activity was not modified by plasminogen. SP-25 was first detected in neonatal forebrain, but was below detection level in adult forebrain. It was exclusively associated with particulate vs. soluble fractions. SP-25 was not extractable from particulate fractions by Zwitterionic and nonionic detergents, nor by subsequent low-salt or high-salt treatment of the detergent-insoluble pellets. These characteristics suggest a tight association of the protease with cytoskeletal elements. SP-25 activity was partially inhibited by several divalent cations, including Ca^{++}. Although the functional significance of this brain protease is unclear at present, recent immunohistochemical studies indicate the presence of an inhibitor of chymotrypsin-like enzymes (α-1-antichymotrypsin) in both normal CNS and as a core component of neuritic plaques in Alzheimer's disease. The present evidence is the first to describe a chymotrypsin-like enzyme in brain which might interact with α-1-antichymotrypsin, and raises the possibility that SP-25 or similar proteases still to be found in brain may directly participate in certain CNS pathologies.

135.10

ELECTRON MICROSCOPIC LOCALIZATION OF CHOLINESTERASES IN ALZHEIMER BRAIN TISSUE. K.A. Carson, C. Geula and M-M. Mesulam. Harvard Medical School, Boston, MA; Old Dominion University and Eastern Virginia Medical School, Norfolk, VA.

Light microscopic examination demonstrated that neurofibrillary tangles (NFTs) in Alzheimer's Disease (AD) appeared to have acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activity (Mesulam and Moran, Annals of Neurol., 1987). The studies reported here were undertaken to reveal the subcellular site of this enzymatic activity. Frozen sections from fixed AD brains were used to demonstrate AChE and BChE activity with appropriate specific inhibitors. Following light microscopic examination the tissue was osmicated, embedded in epoxy resin and thin sectioned. In areas that contained AChE- or BChE-positive NFTs by light microscopic examination, the electron microscope showed that the reaction product was specifically associated with dense bundles of tangle-like intraneuronal filaments. Extraneuronal (ghost) tangles were often unstained. In contrast to tangle-bearing neurons, normal AChE-positive neurons contained reaction product in the nuclear envelope and rough endoplasmic reticulum, but not associated with neurofilaments. These observations confirm our earlier reports that NFTs have cholinesterase (AChE and BChE) activity and that this represents a major deviation from the subcellular distribution of cholinesterases in normal neurons.

135.11

CASEIN KINASE II IS ASSOCIATED WITH NEUROFIBRILLARY TANGLES IN ALZHEIMER'S DISEASE. D. Iimoto, E. Masliah*, and T. Saitoh, University of California, San Diego, School of Medicine, Neuroscience Dept., M-024, La Jolla, 92093, U.S.A.

Alzheimer's disease (AD) is characterized by the formation of structures known as neurofibrillary tangles (NFT) and neuritic plaques (NP). The composition of these structures is just now being elucidated. Because there are several aberrantly phosphorylated proteins, tau and neurofilaments, that are associated with NFT and the neuritic component of NP, it is likely that there is a kinase and/or phosphatase responsible for this abnormal post-translational modification. Yet none of the kinases that have been shown to phosphorylate tau have been proven to be responsible for tau phosphorylation in AD.

Casein kinase II (CK-II) was shown to be aberrant in AD in that its amount as measured by its immunoreactivity on a Western blot was 60% lower in AD than in control samples. We have evidence that the localization of CK-II is also altered in AD. Rabbit anti-CK-II serum reacts with NFT and the neurite component of plaques in AD. Furthermore, the anti-CK-II antibodies recognize NFT from a variety of other dementias including Picks' bodies, progressive supranuclear palsy, and Kuf's disease. Using Proteinase K treatment, the antibody staining pattern differed from the antibody staining of the other major components of NFT. These include anti-tau, anti-ubiquitin, and Alz-50. The colocalization of tau and CK-II on NFT suggests that CK-II may be responsible for phosphorylating tau and/or neurofilaments in AD.

135.12

HUMAN ANTIBODIES PREFERENTIALLY REACTIVE WITH BRAIN OR WITH TEMPORAL CORTEX IN ALZHEIMER'S DISEASE. F. Gaskin, M.R. Farlow*+ and S.M. Fu*, Univ. of Virginia Sch. of Med., Charlottesville, VA 22901 and +Indiana Univ. Sch. of Med., Indianapolis, IN 46223.

Monoclonal or oligoclonal Epstein Barr Virus transformed cell lines from patients with Alzheimer's disease (AD), Gerstmann-Straussler-Schreinker Syndrome-AD (GSS-AD), post stroke and age-matched controls were established. In our initial study, antineurofibrillary tangle (NFT) and antineural antibodies (Abs) were present in cell lines from AD and control groups. We have extended our studies. To date, 2489 cell lines have been screened for their reactivity on the temporal cortex of three AD brains and four age-matched control brains, HeLa cells and human fibroblast and neuroblastoma cell lines. We found 13 Abs that react only with AD brain and an additional eight Abs that react only with brain. These cell lines were derived from one patient with a neuropathological confirmation of AD, one patient with GSS-AD, one control aged individual and two patients with a history of stroke. Reactive structures included NFT, plaque neurites, neuritic processes, astrocytes, filaments and nuclei. These Abs constitute an unique resource to explore NFT, plaques and astrocytes in AD and other neurodegenerative diseases. Supported by AG-06348 and the Eleanor Naylor Dana Trust (New York).

136.1

ISOLATION OF A PEANUT AGGLUTININ-BINDING GLYCOPROTEIN FRACTION WHICH INHIBITS AXON GROWTH IN THE CHICK EMBRYO. J.A. Davies*, S.A. Wajed*, G.M.W. Cook*, C.D. Stern* and R.J. Keynes. Department of Anatomy, University of Cambridge, Cambridge CB2 3DY, U.K., and Department of Human Anatomy, South Parks Road, Oxford OX1 3QX, U.K.

The segmented pattern of peripheral spinal nerves in higher vertebrates is generated by interactions between growing nerve cells and somites. Neural crest cells, motor axons and sensory axons grow preferentially through the anterior half of each successive somite-derived sclerotome, and are excluded from posterior (P) half-sclerotome. In chick embryos, P cells bind the lectins peanut agglutinin and jacalin. By affinity chromatography with immobilised lectins we have isolated a glycoprotein fraction from chick somites with inhibitory activity for axon growth. Using a method devised by Dr J.A. Raper (University of Pennsylvania), in which liposomes containing the fraction are applied to cultures of chick sensory neurons, growth cones are found to undergo abrupt collapse, recovering within 8 hours of liposome removal. By SDS-PAGE the active fraction has two major components of apparent M_r 48,000 and 55,000. Rabbit polyclonal antibodies directed against these components recognise P cells only, consistent with a role for this material in excluding axons from P-sclerotome in vivo. Collapsing activity is attenuated following treatment with various endo-glycosidases, suggesting that N-linked glycan(s) may participate in the inhibitory mechanism.

136.2

TIME-LAPSE OBSERVATIONS OF NEURITE GROWTH BY SYMPATHETIC PREGANGLIONIC NEURONS *IN VITRO*. S.J. Moorman* and R.I. Hume (SPON: J. Walker) Dept. of Biology, University of Michigan, Ann Arbor, MI

Time-lapse microscopy studies have shown that neurons from chick embryos can respond in several different ways to the same extracellular matrix molecules, and to molecules on the cell surfaces of other neurons. This suggests that neurons respond in a cell-type specific manner to environmental signals. We previously developed methods, using the fluorescent dye di-I, that allow us to unambiguously identify particular classes of CNS cells in culture. Here, we examine the growth and interactions *in vitro* of neurites from an identified type of CNS cell, sympathetic preganglionic neurons.

Spinal cord cells from stage 30-32 chick embryos were plated onto laminin coated glass cover slips, and the growth of neurites from preganglionic neurons was observed beginning 24 hours after plating. The growth cones of all neurites of each cell were tracked for 2 to 6 hours using a digital image acquisition/analysis program. Our initial analysis included only cells whose growth cones did not encounter other cells or cell processes during the entire observation period. The growth rate of preganglionic neurites averaged 42.7 um/hr at 37° C, but when the growth of all of the neurites of a single cell was examined it became clear that the dynamics of growth are very complex. A variety of behaviors were observed, including; all neurites extending at the same time but at different rates, some neurites extending and some retracting at the same time, and one neurite growing while the others neither grow nor retract. We conclude from these observations that the growth of the different neurites of a single cell is relatively autonomous.

We also determined the effect of cell-cell contact on the rate of neurite extension. We found that when preganglionic neurites encountered the cell bodies and processes of their normal target cells, sympathetic ganglion neurons, they typically crossed them, although the rate of growth often transiently slowed. This behavior is very different from the growth cone collapse that has been reported by others to be the most common interaction *in vitro* between central and peripheral neurons that do not normally contact each other.

136.3

INTERACTION OF GOLDFISH RETINAL AXONS WITH FISH OLIGODENDROCYTES *IN VITRO* M. Beckmann* M.Bastmeyer and C.A.O.Stuermer, Friedrich-Miescher-Lab. der Max-Planck-Ges., Tübingen, FRG.

Mammalian oligodendrocytes impair the growth and regeneration of mammalian neurites *in vivo* and *in vitro*. These cells also affect goldfish retinal axons: Upon contact with mammalian oligodendrocytes, 60% of the fish growth cones collapsed, 40% avoided the cells, but never grew over the cells (Bastmeyer et al., 1988). To test whether the properties of fish oligodendrocytes differ from mammalian oligodendrocytes, we monitored the encounter of goldfish retinal axons with fish oligodendrocytes using time lapse videomicroscopy. Fish glial cells were cultured either from regenerating goldfish optic nerves or from brains of juvenile zebrafish. Oligodendrocytes were identified by the antibody O1.

1) Oligodendrocytes from optic nerves: Cells were bi- or multipolar and proliferated over several weeks. From 10d *in vitro* onwards most cells were $O1^+$, and, unexpectedly, coexpressed GFAP. Goldfish retinal axons grew readily over or even on these cells.

2) Oligodendrocytes from zebrafish brains: $O1^+$ cells were either of stellate or (like mature mammalian oligodendrocytes) of flat morphology and rarely $GFAP^+$. When confronted with fish oligodendrocytes of stellate morphology roughly 80% of the axons grew over the $O1^+$ cells. However, more than 20% of the growth cones retracted and 55% grew around the $O1^+$ cells of flat morphology, suggesting that these cells are not growth permissive.

Whether fish oligodendrocytes with flat morphology possess inhibitory proteins typical for mammalian oligodendrocytes remains to be investigated.

136.4

IN VITRO REGENERATION OF ADULT RAT RETINAL GANGLION CELL AXONS ON ASTROCYTES: GROWTH CHARACTERISTICS AND MOLECULAR MECHANISMS. M. Baehr[1], M. Schachner[2] and R.P. Bunge[3]. [1]Max-Planck Institut fur Entwicklungsbiologie, Tuebingen, FRG; [2]Univ. Heidelberg, FRG; [3]Dept. of Anatomy & Neurobiology, Washington Univ. Sch. Med., St. Louis, MO.

The adult rat optic nerve, which contains astrocytes and oligodendrocytes, is non-permissive for regenerating axons in vivo and in vitro. In order to investigate the influences of characterized populations of astrocytes on injured retinal ganglion cells (RGC) of the adult rat, we have studied RGC survival and axon regrowth in vitro. Retinal explants were obtained from adult rats 5 days after optic nerve crush and cultured with preparations of Type I cortical astrocytes prepared from newborn rats. Astrocyte cultures were used 7-10 days after purification (immature astrocytes expressing a flat morphology) or after subculturing for more than 3 weeks and shifting to defined medium (mature astrocytes expressing a stellate morphology). Neither flat (immature) or stellate (mature) astroglia, enhanced RGC survival in vitro. Both astroglia preparations, however, were permissive for the growth of regenerating RGC axons. The growth rate of axons on flat astrocytes was significantly higher than on stellate astrocytes. Polyclonal antibodies against murine sarcoma laminin, which decorated the surface of flat astrocytes, reduced the growth of RGC axons by more than 60% compared to controls. Growth of adult RGC axons on astrocytes was not significantly affected by antibodies against the cell adhesion molecules L1 or N-CAM, or the surface antigen Thy-1. From these studies of adult mammalian retinal tissue we conclude that the extracellular matrix component laminin, when expressed by astrocytes, is involved in mediating the regeneration of RGC neurites obtained from adult rats. [Support: Max Planck Inst. (MB), BMFT0701771/4 (MS), NS09923 (RPB)]

136.5

ANTIBODIES AGAINST THE CELL ADHESION MOLECULE L1 DO NOT BLOCK GROWTH OF ADULT RAT RETINAL GANGLION CELL AXONS ON SCHWANN CELLS. J.M. Hopkins, M. Schachner[1] and R.P. Bunge, Dept. Anat. & Neurobiol., Washington Univ., St. Louis, MO, USA; [1]Dept. Neurobiol., Univ. Heidelberg, Heidelberg, FRG.

Previous in vitro studies have demonstrated that isolated Schwann cells (SC) as well as Schwann cells with their basal lamina (SC+BL) support regeneration of axons from adult rat retinal ganglion cells (RGC). As part of a search for cell surface molecules responsible for the support of adult RGC axon regeneration, we have cultured explants of adult rat retina (5 days after optic nerve crush) on SC and SC+BL in the presence of antibodies against laminin or L1. Two measures of neurite growth were examined; the number of neurites which emerged from the explant to grow on the substratum (neurite access) and the rate of neurite elongation on the substratum (rate of growth). Antibodies against L1 did not reduce neurite access to either SC or SC+BL. The rate of growth on SC or on SC+BL was not significantly reduced by anti-L1. Antibodies against laminin did not reduce neurite access to SC, but did reduce access of neurites to SC+BL by 90%. Anti-laminin reduced the apparent rate of growth on SC+BL, but not on SC. These results suggest that the isolated SC surface supports growth of adult RGC axons via mechanisms that do not rely primarily on L1 or laminin alone, and that laminin may be involved in growth on SC+BL. The reduction of neurite access to SC+BL substrata by anti-laminin suggests that regenerating adult RGC neurites interact with laminin in the BL before gaining access to the underlying SC surface. Since it has recently been shown that the growth of embryonic rat retinal neurites on SC is dependent on L1, our current results reveal a fundamental difference between neurite growth mechanisms during development and regeneration in the mammalian visual pathway. [NS09923 (RPB), EY06073 (JMH), BMFT 0701771/4 (MS)]

136.6

AXOTOMY INDUCES NEURITES FROM RAT SEPTAL-BASAL FOREBRAIN EXPLANTS TO PREFERENTIALLY ELONGATE ON LAMININ. M. Schinstine & C.J. Cornbrooks, Dept. of Anatomy & Neurobiology., Univ. of Vermont, Burlington, Vt. 05405.

We have previously reported that Schwann cell-derived basal lamina acts as a preferred substratum for the elongation of neurites from embryonic day (E)18, but not E15, CNS neurons (Dev. Brain Res.,(1988) 43:23-37). These observations led to the hypothesis that regenerating (E18) and newly differentiated (E15) axons differentially respond to a laminin substratum. The present study was conducted to determine if rat, embryonic septal-basal forebrain neurons would preferentially elongate on a laminin substratum after axotomy. The length of neurites in E15 primary explant cultures was similar on laminin and collagen. In contrast, neurites from E18 SBF neurons grew significantly longer on laminin as compared to a collagen substratum. E15 explants could be induced to extend longer processes on laminin if they were axotomized after 5 days in primary culture and replated onto a laminin substratum in secondary culture. Under these conditions, neurites from axotomized E15 explants grew 27% longer on laminin as compared to growth on collagen. Control E15 explants, maintained in primary, suspension culture to avoid axotomy, demonstrated similar growth rates on laminin and collagen in secondary culture. These results suggest that embryonic, SBF neurons become more responsive to a laminin substratum subsequent to axotomy. Supported by NS 21811.

136.7

EVIDENCE FOR INHIBITION OF AXON OUTGROWTH BY KERATAN SULFATE/CHONDROITIN SULFATE PROTEOGLYCAN IN VIVO AND IN VITRO. D. M. Snow, V. Lemmon and J. Silver. Center for Neurosciences, Case Western Reserve University, Cleveland, Ohio 44106.

It has been suggested that inhibition of axonal growth cones may be as important a mechanism in the guidance of axons during development as is attraction. In a previous abstract (Snow et al., Soc. Neurosci. Abst., 1987), we presented in vivo data which suggested that keratan sulfate is present in the roof plate of the developing spinal cord at a time period when the dorsal column axons and the ventral commissural axons are elongating near the roof plate but do not cross the dorsal midline. We noted that other structures or regions which contain keratan sulfate are also not innervated, such as cartilage, epidermis and the dorsal midline of hamster tectum.

We report here that in an in vitro bioassay using intersecting stripes of various substrates bound to nitrocellulose-coated tissue culture dishes (Lagenaur and Lemmon, 1987), embryonic chick retinal neurites were inhibited from proceeding along the permissive substrate, laminin, when presented with a crossing stripe of keratan sulfate/chondroitin sulfate proteoglycan (KS/CS-PG). Retinal neurites grew robustly up to the edge of the KS/CS-PG then consistantly stopped abruptly or turned away and grew along the permissive substrate.

These data suggest that KS/CS-PG may function, in part, to inhibit axonal growth cones in vivo during development.

136.8

REMOTE TARGET CONTROL OF INDUCTION AND DIRECTIONAL GROWTH OF AXON COLLATERALS IN THE MAMMALIAN BRAIN. C.D. Heffner, A.G.S. Lumsden & D.D.M. O'Leary (SPON: L. Harris) Dept. of Neurosurgery, Washington Univ Sch of Med, St. Louis, MO 63110

Individual neurons in the brain send axons over considerable distances to multiple targets. An amenable system for studying axon growth and target selection is the mammalian corticopontine projection. This major connection develops from parent corticospinal axons that days before have grown past the pons, by a delayed interstitial budding of collaterals which then grow directly into their target, the basilar pons (O'Leary & Terashima, 88, Neuron 1:901). Thus, in this system, unlike others characterized to date, the target is not recognized by the growth cones of the primary axons, but the budding of collaterals at stereotypic positions suggests that cues do identify the basilar pons as a cortical target. Here we present evidence that the maturing basilar pons produces a diffusible signal that acts on the overlying cortical axons and induces the budding and directed ingrowth of collaterals. Basilar pons, co-cultured with appropriately aged explants of cortex in 3-D collagen matrices (which enable the detection of diffusible activities - see Lumsden & Davies, 86, Nature 323:538), attracts growing cortical axons by growth cone turning from a distance of 200-300 um, and also elicits the formation of collaterals within the cortical explant and their directional growth across the intervening matrix, as revealed by retrograde DiI filling. Control explants (olfactory bulb, mammillary bodies, hypothalamus, neocortex) do not elicit these effects. Thus, this influence appears to be target specific. Our in vitro evidence that a signal derived from the pontine target can operate over a distance and affect the elaboration of a major projection suggests an important role for diffusible molecules in the establishment of connections in mammalian brain. (Supported by NEI grant EY07025, the McKnight Foundation, and a Sloan Foundation Fellowship)

136.9

J1/TENASCIN IS AN INHIBITORY SUBSTRATE FOR CEREBELLAR NEURONS. A. Faissner* (SPON : D. Ganten). Dept. of Neurobiology, University of Heidelberg, Im Neuenheimer Feld 364 6900 Heidelberg, Germany (FRG).

The functional properties of J1/tenascin were investigated in vitro. J1/tenascin is an extracellular matrix (ECM) constituent consisting of two glycoproteins of 200 kD and 220 kD apparent molecular weight. It is transiently expressed by astrocytes during CNS development and mediates neuron-astrocyte interactions. We have prepared monoclonal antibodies against J1/tenascin and used J1/-tenascin immunoaffinity purified from early postnatal mouse brains to analyze its effects on the behaviour of defined neural cell types. Patterned substrates were constructed by absorbing J1/tenascin or other ECM components to poly-ornithine (PORN) treated glass coverslips and removing the glycoproteins from selected areas. Cerebellar granule cell neurons plated onto these substrates avoided J1/tenascin containing regions, whereas they grew well on PORN alone or PORN covered by laminin or fibronectin. The inhibitory activity of J1/tenascin was inactivated by heat or UV irradiation. In contrast to neurons, mouse cerebral astrocytes, the major source of J1/tenascin in the CNS, behaved normally on J1/tenascin carrying PORN surfaces. In view of these results and the pattern of expression of J1/tenascin during cortical barrel field formation (Steindler et al., Dev. Biol. 131, 243-260 (1989)), we propose that J1/tenascin is involved in CNS pattern formation.

136.10

LOCALIZATION OF A DEVELOPMENTALLY REGULATED MOLECULE DEFINED BY OZ42 ON ISOLATED CEREBELLAR GRANULE CELLS FROM EARLY POSTNATAL MICE. L.B. Pickford,[1]* P.E. Sheehy,[2]* D.A. Brown[2]* & R.V. Rouse[1]. Dept. of Pathology, Stanford Univ. School of Medicine, Stanford[1] & Veterans Admin. Hospital, Palo Alto[2], CA. (SPON: L.F. Eng)

Cerebellar granule cells (GC) complete their development postnatally undergoing proliferation, migration, axon and dendrite growth and synaptogenesis. OZ42 is a monoclonal antibody which specifically recognizes the deep region of the transitory external granular layer which contains postmitotic, premigratory GC (Pickford et al., (1989) J. Neurocytol., 18, in press). The temporal and spatial pattern of reactivity to OZ42 suggest an association with early axon extension by GC or with initiation of migration across the cerebellar molecular layer. We have examined the immunoreactivity of isolated GC cultured for different time periods and in cytospin preparations of freshly isolated GC. GC suspensions were prepared by mechanical disruption, incubated on poly-D-lysine coated glass microwell slides or coverslips, then acetone fixed and stained with antibodies. The reactivity of the GC was determined by immunoperoxidase, fluoresence and immunoelectron microscopy (IEM), using both single and double labelling methods to identify reactive cell types. In cytospin preparations small round cells with large nuclei (GC) had extranuclear reactivity with OZ42 in partial or complete rings. The proportion of reactive to unreactive small cells at different postnatal ages from P0-P20 correlated with the relative amount of reactivity in tissue sections at the same ages. Antibodies reactive with other cell types, including Thy1, glial, macrophage and endothelial cells showed distinct patterns of reactivity. Primary cultures developed networks of processes from both glial and neural cells. The presumptive GC neurites were very fine in cross section but were readily detectable with transmission and scanning IEM 24 hours after plating. A time course for this reactivity from 0-72 hours after plating of GC suspensions from mice of ages P0-P20 will be presented. This study indicates that the molecule recognized by OZ42 is expressed during GC axon development.

136.11

APLYSIA PROTEIN AP-100 IS A NEURON-SPECIFIC MEMBRANE PROTEIN ENRICHED IN SYNAPTOSOMES. F. Keller and S. Schacher, Ctr. for Neurobiol. & Behav., CPS, Columbia University and NYS Psych. Inst., New York, NY 10032.

We have generated a panel of monoclonal antibodies (Mabs) directed against membrane proteins in the CNS of juvenile *Aplysia californica*, with the aim of identifying and isolating membrane molecules involved in neuritic outgrowth and synapse formation. Several Mabs recognize antigens which are enriched in the neuropil (tissue sections), and in synaptosomes (sucrose-gradient fractionation). One antibody, Mab 4E8, appears to recognize a 100 kDa integral membrane protein, which is only present in the nervous system, is localized in a subset of neurons, and is enriched in synaptosomes. The protein was immunoprecipitated and is presently being sequenced with the method described by Kennedy et. al. (PNAS 85:7008, 1988). Interestingly, other protein bands are co-precipitated with the 100 kDa protein but do not crossreact with the antibody. Their identity and association with the 100 kDa protein is currently being investigated. Mab 4E8 appears to specifically perturb the pattern of neuritic growth in culture (S. Schacher and F. Keller, Abstract presented at this meeting). Furthermore, we are investigating how these membrane proteins might be associated with the cytoskeleton and with intracellular second messenger systems which are thought to shape developing axons and synapses.

136.12

MONOCLONAL ANTIBODY (MAB) THAT RECOGNIZES APLYSIA PROTEIN AP-100 ALTERS PATTERN OF NEURITE OUTGROWTH BY IDENTIFIED APLYSIA NEURONS IN CULTURE. S. Schacher and F. Keller. Ctr. for Neurobiol. & Behav., Columbia CPS & NYS Psych. Inst., New York, NY 10032.

Mab 4E8 recognizes a neuron-specific membrane protein that is localized to axons and terminal regions in the intact CNS of Aplysia (F. Keller and S. Schacher, Abstract in this volume). A similar pattern is observed for abdominal ganglion cells in culture. All identified cells examined thus far by light microscopic immunofluorescence, with the exception of the right upper quadrant cells (R3-R13), are stained positively with 4E8. Positive reaction is restricted to axons and regenerated neurites and appears to be enriched at growth cones. To explore the role of the antigen recognized by 4E8, we compared the pattern of neurite outgrowth for a number of identified cells maintained continuously in the presence of 4E8 to that obtained for cells maintained either without antibodies or with various control antibodies. Compared to the other treatments, 4E8 appeared to defasciculate extending neurites into a large number of finer diameter processes. The antibody had no significant effect, however, on the time required to initiate neurite outgrowth or on the rate of neurite extension. The consequences of 4E8 application on the structure and motility of growth cones, and on the cell-cell interactions associated with synapse formation are being investigated.

137.1

CHOLINERGIC NEURON ATROPHY AND SEVERE SPATIAL MEMORY IMPAIRMENTS IN AGED RATS ARE PARTIALLY AMELIORATED BY CHRONIC INFUSION OF NGF. (SPON: G. Doucet)
W. Fischer*, K. S. Chen§, F. H. Gage§ and A. Björklund, Dept. Med. Cell. Res., University of Lund, Sweden and §Dept. Neurosci. UCSD, 92093, USA.

In a previous experiment, we showed that continuous intracerebral infusion of NGF improved retention of a spatial memory test in aged (23-25 month) female Sprague Dawley rats and partially ameliorated cholinergic neuron atrophy, as visualized by AChE histochemistry. In the present study we have pursued this NGF-effect further using a combination of ChAT and NGF receceptor immunocytochemistry in behaviorally severely impaired 28-30 month old rats. NGF was administered during 4 weeks into the lateral ventricle using osmotic minipumps (0.25 ug/day). As previously, the NGF treated rats showed improved retention of place navigation in the Morris' watermaze. Using a double labelling procedure, neurons which were stained for ChAT, NGF-receptor or both, could be detected and analyzed. The mean number of measured ChAT positive neurons was about 560 in the aged group and 940 in the young group. Proportionally, about 15% of all the ChAT positive neurons in the NBM in the aged group and about 20% in the young group only stained for ChAT. Cell size: In the vehicle-treated group, size of double labelled neurons was reduced by about 15% compared to the young animals. The single labelled neurons showed a further reduction to about 25% compared to the young group. This was also observed on the contralateral side of the infusion in the NGF-treated animals. On the NGF-infused side, an increase in cell size compared to the contralateral (nonimfused) side of about 25% of double labelled and single labelled neurons was observed. Cell numbers: An approximate 30% loss of double labelled neurons, compared to the young group was observed in both aged groups. A reduction in the number of single labelled ChAT-positive neurons of about 60% was detected in the NGF- as well as the vehicle treated rats. Taken together with previous results, these findings further support the notion that the cholinergic neurons in the NBM are affected by the ageing process and that NGF exerts an important trophic effect on these atrophic neurons.

137.2

RESTITUTION OF STRUCTURE IN HEMISECTIONED RAT SPINAL CORD BY X-IRRADIATION.
N. Kalderon#, J. Palmer,# A. Alfieri*, J.H. Kim* and Z. Fuks*. Rockefeller Univ.# and Radiation Oncology Dept., Memorial Sloan-Kettering Cancer Cnt., NY, NY 10021.

The potential use of x-irradiation in the management of CNS injury is being studied. It was established in our studies that irradiation of severed olfactory bulbs (OBs) resulted in reduction/elimination of reactive astrocytes at the site of incision, structural healing with partial anatomical regeneration, rescue of axotomized primary neuronal (mitral) cells and salvage of the tissue from degeneration (Kalderon et al., Proc. Natl. Acad. Sci., 1989 submitted). These results suggest that irradiation eliminates cells which obstruct and impair the cascade of recovery processes in injured CNS. We report here effects of irradiation on transected spinal cord. Experiments were performed on adult rat spinal cords which were hemisectioned at thoracic level T11-T12. Radiation was delivered at 16-18 days postinjury (total dose 10 Gy), and animals were analysed 60 days after injury. Segments of the lesioned cords (15-20mm) were cryostat-sectioned and analysed by histological and immunocytochemical techniques. Results so far show that as in the OB, x-irradiation is effective in averting some of the deleterious consequences of transection injury to the adult rat spinal cord. Irradiadion of the hemisectioned cords led to partial restitution of structural integrity along the site of incision, preservation of neurons and extensive neurite growth (neurofilament staining) around the cut.
Supported by NIH, NS 23064.

137.3

PREFERENTIAL MOTOR REINNERVATION:PATHWAY REGULATION.
T.M.Brushart.Dept. of Neurology, Johns Hopkins Univ., and the Curtis Hand Center, Baltimore Md. 21218.

When regenerating motor axons are given equal access to sensory and motor branches of the rat femoral nerve, they preferentially reinnervate the motor branch (J. Neurosci. 8:1026-31). Pruning axon collaterals from inappropriate distal pathways may contribute to generation of this specificity (Neurosci. Abst. 14:166). The present experiments evaluate the role of the pathway in this process. Both femoral nerves of 30 3 week old female Sprague Dawley rats were severed proximally and the stumps separated by 1/2mm within silicon tubing to eliminate mechanical axon alignment as a source of specific regeneration. The distal sensory and motor branches of these nerves were severed at the level of quadriceps innervation,ligated, and enclosed within silicon caps to deny regenerating axons end organ contact. Groups of 10 animals were evaluated at 2,3,and 8 weeks by application of HRP to one femoral branch and Fluoro Gold to the other. The mean number of motoneurons projecting axons into the motor and sensory branches was not significantly different at 2 wks (M-106,S-96), but at 3 wks (M-142,S-82) and 8wks (M-175,S-70) a significant difference was found (p=.01). The mean number of double-labeled neurons decreased significantly (p=.01) from 62 at 2wks to 43 at 3 wks and 26 at 8 wks. Regenerating motor axons demonstrated no preference for motor or sensory Schwann cell tubes at 2 wks. Neurotropic guidance of axons to "correct" Schwann cell tubes is thus unlikely. Previous work has shown the pruning of axon collaterals to accompany the generation of specificity. In the present experiments this occurred even when end organ contact was denied. There is thus a specific interaction between regenerating motor axons and old motor pathways which is probably trophic in nature.
Supported by Johns Hopkins University and the Curtis Research Foundation.

137.4

REGENERATION OF THE GOLDFISH MAUTHNER CELL DOES NOT UNDERLIE BEHAVIORAL RECOVERY OF THE STARTLE RESPONSE.
S.I. Zottoli, S.C. Northen*, and T.L. Scalise*. Dept. of Biology, Williams College, Williamstown, MA 01267.

The goldfish central nervous system is capable of functional regeneration after spinal cord injury. Although many behaviors including swimming return by 3 months, a startle response (C-start) does not return until about 6 months postoperatively. To test whether functional regeneration of the Mauthner cell (M-cell) could underlie the return of a C-start, we have studied the EMG responses elicited by intracellular M-axon stimulation in goldfish. Ten control and 7 experimental fish were pretested for startle responses with a sound pulse. The animals responded with C-starts in approximately 80% of the trials. The spinal cords of the experimental fish were crushed at the spinomedullary level. After injury, these animals all regained swimming, and equilibrium in 2-3 months. They all displayed C-starts beginning at about 6 months. 421-468d postoperatively these fish were restrained and EMG electrodes were placed bilaterally in the trunk and tail musculature. The M-axon was activated by intracellular stimulation, EMG responses were recorded, and visual observations of trunk and tail muscle movements were made. The EMG responses and movement of trunk musculature of the experimental fish were significantly smaller than those of controls. Although the M-cell is capable of regeneration caudal to a wound site, we suggest this regrowth does not underlie the return of a C-start. Therefore, we hypothesize that the C-start recovery results from functional regeneration of another neuronal system. Supported by NSF grant BNS 8809445.

137.5

ADULT SYMPATHETIC AXONS REGENERATE INDEPENDENTLY OF NGF.
Andrew Gloster and Jack Diamond. Dept. of Biomedical Sciences, McMaster University, Hamilton, Ontario CANADA L8N 3Z5.

In the adult rat the functional regeneration of crushed sensory nerves, but not the collateral sprouting of undamaged ones, occurs independently of endogenous NGF (PNAS 1987; 84, 6596). We have now studied sympathetic neurons, which retain their need for NGF throughout life (Gorin and Johnson, 1980; Brain Res. 198, 27). Sympathetic axons were revealed by amine fluorescence, and their cutaneous fields by evoked pilomotor responses. As expected, daily anti-NGF treatment appeared to prevent sympathetic collateral sprouting into surrounding denervated skin; it also reduced the fluorescence of the dermal axons to undetectable levels, although fluorescent axons were visible within the nerve trunk. In normal animals, sympathetic nerves regenerating after a crush restored pilomotor function to denervated skin at about the same rate as regenerating C fibers restored heat-nociception. We tested how daily anti-NGF treatment would affect this. Not only did regeneration of sympathetic axons occur along the nerve trunk, as evidenced by their fluorescence, but in a significant number of instances pilomotor responses were restored to the skin. It seems that, like sensory axons, sympathetic fibers regenerate independently of NGF. However, prolonged anti-NGF treatment reduces amine levels, and may compromise pilomotor function. We are now examining whether enhancement of amine content will improve the incidence of functional recovery by regenerating axons.

137.6

MUSCLE REINNERVATION BY CNS AXONS REGENERATING VIA VENTRAL ROOTS IMPLANTED INTO THE SPINAL CORD.
K.J. Smith[1] & R.T. Kodama[2]* (SPON: C. Morgan). Depts. Anatomy & Cell Biology[1], Urology[2], Eastern Virginia Medical School, Norfolk, VA, 23501, & Neurology[1], U.M.D.S., Guy's Campus, London SE19RT

Central axons can regenerate into a peripheral nerve implanted into the spinal cord, but the ability of these axons then to innervate denervated muscle has been little investigated. To examine this, 17 rats had their T8 ventral root (VR) divided, and a cord stab. In 11 rats, the distal VR stump was implanted into the stab. After 12-15 months, the implant was isolated by severing the ipsilateral T_{7-9} VRs (redividing the T_8 proximal stump) and intercostal nerves (ICN) 4-7 & 9-12. Pharmacological and electrical stimulation (ES) of the spinal cord were used to activate individual motor units in the 8^{th} intercostal muscle. Our criteria for considering a motor unit as reinnervated by central axons included the presence of: a) "giant" potentials, b) with ES, the latency jitter expected from a variable central synaptic delay, and c) the presence of the initiating nerve impulse in the 8^{th} ICN. These criteria were met in implanted animals only. We conclude that central regenerating axons can establish effective synapses on denervated skeletal muscle. Supported by NIH grant NS25118.

137.7

IDENTIFICATION OF CELL TYPES EXPRESSING MHC ANTIGENS IN THE RAT CNS AFTER SUBLETHAL AND LETHAL NEURONAL INJURY. W.J. Streit, M.B. Graeber and G.W. Kreutzberg. Dept. of Neuromorphology, Max Planck Institute for Psychiatry, D-8033 Martinsried, F.R.G.

Recent studies from different laboratories have shown that not astrocytes, but microglial cells function as antigen presenting cells in the brain. In the present study we show that few perivascular and microglial cells carry Ia antigen in normal brain, but that Ia expression is greatly enhanced on these cell types after local neuronal injury. One week after transection of the facial nerve or intraneural ricin injection a striking increase in the number of Ia-positive perivascular cells was observed in the facial nucleus. This increase was more pronounced after ricin treatment than after axotomy. From week 2 to 6 Ia-immunoreactivity was found increasingly on microglial cells while at the same time the number of Ia-positive perivascular cells was decreasing. The changing morphology of Ia-positive cells occured much faster during ricin-induced neuronal degeneration than during regeneration. Ia-positive cells had disappeared completely from the degenerated facial nucleus by week 15, but persisted in the regenerating nucleus in the form of ramified microglia. Based on our observations we suggest that microglia are heterogeneous with regard to their ability to express MHC antigens, and that Ia-positive microglia arise through direct transformation of Ia-positive perivascular cells.

137.8

INDUCTION OF MAJOR HISTOCOMPATIBILITY CLASS I ANTIGENS ON ADULT PRIMATE RETINAL CELLS IN VITRO. *M.R. Burrous and P.R. MacLeish*, Laboratory of Neurobiology, Rockefeller University, New York, New York 10021

The expression of the major histocompatibility (MHC) class I molecules has been reported to be inducible on neonatal CNS neurons by interferon both *in vivo* and *in vitro* (Wong *et al.*, Nature 310: 688 (1984)). We have investigated the expression of MHC class I antigens on cells isolated from the adult monkey retina and maintained with or without human gamma interferon (IFN) in the growth medium. The presence of class I MHC antigen was detected by immunofluorescence labelling with the monoclonal antibody W6/32 (kindly donated by Ralph Steinman) and by observing adhesion and process outgrowth of retinal cells cultured on a monolayer of this class I MHC antibody. When grown in the absence of IFN on W6/32 antibody, retinal cells showed weak labelling by 5-7 days in culture, but a striking enhancement in process outgrowth over cells grown on other substrates (including antibodies to the class II MHC molecules). In cultures treated with IFN (1-100 units antiviral activity/mL), there was a clear dose-dependent increase in the labelling of nearly all cells growing directly on the W6/32 substrate. IFN also effected even more complex elaboration of neuronal processes, especially at the lowest doses. In addition, IFN treatment evoked a striking increase in the number of identifiable Müller cells at all doses. These results suggest that class I MHC molecules are induced on regenerating adult retinal cells, and this expression may be sensitive to physiological doses of IFN. Supported by GM07739-10, EY-05201, and the Javits Center Award.

137.9

TARGETED NERVE LESION INDUCED BY PHOTOSENSITIZING DYES: A NOVEL MEDEL FOR STUDYING NERVE DEGENERATION-REGENERATION. LT Wang-Bennett and DJ Liebl* Dept. of Otolaryngology, Baylor College of Medicine, Houston, TX 77030.

Photosensitizing dyes are excited to an elevated electron state by a specific wavelength of light. In the presence of a reductant the excited dye converts oxygen in the cells into reactive oxygen radicals and causes various cellular damage. Free dyes (Azure-C or mesoporphyrin) or the dye conjugated to a protein carrier (horseradish peroxidase, HRP) are injected into rabbit whisker pad muscles. By retrograde transport these dyes or the dye-HRP conjugates are uptaken into the facial nerve buccal branch and localized centrally in the facial motor nucleus by HRP histochemistry. The absorbance peak of the conjugate or the free dye was at the same wavelength and were activated by a helium-neon laser (λmax=632.8 nm).

EM ultrastructural analysis revealed debris of axon cytoskelton, demyelination, disruption of perineural cells and basal laminal segregation following the irradiation of mesoporphrin injected facial nerve. At longer survival times, the regeneration and sprouting are observed.

In vitro experiment with nerve homogenate incubated with Azure-C or Azure-C-HRP conjugate showed cleavage of a high molecular weight protein (approximate 200 KDa) by laser irradiation.

Supported by Advanced Technology Program grant #1165 from Texas Higher Education Coordinating Board.

137.10

REGENERATION OF SINGLE LASER-ABLATED MOTOR AXONS AND THE REOCCUPATION OF POSTSYNAPTIC SITES FOLLOWED IN LIVING MICE. P. van Mier and J.W. Lichtman. Department of Anatomy and Neurobiology, Washington University School of Medicine, St.Louis, MO 63110.

In order to simplify the study of axonal regeneration, we have used a laser in conjunction with a microscope to selectively damage single motor axons and observe over time regeneration and reinnervation in the sternomastoid muscle of living mice. After microscopic visualization of vitally stained motor axons and their synapses, one axon having a superficial preterminal branch was selected for laser damage. The laser beam focussed to a spot the size of an axon diameter, was positioned over the axon at least 100 μm proximal to the terminal site to avoid direct damage to the junction or the postsynaptic muscle fiber. The axon was then irradiated for 1-5 seconds with 1-100 pulses (pulse length 3 nsec) of blue laser light (λmax. 503 nm). Usually the neuromuscular junction distal to the axonal damage became spontaneously active within seconds causing the postsynaptic muscle fiber to twitch. Vital re-staining of the distal axon and terminal was no longer possible after several hours, and one day after the lesion the distal axon was no longer evident in the Schwann-cell sheath. Within 5 days after laser treatment the axons rapidly regenerated and accurately reoccupied the original synaptic sites. Typically, the regenerating axons are far thinner than normal, even after they have reoccupied the synaptic site. We are presently following the regeneration of single axons and monitoring the behavior of surrounding neuromuscular junctions in response to the denervation of single muscle fibers.

137.11

FUSION OF REGENERATING LEECH AXONS CUT INDIVIDUALLY BY FLUORESCENCE PHOTOINACTIVATION. J.M. Camhi and E. Macagno. Dept. Biol., Columbia Univ., New York, NY 10027.

After cutting or crushing the leech nerve cord, the severed proximal and distal axonal segments are known to be capable of fusion (De Riemer et al. (1983) Brain Res. 272:157-161). This occurs, though, in only 3-9% of the axons, depending on the type of lesion. We have tested whether cutting instead just a single axon, in an otherwise undisturbed nerve cord, increases this rate of fusion.

To cut selectively a Retzius axon, its soma was filled with Lucifer Yellow (LY) and an 80 μm length of axon was then illuminated. Upon refilling 1-2 days later with LY, HRP or DiI, such axons were seen to be cut. Several sprouts had usually developed from the cut end.

When refilled with LY instead after 3-4 days, in 80% of the cells the dye traveled in a single axon, through the cut region and at least 500 μm beyond, finally fading gradually into background. Often there was a constriction and/or sprouting in the region of the cut. Refilling instead with HRP after 3-4 days showed the same features in over 70% of the axons, though the HRP often was visible all the way to the next ganglion. Regenerative growth of sprouts is far to slow to account for these axonal lengths. Since HRP does not pass through known gap junctions, we conclude that axonal fusion has occured.

We are currently studying whether the proximal segment of a cut Retzius axon fuses selectively with its own distal segment when presented with a choice. We are also examining other systems, both invertebrate and vertebrate, for similar instances of fusion.

137.12

MORPHOLOGICALLY DIFFERENT RETINAL GANGLION CELL TYPES FROM ADULT RATS AND CHICKENS REGROW THEIR AXONS IN VITRO. J. Vanselow* and S. Thanos. Max-Planck-Institut für Entwicklungsbiologie, Abt. I, Spemannstr. 35, D-7400 Tübingen, FRG

Retinal explants from adult rats and chickens were cultured to investigate the regenerative ability of distinct retinal ganglion cells in higher vertebrates. Precrushed retinae of 8 rats and 5 chickens were explanted on a poly-lysine/laminin substratum. After 2 days to 2 weeks, the explants were fixed and the cells, whose neurites had grown out, were stained retrogradely with the fluorescent dye Di I by applying crystals of the dye to processes emerging from the explant. In explants from rats, all ganglion cell types (type I, type II and type III) were found having regenerated axons. Some of these cells also showed sprouting of their dendritic processes, which were typically tipped with growth cones. In explants from chickens, 8 different ganglion cell types had regrown their axons. These results indicate that different retinal ganglion cell types of adult rats and chickens have the ability to regrow injured axons in vitro and to maintain their cell type specific morphology for several days.

138.1

CERVICAL SYMPATHETIC NERVE RESPONSES TO BAROCEPTOR LOADING OR UNLOADING IN NEONATAL SWINE. P.M. Gootman, H.L. Cohen, B. W. Hundley*, G. Condemi*, L. Eberle*, M. Brust*, Dept. Physiol. SUNY-Hlth. Sci. Ctr. Bklyn, Brooklyn, NY 11203

We decided to investigate the usefulness of power spectral analyses in the examination of changes in sympathetic activity elicited by changes in baroceptor inputs. In neonatal swine (<1 - 6 weeks of age), lightly anesthetized with Saffan, paralyzed with C-10 and artificially ventilated on 100% O_2, monophasic recordings of phrenic (PHR) and cervical sympathetic (CS) activity were obtained simultaneously with aortic pressure (AoP), EKG, end-tidal CO_2 and intratracheal pressure. Baroceptors were stimulated by increasing AoP with phenylephrine (PE; 20 ug/kg, iv) or inhibited by decreasing AoP with Na nitroprusside (NP; 30 ug/kg, iv). PHR activity was frequently inhibited with baroceptor stimulation. Nerve activity was computer analyzed utilizing the FFT and measures of averaged power. Equi-AoP changes of 30 mm Hg elicited changes in the averaged power of CS activity. Increased AoP resulted in decreased averaged power while falls in AoP had the opposite effect. The magnitudes of the changes in averaged power were equivalent (@ ±30%). Thus equi-AoP changes elicited equi-sympathetic activity changes in neonatal swine. Furthermore, power spectral analyses can be useful in the quantitation of changes in sympathetic activity. (Supported by NIH grant HL-20864.)

138.2

RELATIONSHIPS BETWEEN BASAL DISCHARGES OF SYMPATHETIC NERVES. B. Kocsis, G.L. Gebber and S.M. Barman. Dept. Pharmacol., Mich. St. Univ., E. Lansing, MI 48824.

The coherence function and phase spectrum were used to study the relationships (in the frequency domain) between the discharges of sympathetic nerves (e.g., inferior cardiac and renal) in baroreceptor-denervated cats. The coherence values in the 2- to 6-Hz band were significantly different from zero. The phase spectrum was linear in the coherent frequency band and showed random fluctuations elsewhere. The difference in conduction times from the brain stem to the two nerves was reflected by the slope of the linear portion of the phase spectrum. Taking this into account, two patterns of relationship were discernable. One pattern was characterized by the absence of a time delay between activity in the central circuits controlling both nerves while the other was characterized by the dependency of the time delay on frequency in the 2- to 6-Hz band. In the latter case, the activity of one nerve could lead that of the second at some frequencies and lag at others. The results point to two modes of operation of the central network responsible for 2- to 6-Hz sympathetic nerve activity. In one mode, the components of the network are activated in unison (i.e., without a time delay). Although strong coupling also characterizes the second mode of operation, the phase relations between activity of the components of the central network are variable. (Supported by NIH grant HL13187.)

138.3

COHERENT FREQUENCIES IN SYMPATHETIC NERVE DISCHARGE (SND) AND FRONTAL CORTICAL ACTIVITY (EEG). G.L. Gebber, M.J. Kenney, B. Kocsis and S.M. Barman. Dept. Pharmacol., Mich. St. Univ., E. Lansing, MI 48824.

The coherence function was used to study the relationships (in the frequency domain) between inferior cardiac SND and EEG of chloralose-anesthetized cats. The coherence function contained one or two sharp peaks in the delta and/or theta frequency bands. These peaks were larger in baroreceptor-denervated than in baroreceptor-innervated cats. The coherence of delta-theta frequencies in SND and EEG was reversibly blocked by iv brevital (1-2 mg/kg). The sharp peaks in the coherence function could be attributed to a forebrain influence on sympathetic circuits since midcollicular decerebration eliminated the delta-theta peaks in SND autospectra that cohered to EEG. In contrast, these frequencies persisted in the autospectrum of EEG after decerebration. We also noted that coherence of delta-theta frequencies in SND and EEG was strongest during frontal cortical spindling. Spontaneous spindles were 1-3 s in duration and occurred regularly once every 5 to 10 s. Such spindles could be triggered and entrained by single electrical shocks applied to intralaminar thalamic nuclei. These results support the view that both a forebrain delta-theta rhythm generator and the thalamic spindle generator contribute to SND in chloralose-anesthetized cats. (Supported by NIH Grants HL13187 and HL33266.)

138.4

NONLINEAR DYNAMIC ANALYSIS OF CARDIAC R-R INTERVAL VARIATION FOLLOWING COCAINE ADMINISTRATION. R.K. Harper*, R.C. Frysinger, R.R. Terreberry, A. Garfinkel*, C.A. Richard and R.M. Harper (SPON: R. Strandburg). Brain Research Institute and Depts. of Anatomy & Cell Biology and Kinesiology, UCLA, Los Angeles, CA 90024.

We examined sequencing of cardiac R-R intervals following acute intravenous and intraventricular administration of 3 dose levels of cocaine (5, 7.5, and 10 mg/kg intravenously; .625, 1.25, and 2.5 µg intraventricularly) in intact, freely moving cats. Five cats were instrumented with EEG, ECG, and diaphragmatic EMG electrodes. Following recovery, each cat was allowed to sleep undisturbed in a quiet chamber and then administered cocaine either intravenously or intraventricularly. Cardiac R-R intervals were stored on digital disks. Each cardiac interval was plotted against the successive interval for 10 minutes (a) prior to and (b) following cocaine administration, and the distribution of dependencies was noted. A linear regression was also calculated for each condition. Both intravenous and intraventricular cocaine administration caused an extreme organization of cardiac intervals such that interinterval sequences of correlations of near zero were followed postinfusion by a correlation near 0.8. On one occasion the extreme organization erupted into ventricular tachycardia. We suggest that cocaine predisposes an extreme organization of cardiac rhythm that can deteriorate into chaotic rhythms. Supported by DA04913.

138.5

CHANGES IN HEXOKINASE ACTIVITY IN THE BRAINSTEM AND FOREBRAIN DURING AFFERENT RENAL NERVE (ARN) STIMULATION. J. Schoeneman* and J. Ciriello (SPON: M. Robinson). Dept. of Physiology, Univ. of Western Ontario, London, Canada, N6A 5C1.

Changes in metabolic activity of brainstem and forebrain structures during ARN stimulation were studied using the hexokinase (HK) histochemical method in the rat. ARN stimulation (st-ARN) significantly elevated arterial pressure (AP; 120±6mmHg) compared to the sham stimulated animals (sh-ARN; 97±2 mmHg). In the contralateral brainstem of st-ARN animals increases in HK reaction product were observed in the region of the C1 adrenergic cell group, the commissural and rostral medial subnuclei of the solitary tract, and Kolliker-Fusé nucleus. In the diencephalon increased HK reaction product was observed in the contralateral arcute nucleus, median preoptic nucleus, medial parvo- and magno-cellular components of paraventricular nucleus of the hypothalamus, posterior hypothalamus, supraoptic nucleus, and subfornical organ. Similar results were obtained in st-ARN rats with total autonomic blockade and in which AP was maintained at a level not different from sh-ARN rats. These results have demonstrated that ARN stimulation alters the activity of central structures previously implicated in the regulation of body fluid balance and cardiovascular function. (Supported by HSFO).

138.6

RESPONSES OF MAGNOCELLULAR NEURONS OF THE PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS (PVH) TO ACTIVATION OF RENAL RECEPTORS. J.K. Simon* and J. Ciriello. Dept. of Physiology, Univ. of Western Ontario, London, Canada, N6A 5C1.

Single-unit recording experiments were done in pentobarbital anesthetized rats to investigate the responses of vasopressin (AVP) and oxytocin (OXY) containing cells in the PVH to selective activation of renal receptors. Following the transpharyngeal exposure of the hypothalamus and the neurohypophysis (NH), neurosecretory PVH neurons were identified by antidromic activation from the NH and classified as putative AVP cells if they were phasically firing and/or inhibited by baroreceptor activation or putative OXY cells if they were continuously firing and unresponsive to baroreceptor activation. Renal ischemia excited 3 of 6 AVP cells while renal venous occlusion excited 2 of 6 AVP neurons tested. Intrarenal infusions of bradykinin (2/6), sodium cyanide (2/5), capsiacin (4/6), and adenosine (1/3) excited AVP cells. Renal ischemia did not affect putative OXY cells while renal venous occlusion was found to excite 1/5 OXY cells. Intrarenal infusions of bradykinin (3/4), of sodium cyanide (2/3), and of adenosine (1/2) excited OXY cells. On the other hand, capsiacin was without effect. These data demonstrate that AVP and OXY neurons in the PVH are responsive to activation of different renal receptor populations and suggest that these receptors contribute to the control of AVP and OXY release into the circulation. (Supported by HSFO).

138.7

EFFECTS OF IBOTENIC ACID (IBO)-INDUCED LESIONS IN MEDIAN PREOPTIC NUCLEUS (MnPO) ON CARDIOVASCULAR FUNCTION IN RATS. H. Ohta, J.T. Cunningham, T.G. Beltz,* A.K. Johnson and M.J. Brody, Univ. of Iowa, CV Ctr., Iowa City, IA 52242

The anteroventral third ventricle region contains key structures which participate in drinking behavior and cardiovascular responses induced by angiotensin II (AII) and other centrally acting agents. A previous study showed that drinking behavior induced by systemic administration of AII was abolished in rats with IBO lesions of MnPO. The aim of the present study was to determine whether cardiovascular responses elicited by central and systemic administration of pressor agents are altered in rats twelve weeks after lesions of MnPO produced by microinjection of IBO (5 µg in 1 µl). A guide cannula was implanted into the lateral cerebroventricular (icv) and both femoral artery and vein were catheterized. The pressor response induced by icv injection of AII (100 ng) in MnPO lesioned rats was greater than that in vehicle treated rats (28 ± 3 vs. 19 ± 1 mmHg), whereas the responses induced by intravenous (iv) administration of AII were not altered. Pressor responses induced by icv administration of carbachol (50 ng) and hypertonic NaCl (1M) in both groups were identical. These results indicate that whereas cell bodies in MnPO are required for AII-induced drinking, they are not involved in the pressor response induced by either icv or iv administration of AII.

138.8

BRAIN AND PITUITARY ANP IN EURYHALINE FISH: ITS ROLE IN ADAPTION TO DIFFERENT SALINITIES. Sara M. Galli*, Keping Qian*, Maria Trolliet*, Birgitta Kimura* and M. Ian Phillips. (SPON: P.S. Kalra), Dept. of Physiology, University of Florida, Gainesville, FL 32610

Atrial natriuretic peptide has been reported in brain and neural tissue of most groups of vertebrates and invertebrates, but its physiological function is not known. To study the physiological role of ANP in fish, four species of euryhaline fish were studied; toadfish, mullet, killifish and tilapia. The fish were captured in sea water and given 4 weeks to adapt in sea water tanks. Groups of the fish were then transferred to 50% SW or FW for different periods of time. Blood samples were taken and brains and pituitaries removed after exposure to lower salinity. ANP was analyzed by HPLC and measured by RIA. The the distribution in the brain located by immunocytochemistry. Highest levels of ANP in all species studied was in the diencephalon 180 ng/g tissue with lesser concentrations in telencephalon, metencephalon, myelencephalon, and pituitary. When placed in 50% SW (killifish) ANP brain levels rose >100% within 12 hours and stayed elevated (compared to levels at sea water) for up to 7 days. Pituitary ANP levels also rose (5-25 ng/g tissue). Plasma levels, by contrast, decreased in 50% SW and FW. The decrease was reversed when fish were returned to SW. These results suggest that plasma and brain ANP play a physiological role in the osmoregulation of fish as they move into SW from the lower salinity in estuaries. The opposite changes in brain and pituitary, versus plasma, indicate a reciprocal role in the maintenance and control of Na^+, Cl^-, and volume. (Supported by American Heart Association).

138.9

REDUCED CARDIOPULMONARY REFLEX ACTIVITY IN CONSCIOUS SHR. M.J. Brody, S.J. Lewis* and H. Ohta. Dept. of Pharmacolology and Cardiovascular Center, University of Iowa, Iowa City, IA 52242 USA

The intravenous (iv) injection of 5-HT produces cardiopulmonary afferent-mediated hypotension and bradycardia in rats. The hypotension is produced by vagally-mediated bradycardia and a reduction in sympathetic outflow to the vasculature. This study compared cardiopulmonary reflex (CPR) function in spontaneously hypertensive (SHR) and age-matched (8 week) Wistar-Kyoto (WKY) rats. IV injections of 5-HT in the WKY (1-9µg/kg) produced dose-dependent falls in diastolic arterial blood pressure (DABP) and heart rate (HR) (see table). The SHR were markedly less sensitive to 5-HT (5-40µg/kg). Pretreatment with methylatropine (MA, 0.2 mg/kg, iv) markedly reduced the hypotension and bradycardia in the WKY and abolished these responses in the SHR.

Slopes (Δ parameter/$\log_{10}$dose 5-HT) pre and post MA

	WKY		SHR	
	pre	post	pre	post
HR	-181±11	-12±2*	-57±5†	-1±1*
DABP	-74±5	-23±3*	-28±4†	-4±2*

*p < 0.05 pre vs post, †p < 0.05 WKY pre vs SHR pre

These results demonstrate that CPR function is markedly impaired in the SHR and this impairmment involves both vagal and sympathetic efferent functions.

138.10

ADAPTATION OF BARORECEPTORS WITH ELEVATED STATIC ARTERIAL PRESSURE IS ABSENT OR ATTENUATED WITH PULSATILE PRESSURE. M.W. Chapleau*, G. Hajduczok and F.M. Abboud*. CV Ctr. and Depts. of Internal Med. and Physiol., U. of Iowa Coll. of Med., and VA Med. Ctr., Iowa City, IA 52242.

Most receptors adapt to sustained stimuli. The purpose of this study was to contrast the magnitude of adaptation of baroreceptors (BR) during elevations in static pressure (SP) vs. pulsatile pressure (PP) in the isolated carotid sinus (CS) of dogs anesthetized with chloralose. At an elevated mean pressure of 100 mmHg, whole CS nerve activity (n=9) decreased over a period of 5 minutes by 39±9% during SP and by 12±4% during PP ($P<0.05$ for difference between SP and PP). Single-fiber activity also decreased more ($P<0.05$) during SP (-20±6%) than during PP (-2±6%) (n=6). Relaxation or "creep" of the vessel wall has been proposed as the mechanism of BR adaptation. CS diameter (sonomicrometers, n=6) increased over the 5-minute period of constant elevated pressure (100 mmHg) to the same extent during SP (+37±16 µm) and PP (+37±9 µm). Therefore, other mechanisms besides "creep" of the vessel wall must explain the difference in adaptation. We conclude that adaptation of BR is absent or markedly attenuated during elevated PP in contrast to SP. This may explain the sustained baroreflex inhibition of sympathetic activity seen with PP in contrast to the escape from inhibition reported with SP.

138.11

EFFECTS OF ANGIOTENSIN II (ANG II) INFUSION ON ANG II BINDING SITES IN THE NODOSE GANGLION. Debra I. Diz, Susan M. Bosch*, and Carlos M. Ferrario. Dept. of Brain and Vascular Research, Cleveland Clinic Fdn, Cleveland, OH 44195.

To determine whether the novel high affinity (K_D=0.8 nM) Ang II binding sites present in the nodose ganglion (NG) exhibit ligand-mediated receptor-regulation, male Sprague-Dawley rats (292±3g) were given an ip infusion of Ang II (100 ng/min for 7 days via osmotic minipump). Others have documented that plasma Ang II increases about three-fold using this protocol (Aguilera et al, 1980). In awake rats given Ang II, mean arterial pressure rose by the 6th day of infusion to 142±8 mm Hg (n=5) versus 116±6 mm Hg in control rats (n=3; $p<0.07$). The NG were removed on the 7th day of the Ang II infusion and Ang II binding was assessed over a range of 0.1-1.5 nM using *in vitro* receptor autoradiography (Diz et al; Brain Research Bulletin, 1986). Analyses (EBDA and LIGAND) revealed that neither the binding affinity nor the density were altered in the NG by the Ang II infusion. In the control rats (n=3), a K_D of 1.2±0.3 nM and a Bmax of 25±4 fmol/mg protein was observed. In the Ang II infused rats (n=6), the values were 1.1±0.3 nM and 28±7 fmol/mg protein, respectively. Hill coefficients (control=0.99; infused=0.97) indicate a single population of binding sites. Non-specific binding averaged 44±5% in the NG from infused rats and 38±2% in control rats. These data suggest that low-dose Ang II infusion does not down-regulate receptors in the NG. Thus, in contrast with the regulatory action of Ang II on vascular and adrenal cortex Ang II receptors, neuronal NG receptors may not share the same regulatory mechanisms. Further studies are required to characterize the regulation of Ang II binding in this neural structure. (Supported in part by HL-6835, HL-38535, and the Reinberger Fdn.)

138.12

ROLE FOR NEUROKININS IN THE PERIPHERAL REGULATION OF THE CARDIOVASCULAR SYSTEM. R. Couture, H. Hasséssian and C. Guimond*. Dept. of Physiology, Faculty of Medicine, University of Montréal, Montréal, Qué., Canada.

This study characterized the neurokinin (NK) receptor subtype and mechanism mediating the cardiovascular responses evoked by the i.v. administration of NKs. In urethane anesthetized Wistar rats, substance P (SP), neurokinin A (NKA), neurokinin B (NKB), and seven NK receptor subtype selective agonists: [Sar^9, $Met(O_2)^{11}$]SP, (A); [Pro^9, $Met(O_2)^{11}$]SP, (B); [β-Ala^4, Sar^9, $Met(O_2)^{11}$]SP (4-11), (C); NKA(4-10), (D); [succinyl-Asp^6, $MePhe^8$]SP (6-11), (E); [β-Asp^4, $MePhe^7$]NKB (4-10), (F); [$MePhe^7$]NKB, (G) were studied on blood pressure and heart rate (HR). Vasodepressor responses were obtained using 0.65 to 65 nmol/kg doses, with the following rank order of potency: A = B > C = E = F = G > SP > NKB = NKA >> D. NKA and the NK-2 selective agonist (D) stimulated HR, while NKB and NK-3 selective agonists (E, F, G) inhibited HR. SP and NK-1 selective agonists (A, B, C) produced a rise in HR, which was followed by a modest bradycardia. These results and our additional pharmacological investigation lead us to conclude: a) NKs act on NK-1 receptors of blood vessels to reduce blood pressure, b) activation of NK-2 receptors on cardiac sympathetic fibers and/or adrenal medulla causes HR acceleration, c) activation of the vagus via NK-3 receptors produces a bradycardia, d) some NK-1 agonists can also affect cardiac function by releasing prostaglandins. (Supported by the MRC of Canada).

139.1

TOXIN I, A MAMBA SNAKE DENDROTOXIN, INDUCES LONG-LIVED SUB-CONDUCTANCE EVENTS WHEN APPLIED TO THE INTRACELLULAR SIDE OF MAXI K(Ca) CHANNELS. K.J. Lucchesi* and E.G. Moczydlowski, Dept. Pharmacology, Yale Univ. Sch. Med., New Haven, CT 06510. Dendrotoxins have previously been shown to block a variety of voltage-dependent K-channels at peripheral nerve terminals, frog node of Ranvier and brain neurons. We examined the effect of Toxin I from Dendroaspis polylepis on maxi K(Ca) channels from rat muscle incorporated into planar lipid bilayers. 40 nM Toxin I produces novel subconductance blocking events when applied to the internal side of the channel. In contrast, 5 uM Toxin I has no effect on the external side. The induced subconductance behavior is highly complex, involving transitions between at least four discrete current levels. A primary effect is the appearance of long-lived 2.8 pA states in single channel records that normally fluctuate between 0 and 4.1 pA under control conditions (50 mM symmetrical K+, +20 mV holding potential). The toxin dose-dependence indicates that the long-lived subconductance events correspond to the dwell time of bound toxin. A kinetic analysis based on a one-site model gives a Kd_5 of 93 nM for Toxin I with koff = 0.042 s^{-1} and kon = 4.5 x 10^{5} s^{-1} M^{-1} at 50 mM symmetrical K+. Kon varies inversely with internal ionic strength and the duration of the substate induced by bound Toxin I is lengthened by depolarization. These results add to growing evidence for homology among classes of K-channels and provide a unique perspective on K-channel structure and function. We thank Alan Harvey for Toxin I. Supported by NIH HL38156.

139.2

SOLUBILIZATION AND PURIFICATION OF RAT BRAIN DENDROTOXIN RECEPTORS ASSOCIATED WITH VOLTAGE-GATED POTASSIUM CHANNELS. R.G. Sorensen. Dept. Med., Div. Environ. Med. and Toxicol., Jefferson Medical College, Philadelphia, PA 19107.

The venom of the green mamba, Dendroaspis angusticeps, contains four polypeptides (dendrotoxins, DaTX), that block brain voltage-gated K channels. The receptors for two of the dendrotoxins, α-DaTX and β-DaTX, which preferentially block inactivating and non-inactivating K channels, respectively, have been solubilized and partially purified from rat brain.

The dendrotoxin receptors were extracted from brain membranes in the presence of 150 mM KCl, 2 mM $MgCl_2$, 10 mM HEPES, pH 7.0, containing 0.8% zwittergent 3-12 and 0.25% lecithin. Toxin binding to the soluble receptors was then measured by a spun column method. The addition of lecithin increased the recovery of soluble toxin binding activity by 2-fold. Potassium ion optimally preserved soluble toxin binding, which was reduced as K was replaced in the order: K > Rb > Cs > Li > Na.

The affinity of [^{125}I]α-DaTX decreased 10-fold (K_D = 7 nM), and of [^{125}I]β-DaTX decreased 3.5-fold (K_D = 124 nM), after receptor solubilization. The molecular weight of the solubilized receptors was estimated to be 270,000 by sucrose density gradient centrifugation.

Partial purification of the dendrotoxin receptors has been achieved by DaTX-affinity chromatography. The purification of the receptors will be discussed. (Supported by NIH grant NS 27533).

139.3

IMMUNOLOGICAL EVIDENCE FOR A RELATIONSHIP BETWEEN THE DENDROTOXIN BINDING PROTEIN AND THE MOUSE BRAIN POTASSIUM CHANNEL GENE, MBK1. R. A. Newitt*, H. Rehm* and B. L. Tempel. GRECC, VA Medical Center, Seattle, WA 98108 and Dept. of Pharmacology, Univ. of WA, Seattle, WA 98195

We have asked whether the cloned potassium channel gene, MBK1 (Tempel et al, 1988) is related to the putative potassium channel protein purified from rat and bovine brain using dendrotoxin as an affinity ligand (Rehm and Lazdunski, 1988). Polyclonal antibodies were raised against synthetic peptides from different parts of the predicted amino acid sequence of the mouse potassium channel gene and used to probe purified putative potassium channel proteins (DMB proteins) from rat and bovine brain membranes on Western blots following SDS-PAGE. DMB proteins of both species consisted of a toxin binding subunit of mol. wt. of 90 Kd (rat) and 83 Kd (bovine) and a smaller 38 Kd subunit. Antibodies recognized specifically the dendrotoxin binding subunit. The difference in molecular weight of this toxin binding protein subunit between the species was at least partially due to differential glycosylation. The 38 Kd subunit did not bind antibody nor did it appear to be appreciably glycosylated. Presently, the sequence of both of these subunits is being determined to elucidate their function and compare with those of other ion channel proteins.

139.4

TISSUE SPECIFIC EXPRESSION OF MBK1, A POTASSIUM CHANNEL GENE. B. L. Tempel, M. A. Miller*, J. H. Urban and D. M. Dorsa (SPON: R. Malenka). GRECC, VA Med. Ctr., Seattle, WA, and Depts. of Pharmacology and Medicine, Univ. of WA, Seattle, 98108

The molecular mechanisms underlying K+ channel diversity are not known. Questions remain regarding the number of genes involved or the distribution of expression of members of this gene family. We are studying the tissue specific expression of a mouse brain K+ channel gene, MBK1, which induces the appearance of a non-inactivating K+ conductance when expressed in frog oocytes (see Houamed, et al, these abstracts).

Sequences derived from both coding and non-coding regions of MBK1 were used at high strengency to probe poly A+ RNA blots from various tissues of rat and mouse. A single 8 Kb transcript was seen in brain stem, cortex, hippocampus, cerebellum and, at a much lower level, in heart and skeletal muscle. Oligonucleotide probes were used for in situ hybridization studies to determine the cellular distribution of MBK1 in the CNS. Specific hybridization was widely distributed but varied in intensity from region to region. For example, we found heavily labeled cells in the area of the cranial motorneurons in the brain stem and a much higher level of expression in the CA3 pyramidal cells than in other hippocampal areas.

139.5

SITE-DIRECTED MUTAGENESIS OF THE S4 SEQUENCE OF THE SHAKER K^+ CHANNEL. D.M. Papazian*, L.C. Timpe, Y.N. Jan, and L.Y. Jan. HHMI and Dept. of Physiology, UCSF, San Francisco, CA 94143.

The A-type K^+ channel encoded by the Shaker gene of Drosophila contains an S4 sequence similar to those in Na^+ and Ca^{2+} channels. S4 sequences, which consist of repeated triplets of one basic (arginine or lysine) and two hydrophobic amino acids, have been proposed to function as transmembrane voltage sensors. This proposal has been tested by site-directed mutagenesis of the S4 arginine and lysine residues in the Shaker cDNA clone, ShB1. Three types of single amino acid mutations have been made. Basic residues have been switched to 1) glutamine, 2) glutamate and 3) arginines have been changed to lysines, and vice versa. Mutant A channels have been expressed in Xenopus oocytes and analyzed by the two electrode voltage clamp technique.

Mutations of basic amino acids in the middle positions of the S4 to glutamine or glutamate eliminated channel activity, whereas all other mutations gave A currents. Although some of these mutant channels conducted normal currents, the others affected a subset of the functional properties of the channel. 1) The conductance-voltage and steady state inactivation-voltage relationships were shifted along the voltage axis in the depolarized direction. The slopes of these curves were affected in at least one mutant. 2) The rate of macroscopic inactivation was slowed. None of the mutations had dramatic effects on the K^+ selectivity of the channel, or on its rate of recovery from inactivation. These results indicate that the S4 sequence is important for the voltage-dependence and kinetics of the K^+ current.

139.6

Shaker Potassium Channels: a Leucine Zipper Motif may Indicate a Site for Subunit Interaction and Gating. K. McCormack*§, M. Ramaswami *§, M.K. Mathew*§, M. A. Tanouye§, L. Iverson*‡,T. McCormack*† & B. Rudy † (SPON B. Trevarrow) § Division of Biology, California Institute of Technology, Pasadena,CA; ‡ Division of Neurosciences, City of Hope,Duarte,CA; † Department of Physiology, New York University, New York, NY.

Recent work on a class of DNA-binding proteins, including *fos* and *jun* , has shown that dimerization is mediated by a "leucine zipper" motif. We report the presence of this motif in a family of potassium channels, including *Shaker* (*Sh*), which are thought to form functional multimers. The *Sh* motif is a heptad repeat containing 5 leucine residues. This region indicates a likely site for subunit interactions which, in turn, could be a major determinant in channel structure.

Interestingly, the leucine zipper motif is immediately adjacent to the S4 domain: this proximity suggests that S4 translocation may affect the association and/or dissociation of subunits. We propose that subunit association/disassociation through the leucine zipper motif acts as the transduction mechanism for the voltage-sensing S4 domain in determining open and/or closed states of the channel. A similar motif is present in sodium and calcium channels suggesting that this may be a common mechanism for gating in all voltage-gated channels. Mutations of amino acid residues within the *Sh* zipper motif have been constructed to test this model in the *Xenopus* oocyte expression system.

139.7

MUTATIONS IN THE AMINO TERMINAL VARIABLE DOMAIN ALTER INACTIVATION OF *SHAKER B* POTASSIUM CHANNELS IN *XENOPUS* OOCYTES T. Hoshi*, W. N. Zagotta* and R. W. Aldrich, Dept of Neurobiology, Stanford University, Stanford, CA 94305.

We have compared the properties of single channels in *Xenopus* oocytes injected with mRNA from the *Shaker B* variant and from variants generated by *in vitro* mutagenesis. We have found that all of the molecular transitions after first opening, including the inactivation transition, are voltage independent and therefore not associated with charge movement through the membrane. A partially coupled model accurately reproduces all of the single-channel and macroscopic data. Comparison of microscopic inactivation rates of channels that vary in amino-acid sequence in a putative cytoplasmic domain near the amino terminus suggests an involvement of this region in the inactivation process. We have further examined this by constructing and analyzing the gating of mutant channels, generated *in vitro*, that delete different portions of the sequence in this region. The inactivation rate is profoundly slowed in mutants that delete amino acids beginning at the 6th position. The gating and conductance properties of these mutant channels closely resemble those of delayed rectifier channels in *Drosophila* muscle, suggesting that only small structural differences may exist between A-type and delayed potassium channels. Deletion of amino acids in a region closer to the splice junction leave inactivation intact, and in some cases increases the microscopic inactivation rate. Treatment of channels in inside-out patches with internal trypsin mimics the mutations that slow inactivation, suggesting that the amino terminal is cytoplasmic. External trypsin has no effect. The lack of voltage dependence of inactivation rates, the ability of internal proteolytic agents to modify inactivation, and the effects of mutations that slow or speed inactivation suggest a mechanism of inactivation similar to the ball and chain model originally proposed by Armstrong and Bezanilla for voltage-gated Na channels. Supported by NS 23294, NS07158 and the American Heart Association.

139.8

PROPERTIES OF ShB A-TYPE K^+ CHANNELS EXPRESSED IN *SHAKER* MUTANT *DROSOPHILA* BY GERMLINE TRANSFORMATION. W. N. Zagotta*, S. E. Germeraad*, S. S. Garber and R. W. Aldrich (SPON: D. A. Baylor). Dept. of Neurobiology, Stanford University, Stanford, CA 94305.

The *Shaker* gene codes for a large variety of alternatively spliced mRNAs, many of which have been shown individually to produce functional K^+ channels when injected into *Xenopus* oocytes. These channels differ in gating and pharmacology from the native channels normally expressed in *Drosophila* myotubes. To begin to investigate *Shaker* variants in their native environment we have used P-element mediated germline transformation to express ShB channels in *Drosophila* cells. ShB cDNA under the control of a heat shock promoter (*hsp70*) was transformed into a host strain of *Drosophila* mutant in the endogenous *Shaker* gene. Northern blots reveal that the transformed DNA is efficiently transcribed in response to heat shock. The transformant homozygotes, however, continue to shake as vigorously as the *Shaker* host strain. Primary cultures of myotubes were prepared from the transformant embryos and heat shocked for 30 to 60 min at 37°C. Whole-cell patch clamp of the cultured myotubes reveals large A-type potassium currents in the heat-shocked cultures, but only delayed potassium currents in the non-heat-shocked cultures and cultures made from the host strain. While qualitatively similar to the native *Shaker* A-currents expressed in wild type myotubes, the transformant A-current inactivates more rapidly and recovers from inactivation more rapidly, similar to the ShB channels expressed in *Xenopus* oocytes. Unlike the channels in oocytes, however, the transformant A-current was present in 50 nM charybdotoxin. The *Shaker* transformants will allow us to study the interactions of the ShB protein with other *Shaker* variants and other gene products in their native cells. Supported by NS23294, NS07158, and a National Cystic Fibrosis Foundation fellowship.

139.9

EXPRESSION OF VOLTAGE-GATED POTASSIUM CHANNELS USING RECOMBINANT VACCINIA VIRUS. R.J. Leonard[1], A. Karschin*[1], J. Aivar*[1], N. Davidson[1], M.A. Tanouye[1], L. Thomas*[2], G. Thomas*[2] & H.A. Lester[1]. Div. Biology, [1]Caltech, Pasadena, CA 91125 *and* [2]VIABR, Oregon Health Sciences Univ. Portland, OR 97201.

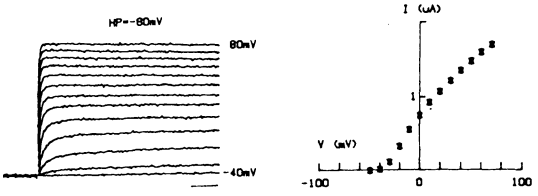
Vaccinia virus (VV) is a useful tool for the transient expression of foreign proteins, because it replicates in the cytoplasm and has a broad host range. We constructed a recombinant vaccinia virus containing the coding region of the *Shaker* H4 cDNA, a member of the Drosophila "A-current" channel family. The VV:H4 virus was used to infect four types of cells in culture: NIH-3T3 fibroblasts; rat basophilic leukocytes (RBL-1); undifferentiated PC-12 cells; and monkey kidney CV-1 cells. Whole-cell patch clamp records one day post-infection revealed robust (>2 nA peak) transient outward currents in all four cell types. The induced currents possessed the hallmarks of Drosophila *Shaker* A-currents: They were activated by depolarizations above -40 mV. The mid-point for inactivation by a depolarizing prepulse was -30 mV. Recovery from inactivation was rapid ($t_{1/2}$= 200ms). Both activation and inactivation rates increased with depolarization. Tail currents reversed at the potassium equilibrium potential. We estimate >4 functional channels/μ^2 in RBL-1 cells. Currents were blocked by 4-AP and (like H4 expressed in oocytes) charybdotoxin. No such currents were observed in recordings from uninfected cells. Endogenous voltage-activated currents in PC-12 cells were not suppressed by the infection. Vaccinia should be a useful tool for expressing cloned ion channels and neurotransmitter receptors, and may provide a vehicle for the study of cell-specific modulation of channel function. Support: GM-10991 & GM-29836 (NIH) and The Cystic Fibrosis Foundation.

139.10

CLONING AND EXPRESSION OF DELAYED RECTIFIER K^+ CHANNELS FROM RAT BRAIN AND SOLEUS MUSCLE. G. Koren*, E.R. Liman*, D.E. Logothetis*, P. Hess*, B. Nadal-Ginard* (SPON: M. Plummer). Dept. of Cardiology, Childrens Hospital, Dept. of Physiology and Program in Neuroscience, Harvard Medical School, Boston, MA. 02115.

Using oligonucleotide probes from published rat brain potassium channels we have isolated a clone from a rat brain cDNA library that is identical in the coding region to RCK1 (Baumann et. al., EMBO 7:2457, 1988), but differs in the 5' untranslated region. The S4-S6 region of this clone was used to screen a rat soleus cDNA library. High stringency hybridization resulted in the isolation of 3 clones. Partial sequencing and restriction map analysis indicated that these clones are most likely to be identical to the brain clone. Injection of in vitro transcribed mRNA from one of the clones (RSK1) into xenopus oocytes resulted in the expression of a delayed rectifier K^+ current, which was never observed in uninjected oocytes. Low stringency hybrization of the same cDNA library resulted in the isolation of 2 different clones that are 80% homologous at the nucleotide level to the rat brain cDNA clone.

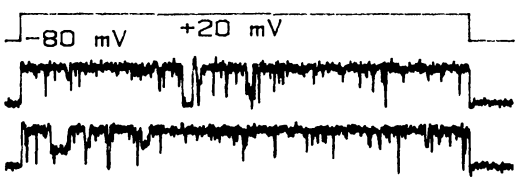
These results suggest that both brain and muscle express identical delayed rectifier K+ channels.



139.11

STABLE EXPRESSION IN A MAMMALIAN MUSCLE CELL LINE OF A CLONED DELAYED RECTIFIER K^+ CHANNEL FROM RAT BRAIN . E. Liman*, G. Koren*, B. Nadal-Ginard*, P. Hess* (SPON:M. Segal). Dept. of Physiology and Program in Neuroscience, Harvard Medical School, and Dept. of Cardiology, Childrens Hospital, Boston, MA. 02115.

Mouse SOL 8 cells were transfected with a plasmid containing a brain K^+ channel identical to RCK-1 (Baumann et. al., 1988) under the control of a mouse metallothionein-I promoter. The colonies were cloned by limited dilution. Out of seven colonies examined, one expressed large delayed rectifier type potassium currents (up to 178 pA/pF at 0 mv) that differed both in their magnitude and kinetics from currents seen in control cells (n = 20) The current was potassium selective, exhibited steady state inactivation at potentials greater than -50 mv, and was sensitive to Charybdotoxin. Single channels recorded in the cell attached configuration from this cell clone (5mM KCl in the pipette) had a slope conductance of 14 pS, activated steeply with the boltzman parameters of V1/2 = -50mV and k = 5 mV, and displayed multiple sub conductance levels. Periods of channel opening were punctuated by long intervals (upto several minutes) when the channel did not open.



139.12

K CHANNEL mRNA REGULATED BY ESTROGEN IN MYOMETRIUM MAY BE UNRELATED TO SHAKER FAMILY. M. Pragnell*, L.K. Kaczmarek, and M.B. Boyle. Dept. of Physiol. & Biophys., Univ. of Iowa, Iowa City, IA 52242 and Depts. of Pharmacol. & Physiol., Yale Univ., New Haven, CT 06510.

The enhancement of myometrial excitability by chronic exposure to estrogens may involve the regulation of K channel mRNA. A very slow voltage-dependent K current is expressed in Xenopus oocytes injected with myometrial RNA from estrogen-treated but not estrogen-deprived rats. Injection of size-fractionated RNA suggested that an mRNA species similar in size to the 18S ribosomal RNA, about 2 kb, was responsible for the expression of the slow K current. We now report the results of Northern blot analysis with probes showing sequence homology with the Shaker potassium channel clones. A rat brain cDNA clone (K41) detected mRNA species of about 3 and 6 kb from 12-day-old rat brain. Bands at 3 kb only were apparent in the lanes containing uterine RNA. This brain cDNA clone has previously been used to isolate a small uterine cDNA clone found to be identical in sequence with the 3' end of the brain clone. Although these results are consistent with the identification of homologous ion channel mRNA species in the brain and myometrium, the absence of the 6 kb band from uterus implies some difference in the regulation of expression. The intensity of the 3 kb bands did not differ substantially between uterine RNA from estrogen-treated and estrogen-deprived rats. Our results suggest that the mRNA species regulated by estrogen is unrelated to Shaker. We are now testing the hypothesis that the estrogen-regulated uterine mRNA species may be similar to a small kidney mRNA species (Takumi et al., Sci. 242:1042) which is capable of inducing a very slow K current in RNA-injected oocytes.

140.1

SYNCHRONIZATION OF INHIBITORY POTENTIALS IN THE NEOCORTEX IS RESISTANT TO EXCITATORY AMINO ACID BLOCKERS. J.A. Aram* & R.K.S. Wong. Dept. of Neurology, Columbia University, N.Y. N.Y. 10032.

Excitatory synaptic transmission can be blocked in neocortical slices by bath application of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 5-20uM) and D-2-aminophosphonovaleric acid (AP5; 10-30uM). In these conditions 4-aminopyridine (4-AP; 30-100uM) elicited spontaneous membrane depolarizations which occurred at a frequency of 2-4 min^{-1}. These events represented synaptic potentials resistant to CNQX and AP5 as they were reversibly blocked in low Ca^{2+} perfusate (0.5mM Ca^{2+}/10mM Mg^{2+}). Application of bicuculline or picrotoxin (10-25uM) reversibly abolished the synaptic potentials. In contrast phaclofen (1mM), a $GABA_B$ antagonist, had little effect on the occurrence or time course of the event. Varying membrane potential revealed two components of the event with reversal potentials of -68mV and -75mV. These results suggest that the synaptic potentials resistant to excitatory amino acid blockers were elicited via the activation of GABAergic interneurons.

At RMP (mean =-73mV) the synaptic potentials were 6-15mV in amplitude and lasted 1-3 seconds. These values are significantly larger than those of unitary i.p.s.p.s. Two actions of 4-AP are possible. Firstly 4-AP causes bursting and enhanced release in individual inhibitory neurons to bring about these synaptic events. Or secondly 4-AP uncovers a synchronizing mechanism for GABAergic interneurons that is not dependent on glutamatergic synapses. Our data suggests that the events were synchronized as (i) coupling of potentials existed between two recording locations of up to 3mm apart, and (ii) stimulation of the white or grey matter evoked similar events in an all-or-none manner with variable latencies close to threshold. The results suggest that functional coupling may exist between inhibitory neurons that is resistant to excitatory amino acid blockers and that 4-AP enhanced such coupling to bring about spontaneous synchronized potentials.

140.2

ECTOPIC ACTION POTENTIAL GENERATION INCREASES WITH KINDLING -LIKE INDUCTION OF ELECTROGRAPHIC SEIZURES IN VITRO. S. F. Stasheff and W. A. Wilson. Depts. of Pharmacology and Medicine, Duke Univ. and V.A. Medical Centers, Durham, N.C.

We have previously described an in vitro model of kindling-like epileptogenesis in which electrographic seizures (EGSs) are induced in rat hippocampal slices. We now report that EGS induction is accompanied by a marked increase in the occurrence of a novel type of action potential which arises sharply from the baseline membrane potential, without preceding depolarizing potentials. We call such action potentials "baseline spikes."

EGSs were induced in rat hippocampal slices with repeated stimulus trains. Twenty-seven CA3 pyramidal cells were continuously recorded before, during and after the induction of EGSs. Baseline spikes were present in ten of these cells (37 %) prior to EGS induction, and in 23 cells (85 %) after induction. Furthermore, in 21 of these 23 cells displaying baseline spikes (91 %), the frequency of baseline spike firing after EGS induction was markedly greater than before induction.

In a number of cells, the soma was hyperpolarized by 10 to 20 mV, to reveal any underlying depolarizing potentials which might be difficult to detect near resting membrane potential. Baseline spikes continued to occur, without such prepotentials. This lack of preceding depolarizations, as well as the inability of soma hyperpolarization to block baseline spikes, suggest that baseline spikes are generated at an "ectopic" site remote from the soma.

140.3

MODULATION OF KAINIC ACID-INDUCED BEHAVIORAL SEIZURES IN RAT PREPIRIFORM CORTEX BY ADENOSINE RECEPTOR ACTIVATION AND ANTAGONISM. G. Zhang*, P. H. Franklin and T. F. Murray. College of Pharmacy, Oregon State University, Corvallis, OR 97331.

We have previously shown that adenosine receptors in rat prepiriform cortex (PC) play a fundamental role in suppression of seizures induced by bicuculline (Franklin, P. H. et al., Eur. J. Pharmacol., 150:207, 1988). Kainic acid is a neurotoxicant which stimulates glutamate and aspartate release via presynaptic receptors in CNS (Ferkany, J. W. et al., Nature, 298:757, 1982). The aim of the present study was to investigate the possibility of adenosine receptor involvement in the modulation of seizure activity elicited by kainic acid in the PC.

Kainic acid (100, 150 and 200 pmol/rat) microinjected unilaterally into the rat PC evoked bilateral motor seizures. Co-injection of the adenosine receptor agonist N-ethylcarboxamidoadenosine (NECA) afforded protection against kainic acid (200 pmol)-induced seizures in the rat PC. The ED_{50} value for the anticonvulsant effect of NECA was 25.6±2.1 pmol/rat. In addition, this seizure suppressant effect of NECA (81 pmol) was completely abolished by the adenosine receptor antagonist 8-p(sulfophenyl)theophylline (8pSPT) indicating adenosine receptor activation underlies the seizure suppressant action of NECA. Moreover, the severity of seizures induced by kainic acid (100 pmol/rat) was significantly enhanced by co-administration of 8pSPT (1.6 nmol) as compared to kainic acid alone. Taken together, the anticonvulsant effect of NECA and proconvulsant effect of 8pSPT on kainic acid-induced seizures support the involvement of adenosine receptor modulation of presynaptic excitatory amino acid release in the observed anticonvulsant actions of adenosine analogs in the prepiriform cortex. (Supported by HHS Grant NS23227).

140.4

DENTATE GRANULE CELL NEUROPHYSIOLOGY IN HUMAN EPILEPTIC HIPPOCAMPUS *IN VITRO*. Masako Isokawa-Akesson[1], David M. Finch[1], Giuliano Avanzini[2], Michel F. Levesque[1], and Thomas L. Babb[1] [1]Brain Research Institute and Dept. of Neurology & Neurosurgery, UCLA, Los Angeles, CA 90024, USA and [2]Istituto Neurologico C. Besta, Milano 20133, Italy.

Neuronal excitability was studied in hippocampal slices prepared from human epileptic patients who underwent surgical treatment for intractable seizures. Intracellular recordings were obtained from dentate granule cells. Satisfactory penetration provided the average resting membrane potential of -51.8 mV ± 0.67 SD, the average spike amplitude of 62.7 ± 16.4 mV and the average membrane resistance of 43.9 ± 1.98 MΩ based on I-V curves. The recordings showed features such as spontaneous EPSPs and depolarizing afterpotentials that are characterisitic of these cells. Perforant path stimulation commonly produced EPSP-IPSP sequences, and the IPSPs lasted about 300 msec. In 2 additional neurons (ave.spike amp.: 30.8 mV, ave.rest.mem.pot.: -37.9 mV), giant EPSPs (>15 mV, >150 msec) were observed upon perforant path stimulation. Application of APV (100 μM) reduced the amplitude of the EPSP in 10 min and eliminated them in 20 min, indicating that NMDA receptors were involved in their generation. Histochemical analysis of the tissue showed that the giant EPSPs were always accompanied by mossy fiber sprouting. This suggests that sprouting may be a morphological substrate for temporal lobe epilepsy, and that it may play a role in inducing abnormal NMDA responsivity. Further studies of the correlates of dentate hyperexcitability are in progress. Supported by NIH Grant NS 02808.

140.5

LAMINAR INTERACTIONS IN RAT MOTOR CORTEX DURING CYCLICALLY OCCURRING SEIZURE BURSTS

DANIEL S. BARTH[1,2], CHRISTOPH BAUMGARTNER[1,4] and SHI DI[1,2,3] *Departments of [1]Neurology and [2]Psychology, University of California, Los Angeles, Los Angeles, CA 90024 (U.S.A), [3]Mental Health Institute, Beijing Medical University (China) and [4]Neurological University Clinic, Vienna (Austria)*

Laminar interactions between neurons in rat motor cortex during cyclical seizure episodes in the penicillin focus were studied using a combination of current source-density (CSD) and principal component analysis (PCA), combined with computer based physical modeling. These data suggest that polyspike bursts (PSB) during seizures are produced by interactions between two distinct populations of neurons, the same neuronal circuits previously reported to give rise to the direct cortical response (DCR) and electrically evoked interictal penicillin spikes (EIIS). The first population consists of small pyramidal cells in the supragranular layer, and the second population consists of larger pyramidal cells in the infragranular layers with apical dendrites extending to the cortical surface. The supragranular cells serve as a trigger zone for initiating PSB. Fast activity in the supragranular cells is typically followed by a hyperpolarizing slow wave that may be the result of Ca^{++} activated K^+ currents. This slow wave increases during seizures, possibly reflecting changes in Ca^{++} associated with seizure onset and termination. The response of infragranular cells is similar in polarity and morphology to the intracellularly recorded paroxysmal depolarization shift (PDS) and may indicate that these deeper neurons are mainly responsible for this phenomena in neocortex.

140.6

SEIZURE AND OSMOLALITY: OF SLICE AND MEN. R.D. Andrew. Dept. of Anatomy, Queen's University, Kingston, Ontario. K7L 3N6.

Over 60 years ago it was found that antidiuretic hormone (ADH) administration and water loading promoted grand mal seizure in patients and experimental animals. Rapid reduction of plasma osmolality (normally 287 mOsm/kg) can also induce seizure in several clinical situations. 1) Compulsive water drinking occurring in 8-17% of schizophrenics; ADH levels are elevated. 2) The syndrome of inappropriate (elevated) ADH secretion presenting with hyponatremia. 3) Dialysis disequilibrium syndrome, where rapid urea clearance shifts water from plasma to brain. 4) Rapid rehydration of dehydrated patients, again causing a shift in water from plasma to brain. In all cases, raising plasma osmolality inhibits seizure onset.

It is not clear how overhydration and seizure are related. We studied this question using hippocampal and neocortical brain slices from rat and found that hyposmolar saline (240-270 mOsm) promotes electrographic seizure in slices bathed in zero-Mg^{2+} saline by a) increasing excitatory postsynaptic potential (EPSP) amplitude as measured intracellularly, whether spontaneous or evoked and b) increasing population spike amplitudes and so presumably, field (ephaptic) effects. Both responses are inversely proportional to osmolality and are not the result of Na^+, Ca^{2+} or Mg^{2+} dilution in the hyposmotic media. Hyposmolality has no effect on intrinsic neuronal properties in rat cortex; rather it appears to strengthen excitatory interactions among neurons. Direct osmotic effects, rather than brain injury caused by swelling or edema, can help explain the clinical relationship between overhydration and seizure noted above.

Supported by the Medical Research Council of Canada.

140.7

PHENOTYPIC CHANGES OF HIPPOCAMPAL NEURONS AND DYNORPHIN IN HUMAN EPILEPSY. N.C. de Lanerolle, S. Sundaresan*, M.L. Brines* and D.D. Spencer. Sections of Neurosurgery and Neuroendocrinology, Yale Univ. Sch. Med., New Haven, CT 06510.

The imunocytochemical distribution of dynorphin-A (DYN) was examined in surgically removed hippocampi of patients with medically intractable tumor associated (TTLE) and cryptogenic or non-tumor related (CTLE) temporal lobe epilepsy. In TTLE, DYN immunoreactivity was in the somata of dentate granule cells and in their mossy fiber axons which terminate on CA4 and CA3 neurons. There was no immunoreactivity in the dentate molecular layer (ML). This pattern is similar to that reported in non-epileptic human tissue and animals. In the CTLE hippocampi DYN was localized within the somata of granule cells and mossy fiber axons/terminals as in TTLE. Additionally, DYN immunoreactivity was observed in two new locations. (1) There was a dense band of immunoreactivity confined to the inner part of the ML, perhaps reflecting axon collateral "sprouting" from granule cells into this region. Such collateral sprouting has been reported in animal models of epilepsy (Science 239:1147, 1988). (2) Immunoreactivity was present within the cell bodies of large hilar neurons. Such neurons were present in TTLE but did not manifest DYN. In situ hybridization using anti-sense [35S]-prodynorphin mRNA oligonucleotide probe has been employed to determine the potential of hilar neurons in TTLE to express DYN. These changes in CTLE may be associated with increased excitability of hippocampal neurons if DYN functions as an excitatory neurotransmitter (Life Sci. 31: 1785, 1982).

140.8

A HUMAN DIALYTRODE: IN VIVO MEASUREMENTS OF NEUROACTIVE SUBSTANCES IN THE HUMAN HIPPOCAMPUS WITH SIMULTANEOUS DEPTH EEG RECORDINGS. M.J. During, G.M. Anderson*, R.H. Roth, D.D. Spencer. Departments of Neurosurgery, Pharmacology, Psychiatry and Laboratory Medicine, Yale School of Medicine, New Haven, CT 06510.

A combination microdialysis probe and depth electrode for human use was developed and characterized in vitro. Probes were stereotactically implanted (using MRI-derived coordinates) into the left and right hippocampi of patients with intractable epilepsy. The dialytrode enabled simultaneous monitoring of the depth EEG with hippocampal neurotransmitter release and ECF composition. During four seizures, in which microdialysis perfusion was pooled at 3 min intervals, neuroactive amino acid concentrations were elevated. GLU increased by 77±25% (basal levels 0.59±0.05μM); ASP increased by 43±26% (basal levels 1.58±0.13μM); TAU increased by 61±17% (basal levels 0.16±0.03 μM); both SER and GLN levels were unchanged. In addition, interictal spike activity correlated with dialysate lactic acid concentration - a measure of local glucose metabolism. Hippocampal ECF levels of anticonvulsants were detectable and showed pharmacokinetics similar to the plasma compartment. This technique makes possible the quantification of the neurochemical concomitants of regional brain activity, and a means to determine basal, diurnal, ictal and stimulated neurotransmitter release, glucose metabolism, ionic concentrations and drug monitoring. Implantation of bilateral probes enables comparison of the in vivo neurochemistry of the lesion (epileptic focus) versus nonlesion side in selected patients.

140.9

LAMINAR AND CIRCUMFERENTIAL ALTERATIONS IN INHIBITORY AND EXCITATORY NEUROTRANSMITTER RECEPTOR DENSITY IN THE DEVELOPING EPILEPTIC FOCUS USING THE ALUMINA PRIMATE MODEL. RAE Bakay, MS Fiandaca, D Levy*, GP Brinkley* & CM Epstein*. Yerkes Regional Primate Research Center of Emory Univ. & Veterans Admin. Medical Center, Atlanta, GA 30322.

Fourteen juvenile monkeys (M. mulatta) received alumina injections into the left pre-central gyrus to produce a focal motor epilepsy. Electroencephalographic recordings were used to determine the stage of epileptogenisis of the subject prior to sacrifice. Four pre-epileptic animals with only slowing on EEG were sacrificed from 2 to 4 weeks following alumina injection. Two of the subjects demonstrated an increase in the number of muscimol (MUS) and flunitrazepam (FNZP) receptors in the area adjacent to the alumina. Four acutely epileptic subjects (seizures < 1 wk) demonstrated loss of multiple neurotransmitter receptors in the epileptic focus. Six mature epileptic animals (seizures > 1 mo) demonstrated further loss of receptors but only the MUS and FNZP receptors were reduced to background. Although the total number was decreased, the relative density of glutamate receptors was increased in the area of the epileptic focus. A relative increase in MUS receptors in the cortex adjacent to the epileptic focus was suggestive of an "inhibitory surround." Saturation binding studies indicate that the changes in the focus are due to loss of receptors without significant change in affinity. This work was supported by the Medical Research Service of the Veterans Admin. and the Yerkes NIH Core Grant (RR-00165).

140.10

SLOW DEPOLARIZATIONS FOLLOWING PENICILLIN-INDUCED PAROXYSMAL DEPOLARIZATIONS IN THE MOTOR CORTEX

O.W. Witte, S. Uhlig* and E. Valle*. Neurologische Universitätsklinik, Moorenstr. 5, D-4000 Düsseldorf, F.R. Germany

Depolarizing afterpotentials of penicillin-induced paroxysmal depolarizations (PDS) were further investigated in the present study. The experiments were performed on the motor cortex of the rat in vivo using standard intracellular recording techniques.

Neurones with high resting membrane potential (-84.9 mV, n=21) displayed slow afterdepolarizations with a duration of 1800 ±200 ms. Similarity of duration, time course and changes with epicortical stimulation to extracellular K^+ accumulation suggested that these afterpotentials were caused by K^+ accumulations in the extracellular space.

Reduction of neuronal K^+ conductance by intracellular injection of large amounts of Cs^+ revealed afterdepolarizations which peaked 700 ms following PDS onset and increased in amplitude with membrane depolarization. The potential dependence was caused by membrane rectification.

It is concluded that in the penicillin focus in vivo excitatory postsynaptic potentials as well as passive membrane depolarizations caused by extracellular K^+ accumulation can be demonstrated to follow the PDS.

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140.11

$GABA_A$- AND $GABA_B$-DEPENDENT IPSPs FOLLOWING PENICILLIN-INDUCED PAROXYSMAL DEPOLARIZATIONS IN THE HIPPOCAMPAL SLICE

R. Domann*, T. Dorn* and O.W. Witte (SPON: ENA). Neurol. Klinik, Univ. Düsseldorf, Moorenstr. 5, D-4000 Düsseldorf, F.R.Germany

Penicillin-induced paroxysmal depolarizations are followed by pronounced afterhyperpolarizations caused by synaptically and intrinsically evoked inhibitory potentials. In this study the participation of inhibitory postsynaptical potentials (IPSPs) was further analyzed.

The study was performed on slices of rat hippocampus, using standard preparation techniques. Intracellular recordings were obtained from 47 CA1 pyramidal cells.

The afterhyperpolarization lasted 2100 ms (S.D.=300, n=21). An early component of about 400 ms duration was Cl^--dependent as revealed by intracellular injection of KCl. Bath application of the $GABA_A$-antagonist bicuculline blocked this component. A second component of the afterhyperpolarization which lasted for about one second was blocked by bath application of the $GABA_B$-antagonist phaclofen. The reversal potential of this component indicated K^+-dependence.

It is concluded that penicillin-induced paroxysmal depolarizations are followed by $GABA_A$- and $GABA_B$-mediated IPSPs. The $GABA_A$-mediated IPSP in the penicillin superfused slice is larger than that in normal tissue.

This work was supported by grants DFG Wi 830 1-1, SFB 200 C9 and Ministry for Education and Science NRW IV B5-400 04286.

140.12

CONNEXIN mRNAs ARE ELEVATED IN EPILEPSY. C.C.G. Naus and J.F. Bechberger*. Dept. of Anatomy, University of Western Ontario, London, Canada, N6A 5C1.

To determine a possible role for gap junctions in epilepsy, the level of mRNA coding for the gap junction proteins, connexin32 and connexin43, was examined in RNA isolated from cerebral tissue samples obtained at the time of surgical resection for the treatment of intractable seizure disorder. Pathological examination revealed mild gliosis and cytoarchitectural changes such as ectopic neurons and focal neuronal loss. No neoplastic characteristics were found in these samples. Northern blot analysis of total cytoplasmic RNA isolated from these samples indicated a much higher level of the connexin43 mRNA in the seizure tissue than in similar peritumoural tissue obtained during surgical removal of cerebral tumours. In contrast, there was only a slight increase in the level of connexin32 mRNA in the epileptic tissue.

In order to examine the expression of gap junction mRNA at the cellular level, we are pursuing in situ hybridization and immunocytochemistry on sections of similar brain tissue.

Supported by MRC and Ministry of Community and Social Services Lottery Grant Program, Ontario Mental Health Foundation.

141.1

AN ANIMAL MODEL OF GLOBAL ISCHEMIA IN THE MONKEY: NEUROPATHOLOGICAL AND BEHAVIORAL FINDINGS. S.Zola-Morgan, L.R. Squire, D.G. Amaral, J.E. Fleischer,* M.S. Scheller,* and M.H. Zornow.* V.A. Medical Center, San Diego, Dept. of Psychiatry, UCSD School of Medicine, La Jolla, CA 92093, Salk Institute, La Jolla, and Dept. of Anesthesiology, UCSD School of Medicine, La Jolla.

Patient R.B. exhibited marked anterograde amnesia following an ischemic episode. The principal histological finding was a bilateral lesion involving the entire CA1 field of the hippocampus. We have developed a model of global ischemia in the monkey using pharmacologically-induced hypotension in combination with abrupt inflation of a neck cuff. Six cynomolgus monkeys were subjected to ischemia lasting 14-17 min. Following a two-week survival, neurohistological examination revealed bilateral symmetrical neuronal loss within the CA1 field of the hippocampus. Cell loss was most extensive at caudal levels of the hippocampus. There was also substantial loss of somatostatin-staining cells in the dentate gyrus. In four additional monkeys the cognitive effects of a 15-min period of ischemia were evaluated. On delayed non-matching to sample, the ischemic monkeys performed normally at delays of 8s and 60s, but exhibited a marked impairment at a delay of 10 min. This model makes it possible to compare the severity of memory impairment associated with ischemia to the memory impairment associated with surgical lesions of the medial temporal region.

141.2

REDUCED SIZE OF HIPPOCAMPAL FORMATION IN AMNESIC PATIENTS: A MAGNETIC RESONANCE IMAGING STUDY. L.R. Squire, D.G. Amaral, G. Press. VA Med. Ctr. San Diego, CA, Dept. Psychiatry, UCSD, LaJolla, CA, Salk Institute, La Jolla, CA and Dept. Radiology, UCSD, La Jolla, CA.

Neuropathological study of a well-characterized amnesic patient (R.B.) demonstrated that damage limited to the hippocampus can cause a clinically significant memory impairment. This case, together with a few others, provides the only available information about the anatomy of amnesia in humans. In an effort to obtain improved anatomical information from living patients, we developed a new high-resolution protocol for imaging the human hippocampus. The images were obtained with a 1.5 T magnet using a T1-weighted sequence (TR=400, TE=20, 6 NEX, FOV=16, matrix = 256x256). Six interleaved, 5mm-thick slices were obtained in the coronal plane, with the patient's head tilted posteriorly so that the hippocampus was imaged precisely perpendicular to its longitudinal axis. In normal subjects, the hippocampal formation could be visualized in considerable cytoarchitectonic detail. Specifically, it was possible to identify the pyramidal cell layer of the hippocampus and the subiculum, the perforant path, the fimbria, the alveus, and the stratum lacunosum-moleculare. In three amnesic patients, one who had suffered a respiratory arrest, and two of unknown etiology, the area comprising the dentate gyrus, the hippocampus, and the subiculum was markedly reduced bilaterally. In the patients this area was 49% of the corresponding area in age-matched control subjects ($t[5]=5.3$, $p<.01$). The size of the temporal lobe was the same in the two groups. These findings provide the first magnetic resonance evidence from amnesic patients of bilateral medial temporal lobe damage. The method provides a means for classifying memory-impaired patients independently of their behavioral test scores and a way to understand quantitative differences among patients.

141.3

THE STRUCTURES DAMAGED IN AMNESIA CONTRIBUTE SIMILARLY TO RECALL AND RECOGNITION MEMORY. F. Haist, A.P. Shimamura, and L.R. Squire, VA Med. Ctr., and Dept. of Psychiatry, UCSD, La Jolla, CA 92093.

The rate of forgetting in recall and recognition memory was assessed in seven patients with Korsakoff's syndrome, six other amnesic patients, and two groups of control subjects. Subjects participated in 12 separate learning/test sessions, each of which involved a different study list of 20 words and a subsequent retention test. Six of the 12 sessions assessed free recall followed by cued recall from 3-letter stems, and 6 sessions assessed recognition memory using a two-alternative, forced-choice procedure. The retention interval between word-list learning and testing ranged from 15 s to 2 weeks for amnesic patients, and from 15 s to 8 weeks for control subjects. Patients with Korsakoff's syndrome and non-Korsakoff amnesic patients were impaired on all 3 tests: recognition, recall, and cued recall. In order to compare the severity of impairment in recall and recognition memory, recognition memory scores were equated between amnesic patients and control subjects by matching shorter retention intervals for amnesic patients to longer retention intervals for control subjects. When levels of recognition memory were equated in this way between amnesic patients and control groups, levels of recall were also virtually the same for amnesic patients and control subjects. The results support the conclusion that recall and recognition memory are linked deficits of declarative memory that reflect damage to the same functional system.

141.4

INTACT LEARNING OF SPECIFIC TEXT BY AMNESIC PATIENTS AS MEASURED BY READING SPEED G. Musen*, A. P. Shimamura and L. R. Squire, V.A. Med. Ctr., and Dept. of Psychiatry, UCSD, La Jolla, CA 92093

Amnesic patients exhibit preserved learning abilities in certain skill-based tasks such as the reading of mirror-reversed words. It has been unclear whether this learning ability reflects a general practice effect or a specific learning of the particular words read. Efforts to address this question have been complicated by differences in baseline reading speed between memory-impaired and normal groups. We measured reading speed for passages of text in 8 amnesic patients (four with Korsakoff's syndrome and four other amnesic patients) and 9 age-matched control subjects. Subjects read a text (20 lines) three consecutive times and then read a different text three times. The order of the two texts was counterbalanced across subjects. The amnesic patients exhibited the same initial reading speed as the control subjects (70s vs. 68s) and decreased their reading speed across the three trials at a similar rate. Importantly, on the first reading of the second passage, the reading speed of both groups returned to the same level that had been achieved on the first reading of the first passage. Finally, the amnesic patients were impaired in answering questions about the content of the passages. There was no difference in the reading speed performance between patients who performed well on the comprehension test and patients who performed poorly. These results show that improved reading speed in amnesic patients is specific to the words and to the associations between words that occur in particular texts. Thus, facilitatory effects of previous exposure to specific items can be demonstrated in skilled behavior independently of declarative memory and independently of the structures damaged in amnesia.

141.5

DIRECT CONNECTIONS FROM HIPPOCAMPUS TO PREFRONTAL CORTEX: A TOPOGRAPHIC STUDY IN CAT AND MACAQUE MONKEY. C. Cavada and F. Reinoso-Suárez. Dept. Morfología, Fac. Medicina, Univ. Autónoma de Madrid, Spain.

In the course of a broader study on the connectivity of the prefrontal cortex (PFC), we have uncovered the existence of heavy direct projections between two structures involved in memory function, the hippocampus and the orbitofrontal sector of PFC.

Injections of the retrograde tracers HRP, Fast Blue and Diamidino Yellow were placed in various sectors of PFC in cats and macaque monkeys. Large numbers of retrogradely labeled neurons were observed in the hippocampus ipsilateral to the injections only following orbitofrontal cortex deposits. The labeled neurons were located principally in field CA1. In cat, the rostral two-thirds of the hippocampus contain 75% of the labeled neurons. In macaque, however, over 70% of hippocampal cells projecting to orbitofrontal cortex are located in the rostral third.

A double labeling experiment carried out in both hemispheres of a macaque monkey revealed that Walker's cytoarchitectonic areas 14 and 10-11 are the targets of hippocampal projections. Interestingly, ~60% of the cells that project to caudomedial area 14 are located in the rostral third of the hippocampus, whereas the caudal third contains ~60% of the hippocampal neurons that project to rostal areas 10 and 11.

The prominent connectional links between the hippocampus and orbitofrontal cortex, and their remarkable topographic specificity open new questions regarding the neuronal circuitry involved in memory processing.

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141. 6

AUDITORY-VISUAL ASSOCIATIONS, HEMISPHERIC SPECIALIZATION, AND TEMPORAL-FRONTAL INTERACTION IN THE RHESUS MONKEY. D. Gaffan and S. Harrison*. Dept. Exptl. Psychology, Oxford University, OX1 3UD, England.

The left superior temporal gyrus (STG) is more important than the right STG for audition in the monkey. The prefrontal cortex (PFC) is important for cross-modal association. The left PFC is specialized for language in man. Therefore, we investigated the involvement of the left and right PFC in auditory-visual association in the monkey. Each of 6 noises was the instruction to choose one of 6 visual stimuli. Six normal animals achieved a performance level of 12.5 percent errors in 2-choice trials (chance = 50 percent) and were divided into 2 groups for surgery. Group 1: unilateral right PFC ablation left error rate nearly unchanged, at 13 percent. Addition of a left unilateral STG ablation to this group reduced their performance nearly to chance. Group 2: unilateral left PFC ablation doubled error rate, to 24 percent. Addition of right STG ablation produced no further impairment. Further addition of forebrain commissurotomy reduced their performance nearly to chance. Thus, auditory-visual association requires a temporal-frontal cortical interaction for which the left hemisphere is specialized, and the forebrain commissures are important in recovery from left prefrontal lesions.

141.7

EXPERIMENTAL MEMORY CHANGE AFTER FRONTAL AND TEMPORAL LOBE LESIONS. P.J Eslinger and L.M. Grattan,* Neurobehavioral Lab, Brown Univ. Rhode Island Hospital, Providence, R.I.

We probed the nature of visual memory processing after brain injury with an experimental technique that may influence organization and encoding of information. Nineteen adult patients with focal frontal and temporal lobe lesions were studied and compared to 7 patients with focal posterior (parietal-occipital) lesions. Each subject copied complex figures in standard format (self-directed copy of full figure) and experimental format (examiner-directed sequential presentation of elements comprising figure). Stimuli and formats were randomly chosen for 2 sessions separated by 48 hours. Twenty minute delayed visual recall (measured relative to initial copy scores and treated as "percentage recall from copy") indicated that memory was more accurate under experimental conditions for the following lesion groups: inferior, mesial frontal, right dorsolateral frontal, and temporal lobe. The experimental format led to dissociations between superior and inferior mesial frontal groups, and between left and right dorsolateral frontal groups.

Findings indicate that the system of cerebral structures subserving visual memory are selectively amenable to sequential stages for perceiving, encoding and retaining complex visual information. Results are discussed within a framework of how experimental formats of information processing may affect visual memory. Supported in part by NINDS Grant NS 26985 Memory Disorder Research Center.

141.8

MONKEYS DEMONSTRATE HIGH LEVEL OF RECOGNITION MEMORY IN DELAYED NON-MATCHING TO SAMPLE WITH RETENTION INTERVALS OF 6 WEEKS. R.C. Saunders, NIMH, Washington, D.C. 20032.

In humans, long term retention of visual 'habits' and 'memories' has been well documented even with delays of many weeks. In monkeys, while long term retention of 'habits' has been readily demonstrated, high level long term retention of 'memories' has not. The present study assessed long term recognition memory in monkeys using a modified delayed non-matching to sample task. Rhesus monkeys were presented two identical sample objects over two lateral wells and allowed to view them for 30 s. After a 10 s interval the sample object was presented together with a new object for a total of 20 s. Recognition memory was assessed by comparing the viewing time of the novel object with that of the more familiar sample object. Because monkeys are attracted to novel stimuli they tend to view the novel object longer than the previously presented sample object at the retention test. For long retention intervals of 1 to 6 wks, the object to be remembered was presented as a sample object in 6 trials during 2 consecutive testing sessions. For the retention test the sample was presented only in a retention trial together with a novel object. Retention was assessed for delays of 10 s, 24 hrs, 48 hrs, 1 wk, 2 wks, 3 wks and 6 wks. Monkeys were capable of discriminating novel from familiar objects on nearly 80% of the test trials even at a delay of 6 wks.

141.9

EFFECTS OF RHINAL CORTICAL LESIONS ON VISUAL RECOGNITION MEMORY IN RHESUS MONKEYS. E.A. Murray, J. Bachevalier, and M. Mishkin. Lab. Neuropsychology, NIMH, Bethesda, MD 20892.

Earlier work had provided indirect evidence that the rhinal cortex (Rh) is a third temporal-lobe component of a limbic memory system (Murray et al., 1985, Soc. Neurosci. Abstr., 11: 461). In order to directly assess the contribution of the Rh to recognition memory, we have now examined the behavioral effects of removal of this region. Naive rhesus monkeys were trained on the trial-unique version of delayed nonmatching-to-sample (DNMS) with 10s between sample presentation and choice test until they attained the criterion of 90 correct responses in 100 trials. They then received an Rh lesion that included the entorhinal cortex, which occupies the medial bank of the rhinal sulcus and the ventral surface cortex medial to it, and the perirhinal cortex, which occupies the lateral bank of the rhinal sulcus. Following a 10-14 day recovery period, the monkeys were retrained on DNMS. When they reattained criterion, they were given a performance test that taxed their recognition memory by requiring them to remember either single objects for increasingly longer periods of time or a list of objects. Monkeys with Rh ablations required about 400 trials to relearn DNMS and attained a mean of 68% correct responses on the performance test whereas the controls relearned immediately and attained 92% correct responses. These preliminary results indicate that the Rh itself makes a critical contribution to recognition memory, either directly, by virtue of its connections with the neocortex and diencephalon, or indirectly, by relaying sensory information from modality-specific neocortical areas to the other medial temporal lobe limbic structures known to be important for memory.

141.10

CORTICAL AFFERENTS TO THE POSTERIOR PARAHIPPOCAMPAL GYRUS OF THE RHESUS MONKEY. I. INPUT FROM LIMBIC, MULTIMODAL AND UNIMODAL AREAS IN TEMPORAL, INSULAR, PARIETAL AND OCCIPITAL CORTICES. G.J. Blatt, D.L. Rosene and D.N. Pandya. Boston University School of Medicine, Department of Anatomy, Boston, MA, 02118 and Edith Nourse Rogers Memorial Veterans Administration Hospital, Bedford, MA 01730.

The posterior parahippocampal gyrus (PPHG) receives input from limbic, auditory, visual and somatosensory association cortices as well as from multimodal cortices. The present study investigated the organization of this cortical input to areas TH, TLc, TF, THO, TLO and TFO using injections of fluorescent retrograde tracers in five rhesus monkeys. Injections into area TH or THO labeled cells in the lateral part of the rostral bank of the superior temporal gyrus (auditory association areas TS2 and TS3), multimodal area TPO of the superior temporal sulcus (STS), visual area PGm, areas 23, 29, 30 of the cingulate gyrus (CG) and the insula. In contrast, injections in area TF or TFO in the medial bank of the occipitotemporal sulcus labeled cells in multimodal areas PGA, IPa and TPO and visual areas TEA and TEM in the STS, visual areas in the parietal and occipital lobes (areas PGm, 7a, LIP, 19, V3A and V4d), areas 23 and 29 of CG and in the agranular insula (AgI). In between TH and TF lies another area that we have designated TL. While it receives inputs from some of the same areas as TH and TF, the relative absence of cortical inputs is striking. Thus, while the medial (TH, THO) and lateral (TF, TFO) parts of the PPHG receive some similar cortical inputs, most originate from different limbic, unimodal or multimodal association cortices, e.g., medial PPHG receives unimodal input from auditory association areas while the lateral PPHG receives unimodal input from visual areas. It is interesting that different longitudinal strips of projection neurons in CA1 of the hippocampal formation differentially project to area TH, TL or TF. Taken together these data suggest that specific areas in the PPHG receive differential cortical and hippocampal inputs that may contribute to memory processes in the rhesus monkey brain. (Supported by NIH grants NS16841 and NS19416).

141.11

PROJECTIONS FROM INFERIOR TEMPORAL CORTEX TO WIDESPREAD PERIRHINAL AREAS IN INFANT MONKEYS. M.J. Webster, L.G. Ungerleider, and J. Bachevalier. Lab. Neuropsychology, NIMH, Bethesda, MD 20892.

Visual recognition memory depends on interactions of inferior temporal cortex (IT) with both the amygdala, via direct connections, and the hippocampus, via indirect connections through entorhinal cortex. Last year we reported a transient projection from area TEO (posterior IT) to the amygdala in infant monkeys. We now have evidence in the same animals for an exuberant but transient projection from area TE (anterior IT) to the perirhinal cortex. To investigate the development of IT-limbic connections and their relationship to the ontogeny of memory, we injected tritiated amino acids into area TE and WGA-HRP into area TEO, or vice versa, in a total of 6 adult and 4 infant rhesus monkeys. Previous studies have shown that area TE has access to the hippocampus via a two-stage pathway involving both perirhinal areas 35 and 36 and entorhinal area 28. Our results for the adult are consistent with those studies and indicate that neither area TE nor TEO is connected directly to area 28. In addition, we found area TE to be reciprocally connected to areas 35 and 36, primarily along the anterior extent of the rhinal sulcus. In contrast, area TEO does not project directly to areas 35 and 36, although it does receive a projection back from these areas. This asymmetry parallels the one shown for TEO-amygdala connections (Iwai and Yukie, '87). Projections in the infant monkey resemble those in the adult, although the projection from area TE to areas 35 and 36 is much more extensive in the infant, terminating along the entire length of the rhinal sulcus. These transient projections may become stabilized in infants with brain injury, thus explaining recovery of memory function in such cases.

141.12

LIMBIC-DEPENDENT MEMORY: EARLY OR LATE DEVELOPING? Adele Diamond, Psychology Dept, U of P, 3815 Walnut, Phila, PA

Success on Delayed Non-Match to Sample (DNMS) does not appear until late in infancy (4 months in rhesus monkeys, over 12 months in human infants). This has been taken as evidence that the limbic-dependent memory system matures late, as DNMS is dependent on hippocampal function. However, success on Visual Paired Comparisons (VPC), which is similar to DNMS, appears very early (by 15 days in monkeys, 2 months in humans). In both DNMS & VPC, the subject is presented a sample, a delay is imposed, then the sample is paired with a novel stimulus. On both tasks, memory of the sample is inferred from subject's choice of the novel (non-matching) stimulus. Performance on VPC is disrupted by hippocampal-amygdala lesions even at 15 days. Thus, while DNMS results suggest late maturation of limbic memory system; VPC results suggest early maturation.

Might success on DNMS appear late because of requirements of the task other than memory? In VPC, the subject looks at whichever stimulus is interesting; reward = the stimulus. In DNMS, the subject displaces the stimulus to retrieve the food reward beneath; reward = the food, not the stimulus. Perhaps the indirect, means-end response required by DNMS is what accounts for the late appearance of success on the task.

120 human infants were tested; 60 on VPC & 60 on DNMS. On both tasks the same trial-unique objects were used and the stimulus served as its own reward. Each subject received 2 trials at delays of 0, 10, 60, 180, & 600 sec. Infants of 6 & 9 months succeeded on DNMS & withstood delays on DNMS fully as long as those on VPC (180 sec at 6 mo & 600 sec at 9 mo). Conclusion: success on DNMS appears very early when subjects reach, not in order to obtain something else, but to obtain the stimulus itself. This is consistent with an early maturational timetable for limbic-dependent memory. (Supported by NIMH R01 MH/HD41842 & NIH BRSG S07-RR-07083.)

141.13

DIFFERENTIAL FORGETTING OF PAIRED ASSOCIATES, PROSE, AND FIGURAL STIMULI IN DEMENTIA. A. Tröster*, D. Jacobs*, D. Salmon, and N. Butters (SPON: P. Langlais). Dept. of Psychiatry, Univ. of California, San Diego, La Jolla, CA 92093 and VA Medical Center, San Diego, CA 92161.

Studies of forgetting rates in Alzheimer's disease(AD) disagree whether or not AD patients forget more rapidly than do normals (NC).These conflicting results may be due to different methodologies employed (i.e., repeated vs. single learning trials). This study compared forgetting rates of AD, Huntington disease (HD), and NC subjects on the WMS-R Verbal and Visual Paired Associates (VerPA and VisPA) (repeated trials), and Logical Memory (LM) and Visual Reproduction (VR) (single trials) tests.

AD and HD patients forgot LM and VR materials more quickly than did age and education matched NCs.LM and VR, but not VerPA and VisPA forgetting rates, differentiated AD and HD patients in both the early and later stages of the diseases. A discriminant function based on LM and VR savings scores correctly classified 84% of the 176 subjects. The addition of VerPA and VisPA savings scores to the discriminant function failed to enhance classification accuracy. These results suggest that the use of repeated learning trials may lead to overlearning in some patient groups and thereby obscure important differences among patient groups' forgetting rates. Supported by the Medical Research Service of the VA and by NIA grants AG-05131 and AG-08204.

141.14

PICTORIAL PRIMING AND WEIGHT BIASING IN DEMENTIA. W. Heindel*, D. Salmon and N. Butters. Depts. of Psychiatry and Neurosciences, Univ. of Cal., San Diego, La Jolla, CA 92093 and VA Medical Center, San Diego, CA 92161.

Alzheimer's Disease (AD) and Huntington's Disease (HD) patients have been shown to display different patterns of performance on motor skill learning and verbal priming tasks. The present study compares AD and HD patients on two other tests of "implicit" memory: (1) A pictorial priming test in which patients were asked to "say the first thing you think of" when shown incompletely drawn pictures; and (2) A weight biasing task in which subjects first were exposed to either a relatively heavy or a relatively light series of weights, and later were asked to rate the heaviness of a standard set of weights. HD patients, but not AD patients, demonstrated a normal increase in their ability to identify fragmented versions of previously seen pictures relative to novel pictures. In contrast, AD patients', but not HD patients', ratings of the standard set of weights were significantly influenced by the relative heaviness of the first series of weights. These results suggest that pictorial priming, like verbal priming, is dependent upon the neocortical association areas involved in the storage of semantic memory, whereas the biasing of weight judgments, like motor skill learning, may be dependent upon the integrity of the neostriatum. Supported by the Medical Research Service of the VA and by NIA grants AG-05131 and AG-08204.

mRNA REGULATION I

142.1

PRODYNORPHIN mRNA LOCALIZATION AND REGULATION IN THE RAT PITUITARY. R. Day, M.K.-H. Schafer*, S.J. Watson and H. Akil. Mental Health Res. Inst., Univ. of Michigan, Ann Arbor , MI, 48109-0720.

The presence of prodynorphin (proDyn) related opioid peptides in the pituitary is well established. By immunohistochemical and radioimmunological techniques, proDyn peptides were co-localized in gonadotrophes with LH and FSH. ProDyn peptides are also known to exist in the neurosecretory terminals of the posterior pituitary which originate in is the magnocellular cells of the hypothalamus. Our study has re-examined the distribution of proDyn in the pituitary by detection and quantification of its mRNA. *In situ* hybridization and Northern blot analysis were used in this study. The distribution of ProDyn mRNA in the anterior lobe as observed by *in situ* hybridization correlates with the previous observation of localization within gonadotrophes. In addition, we provide evidence that all the melanotrophes of the intermediate lobe contain proDyn mRNA. These are the same cells which are well known for their high abundance in proopiomelanocortin (POMC) peptides and mRNA. This co-expression of opioid precursors is unique and the functional significance needs to be further explored. By Northern gel analysis intermediate lobe proDyn mRNA is more abundant than anterior lobe proDyn mRNA, but proDyn mRNA from both tissues are the same size as proDyn mRNA from other sources such as hippocampus and striatum (~ 2.4 k base pairs). Since melanotrophes are known to be under dopaminergic control (POMC mRNA has been shown to be modulated by dopaminergic compounds), this study has also examined the effects of dopaminergic agonists and antagonists on proDyn mRNA in the intermediate lobe. The results will be discussed in the context of co-regulatory aspects with POMC. Supported by the Theophile Raphael Fund and NIMH grant MH 422251. R.D. is a fellow of the Medical Research Council (MRC) of Canada

142.2

NUCLEUS CAUDALIS PREPROENKEPHALIN mRNA: BIPHASIC ENHANCEMENT BY AFFERENTS. T. Nishimori*, G. Buzzi*, M. Moskowitz, and G.R. Uhl. NIDA/ARC and Depts. of Neurology & Neuroscience, JHUSM, Baltimore, MD 21224, and MGH & HMS, Boston, MA 02114.

Preproenkephalin mRNA in neurons of laminae I and II of the nucleus caudalis of the trigeminal may play an important role in modulating pain. Large- and small-caliber primary afferents may regulate the function of these segmental neurons. To investigate the impact of different inputs, we have used *in situ* hybridization to assess the influences of high- and low-intensity stimulation and capsaicin lesions of the trigeminal nerve on levels of preproenkephalin mRNA expression in nucleus caudalis neurons.

0.1 mA stimulation, activating large-caliber fibers, increases the number of neurons expressing preproenkephalin mRNA immediately after the end of 1 hour stimulation and at 6-24 hrs. Expression was less altered 2 hrs. following stimulation. 1.0 mA stimulation, activating both large and small caliber fibers, failed to increase the number of preproenkephalin expressing neurons until 6 hours following the stimulus in normal animals. Animals that were subjected to selective small-fiber capsaicin lesions displayed more hybridizing neurons both immediately and 6 hours after the end of 1.0 mA stimulation.

Primary afferent information can thus produce a time-dependent induction of preproenkephalin gene expression. A "rapid response" (0-1 hr) appears to be induced by large caliber inputs but suppressed by simultaneous stimulation of small caliber fibers. A "later response" (6 hr) is found when either large diameter or large- and small- caliber fibers are stimulated.

142.3

AGE-RELATED DECREASE IN PROOPIOMELANOCORTIN (POMC) GENE EXPRESSION IN THE ARCUATE NUCLEUS (ARC) OF THE MALE RAT. D. A. Gruenewald* and A. M. Matsumoto. VAMC, GRECC, and Univ. of Washington Sch. of Med., Seattle, WA 98108.

Hypothalamic β-endorphin (βE) activity has been hypothesized to increase with age, inhibiting gonadotropin releasing hormone (GnRH) and gonadotropin secretion. We determined the effects of aging on gene expression of the βE precursor POMC in male rats. Three 20μm coronal sections from the rostral ARC of 3-month (n=6) and 23-month old (n=4) F344 rats were anatomically matched, and POMC mRNA levels were quantitated by in situ hybridization, using a ^{35}S-labeled oligodeoxynucleotide probe complementary to a portion of rat POMC cDNA and a computerized image analysis system. The number of silver grains/cell and cells/ section were used as indices of cellular POMC mRNA content and number of neurons expressing the POMC gene, respectively. Results (mean ± SEM):

	Grains/Cell	Cells/Section
3-month old	73 ± 4	72 ± 6
23-month old	59 ± 3 (p<0.05)	57 ± 9 (p<0.20)

There was an age-related reduction in rostral ARC POMC mRNA content and a trend towards fewer neurons expressing the POMC gene. We conclude that in the male rat, aging is associated with reduced POMC gene expression in rostral ARC neurons, suggesting that age-related reduction in GnRH activity is not related to increased synthetic capacity of βE neurons.

142.4

TISSUE-SPECIFIC CHOLINERGIC MECHANISMS REGULATE PREPRO-ENKEPHALIN mRNA. J. DeCristofaro, G. Weisinger*, E. La Gamma Depts of Peds and Neurobio, Stony Brook, NY 11794-8111.

Adrenal enkephalin (ENK) peptide and prohormone levels increase 3-fold after combined cholinergic-nicotinic and muscarinic treatments (Neurosci, in press). To determine whether mRNA levels for prepro-ENK also increase and whether this effect is tissue-specific, groups of rats were treated for four days with nicotine (5 mg/kg sc q12h), oxotremorine (muscarinic agonist, 1 mg/kg sc q12h), both agents, or an equal volume of saline vehicle, or not treated (control). Twelve hours after the last injection, total RNA was extracted from the adrenal medulla and the brain striatum. Northern blots were performed using a 435 bp Pvu II fragment of pRPE2 (entire translated region of rat prepro-ENK). Northern blot analysis revealed that nicotine treatment increased adrenal prepro-ENK mRNA levels 20-fold over saline treated or control groups. Oxotremorine treatment resulted in a 50-fold rise and combined cholinergic treatment >100-fold rise. Striatal prepro-ENK mRNA increased only slightly (2-3 fold) with each of these treatments including saline. Saline treatment alone did not alter adrenal prepro-ENK mRNA levels. S1 analysis showed that these treatments alter transcription at the level of initiation (see Weisinger et. al. Neurosci abstr, 1989). These data suggest that cholinergic interactions regulate prepro-ENK mRNA in a tissue-specific manner and that transduction mechanisms may be cell type-specific. Supported by NSF #BNS8719872.

142.5

VASOPRESSIN mRNA IN THE NEUROHYPOPHYSIS. S.L.Lightman, D.Murphy*, A.Levy* & D.Carter*.Medical Unit, Charing Cross & Westminster Med.Sch.London, UK & Neuropeptide Laboratory, Inst.of Molecular & Cell Biology, Nat.Univ.of Singapore.

Vasopressin (VP) transcripts were detected by Northern blot analysis of RNA extracted from both rat and mouse pituitary neurointermediate lobes (NILs). These VP transcripts were shorter than those in the hypothalamus. Following digestion with RNAase H in the presence of Oligo(dT) the hypothalamic and neurointermediate lobe mRNAs co-migrated on Northern analysis. Adrenalectomy had no effect on NIL VP mRNA, but following osmotic stimulation with 2% saline drinking water there was an 8-fold increase in NIL VP transcript levels at 5 days and a 25-fold increase at 10 days. The anatomical distribution of the VP mRNA was investigated by *in situ* hybridization. In the basal state VP mRNA was only seen in the intermediate lobe. Saline loading, however, did not alter intermediate lobe VP mRNA - the marked increase detected by Northern blotting was found to be due to the dramatic appearance of abundant VP transcripts in the neural lobe. The origin of this mRNA is uncertain but is likely to be the neural lobe glial cells - the pituicytes, although the possibility of transport into the neurosecretory endings of hypothalamic supraoptic and paraventricular neurons cannot be discounted. The potential role of locally synthesized VP is also a matter for conjecture, but it may be involved in local paracrine control.

142.6

PROTRH GENE EXPRESSION IN THE RAT HYPOTHALAMIC PARAVENTRICULAR NUCLEUS (PVN) IS NOT REGULATED BY GLUCOCORTICOIDS. R.M.LECHAN, E.M. DYESS* AND S.L. LEE.* Div. of Endo., New England Med. Cntr. , Boston, MA 02111.

At pharmacologic doses, glucocorticoids reduce TSH secretion in both experimental animals and man (Wilber & Utiger, JCI 48:2096,1969). Since a glucocorticoid-receptor binding consensus sequence is present in the 5'-flanking region of the TRH gene (Lee et al., JBC 263:16604, 1988), we determined whether dexamethasone (dex) regulates proTRH mRNA levels in the hypophysiotropic neurons of the hypothalamic PVN. Adult male adrenalectomized (adx) Sprague-Dawley rats were treated with dex (100 ug/100 g B.W. i.p.) or vehicle. After two weeks, the brains were fixed by intracardiac perfusion with 4% paraformaldehyde and sectioned coronally on a cryostat through the PVN. Serial sections were hybridized with a 35S-labeled cRNA probe to proTRH or proCRH (gift of R.C. Thompson) and autoradiographed. PVN cell densities were analysed by computer imaging and compared to age matched normal controls. ProCRH mRNA content increased in adx animals compared to normal controls, whereas dex treatment resulted in suppression (Normalized Density Units (NDU) ± S.E.M.: Adx 3.5± .41; Cont. 1.99± .64; Dex 1.26 ± .29). In contrast, no significant alterations were observed in total proTRH mRNA content (NDU ± S.E.M.: Adx 1.31 ± .16; Cont 1.12± .20; Dex 1.34 ± .18). These studies indicate that glucocorticoids do not exert a significant effect on proTRH gene expression in the hypothalamic tuberoinfundibular system. The suprahypophysial effects of glucocorticoids on TRH secretion, therefore, may occur as a result of post-translational modification of proTRH gene products or are mediated by changes in other hypophysiotropic substances.

142.7

VASOPRESSIN AND OXYTOCIN SYNTHESIZING NEURONS RESPOND DIFFERENTIALLY TO INCREASE OF cAMP IN PRIMARY CULTURE .H. Schmale[1], K. Schilling*[2] and P. Oeding*[1] Inst.of Cellbiochem.[1],Univ.of Hamburg,2000 Hamburg 20 and Dept.of Anatomy and Cellbiol.[2],Univ.of Ulm, 7900 Ulm, Federal Republic of Germany.

Vasopressin(VP) and oxytocin(OT) genes are expressed in mutually exclusive sets of magnocellular neurons in the hypothalamus. Cell specificity and regulation have to be controlled by extra - and intracellular signals acting on one or the other gene. In order to identify such factors , we have used primary hypothalamic cultures prepared from 14 day old rat embryos to monitor specific gene expression by combined immunocytochemistry and in situ hybridization. Treatment of the cultures with either forskolin, the phospodiesterase inhibitor IBMX, or a combination of both, increased the number of VP expressing cells up to tenfold, whereas the number of cells expressing OT remained unaltered. Drug application had no mitogenic effect on VP-synthesizing neurons as determined by ^{3}H-thymidine labelling in combination with specific immunocytochemistry. The results show that increase of intracellular cAMP in hypothalamic primary cultures affects the expression of VP and OT genes in a differential fashion. The presence of putative response elements for the transcription factor AP2 in the 5'promoter regions of all VP genes sequenced so far but not in OT genes may provide the basis for the observed cAMP effect.

142.8

DETECTION OF VASOPRESSIN mRNA IN THE POSTERIOR PITUITARY BY SOLUTION HYBRIDIZATION AND NORTHERN BLOTTING. J.T.McCabe, E. Lehmann*#, J. Hänze*#, R. Lang*#, D. Ganten*# and D.W. Pfaff. Neurobiology and Behavior Laboratory, Rockefeller University, NY, NY 10021-6399 and #Department of Pharmacology, University of Heidelberg, Heidelberg, FRG.

Vasopressin messenger ribonucleic acid (VP mRNA) has been detected in several peripheral organs as well as in different areas of the brain. Results initially found by Lehmann indicate VP mRNA is present in the posterior pituitary as well (Lehmann, unpublished dissertation 1988, U. Heidelberg). We have employed solution hybridization and Northern blotting to analyze VP mRNA in hypothalamus, cortex, cerebellum, whole pituitary, and in separate anterior/intermediate lobe and posterior lobe samples (procedures: McCabe, et al., Neuroscience, 27:159, 1988). A low, but reliably detectable level of VP mRNA was present in the whole pituitary and posterior lobe tissue samples. In rats drinking 2% NaCl-water for 0, 2, 4, or 10 days, or 10 days and then 14 days of tap water, the levels of VP mRNA in the posterior pituitary were altered in a fashion that paralleled changes seen in the hypothalamus. The results from Northern blotting suggest VP mRNA in the pituitary is similar, but slightly shorter, than the message in the hypothalamus. To date, we have not determined, by in situ hybridization, whether VP mRNA is synthesized in pituicytes.

142.9

Sex steroids and fos expression in the rat brain and uterus. R.B. Gibbs, C. Mobbs, & D. Pfaff. Laboratory of Neurobiology and Behavior, Rockefeller University, N.Y., N.Y. 10021

Fos expression in the adult rat brain and uterus was examined following systemic treatment with estrogens and progesterone. Fifty female Sprague-Dawley rats were ovariectomized at least two weeks prior to steroid treatment. Animals received a subcutaneous injection of either estradiol (E: 5 µg E_2 in 10% ethanol/saline + 5 µg EB in oil), progesterone (P: 1 mg in oil), estradiol followed 24 hr. later with progesterone (E+P: same doses as above), or vehicle. Relative concentrations of fos mRNA in the uterus and ventromedial hypothalamus (VMH) were determined. Immunocytochemistry was also performed using an affinity purified rabbit IgG raised against fos amino acids 127-152 (provided by Dr. Tom Curran).

Fos mRNA within uterine tissue increased several fold relative to controls within 3 hr. after receiving E (consistent with Loose-Mitchell et al., Molec. Endocrin. v2, 1988 and Weisz et al., Molec. Endocrin. v2, 1988). Fos-like immunoreactivity (IR) was barely detectable in the uteri of untreated animals. However, IR within epithelial cells (but not myometrial or stromal cells) increased dramatically within 3 hr. after receiving E. This effect was dose-dependent and steroid-specific – IR did not increase in response to P, nor in response to a low concentration of E (0.13 µg E_2 + 0.13 µg EB) followed 24 hr. later with 1 mg P.

No induction of fos mRNA in the VMH was observed. Likewise, no effect of E, P, or E+P on fos-like IR was detected in the VMH, amygdala, hippocampal formation, or midbrain central gray. As a positive control, increases in fos-like IR in the PVN, SON or hippocampal formation following water deprivation or metrazol-induced seizures, were sucessfully detected as described by others (Morgan et al, Science v237, 1987 and Sagar et al., Science v240, 1988).

These data demonstrate that the regulation of fos by estrogens is both cell- and tissue-specific, and suggest that estrogenic effects in the adult brain are not mediated *via* an induction of fos mRNA or fos-related immunoreactive molecules.

142.10

TISSUE SPECIFIC EXPRESSION OF RAT ANDROGEN RECEPTOR mRNA. R. I. McLachlan*, B. L. Tempel, M. A. Miller*, D. B. Lubahn*, D. R. Joseph*, F. S. French*, E. M. Wilson*, W. J. Bremner*, D. M. Dorsa (SPON: D. Bredeson). Dept. Med. & GRECC, VAMC, Univ. Wash., Seattle, WA. 98108, & Lab. Reprod. Biol. Univ. N. Carolina, Durham, NC 27599.

We have used two probes from the rat androgen receptor (AR) cDNA (Tan et al, Mol. Endo., 1988:2, 1276) to assess tissue specific expression of AR mRNA. The probes were a 962 bp fragment [AR1] from the 5' untranslated region and a 532 bp fragment from the 5' end of the coding region (AR2). Poly A^+ selected RNA was isolated from adult male Wistar rats and was size-fractionated on a 0.75% agarose, 18% formaldehyde gel, partially hydrolyzed in 50 mM NaOH, transferred to a Nytran membrane and hybridized with ^{32}P-labeled cDNA probes. With the AR1 probe a single band of approx. 12Kb was detected in mRNA from the prostate and epididymis with lower levels apparent in the testis, kidney, skeletal muscle, and liver whilst transcript was just detectable in whole brain and spleen. With the AR2 probe a similar 12 kb band was apparent in the prostate, epididymis, kidney, and testis. In situ hybridization with ^{35}S-labeled AR1 riboprobe showed labelling of cells in the medial preoptic area and in the hippocampus. The latter two regions have been shown to specifically bind radiolabelled androgens. This AR probe should prove useful in further studies on the localization and physiological relevance of AR receptors in the brain.

142.11

THE DISTRIBUTION AND REGULATION OF ANDROGEN AND ESTROGEN RECEPTOR mRNA IN THE RAT BRAIN. R.B. Simerly and L.W. Swanson. The Howard Hughes Medical Institute and Salk Institute, La Jolla, CA 92138.

Recent studies suggest that steroid hormone receptors mediate the actions of their respective hormones by functioning as ligand-dependent nuclear transcription factors which alter the expression of specific genes or gene networks (see Evans, Science 240:889, 1988). A prerequisite to understanding the impact of these transcription factors on neural function is a clear understanding of the organization and regulatory profile of the neural systems in which steroid hormone receptors are expressed. We have begun to address these questions by applying *in situ* hybridization histochemistry and examing the distribution of neurons that express detectable levels of androgen (AR) and estrogen (ER) receptor mRNA-containing cells in the rat brain. ^{35}S-labeled RNA probes complementary to AR and ER mRNA were synthesized from cDNA inserts generously provided by Drs. C. Chang & S. Liao (Univ. Chicago) and Drs. M. Muramatsu & S. Koike (Univ. Tokyo), respectively. In both male and female animals, AR mRNA-containing neurons were found to be widely distributed in the forebrain, but the distribution of ER mRNA-containing cells was limited to a subset of the regions that contained AR mRNA-containing cells. Both ER and AR mRNA expression was particularly abundant in several sexually dimorphic nuclei including the anteroventral periventricular nucleus of the hypothalamus (AVPv), the medial preoptic nucleus, the encapsulated part of the bed nucleus of the stria terminalis (BSTe), and the posterodorsal part of the medial nucleus of the amygdala (MeAp). In addition, two other nuclei which share strong connections with these sexually dimorphic nuclei, namely the amygdalo-hippocampal area (AHZ) and the ventral premammillary nucleus, contained heavily labeled AR mRNA-containing neurons, but only the AHZ contained a high density of ER mRNA-containing cells. Nevertheless, the extensive overlap of the distributions of AR and ER mRNA-containing cells suggests that in many regions individual cells may express both receptors.

In order to evaluate the possible regulation of AR and ER mRNA levels within cells in sexually dimorphic nuclei we compared the density of AR and ER labeling over selected nuclei in intact and gonadectomized (28 days) male and female rats. In each sex, gonadectomy reduced levels of AR mRNA, but ER mRNA levels were significantly reduced only in ovariectomized female rats. These findings suggest that AR expression may respond to either testosterone or estrogen treatment, but that ER expression is selectively autoregulated.

142.12

IDENTIFICATION OF ESTROGEN-INDUCIBLE GENES IN RAT HIPPOCAMPUS VIA SUBTRACTIVE LIBRARIES. A. Maggi and E. Bettini*. MPL, U. of Milano, Piazza Durante 11, 20131 Milano, Italy.

We have recently demonstrated the presence of estrogen receptors in the rat hippocampus. The physiological significance of an action of this female sex hormone in this brain area is still unclear: the identification of genes induced by the hormone in this area might help in the understanding of the role played by estradiol in the control of this important area of the limbic system. To this aim, a cDNA library was constructed with mRNA isolated from the hippocampus of rats ovariectomized and injected with E_2. The library was probed with ^{32}P cDNA synthesized on templates of poly(A+) RNA extracted either from controls or E_2-treated rats. At the moment, a clone (13.4.3) of 600 bps has been isolated which hybridizes with a mRNA of ≈8000 bp present in hypothalamus, cerebellum, mid-brain, hippocampus. The induction of the 13.4.3 mRNA occours as soon as at 3 hours following the subcutaneous administration of the hormone. Preliminary analysis of the 13.4.3 sequence indicates a strong homology with the rat mitochondrial gene cytochrome oxidase subunit III

PEPTIDES: RECEPTORS I

143.1

EXPRESSION IN XENOPUS OOCYTES OF FUNCTIONAL BOMBESIN/GRP RECEPTORS TRANSCRIBED FROM A BACTERIOPHAGE cDNA LIBRARY. E.R. Spindel, T.P. Segerson*, P. Brehm, and R.H. Goodman*, Div of Neuroscience, Oregon Regional Primate Research Center, Beaverton OR 97006 and Div of Molecular Medicine, New England Med Center, Boston, MA 02111.

Bombesin and its mammalian homolog, gastrin-releasing peptide (GRP) are potent growth factors, neurotransmitters and paracrine regulators of GI function. To begin to characterize the diverse physiologic actions of bombesin-like peptides, we have examined the expression of mRNA encoding the bombesin receptor in *Xenopus laevis* oocytes. We have previously reported that oocytes injected with poly(A) RNA from murine Swiss 3T3 fibroblasts express functional bombesin receptors. Using this RNA, a size-selected cDNA library was prepared in the RNA expression vector lambda ZapII. Phage DNA was prepared from pools of 50,000 clones, linearized with the restriction enzyme *Not*I to produce sense-strand templates, and transcribed with T7 RNA polymerase. To each reaction, 1 ng of the serotonin Ic receptor cDNA was added as an internal control. Transcripts from 16 pools were screened by two-electrode voltage clamp analysis of microinjected oocytes treated with 2 μM bombesin. One pool of 50,000 transcripts produced a 20 nA peak inward current flow in response to bombesin. The pool of 50,000 bacteriophage clones was subdivided twice to enrich for the bombesin receptor cDNA, resulting in a pool of 7500 clones which gave a 50 nA peak response to 1 μM bombesin. It is likely that the bombesin receptor is encoded by a single cDNA within this pool.

143.2

EXPRESSION OF FUNCTIONAL PITUITARY SOMATOSTATIN RECEPTORS IN *XENOPUS* OOCYTES Michael M. White and Terry Reisine. Dept. of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

Functional somatostatin (SRIF) receptors were expressed in *Xenopus* oocytes following injection of RNA isolated from the anterior pituitary cell line AtT20. SRIF receptors were detected by measuring the ability of SRIF to inhibit cAMP formation stimulated by β2-adrenergic agonists in individual oocytes. β2-adrenergic receptors (β2AR) were expressed in oocytes by co-injecting RNA prepared by *in vitro* transcription of a β2AR cDNA clone with the AtT20 cell RNA. Uninjected oocytes do not express detectable levels of either β2ARs or SRIF receptors. In oocytes injected with both β2AR and AtT20 RNA, on the other hand, isoproterenol tretment led to a 2-3 fold increase in cAMP levels, while co-treatment with SRIF reduced this accumulation by 50-60%. The SRIF precursor somatostatin-28 and the cyclopeptide agonist MK678 also inhibited cAMP accumulation, whereas the biologically inactive *N*-terminal 14-amino acid fragment of somatostatin-28 was ineffective. The ability to detect changes in cAMP levels in individual oocytes provides a simple procedure for the expression cloning of SRIF receptor cDNAs and those for other receptors functionally coupled or stimulation or inhibition of adenylate cyclase. Supported by Sloan Fellowship BR-2733, NIH grants NS23885 and GM34781, and ONR grant N00014-88-K-0048.

143.3

EXPRESSION OF ANGIOTENSIN II RECEPTORS FROM MURINE NEUROBLASTOMA N1E-115 RNA IN XENOPUS OOCYTES. S.J. Fluharty, J.S. Camardo, R. Mir and M.M. White. (Spon: P.J. Hand) Depts. of Animal Biology and Pharmacology, and Institute of Neurological Sciences, Univ. of Pennsylvania, Phila., PA 19104.

Angiotensin II receptors (AngII-R), which are coupled to the mobilization of intracellular Ca^{2+}, are found on murine neuroblastoma N1E-115 cells. In the present report we compare the properties of AngII-R on N1E-115 cells with those expressed in Xenopus oocytes after translation of N1E-115 RNA. Injection of N1E-115 RNA (50 ng) into oocytes resulted in the appearance of surface receptors for the Ang II antagonist [^{125}I]-SARILE. These expressed binding sites exhibited a similar specificity for Ang II related peptides as that observed in N1E-115 cells. Moreover, in vitro differentiation of N1E-115 cells, which resulted in a 12-fold increase in AngII-R density on intact cells, substantially increased the levels of expressed binding sites as well. Coincident with the expression of these AngII-R, Ang II produced a dose-dependent modulation of endogenous chloride currents characterisitic of Ca^{2+} mobilization in oocytes. These responses were not observed in uninjected oocytes. As was the case in N1E-115 cells, the magnitude of the Ca^{2+} mobilization response was similar after injection of RNA from undifferentiated or differentiated cells. These results suggest that the Xenopus oocyte expression system is well suited for cloning the cDNA which codes for the N1E-115 AngII-R. Supported by NS 23986, MH 43787, and Alfred P. Sloan Research Fellowship BR-2733.

143.4

SPECIFIC EXPRESSION OF THE NGF RECEPTOR GENE IN BASAL FOREBRAIN NEURONS OF TRANSGENIC MICE. Nila Patil*, Elizabeth Lacy*, and Moses Chao* (SPON: J. Adler). Cell Biology and Molecular Biology program, Cornell University Graduate School of Medical Sciences, Medical College division, 1300 York Avenue, New York, NY 10021

The receptor for nerve growth factor(NGF) is expressed on neuronal and nonneuronal derivatives of the neural crest in a highly regulated manner during development. The human NGF receptor gene contains 6 exons spanning over 23 kilobases(kb) with a promotor sequence characteristic of constitutively expressed genes. To study the regulation of the receptor gene, we have generated six independent lines of transgenic mice carrying a 35 kb cosmid clone encoding the human NGF receptor gene. The number of integrated copies ranged from 10 to over 100 per genome. Expression of human receptors in the transgenic mice was investigated by (1) using a monoclonal antibody(ME20.4) specific for the human receptor in immunocytochemistry experiments, (2) immunoprecipitation of affinity crosslinked receptor, and (3) detection of receptor mRNA by S1 nuclease protection. Our results indicate that the human gene is specifically expressed in cholinergic neurons from the basal forebrain and Purkinje cells of the cerebellum. This suggests that the cis-acting sequences necessary to direct specific expression of the NGF receptor gene lie within the 35 kb fragment used to generate the transgenic mice.

143.5

Characterization of a High Affinity Neuropeptide γ Binding Site in Rat Duodenum, Urinary Bladder and Vas Deferens.
Y. Takeda and J.E. Krause, Department of Anatomy & Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

Two N-terminally extended derivatives of Neurokinin A, called Neuropeptide γ [NPγ;γ-PPT-(72-92)-NH_2] and Neuropeptide K [NPK; β-PPT-(72-107)-NH_2] can be derived from the γ- and β-preprotachykinin (PPT) precursors, respectively. These peptides are differentially expressed in rat tissues and are potent in certain tachykinin-sensitive bioassays. In this study, we have characterized a high affinity NPγ binding site that has the characteristics of a tachykinin receptor.

^{125}I-NPγ was prepared by chloramine T iodination and was purified by HPLC. Radioligand binding in duodenal (D), urinary bladder (UB) and vas deferens (VD) membranes was performed using a rapid filtration assay. Specific ^{125}I-NPγ binding to membrane fractions was saturable, temperature dependent and reversible. Scatchard analysis indicated that binding was due to a single class high affinity binding component. K_d and B_{max} values ranged from 0.14 to 0.26 nM and from 16 to 60 fmol/mg membrane protein for the three preparations. The rank order of tachykinin peptide potency was NPγ=NPK> or =NKA>NKB>SP, suggesting that ^{125}I-NPγ binds predominantly to the NK-2 receptor subtype. The potency of NKA on the displacement of ^{125}I-NPγ binding was similar to NPγ and NPK in UB and VD membranes, however NKA was 8 to 10-fold less potent than NPγ and NPK in D membranes. In addition, NPγ had potent smooth muscle contractile activity in these tissues.

We conclude that NPγ and NPK specifically bind with a high affinity to the NK-2 type receptor in peripheral tissues, and that the properties of ligand binding in rat D appear to be somewhat different from those in rat UB and VD. Thus, differential posttranslational processing of β-and γ-PPT into NPK and NPγ, compared to NKA, can dramatically alter ligand binding and biological activity of these tachykinin peptides.

143.6

AXONAL TRANSPORT OF PEPTIDE YY BINDING SITES IN THE RAT VAGUS NERVE. E.S Corp*, and G.P. Smith (SPON:J. Sechzer). E.W. Bourne Laboratory, Dept. of Psychiatry, NYH-Cornell Medical Center, White Plains, NY 10605.

We used quantitative in vitro autoradiography with computerized (film) image analysis to characterize parameters of [^{125}I]-PYY (NEN-Dupont) equilibrium binding to slide-mounted sections of cervical vagus nerve which had been ligated 24 h. prior to extraction. Accumulations of saturable PYY binding sites were measured on the proximal side of ligatures (approx. 0.40 mm^2), with smaller accumulations detected on the distal side (approx 0.1 mm^2). Analysis (LIGAND) of preliminary binding data resolved [^{125}I]-PYY binding to a single class of vagal sites with a Kd of 1.71 $\pm$ 0.4 nM and a Bmax of 173 $\pm$ 43 fmol/mg tissue. Under these same binding conditions, [^{125}I]-PYY bound with similar affinity to sites in the area postrema (Kd=0.93 $\pm$ 0.06 nM, 41 $\pm$ 2 fmol/mg tissue).

These findings suggest that PYY receptors undergo anterograde and retrograde transport along fibers of the vagus nerve. The localization of these sites to visceromotor or sensory components of the vagus, their distal terminus, and their functional significance is not yet determined. [Supported by St. Lukes\Roosevelt Obesity Core Center (ESC) and NIH MH 40010 (GPS).]

143.7

SOLUBILIZATION AND CHARACTERIZATION OF SUBSTANCE P RECEPTORS FROM PORCINE STRIATAL MEMBRANES: POSSIBLE NATURE OF THE CARBOHYDRATE COMPOSITION OF THE RECEPTOR. Y.F. Liu* and R. Quirion, (Spon: M. Dalpé) Dept Pharm. McGill Univ. & Douglas Hosp. Res. Center, Montreal, Quebec, Canada H4H 1R3

Substance P receptors were solubilized from porcine striatal membranes using 10 mM (3-[(choramidopropyl)-dimethylammoniol]-1-propanesulfate, CHAPS) (yield of solubilization: 75-85%). In solubilized preparation, [^{3}H] substance P (SP) apparently bound to a single class of high affinity sites (K_D= 0.82 $\pm$ 0.13 nM), data which are comparable to values obtained in membrane homogenates. The ligand selectivity pattern observed in both membrane and solubilized receptor preparations indicated that [Sar^9, Met $(O_2)^{11}$]SP = SP >> [MePhe^7] neurokinin B = [Nle^{10}] neurokinin A (4-10). This suggests the selective labelling of the NK-1 receptor sub-type. Solubilized receptor preparations were then applied to various agarose affinity columns with different mobilized lectins. The solubilized receptors were retained by lectins that bind galactose (Recin I and Recin II; most potent ones), mannose (concanavalin A and lectil), N-acetylglycosamine (wheat germ) but not by lectins that bind fucose (lotus A) and N-acetylgalactosamine (dolichos biflorus A). After desorption, [^{3}H] SP binding was enriched between 20-50 folds. In summary, [^{3}H] SP binding sites can be solubilized with CHAPS and they appears to be enriched with carbohydrate moieties, galactose being presumably predominant. (MRC, Canada).

143.8

SUBSTANCE P RECEPTORS ON HUMAN ASTROCYTOMA CELLS : STUDIES ON INOSITOL PHOSPHOLIPID TURNOVER AND TAURINE RELEASE. C.M. Lee and W.L. Tung*. Department of Biochemistry, Chinese University of Hong Kong, Shatin, Hong Kong

We have recently demonstrated the presence of NK-1 tachykinin receptors on a human astrocytoma cell line U-373MG (Lee C.M. , Kum W., Cockram C.S., Teoh R. and Young J.D., Brain Res., in press, 1989). The binding of ^{125}I-Bolton Hunter conjugate of substance P to these receptors can be modulated by guanyl nucleotides which suggests that the NK-1 receptors may be coupled with a G-protein regulated biochemical process. In the present study we have examined the effects of substance P on inositol phosphates and cyclic AMP formation in these cells. While substance P (1 μM) did not alter the basal or isoproterenol-stimulated cyclic AMP levels, it did cause a concentration dependent accumulation of inositol monophosphate with an EC_{50} of 0.3 nM. This stimulatory effect of substance P on inositol phospholipid turnover can be inhibited by spantide (1 μM), a substance P receptor antagonist. The activation of NK-1 receptors on U-373MG cells stimulated the incorporation of uridine into nucleic acids as well as the release of taurine. The latter effect is distinct from that of a rat glial cell line LRM 55 where activation of substance P receptor was shown to inhibit cyclic AMP dependent beta-adrenergic stimulated taurine release (Perrone M.H., Lepore R.D. and Shain W., J.Pharmacol. Exp. Ther., 238: 389, 1986).

143.9

IDENTIFICATION AND CHARACTERIZATION OF THE ZETA (ζ) OPIOID RECEPTOR IN NEUROBLASTOMA CELLS IN CULTURE. I.S. Zagon, S.R. Goodman and P.J. McLaughlin. Dept. Anatomy, Penn. State Univ. Coll. Med., Hershey, PA 17033 and Dept. Structural and Cellular Biology, Univ. South Alabama, Mobile, AL 36688.

Endogenous opioid systems are involved in the growth of normal and neoplastic cells/tissues of human and animal origin. Utilizing murine S20Y neuroblastoma (NB), tumor transplantation and tissue culture studies have revealed that [Met^5]-enkephalin is the most potent opioid peptide associated with growth. Binding assays using NB tissue and [^{3}H]-[Met^5]-enkephalin have demonstrated the presence of a new opioid receptor - zeta - which is involved in growth. To elucidate the zeta receptor in NB cells in culture, binding assays with [^{3}H]-[Met^5]-enkephalin were performed. Specific and saturable binding was detected in cell homogenates, with a K_d of 1.6 nM and a B_{max} of 48.1 fmol/mg protein. Binding was dependent on protein concentration, time, temperature, and pH, and was sensitive to Na^+, Ca^{++}, and Mg^{++}, but not GppNHp. Displacement experiments indicated that [Met^5]-enkephalin was the most potent displacer of [^{3}H]-[Met^5]-enkephalin. The results of these studies conducted with cells in culture are consistent with earlier findings which have identified the zeta receptor in neuroblastoma tissue.

Supported by NIH Grants NS-20623 and NS-20500.

143.10

Quantitative In Vitro Autoradiography of Transferrin Receptors in the Striatum of MPTP Treated Mice. I. Pablo, M. Basile*, S. M. Efange*, I. Sanchez-Ramos*, W. J. Weiner, and D. C. Mash, Dept. of Neurology, University of Miami School of Medicine, Miami, FL. 33101 and the Dept. of Radiology, University of Minnesota, Minneapolis, MN. 55455.

We have recently demonstrated a loss of transferrin binding sites in the putamen in Parkinson's disease (Neurology *suppl.* 39: 424, 1989). The reduced number of transferrin receptors in Parkinson's disease may result from either primary and/or secondary degenerative changes occurring in the striatum. The apparent selective reduction in transferrin binding in the putamen may reflect the differential loss of striatal dopaminergic terminals. However, the cellular location of transferrin binding sites in the CNS is, at present, unknown. One possibility is that ferrotransferrin-receptor complexes are transported down the axon to the dopaminergic terminals fields. Iron bound to transferrin may be internalized via transferrin receptors which are located on nigral cell bodies and dendrites. We have addressed this possibility in animal studies by using high resolution in vitro autoradiography of transferrin receptors in brains of mice treated with the dopaminergic neurotoxin, MPTP. Our results suggest that [^{125}I]-transferrin binding sites are significantly reduced in the caudate-putamen at 7 and 10 days post lesion. At 28 days after an injection of MPTP, the density of transferrin receptors in the caudate-putamen were elevated as compared to control brains (~ 8 pmol/g tissue). This increase in [^{125}I]-transferrin binding appeared to correlate with the recovery of dopaminergic innervation in the striatum as assessed by differential [^{3}H]-mazindol autoradiography performed on adjacent slide-mounted sections. These results suggest that striatal transferrin receptors may be located, in part, on dopaminergic terminals.

Supported by the National Parkinson Foundation.

143.11

IDENTIFICATION OF NEUROTENSIN AUTORECEPTORS IN THE VENTRAL MIDBRAIN TEGMENTUM OF THE RAT. J. Woulfe* and A. Beaudet (SPON: D. Baxter). Neuroanatomy Laboratory, Montreal Neurological Institute, Montreal, Quebec, H3A 2B4. Canada.

The presence of D2 autoreceptors confers upon midbrain dopaminergic neurons the capacity for autoinhibition in response to local, dendritically-released dopamine. Many of these neurons also contain and release neuroactive peptides. Whether these peptides are capable of similar autoregulation has not been established. In the present study, the possibility that neurotensin-containing neurons in the ventral midbrain bear autoreceptors was investigated in adjacent 5μm sections alternately processed for the immunohistochemical demonstration of endogenous neurotensin or for the autoradiographic localization of ^{125}I-neurotensin binding sites. Within the midbrain, neurotensin immunoreactive perikarya were consistently identified in the parabrachial pigmented and paranigral nuclei of the ventral tegmental area, the interfascicular nucleus, the caudal linear raphe nucleus, and occassionally in the rostral linear raphe nucleus, central grey and substantia nigra. A proportion of these neurons exhibited ^{125}I-neurotensin binding sites in the adjacent 5μm autoradiograph. Whether these binding sites represent functional autoreceptors at the level of the perikaryal and dendritic plasma membrane or intracellular receptors undergoing storage, synthesis, degradation, and/or transport to more remote sites remains to be elucidated. Supported by MRC.

NEUROETHOLOGY I

144.1

AUDITORY PROPERTIES OF SONG NUCLEI OF ESTRILDID FINCHES. A.J.Doupe and M.Konishi. Div. of Biol. 216-76, Calif. Inst. of Technology, Pasadena, CA 91125

The importance of auditory experience and feedback in song development suggests that there must be links between the auditory and vocal motor systems. To identify the location and physiological nature of such connections, we have studied estrildid finch song nuclei using single unit recordings and anatomical tract tracing. Both nuclei MAN and X of adult male finches have auditory responses. In area X, the auditory units have latencies of 20 msec or more, are broadly tuned to frequency, and prefer complex stimuli such as white noise and finch song. In MAN, some of the units show inhibition or no response to white noise and pure tone bursts, but fire briskly to presentation of the bird's own song and more weakly to finch songs from other individuals.

Tracing of inputs to the auditory neurons in MAN and X with horseradish peroxidase confirm a recently described circuit from X to the medial dorsolateral thalamic nucleus (DLM) and back to MAN. Preliminary results of direct recordings in DLM suggest that this nucleus also contains long latency auditory units. These data provide clear evidence for auditory responsiveness in the song nuclei MAN and X, support the neuronal circuit from X to DLM to MAN suggested on the basis of earlier connnectivity studies, and raise the possibility that this circuit carries auditory information.

AJD is a Monsanto Fellow of the Life Sciences Research Foundation.

144.2

NMDA RECEPTORS MEDIATE SYNAPTIC TRANSMISSION IN A ZEBRA FINCH SONG CONTROL NUCLEUS. R. Mooney* and M. Konishi. Div. of Biology 216-76 Caltech, Pasadena, CA 91125 (Sponsor: S. Volman).

We are interested in studying synaptic properties which may be correlated with song learning in passerine birds. The forebrain song nucleus RA is in a descending motor pathway implicated in vocal control during song. It is known to receive input from two other song control nuclei, HVc and MAN. HVc axons "wait" in an area dorsal to RA from day 15 to day 30 posthatch, after which they enter RA. MAN terminals, on the other hand, are present within RA from day 15 onward.

We have characterized afferent input to RA in acute brain slices prepared from 15-30d birds. Electrical stimulation in an area lateral to RA, known to contain MAN axons, elicits EPSPs in RA neurons. These EPSPs are antagonized by kynurenic acid and often display longer latency components which are blocked by AP5 or by hyperpolarization of the postsynaptic membrane. These results suggest that as early as day 15, MAN provides afferent input onto RA which is glutamatergic and has properties associated with NMDA receptor-mediated transmission.

After day 35, stimuli applied to the HVc and MAN axonal tracts entering RA generate EPSPs at the same postsynaptic RA neuron. These EPSPs summate when stimuli to HVc and MAN axons are paired. Therefore, HVc and MAN provide convergent excitatory synaptic input onto single neurons within RA.

144.3

LHRH-LIKE IMMUNOREACTIVITY IN THE BRAIN OF A SEX REVERSING FISH: MODULATION BY GONADAL STEROID HORMONES. M.S. Grober* and A.H. Bass (SPON: P. BRODFUEHRER). Section of Neurobiology and Behavior, Cornell University, Ithaca, N.Y. 14853.

The sex-reversing fish, *Thalassoma bifasciatum*, exhibits two color morphs (primary phase, PP, and terminal phase, TP). PP fish are born either male or female, while TP males result from the transformation of PP males or females. This analysis initiates immunocytochemical studies of hormone binding in the central nervous system (CNS) of these fish and describes the possible role of gonadal steroid hormones on the binding of gonadotropin releasing factors in the CNS.

LHRH immunoreactive (IR) fibers were found widespread throughout regions of the forebrain and brainstem, although there were no differences in binding between the different sexual phases. Areas of dense labeling included the olfactory tract and bulb, area ventralis of the telencephalon, midbrain tectum and tegmentum, posterior tuber region of the diencephalon, and ventral brain stem. LHRH-IR somata were located in the olfactory nerve and probably comprise a nervus terminalis system. More suprising however, was the presence of LHRH-IR in cranial and spinal motor nuclei. Intra-peritoneal implants of 11 Ketotestosterone into PP (male and female), transitional (sex undetermined), and TP fish produced a marked increase in LHRH-IR both in cranial and spinal motor nuclei, and in a group of small cells surrounding the nucleus glomerulus of the hypothalamus. Typically, a granular-like precipitate was found throughout the cytoplasm surrounding the nucleus. Lower levels of this characteristic binding are seen in field collected specimens or fish maintained in the laboratory for periods of up to 4 months, suggesting that LHRH-IR in motor nuclei is a naturally occuring event and thus may play an important role in the hormonal control of sex reversal and subsequent behavioral transformations. Supported by a NIH Postdoctoral Training Grant (MG) and NIH / NSF Grants (AHB).

144.4

VISUAL ACTIVATION OF THE OCTAVOLATERALIS EFFERENT SYSTEM DEMONSTRATED IN THE LATERAL LINE OF FREE-SWIMMING TOADFISH. T. C. Tricas and S. M. Highstein. Wash. Univ. Sch. Med., Dept. Oto., St. Louis, MO 63110.

The octavolateralis efferent system (OES) can be activated by multimodal sensory stimuli in the toadfish (Highstein and Baker, 1985, J. Neurophys. 54:370) and has a predominantly inhibitory action on the firing of lateral line (LL) primary afferent fibers in fishes. We studied the OES in a semi-natural setting to determine if visual stimuli of biological significance can activate it. Single LL afferents were recorded chronically with metal microelectrodes for periods up to 9 days in toadfish swimming freely in a small tank. Trains of bright photic stimuli (10 flashes/sec for 3 sec) resulted in a 20% increase in spontaneous spike rates of afferents for the stimulus duration with an onset delay of about 1 sec. In contrast, flashes were often followed by decreased mechanically evoked afferent firing rates. When small prey fish in a clear sealed chamber were presented to toadfish, some LL fibers decreased their spontaneous activity while others did not change. Mechanically evoked firing (by mild 25-90 Hz vibrations of the tank) decreased following prey presentation and persisted up to 120 sec. OES axons to the LL are spontaneously active and modulated by visual stimuli as confirmed by direct chronic recording from one putative efferent fiber. Thus, the OES modulates the sensitivity of the LL system via visual input pathways. This modulation may be behaviorally significant for the detection of water motion or capture of nearby prey.

144.5

SUPPRESSION OF 'COMMON-MODE' SIGNALS IN THE CENTRAL ELECTROSENSORY SYSTEM. G.J. Rose. Dept. of Biology, Univ. of Utah, Salt Lake City, UT 84112.

Gymnotiform fish generate electrical discharges via an organ located in the tail and sense these signals with electroreceptors distributed over their body surface. Since a variety of motor activities can result in modulations of the discharge properties of primary afferents (e.g. ventilation, courtship signaling), the problem arises as to how such 'reafferent' information is distinguished from 'exafferent' (environmental) sensory input. During ventilation, electroreceptors on corresponding regions of the left and right opercula should experience similar modulations of activity; in courtship behavior the entire body surface experiences modulations in the amplitude and/or frequency of electric organ discharges (EODs).

One potential mechanism for discriminating between such reafferent input and exafferent information is to suppress activity resulting when antipodal areas of the body surface experience similar modulations of the EOD amplitude. Evidence for this 'common-mode' suppression mechanism has been obtained for the little skate (New, J.G. and Bodznick, D., Neurosci. abst., 114.10, 1988). In the present study, evidence in support of such a mechanism was found in the electrosensory lateral line lobe of Eigermannia. 'E-' and 'I-type' units in this structure increase their discharge rates during rises or falls of signal amplitude, respectively. Units of these types were found that discharged strongly to stimuli in which particular antipodal areas of the body surface experienced opposite patterns of amplitude modulation, but responded poorly when the entire body surface experienced identical modulations.

144.6

FREQUENCY AND TEMPORAL SELECTIVITY OF SINGLE AUDITORY NERVE FIBERS IN THE TOKAY GECKO. F. Dodd and R. R. Capranica, Section of Neurobiology & Behavior, Cornell University, Ithaca NY 14853.

Auditory nerve fibers in the Tokay gecko have sharp V-shaped tuning curves with $Q_{(10dB)}$'s from 2-10. The best excitatory frequencies (BEF) range from 200 to 5000 Hz with thresholds from 4-35 dB SPL. All fibers exhibit pronounced two-tone suppression on the high frequency side, but not on the low frequency side.

We measured the spike rate contour for single fibers as a function of frequency with the intensity held fixed. These iso-intensity functions divided the population of nerve fibers into 3 groups. Units with BEF from 250-950 Hz had peaked iso-intensity contours, which did not change shape with increasing sound intensity and the responses were phase-locked from 100-700 Hz. For units with BEF 950-2250 Hz the peak of their spike-rate contour shifted to lower tonal frequencies with increasing sound intensity, reaching their maximum firing around 600 Hz. For this group the phase-locking was also observed from 100-700 Hz, but only at high intensities. Cells with BEF 2250-5000 Hz have peaked iso-intensity contours at intensities from 10-30 dB above threshold and do not show phase-locking. At higher intensities these contours flatten and become bell-shaped, centered around their BEF. The flattening occurs even though saturation has not yet been reached. All units with BEF 200-2250 Hz responded below 600 Hz to the waveform periodicity in a 1:1 manner, i.e. one spike per cycle, and the individual unit is thus constrained by the periodicity of the stimulus and can therefore not encode intensity changes in this frequency range.

The natural vocalizations of these geckos contain complex harmonic structure with a fundamental frequency around 375-450 Hz and these units can therefore reliably encode both the waveform periodicity and spectral patterns of these signals over a wide intensity range. This work was supported grants form NIH, the Danish Research Academy and the Carlsberg Foundation.

144.7

INTERACTION OF SOUND AND VISUAL CUES IN ORIENTATION BEHAVIOR OF CRICKETS. K.Schildberger and H. Böhm. MPI f. Verhaltensphysiologie Seewiesen FRG.

A female cricket can find a singing male by orienting toward the sound source without any other orientational cues. In nature crickets perform partner finding under more complex situations. The influence of visual signals on the phonotaxis was studied under open-loop condition.

Firstly, the turning tendency was determined for various sound directions in the dark and in the presence of a patterned environment. The precision and strength of the phonotaxis is increased with visual cues available. Secondly, the turning tendency was measured while moving a stripe drum. This optomotor response increases for contrast frequencies up to 2/s. Thirdly, sound and movement stimuli were combined. The visually and acoustically induced turning tendencies are additatively superimposed, i.e. the optomotor response curve is shifted by the contribution of the sound signal and vice versa.

It might be concluded that the optomotor system is used to enhance the precision of orientation to a conspecific calling song.

144.8

THE NEUROETHOLOGY OF SEX AND DEATH IN CRICKETS: A ONE-NEURON "SWITCH" THAT DEPENDS ON BEHAVIORAL CONTEXT. R.Hoy and F.Libersat, Section of Neurobiology & Behavior, Cornell University, Ithaca, NY 14853

Hearing in crickets mediates both sexual behavior and predator (bat) detection. Males sing courtship songs (CS) to facilitate copulation. In two species, T.oceanicus and G.bimaculatus the power spectrum of CS sound pulses show peaks at the fundamental frequency (4.5 kHz) and its harmonics (9,13.5, 18 kHz). Yet, the high frequencies can trigger an escape response, during flight. An identified neuron, int-1, mediates the escape response, and here we investigate its possible role in courtship behavior. For the CS of T.oceanicus the carrier frequency, 4.5 kHz (95dB) is always more energetic than higher harmonics (13.5 kHz (82dB). When males are muted by wing amputation, their courtship success decreases by 80%. Playback of tape-recorded courtship songs (natural CS or a 4.5 kHz model) during courtship restores a muted male's courtship success, but not playback of 13.5 kHz. Thus in ocean. where 5 kHz is the major component of CS and 13.5 the minor, the 13.5 kHz does not promote mating. For the CS of G.bimaculatus the dominant frequency is 13.5 kHz (104 dB) and always exceeds the carrier, 4.5 kHz (89dB). Murray & Hoy (Neurosci.abst 14:310) showed that in G.bimac.a 13.5 kHz model CS restores courtship success but a 4.5 kHz does not.

In both species, 13.5 and 30 kHz ultrasound elicits avoidance steering in tethered flight. When 30 kHz CS models were played during the courtship of mute males,females ignored them. While int-1 signals the presence of ultrasound to the brain in both courtship and escape contexts, the behavioral consequences of its activation depends on the activation of other parallel neural systems.

144.9

COLLISION AVOIDANCE BEHAVIOUR IN THE LOCUST. R.M. Robertson and D.N. Reye*. Department of Biology, Queen's University, Kingston, Ontario, K7L 3N6, Canada.

The visual properties of the locust DCMD neurons and their synaptic connections to flight motoneurons suggest that they are involved in directing flying locusts around objects with which they are about to collide.

We have characterized evasion manoeuvres of tethered locusts flying in a wind-tunnel by videotaping their responses to rapidly looming objects which were directed toward the head of the animal and on either side of the midline. Locusts respond to such stimuli with brief and rapid movements of the abdomen and the wings. The abdomen either bent always to the same side regardless of the target position or bent such that the animal would have turned away from the approaching target. At the same time the relative phasing of the wings, the amplitude of wing movement and angle of attack of the wings were altered. On the inside of the turn the wing pronated and the stroke amplitude increased. A reciprocal relationship existed so that changes in wing parameters depended on the side of the animal that the target approached.

The results show that the locust possesses a robust behaviour for avoiding collision with objects during flight. There is a good correlation of the motor output with the visual and synaptic properties of the DCMD neurons, supporting the hypothesis that these neurons play a major role in mediating this behaviour.

144.10

NEUROETHOLOGICAL ASPECTS OF ULTRASOUND AVOIDANCE IN KATYDIDS. F. Libersat and R. R. Hoy, Section of Neurobiology & Behavior, Seeley G Mudd Hall, Cornell University, Ithaca NY, 14853.

Several nocturnally flying insects including lacewings, moths, mantises, and crickets detect and avoid ultrasound such as that produced by echolocating insectivorous bats . Bats are likely to exert strong predation pressure on other insect groups as well. Katydids (Tettigoniidae) are nocturnally active insects not previously known to avoid ultrasound. In the present work we show that Katydids respond both physiologically and behaviorally to ultrasound.

Suspended freely flying animals were tested. One species of katydid (*Neoconocephalus ensiger*) responded to ultrasonic pulses (100ms, 90dB) by folding its wings as lacewings do. This results in a sudden drop in the flight path . We further characterized the behavioral response of *N. ensiger*, using a photocell to monitor wing movements in tethered flight. When single tones with frequencies ranging from 10 to 80 kHz (100ms, 90dB) were presented to the tethered flying katydid, escape responses were triggered only by frequencies ranging from 20 to 60 kHz. The threshold for the escape response at 30 kHz was 80 dB. This response latency was short and ranged between 30ms and 90ms, averaging 57±14 ms.

We also investigated the underlying neural correlates of ultrasound avoidance. An auditory interneuron was found, a T-neuron, whose physiology suggests that it is involved in the escape behavior. The response latency of this neuron to single tones (3ms, 30 kHz, 90dB) was 12.3±0.6ms Its lowest threshold was below 70 dB for frequencies ranging from 20 to 60kHz. Moreover, the number of spikes elicited in response to single tones (3ms, 90dB) for frequencies from 20 up to 60 kHz was at least twice that to frequencies of 5,10,70 and 80 kHz. Its average discharge frequency was 390±70 Hz. Therefore, the T-neuron is a good candidate for mediating the ultrasound avoidance in katydids. This neuron has its soma located in the prothoracic ganglion and sends two relatively large axons both rostrally and caudally. Its anatomy suggests that it may have a direct access to the thoracic flight circuitry. Work is now in progress to determine wether T-neuron mediates bat avoidance in Katydids.

144.11

MULTISENSORY CONTROL OF COCKROACH ESCAPE: SOME PREDATORS ARE DETECTED BY ANTENNAL AND OTHER NON-CERCAL SENSORY SYSTEMS. C.M. Comer, M.E. Getman*, M.C. Mungy* and J. Plishka*. Dept. Biol. Sciences & Committee on Neuroscience, Univ. of Illinois, Chicago, IL 60680

Some natural predators of cockroaches (P. americana) generate wind cues as they strike, and these cues are used by the cercal-to-GI system to control escape. We have described tactile sensory pathways associated with the antennae, and provided evidence that antennal stimulation may lead to escape (Brain Res. 445:370). To understand the significance of such non-cercal pathways we studied interactions of cockroaches with small predators that might generate less wind during attack: rodents (mice), arachnids (wolf spiders) and insects (mantids). Behavior was recorded by high-speed videography. Also, responses of cockroaches with either cerci or antennae removed were compared with intact animals.

Many features of these interactions suggested involvement of tactile cues rather than, or in addition to, wind cues. These included: 1) evasive turning often did not begin until contact was made with the antennae or body. 2) When predators struck from one side, but went over the cockroach and made contact on the opposite side, turns were directed away from the side of contact not the side from which the predator lunged. 3) Antennal removal significantly increased the probability of a cockroach being captured, while cercal ablation did not cause a comparable increase. We suggest that tactile sensory pathways may be as important as wind pathways for understanding the evolution and neural control of escape. (Supported by NSF grant #BNS-8617393 to C.M.C.)

144.12

DEVELOPMENT OF SWIMMING BEHAVIOR IN THE MEDICINAL LEECH. S.A. Reynolds* and W.B. Kristan, Jr. (SPON: W.A. Harris) Dept. of Biology, UCSD, LaJolla, CA 92093

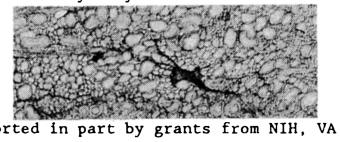
One prerequisite for studying how neurons make appropriate connections with one another to produce coordinated behavior is a detailed description of the normal course of behavioral development. Therefore, we have characterized the development of swimming behavior in the medicinal leech, *Hirudo medicinalis*. Videotapes of swimming leech embryos were analyzed frame-by-frame, and the changes in various components of the behavior were followed over embryonic time. The major increase in normalized swimming velocity (rate of forward progress/body length) occurred during the last 10 days of embryonic development. 20% of this increase was due to a rise in swim cycle frequency, and the remaining 80% was due to increased efficiency of swimming. Factors that were important for increased swimming efficiency included the flattening and widening of the animal prior to swimming, how far along the animal the body wave was propagated, and the amplitude of the tail stroke during swimming. Factors that could potentially have affected swimming, but did not, include the rate of wave progression through the body, coordination of different parts of the body, variability in swimming behavior, and the amplitude of the body wave at the head. These studies set the stage for determining how the pattern of synaptogenesis between members of the neuronal swim circuit gives rise to this observed pattern of behavioral development. (Supported by NIMH research grant MH43396.)

NEUROGLIA: MYELIN AND MYELIN-FORMING CELLS

145.1

IN SITU DEMONSTRATION OF OLIGODENDROCYTES IN THE ADULT CNS WITH A NEW MONOCLONCAL ANTIBODY, RIP. B. Friedman, J.A. Black, S. G. Waxman, Dept. Neurology, Yale Univ. Sch. med. and VAMC, West Haven, Ct. 06516

Monoclonal antibody Rip is a new marker for oligodendrocytes that appears to stain pre-ensheathing, ensheathing and myelin related oligodendrocytes. Double-labelling experiments with Rip and anti-GFAP antibodies indicate that Rip does not recognize astrocytes in the mature CNS. In adult rat spinal cord, Rip strongly stains oligodendrocytes and their cytoplasmic processes (see figure). Myelin is only lightly stained in nonpermeabilized adult tissue. The arborization patterns of mature oligodendrocytes vary but are distinct from branching patterns of immature pre-ensheathing oligodendrocytes. However, in adult rat neocortex, oligodendrocytes with both mature and immature arborization patterns were observed. Further studies of Rip staining in 1-2 year old rats will examine if immature cells persist and if they can be triggered to differentiate by demyelination.



(Supported in part by grants from NIH, VA, and the NMSS)

145.2

S-100 IS PREFERENTIALLY DISTRIBUTED IN MYELIN FORMING SCHWANN CELLS. D.J.Fink, D.Alessi* and M.Mata. University of Michigan and VA Medical Center, Ann Arbor, MI, 48105

The S-100 proteins are a group of abundant low molecular weight proteins, members of the large family of calcium binding proteins of the EF hand structure, which are highly enriched in nervous tissue. In order to gain some insight into the role of S-100 in the peripheral nervous system *in vivo*, we used EM immunocytochemistry to study the localization of S-100 in the peripheral nervous system of the rat.

Male Sprague Dawley rats were used in all experiments. The animals were sacrificed by intracardiac perfusion with 0.5% glutaraldehyde and 4% paraformaldehyde in phosphate buffer, and the sciatic nerve and the cervical sympathetic trunk placed in the same fixative for an additional 2 hrs, then embedded in LR White. Ultrathin sections were exposed to anti S-100 antibody (Dako) in dilutions from 1:300 to 1:1000 for 2 hr, followed by goat anti-rabbit IgG bound to colloidal gold.

In normal nerve, S-100 immunoreactivity was found predominantly in Schwann cells, but was differentially distributed. Most of the S-100 immunoreactivity was found in the cytoplasm and over the nucleus of Schwann cells of large myelinated fibers, less S-100 immunoreactivity was found in the Schwann cells of smaller myelinated fibers, and very little was found in those Schwann cells surrounding unmyelinated axons. Within the Schwann cells the immunoreactivity was seen in the nucleus, the perikaryal cytoplasm, as well as in the Schmidt Lanterman clefts and in the paranodal loops at the node of Ranvier. Using these antibodies we did not see intra-axonal immunoreactivity in normal nerve. These results suggest that in Schwann cells S-100 expression may be related to axon diameter and degree of myelination.

Supported by grants from the JDF, the NINDS, and the VA.

145.3

EXPRESSION AND PURIFICATION OF MYELIN-ASSOCIATED GLYCOPROTEIN. J. Attia*, P. Johnson*, J. Roder, and R.J. Dunn. Dept. Medical Genetics, University of Toronto, Toronto, Ontario. M5S 1A8.

Myelin-associated glycoprotein (MAG) is expressed by glial cells of the PNS and CNS during development (in uncompacted myelin) and in the mature animal (inner and outer mesaxons, Schmidt-Lanterman incisures, and especially in the periaxonal space). By virtue of its location, its membership in the immunoglobulin gene superfamily, and the results of MAG liposome/DRG binding assays, MAG is believed to be an adhesion molecule involved in the initiation of myelin wrapping.

Since MAG constitutes less than 1% of myelin proteins, the baculovirus expression system has been used to overexpress a soluble form of MAG. Differing multiplicities of infection, with constructs varying in 5' non-coding region, are being tested for optimization of the expression level. A simple purification involving ammonium sulfate precipitation and lentil-lectin chromatography has been established. The purified protein has been used in a number of binding assays, e.g. binding to membrane preparations and to whole cells, ligand blotting, and emulsion autoradiography.

Future work on MAG will focus on physical and structural analyses, including crystallization.

145.4

MAPPING OF FUNCTIONAL DOMAINS OF THE MYELIN-ASSOCIATED GLYCOPROTEIN. A. Meyer, P. Fischer+*, K. Beyreuther+*, M. Tropak++, J. Roder++ and M. Schachner. Dept. Neurobiology and +ZMBH, University of Heidelberg, and ++Mount Sinai Hospital Research Institute, Toronto.

The myelin-associated glycoprotein (MAG) is a cell adhesion molecule belonging to the carbohydrate-based L2/HNK-1 family and the immunoglobulin superfamily. It has been implicated in axon-myelinating cell interactions and binds to different types of collagens and heparin. To map the molecular domains responsible for these functions, cyanogen bromide fragments of immunoaffinity-purified MAG from adult mouse brain were obtained and assigned to the primary protein structure by partial aminoacid sequencing and alignment with the cDNA-deduced primary aminoacid sequence. In addition, monoclonal antibodies recognizing distinct epitopes on the molecule were used. The binding to heparin and collagen types I and III was located by radio-ligand binding and interaction with heparin-agarose to the fragments containing the first two immunoglobulin domains. Binding of MAG-containing liposomes to cultures of spinal cord neurons could be prevented by antibodies recognizing epitopes localized to the immunoglobulin domains III, IV and V. These observations indicate that the heparin- and collagen-binding domains of MAG are different from those involved in cell binding.

145.5

EXPRESSION OF P_0 mRNA and P_0 GLYCOPROTEIN DURING RAT PERIPHERAL NERVE DEVELOPMENT. P.L. Baron*, M. Shy*, H. Honda*, J. Sladky*, M. Sessa*, G. Conti*, D. Pleasure and J. Kamholz, Neurology, Univ. of Pennsylvania, Phila. PA 19104

The integral membrane glycoprotein P_0 is the major protein in PNS myelin. We investigated transcription and translation of P_0 during development of rat sciatic nerve, using frozen sections prepared from day 1, 5, 10, and 42 postnatal rats and from E16 and E18 rat embryos. In situ hybridizations were performed with uniformly labelled sense and antisense single stranded RNA probes transcribed from a 1.9 kb P_0 cDNA. A rabbit antiserum against rat P_0 glycoprotein and an avidin-biotin system were used to detect P_0 glycoprotein in the sections. P_0 message was detected in nerves of all ages studied form E18 onward. Grain density within endoneurium was highest between postnatal days 5 and 10, consistent with Northern blot estimates of P_0 mRNA abundence in rat sciatic nerve. Silver grains were found in clusters around Schwann cell nuclei. The proportion of Schwann cells expressing detectable levels of P_0 mRNA decreased by 42 days postnatally, but some Schwann cells remained intensely positive. Immunohistologically demonstrable P_0 glycoprotein was present in occasional Schwann cells on day E18, and had become much more abundant by day 5 postnatally. The results of this study indicate that transcription and translation of P_0 glycoprotein in rat sciatic nerve commence prenatally, and that active transcription of P_0 continues within a subset of Schwann cells in mature nerves.

145.6

A FUNCTIONAL ROLE FOR MYELIN Po PROTEIN AS AN ADHESION MOLECULE. M.T. Filbin*, F.S. Walsh and G.I. Tennekoon* (SPON: R.T. Johnson). The Johns Hopkins Sch. of Med., Baltimore and Inst. of Neurol., London, England.

The major glycoprotein of peripheral myelin, Po, is postulated to be involved in the compaction of myelin through homophilic interaction of its extracellular domains. To assess the ability of Po to interact with itself we compared adhesion of Chinese hamster ovary (CHO) cells transfected with the rat Po-cDNA and control transfected cells (without Po-cDNA). Cells expressing an abundance of Po (both RNA and protein) were selected by the dhfr/MTX gene amplification strategy. Po expressed by these cells was glycosylated and reached the cell surface. Adhesion was assayed by allowing a single cell suspension to reaggregate at 37°C in a couette viscometer. By visual examination (light microscopy) within 70 min the Po-expressing CHO cells had formed large clumps, whereas control transfected cells had formed only doublets and triplets. In agreement with this observation, when assessed with a Coulter counter, the total particle number in the reaggregating Po-expressing cells decreased to 20% of the starting number while control transfected cells decreased to only 50%. Furthermore, reaggregation was independent of the calcium concentration. These results suggest that Po increases the aggregation of CHO cells, which in turn indicates that Po is behaving as an adhesion molecule. (Supported by grants NS 26242, NS 21700, and BNS 8720070.

145.7

COMPARATIVE STUDY OF MYELINATED NERVES USING TETRACYCLINE DERIVATIVES. by P.M. Pereyra * and B.I. Roots. Department of Zoology, University of Toronto, Toronto, Canada.

We have previously reported that tetracycline (TC) forms a fluorescent adduct with myelin membranes in vertebrates as well as with the myelin-like membranes in invertebrates (e.g. Lumbricus; Brain Res. 458:377-382 (1988)). Despite the fact that invertebrate "myelin" seems to have no molecular relationship with vertebrate myelin (Neurochem. Res. 13(9):893-901 (1988)), the fluorescent staining by tetracycline and the presence of an intrinsic blue-green fluorescence seem to be two factors associated with all myelinated nerves (ISN Abstracts 14:584 (1988), Trans. ASN 20:260(1989)) Under in vitro myelinated nerves will show a TC fluorescence associated with axonal mitochondria as well as with a series of discrete compartments associated with myelin or surrounding glial cells: In vertebrate PNS myelin, the fluorescence is exclusively associated with the paranodes, while in fish spinal cord, it is associated with unidentified cytoplasmic compartments under low extracellular Ca+2 and with the myelin sheath under high (>3mM) extracellular Ca+2. In vitro incubation of earthworm nerve cords shows TC fluorescence associated with two intracellular compartments, one related to the "myelinating" glial cells, and a second one related to one of the three components of the limiting sheath around the nerve cord. Inhibition of oxidative metabolism (e.g. addition of KCN) produces an immediate shift in the TC fluorescence to the compact myelin compartment. These results are taken to indicate that the relationship of Ca+2 with myelin is an active one, as already implied from previous X-ray studies (Blaurock et al. (1986) Neurochem Res. 11:1103-1129) and not only due to electrostatic interactions of Ca+2 and myelin phospholipids. In vitro experiments using a series of TC derivatives with different binding affinities for cations as well as a different membrane permeability has reinforced the idea that Ca+2 is actively extruded from myelin. The implications of our observations will be discuss with respect to axonal injury, axon-myelin stability and theinterpretation of X-ray studies.

145.8

PRESENCE OF Ca^{++}/CALMODULIN-DEPENDENT PROTEIN KINASE IN A PURIFIED MYELIN ISOLATED FROM ADULT RAT FOREBRAIN. Y. Huang*, M. Stanley, and K. Wu* (SPON: P. Siekevitz).Div. of Neuroscienc, New York State Psychiatric Inst., New York, N.Y. 10032, and Dept. of Neurology, Cornell Univ. Medical Center, New York, N.Y. 10021.

Our previous studies indicated that myelin fraction purified from forebrain of adult rat contains Ca^{++}/CaM-dependent protein kinase. Phosphorylation of 48/50 kDa and 56/58 kDa polypeptide doublet were observed in a Ca^{++}/CaM-dependent manner. The 50 kDa phosphprotein was not detectable in synaptic plasma membrane fraction and other subcellular fractions studied in parallel, suggesting that this phosphoprotein is specifically located in the purified myelin fraction. In the present study, we examined the proteins in the purified myelin fraction which bind CaM in the presence of Ca^{++}. To this end, the proteins in purified myelin fraction was separated by SDS-PAG electrophoresis, and ^{125}I-CaM binding to the proteins in the gel was carried out as described by Carlin et al.(1981). It was found that there are about 5 major CaM binding polypeptide bands in the myelin fraction. Polypeptide bands of 48 kDa and 58 kDa regions also bind CaM, although to a lesser extent. Our results suggest that the phosphoprotein bands of 48 kDa/58 kDa are either protein kinase themselves or are substrates of protein kinase. We are currently investigating these possibilities.

145.9

CHARACTERIZATION OF NEURAMINIDASE ACTIVITIES IN RAT OLIGODENDROGLIA. R.K. Yu, M. Saito*, and C. Sato*. Department of Biochemistry, Medical College of Virginia, VCU, Richmond, VA 23298.

Myelin has an intrinsic neuraminidase (myelin-associated neuraminidase), which is probably synthesized in oligodendroglial cell bodies (oligos) and is transported to the myelin membrane. In this study, neuraminidase activities in rat oligos were examined. The enzyme activity toward the ganglioside GM3 (GM3-Nase) in oligos increased continuously after the onset of myelination and reached to the adult level (636 pmol/mg prot/min), which was much higher than those in neurons and strocytes (40 and 4 pmol/mg prot/min). The GM3-Nase activity in myelin also increased in the active myelination period, but decreased thereafter to the adult level. In myelin subfractions, the enzyme activity was distributed evenly in all fractions at the younger age, but was enriched in "heavy" myelin fraction at the adult age. These results suggest that the GM3-neuraminidase in oligos may play an important role in the formation and maintenance of the myelin sheath. (supported by USPHS grants NS 23102 & NS 11853)

145.10

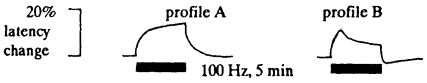
OLIGODENDROCYTE SUBPOPULATIONS ARE DIFFERENTIALLY AFFECTED IN THE MYELIN DEFICIENT RAT. A. Espinosa de los Monteros*, M. Zhang*, M.N. Gordon, S. Kumar*, S. Scully* and J. de Vellis. Mental Retardation Research Center, UCLA School of Medicine, Los Angeles, CA 90024-1759.

The myelin deficient (*md*) mutant rat is a model that offers the possibility for a better understanding of abnormalities in oligodendrocyte development and function. Recently, we proposed a model of oligodendrocyte development based on double immunocytochemistry for transferrin (Tf) and myelin basic protein (MBP) from studies done in vitro and in vivo (freshly dissociated cells). In the present study, we compared the distribution pattern of these oligodendrocyte markers, Tf and MBP, in freshly-dissociated cells from the *md* and wild-type rat brain. The *md* mutant rat possessed oligodendrocytes displaying the three different phenotypes previously described: Tf+/MBP-, Tf+/MBP+ and Tf-/MBP+. However, the distribution of cells among these three phenotypes was altered compared with control littermates. Tf expression was delayed in the *md* rat brain, and Tf+/MBP- oligodendrocytes were larger than Tf+/MBP+ cells from the same animal. Tf+/MBP+ cells were the expected size. Finally, cells with the Tf-/MBP+ phenotype were reduced in number, but of normal size. RNA hybridization (Northern) blots also demonstrated a developmental delay in the appearance of Tf mRNA, and decreased mRNA levels, in the *md* brain compared with unaffected littermates. In conclusion, these results suggest that a major abnormality in oligodendrocyte development in the *md* rat brain coincides with the Tf+ phenotype.

145.11

GENETIC DELETION OF MYELIN BASIC PROTEIN IS ASSOCIATED WITH ABNORMAL LATENCY SHIFTS DURING REPETITIVE IMPULSE DISCHARGE. S.C. Steffensen, S.A. Raymond, A.L. Ganser, and G.R. Strichartz. Anesthesia Research Laboratories, Brigham & Women's Hospital, & Neurology Research, Children's Hospital, Harvard Medical School, Boston MA 02115.

The latency of spike conduction in single myelinated fibers from excised mouse sciatic nerves was recorded during trains of 100Hz stimulation lasting 5-10 min. In normal controls, the latency increased throughout the stimulation for 12 of 13 fibers studied at 36 °C and for 9 of 10 fibers at 26 °C (profile A). In shiverer mice (age-matched and genetically-matched to controls except for a deletion in the gene for myelin basic protein "MBP"), the latency (N=17) showed a different profile (B), with a similar initial slowing but without progressive slowing during the maintained high-frequency discharge. Instead, conduction actually became more rapid. After the train, recovery in shiverer fibers was marked by a transient speed-up in conduction. Conduction velocities in normals at 36 °C (range 12-37 m/s, avg 29) overlapped extensively with that of the fiber population from shiverer (range 17-30 m/s, avg 25), leaving the absence of MBP to account for the difference in profiles. Normal axons showed the shiverer profile (B) after exposure to phorbol di-acetate at concentrations (14-28 μM) that are known to activate protein kinase C, and that did not change profiles of shiverer fibers. These results implicate myelin, and specifically phosphorylation events contingent on presence of MBP, in active processes that modulate impulse conduction during sustained impulse activity.



145.12

HYPOMYELINATION IN A NEW NEUROLOGICAL RAT MUTANT
I.D. Duncan, University of Wisconsin, Madison WI, B. Holmgren*, R. Urba-Holmgren*, and L. Riboni*, Universidad Autonoma de Puebla, Puebla, Mexico

A spontaneous mutation in a colony of Sprague-Dawley rats has been detected, which results in progressive neurological disturbances from 1 month of age onwards. Affected rats develop a fine tremor, ataxia (4 months), immobility episodes, and seizures and paralysis (after 10 months). Selective breeding suggests that it may be an autosomal recessive trait. There are no gross abnormalities of the CNS but weights of both the cerebrum and the cerebellum are significantly reduced in the mutant. Affected rats at 2, 3 and 6 months and normal littermates were perfused with Karnovsky's fixative and the tissue collected from the corpus callosum, cerebellum and spinal cord, for light microscopy, EM and immunocytochemistry. In the spinal cord there was marked hypomyelination in all funiculi, most notably at 6 months in the dorsal columns. This myelin abnormality was more marked at 6 months than at 2 months in the cord and cerebellum. Preliminary counts of glial cell number in the thoracic spinal cord show a marked increase in the mutant compared with controls at all ages. EM observations showed a widespread microtubular proliferation in oligodendrocytes. As microtubules are thought to play a role in vesicular transport of myelin constituents it is possible that this cytoplasmic abnormality plays a role in the myelination disorder. (Supported by NIH grant NS23124 and NMSS grant RG1791)

145.13

THE FACTOR(S) IN GLIAL CONDITIONED MEDIUM RESPONSIBLE FOR MYELIN EXPRESSION IN JIMPY CULTURES IS A PROTEIN. W.P. Bartlett. Dept. of Anatomy, M.S. Hershey Medical Center, Hershey, PA 17033.

Jimpy is a sex-linked mutation that results in severe hypomyelination in the CNS of affected male mice. In culture, jimpy oligodendrocytes express the mutation in a manner similar to that found in vivo. The number of oligodendrocytes and the expression of myelin-related features is significantly reduced in cultures of jimpy glial cells compared to controls. However, when cultures of jimpy glial cells are maintained in medium that has been "conditioned" by normal glial cells (GCM), there is a dramatic increase in the number of oligodendrocytes, synthesis of myelin-like membrane and expression of myelin basic protein. In order to determine whether the factor(s) in GCM responsible for myelin expression in jimpy cultures is a protein, GCM was subjected to trypsin and heat treatment. The trophic activity of GCM was abolished by both trypsin digestion (0.1% for 30' at 37°C, followed by 0.2% trypsin inhibitor) and heating (100°C for 4'). In these experiments, the normal cultures used to condition medium consisted principally of astrocytes (>95% GFAP+). These results suggest that the factor(s) responsible for myelin expression in jimpy cultures is a protein synthesized by astyrocytes. (Supported by National Multiple Sclerosis Society, Grant 1998.)

NEUROGLIA: ACTIVE MEMBRANE RESPONSES

146.1

STIMULATION OF α_2-ADRENERGIC RECEPTORS INCREASES $CA^{++}{}_i$ IN TYPE I ASTROGLIA. A. K. Salm and Ken D. McCarthy*. Dept. of Pharmacol. & Curriculum in Neurobiol., Univ. N. Carolina, Chapel Hill, NC, 27599.

Putative α adrenergic receptor (α AR) subtypes which influence $Ca^{++}{}_i$ in cultured rat type I astroglia were pharmacologically characterized using the fluorescent Ca^{++} probe fura-2. Spectrofluorometric and video imaging systems were used to monitor single cell and population $Ca^{++}{}_i$ responses in astroglia after exposure to α AR agonists and antagonists. Nearly 80% of astroglia (~200 cells) exhibited increased $Ca^{++}{}_i$ in response to norepinephrine. In experiments carried out in the presence of 1 uM propranolol to block β ARs, the majority of these responses appeared to be mediated via α_1ARs, e.g., they were more potently blocked by the α_1 AR selective antagonist prazosin than by the α_2 AR selective antagonist yohimbine. 10 μM of the α_1 AR selective agonist l-phenylephrine (l-phen) was also effective in eliciting increased $Ca^{++}{}_i$. These results were expected as α_1ARs are linked to 2nd messengers controlling the release of intracellular Ca^{++}. Surprisingly however, yohimbine was equally or more potent than prazosin in blocking the NE-evoked $Ca^{++}{}_i$ response in a number of cells. In some of these cases 10 uM of the α_2 AR selective agonist clonidine was also able to increase $Ca^{++}{}_i$. Testing of populations of cells in the presence of propranolol, 1 uM prasozin and 10 uM l-phen to block and then desensitize residual α_1ARs showed that a subpopulation of cells responded to the highly selective α_2 AR agonist UK 14,304. When the procedure was reversed, i.e., testing of l-phen following incubation with yohimbine and UK 14,304, it was seen that the populations of cells which expressed pharmacological characteristics of α_1ARs and α_2ARs only partially overlapped. Thus, it appears that subpopulations of type I astroglia exist: those that express α_2ARs and/or α_1ARs which regulate $Ca^{++}{}_i$. To our knowledge, this is the first indication that α_2ARs in brain may regulate Ca^{++}. (NS20212).

146.2

DISTINCT SUBSETS OF ASTROGLIA CAN BE IDENTIFIED BY THEIR CALCIUM RESPONSE TO DIFFERENT NEUROLIGANDS.
Ken D. McCarthy* and A.K. Salm (SPON: Barry Pallotta).
Dept. Pharmacology, Univ. North Carolina, Chapel Hill, NC 27599

The combined results from many different laboratories indicate that in culture, polygonal (type 1) astroglia exhibit in excess of twenty different neuroligand receptor systems. Most of these studies were completed using large numbers of purified astroglia and examining either second messenger responses or radioligand binding of the total population. Using these methods it has been difficult to establish whether pharmacologically-distinct subsets of astroglia are present in astroglial cultures. To address this question we have developed a video imaging system to simultaneously study the effect of neuroligands on multiple individual astroglia loaded with the indicator dye, fura-2. Images were stored within 2 sec of drug application and every 6 sec over the subsequent 1 to 2 min. Greater than 95% of cells in these cultures stain for GFAP. In several cases it was possible to immunocytochemically-identify studied cells as $GFAP^+$ polygonal astroglia. Distinct subsets of astroglia responded to different receptor agonists with an increase in calcium levels. Among six different ligands examined, stimulation of P_{2Y} purinergic receptors increased calcium in the largest percentage of astroglia. Histamine, carbachol, serotonin, glutamate, and phenylephrine each stimulated a subset of astroglia when added at their maximally effective concentration. While there was some overlap among cells responding to the different ligands, there were many examples of cells responding to a subset of the ligands. Cells also varied in the magnitude and time course of their calcium response to a given drug. The results of these studies indicate that, like neurons, astroglia consist of subpopulations of cells that respond uniquely to different receptor agonists. (Supported by NS 20212)

146.3

TYPE 2 ASTROGLIA EXHIBIT A NUMBER OF DIFFERENT NEUROLIGAND RECEPTORS THAT REGULATE INTRACELLULAR CALCIUM LEVELS.

Vijendra Dave* and Ken D. McCarthy* (SPON: P. Maness). Dept. Pharmacology, Univ. North Carolina, Chapel Hill, NC 27599

Type 2 astroglia appear to represent a distinct lineage of astrocytes which can be morphologically and immunocytochemically distinguished from type 1 astroglia. Problems in obtaining large numbers of purified type 2 astroglia have made it difficult to examine the expression of neuroligand receptors by these cells. In order to address the question of receptor expression by type 2 astroglia, we have developed a video imaging system to simultaneously study the effect of neuroligands on calcium levels in multiple individual astroglia loaded with the calcium indicator dye, fura-2. Type 2 astroglia were isolated from rat cerebral cortical tissue by the method of McCarthy and de Vellis and maintained in medium containing 10% FCS. Cells were loaded with 2.5 uM fura-2 AM for 30 min prior to analysis. Images were stored within 2 sec of drug application and every 6 sec over the subsequent 30 to 60 sec. Studied cells were marked and processed for GFAP immunostaining. Only results from GFAP+ process-bearing astroglia were analyzed. Agonists examined to date include norepinephrine, carbachol, histamine, 2-methyl-thio-ATP (a P2Y purinergic receptor agonist) and bradykinin. Treatment with any one of these agonists in uM concentrations led to a rapid rise in intracellular calcium. The magnitude and duration of the rise in calcium varied among cells. Type 2 astroglia also appeared to exhibit spontaneous oscillations in their calcium levels whose frequency could be influenced by certain neuroligands. Where it was possible to sequentially examine a series of neuroligands on a group of type 2 astroglia, it seemed that the ability of these cells to respond to the different drugs varied. These experiments indicate that like type 1 polygonal astroglia, type 2 astroglia exhibit a variety of neuroligand receptors that regulate their levels of calcium. (NS 20212).

146.4

ASTROGLIAL CALCIUM CONCENTRATION FALLS DURING SPREADING DEPRESSION. R.P. Kraig. Department of Neurology, Univ. of Chicago, Chicago, Il 60637

Astroglia are structurally and functionally robust. The molecular mechanisms by which this dynamism is accomplished are unknown, though fluctuations in cellular pH and Ca2+ are likely. Indeed, a significant pH rise occurs in these cells when they are depolarized &, i.e., can reach 7.6-7.8 pH during spreading depression (SD) (see refs below). To begin to assess any interrelation between pH & Ca2+ in astrocytes, Ca2+ measurements were made during SD. Rats (n=9) were anesthetized with halothane, & experiments done as previously described (Chesler & Kraig, Am J Physiol, '87: J Neurosci, '89). Ca2+ measurements were made with electrodes whose detection limit was 7-8 pCa2+ & showed the typical absence of change in astrocytic (& variable rise in neuronal) Ca2+ during stimulation (Morris et al., Neurosci, '85). Surprisingly, astrocytic Ca2+ fell during SD from about a uM to the detection limit of the electrodes (n=12). Since astrocytic pH rises during SD part of the fall in Ca2+ seen may be due to passive, physicochemical buffering of Ca2+ though active uptake into organelles is also likely. These results raise the possibility that astrocytic Ca2+ (like pH?) may be modulated from some resting level when cells are activated.

146.5

ATP-DEPENDENT CALCIUM INFLUX STIMULATES PROTEIN PHOSPHORYLATION/DEPHOSPHORYLATION IN ASTROCYTES. J.T. Neary, J. Blicharska*, L.O.B. Norenberg* and M.D. Norenberg, Lab. Neuropath., Vet. Admin. Med. Ctr. & Univ. of Miami/Jackson Mem. Hosp., Miami, FL 33101.

We have recently shown that ATP and other purines stimulate calcium uptake in astrocytes (Biochem Biophys Res Commun 157: 1410, 1988). Since some actions of calcium are mediated by protein phosphorylation, we have now investigated the effect of the ATP-stimulated increase in intracellular calcium on protein phosphorylation in astrocytes. Primary astrocyte cultures were prepared from newborn rat cortices. Following incubation of the cells in physiological salt solution containing 32-Pi for 1.5 hr, ATP (1 uM-1 mM) was added for 0.5-1.5 min. We found 4- and 2-fold increases in phosphorylation in 55 and 52 kDa proteins, respectively; the latter protein co-migrated with glial fibrillary acidic protein. We also noted 50% decreases in phosphate incorporation in 24 and 21 kDa proteins. These effects were time- and dose-dependent. The changes were completely blocked by $LaCl_3$ and partially reduced by EGTA, thereby suggesting that the ATP-induced changes in 32-P incorporation were dependent on increased levels of intracellular calcium. Cultures previously treated with dibutyryl cyclic AMP have increased calcium-stimulated phosphorylation and elevated levels of 32-P incorporation in 55 and 52 kDa proteins (Neary et al, Brain Res 410: 164, 1987); addition of ATP to these cells had little effect on the phosphorylation of these proteins but did decrease 32-P incorporation in the 24 and 21 kDa proteins. In summary, these results suggest that calcium-dependent protein kinases and phosphatases transduce the effects of the calcium signal brought about by activation of purinergic receptor-operated calcium channels in astrocytes.

146.6

EFFECTS OF BRAIN NATRIURETIC PEPTIDE (BNP) AND ATRIOPEPTINS ON RAT CEREBROCORTICAL ASTROCYTES. K. Beaumont and P.K. Tan. Dept. of Medicine, UCSD, La Jolla, CA 92093.

BNP is a peptide recently isolated from porcine brain that has high sequence homology to atrial natriuretic factor (ANF). The separate distribution of BNP and atriopeptin III immunoreactivity in rat CNS (Saper et al., Neurosci. Lett. 96:29, 1989) indicates that these structurally-related peptides have separate functions. Prior studies (Friedl et al., J. Neurochem 52:589, 1989; Fiscus et al., ibid, 48:522, 1987) have demonstrated that atriopeptins elevate cyclic GMP levels in glial cultures and C6 glioma cells. We determined the effects of porcine BNP (pBNP) and ANF-related peptides upon astrocytes from rat cerebral cortex, a region with high BNP and low atriopeptin levels, in order to identify possible distinct actions of these peptides.

In astrocyte cultures (21-26 days) prepared from 1-2 day rat pup cerebral cortices, 1 μM rat ANF (rANF) increased cyclic GMP levels over 100-fold to 31 pmol/mg. Lowest concentration of rANF producing stimulation was 1 nM. Atriopeptin III and auriculin B were somewhat less potent than rANF, while atriopeptin I was significantly less effective and ring-deleted C-ANF(4-23) had no effect. BNP was less potent than atriopeptin III at concentrations below 100 nM, but produced greater stimulation than rANF at 1 μM. In preliminary studies, rANF did not alter basal potassium transport in astrocytes. Astrocyte cultures contained relatively high densities of 125-I-rANF binding sites (10 fmol/mg at 150 pM). Competition curves with rANF and BNP were biphasic, indicating presence of multiple sites. Potent competition by C-ANF(4-23) indicates the presence of the C-ANF (clearance) receptor subtype in astrocytes in addition to guanylate-cyclase coupled (B-ANF) receptors.

146.7

IMMUNO-ULTRASTRUCTURAL LOCALIZATION OF SODIUM CHANNELS AT NODES OF RANVIER AND ASTROCYTES IN RAT OPTIC NERVE. J.A. Black, B. Friedman, S.G. Waxman, L.W. Elmer[1*], and K.J. Angelides[1*]. Dept. Neurology, Yale Univ. Sch. Med. and VAMC, West Haven, CT. 06516 and Dept. [1]Physiol. and Molec. Biophys., Baylor Col. Med., Houston, TX 77030.

Voltage-dependent sodium channels are concentrated within the axon membrane at nodes of Ranvier and are in much lower density within the axon membrane beneath the myelin sheath. The distribution of sodium channels within rat optic nerve was examined ultrastructurally with anti-sera 7493, which is directed against purified sodium channels from rat brain and which recognizes a single band of Mr 260 kDa in immunoblots.

Rat optic nerve sections incubated with anti-sera 7493, and then reacted with biotin-avidin-HRP, exhibited intense immunostaining of the axon membrane at nodes of Ranvier; notably, axon membrane beneath the myelin sheath did not display immunoreactivity. Perinodal astrocyte processes, as well as somata and major longitudinally-oriented processes of astrocytes, were immunostained by anti-sera 7493. Astrocyte processes forming the glia limitans and surrounding blood vessels generally exhibited limited immunostaining with anti-sera 7493.

These observations suggest a role for astrocyte in the aggregation/maintenance of sodium channels within the axon membrane at the node of Ranvier.

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146.8

TUMOR NECROSIS FACTOR PRODUCES PROCESS RETRACTION AND POTASSIUM CURRENT INHIBITION IN OLIGODENDROCYTES. B. Soliven*, S. Szuchet*, and D.J. Nelson* (SPON: S. Bursztajn). University of Chicago, Department of Neurology, Chgo, Il. 60637.

Tumor necrosis factor (TNF-α), a cytokine secreted by activated macrophages, has been reported to induce a delayed-onset oligodendrocyte (OLG) necrosis and demyelination *in vitro* (Selmaj & Raine, Ann. Neurol. 23:339-346, 1988). We used the whole-cell patch-clamp technique to investigate whether dysfunction of ion channels in OLGs might contribute to these pathological changes. Adult ovine OLGs after 2-3 weeks in culture were incubated with recombinant human TNF (10^4 U/ml) for 48-72 hrs. These cells remained viable as determined by dye exclusion, but some of them had either partially or completely retracted processes. Resting membrane potential (RMP) was estimated as the zero current potential from current-voltage plots. Cells that had retracted processes (grp.A) were found to have a depolarized RMP: -37.3 ± 2.0 mV (n=18), as compared to -62.4 ± 2.3 mV (n=8) in cells that retained normal processes (grp.B). RMP in untreated cells (grp.C) was -68.6 ± 1.5 mV (n=16). Mean amplitude of inwardly rectifying K^+ current measured at -120 mV was -63 ± 12.5 pA in grp.A; -185.8 ± 35.8 pA in grp.B, and -507 ± 126 pA in grp.C. There was no significant difference in the cell membrane capacitance (grp.A = 16.6 ± 2.1 pF, n=8; grp.C = 20.1 ± 3.1 pF, n=7). Preliminary data from OLGs incubated with IL-2 showed no reduction in RMP or inward current amplitude. We conclude that TNF-treated OLGs exhibit reduced mean current amplitude. Resting membrane potential is reduced in cells with retracted processes. We postulate that dysfunction of ion channels may precede process retraction based on our finding that current amplitude was also reduced in TNF-treated cells that appeared morphologically normal. Supported by NIH grant PO1 NS24575. B.S. is a fellow of Natl. M.S. Soc.

146.9

INWARD RECTIFYING POTASSIUM CHANNELS IN RETINAL GLIAL (MÜLLER) CELLS. Eric A. Newman, Eye Research Inst. and Dept. Ophthalmol., Harvard Med. Sch., Boston, MA 02114.

Current-voltage (I-V) relations of dissociated salamander (*Ambystoma tigrinum*) Müller cells were determined with two-electrode, whole-cell voltage clamp. Patch electrodes contained 88 mM K^+; cells were bathed in a solution containing 88 mM K^+ and 2 mM Cd^{2+} (to block Ca^{2+} and Ca^{2+}-activated K^+ currents).

The I-V relation of cells whose endfeet had been shorn off during the dissociation process showed strong inward rectification. Cell reversal potential was near 0 mV, the K^+ equilibrium potential. For hyperpolarizing command pulses, the evoked current showed partial inactivation. The I-V relations of Müller cells with their endfeet intact were almost identical to those of cells lacking endfeet in the degree of rectification and in the time course of inactivation. They differed only in the magnitude of the current, which was 8.8 times larger for cells with endfeet.

In cell-attached patch clamp recordings from the soma region, multi-channel currents showed strong inward rectification. Much larger currents showing similar rectification were recorded from patches on the endfoot. Currents of single, inward rectifying channels were recorded from soma patches. However, single channel currents could not be resolved from endfoot patches due to the high density of channels in this cell region.

These results indicate that the principal conductance of amphibian Müller cells in all cell regions is mediated by K^+ inward rectifying channels. Greater channel density in the endfoot is responsible for the high conductance of this cell region. Supported by grant EY04077.

146.10

EXCITATORY AMINO ACID AGONIST-EVOKED TAURINE RELEASE FROM CULTURED ASTROCYTES. G.R. Dutton, R.A. Philibert, M.L. Simmons*, R. Miyazaki* and J. Eastburn*. Department of Pharmacology, College of Medicine, University of Iowa, Iowa City, IA 52242.

Cultured cerebellar astrocytes from 7-9 day rats and grown 17-19 days in vitro release taurine in response to elevated K^+ levels in a dose-dependent fashion requiring Ca^{2+} and Na^+. Since these cells are reported to possess excitatory amino acid receptors, we began studies to determine if elevated concentrations of glutamate, or its receptor agonists, produced similar increases in efflux.

Quisqualate (1-100 μM) and N-methyl aspartate (0.1-1000 μM) had no effect on the basal levels of taurine efflux. However, kainate (10-1000 μM) increased release approximately 3-500% at concentrations >200 μM. In addition, L-glutamate produced a dose-dependent, 4-fold increase in taurine efflux (EC_{50} = 100 μM) over the range 10-1000 μM. The kainate-evoked taurine efflux may not be receptor mediated since the antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 200 μM) did not attenuate release evoked by 500 μM kainate.

Thus, these and our previous results suggest, that in cultured astrocytes increased taurine efflux produced by elevated K^+, glutamate and kainate may be related to a recovery phase following cellular swelling during which taurine is expelled into the extracellular space.

This work was supported by NIH grant NS 20632.

146.11

THE EFFLUX OF PRELOADED GABA FROM CEREBRAL ASTROCYTES IS STIMULATED BY ELEVATED POTASSIUM BUT NOT BY VERATRIDINE AND OUABAIN. J. Albrecht* and M.D. Norenberg. Laboratory of Neuropathology, Veterans Adm. Med. Ctr. and Univ. Miami Sch Med/Jackson Mem. Hosp., Miami, FL 33101.

Studies on mixed glial preparations have indicated that glia release GABA upon treatment with depolarizing agents (c.f: Sarthy, J.Neurosci. 3, 2494, 1983). However, in cerebellar astrocytes potassium-stimulated GABA release was not detected (Pearce et al., Brain Res., 206, 485, 1981), casting doubt on its ubiquity in glia. In the present study, GABA release was investigated in cultured astrocytes derived from neonatal rat cerebral cortex. The cells (21-30 DIV) were preloaded with [^{3}H] GABA and subjected to superfusion in Krebs-Ringer medium followed by a change to stimulation media. GABA efflux was stimulated by K^+ in a dose-dependent manner from 7.5-120 mM. The effect of K^+ appeared Ca^{2+}- and Mg^{2+}-independent, suggesting a non-vesicular mechanism of GABA release. Veratridine (100 uM) and ouabain (1 mM), which in synaptosomes stimulate Ca^{2+}-independent GABA release by collapsing the Na^+ electrochemical potential, evoked in astrocytes a transient decrease of GABA efflux. The results indicate that astrocytes derived from neonatal rat cerebral cortex are capable of GABA release upon treatment with depolarizing concentrations of K^+. While the mechanism of K^+-induced, Ca^{2+}-independent GABA release from astrocytes remains to be elucidated, the results suggest that, unlike that in synaptosomes, it must occur by a process other than a reversal of the Na^+-driven GABA uptake.

146.12

HYPOOSMOTIC MEDIA-INDUCED RELEASE OF AMINO ACIDS FROM ASTROCYTES. H.K. Kimelberg, S. Goderie* and †R. Waniewski. Div. of Neurosurgery, Albany Medical College and †Wadsworth Center for Labs and Res., N.Y. State Dept. of Health, Albany, New York

During exposure of primary astrocyte cultures to hypo-osmotic media the cells rapidly swell and then return to normal values, a process termed regulatory volume decrease or RVD. We have found that under these conditions the cells lose label after loading them with [^{3}H] D-aspartate, [^{3}H] L-glutamate or [^{3}H] taurine, as previously reported for [^{3}H] taurine (Pasantes-Morales & Schousboe, J. Neurosci. Res. 20, 505, 1988). There is also comparable loss of endogenous L-glutamate, glutamine or taurine as measured by high pressure liquid chromatography. We have found that this release, measured by loss of label, can be inhibited by the anion exchange transport inhibitors SITS and furosemide, but not by bumetanide a specific inhibitor of $Na^+ + K^+ + 2Cl^-$ co-transport. A novel fluorenyl compound, L-644,711, an effective anion exchange inhibitor, also inhibits release of [^{3}H] L-glutamate and [^{3}H] taurine and this compound has been shown to improve recovery from a brain trauma/hypoxia injury in which astrocytic swelling occurs (Kimelberg et al. Central Nervous System Trauma, 4,3, 1987). It now seems possible that part of this beneficial effect may be due to inhibition of release of excitotoxins such as L-glutamate from swollen astrocytes.
(Supported by grant NS23750 to H.K.K.).

146.13

IONIC DEPENDENCE OF [^{3}H]ADENOSINE UPTAKE INTO CULTURED ASTROCYTES. A.S. Bender, D.M. Woodbury, and H.S. White*. Dept. Pharmacol. & Toxicol., Univ. of Utah, Salt Lake City, UT 84112.

Astrocytes in primary culture possess a high-affinity uptake system for the inhibitory neurotransmitter adenosine that is equivalent to that of cultured neurons. Removal of either Na^+, Cl^- or Ca^{2+} significantly reduced [^{3}H]adenosine uptake into cultured mouse astrocytes by 42, 27 and 13%, respectively. Pretreatment with ouabain (1 mM), DIDS (1 mM) or the Ca^{2+} ionophore A23187 (1 μM) also reduced uptake by 50, 33 and 15%, respectively. In addition, uptake was blocked by the proton pump inhibitor omeprazole, the K^+ channel activators BRL 34915 and nicorandil and the Na^+/K^+-Cl^- exchange inhibitor furosemide (IC50's: .047, .075, .696 and 2.2 mM, respectively). The potent inhibition of uptake by BRL 34915 probably represents a direct effect on the adenosine carrier as opposed to the K^+ channel since its effect was not antagonized by glyburide and since the removal of K^+ or the addition of excess K^+ failed to affect [^{3}H]adenosine uptake. These results demonstrate that astrocytic adenosine uptake is under complex regulation and may represent a potential target for CNS-acting drugs such as vasodilators and anticonvulsants. Supported by NIH grant NS-22200 from the NINDS (HSW) and a fellowship from the Medical Research Council of Canada (ASB).

146.14

PH REGULATION IN GLIAL CELLS OF NECTURUS OPTIC NERVE. M.L. Astion*, A. Chvatal*, and R.K. Orkand. Inst. of Neurobiology, Univ. of Puerto Rico Medical Sciences Campus, San Juan, PR 00901.

Glial cells from intact Necturus optic nerve were impaled with double-barreled pH-sensitive microelectrodes. At a bath pH of 7.5, the mean intracellular pH (pH_i) was 7.32 (S.D.=0.03, n=6) in nominally HCO_3^--free solution and 7.39 (S.D.=0.1, n=6) in HCO_3^-/CO_2. Superfusion (4 min) followed by withdrawal of 15 mM NH_4^+ induced an acidification of less than 0.4 pH unit after which the pH_i recovered toward the original baseline. In the absence of HCO_3^-/CO_2, the recovery from acidification was Na^+-dependent, and blocked by 1 mM amiloride. The recovery was stimulated by HCO_3^-/CO_2 which also hyperpolarized the membrane. In HCO_3^-/CO_2, the recovery from acidification was Na^+-dependent, inhibited by 0.5 mM SITS, and associated with a hyperpolarization of the membrane. Removal of Na^+ or addition of SITS induced a depolarization which accompanied the inhibition of the pH_i recovery. The data strongly suggest that the relatively alkaline pH_i of glial cells is maintained by at least two mechanisms, Na^+/H^+ exchange and electrogenic Na^+/HCO_3^- cotransport. Supported by NIH grants NS07464, GM07170, and RII8705802 (NSF).

147.1

CAPSAICIN INHIBITS VOLTAGE-ACTIVATED CALCIUM CURRENTS IN A SUB-POPULATION OF ADULT RAT DORSAL ROOT GANGLION NEURONES. *B. Robertson*[T], *R.J. Docherty* and S. Bevan** (SPON: I. Forsythe) Sandoz Institute for Medical Research, 5 Gower Place, London WC1E 6BN, UK.

Capsaicin opens a cation (Ca^{2+}, Na^+, K^+) permeable channel in a sub-population of DRG neurones. In addition, capsaicin has been reported to increase the inactivation rate of voltage gated calcium currents in guinea pig DRG neurones, where it had no agonist action.

We have examined whole cell calcium currents in freshly plated rat DRG neurones, which show the agonist effect of capsaicin. In calcium (2.5-10mM) containing solutions, capsaicin (0.5-30μM) evoked an inward calcium current (V_H=-80mV) in some neurones. In such cells, voltage activated calcium currents were inhibited during exposure to the agonist. With brief exposures (<10secs) some recovery of the calcium current was noted whereas longer exposure always resulted in an irreversible inhibition or abolition. In cells where capsaicin failed to elicit an inward current the calcium currents were either unaltered or showed an apparent increase in inactivation rate, which was reversible. In solutions with either Ba^{2+} (10mM) or Na^+ (140mM + 1μM TTX) as the charge carrier, capsaicin no longer produced an irreversible depression of the voltage activated current even though a large agonist induced current was elicited.

We conclude that the irreversible effects of capsaicin on voltage activated calcium currents are secondary to increases in intracellular calcium and are unlikely to be due to a direct effect of capsaicin on calcium channels.

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147.2

DEPOLARIZING ACTION OF DANTROLENE Na ON CA1 HIPPOCAMPAL NEURONS. K. Krnjević and Y. Z. Xu. Anaesthesia Research Department, McGill University, Montréal, Québec, Canada.

At a concentration of 10-20 μM dantrolene (DAN) consistently depolarized pyramidal cells in slices from Sprague-Dawley rats (by 5-30 mV). This effect which was rapidly reversible after washing-out DAN, was accompanied by no change or some apparent reduction in input resistance and a marked depression of slow after hyperpolarization (both measured at initial resting potential). Neither the depolarization nor the corresponding slow inward shift recorded by single electrode voltage clamp was blocked by tetrodotoxin: they were therefore unlikely to be mediated by Na currents. Similarly they were not affected by 4 mM Cs, which fully blocked anomalous rectification and the time-dependent Q-current. Being equally well defined when recording with KCl or K acetate-filled microelectrodes, the DAN-evoked depolarization also cannot be due to activation of a Cl^- current. We therefore conclude that the inward current that is either activated or uncovered by DAN is probably carried by Ca^{2+}. Such an effect could be produced by diminished Ca^{2+} release from internal binding sites. A direct action of DAN on surface membrane channels has not been excluded. Supported by Medical Research Council of Canada.

147.3

CALCIUM SPIKES ARE REDUCED BY SOMAN IN BULLFROG SYMPATHETIC GANGLION CELLS. T.J. Heppner* and J.F. Fiekers (SPON: B. Kapp). Dept. Anat. and Neurobiol., Univ. of Vt. Coll. Med., Burlington, VT 05405.

The action of soman on Ca^{++} spikes was examined in sympathetic ganglion cells located in the isolated 9th and 10th paravertebral ganglion of the bullfrog Rana catesbeiana. Intracellular recordings were obtained from ganglion cells superfused with high Ca^{++} saline (Ca^{++}, 9.0 mM; TTX, 1.5 uM; TEA, 20 mM). Prolonged action potentials (~70 mV; 23ms-43ms) were recorded in response to brief depolarizing current pulses. Soman (0.1 uM) decreased the duration of the Ca^{++} spike to 37% of pretreatment values. Similar results were obtained when Ca^{++} was replaced with 2 mM Ba^{++}. Although the spike duration was greatly prolonged in Ba^{++} (ranging from 57 ms to 12 sec), soman (0.1 uM) reduced the Ba^{++} spike duration to 23% of pretreatment values. Both Ca^{++} and Ba^{++} spikes fully recovered within 6 min of a wash period. The membrane input resistance measured with hyperpolarizing pulses was not significantly altered by soman (0.1 uM). Pretreatment with atropine (5 uM) or hexamethonium (10 uM) did not prevent the soman-induced reduction in spike duration, suggesting that this action of soman was not mediated through nicotinic or muscarinic receptors. These results suggest that soman alters Ca^{++} channel properties in bullfrog sympathetic neurons. This work supported in part by the USAMRDC, Contract No. DAMD17-86-C-6031.

147.4

A DEVELOPMENTAL PROFILE OF RECEPTORS FOR PHENYLALKYLAMINE CALCIUM CHANNEL LIGANDS IN *DROSOPHILA*. Robert M. Greenberg[1]*, H. Glossmann[2]*, and Linda M. Hall[1] (SPON: D.Gil). [1]Dept. of Mol. Genetics, Albert Einstein Coll. Med., Bronx, NY 10461 and [2] Institut für Biochemische Pharmakologie, Innsbruck, Austria.

We are interested in establishing a molecular genetic system for studying voltage-sensitive calcium channels in *Drosophila*. As a first step, we defined receptors for calcium channel blockers in *Drosophila* head membranes. Phenylalkylamine calcium channel ligands bind to high and low affinity receptors in membranes from *Drosophila* heads. These receptors are associated with 136 kDa (high affinity) and 27 kDa (low affinity) proteins. Receptors for other classes of calcium channel ligands (dihydropyridines, benzothiazepines) are barely detectable in this preparation. The phenylalkylamine binding activity has been solubilized in 1% digitonin and the digitonin/receptor complex runs on sucrose gradients with an apparent molecular weight of 260,000 (Greenberg, R.M. *et al.*, *Insect Biochem.*, in press). We have been examining the tissue specificity and developmental profile of each receptor. Both the stereoselective, high affinity receptor and the low affinity receptor are enriched in membranes from *Drosophila* heads compared with bodies. Thus, both receptors are likely to be components of the nervous system. The B_{max} of the high affinity receptor is very low during embryogenesis and then increases dramatically. The density of the receptor then remains relatively constant during later developmental stages. In contrast, the low affinity receptor is present at high levels in embryonic stages and remains at these levels throughout development. Thus, the two receptors for phenylalkylamine calcium channel blockers in *Drosophila* are not coordinately regulated during development.

147.5

IONIC BASIS OF THE CARDIAC ACTION POTENTIAL IN THE MOTH: EVIDENCE FOR A TTX-SENSITIVE CALCIUM CHANNEL. D.L. Brink and N.J. Tublitz. Inst. Neurosci., U. Oregon, Eugene, OR 97403.

We are investigating the mode of action of 2 cardioregulatory neuropeptides on the heart of the tobacco hawkmoth, M. sexta. As part of this study, the ionic mechanisms underlying the cardiac action potential were analysed using standard intracellular recording of spontaneously contracting heart cells. The moth heart action potential is characterized by a slow pre-potential leading to a fast, overshooting depolarization, followed by a pronounced, 100-200 ms long depolarizing plateau and terminated by a rapid repolarization. Ion substitution experiments, in which $[Na^+]_{out}$ was replaced by isotonic dextrose, had no detectible effect on action potential shape or duration. In contrast, the action potential was markedly affected by manipulation of $[Ca^{2+}]_{out}$. Elevated $[Ca^{2+}]_{out}$ increased pre-potential duration, while reduced extracellular Ca^{2+} diminished action potential amplitude and duration. Exposure to Ca^{2+}-free saline or saline containing 5 mM Co^{2+} reversibly eliminated all spontaneous electrical activity whereas replacement of $[Ca^{2+}]_{out}$ by equimolar Ba^{2+} substantially prolonged the depolarized plateau and prepotential. The organic Ca^{2+} channel blockers verapramil and w-omega conus toxin were totally ineffective. Surprisingly, the Na^+ channel blockers TTX (1 uM) and STX (1 uM) applied in a Na^+-free saline reversibly eliminated the action potential. These results suggest the hypothesis that the Manduca heart muscle contains a class of cation channels with the ionic selectivity of a calcium channel and the pharmacology of a voltage sensitive sodium channel. We are currently testing this hypothesis with patch-clamp techniques.

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147.6

DIFFERENTIAL INHIBITION BY ω-CONOTOXIN OF cGMP PRODUCTION IN CULTURED CEREBELLAR NEURONS. P.G. Lysko* and G.Z. Feuerstein (SPON: K.M. Woodbury). Dept. of Neurology, USUHS, Bethesda, MD 20814.

Cultured cerebellar granule cells have previously been shown to have dihydropyridine binding sites which regulate Ca^{2+} influx through voltage-sensitive Ca^{2+} channels. Ca^{2+} influx has been shown to be inhibited by nifedipine and the inorganic ion Cd^{2+}, and stimulated by the Ca^{2+} channel agonist Bay K 8644. The excitatory amino acid glutamate, the analog kainate, and the alkaloid veratridine are all known to cause depolarization leading to Ca^{2+} influx and cGMP production. Here we measured cGMP production in granule cells exposed to these different depolarizing agents and examined their sensitivity to the Ca^{2+} channel blocker ω-conotoxin GVIA (ω-CT). Only glutamate-stimulated cGMP production was significantly inhibited (25-30%) by ω-CT. Inhibition by ω-CT occurred over a narrow dose-response range, with no inhibition at 2 μM and maximal inhibition at 20 μM. At these high concentrations, ω-CT displayed no agonist activity. Inhibition of glutamate-induced cGMP production by ω-CT was dependent on incubation conditions; the greatest inhibition occurred in buffer containing Mg^{2+} but not glucose, conditions which enable glutamate to become excitotoxic in these cells. The results suggest that glutamate mediates Ca^{2+} influx via different channels than other stimuli, which may be useful in understanding glutamate-induced excitotoxicity.

147.7

IFENPRODIL TARTRATE INHIBITS SLOW PHASE OF VOLTAGE-DEPENDENT $^{45}Ca^{2+}$ UPTAKE INTO SYNAPTOSOMES. H. Honda[1'2], T. Shibuya[1'2], B. Salafsky[1], and B. Curtis[1] 1: Department of Biomedical Sciences University of Illinois College of Medicine at Rockford, Rockford, IL 61107-1897. 2: Department of Pharmacology, Tokyo Medical College, Tokyo 160, Japan.

Ifenprodil tartrate (IFT), which has been used in the treatment of human cerebrovascular disease, has both α-adrenergic blocking [1] and vasorelaxing actions [2]. IFT also has the action of increasing cerebral blood flow [3]. We have previously suggested that the vascular myorelaxing action of IFT is due to the inhibition of Ca^{2+} mobilization in the cell [2,3]. We report here the effect of IFT on fast and slow phases of voltage-dependent $^{45}Ca^{2+}$ uptake into cerebrocortical synaptosomes of rats. IFT (10^{-8} to 10^{-4}M) induced dose-related inhibition of voltage-dependent $^{45}Ca^{2+}$ uptake during the slow phase. But IFT had little affect on the fast phase of $^{45}Ca^{2+}$ uptake. Results suggest that IFT specifically inhibits the slow phase of voltage-dependent $^{45}Ca^{2+}$ uptake into cerebrocortical synaptosomes of rat.

[1] Honda et al. (1988) Arch. Int. Pharmacodyn. 292: 112.
[2] Honda and Sakai (1987) Arch. Int. Pharmacodyn. 285: 211
[3] Shibuya et al. (1989) Int. J. Clin. Pharmacol. Ther. Toxicol. 26: 534.

Supported by a grant of the Monbusho International Scientific Research Program (1988).

147.8

INHIBITION OF DEPOLARIZATION-INDUCED CALCIUM UPTAKE IN RAT BRAIN SYNAPTOSOMES BY TRICYCLIC ANTIDEPRESSANTS. G. Beauchamp*, P.-A. Lavoie and R. Elie*. Dept. Pharmacology, Univ. of Montreal, Montreal, Canada.

We have used a choline-based medium, so that the increased calcium uptake due to replacement of choline by K^+ was only a reflection of calcium channel activation by depolarization. Brain cortex synaptosomes were preincubated for 20 min at 30°C in medium rich in choline (145 mM), with or without imipramine, desipramine, chlorimipramine, amitriptyline, or diltiazem (classical calcium channel antagonist). A 100 µl aliquot of preincubated synaptosomes was then added to an equal volume of either choline-based or potassium-based medium containing ^{45}calcium, and incubated for 10 sec at 30°C. Calcium uptake was terminated by addition of 5 ml cold calcium-free choline buffer containing 1 mM ethyleneglycol-bis-(β-aminoethyl ether)-N,N'-tetraacetic acid, followed by rapid filtration on Whatman GF/C filters. The filters were rinsed, and then immersed in Aquasol for liquid scintillation counting. Basal uptake (5 mM K^+) was essentially unaffected by drug concentrations ranging from $2X10^{-6}$ M to $2X10^{-4}$ M. In contrast, stimulated uptake (60 mM K^+) was dose-dependently reduced, which may indicate an antagonist activity at the neuronal voltage-dependent calcium channels; all drugs tested appeared equipotent, and virtually complete inhibition was achieved at $2X10^{-4}$ M. (Supported by CAFIR fund of University of Montreal).

147.9

THE HIGH-AFFINITY RECEPTOR FOR ω-CONOTOXIN IN BRAIN DIFFERS FROM THE DIHYDROPYRIDINE RECEPTOR. T. Abe, N. Hayakawa*, T. Yamaguchi*, T. Morita*, H. Saisu* and H. Mitsui*[1]. Dept. of Neurochem, Brain Res. Inst. and [1]Dept. of Biol., Faculty of Sci., Niigata Univ., Niigata 951, Japan.

Multiple types of voltage-sensitive calcium channels exist in the nervous system. However, the molecular basis of the diversity is not known. We prepared monoclonal antibodies (mAbs) to the dihydropyridine (DHP) receptor purified from the rabbit skeletal muscle to identify calcium channel molecules in the brain. One mAb immunoprecipitated practically all the DHP binding activity in the digitonin extract of the skeletal muscle microsomes. It could also precipitate most (>80%) of the high-affinity DHP binding activity in the digitonin extracts of the bovine cardiac muscle and brain membranes However, it did not significantly precipitate the receptor for ω-conotoxin GVIA (GVIA) in the digitonin extract of the bovine brain membranes. These results indicate that most, if not all, of the GVIA receptor in the brain differs from the DHP receptor (L-type channels). Since GVIA probably does not affect T-type channels, the high-affinity GVIA binding site in the brain most likely represents N-type calcium channels.

147.10

CALCIUM CHANNEL BLOCKING ACTIONS OF OCTANOL. D.A. Twombly* and T. Narahashi (SPON: D.L. Colbern). Dept. Pharmacology, Northwestern Univ. Sch. Med., Chicago, IL 60611.

Octanol has been reported to produce specific block of low-threshold calcium conductances in inferior olivary neurons (Llinas and Yarom, Soc. Neurosci. Abstr., 1986) and reticular neurons (Leonard & Llinas, Soc. Neurosci. Abstr., 1987). The objective of our study was to determine whether the block of low-threshold channels could involve modification of inactivation mechanisms.

Whole-cell voltage clamp experiments were performed on N1E-115 neuroblastoma, cells which possess low-threshold "type I" calcium channels as well as high-threshold "type II" channels. At concentrations of 20 - 300 µM, octanol reversibly suppressed the amplitudes of both types of calcium channel currents, without altering the voltage dependence of activation. Type II currents were somewhat more sensitive to octanol than were type I currents. After exposure to 100 µM octanol, for example, peak type I and type II currents were reduced by 27% and 35%, respectively. Octanol also produced changes in type I channel inactivation, including accelerated inactivation kinetics and 5 - 8 mV hyperpolarizing shifts in the voltage dependence of steady-state inactivation. During repetitive stimulation at 0.5 - 8.0 Hz, the peak amplitudes of type I currents decreased less in the presence of octanol than in control. The differences in use-dependent behavior were partly due to more rapid recovery from inactivation.

These experiments have confirmed the ability of octanol to block voltage-dependent calcium channels. The potency of octanol was approximately 1000-fold greater than that of ethanol, which also blocks type I and type II currents (Twombly *et al.*, Alcohol. Clin. Exp. Res., 1988). Octanol did not prove to be selective for low-threshold channels in this preparation. However, it exerted specific effects on type I channel inactivation which could modulate the block as a function of holding potential and cell activity (supported by grant AA07836).

147.11

CHLORODIAZEPOXIDE BLOCKS TWO TYPES OF Ca^{2+} CHANNELS IN NEUROBLASTOMA CELLS. E. Reuveny and T. Narahashi. Dept. of Pharmacol., Northwestern Univ. Med. Sch., Chicago, IL 60611.

Benzodiazepines (BDZ) in µM concentrations inhibit voltage-dependent Ca^{2+} uptake in mammalian nerve terminal preparations and Ca^{2+} conductance in leech and sea snail neurons. In synaptosomes Ca^{2+} uptake is regulated by µM affinity BDZ binding site rather than the Cl^- conductance site. These two binding sites have been distinguished on the basis of kinetic and pharmacological studies and the inhibition of electric shock-induced seizures. We studied the effect of chlorodiazepoxide (CDZ), a BDZ with anticonvulsant properties on voltage-gated Ca^{2+} channels. CDZ was slightly more potent in producing resting block of type I (K_i=350 µM) than type II (K_i= 420 µM) Ca^{2+} channels in a dose-dependent and reversible manner. CDZ block of type II channels was also time- and voltage-dependent, increasing with larger depolarizations. In addition, CDZ produced use-dependent block of type I channels and shifted the steady-state inactivation curve by 7.4 mV in the hyper-polarizing direction. Closed and open channel block by CDZ of the two types of Ca^{2+} channels may be correlated with the inhibition of electric shock-induced seizures, by either inhibiting Ca^{2+}-dependent transmitter release or decreasing the peak and duration of the Ca^{2+} action potential. Supported by NIH grant NS14144.

147.12

PHYLOGENETIC AND SUBTYPE SPECIFICITY OF ω-CONOTOXINS FOR CALCIUM CHANNELS. L. J. Cruz, J. S. Imperial*, G. C. Zafaralla*, J. Rivier* and B. M. Olivera. Dept. of Biology, Univ. of Utah, Salt Lake City, UT 84112, Salk Institute, La Jolla, CA 92307 and Marine Sci. Inst. and Dept. of Biochem., Univ. Philippines, Metro Manila.

ω-Conotoxin GVIA was first demonstrated by McCleskey, et al. (*PNAS* 84:4327, 1987) to block both the N and L subtypes of calcium channels in chick dorsal root ganglia. So far, eleven ω-conotoxins have been isolated and sequenced from several fish-hunting *Conus* species. In addition, a number of ω-conotoxin-like peptides have been purified but these do not induce the same in vivo symptomatology in mammals. Recently, a number of investigators have used GVIA as a pharmacological tool for determining the calcium channel subtypes present in neuronal tissues of mammalian species. Results obtained in a number of laboratories however suggests that caution must be exercised in interpreting interaction with ω-conotoxins as proof for the presence or absence of N and L type channels. In the mammalian CNS, ω-conotoxin GVIA apparently binds only with a small subset of voltage sensitive Ca channels; less than 50 percent of ^{45}Ca uptake by synaptosomal preparation is inhibited by GVIA. In contrast, in avian systems the spectrum of targets is much wider and almost all ^{45}Ca uptake is inhibited. Comparison of in vivo effects in fish, frogs, chicks and mice and binding studies using radioiodinated derivatives of the toxins have indicated not only phylogenetic and channel subtype specificity but also the dependence of interaction on the particular ω-conotoxin used. Biochemical characterization of calcium channel subtypes is presently in progress using different ω-conotoxins. (Supported by Grant GM22737 to BMO and AM 26741 to JR).

147.13

THE INHIBITION OF 125I OMEGA CONOTOXIN GVIA BINDING BY NEOMYCIN TO NEURONAL MEMBRANES IS MODULATED BY GTP-γ-S. R.J. Stumpo*, L.M. Pullan, B.A. Meiners and A.I. Salama. Dept. of Pharmacology, ICI Pharmaceuticals Group, ICI Americas Inc., Wilmington, DE 19897.

Omega conotoxin GVIA (ωCT) blocks calcium currents at the L and N-type calcium channels. Omega conotoxin and neomycin, an aminoglycoside antibiotic, have been shown to inhibit 45Ca2+ uptake and to block neurotransmitter release, presumably acting at the N-type calcium channel. We have previously shown that neomycin inhibits dihydropyridine insensitive [125I] ωCT binding to neuronal membranes. We demonstrate here that the inhibition curve of neomycin against [125I] ωCT binding is shifted to the right by GTP-γ-S or GMP-PNP, increasing the IC_{50} of neomycin from 0.9 μM to 3.6 μM. The ωCT inhibition curve is unaffected by the GTP analogues. The effect is specific for GTP analogues; GDP, cGMP, ATP, ADP and cAMP did not modulate neomycin or ωCT potency at the [125I] ωCT binding site. Sodium fluoride, which stimulates G-protein activity, also reduced the potency of neomycin for the [125I] ωCT binding site. These results may indicate that the [125I] ωCT binding site of the N-channel may be coupled to a G-protein.

147.14

MPP^+ CAUSES A LONG-LASTING EFFLUX OF STRIATAL DOPAMINE IN VIVO VIA A NIMODIPINE SENSITIVE L-TYPE NEURONAL CALCIUM CHANNEL. C.C. Chiueh and H. Miyake*. NIMH, Bethesda, MD 20892.

Intracerebral administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPP^+) causes an accumulation of ^{45}Ca (Sun et al., 1988). MPP^+ produces also an increase in the release of dopamine (DA; Rollema et al., 1986; Ozaki et al., 1987) and serotonin (Miyake and Chiueh, 1989). Microdialysis perfusion of the rat striatum with MPP^+ caused a dose-dependent increase in the efflux of DA. The MPP^+-induced release pattern of striatal DA appears to be **'biphasic'**. In addition to a fast releasing peak of DA, a **slow releasing peak** of DA appeared at 20 min after the termination of MPP^+ perfusion (10^{-3}M, 5min). MPP^+ produced only the fast release peak of DA when the striatum was perfused with a Ca^{++}-free artificial CSF containing 10 mM EDTA or EGTA. Nimodipine (L-type Ca^{++} channel antagonist) inhibited the MPP^+-induced late DA releasing peak but not of the initial fast DA releasing peak. These results indicate that MPP^+ produces a biphasic increase in the DA efflux from the striatum via two different mechanisms of action. The fast DA releasing action of MPP^+ resembles that of d-amphetamine (Ca^{++} insensitive). The long-lasting late DA releasing action of MPP^+ is a Ca^{++} dependent process which involves a L-type neuronal Ca^{++} channel.

147.15

BLOCKADE OF HIGH-VOLTAGE-ACTIVATED CALCIUM CURRENTS BY PHENYTOIN IN ACUTELY DISSOCIATED GUINEA-PIG HIPPOCAMPAL NEURONS. J. Black, L.J. Larson-Prior and N.T. Slater. Department of Physiology, Northwestern University Medical School, Chicago, IL 60611 U.S.A.

Anticonvulsant agents currently employed in the treatment of partial complex seizures have been proposed to exert their therapeutic effects through actions on neuronal sodium and calcium currents. However, little biophysical evidence has been obtained to evaluate this hypothesis in neurons in the limbic or cerebral cortex, the sites of initiation of generalized seizures. We have studied the effects of phenytoin on high-voltage-activated calcium currents in enzymatically dissociated neurons from the CA1 and CA3 regions of adult guinea-pig hippocampus using whole-cell patch-clamp recording techniques. 3 mM external barium was employed as the charge-carrying cation; the internal solution included 5 mM CsBAPTA and MgATP. The kinetic properties of the macroscopic calcium currents were studied using 10 to 2000 ms command steps to potentials of -80 to +60 mV from holding potentials (V_h) of -100 to -40 mV. Phenytoin (10 - 200 μM) was applied by rapid microperfusion using a five-barreled pipette array. No evidence for use-dependent block of the barium current (I_{Ba}) by phenytoin was observed, but the blockade of I_{Ba} was extremely sensitive to V_h, 100 μM phenytoin producing only 30% block at V_h = -100 mV, and >90% block at -40 mV. The tail current amplitude was twice as sensitive to phenytoin as the peak I_{Ba} evoked by depolarizing command steps, but no change in the I-V relations of either was observed. Phenytoin also produced an enhancement of the apparent rates of deactivation and inactivation, but had no effect on the kinetics of activation of I_{Ba}. These results suggest that a voltage-, but not use-dependent block of neuronal calcium currents recruited during paroxysmal discharges may substantially contribute to the therapeutic effectiveness of phenytoin in the treatment of generalized seizure disorders. Supported by NS25682 & NS23482.

147.16

EFFECTS OF ORGANIC CALCIUM ANTAGONISTS ON CALCIUM CURRENTS IN ACUTELY DISSOCIATED CA1 NEURONS: LACK OF EFFECT OF GTP-γ-S. J.M.H. ffrench-Mullen, J. Black, J.L. Barker and N.T. Slater. Dept. of Physiology, Northwestern University Medical School, Chicago, IL 60611 and Lab. of Neurophysiology, NINDS, NIH, Bethesda, MD 20814 U.S.A.

Activation of G-proteins by internal GTP-γ-S has been demonstrated to modulate both the gating kinetics of calcium currents and the responsiveness to organic calcium antagonists of peripheral sensory neurons (Scott & Dolphin, 1987). We have examined the effects of internal GTP-γ-S on the kinetics of the macroscopic calcium current in acutely dissociated CA1 hippocampal neurons, and their modulation by organic calcium antagonists. Neurons were enzymatically dissociated from the CA1 region of adult guinea-pig hippocampus and calcium currents were recorded using whole-cell patch-clamp methods with 3mM Ba as the external charge-carrying cation. Drugs were applied by rapid microperfusion from a multi-barrel array. The effects of the organic antagonists on the kinetic properties of the barium current (I_{Ba}) were assessed using holding potentials of -80 to -40 mV. D-600 and verapamil produced a highly voltage-dependent block, the degree of blockade increasing with membrane hyperpolarization. The blockade by nifedipine displayed the opposite voltage dependence, whereas blockade by diltiazam was not voltage-dependent. The potency ratio for blockade was (K_D, μM): nifedipine (5) > ±verapamil (10) > ±D-600 (50) > diltiazam (100). In the presence of internal GTP-γ-S (500 μM), no effects on the kinetic properties of I_{Ba}, and no apparent change in the potency or voltage dependence of blockade by the antagonists were observed. These results indicate that organic calcium antagonists exhibit similar mechanisms of blockade of hippocampal calcium currents to those previously observed in peripheral neurons. However, neither the calcium current kinetics, nor the effects of antagonists were modified by internal GTP-γ-S in CA1 neurons, suggesting differences exist in the regulation of calcium channels by G-proteins in central and peripheral neurons.

147.17

PEPTIDE TOXINS FROM AGELENOPSIS APERTA SPIDER VENOM BLOCK DEPOLARIZATION-INDUCED INCREASES IN CYTOSOLIC FREE CALCIUM IN RAT CEREBELLAR GRANULE NEURONS. L. D. Artman[1]*, N. A. Saccomano[2]*, R. A. Volkmann[2]*, E. F. Nemeth[1] and T. N. Parks[1] (SPON: W. Stevens). Natural Product Sciences Inc., Salt Lake City, UT 84108[1] and Pfizer Central Research, Groton, CT 06340[2].

Spider venoms contain several classes of toxin with a variety of targets in excitable tissues (Ann. Rev. Neurosci. 12; Ann. Rep. Med. Chem. 24,1989). Peptide toxins from the funnel-web spider A. aperta have been reported to act as calcium antagonists in vertebrate and invertebrate neurons and potently block synaptic transmission in chick cochlear nucleus and rat hippocampus (op. cit.). We studied the ability of five purified and sequenced 4200-5500 dalton peptide toxins (H1, H2, I, J, K) to inhibit depolarization-induced increases in cytosolic free calcium concentration ([Ca]i) in cultures of neonatal rat cerebellar granule neurons studied by fura-2 fluorimetry. In this preparation, increases in [Ca]i to depolarization with 10^{-2} M KCl or treatment with the dihyrdopyridine calcium agonist Bay K8644 (10^{-6} M) are completely blocked by 10^{-6} M nifedipine (see Nemeth and Parks, this meeting). Dose-response studies showed some of the spider toxins to be very potent in blocking depolarization-induced increases in [Ca]i; IC_{50}'s for the different toxins ranged from 25 nM to >1 μM. Thus, these toxins may be useful in studying voltage-sensitive ion channels in mammalian neurons (see also Albensi et al., this meeting).

147.18

ALTERATIONS OF RELATIVE DIVALENT CATIONIC INFLUX AND Ca^{2+}-CHANNEL LIGAND BINDING IN SYNAPTOSOMES BY METHYLMERCURY. T.J. Shafer, M.L. Contreras and W.D. Atchison. Dept. of Pharm./Tox., Mich. State Univ., E. Lansing, MI 48824.

Depolarization-induced influx of ^{45}Ca, ^{85}Sr and ^{133}Ba into rat forebrain synaptosomes was measured to determine the effects of MeHg on the relative influx of divalent cations through Ca^{2+} channels. In addition, the effects of MeHg on binding of the Ca^{2+} channel ligands [^{3}H]-nitrendipine and [^{125}I]-ω-conotoxin GVIA were measured in synaptosomes. When synaptosomes were depolarized in 42.5 mM K^+ solutions, the relative influx of ^{45}Ca:^{85}Sr:^{133}Ba was altered from 6:2:3 in the absence of MeHg to 6:1:3 in the presence of 100 μM MeHg. Equilibrium binding of [^{3}H]-nitrendipine was measured in non-depolarizing (5 mM) and depolarizing (77.5 mM) K^+ solutions and respective Scatchard values for K_D of 634±155 and 1092±299 pM were obtained. In the presence of 100 μM MeHg, the respective values for K_D were 2515±633 and 5327±2095 pM. After three experiments in non-depolarizing K^+ solutions, the K_D values for [^{125}I]-ω-conotoxin binding were 373±83 pM in the absence and 262±17 pM in the presence of 100 μM MeHg. MeHg did not alter B_{max} for either ligand. These results indicate that: 1) MeHg alters the relative influx of divalent cations through Ca^{2+} channels, and 2) concentrations of MeHg which block Ca^{2+} influx into synaptosomes also bind competitively at a dihydropyridine-sensitive binding site. (Supported by NIH grant ES03299; TJS is the recipient of a Student Fellowship sponsored by Hoffman-LaRoche, Inc.)

147.19

EFFECT OF IONIZING RADIATION ON CALCIUM CHANNELS IN RAT ILEUM MYENTERIC PLEXUS SYNAPTOSOMES. S.B. Kandasamy, W.A. Hunt* and A. Dubois.* Behavioral Sciences Department, Armed Forces Radiobiology Research Institute and Department of Medicine, Uniformed Services University of the Health Sciences, Bethesda, MD 20814-5145.

Radiation exposure induces increased gastrointestinal (GI) motility which is correlated with an increase in prostaglandin (PG) levels. The myenteric plexus (MP), which lies between longitudinal and circular smooth muscles of the gut, is the most important independent neural network responsible for GI motility in the absence of extrinsic innervation. Although MP is a part of the peripheral nervous system, it resembles in many ways the central nervous system. Exposure to ionizing radiation has been shown to reduce voltage-dependent sodium and calcium uptake into rat brain synaptosomes. The effect of ionizing radiation on voltage-dependent calcium influx was studied by measuring KCl-stimulated Ca-45 uptake into rat MP synaptosomes. Gamma irradiation (Co-60) (1-30 Gy) reduced calcium uptake after 3 sec in a dose-dependent manner similar to that found for brain synaptosomes. However, unlike in brain synaptosomes, PGE2, PGF2α and PGD2 (10 nM-1μM) increased the entry of calcium into non-irradiated and irradiated MP synaptosomes. These results suggest a radioprotectant effect of prostaglandins in reversing the reduced calcium uptake induced by radiation.

ISCHEMIA: EXCITABILITY AND NEUROTRANSMISSION

148.1

EXCITABILITY CHANGES OF RODENT HIPPOCAMPAL NEURONS AFTER TRANSIENT FOREBRAIN ISCHEMIA -IN VITRO STUDY. S. Miyahara *, N. Hori *, N. Doi * and K. Kinoshita *. (SPON: H. Kannan). Dept. of Neurosurgery, Miyazaki Med. College, Miyazaki 889-16 and Dept. of Pharmacology, Kyushu Univ., Fukuoka 812, JAPAN.

In order to clarify the mechanisms of delayed neuronal death of hippocampal CA1 neurons, we recorded responses of CA1 and CA3 neurons to orthodromic electrical stimulations in slice preparations obtained from rats and gerbils subjected to transient forebrain ischemia. Twenty-four hours after ischemia mossy fiber stimulations produced epileptiform burst discharges of CA3 neurons and Schaffer collateral stimulations evoked multiple population spikes superimposed on field EPSP in CA1 area. Seven days after ischemia evoked epileptiform burst discharges were still recorded in CA3 region and no responses in CA1 region where pyramidal cells were lost. In gerbils similar responses were obtained in CA1 and CA3 regions to those in rats after ischemia. However epileptiform burst discharges were recorded in CA3 region in sham-operated control gerbils. These results indicate epileptiform burst discharges of CA3 neurons after transient cerebral ischemia may cause delayed death of CA1 pyramidal neurons.

148.2

CHANGES IN MEMBRANE RESISTANCE AND CAPACITANCE INDUCED BY EXPERIMENTAL ISCHEMIA IN HIPPOCAMPAL SLICE. R.K. Rader, R.S. Wilkinson+ and T.H. Lanthorn (Spon: H. Sato+). CNSDR, G.D. Searle & Co., St. Louis, MO 63198 and Dept. of Cell Biology+, Washington Univ. School of Medicine, St. Louis, MO 63110.

We are utilizing a model of experimental ischemia which involves exposure of hippocampal slices to both hypoxia and hypoglycemia (2mM D-glucose). Synaptic responses of CA1 neurons, recorded intracellularly, fail within two minutes from the onset of hypoxia and this is followed by depolarization of the membrane potential toward zero (anoxic depolarization, AD). During reoxygenation, following AD, synaptic failure persists and the membrane potential ordinarily remains depolarized. This persistent depolarization can be blocked by NMDA antagonists.

Hyperpolarizing intracellular current pulses (0.1-0.6 nA) were used to construct an I/V plot from which membrane resistance of cells was measured. In addition, membrane impedance was estimated from frequency domain analysis (2-2000 Hz) of membrane responses to sinusoidal current (5-100 pA rms) measured with a phase-locked amplifier. Resting membrane potential (> -60mV, RMP) and resistance (45 ± 7 Mohms, Rin) were measured prior to hypoxia while in 2mM Glucose ACSF (n=13). At 15 min post-AD, RMP was -3.5 ± 2.4 mV and Rin was 110 ± 6% of its pre-hypoxic values. Similarly, at 30 min post-AD, RMP was -5.3 ± 4.6 mV and Rin was 101 ± 7% of control. Impedance measurements confirmed that Rin increased following AD, and indicated that the capacitive component declined from the onset of hypoxia, falling precipitously at AD. Membrane properties, including capacitance, recovered to pre-hypoxic values if the NMDA antagonist CPP (100 uM) was present. Our data suggest that although NMDA receptors are involved at the onset of AD, no significant increase in membrane conductance persists. Thus the failure of cells to repolarize following AD is not due to the continued opening of NMDA-associated ion channels.

148.3

SPREADING DEPRESSION-LIKE DEPOLARIZATION AND OTHER EFFECTS OF HYPOXIA ON MOUSE SPINAL CORD *IN VITRO*. G. Czéh* and G.G. Somjen, Dept. Physiol. Medical Univ. of Pécs, Hungary, and Div. Physiol. Duke Univ. Durham, NC 27710.

Spreading depression (SD) of Leão has been observed in almost all parts of the central nervous system of mammals, except the spinal cord. The reason for the "immunity" of spinal cord tissue is not known. We studied the effect of oxygen deprivation on isolated spinal cords of 9-16 day old mice maintained at 24°C. When exposed to N_2-saturated artificial CSF (ACSF), synaptic transmission was suppressed in 15 minutes except when the cord was bathed in ACSF with elevated $[Ca^{2+}]_o$ (2.4-3.6 mM) in which case synaptic failure was delayed to 30 min. Following 45 min hypoxia, transmission recovered to normal levels in 20-50 min. An hour or more after reoxygenation spontaneous discharges erupted in about 40% of the spinal cords. During hypoxia a 10-20 mV negative shift of extracellular potential, elevation of $[K^+]_o$ to 25-40 mM, and loss of axonal conduction, indistinguishable from SD, was seen in 1 out of 3 spinal cords. Reoxygenation restored V_o and $[K^+]_o$. SD occurred more frequently when O_2 was withheld for a second time in the same cord. We conclude that: 1. excess $[Ca^{2+}]_o$ retards hypoxic failure of synaptic transmission; 2. SD can, under certain circumstances, occur in spinal gray matter.

(Supported by NIH grants NS 17771, NS 06233)

148.4

EFFECTS OF NMDA RECEPTOR ANTAGONIST ON POSTISCHEMIC SYNAPTIC PHYSIOLOGY OF CA1 HIPPOCAMPAL PYRAMIDAL CELLS. L. Urban*, K.H. Neill*, B.J. Crain, J.V. Nadler and G.G. Somjen (SPON: J.P. Vicedomini). Depts. Cell Biology, Pathology, Neurobiology and Pharmacology, Duke Univ. Med. Ctr., Durham, NC 27710.

Transient forebrain ischemia in the Mongolian gerbil leads to delayed degeneration of CA1 hippocampal pyramidal cells. Earlier experiments with hippocampal slices cut from previously ischemic brains showed that ischemia enhanced excitatory synaptic transmission in area CA1 before it abolished synaptic function. In the present study we tested the hypothesis that these effects require the activation of NMDA receptors during the postischemic period.

Gerbils were subjected to a 5-min occlusion of both common carotid arteries. Hippocampal slices were prepared 30-40 min or 4 h after the occlusion and superfused at 34.5 °C. Schaffer collateral-commissural input-output curves were constructed from recordings made every 30 min for 10-24 h.

Inclusion of the NMDA receptor antagonist CPP (2 or 10 uM) in the superfusion medium considerably prolonged the lifetime of control slices and it progressively increased both the initial slope of the focally-recorded EPSP (fEPSP) and the amplitude of the orthodromic population spike. In slices from animals subjected to transient ischemia, CPP completely prevented the loss of pyramidal cell excitability, even when the slices were prepared 4 h after ischemia. CPP also prevented the progressive increase in fEPSP duration normally observed after ischemia. Thus CPP both enhanced the viability of the preparation and halted the progression of ischemic damage. These results support the hypothesis that NMDA receptor activation during the postischemic period is one of the etiological factors in the delayed degeneration of CA1 pyramidal cells. (Supported by NIH grant NS 06233.)

148.5

EFFECTS OF THREE NMDA RECEPTOR ANTAGONISTS IN THE GERBIL CAROTID OCCLUSION MODEL OF TRANSIENT FOREBRAIN ISCHEMIA. M.A. Warner*, J.V. Nadler and B.J. Crain. Depts. Pharmacology, Neurobiology and Pathology, Duke Univ. Med. Ctr., Durham, NC 27710.

Excessive activation of the NMDA receptor has been suggested to play a key role in ischemic brain damage. We have tested the ability of three NMDA receptor antagonists, MK-801, CGS 19755 and MDL 27,266, to attenuate brain damage produced by transient forebrain ischemia in the Mongolian gerbil.

Animals were anesthetized with 2.5% halothane and both common carotid arteries were occluded for 5 min. When rectal temperature was maintained at 36-37 °C, transient ischemia consistently destroyed an average of 96% of the CA1 hippocampal pyramidal cells and damaged 32% of the striatum.

NMDA receptor antagonists were administered i.p. either 30-60 min before ischemia or after ischemia. Doses tested were: 1 and 10 mg/kg of MK-801 (pre- or postischemia); 30 mg/kg of CGS 19755 preischemia; four 25 mg/kg doses of CGS 19755 administered between 0.5 and 6.5 hours postischemia; and 40 mg/kg of MDL 27,266 (pre- or postischemia). All drug treatments markedly attenuated striatal damage, but only 10 mg/kg MK-801 (pre or post) and 40 mg/kg MDL 26,277 (pre only) significantly reduced the extent of CA1 pyramidal cell degeneration. CA1 damage varied widely from case to case and only 34-48% of the pyramidal cells, on average, was spared. The effects of NMDA receptor antagonists resembled those of hypothermia (29-32 °C). Most of the CA1 pyramidal cells that were protected from degeneration by an antagonist appeared abnormal, as judged by silver impregnation. From our electrophysiological work, it seems possible that these neurons were so damaged that, although viable, they were essentially non-functional. Our results therefore suggest that NMDA receptor antagonists will be less than ideal anti-ischemic drugs, at least when given by themselves. (Supported by NIH grant NS 06233.)

148.6

NON-COMPETITIVE NMDA ANTAGONISTS AND SIGMA-OPIATE LIGANDS BLOCK GLUTAMATE RELEASE FROM THE RAT HIPPOCAMPAL SLICE DURING ISCHEMIA. D. Lobner* & P. Lipton. Dept. of Physiol., Univ. of Wisconsin, Madison, WI 53706.

Exposure of rat hippocampal slices for 5' to buffer equilibrated with 95% N_2-5% CO_2 and devoid of glucose (ischemia) causes an ~500% increase in release of endogenous glutamate. This release is inhibited by high levels of the non-competitive NMDA receptor blockers (PCP EC_{50} ~50 μM, ketamine EC_{50} ~100 μM, and MK-801 EC_{50} ~100 μM). It is also inhibited by the sigma-opiate ligands ditolylguanidine (DTG) EC_{50} ~50 μM and haloperidol EC_{50} ~10 μM. The non-competitive NMDA receptor blockers also have sigma-opiate receptor affinity. The rank order of efficacy in inhibiting release by the above compounds is the same as the order of their binding affinities to the sigma-opiate receptor. We suggest the non-competitive NMDA receptor blockers inhibit ischemic glutamate release by acting at the sigma-opiate receptor.

The ability of different NMDA receptor blockers to protect against ischemic transmission damage in the slice depends on blocking both glutamate release and the NMDA receptor; that is, blockade of the NMDA receptor alone does not provide good protection while blockade of glutamate release and NMDA receptors protects very well. Therefore, we conclude that at glutamate levels reached during severe ischemia, damage occurs via both NMDA and non-NMDA glutamate receptors.

148.7

INCREASED CONCENTRATIONS OF GLUTAMATE AND ASPARTATE IN SUPERNATANTS OF HYPOXIC CNS CULTURES. I.E. Madl, D.H. Smullin, S.R. Skilling,* A.A. Larson and W. Raabe. Depts. of Vet. Biology and Neurology, U. of Minnesota, St. Paul, MN 55108.

Increased concentrations of extracellular Glu and Asp have been reported during ischemia *in vivo*, and are believed to mediate neuronal damage. The accumulation and possible origins of these excitotoxins was examined in cell cultures. Hippocampal and cerebellar cultures were obtained from 19 day rat fetuses and maintained *in vitro* for 17-18 days. Cells were washed with an electrophysiologic recording medium (ERM; in mM: NaCl 145, KCl 5, $CaCl_2$ 2, $MgCl_2$ 1, HEPES 10, glucose 5, pH 7.4, osmolarity 325) and then incubated in 91% N_2 with 9% CO_2 or ERM containing NaCN. In cultures exposed to hypoxia or NaCN, the concentrations of Glu and Asp in the ERM were increased more than 10 fold above controls while the concentrations of Gly, Asg and Gln increased less than 2 fold. The increases of Glu and Asp preceded neuronal cell death, as measured by trypan blue exclusion, and were potentiated by glucose deprivation. The increases were not inhibited by 1 μM tetrodotoxin or 10 mM Mg++, suggesting that the increases were not due to synaptic release. Hypoxia- and CN-induced increases in Glu and Asp were also obtained in C6 rat glioma and human astrocytoma cultures. The results indicate that glia contribute to hypoxia-induced increases in extracellular Glu and Asp *in vitro* and suggest that glia may play a major role in the extracellular accumulation of Glu and Asp during ischemia *in vivo*. Supported by NS01105, DA04090, DA04190, DA00124, CA01342.

148.8

CONCOMITANCE AND DEPENDENCE OF MASSIVE POTASSIUM FLUX TO EARLY GLUTAMATE RELEASE DURING CEREBRAL ISCHEMIA IN VIVO. Y.KATAYAMA, T.TAMURA*, T.KAWAMATA* AND D.P.BECKER. Division of Neurosurgery, UCLA Sch. of Med., Los Angeles, CA 90024.

An increase in the extracellular concentration of glutamate ([glu]e) during 10-min cerebral ischemia has been demonstrated by microdialysis in vivo. We tested the hypothesis that this early increase in [glu]e is responsible for massive potassium flux across the plasma membrane which is seen during the same period of ischemia. Changes in the extracellular concentration of potassium ($[K^+]e$) and [glu]e during initial 10 min period after the onset of decapitation ischemia were determined by 1-min fractions of microdialysis in young rats. An increase in [glu]e (1.8±0.2 fold baseline, n=5) occurred concomitantly with the sudden large increase in $[K^+]e$ which was observed 2-4 min (3.3±0.5 min) after the onset of ischemia, [glu]e decreased transiently thereafter. The onset of the massive increase in $[K^+]e$ was significantly delayed (1.3±0.2 min, n=8) by in situ administration of kynurenic acid (10 mM), an broad-spectrum antagonist of glutamate, through the dialysis probe. These results suggest that glutamate mediates a major component of massive potassium flux across the plasma membrane during cerebral ischemia in vivo.

148.9

DETECTION OF ISCHEMIC CELLULAR SWELLING IN VIVO WITH BRAIN MICRODIALYSIS: CHANGES IN CONCENTRATION OF ^{14}C-SUCROSE PERFUSED INTO THE EXTRACELLULAR SPACE. T.TAMURA*, Y.KATAYAMA, D.P.BECKER, AND T. KAWAMATA*. (Spon. N.Hayashi) Division of Neurosurgery, UCLA Sch. of Med., Los Angeles, CA 90024.

A cellular swelling and shrinkage of the extracellular space (ECS) during cerebral ischemia have been shown in vivo as an increase in concentration of ECS markers that can be continuously measured by electrodes. We attempted to detect the cellular swelling with brain microdialysis using similar principles. A dialysis probe was placed in the hippocampus of the rat, and perfused for 20 min with ^{14}C-sucrose (10 mM) as an ECS marker. The probes were perfused with Ringer solution thereafter, and $[^{14}C\text{-sucrose}]_e$ were determined from dialysis fractions. Concomitant with an abrupt increase in $[K^+]_e$ which occurred few minutes after the onset of decapitation ischemia, $[^{14}C\text{-sucrose}]_e$ was also elevated. This was taken as evidence of the shrinkage of ECS, i.e., water movement into the cells leaving the ECS marker behind. With in situ administration of Mg^{2+} (10 mM) through the probe, which blocks transmitter release, the onset of $[K^+]_e$ increase and the concomitant $[^{14}C\text{-sucrose}]_e$ increase were both delayed. Thus, the transmitter release appears to be involved in the ischemic cellular swelling.

148.10

ALTERATIONS AT THE N-METHYL-D-ASPARTATE (NMDA) RECEPTOR COMPLEX IN GERBIL HIPPOCAMPUS FOLLOWING SHORT-TERM (7 DAYS) AND LONG-TERM (32 DAYS) RECOVERY FROM 10 MIN FOREBRAIN ISCHEMIA. L.P. Miller* and T.R. Insel (SPON: D.C. Perry) Vet Adm Med Ctr, Wash D.C. 20422, Dept. Pharm., Georgetown Univ. Sch. Med., Wash D.C. 20007 & Lab. Clin. Sci., NIMH, Poolesville, Md. 20837.

It is well known that glutamate/aspartate act as excitatory amino acid (EAA) neurotransmitters in the CNS. In addition, there is support for the NMDA-receptor complex as a major site of action of EAAs. This complex has been shown to consist of: (a) glutamate recognition site, (b) glycine modulation site and (c) Ca^{++} channel site. All three sites have now been described using receptor binding technology in combination with autoradiography. The present series of experiments utilizes this combined technology to examine the individual components of the NMDA-receptor complex at the 7 and 32 days following a 10 min ischemic insult to gerbil forebrain. In addition we examined the other EAA receptor subtypes; quisqualate (QA) and kainate (KA). Ischemia in 10 - 12 week old female gerbil was induced by bilateral clamping of the common carotids for 10 min under 2.5% halothane anesthesia. At 7 and 32 days post-ischemia the animals were sacrificed by decapitation, brains removed and immediately frozen in isopentane/dry ice. Using 12 micron coronal sections EAA receptors and components of the NMDA-receptor complex were characterized in various subregions of dorsal hippocampus as: a) NMDA-displacable 3H-glutamate binding to the NMDA recognition site, b) 3H-glycine binding to the modulation site, c) 3H-TCP binding to the CA^{++} channel portion of the complex, d) 3H-AMPA binding to the QA subtype and e) 3H-KA binding to the KA subtype. Quanatitative autoradiography was performed using a LOATS RAS 1000 image analysis system. Within the strata radiatum our results expressed as percent of control were NMDA 75 & 40, TCP 79 & 49, Glycine 54 & 52, AMPA 62 & 58, KA(CA3 subfield) 88 & 97 at 7 and 32 days post-ischemia, respectively. Alterations within other hippocampal subregions will also be presented.

148.11

THE EFFECTS OF ISCHEMIA AND NMDA RECEPTOR BLOCKADE IN ORGANOTYPIC CULTURES OF RAT HIPPOCAMPUS. D. Newell*, A. Malouf and J. Franck. Department of Neurological Surgery, University of Washington, Seattle, WA 98195.

Hippocampal pyramidal cells, and particularly those in subfield CA1, are selectively vulnerable to experimental and clinical ischemia. In the whole organism, a variety of systemic, circulatory and endogenous neurotoxic factors may interact to confer this unique vulnerability. Likewise, agents which have been shown to block this cell death may do so on the basis of systemic effects and/or direct cellular effects. We are examining the effects of ischemia in organotypic roller tube cultures of hippocampal slices to determine if discrete vulnerability of specific populations of hippocampal neurons is maintained in a system where systemic effects are not operational.

Organotypic cultures were prepared from 4-day-old rat hippocampi according to the method of B.H. Gähwiler (*J. Neurosci. Methods*, 1981, 4: 329-342). Two- to four-week-old cultures were placed in glucose-free medium, and exposed to varying periods of anoxia. Following "ischemia" *in vitro* (anoxia + no glucose), the cultures were returned to normal growth medium for 48 hours. Cultures were then examined histologically for neuronal death. Additional cultures were made ischemic in the same fashion except the glucose-free medium also contained 30 μM MK-801 to block NMDA receptors. Our preliminary data suggest that (1) a gradient of vulnerability of hippocampal neurons to ischemia is maintained in this organotypic culture system, and (2) this vulnerability, as *in vivo*, may be dependent on activity at the NMDA-type glutamate synapse.

Supported by NIH, NINDS grants NS 20482, NS 25155, and NS 07144.

148.12

SELECTIVE SPARING OF NADPH-DIAPHORASE-SOMATOSTATIN-NEUROPEPTIDE Y NEURONS IN ISCHEMIC GERBIL STRIATUM. Y. Uemura*, N.W. Kowall, M. F. Beal, M. A. Moskowitz (SPON: J.H. Sandell). Neurology and Neurosurgery Services, Mass. General Hospital, Boston, MA 02114.

An increasing body of evidence has linked ischemic brain damage to a neurotoxic effect of glutamate acting at the N-methyl-D-aspartate (NMDA) receptor. In the present study we investigated the relative vulnerability of neuronal subsets in the gerbil striatum to ischemia induced by bilateral transient ligation of the common carotid. After 4 days survival, brains were evaluated histologically with histochemical methods (NADPH diaphorase and silver degeneration procedures) and radioimmunoassay for somatostatin (SS), neuropeptide Y (NPY) and substance P (SP) and measurements of amino acids and catecholamines using HPLC with electrochemical detection. NADPH diaphorase neurons were strikingly preserved in the ischemic dorsolateral portion of the striatum. NPY and SS concentrations were unchanged despite a 25% depletion of substance P and GABA. Aspartate, glutamate, taurine and dopamine concentrations were unchanged while homovanillic acid was significantly increased. Previous studies of NMDA excitotoxin lesions in striatum have shown a sparing of NADPH diaphorase-somatostatin neuropeptide Y neurons. The similar sparing of these neurons following ischemic lesions in gerbil striatum suggests that NMDA receptor activation plays a role in ischemic injury.

148.13

BLOOD AND BRAIN PHARMACOKINETICS OF MK-801 IN NEONATAL HYPOXIA/ISCHEMIA. P.H. Schwartz, L.M. Adams*, and C.G. Wasterlain. Epilepsy Res Lab, VAMC, Sepulveda, CA 91343 and Ped ICU Res, Child Hosp, Los Angeles, CA 90027.

Cardiopulmonary arrest in the adult rat decreases the clearance of MK-801 from plasma (Neurology 39(Suppl. 1):387, 1989). We examined the pharmacokinetics of MK-801 in both plasma and brain in a neonatal model of hypoxia/ischemia. 7-Day old rats underwent sham surgery or ligation of both carotid arteries under methoxyflurane anesthesia followed by a one hour exposure to 8% O_2. Sham animals received a 10 mg/kg i.p. injection of MK-801 5 hours after surgery while the animals exposed to hypoxia received it immediately after hypoxia. Trunk blood and brains were collected 2 to 240 minutes after the injection and the concentration of MK-801 in serum (μmol/L) and parietal cortex (μmol/kg) were determined by GC-NPD. Peak serum levels of MK-801 (3.3±1.3 μM) were attained between 20 and 30 minutes after i.p. administration. Neither the rate of appearance of MK-801 in the plasma nor the rate of elimination were affected by a previous hypoxic exposure although there was a non-significant trend towards a decreased elimination in the hypoxic animals. The half-life of elimination was approximately 100 min. Brain concentrations (21.6±7.1 μM) of MK-801 were significantly greater than plasma concentrations at all time points measured (6.8±1.6-fold) and a previous hypoxic exposure had no effect on this gradient. Thus, in a neonatal model of hypoxic/ischemic brain injury access to the brain by i.p. MK-801 is not hindered.

148.14

EXCITOTOXIC NEUROTRANSMITTER RELEASE IN AN INFANT CEREBRAL ISCHEMIA MODEL. JK Deshpande, SE Haun*, G Tsai, M Helfaer*, J Kirsch*, RJ Traystman, JT Coyle. Depts. of Anesthesiology & Neurosciences, The Johns Hopkins Medical Institutions, Baltimore, MD 21205

Intracerebral microdialysis was performed in dorsal hippocampus in pigs (8-12 mo; n=4) and piglets (2-3 wk; n=5) which underwent 10 min of global ischemia (ISC) by aortic cross clamp, and 1-2 hr recovery. Dialysis fractions were collected over 10 min periods: immediately after insertion (-60 min); 20 min and 10 min prior to ISC; ISC fraction; and every 10 min until the animal was sacrificed. Samples were assayed for aspartate (ASP), glutamate (GLU) and N-acetylaspartylglutamate (NAAG). ASP changed minimally during ISC and recirculation in both groups. GLU concentration increased in pigs from 2.8 to 19.4 uM (ISC), but changed minimally in piglets (8.2 to 6.4 (ISC) and 7.6 uM (10'recovery)). NAAG increased in pigs during ISC (172.7 to 296.8 nM), whereas the NAAG rise occurred in 10' and 20' recovery in piglets (89.0 to 252.7 and 333.0 nM). Our results in vulnerable brain regions varies with age. This finding may account for the disparity in outcome after global ISC in infants versus adults.

148.15

CHARACTERIZATION OF THE UPREGULATION OF LAMBDA OPIATE BINDING IN GERBIL HIPPOCAMPUS FOLLOWING FOREBRAIN ISCHEMIA IN GERBILS. D.C. Perry[1] & L. P. Miller*[2] (SPON. R. J. Walsh). [1]Dept. Pharmacology, George Washington Univ., 20037 & [2]Vet. Admin. Med. Ctr., 20422 & [2]Dept. Pharmacology, Georgetown Medical School, Washington, D.C.

We (Miller & Perry, Brain Res., in press) have shown that lambda opiate binding in mossy fiber terminal field of gerbil hippocampus is upregulated 7 days following forebrain ischemia. Ischemia was induced in 10-14 week old female gerbils by bilateral clamping of the common carotid arteries for 10 min under halothane anesthesia. Seven days post-ischemia brains were removed and frozen sections were cut. Lambda autoradiography was done with [^{3}H]naloxone plus 300 nM diprenorphine (Perry & Sadee, Eur. J. Pharmacol. 129: 147, 1986) using a Loats RAS1000 image analysis system. We examined binding in a coronal plane at six different regions along a rostral-caudal axis. The greatest increase in lambda binding with ischemia (41.2% greater than sham controls, $p<0.05$) was in the most rostral sections. More caudal regions showed smaller increases (14.5-31.7%). In a separate experiment we examined lambda binding in rostral hippocampus 32 days post-ischemia: the increase in lambda binding was still apparent (28.7%, $p<0.01$) but somewhat smaller than at 7 days. These studies support the possibility that endogenous opioids play a role in ischemia-induced neuronal damage. Supported by DA04191 (DCP) and VAMC (LPM).

149.1

GRADED HYPOTHERMIA PROTECTS AGAINST ISCHEMIC INJURY IN GERBIL HIPPOCAMPUS. F.A.Welsh and R.E.Sims*. Div. of Neurosurgery, Univ. of Penna. Sch. of Med., Philadelphia, PA 19104.

To define the degree of hypothermia required to ameliorate ischemic injury, we regulated brain temperature at 37°C, 35°C, or 32°C in three groups of gerbils during 5 min bilateral carotid artery occlusion. Normothermia was restored upon reperfusion, and the animals were permitted to recover for 1 week. The brain was quick-frozen, and alternate thin sections were stained for histologic scoring or freeze-dried for microassay of ATP and adenylate kinase. By all three endpoints, injury was reduced in CA_1 hippocampus of gerbils cooled to 35°C, compared to those at 37°C. Cooling to 32°C completely prevented CA_1 injury, as judged by each of the endpoints. Preliminary histologic evidence indicated that postischemic hypothermia (32°C) did not reduce CA_1 injury. These results demonstrate that hypothermia of 2°C during ischemia is sufficient to ameliorate injury in this model. In addition, ATP content and adenylate kinase activity are excellent measures by which to quantitate ischemic injury.

149.2

HYPERTHERMIC BRAIN ISCHEMIA: ACUTE MICROVASCULAR AND NEURONAL ALTERATIONS. M. Halley*, I. Valdes*, R. Busto and W. D. Dietrich. Cerebral Vascular Disease Research Center, Department of Neurology, University of Miami School of Medicine, Miami, FL 33101, U.S.A.

We have determined whether variations in brain temperature influence the acute response of microvessels and neurons to transient cerebral ischemia. Six animal subgroups were investigated, including rats whose brain temperatures were maintained at 30, 33, 36, or 39°C during 20 minutes of 4-vessel occlusion. Following a 1-hour postischemic recirculation period, rats were injected i.v. with horseradish peroxidase (HRP) and perfusion-fixed. Nonischemic control rats (36 or 39°C) showed no extravasation of HRP. Likewise, postischemic rats in which brain temperature was controlled at either or 30 or 33°C failed to demonstrate blood-brain barrier (BBB) alterations. In contrast, focal sites of HRP extravasation were seen in rats where brain temperature was maintained at either 36 or 39°C. Permeability alterations were most evident in the 39°C ischemic group, occurring in cortical, thalamic and hippocampal regions. In this ischemic group, dark-shrunken neurons were also observed in deeper cortical layers and CA1 hippocampus. These results demonstrate that brain temperature is a critical factor in determining whether BBB dysfunction is an acute consequence of a transient cerebral ischemic insult. In contrast to normothermic ischemic conditions, morphological indicators of acute neuronal injury are seen when intraischemic brain temperature is elevated.

149.3

HYPOTHERMIA BLOCKS ISCHEMIA-INDUCED INHIBITION OF CaM KINASE II. S.B. Churn,* R.E. Blair,* W.C. Taft and R.J. DeLorenzo. Dept. of Neurology, Medical College of Virginia-VCU, Richmond VA 23298.

Five minutes of bilateral carotid occlusion in the gerbil produces a brief episode of global cerebral ischemia and causes uniform death of hippocampal CA1 pyramidal neurons. An early event in the development of ischemia-induced cell death in this model is a pronounced and prolonged decrease in CaM kinase II activity (Brain Res. 447,159,88). In order to further investigate the relationship between CaM kinase II and ischemia-induced cell death we have examined the effect of hypothermia, a cerebroprotectant, on these two parameters.

Four groups of gerbils were subjected to 5 min of bilateral carotid occlusion. In all experiments, hippocampal temperature was directly measured by inserting an Omega hypodermic thermocouple probe through a guide cannula which was stereotaxically implanted in the hippocampus one day prior to ischemic insult. The groups studied were naive animals (unoperated), sham-operated animals (no ischemia), ischemic normothermic animals, and ischemic hypothermic animals. Two hours after ischemia, the animals were sacrificed and CaM kinase II activity was measured in vitro in forebrain homogenates. Percent phosphorylation of the 50 kDa subunit of CaM kinase II in each group was: naive (100.0), sham (87.8), ischemic normothermic (12), and ischemic hypothermic (109.7). Thus, CaM kinase II activity is substantially reduced by ischemia in normothermic animals, and this reduction is completely prevented by a decrease in intracerebral temperature to 32°C during ischemia. Cell counts showed total loss of CA1 pyramidal neurons in ischemic normothermic animals and complete preservation of neurons in the ischemic hypothermic group.

149.4

Chemical Adrenalectomy Protects Hippocampal Cells following Ischemia. J. K. Morse and J. N. Davis, V.A. Medical Center and Duke University Medical Center, Durham, NC 27710

It is well known that transient forebrain ischemia leads to a delayed hippocampal CA1 neuronal death. This delayed cell loss can be exacerbated by glucocorticoids and ameliorated by adrenalectomy. Previously we reported no hippocampal damage in 92% of hemispheres from adrenalectomized gerbils. We believe the sparing of hippocampal cells is in part due to the absence of adrenal glucocorticoids. To test this hypothesis an anti-glucocorticoid, metapyrone, was used in the present study. We used three different doses of metapyrone: 25 mg/Kg,50 mg/Kg, and 100 mg/kg (n=8 for all groups). Metapyrone was injected at 0 hours, 24 hours, and 48 hours after carotid occlusion. At 72 hours (day 4) all animals were killed. Increasing doses of metapyrone effectively reduced hippocampal cell loss. 69% of hemispheres had no hippocampal CA1 cell loss in the 100 mg/Kg group. In the 50 mg/Kg group 31% of the hemispheres were spared, while in the 25 mg/Kg group 6% of hemispheres were undamaged. These results imply that an anti-glucocorticoid, such as metapyrone, may be an effective treatment following ischemic injury. (Sponsored by NSO7274)

149.5

DEXAMETHASONE AFFECTS CEREBROSPINAL FLUID PRESSURE (CSF-p). B.A. Morrow*, V.P. Starcevic*, L.C. Keil* and W.B. Severs (Spon: J. Summy-Long). Dept. Pharmacol., Col. Medicine, Pennsylvania State University, Hershey, PA 17033

Dexamethasone (dex) is often used to treat elevated CSF-p. We examined it's effect on CSF-p elevated by a low rate infusion of artificial cerebrospinal fluid in conscious, relatively unrestrained adult male S-D rats. Rats were infused for 10 min with 4 µl/min into each lateral cerebroventricle; after a 10 min rest, when CSF-p was normal, they were infused with 0.25 µl/min into each lateral cerebroventricle for 8 h. CSF-p's (cmHOH) were similar to those previously reported in non-infused rats for about 2 h (Brain Res. 462:112,1988). Then, CSF-p rose during the 8 h period to levels about 3 x higher than at 1 h. Dex (0.04 mg i.m.) was given just prior to the preparatory infusion (20 min before starting the 1st h). CSF-p's of dex rats were lower throughout the entire period but still rose after the 2 h lag period. Dex may reduce an inflammatory response that contributes to the elevated CSF-p without affecting the temporal pattern of the infusion-induced increase in CSF-p. (NASA NCC-2-127, NIH TWO343-01)

CSF-p ± SEM	1st h	2nd h	4th h	8th h
Inf. (n=13)	15 ± 1	16 ± 2	35 ± 5†	46 ± 7†
Inf./dex (n=8)	10 ± 1*	9 ± 1*	19 ± 2*†	27 ± 3*†

2-way ANOVA (rep. measure) F(treatment) = 6.7, p < 0.05; F(subjects)= 35, p < 0.001; F(interaction)= 2.8, p < 0.05
* = p < 0.05 vs Inf., † = p < 0.05 vs 2 h CSF-p.

149.6

NEURONAL AND GLIAL NA,K-ATPases LOSSES: INDICATORS OF MEMBRANE FAILURE IN IRREVERSIBLE FOCAL CEREBRAL ISCHEMIA . V.A. Bharucha*, C.G. Wakade, S.E. Karpiak, and S.P. Mahadik. Div Neurosci NYS Psychiat Inst, Depts Psychiat, Biochem & Mol Biophys, P&S, Columbia U, NY, NY

Cerebral focal ischemia in rat alters tissue water, Na+ K+ and Ca++ levels and functional parameters. Since these changes reflect membrane failure we have analyzed the activities of neuronal and glial Na,K-ATPases since they are sensitive to membrane integrity. Enzyme activities were computed by their differential sensitivity to strophantin. Rats (4-7/group) were sacrificed at 24, 72, 120hrs after focal cortical ischemia (MCAo+CCAo). At 24hrs after ischemia there were no changes in glial or neuronal ATPase in the primary or peri-infarct cortical areas. After 72hrs there were significant decreases in both neuronal (-55%) and glial (-60%) ATPase activity in the primary infarct area. In peri-infarct areas at 72hrs decreases in enzyme activity were small while at 120hrs the enzyme activities were comparable to sham controls. Data indicate that there is irreversible failure of plasma membrane function in the primary infarct area, but recovery is seen in peri-infarct areas >72hrs after ischemia. Further, there is no gliosis in the primary infarct area since losses in glial ATPase persist. These effects of GM1 ganglioside on ATPase losses is being studied since GM1 has been shown to protect membrane changes following global ischemia [NINCDS NS-2525856].

149.7

FGF LEVELS AFTER STROKE. S.P. Finklestein, C.G. Caday*, M. Kano*, J. Foster*, C.Y. Hsu, P.H. Liu, M.A. Moskowitz, and M. Klagsbrun*. Massachusetts General Hospital, Boston, MA 02114.

We measured changes in levels of fibroblast growth factors (FGF) following focal cerebral infarction in the rat. Acidic and basic FGF are heparin-binding polypeptides with potent trophic effects on brain neurons, glia, and endothelial cells. Focal infarcts were made in the right cerebral cortex of mature male Long-Evans rats as per Chen et al. (Stroke, 17:738-743, 1986). At various times (0,3,7,14,21,30, and 60 days) after infarction, animals were sacrificed, and the right cerebral cortex was removed and assayed for total FGF. FGF levels were estimated as mitogenic activity on 3T3/Balb C cells in vitro, and expressed as mitogenic units/mg protein. More than 90% of this activity bound to a heparin-affinity column and reacted with specific anti-FGF antisera. We found a 1.5 - 2 fold increase in FGF levels in the first three weeks after stroke; this increase was sustained for at least two months after infarction. Given their multipotential trophic effects, such increases in FGF levels may play an important role in glial proliferation, neovascularization, and neural sprouting after stroke.

149.8

RETINAL GANGLION CELL VULNERABILITY AFTER ISCHEMIA IN THE CAT. EFFECTS OF NGF. R. Siliprandi*, R. Canella*, R. Zanoni* and G. Carmignoto* (SPON.: L. Facci). Fidia Research Laboratories, 35031 Abano Terme (PD), ITALY.

Electroretinographic (ERG) and morphological results obtained in this study 30 days following the induction of an episode of complete retinal ischemia (ranging from 60 to 90 minutes) in the cat, indicate that retinal ganglion cells (RGCs) are more vulnerable to ischemia as compared to the more distal retinal neurons.

Since intraocular (i.o.) administration of NGF has been proven to enhance the survival of RGCs following section of the optic nerve in the adult rat (Carmignoto et al., J. Neurosci, in press), we also assessed whether NGF could be effective in protecting RGCs from the ischemic damage. In order to address this issue, NGF was injected intraocularly in cats 30 minutes prior to the induction of the ischemic episode and following the ischemic injury every other day for 30 days, at dose of 30 μg, i.o.

Results obtained indicate that in NGF-treated cats, the ERG in response to a patterned stimulus, which reflects the functionality of RGCs, is less reduced as compared to controls. In addition quantitative morphological analysis performed on Nissl-stained whole-mounted retinae indicate that RGC loss is also reduced in NGF-treated cats, thus suggesting that NGF can in part prevent the neuronal damage following ischemia.

149.9

IMMUNOELECTRON MICROSCOPIC STUDY OF TUBULIN AND MAPS IN GERBIL BRAIN DURING PROGRESSIVE ISCHEMIA AND REPERFUSION. H. Tomimoto* and T. Yanagihara. Dept. Neurol., Mayo Clinic, Rochester, MN 55905

We previously observed prompt loss of the immunohistochemical reaction for tubulin and recently observed more prompt loss of micro-microtubule associated proteins (MAPs) in the vulnerable areas of the ischemic brain. We therefore investigated the immunocytochemical reaction for tubulin and MAPs (MAP1A and MAP2) by using immunoelectron microscopy. Mongolian gerbils were subjected to bilateral carotid occlusion for 10 to 30 min and reperfusion for up to 72 hours following ischemia for 10 min. After ischemia for 10 min, some dendrites in the stratum moleculare of the subiculum-CA1 region lost electron-dense precipitates for α-tubulin and MAPs associated with microtubules. Loss of the reactions advanced further and spread to the medial CA1 region during progressive ischemia for 30 min. In some dendrites electron-dense precipitates for MAPs were observed in the cytoplasm with little reaction product on microtubules but without alteration of the reaction for α-tubulin. After recirculation, loss of electron-dense precipitates for tubulin and MAPs as well as disintegration of microtubules propagated further to the medial CA1 region and to the proximal dendrites. The present study demonstrated prompt disintegration of microtubules and rapid disappearance of the reaction for MAPs which was probably caused by detachment of MAPs from microtubules.

149.10

CEREBRAL ISCHEMIA TRIGGERS NMDA RECEPTOR-LINKED CYTOSKELETAL PROTEOLYSIS IN HIPPOCAMPUS. P.Seubert*, K.S.Lee and G.Lynch (SPON: D.Arst). CNLM, Univ. of California, Irvine, CA 92717 and Dept. of Anat., Thomas Jefferson Med.School, Philadelphia, PA 19107.

Calcium-activated proteolysis of brain spectrin is detectable by immunoblot analysis following various neural insults including the activation of N-methyl-D-aspartate (NMDA) receptors. Using this assay we examined the effects of a 10 minute bilateral carotid artery occlusion on spectrin in various brain regions of the gerbil.

In common with other neurodegenerative events a massive proteolytic breakdown of spectrin occurred during the delayed cell death phase of hippocampal region CA1. In this vulnerable region 36% of the total spectrin immunoreactivity is present as the 155kDa and 150kDa spectrin breakdown products at 4 days post-ischemia. Interestingly, substantial breakdown (7.7%) is found in CA1 within 15 minutes of removal of the vascular clamps. Proteolysis is significantly higher in this region compared to other brain structures.

Since NMDA receptor activation has been implicated in both ischemic injury and spectrin proteolysis we examined the effects of MK-801, an NMDA antagonist with reported protective effects against ischemia. MK-801 significantly reduced both phases of proteolysis at a neuroprotective dosage (10 mg/kg) administered prior to the occlusion. These results suggest that NMDA receptor-linked proteolytic events may predispose vulnerable neurons to delayed cell death.

149.11

POST-ISCHEMIC MODIFICATION OF SYNAPTIC FACILITATION AND ADENOSINE INHIBITION IN THE CA1 REGION. K.S. Lee. Dept. of Anatomy, Thomas Jefferson University, Philadelphia, PA

Delayed neuronal death of CA1 pyramidal cells is a hallmark of transient forebrain ischemia. Increased neuronal activity during the post-ischemic period is thought to be a critical event contributing to the process of delayed neuronal death. The basis for this increased activity is unknown but is likely to reside in a disturbance of one or more of the endogenous factors which normally serve to control the output of CA1 neurons. Synaptic facilitation was examined in the stratum radiatum of CA1 in slices from control animals and from animals subjected to a 10 minute period of bilateral carotid artery occlusion and allowed to survive for 1-2 hours. Paired-pulse stimuli were delivered to the Schaffer collateral/commissural afferents with interpulse intervals ranging from 20 to 500 milliseconds. Paired-pulse facilitation was greatly reduced in slices from post-ischemic animals. As previously described (Urban et al., Neurosci. Abs. 14, 1988), single pulse evoked responses are enhanced in slices from post-ischemic animals, i.e. synaptic responses were larger for a given stimulus intensity during the early post-ischemic period. These data suggest that a modification of presynaptic mechanisms could be involved in altered neuronal activity following transient ischemia.

The effect of adenosine on synaptic facilitation was also examined in control and post-ischemic slices. Adenosine is a potent neuromodulator in the CA1 region which has been shown to inhibit evoked synaptic responses and to increase synaptic facilitation (Dunwiddie and Haas, J. Physiol. 369, 1985). The enhancement of synaptic facilitation by adenosine was reduced in slices from post-ischemic animals. It is proposed that reduced inhibition by adenosine at presynaptic sites contributes to enhanced synaptic responses and increased neuronal activity following transient ischemia. (Supported by NIH grant NS 24782 01A2).

149.12

PROTEIN SYNTHESIS INHIBITORS CAN PREVENT ISCHEMIC NEURONAL DEATH IN GERBILS. D.M. Ragan*, R.K. Rader and T.H. Lanthorn (SPON: J.M. Farah, Jr.). CNS Diseases Research, G.D. Searle & Company, 700 Chesterfield Village Parkway, St. Louis, 63198.

We have found that experimental ischemia, in vitro, induces a persistent depolarization which may be an early marker of impending neuronal death. The induction, but not the maintenance, of this persistent depolarization can be blocked by NMDA antagonists. Long-term potentiation (LTP) is another persistent process in which the initiation, but not the maintenance, can be blocked by NMDA antagonists. LTP can also be blocked by inhibitors of protein synthesis. We have investigated the possibility that compounds that inhibit protein synthesis can prevent ischemic neuronal death. Ischemic death of CA1 pyramidal neurons was induced by 5 min of bilateral carotid occlusion in gerbils and was assessed by cell counts on sections taken from the hippocampus 7 days following the ischemic insult. Anisomycin and emetine were able to significantly reduce the neuronal death when administered 30 min prior to, but not 24 or 48 hrs after, the ischemic insult. With 150 mg/kg anisomycin (30 min prior) there were 76.5 ± 10.2 (n=34) neurons remaining per field vs. 24.0 ± 6.8 (n=41) for controls ($p<.0001$). With 30 mg/kg emetine (30 min prior) there were 49.2 ± 14.8 (n=10) neurons remaining vs. 22.2 ± 14.4 (n=10) for controls ($p<.02$). These results suggest that the NMDA-initiated events underlying ischemic neuronal death and LTP may involve similar processes.

149.13

CHARACTERIZATION OF NOVEL PROTEINS IN THE HIPPOCAMPUS FOLLOWING ISCHEMIA. R.M. Leimgruber*, R.K. Rader, D.M. Ragan* and T.H. Lanthorn. CNS Diseases Research, G.D. Searle & Co., St. Louis, MO 63198.

In order to determine if protein synthesis may be involved in neuronal death, we employed two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) to look for novel proteins in the brain after an ischemic insult. Proteins from brain extracts were fractionated by either isoelectric focusing or non-equilibrium pH gradient electrophoresis for the first dimension and by molecular weight using SDS-PAGE for the second dimension. Protein was obtained from the hippocampus of gerbils one hour following five minutes of bilateral carotid occlusion. Protein was also obtained from in vitro rat hippocampal slices one hour after anoxic depolarization induced by hypoxia plus decreased levels (2 vs. 10 mM) of D-glucose. The two-dimensional gel protein patterns obtained from the gerbil hippocampal slices were almost identical to those obtained from the rat hippocampal slices for all experimental conditions. Ischemia, or an in vitro model of ischemia, induced several proteins in the 20-45 KDa and 60-75 KDa molecular weight regions. These "novel" proteins were either absent or present in negligible amounts in untreated controls and in brain tissue which had been protected from the effects of ischemia by the use of the NMDA antagonist, MK-801, or the protein synthesis inhibitor, anisomycin.

149.14

EARLY IMPAIRMENTS IN HIPPOCAMPAL PROTEIN SYNTHESIS FOLLOWING GLOBAL CEREBRAL ISCHEMIA IN NEONATAL PIGS. J.M. Gidday and T.S. Park. Department of Neurosurgery, University of Virginia, Charlottesville, Virginia 22908.

There is evidence that protein synthesis shows a prolonged period of impairment following cerebral ischemia in adult animals even though energy metabolism and glucose utilization recover relatively rapidly. To investigate this phenomenon in the neonate, we assessed by autoradiography the regional incorporation of ^{14}C-leucine in hippocampal substructures of 6 isoflurane-anesthetized piglets (less than 5 days of age) following 10 minutes of global cerebral ischemia induced by subclavian and brachiocephalic arterial occlusion. After a 6 hour reperfusion period, a tracer dose of leucine (100μCi/kg) was given i.v. and the brains were transcardiac perfusion-fixed 30 minutes later. 30μm coronal sections were obtained at -20°C. Densitometric analyses of the autoradiographs revealed a depression in amino acid incorporation relative to controls of 22% in the granule cell layer of the dentate gyrus, 55% in the dentate hilus, and 57% in the pyrimidal cell layer of CA3. The greatest depression, 67%, occurred in the pyrimidal cell layer of CA1. Such early derangements in protein metabolism may play a key role in the delayed neuronal death experienced by these hippocampal cells following ischemic insults. (Supported by American Heart Association Virginia Affiliate; NS 00924 and NS 21045).

149.15

THE PHOSPHATIDYLINOSITOL SECOND MESSENGER SYSTEM AFTER TRANSIENT GLOBAL ISCHEMIA: AN AUTORADIOGRAPHIC STUDY. J.Deckert, M.B.Jorgensen* and D.C.Wright*. BPB, NIMH and SNB, NINCDS, NIH, Bethesda, Md. 20892, USA.

The hypothesis of excitotoxic neuronal cell death postulates increased intracellular Ca-concentrations induced by glutamate. This could occur due to increased phosphatidylinositol(PI)-hydrolysis and subsequent formation of inositoltrisphosphate (IP3) and diacylglycerol (DAG). We therefore decided to investigate binding sites for IP3 and DAG using a quantitative autoradiographic method and as ligands (3H)IP3 and (3H)phorboldibutyrateester ((3H)PDBU).

Male Sprague-Dawley rats were subjected to transient forebrain ischemia using a modification of the four-vessel occlusion model. (3H)IP3 (in the absence of calcium) and (3H)PDBU (in the presence of calcium) autoradiography were performed as originally described.

An early decline of (3H)IP3 binding after 6 hours of about 40% was measured in the CA1, the CA3, the Molecular Layer of the Dentate Gyrus and the Parietal Cortex. (3H) PDBU binding decreased by 20%-40% after 12 hours in the CA3 and the Molecular Layer of the Dentate Gyrus and only after 72 hours in the CA1 and the Parietal Cortex. Cell death was observed after 48 hours in the Dentate Hilus and after 72 hours in the CA1.

These data clearly implicate a role for the PI-second messenger system in the early stages of excitotoxic neuronal cell death.

149.16

SEPARATION OF INOSITOL PHOSPHATES IN RAT BRAIN BY CHROMATOGRAPHY. T.N. Lin*, G.Y. Sun, N. Premkumar*, R.A. MacQuarrie* and S. Carter* (SPON: Robert T. Zoeller). Biochem Dept. and Sinclair Research Farm, Univ. Missouri-Columbia; School of Basic Life Sci., Univ. Missouri-Kansas City; and Dionex Corp., Sunnyvale, CA.

In spite of great interest in understanding the signal transduction mechanism involving turnover of phosphoinositides and generation of inositol phosphates as second messengers, an inherent limitation to this type of study is the lack of sensitive method for quantitation of the inositol phosphates and isomers. To this end, a HPLC procedure has been developed in our laboratory for separating the inositol phosphates (IP, IP_2 and IP_3) and their isomers in brain. This procedure involves an Ion Pac AS5A-5u column together with an Anion Micro-Membrane Suppressor and subsequent detection using an Ion Conductivity detector (Dionex Corp.). In a typical study, Sprague-Dawley rats were killed by decapitation and the brain cortex removed and homogenized in chloroform-methanol (2:1, v/v). The homogenate was centrifuged to separate the protein layer and the organic solvent which was later discarded. The pellet was dried under nitrogen and resuspended in deionized water. Samples were subjected to a clean-up cartridge prior to injection into the column. Samples were eluted isocratically with 150 mM NaOH and H_2O in different proportions. Using this separation condition, the amount of IP, IP_2 and IP_3 observed in normal brain was 30-50, 150-200, and 4-7 nmole/g wet wt, respectively. Lithium treatment resulted in a 10-40 fold increase in the level of IP (depending on dose and time of treatment) but did not appreciably alter the levels of IP_2 or IP_3. Ischemic treatment also resulted in a time-dependent change in the levels of the inositol phosphates in brain. (Supported in part by NS 20836 from NINCDS and AA06661 from NIAAA).

149.17

TIME-DEPENDENT CHANGES IN PROTEIN KINASE C MEDIATED *IN VITRO* PHOSPHORYLATION IN ISCHEMIC SPINAL CORD. A. Kochhar*, J. A. Zivin, and T. Saitoh, Dept. of Neurosciences, School of Medicine, Univ. of California, and VA Medical Center, La Jolla, CA 92093.

Ischemia initiates a series of biochemical events that can eventually lead to cell death. We have previously shown that extended periods of ischemia (60 min) result in reduced *in vitro* phosphorylation of endogenous proteins by endogenous protein kinase C (PK-C). This ischemia duration produces irreversible neurological damage. In the present study, we examined the effects of shorter durations of ischemia on PK-C-dependent phosphorylation in rabbit spinal cord. Ten min of ischemia does not produce irreversible neurological damage, while 30 min of ischemia produces irreversible damage in 50% of the animals. Spinal cords were rapidly excised from control and ischemic rabbits and frozen. Tissue samples were homogenized and centrifuged. In *in vitro* endogenous protein phosphorylation assays, membrane and cytosolic fractions were incubated under phosphorylating conditions, in the absence or presence of PK-C activators, 4ß-phorbol 12-myristate 13-acetate and phosphatidyl serine. Proteins were separated by SDS-polyacrylamide gel electrophoresis, stained with Coomassie Blue, and phosphorylated proteins were detected by autoradiography. Ten min of ischemia did not affect endogenous protein phosphorylation mediated by PK-C in either fraction. However, the endogenous protein phosphorylation pattern under PK-C activating conditions was reduced 25 to 50% in both fractions after 30 min of ischemia. These studies show a temporal effect of ischemia on endogenous PK-C phosphorylation system. Short durations of ischemia (10 min) do not affect PK-C mediated phosphorylation, intermediate ischemia durations (30 min) can reduce protein phosphorylation by 50%, while long ischemia durations (60 min) result in almost complete loss of protein phosphorylation.

149.18

PROTEINKINASE C IS TRANSLOCATED TO THE PLASMAMEMBRANE DURING CEREBRAL ISCHEMIA. M. Cardell*, T. Saitho, J. Zivin, T. Wieloch* (SPON: B. Ehinger). Lab. for Exp. Brain Res., Lund University Hospital, 221 85 Lund, Sweden, Dept. of Neurosciences, UCSD Med School, La Jolla, CA 92093.

Transient periods of cerebral ischemia lead to selective neuronal necrosis in certain regions of the brain, including cerebral neocortex, hippocampus and striatum. We investigated protein kinase C (PKC) activity and PKC immunoreactivity (PKC-IR) in the membrane and cytosolic fractions from rat cerebral cortex and striatum. Activity of PKC was measured by incorporation of [32P] into histone III and endogenous phosphoproteins, and the amount of PKC was determined by Western blotting. Polyclonal antibodies directed against PKC α,β, or γ was used. Ischemia was induced either by cardiac arrest, or by bilateral occlusion of both common carotid arteries combined with hypotension.

Results:

1. During ischemia PKC-IR in cortex increased progressively in the membrane fraction and decreased in the cytosolic fraction.

2. Free arachidonic acid increased with time of ischemia.

3. *In vitro* phosphorylation of histone III and endogenous proteins in striatum decreased in the membrane fraction but was unaffected in the cytosolic fraction. No evidence for the formation of proteinkinase M (PKM) was seen.

Conclusions:

No partial proteolysis of PKC leading to the formation of PKM takes place during ischemia, despite elevated intracellular calcium concentrations. Proteinkinase C is translocated during cerebral ischemia to the cellular membranes possibly due to the accumulation of arachidonic acid, while the enzyme activity decreases which may be due to an inhibitor. Translocation of PKC could lead to changes in ion channel properties and gene expression, that may be of pathological significance in ischemia.

Supported by United States PHS (NS 25302) and Swedish MRC (14X-08644).

149.19

EFFECTS OF HYPOXIA ON ORNITHINE DECARBOXYLASE ACTIVITY IN BRAIN REGIONS OF NEONATAL RATS. A. Chicz-DeMet, V. Bjelajac*, B. Stoveken*, A. Gurnani*, and E.M. DeMet. State Dev. Rsch. Inst., Costa Mesa, CA 92626, Dept. Psychiatry, Univ. of California, Irvine, CA 92717

Ornithine decarboxylase (ODC), a rate limiting enzyme in the synthesis of polyamines, is normally induced during cellular replication and differentiation. Higher than normal levels may be induced in rats under conditions of stress, eg. separation from dam, toxic insult, or hypoxia, with maximum sensitivity on postnatal (pn.) day 8. Therefore, ODC levels may represent a sensitive measure of hypoxic exposure. The present study compared ODC responses to a 2 hr/7% oxygen exposure in male and female Sprague Dawley rats. Rats treated on day 8 pn. were sacrificed immediately following exposure and on days 13 and 20 pn. Brains were dissected into 9 regions and stored at -70°C for assay. Preliminary results of 5 brain regions confirm an earlier report that hypoxic treatment elevated ODC levels in all of these regions. Maximal increases were found on day 8 pn. in the hippocampus, midbrain, brainstem and cerebellum whereas levels in the cortex peaked on day 13 pn. Untreated female controls generally had higher levels than males, and ODC increases in treated females were greater than in males on days 8 and 13 pn. Treated rats of both sexes had lower than control levels on day 20 pn. with significant decreases only in the males. The possible relationship between ODC changes and neuronal damage will be discussed.

149.20

RELATION OF BLOOD-BRAIN BARRIER (BBB) CHANGES TO CALCIUM UPTAKE IN CEREBRAL ISCHEMIA. G.Nagashima*, J.Ikeda*, T.S.Nowak,Jr, F.Joo*, G.Mies*, C.Ruetzler*, J.Lohr* and I.Klatzo Lab. of Neuropathology and Neuroanatomical Sciences, NINDS, NIH, Bethesda, MD 20892.

The purpose of this study was to gain further insight into the nature of pathomechanisms operative following cerebral ischemia. Mongolian gerbils were subjected to repeated bilateral common carotid artery occlusions at 1 h intervals. The uptake of calcium in brain tissue was assessed by autoradiography using 45-Ca injected 7 h before sacrifice and by electron microscopy using an oxalate-pyroantimonate precipitation method. The permeability of the BBB was assessed with horseradish peroxidase or by immunostaining using a rabbit anti-gerbil albumin antibody. Autoradiographic observations after repeated insults revealed, in addition to hippocampus, conspicuous Ca uptake in the striatum, thalamus, medial geniculate and substantia nigra. The areas of calcium accumulation showed frequent association with immunocytochemical evidence of albumin extravasation in those regions. Electron microscopy revealed a pattern of abnormal calcium accumulation primarily in dendritic structures, similar to that described following convulsive seizures associated with neuroexcitation. Our studies suggest that neuroexcitatory mechanisms associated with cerebrovascular permeability changes and an increased calcium uptake may play an important role in development of postischemic lesions.

149.21

THE ROLE OF CALCIUM IN GLUTAMATE-ENHANCED HYPOXIC NEURONAL DAMAGE A. Schurr, C.A. West* and B.M. Rigor. University of Louisville School of Medicine, Louisville, KY 40292

Recently, the pathophysiology of cerebral hypoxia-ischemia has been attributed to neurotoxicity of the excitatory amino acids glutamate (Glu) and aspartate (Asp). Here we attempted to determine the role of Ca^{2+} in the enhancement of hypoxic-ischemic neuronal damage (HIND) by Glu, Asp and their analog NMDA. Extracellular recordings of orthodromic responses (population spikes) evoked in the CA1 stratum pyramidale of hippocampal slices were used as a measurement of neuronal function. Under normoxic conditions Glu and Asp (0.1-3.0 mM) had no adverse effect on neuronal function. However, when hypoxia was applied for 10 min, the recovery rate of neuronal function upon reoxygenation decreased with increasing concentration of Glu. In contrast to Glu and Asp, NMDA depressed normoxic neuronal function at concentrations above 50 uM. Under hypoxic conditions, 10 uM of NMDA induced a degree of neuronal damage equal to that of 3000 uM Glu. Elimination of Ca^{2+} from the perfusion medium during hypoxia completely abolished the neurotoxic effects of Glu, Asp and NMDA. Nevertheless, HIND could be detected in the presence of a standard concentration of Ca^{2+} (2.5 mM) and in the absence of an excitotoxin. APV, an NMDA antagonist, attenuated the NMDA-enhanced HIND. We concluded that Ca^{2+} influx and its intracellular accumulation are the major processes leading to HIND, whereas Glu and Asp, although enhancing such influx and damage, are by themselves innocuous.

149.22

ISCHEMIC CONDUCTION FAILURE IN RAT PERIPHERAL NERVE. J.D. Schmelzer* and P.A. Low. Neurophysiology Laboratory, Dept. of Neurology, Mayo Foundation, Rochester, MN 55905

Our rat model of severe nerve ischemia, produced by the temporary occlusion of the abdominal aorta and both iliac arteries for 1-3 hrs, consistently produced ischemic conduction failure within 30 min in both sciatic-tibial and caudal nerves. In the sciatic-tibial nerve, when submitted to 1 hr of ischemia, the muscle CAP recovered to >50% upon 3 hrs of reperfusion but when submitted to 3 hrs of ischemia the muscle CAP recovered to only 10% and 35% after 3 hrs and 1 wk of reperfusion, respectively. Three hrs of ischemia resulted in the recovery of nerve CAP to ~40% and ~60% after 3 hrs and 1 wk of reperfusion, respectively. In caudal nerve, following 1 hr of ischemia, recovery of the nerve CAP was prompt and 80% complete upon 2 hrs reperfusion and 90% upon 1 wk reperfusion. In contrast, muscle CAP recovered to only 20% and 30% upon 2 hrs and 1 wk of reperfusion. Following 3 hrs of ischemia, recoveries of the nerve CAP were 10%, 50%, and 80% upon 2 hrs, 1 wk, and 1 month of reperfusion. In contrast, the recoveries of the muscle CAP were 0%, 0%, and 28% upon 2 hrs, 1 wk, and 1 month of reperfusion. We conclude that the electrophysiological consequences of ischemia and reperfusion are better explained by conduction failure in muscle and/or nerve terminal rather than in peripheral nerve.

149.23

EFFECT OF CORTICAL BARREL-FIELD INFARCTION ON CYTOCHROME OXIDASE HISTOCHEMISTRY IN THE RAT. W.D. Dietrich, O. Alonso, and M. Halley*. Cerebral Vascular Disease Research Center, University of Miami, School of Medicine, Miami, FL, 33101.

Cytochrome oxidase (CO) histochemistry was used to obtain evidence for anatomical reorganization following focal brain injury. Young rats (25 days) underwent photochemically induced infarction of the left cortical barrel-field (BF). Three months later, rats were perfusion-fixed and processed for the light and electron microscopic visualization of CO histochemistry. In cleared Vibratome sections, abnormally dense CO staining was demonstrated in layers 4-6 of the somatosensory cortex contralateral to the BF infarct. Ultrastructural analysis of this area demonstrated a high frequency of large and darkly reactive dendritic mitochondria. The somata of many cortical neurons also contained large numbers of densely reactive mitochondria, while other neurons appeared dark with increased numbers of ribosomes. Astrocytes appeared swollen with clumped nuclear chromatin. Focal brain injury results in morphological indicators of altered mitochondrial function and protein synthesis in remote brain regions. Degeneration of transcallosal fibers following cortical infarction may lead to denervation of the contralateral hemisphere and subsequent neuronal reorganization at this relatively late post-injury period.

149.24

DENSE DEPOSITS IN RAT HIPPOCAMPAL CA1 NEURONS DURING POST ISCHEMIC DELAYED NEURONAL DEATH K Magnusson*, J Deshpande, T Linden*, H Kalimo* and T Wieloch*. (Spon: MC Rogers) Dept. of Anesthesiology, The Johns Hopkins Hospital, Baltimore, MD 21205 and Lab. for Experimental Brain Research, Lund University Hospital, Lund, Sweden.

Ultrastructural changes in hippocampal CA1 neurons were studied 1h, 6h, 24h, 48h, & 72h 10 min after cerebral ischemia with the 2 v-o model. Delayed neuronal death was observed on light microscopy. Until the appearance of frank necrosis, mitochondria appeared intact and nuclear chromatin was dispersed; nucleolus was unremarkable, the nucleolemma was irregular but no bleb formation of plasma membrane or cell fragmentation was observed on EM. At 6h, electron-dense fluffy material (DM) associated with tubular saccules appeared in soma and in dendrites immediately beneath the plasma membrane. The amount of DM increased progressively with recirculation. At 48h there was a gradient of DM with high density in soma and in the major trunks of dendrites and seldom in spines. DM did not correspond to Ca precipitates on oxalate-pyroantimonate stained sections. HSP-70 immunoreactivity (IR) was not observed in controls, but appeared at 24h and 48h. Ubiquitin IR found in control animals disappeared and never recovered in CA1. We propose that the DM represents aggregates of high turnover proteins which may induce disruption of cellular structure and function, ultimately leading to cell death.

149.25

FUNCTIONAL INTERACTIONS BETWEEN GABA AND GLIAL CELLS IN THE GERBIL FOREBRAIN AFTER TRANSIENT ISCHEMIA. C.S.Lin, K.Polsky and B.J.Crain Department of Physiology & Biophysics, Hahnemann University, Phila.,Pa. 19102 and Department of Neurobiology and Pathology, Duke University Medical Center, Durham, NC 27717

The present study was designed to investigate the functional relationships between the GABAergic neurons and the glial cells in the gerbil forebrain after transient ischemia. We used the immunostaining of the glial cells with GFAP and the GABAergic neurons with GABA and/or GAD to assess the interaction between GABA and glial cells by varying the survival time-i.e. from 1 day to 4 weeks after a 5 minute bilateral common carotid occlusion. An increase of GFAP reactivity after 4 days post-ischemic survival was noted mainly centered in the lateral septal nucleus, the dorso- lateral region of the striatum, the CA_1 sector of the hippocampus and the layer 3 of the somatosensory and auditory cortices. This reactive astrocytotic action was clearly noted beginning at 4 days and persisted up to 4 weeks. Further, the adjacent sections immunostained with GABA antiserium displayed a glial cell-like reactive change found in those most severely damaged areas after a 7 days survival. This glial cell-like reaction extended up to 4 weeks. However, this reactive change was not found in the adjacent sections stained with GAD antiserum. Our present finding suggests an intimate interaction between GABA and glial cells in the ischemic damaged gerbil forebrain. The functional significance of this phenomenon will be discussed. Supported by NIH NS 06233 and 1-SO7RR07241.

TRANSMITTERS IN INVERTEBRATES II

150.1

SUBSTANCE P-LIKE IMMUNOREACTIVITY IN A SUBPOPULATION OF THE FMRFamide-LIKE IMMUNOREACTIVE NEURONS OF *DROSOPHILA* AND *CALLIPHORA* D. R. Nässel and T. Lundquist*. Dept. Zoology, University of Stockholm, S-10691 Stockholm, Sweden.

For the understanding of peptidergic transmission in the insect CNS detailed mappings of neuron morphology and projections may be helpful. We have selected some peptidergic systems for such analysis. An antiserum against substance P immunolabels a small distinct population of neurons in the flies *Drosophila* and *Calliphora*. Double labeling shows that the substance P-like immunoreactive (SPLI) neurons constitute a subpopulation of the numerous FMRFamide-immunoreactive (FLI) neurons. In *Drosophila* there are 10 SPLI neurons in the brain and 10 in the fused thoracico-abdominal ganglia. In *Calliphora* the same 20 SPLI neurons are seen with about 30 additional smaller SPLI neurons. Six of the SPLI neurons in both species are thoracic neurosecretory neurons with terminals in a neurohaemal release site in the dorsal ganglion sheath and central processes connecting the thoracic neuromeres with the suboesophageal ganglia. In the brain SPLI neurons are projection neurons and large field amacrine neurons. Subpopulations of the FLI neurons are also recognized by antisera against CCK and enkephalins. Probably the FMRFamide- and CCK-antisera react with drosulfakinins [CCK-like peptides with the -MRFamide C-terminus in common with the *Drosophila* peptide DPKQDFMRFamide (Nichols et al., *J. Biol. Chem.* **25:** 12167, 1988; Schneider and Taghert, *Proc. Natl. Acad. Sci.* **85:** 1993, 1988)]. The FLI neurons may hence contain peptides similar to DPKQDFMRFamide and drosulfakinins, and some have colocalized substance P-like peptide. Functionally different types of neurons may use these peptides as neurotransmitters, modulators or neurohormones.

150.2

ISOLATION AND CHARACTERIZATION OF A **DROSOPHILA** AKH. M.H. Schaffer, B.E. Noyes*, C.A. Slaughter*, G.C. Thorne*, and S.J. Gaskell*. Depts. of Psychiatry and Biochemistry, U. Texas Southwestern Medical Center, Dallas, Tx., 75235-9070 and Center for Experimental Therapeutics, Baylor College of Medicine, Houston, Tx.

A member of the AKH - RPCH family of neuropeptides was isolated from **Drosophila melanogaster** based on the prediction that the **Drosophila** peptide would have physical properties and bioactivity on the grasshopper extensor tibialis similar to other family members. A peptide with these properties was extracted from thorax into methanol, acetic acid, water and purified by three steps of reverse phase HPLC. Its structure has been determined by a combination of automated Edman degradation sequencing (150 pmoles) and fast atom bombardment hybrid mass spectrometry with low energy collisional activation (150 pmoles). The structure has been confirmed by comparison of the fragmentation pattern to that of a synthetic standard. While the **Drosophila** peptide appears to be a typical family member in most respects, it is remarkable for the presence of an asp rather than an asn residue. Control experiments are required to document that this is not the result of hydrolysis during the purification, but this is unlikely since other family members subjected to the same purification are not degraded. Despite this change, the **Drosophila** peptide is clearly active on on the grasshopper leg.

150.3

ISOLATION, CHROMOSOME LOCALIZATION, AND EXPRESSION OF A cDNA CLONE FROM *DROSOPHILA MELANOGASTER* WHICH CODES FOR A PUTATIVE OCTOPAMINE RECEPTOR. [1]D.A. Urquhart, [1]L. M. Hall, [2]S. Arakawa* and [2]J. C. Venter*. [1]Dept. of Mol. Genetics, Albert Einstein Coll. of Med., Bronx, NY 10461 and [2]Sect. of Receptor Biology, NINDS, Bethesda, MD 20892.

Octopamine (OCTO) functions as a neurotransmitter and a circulating neurohormone in insects. At least two classes of receptors for OCTO can be distinguished pharmacologically; these receptors display properties similar to vertebrate a_2-adrenergic receptors (AR). Using a cDNA clone encoding a human b_2-AR, *Drosophila* genomic and cDNA libraries were screened under reduced stringency (Arakawa and Venter). A 3.3 kb cDNA clone encoding a protein of 601 amino acids was isolated. This protein expressed in CHO cells displayed high affinity ^{3}H-yohimbine binding (K_d = 6 nM) with a B_{max} = 1.5 pmol/mg membrane protein. Octopamine, synephrine, and clonidine were essentially equipotent in inhibiting yohimbine binding (K_i = 15-30 mM) while epinephrine, dopamine and isoproterenol were significantly less potent, suggesting that the protein encoded by this cDNA clone may be a *Drosophila* OCTO receptor. *In situ* hybridization studies using a biotinylated genomic clone has shown that this receptor gene maps to position 99A10-B1 on the right arm of the third chromosome. The relationship of this gene to exisiting mutants and chromosome aberrations in this region will be presented.

150.4

IDENTIFICATION OF GLUTAMATE NEURONS IN THE ABDOMINAL NERVOUS SYSTEM OF THE MOTH MANDUCA SEXTA. S.E. Fahrbach, Dept.Entomol., Univ. of Illinois, Urbana, IL 61801.

Glutamate (GLU) is the short-latency excitatory neurotransmitter at the insect NMJ. In studies of developmental neuron death in Manduca sexta, selective cellular markers would facilitate identification of neurons fated to die. (Of the cells that die in each abdominal ganglion after adult emergence, approximately 80 are motoneurons.) I have used a polyclonal antibody specific for GLU to identify GLU-IR neurons in whole ganglia and frozen sections of the moth abdominal nervous system. These studies have demonstrated not only that this antibody can recognize putative GLU-containing motoneurons in the moth nervous system, but have also revealed the surprising extent to which GLU must serve as an excitatory transmitter within the segmental ganglia, since it is expressed in numerous interneurons whose processes do not leave the CNS. The pattern of staining was reproducible from ganglion to ganglion. Staining for GLU should provide a label for this neuronal population as it undergoes a dramatic (50%), hormonally-regulated reduction in cell number.

150.5

THE DISTRIBUTION OF GABAERGIC LINEAGES IN INSECTS. J.L. Witten and J.W. Truman. Dept. of Zoology, University of Washington, Seattle, WA. 98195.

Using immunochemical methods (antiserum supplied by J. Hildebrand, U. Arizona), we have localized GABA to six identifiable postembryonic lineages in the thoracic ganglia of the moth, *Manduca sexta*. There are three ventral (K,M,N) and two dorsal (E,T) paired lineages and one dorsal unpaired (X) lineage. All the neurons within each lineage express the GABAergic phenotype. We found the same number of clusters of GABA - immunoreactive neurons in similar positions in the adult ganglia of the firebrat, grasshopper and beetle. In addition, the primary processes of cells in clusters that are in similar positions insert into the same tracts. These observations strongly suggest that these six clusters in the different insects are homologous lineages.

We are using this comparative approach to test the hypothesis that homologous lineages may serve similar functions in adult specific behaviors such as walking or flight. The size of individual lineages varies in different insects and we are trying to correlate this size difference with these particular species specific adult behaviors.

Supported by NIH grants NS 07936(JLW) and NS 13079(JWT).

150.6

NEURAL REGULATION OF SEX PHEROMONE PRODUCTION IN FEMALE MOTHS. I. MORPHOLOGY. H. Itagaki, T.A. Christensen, and J.G. Hildebrand. ARL Div. of Neurobiology, Univ. of Arizona, Tucson, AZ 85721.

Recent work by Teal and Tumlinson (PNAS, in press) indicates that sex pheromone biosynthesis in female *Heliothis* moths is regulated by a two-step process. Pheromone biosynthesis-activating neuropeptide (PBAN, Raina & Menn, 1987) appears to be transported down the ventral nerve cord (VNC) from the subesophageal ganglion (SEG) to the terminal abdominal ganglion (TAG), where it acts on a second neural element that stimulates pheromone biosynthesis by the pheromone gland (PG), a specialized monolayer of cells in the intersegmental membrane at the abdominal tip. Using standard techniques, we have attempted to define the cellular elements of this system in female *Heliothis* moths.

Using paraldehyde-fuchsin staining for neurosecretory cells, we have identified one or two candidate PBAN-transporting cell(s) linking the SEG and the TAG. Specific staining was found in one axon in each connective, with terminals located in the pterothoracic ganglion and the TAG. We do not yet know if one or two cells are involved, as no soma(ta) have been found linked to these axons. We are planning further work using a PBAN-specific antibody to clarify this question.

We have also used cobalt staining followed by silver-intensification to characterize the neurons innervating the PG. Cutting and staining of TAG nerves revealed fibers projecting toward, and fine arborizations associated with, the PG. Cobalt backfilling from the PG toward the TAG has stained several small groups of cells located on the posterior margin of the TAG. Some of these cells also are stained by the vital dye Neutral Red, suggesting the possible presence of biogenic amine(s). This staining has led to a hypothesis about the transmitter used by TAG neurons to control pheromone biosynthesis (see next abstract). We are now trying to identify specific cells innervating the PG by intracellular staining using microelectrodes. [Supported by USDA, NIH, and Monsanto Co.]

150.7

NEURAL REGULATION OF SEX PHEROMONE PRODUCTION IN FEMALE MOTHS. II. PHYSIOLOGY AND NEUROCHEMISTRY. T.A. Christensen, H. Itagaki, P.E.A. Teal*, and J.G. Hildebrand. ARL Div. of Neurobiol., Univ. of Arizona, Tucson 85721, and USDA, Gainesville, FL 32604.

We are using standard anatomical, electrophysiological, and biochemical techniques to study the regulation of sex-pheromone production in the pheromone glands of the female *Heliothis* and *Manduca* moths. Anterograde staining of nerves from the terminal abdominal ganglion reveals several fibers with fine branches that appear to be associated with the gland (Itagaki et al., companion poster). The morphology of these terminals is easily distinguished from that of motorneurons or sensory neurons. Retrograde staining from nerve branches innervating the gland reveals a small cluster of cells at the posterior edge of the ninth neuromere. We are now using intracellular methods to record from and stain individual neurons in this cluster. These neurons stain selectively with the vital dye Neutral Red, suggesting the presence of biogenic amine(s).

Electrical stimulation of the gland nerves during the daylight hours can induce pheromone production in moths that ordinarily would not produce pheromone at this time of day. Several biogenic amines and an amino acid were then tested in isolated glands *in vitro* as candidates for the role of the transmitter mediating this action. We incubated denervated glands with DL-octopamine (OA), dopamine (DA), serotonin (5HT), or L-glutamate for 30 min (100 μg/10 μl saline solution). Only the three amines stimulated pheromone biosynthesis above control levels (OA, 100% above controls; 5HT, 61%; DA, 43%). Glutamate had no effect. Future experiments will focus on the possibility that OA is the efferent transmitter regulating the gland and on the effects of the pheromone biosynthesis-activating neuropeptide (PBAN, Raina & Menn, 1987) on the neurons innervating the gland. [Supported by NIH, USDA and Monsanto Company].

150.8

M1-LIKE MUSCARINIC RECEPTORS MEDIATE CHOLINERGIC ACTIVATION OF AN INWARD CURRENT IN ISOLATED NEURONAL SOMATA FROM LOCUST THORACIC GANGLIA. J.A. Benson. R & D Plant Protection, Agricultural Division, CIBA-GEIGY Ltd., CH-4002 Basel, Switzerland.

The response to acetylcholine in voltage-clamped, thoracic ganglion neuronal somata from *Locusta migratoria* comprises a fast, nicotinic (ACh1) component and a slow, muscarinic (ACh2) component (Benson, J.A. & Neumann, R., *Soc. Neurosci. Abs.*, 13:938, 1987). The current activated via the ACh2 receptor is inward at -30 mV and decreases with hyperpolarisation to zero at -80 to -90 mV. The ACh2 response is activated by muscarine, oxotremorine, arecoline and pilocarpine. It is blocked by quinuclidinyl benzilate and scopolamine at nanomolar concentrations, and atropine acts similarly at concentrations 10-fold higher. Gallamine, a weak nicotinic blocker and a potent antagonist of muscarinic m_2 but not m_1 receptors, and methoctramine, a specific m_2 blocker, are without effect on the ACh2 response at 10^{-4} M. In contrast, pirenzepine, a specific m_1 antagonist, blocks the ACh2 response (EC_{50} = 7.1 $\pm$ 4.5 x 10^{-7} M, m $\pm$ SD, n = 3). The pharmacological profile of the muscarine recognition site of this insect somal receptor thus resembles that of the vertebrate brain m_1 muscarinic receptor. These results are consistent with binding studies on locust neurones that reveal m_1 receptors on somal membranes and m_2 receptors at nerve terminals (Knipper, M. & Breer, H., *Comp. Biochem. Physiol.*, 90C:275, 1988), suggesting that the m_1 binding site corresponds to a functional receptor.

150.9

NEUROTRANSMITTERS OF THE CRAYFISH ABDOMINAL POSITIONING SYSTEM. B.F. Murphy* and J.L. Larimer. Department of Zoology, University of Texas, Austin, TX 78712

The abdominal positioning system of crayfish serves as a model for CNS control of simple behaviors, however, the major neurotransmitters of this system are largely unknown. We perfused isolated crayfish nerve cords with various neurotransmitters as well as some of their agonists and antagonists and monitored the effects by recording from the tonic positioning motor neurons. Each test consisted of a 15 minute prewash, a 30 minute treatment with neurotransmitter and a 15 minute washout period. Inhibition of motor neuron activity followed 10^{-3} M GABA while 10^{-4} M picrotoxin alone was stimulatory. Since neither glycine nor strychnine had an effect, we conclude that GABA probably serves as the major inhibitory transmitter in this system. Other neurotransmitters including glutamate and acetylcholine at 10^{-3} M as well as the agonist of acetylcholine, methacholine showed no effect. The acetylcholine agonist carbachol, however, stimulated the output at both 10^{-3} and 10^{-4} M indicating the presence of some form of acetylcholine receptor. The muscarinic agent atropine was more effective than the nicotinic drugs hexamethonium or curare in blocking the carbachol effect suggesting that the muscarinic receptor type is predominant. (Supported by NIH NS05423, JLL).

150.10

EFFECTS OF NEUROMODULATORS ON PROPRIOCEPTIVE NEURONS IN THE LIMBS OF THE CRAB, CANCER MAGISTER. R. L. Cooper and H. B. Hartman. Biocenter, Univ. of Basel, CH 4056 Basel, Switzerland and Dept. of Biology, Duquesne Univ., Pittsburgh, PA 15282.

Amines and peptides are known to play a variety of roles in crustacean neurophysiology. Serotonin, octopamine and proctolin have been the major compounds of interest in central neuromodulation. Kravitz's group (Beltz and Kravitz, J. Neurosci.,7:533, 1987) has carried out immunocytochemical studies identifing neurons containing these neuromodulators and shown behavioral changes that occur when modulators are injected into lobsters. Pasztor and Bush (Nature, 326:793,1987) have reported on peripheral modulation of mechanosensory neurons of the oval organ in lobster. We chose to study the effects of neuromodulators on the sensory neurons of proprioceptor organs within the limbs of the crab, Cancer magister.

The propus-dactylus (PD) organ was isolated in situ and responses to controlled movement of the organ by individual neurons were recorded using a suction electrode. The output of neurons responsive to joint extension, flexion, and static position was determined when octopamine, serotonin, proctolin, NMDA or GABA were present. The responses were quantified by the statistical index, eta^2. Serotonin (10^{-6}M) caused an increase in activity of the movement sensitive cells. This enhanced activity resulted in a loss of specificity to the stimulus. Octopamine and proctolin (10^{-4} and 10^{-6} M) did not produce any altered responses to the mechanical stimuli. The effects of NMDA and GABA will be described. Supported by NSF Grant BNS-8700506 to H.B.H.

150.11

BIOPHYSICAL AND THEORETICAL CHARACTERIZATION OF THE CURRENTS OF CRAB STOMATOGASTRIC GANGLION NEURONS: PROCTOLIN MODULATION. J. Golowasch, F. Buchholtz, I.R. Epstein, and E. Marder. Brandeis Univ., Waltham, MA 02254.

Many peptides and amines are now known to modulate the pyloric network of the crustacean stomatogastric ganglion (STG). Our aim is to determine the biophysical mechanisms underlying the modulation of identified neurons of the crab STG by the neuropeptide, proctolin. To this end we are using voltage clamp to characterize the time and voltage dependence of the currents in identified STG neurons. The Lateral Pyloric (LP) and Inferior Cardiac (IC) neurons are direct targets of proctolin. Both cells show similar currents. These are: 1) a Ca^{++}-dependent K^+ current, 2) a delayed rectifier-like current, 2) a A-type transient outward current, 4) at least one Ca^{++} current, 5) a slow inward rectifier that activates upon hyperpolarization, and 6) a novel voltage-sensitive current activated by proctolin and carried in part by Na^+ and Cl^-. The experimental data are then fitted with mathematical models to understand how proctolin modifies the excitability of these cells. Supported by NS17813, URI 49620-86-C-0131 and NSF DMB-8604794.

150.12

LOCALIZATION OF PIGMENT-DISPERSING HORMONE (PDH) -LIKE IMMUNOREACTIVITY IN THE CRUSTACEAN STOMATOGASTRIC NERVOUS SYSTEM. Lawrence I. Mortin and Eve Marder. Biology Department, Brandeis University, Waltham, MA 02254.

Pigment-dispersing hormone (PDH) acts to disperse pigments in crustacean chromatophores. We used a polyclonal antibody made against synthetic PDH from the crab Uca pugilator (Cell Tissue Res. 250:377, 1987) to characterize the distribution of PDH-like immunoreactivity in the stomatogastric nervous system of 5 decapod crustaceans: the crabs Cancer antennarius and Cancer borealis, the lobsters Panulirus interruptus and Homarus americanus, and the crayfish Procambarus clarkii. Staining was seen in the neuropil region of the stomatogastric ganglion (STG) of the lobsters and crayfish, but not in the crabs. None of the cells in the STG stained for PDH in any of these species, but cells and neuropil in the paired commissural ganglia (CGs) of all 5 species showed staining; the details of this staining were unique for each species. In each CG, PDH staining was seen in: one large cell in C. borealis; 3 cells, one large, one medium and one small, in C. antennarius; 3-7 medium to large cells in P. clarkii; 1-3 small cells in H. americanus; and 13-17 small cells in P. interruptus. In the lobsters, each CG contained 2 qualitatively different neuropil regions. Fibers from one neuropil region pass into each superior oesopha-geal nerve and travel towards the STG. Lucifer yellow backfills of the inferior oesophageal nerve (ion) combined with PDH antibody staining revealed that the smallest PDH cell in each CG of C. antennarius sends a process through each ion and the oesophageal ganglion (OG) to the opposite CG; these cells may act as bilateral coordinators of CG motor function. None of the cells in the OG showed PDH staining in any of these species. In all instances PDH staining was blocked by preabsorption of the antibody with 100μM PDH; preabsorption with other peptides did not block staining. A PDH-like peptide may act as a neuromodulator of the motor rhythms of the stomatogastric nervous system.

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150.13

MODULATING RHYTHMIC MOTOR ACTIVITY WITH A PROCTOLIN- AND GABA-CONTAINING NEURON. M.P. Nusbaum[1,2], I. Cournil*[3], J. Golowasch[1] and E. Marder[1]. [1]Dept. Biol., Brandeis Univ., Waltham, MA 02254; [2]Dept. Biol., S.F. State Univ., San Francisco, CA 94132; [3]Lab. Neuro. Comparée, CNRS, 33120 Arcachon, France.

The modulatory proctolin-containing neuron (MPN) causes a state-dependent excitation of the pyloric rhythm in the crab stomatogastric ganglion (Nusbaum & Marder, J Neurosci, 9: In Press). Many MPN effects are mimicked by exogenously-applied proctolin. The intensity and time course of these proctolin effects are attenuated by amastatin-sensitive, aminopeptidase activity. One MPN effect that is not fully mimicked by proctolin is the short latency and long duration EPSPs produced by MPN in the LP and IC neurons.

MPN also exhibits GABA-like immunolabeling. Oesophageal ganglia containing Lucifer yellow-filled MPNs were fixed and sectioned. Alternate sections were processed for proctolin or GABA immunoreactivity. MPN showed both proctolin-like and GABA-like staining.

Focally-applied GABA (10^{-4}M) onto the STG neuropil causes short latency and short duration, mixed excitation and inhibition in LP and IC neurons. The $GABA_A$ agonist, muscimol (10^{-3} M), primarily excites these neurons. Depolarizing GABA responses were blocked by picrotoxin (PTX: 10^{-5} M). Hyperpolarizing responses were PTX resistant. In 10^{-4} M PTX, the early peak of the MPN-mediated EPSPs is reversibly reduced, suggesting a role for GABA. The remaining EPSP is likely to be proctolin-mediated. These ongoing studies will help determine how proctolin and GABA contribute to MPNs effects on the pyloric rhythm.

Supported by NS-17813.

150.14

IDENTIFICATION OF ALL GABA IMMUNOREACTIVE PROJECTIONS TO THE LOBSTER STOMATOGASTRIC GANGLION. I. Cournil*, P. Meyrand* and M. Moulins. Lab. Neuro . Phys. Comp., Université de Bordeaux I et CNRS, Place Peyneau, 33120 Arcachon - France.

The rhythmic motor patterns generated in the stomatogastric ganglion (STG) are controlled by modulatory neurons projecting to the STG via the stomatogastric nerve (stn). Recent pharmacological results have suggested that some of these inputs are GABAergic (Cazalets et al, 1987, J. Neurosci. 7: 2884-2893). We have recently identified in rostral ganglia 8 neurons which represent all GABA immunoreactive inputs to the STG. Using GABA antibody and PAP detection on serial paraffine sections, it was found that the stn contains 10 immunoreactive fibers that terminate in the STG neuropile. The cell body location of these fibers have been identified by combining neuroanatomical methods with immunodetection. (1) stn backfills with Lucifer Yellow (LY) were performed to detect double-labelled cell bodies. (2) stn backfills with $NiCl_2$ (which selectively inhibits GABA immunolabelling) permitted detection of GABAergic neurons which do not project to the STG (Cournil and Moulins, in preparation). Identification has been finally confirmed for 4 (of the 8) neurons with electrophysiological techniques coupled with intrasomatic LY injection and subsequent GABA immunodetection.

150.15

SCPb AND FMRFamide IMMUNOREACTIVITIES IN *HOMARUS AMERICANUS* NEURONS: COLOCALIZATION OF TWO PEPTIDES --OR-- COLABELING OF A SINGLE PEPTIDE? Z. Kvitash* and B.S. Beltz. Department of Biological Sciences, Wellesley College, Wellesley, MA. 02181.

Virtually all of the SCPb immunoreactive neurons (ca. 60 cells) in the lobster also contain FMRFamide-like immunoreactivity. Preadsorption controls revealed that staining with each antibody is successfully preadsorbed with its specific antigen, while the normal staining pattern is retained using each antibody following preadsorption with the alternate peptide --potentially leading to the conclusion that these two antibodies are labeling distinct compounds that are colocalized in lobster neurons.

The lobster nervous system does not, however, contain authentic FMRFamide, but rather several FMRFamide-like compounds (Trimmer et al, *J. Comp. Neurol.* 266:16, 1987). The most abundant of these is peptide F, an octapeptide with some sequence homology to FMRFamide. Further preadsorption controls demonstrated that SCPb immunoreactivity is completely preadsorbed by synthetic peptide F, while FMRFamide immunoreactivity is partially blocked by preadsorption with this peptide. Preadsorption controls with met-enkephalin-ARG-PHEamide (an extended opioid peptide containing the FMRFamide sequence) also completely preadsorbs SCPb immunoreactivity. These results indicate that the SCPb antibody can bind to extended forms of FMRFamide-like molecules, and that these antibodies may be colabeling one or more peptide F-like molecules in lobster neurons. (Supported by NSF BNS-8718938 and the Howard Hughes Medical Institute).

150.16

ACTIVATION OF MEMBRANE-BOUND GUANYLATE CYCLASE BY PEPTIDE G_1, A PUTATIVE HORMONE PURIFIED FROM A LOBSTER NEUROSECRETORY GLAND. Michael F. Goy* (SPON: K. Dunlap). Department of Physiology, University of North Carolina, Chapel Hill NC 27599-7545.

The regulation of cyclic GMP metabolism appears to be especially important to the American lobster. The enzymes that control synthesis and breakdown of cyclic GMP, as well as the phosphoproteins and kinase that are targeted by cyclic GMP, are found at relatively high concentrations in many lobster tissues. In an effort to define the hormonal systems that control cyclic GMP metabolism, extracts of lobster neurosecretory glands were screened for endogenous cyclic GMP-promoting agents. Several different peptides were discovered, the most abundant of which has been named peptide G_1.

Peptide G_1 has been purified to homogeneity by sequential anion exchange and reverse phase HPLC. It is a large, hydrophobic peptide (approximately 8,000 daltons) with an acidic isoelectric point. A partial amino acid sequence has been obtained from the purified material, providing more than 60% of the total sequence. Based on this information, peptide G_1 appears to be unique, with no homology to any previously-characterized peptide. It affects cyclic GMP metabolism in many different kinds of tissue. Among the most responsive targets are muscle and hepatopancreas. When tested on muscle homogenates, purified peptide G_1 selectively activates the membrane form of guanylate cyclase, with little or no effect on the soluble cyclase. It shares this property with only a few other peptides, the best studied of which is the vertebrate Atrial Natriuretic Factor.

150.17

LOCALIZATION OF SUBSTANCE P-,SEROTONIN-, AND DOPAMINE-LIKE IMMUNOREACTIVITY IN CENTRAL GANGLIA OF THE HORSESHOE CRAB. Y. McClain* and R.F. Newkirk, Tennessee State University, Nashville, TN 37209-1561.

Evidence has been reported which suggests that neuroactive peptide substance P, serotonin and dopamine may serve as neurotransmitters in the horseshoe crab. In this study we have used techniques of immunocytochemistry to localize these substances in the circumesophageal ganglia. Fixed tissue was cryostat sectioned and reacted with an antiserum against substance P, dopamine or serotonin and the antibody complex was visualized using an indirect method. The results showed specific substance P-like immunoreactivity (SPLI) in fiber-like structures in the neuropil. Neurons were apparent throughout the ganglia also. Serotonin-like immunoreactivity (SLI) had a pattern distinctly different from that of substance P. Of particular note were groups of neurons exhibiting substance P-like immunoreactivity; no similar group was noted in the case of serotonin. Additionally, fibers exhibiting SPLI were noted in the walking leg nerves whereas SLI tended to maintain a central orientation. Dopamine-like immunoreactivity (DLI) revealed a pattern distinctly different from serotonin also. These results suggest substance P, dopamine and serotonin are localized in neurons and nerve fibers in the circumesopohageal ganglia of the horseshoe crab.

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150.18

DETERMINATION OF ENDOGENOUS LEVELS AND LOCALIZATION OF SEROTONIN IN THE VENTRAL NERVE CORD OF LIMULUS POLYPHEMUS. B.S. McAdory* and R.F. Newkirk (SPON: S.M. Fredman). Tennessee State University, Nashville, TN 37209-1561.

The ventral nerve cord of Limulus polyphemus was studied using techniques of immunocytochemistry in an effort to determine the presence and localization of antiserotonin-like immunoreactivity. The chain of paraformeldehyde-fixed abdominal ganglia was exposed to a primary anti-serum (anti-5-HT) followed by treatment with a fluorescent labeled secondary antibody (FITC or rhodamine). The results revealed an extensive plexus of fibers and a pair of anteriorly positioned cell clusters consisting of six cells. A pair of posterior clusters of cells exhibiting less symmetry was also noted. Pretreatment of experimental animals with pargyline, colchicine or 5,7-dihydroxytryptamine (5,7-DHT) significantly enhanced visualization of cells and fibers. HPLC (with electrochemical detection) analysis confirmed the presence of endogenous serotonin in the abdominal ganglia of Limulus. These findings strongly suggest that serotonin is localized in cells and fibers of Limulus polyphemus where it may serve as a central neurotransmitter.

(Supported by NIH Grant #S06RR08092)

150.19

CHARACTERIZATION OF THREE FORMS OF PIGMENT-DISPERSING HORMONE FROM THE SHRIMP PANDALUS JORDANI. K. R. Rao, L. H. Kleinholz*+, and J. P. Riehm*. Dept. of Biology, Univ. of West Florida, Pensacola, FL 32514. + Dept. of Biology, Reed College, Portland, OR 97202.

Although crustacean pigment-dispersing hormone (PDH) occurs in multiple forms (Kleinholz, 1970, 1972), the structural basis of PDH heterogeneity in a given species remained unknown. Utilizing the previously described extraction methods (Fernlund, 1971) and chromatographic procedures (Rao et al., 1985), we have purified three forms of PDH from eyestalks of Pandalus jordani and determined their amino acid sequences. The deduced primary structures have been confirmed by chemical synthesis and by comparison of synthetic and native peptides in bioassays and HPLC. The sequences of these peptides are: NSELINSLLGLPKVMTDAamide, [Leu-8, Thr-16]-β- PDH; NSGMINSILGIPKVMADAamide, [Lys-13, Ala-16, Asp-17]-α-PDH; NSGMINSILGIPRVMTEAamide, α-PDH (identical to DRPH from Pandalus borealis). The β-PDH analog from P. jordani is as potent as β-PDH (Uca/Cancer PDH) in assays for melanophore pigment dispersion in Uca; these two peptides are about 20-fold more potent than α-PDH and 10-fold more active than the α-PDH analog. This indicates that several members of the PDH family, which show differences in relative potency and residue substitutions, account for PDH heterogeneity.

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150.20

RED PIGMENT CONCENTRATING HORMONE MODULATES THE CRAYFISH SWIMMERET RHYTHM. C. M. Sherff and B. Mulloney (SPON: H. Anderson). Dept. of Zoology, UC Davis, Davis, CA 95616.

While swimming, the crayfish beats its swimmerets with alternating power strokes and return strokes controlled by alternating bursts of power-stroke and return-stroke motor neurons. The crayfish can initiate and arrest this rhythm as well as alter the period and intensity of the beating. In isolated nerve cords, stimulation of different command neurons (Wiersma and Ikeda,*Comp. Biochem. Physiol.* 12:509-525, 1964) can activate or inhibit the swimmeret rhythm, but the neural mechanisms by which the animal varies its swimmeret rhythm over a wide range of frequencies and intensities have not been identified.

One transmitter that may be involved in this modulation is Red Pigment Concentrating Hormone (RPCH), a naturally occuring peptide in the crayfish nervous system. Perfusion with RPCH lengthened the period and the duration of the bursts in swimmeret motor neurons in a dose-dependent manner in both isolated nerve cords and semi-intact preparations. Perfusion of 10^{-6}M RPCH through the ventral artery led to changes in period and duration that were significant at the 95% confidence level.

Using an antibody raised against RPCH (gift of A. Madsen and R. Elde), we labeled three pairs of immunoreactive cell bodies in each abdominal ganglion and three pairs of immunoreactive interganglionic axons. These axons send branches into the lateral neuropil, regions that contain much of the circuitry for the generation of the swimmeret rhythm (Paul and Mulloney, *J. Neurophysiol.* 54:28-39). That these interneurons can modulate the swimmeret rhythm has not yet been determined.

150.21

A MAP OF NEURONS WITH HISTAMINE-LIKE IMMUNOREACTIVITY IN THE CRAYFISH NERVOUS SYSTEM. B. Mulloney and W.M. Hall. Zoology Dept., University of California, Davis, California 95616.

Histamine has been demonstrated biochemically to be the transmitter used by interneurons that modulate the stomatogastric ganglion (Claiborne & Selverston, 1984; Sigvardt & Mulloney, 1982), but the numbers and distribution in each segmental ganglion of neurons that use it as a transmitter have not been described. We used a well-characterized antibody to histamine (Panula et al., 1988) to map and to count neurons in the CNS that had histamine-like immunoreactivity (HLI).

To achieve good localization of HLI in these animals, we perfused the CNS for 20 min. through the sternal artery with a 4% EDCDI solution in crayfish saline that lacked Ca^{++}, followed by 4% formaldehyde in PBS. After removing the CNS from the animal, each ganglion was desheathed in 4% formaldehyde and left in that fixative over night. Ganglia were then washed, dehydrated to 70% EtOH, rehydrated, incubated in primary antiserum (1:150) for 24 hrs followed by secondary antiserum for 24 hrs.

Each Commissural Ganglion had one neuron brightly-labeled with HLI, and a dense labeling in its neuropil. The Stomatogastric Ganglion had labeled processes in its neuropil, but no labeled neuronal cell bodies.

The Subesophageal Ganglion and each Thoracic Ganglion had similar patterns of HLI; near the ventroposterior midline of each ganglion, one pair of brightly-labeled neurons were apparent. Each Abdominal Ganglion except the last had two pairs of neurons with HLI. The terminal ganglion had one pair. A few axons labeled brightly in each of the interganglionic connectives.

The antibody used was a gift from Dr. Panula (Helsinki).

151.1

THE GLUTAMATE ANTAGONIST,ϒ-D-GLUTAMYLGLYCINE, BLOCKS PERFORANT PATH STIMULATION-INDUCED INHIBITION OF DYNORPHIN SYNTHESIS IN RAT HIPPOCAMPUS. J.B. Daunais[1], C.W. Xie*, P.H.K. Lee, C.L. Mitchell,J.S. Hong, and J.F. McGinty[1]. [1]Dept. Anat. & Cell Biol. E. Carolina U. Sch. Med. Greenville, NC 27858 and LMIN, NIEHS/NIH, Res.Triangle Pk, NC 27709.

Unilateral stimulation of the perforant path (PPS) elicits wet dog shakes (WDS) and bilateral reductions in dynorphin A (1-8) immunoreactivity (DYN-IR) and prodynorphin mRNA (DYN mRNA) in rat hippocampus (see adjacent abstract). This study examined whether glutamate receptors mediate the above responses. In acute PPS experiments, the ventral hippocampus of rats was bilaterally injected with 25 μg/0.5 μl ϒ-D-glutamylglycine (DGG) or 0.5 μl of artificial CSF (ACSF) 10 min. before 4 consecutive PPS trials to establish the threshold for eliciting WDS. The rats were killed imediately after the last trial to determine DYN-IR in the hippocampus. The ACSF+PPS group had a mean of 73±4 WDS at a stimulus intensity of 0.46±0.03 mA as compared with significantly fewer WDS (x= 45±6, p<0.01) elicited at a significantly higher threshold (0.78±0.05, p< 0.001) in the DGG+PPS group. DYN-IR in the ACSF+PPS group was decreased by more than 40% in both dorsal and ventral hippocampus vs.sham controls. In contrast, the PPS-induced reduction in DYN-IR was antagonized by DGG in ventral but not dorsal hippocampus. In daily PPS experiments, rats received a daily unilateral injection of DGG or ACSF 20 min. prior to PPS delivered once per day for 6 days. In situ hybridization using a S^{35}-labeled oligonucleotide, demonstrated that the extent of decrease in DYN mRNA in dentate granule cells positively correlated with the number of WDS elicited by PPS. In rats treated with DGG before each PPS, no decrease in DYN mRNA was observed even in rats which experienced stage 3-4 seizures. These data indicate that DGG inhibits PPS -stimulated WDS and depletion of DYN-IR and mRNA in dentate granule cells. Supported in part by DA 03982 (JFM).

151.2

DIFFERENTIAL MODULATION OF STRIATONIGRAL DYNORPHIN AND ENKEPHALIN BY DOPAMINE RECEPTOR SUBTYPES. H.K. Jiang*, J.F. McGinty and J.S. Hong. Tri-Service Gen. Hosp., Taipei, Taiwan; Dept. of Anatomy, East Carolina Univ., Greenville, NC; & LMIN, NIEHS/NIH, RTP, NC.

The purpose of this study was to determine which dopamine (DA) receptor subtype(s) mediate the modulatory actions of DA on dynorphin (DYN) and enkephalin (ENK) in the striatonigral regions. Intact or unilaterally 6-hydroxydopamine (6-OHDA) lesioned rats were injected twice daily for seven days with the following compounds either separately or combined: D-1 agonist (SKF-38393, 5 mg/kg), D-2 agonist (LY-171555, 1 mg/kg), D-1 antagonist (SCH-23390, 0.05 mg/kg) and D-2 antagonist (sulpiride, 100 mg/kg). Sixteen hr after the last injection, levels of DYN and ENK in the striatum and substantia nigra were dertermined by RIA.The results indicate that D-1 receptors mediate excitatory control over the biosynthesis of striatonigral DYN and also exert a tonic inhibitory influence on the ENK system. Furthermore, stimulation of the D-2 subtype imposes an excitatory stimulus on ENK biosynthesis but has no effect on DYN.

151.3

MODULATION OF STRIATAL PREPROENKEPHALIN MRNA LEVELS BY THE D2 DOPAMINE RECEPTOR. **A.E. Pollack and G.F. Wooten. Dept. of Neurology, U.Va., Charlottesville, Va. 22908.**

Striatal preproenkephalin (PPE) mRNA levels increase ipsilaterally to a 6-hydroxydopamine (6-OHDA) lesion of the substantia nigra pars compacta both 5 days (d) (Eur. J. Pharm., 130:341, 1986) and 14 d post lesion (dpl) (PNAS 83:9827, 1986). We examined whether administration of quinpirole, a D2 agonist, could attenuate this elevation in striatal PPE mRNA ipsilateral to a nigral lesion. Rats received a unilateral sterotaxic injection of 6-OHDA (2 μl, 4 mg/ml) and their striata processed for RNA extraction. PPE mRNA of ipsi- and contralateral striata were compared by dot blot hybridization with a ^{32}P-labeled 30mer oligonucleotide probe complementary to PPE mRNA. Striatal PPE mRNA ipsilateral to the lesion increased 87% at 7 dpl and 25% at 14 dpl compared to the contralateral striatum. Rats killed 8 dpl that received quinpirole (1 mg/kg/2x day, s.c.) for 7 d demonstrated only a 47% increase in ipsilateral striatal PPE mRNA which was different from the untreated 7 dpl rats (p<0.01). In contrast, 14 dpl rats treated with quinpirole (5 mg/kg/2x day, s.c.) for the last 8 d demonstrated a 38% increase in ipsilateral striatal PPE mRNA which did not differ from controls. These results suggest that occupation of the D2 receptor for 7 d with quinpirole beginning 1 d following a 6-OHDA lesion attenuated the effect of dopamine denervation on striatal PPE mRNA levels, whereas quinpirole treatment for 8 d beginning 6 d following a 6-OHDA did not. This evidence suggests that striatal D2 dopamine receptor occupation regulates PPE gene expression.

151.4

PLASMA HYDROLYSIS OF [LEU]- AND [MET]ENKEPHALIN IN THE MOUSE. S. Shibanoki*[1], S.B. Weinberger[2], G. Schulteis[2], K. Ishikawa*[1], and J.L. Martinez, Jr.[2]. (SPON: C. Bakhit). [1]Dept. Pharmacol., Sch. of Med., Nihon Univ., Tokyo 173, JAPAN; [2]Dept. Psychol., Univ. of Calif., Berkeley, CA 94720.

Using HPLC-ECD, we determined the enzymatic hydrolysis of [leu]enkephalin (LE) and [met]enkephalin (ME) in mouse plasma in vitro by measuring accumulation of Tyr, Tyr-Gly (TG), and Tyr-Gly-Gly (TGG). Hydrolysis of both enkephalins yielded all three metabolites, although only very small quantities of TG were formed from either substrate. Hydrolysis of ME led to greater production of Tyr and TGG than did hydrolysis of LE. LE was hydrolyzed more slowly than was ME; the half-lives of the two enkephalins were 14 and 5 mins, respectively. By contrast, LE and ME have similar half-lives (1.5 mins) in the rat. TGG formation from both enkephalins was reduced approximately 90% by thiorphan (100 μM), an "enkephalinase" inhibitor. Captopril (100 μM), an angiotensin converting enzyme inhibitor, was less effective than thiorphan at reducing TGG formation. In addition, captopril produced a larger reduction in LE than in ME formation of TGG. Although "enkephalinase" does not hydrolyze LE in rat plasma (J. Pharmacol. Exp. Therap. 247, 129, 1988), this enzyme may be involved in the hydrolysis of LE and ME in mouse plasma.

(Supported by NIDA #DA04195, #DA04795, and #DA05334)

151.5

PLASMA HYDROLYSIS OF [LEU]- AND [MET]ENKEPHALIN IN THE RAT. S.B. Weinberger[1], S. Shibanoki*[2], K. Ishikawa*[2], and J.L. Martinez, Jr.[1]. [1]Dept. Psychol., Univ. of Calif., Berkeley, CA 94720; [2]Dept. Pharmacol., Sch. of Med., Nihon Univ., Tokyo 173, JAPAN.

Hydrolysis of [leu]enkephalin (LE) and [met]enkephalin (ME) in rat plasma in vitro was determined using HPLC-ECD to measure Tyr, Tyr-Gly (TG), and Tyr-Gly-Gly (TGG) formation. LE and ME were rapidly hydrolyzed to form Tyr, TG, and TGG, and the half-lives of both enkephalins were approximately 1.5 min. Bestatin (500 μM), an inhibitor of aminopeptidase M, completely blocked formation of Tyr from both LE and ME. Puromycin, an inhibitor of aminopeptidase MII, only reduced Tyr accumulation at a concentration (5 mM) that also reduced TG and TGG formation, suggesting that puromycin was not selective at this concentration and that aminopeptidase MII does not participate in enkephalin hydrolysis in rat plasma. Although LE and ME did not differ in Tyr or TG formation, the formation of TGG from ME hydrolysis was greater than that from LE hydrolysis. Captopril (100 μM), an angiotensin converting enzyme (ACE) inhibitor, reduced TGG formation from both enkephalins, without reducing TG levels. These results suggest that similar enzymes are involved in the hydrolysis of LE and ME in rat plasma, although ACE plays a larger role in the hydrolysis of ME than it does in the hydrolysis of LE. (Supported by NIDA #DA04195 and #DA04795)

151.6

PLASMA HYDROLYSIS OF [LEU]ENKEPHALIN (LE) IN THE CHICK. G. Schulteis, K.A. Nudelman*, E.L. Bennett, M.R. Rosenzweig & J.L. Martinez, Jr. Dept. of Psychology, University of California, Berkeley, CA, 94720.

Prior to initiating studies on the effects of peripherally administered LE on learning and memory in chicks, we measured hydrolysis of ^{3}H-LE in plasma obtained from trunk blood of 2-day-old chicks. TLC was used to separate LE from its metabolites (Behav Neurosci, 102:404, 1988). LE was hydrolyzed with a 1/2-life of 1 min. The aminopeptidase M (Amino M) inhibitor bestatin (1.5 mM) inhibited 95-97% of LE hydrolysis. Either captopril (0.1-1 mM), an inhibitor of angiotensin converting enzyme (ACE), or thiorphan (1mM), an inhibitor of "enkephalinase" ("ENKase"), in combination with bestatin (1.5 mM) inhibited 10% of the remaining LE hydrolysis (<1% of total LE hydrolysis). The Amino MII inhibitor puromycin (5 mM) reduced LE hydrolysis 48-62%; the combination of puromycin (5 mM) and bestatin (1.5 mM) was no more effective in inhibiting LE hydrolysis than bestatin alone, suggesting a nonspecific action of puromycin on Amino M. In chick plasma, LE is degraded almost entirely by Amino M, ACE and "ENKase" activities account for <1% of LE hydrolysis, and Amino MII plays no detectable role in LE hydrolysis. This pattern of enzymatic LE hydrolysis differs from that seen in rat or mouse plasma (Shibanoki et al., Weinberger et al., this volume). (Supported by DA 04795, DA 04195, & DA 05334)

151.7

UPTAKE AND METABOLISM OF ^{3}H-[LEU]ENKEPHALIN AFTER EITHER I.P. OR S.C. INJECTION IN MICE. P.H. Janak, G. Schulteis, & J.L. Martinez, Jr. Dept. of Psychology, University of California, Berkeley, CA 94720.

In vitro studies of [leu]enkephalin (LE) hydrolysis in mouse plasma showed that LE has a half-life of 9.3 min. In order to characterize LE hydrolysis in vivo, mice received either intraperitoneal (IP) or subcutaneous (SC) injections of 30 µg/kg ^{3}H-LE. Trunk blood was collected by decapitation at 1,2,5,7.5,10,20 min after peptide administration. Total amount of ^{3}H was measured in 40 µl of each sample; the percent of LE hydrolysis was determined in another 40 ul of each sample using TLC to separate LE from its metabolites. Plasma uptake of ^{3}H differed between IP and SC injections, peaking at 5 min after IP, and 10 min after SC injection. By 1 min after IP injection, 95% of the ^{3}H in plasma was in the form of LE metabolites, while following SC injection the 95% metabolism level was not reached until 5 min postinjection. These hydrolysis rates are faster than those determined for mouse plasma in vitro and are similar to LE hydrolysis rates measured in the rat in vivo. These results are important for the interpretation of studies examining the behavioral effects produced by peripheral injection of LE. (Spon. by NIDA # DA 04195 and DA 05334)

151.8

CONFORMATIONAL CHARACTERISTICS OF PROENKEPHALIN PEPTIDES E, B, AND F. H.J. Hiddinga*, G.E. Katzenstein*, C.R. Middaugh*, and R.V. Lewis. Dept. of Molecular Biology, Univ. of Wyoming, Laramie, WY 82071.

The conformations of three adrenal medulla enkephalin containing polypeptides (ECPs) were investigated to gain an understanding of their potential structure-activity relationship. Secondary structure characteristics of peptides E, B, and F were examined by circular dichroism (CD) under conditions designed to mimic both their soluble state and the anisotropic environment which exists at the biological effector site. The conformational differences between the three peptides was further determined by Fourier Transform Infrared Spectroscopy (FTIR). Although all three peptides have similar structure and apparently exist in random configurations in aqueous solutions, they exhibit the potential to assume conformations which are unique to the individual peptide. The conformational differences may be important factors in determining their unique biological activities.

151.9

ROLE OF ARG 747 AND ARG 102 IN THE ACTIVE SITE OF NEUTRAL ENDOPEPTIDASE-24.11 (ENKEPHALINASE) INVESTIGATED BY SITE-DIRECTED MUTAGENESIS. A. BEAUMONT*, T. BENCHETRIT*, H. LE MOUAL*†, G. BOILEAU*†, P. CRINE*† and B.P. ROQUES*. (SPON: J.J. Vanderhaeghen). U 266 INSERM, UA 498 CNRS, UER des Sciences Pharmaceutiques, 4 av. de l'Observatoire, 75006 Paris, France. † Lab. Biochimie, Univ. Montreal, Québec H3C 3J7.

Neutral endopeptidase 24.11 (NEP) is a Zn-ectoenzyme which, in the central nervous system, participates in the inactivation of the opioid peptides, methionine and leucine enkephalin. Chemical modifying agents have been used to show the presence of an arginine residue(s) at the active-site of NEP, thought to participate in substrate binding, and Arg747 and Arg 102 have both been proposed to fulfill this role (Benchetrit et al., Biochemistry 27, 5921, 1987, L. Hersh, personal communication).

Met 747 NEP and Met 102 NEP have now been synthesized by site-directed mutagenesis and their activity compared to that of the wild-type enzyme. The Km of the substrate [^{3}H]-D-Ala^{2}-leucine enkephalin was increased for both enzymes as were the IC_{50}s of several NEP inhibitors. The activity of Met 102 NEP however was still affected by the arginine-modifying reagent butanedione, whilst that of Met 747 NEP was unchanged. It is suggested that both of these residues play a role at the active site of NEP.

151.10

A SOLUTION HYBRIDIZATION (SH) METHOD FOR THE QUANTITATION OF RAT PREPROENKEPHALIN mRNA (PPmRNA). Y.S. Zhu*, A.D. Branch*, H.D. Robertson*, T.H. Huang*, S.O. Franklin* and C.E. Inturrisi. Cornell Univ. Med. Coll. and The Rockefeller Univ., New York, N.Y. 10021.

To facilitate study of the regulation of opioid peptide gene expression, a SH method was developed for rapidly quantitating PPmRNA. A 32P-labeled riboprobe for rat PPmRNA was prepared from the pYSEA1 template and purified by use of a CF11 column. This riboprobe hybridized to a single 1.5 kb band using Northern blot analysis of total cellular RNA. For SH the labeled riboprobe is added to a tissue extract containing the mRNA of interest; unhybridized riboprobe is degraded with RNases; protected hybrids are collected by filtration and counted. A PPmRNA sense transcript is used as a calibration standard. Optimal hybridization conditions (16 hrs at 75°C) were defined and did not differ for the calibration standard and the PPmRNA of rat striatum. Both fully protect the 0.97 kb riboprobe. The hybridization conditions produced a linear curve ranging from 1.25 to 160 pg. PPmRNA was measured in total cellular RNA extracts from individual rat tissues that express PPmRNA and in explants of rat adrenal medullae. These results demonstrate the sensitivity and utility of this SH assay and its application to multi-sample analysis. (Supported in part by USPHS.NIDA Grants DA-01457 and DA-05130.)

151.11

POSTTRANSLATIONAL PROCESSING OF PROENKEPHALIN IN A HUMAN NEUROBLASTOMA CELL LINE, SK-N-MC. I.Lindberg and E.Shaw* Dept. of Biochemistry & Mol. Biol.,L.S.U. Med. Ctr., New Orleans, LA 70112

Several groups have recently shown that the SK-N-MC cell line contains enkephalins at concnetrations greater than any cell line yet described. We have analyzed the biosynthesis of enkephalins in this cell line and confirmed this finding. However, enkephalin biosynthesis in this cell line appears to be unusual in several respects. Firstly, these cells store an extremely small proportion of their enkephalins; more than 95% of total enkephalin is detected in the medium rather than in the cells after a two day incubation. Secondly, gel filtration of media samples in combination with radioimmunoassays for all of the penta- to octapeptide enkephalins revealed the presence of only two major immunoreactive species, namely peptides with elution positions corresponding to proenkephalin (2/3 of total) and Peptide B (1/3 total). Only trace quantities of other proenkephalin-derived peptides were observed. SK-N-MC cells thus apparently carry out only limited cleavage of proenkephalin. We also examined the potential phosphorylation and glycosylation of proenkephalin by incubation of cells with labelled inorganic phosphate and methionine followed by immunoprecipitation with Peptide B anitserum. These isotopes were incorporated into 34-38 kDa cellular and secreted polypeptides. The SK-N-MC cell line therefore represents an interesting system for the study of early processing events in enkephalin biosynthesis.

151.12

REGULATION OF CLEAVAGE OF THE N-TERMINUS OF BOVINE PRO-OPIOMELANOCORTIN BY GLYCOSYLATION. N.P. Birch*[1], H.P.J. Bennett*[2], F. Estivariz*[3], P.J. Lowry*[3] and Y.P. Loh[1]. [1]Lab. Dev. Neurobiol., NICHD, NIH, Bethesda, MD 20892, [2] Endo. Lab., Royal Victoria Hosp. and Depart. of Med., McGill University, Montreal, Quebec, Canada,[3] Dept. of Physiol. and Biochem., Univ. of Reading, Reading, England.

The amino terminus of bovine pro-opiomelanocortin (N-POMC1-77) is partially processed in the intermediate lobe of the pituitary to N-POMC1-49 and Lys-γ_3MSH. Seger and Bennett (*J. Steroid Biochem* 25:703, 1986) purified two species of N-POMC1-77 which were either glycosylated or non-glycosylated at Thr45. However, only non-glycosylated N-$POMC_{1-49}$ was detected leading to the proposal that differential O-linked glycosylation of N-POMC1-77 may regulate cleavage at the Arg49-Lys50 processing site. To test this hypothesis purified bovine intermediate lobe N-POMC1-77 enriched in either the O-linked or non-O-linked form was incubated with a purified bovine aspartic protease (POMC converting enzyme) which has previously been shown to cleave POMC at paired basic residues (Loh, Y.P. et al. *J. Biol. Chem* 260:7194, 1985) Analysis of the cleavage products using a γ_3MSH radioimmunoassay showed a 6- to 8-fold greater increase in generation of γ_3MSH immunoreactivity using the non-O-glycosylated substrate compared with the O-glycosylated form. High performance liquid chromotagraphic analysis demonstrated that this material co-migrated with Lys-γ_3MSH, indicating that cleavage occurred between the pair of basic amino acids. These data support a role for differential O-linked glycosylation in the regulation of processing of the N-terminus of bovine POMC.

151.13

THE ONSET OF PROOPIOMELANOCORTIN (POMC) PROCESSING AND IDENTIFICATION OF CLEAVED PRODUCTS IN DEVELOPING MOUSE EMBRYOS. R.A.RIUS*, T.CHIKUMA* and Y.P.LOH (SPON: J.S.GUTKIND). Lab. of Dev. Neurobiol., NICHD, Bethesda, MD 20892.

Previous findings using in situ hybridization have indicated that POMC mRNA expression during mouse development begins at embryonic day (E) 10.5; at the same time, immunocytochemical analysis revealed the presence of POMC-translated products in the CNS. Expression of POMC mRNA was seen in anterior pituitary at E12.5 and greatly increased by E14.5. In the intermediate lobe, POMC mRNA was first detected at E14.5 (Elkabes, S., Dev. Brain Res. 46:85, 1989). The aim of this study was to follow the post-translational modification of POMC during prenatal development. Embryos were obtained from pregnant mice at different gestational days and extracted for analysis of POMC-related materials by SDS-PAGE and h.p.l.c., followed by radioimmunoassay using several antibodies. Analysis with SDS-PAGE indicated that at E10.5 only POMC was present. Processed products were first seen at E11.5 although POMC was also present. By E12.5 only processed products were detectable. H.p.l.c. analysis of ACTH/MSH products present at E11.5 revealed the presence of ACTH 1-39, des-acetyl α-MSH and ACTH 1-14 but no α-MSH. A similar pattern of processed products were also observed at E12.5 except that the amounts were increased. By E14.5 there was a large increase in ACTH 1-39 consistent with the proliferation of anterior pituitary lobe cells seen at this stage. This study indicate that during development there is a time lag between POMC biosynthesis and processing. Proteolytic cleavage of ACTH 1-39 to ACTH 1-14 and formation of des acetyl α-MSH appear to occur as soon as POMC processing begins.

151.14

REGULATION OF OPIOID PEPTIDES AND NEUROTENSIN IN THE RAT BRAIN. M. E. Abood*, J. Eberwine*, E. Erdelyi*, J. D. Barchas and C. J. Evans, Nancy Pritzker Laboratory, Department of Psychiatry, Stanford University, Stanford, CA 94305.

Dopaminergic antagonists increase the levels of opioid peptides in the rat brain striatum and neurotensin in the nucleus accumbens and striatum. We were interested in determining the effect of dopaminergic agonists as well as antagonists on opioid and neurotensin peptide gene expression in these areas. In order to facilitate these studies, we have developed a method to measure peptides and mRNA from the same tissue extracts. Initially, we determined the effects of haloperidol and bromocriptine on preproenkephalin mRNA and total opioid peptides (as measured by an immunoassay directed to the N-terminus of all endogenous opioid peptides) in various rat tissues. We saw significant changes in striatum only. Subsequently, we examined the effect of a selective D1 antagonist, SCH23390, on opioid peptide gene expression in rat brain striatum. We have developed an immunoassay that allows us to measure neurotensin in extremely low concentrations in tissues, and have examined the regulation of neurotensin levels in striata of rats treated with dopaminergic drugs. In addition, we are examining the effects of these drugs on neurotensin mRNA levels in rat brain.

PAIN MODULATION: SPINAL OPIOID MECHANISMS

152.1

THE SPINAL ANALGESIC PROPERTIES OF THE MU AGONIST, DAMPGO, IN ACUTE AND CHRONIC PAIN. L.J. Spanos, J. Stafinsky and T. Crisp. Dept. of Pharmacology, N.E. Ohio Univ. Coll. of Med., Rootstown, Ohio 44272.

The present study: (1) evaluated the validity of computerized activity analysis to determine the spinal analgesic efficacy of the mu receptor-selective agonist, DAMPGO, in arthritic rats and (2) compared tolerance development to the analgesic effects of DAMPGO after repeated intrathecal (i.t) injections in arthritic (AA) and non-arthritic (NA) rats using the tail-flick assay (TFL). Male Sprague-Dawley rats (340-400 g) were cannulated with indwelling PE-10 catheters for i.t. drug injections, and were inoculated with Mycobacteriacum tuberculosis in Freund's adjuvant to induce arthritis. NA control animals were inoculated with incomplete adjuvant. Daily i.t. injections of DAMPGO (0.3 nmol/10μl) were administered to animals from day 7 to day 15 post-inoculation. Drug-treated AA rats displayed increases in locomotor activity when compared to saline-treated AA controls ($p<.05$). TFL scores in response to DAMPGO revealed that NA rats developed tolerance by the 12th injection while AA rats did not show significant tolerance development until the 14th injection of the drug.

152.2

LABELLING OF THE PROJECTION OF THIN AFFERENT FIBERS FROM A SINGLE DORSAL ROOT IN THE SUPERFICIAL LAYERS OF THE RAT DORSAL HORN USING A HIGHLY MU-SELECTIVE OPIOID LIGAND. D. BESSE, M.C. LOMBARD, J.M. ZAJAC, B.P. ROQUES and J.M. BESSON. INSERM U161 and EPHE 2, rue d'Alésia 75014 Paris and INSERM U266 4, av de l'Observatoire 75006 Paris.

Radioautographical and biochemical studies have demonstrated that opioid receptors are, to a large extent, located on thin primary afferent fibers. We have postulated that opioid binding sites could be putative markers to visualize and to quantify the distribution of thin afferent fibers in the rat superficial dorsal horn (laminae I and II). We labelled μ binding sites with (^{3}H)DAGO (3nM) and used a quantitative radioautographic technique. Previously, using the same ligand, we found that in the C7 segment, 76±1% of the total μ binding sites were located presynaptically. The extent of the projections of the C7 root in the spinal segments was evaluated by comparing the binding values in 4 experimental situations: intact rats and rats submitted to a variety of rhizotomies (C4-Th2, C4-Th2 sparing C7 and C7 alone). 8 days after the lesion, the projections of the C7 root, measured every 120 μm over 7 segments were as follows: 4% in C4, 8% in C5, 21% in C6, 42% in C7, 13% in C8, 8% in Th1 and 4% in Th2 segment. These measurements are highly reliable since similar results were obtained when comparing the paired lesions C4-Th2/C4-Th2 sparing C7 and intact/C7 alone. Results will be presented for 1, 2 weeks, 1, 3 months after the lesion.

152.3

INVOLVEMENT OF THE MU-OPIATE RECEPTOR IN PERIPHERAL ANALGESIA. Y.O. Taiwo* and J.D. Levine*. (SPON: T.J. Coderre) Dept. of Medicine, University of California, San Francisco CA 94143

Analgesic effects of opiates injected directly into injured and inflamed tissue suggest that they act at a peripheral as well as a central site. In this study we have evaluated their effect on the hyperalgesia induced by the inflammatory mediator prostaglandin E_2 (PGE_2). The intradermal injection of mu (morphine, DAGO and morphiceptin), kappa (U-50,488H) and delta (DPDPE and DSLET) selective opioid-agonists (10ng-10ug), by themselves, did not significantly affect the mechanical nociceptive threshold in the hindpaw of the rat. Intradermal injection of mu, but not delta or kappa opioid-agonists, however, produced dose-dependent inhibition of PGE_2 (1ng-1ug)-induced hyperalgesia. The analgesic effect of the mu-agonist morphine was dose-dependently antagonized by naloxone (10ng-10ug) and prevented by co-injection of pertussis toxin (1ug). Morphine did not, however, alter the hyperalgesia induced by 8-bromo cAMP (10ng-10ug). We conclude that the analgesic action of opioids on the peripheral terminals of primary afferents is via a binding site with characteristics of the mu-opioid receptor and that this action is mediated by inhibition of the cAMP second messenger system.

152.4

TOLERANCE TO THE ANTINOCICEPTIVE EFFECT OF INTRATHECAL MORPHINE IN INTACT AND CHRONIC SPINAL RATS. **C. Advokat, D. Flershem* and J. Siuciak. Dept. of Pharmacology, University of Illinois, College of Medicine, Chicago, IL. 60612.**

The antinociceptive effect of acute, daily intrathecal morphine injections on the tail flick (TF) withdrawal response was compared in intact rats and rats that were spinally transected for 20-30 days (chronic spinal rats). There was no difference in the initial antinociceptive response of intact and chronic spinal rats to acute intrathecal injections of either 5 μg, 15 μg or 30 μg of morphine. During four successive days of administration the response of chronic spinal rats declined significantly (became tolerant) to each of the three doses of intrathecal morphine. Intact rats did not become tolerant to these treatments. Spinal rats, made tolerant to repeated intrathecal morphine injections were not "cross-tolerant" to a subcutaneous injection of 6.0 mg/kg of morphine. The difference between intact and chronic spinal rats in the development of tolerance to spinal morphine administration suggests that supraspinal mechanisms mediate the development of tolerance to morphine-induced antinociception in the spinal cord.

152.5

ADENOSINE RELEASE FROM THE SPINAL CORD MAY MEDIATE ANTINOCICEPTION BY INTRACEREBROVENTRICULAR MORPHINE. M.I. Sweeney, T.D. White and J. Sawynok. Dept. of Pharmacology, Dalhousie University, Halifax, N. S., Canada. B3H 4H7.

Adenosine release from the spinal cord may mediate antinociception produced by intrathecal (i.t.) morphine (JPET 243:657, 1987). Recently it has been shown that antinociception produced by intracerebroventricular (i.c.v.) morphine is blocked by i.t. theophylline, suggesting that adenosine release also may be involved in antinociception by supraspinal morphine (JPET 239:88, 1986). In this study we have examined this hypothesis directly. For behavioral tests, drugs were injected via chronically implanted i.t. or i.c.v. cannulas in rats, and nociceptive latencies measured using the tail-flick and hotplate tests. In release studies, the i.t. space was perfused with artificial csf, and perfusates assayed for adenosine content using HPLC. Morphine 30μg i.c.v. produced antinociception in the tail-flick and hotplate tests which was inhibited by i.t. 8-phenyltheophylline 0.3μg, 3μg, and theophylline 5μg. However, the effect of theophylline was biphasic, since 50μg potentiated this action of morphine. Morphine 30μg i.c.v. released adenosine from the intact spinal cord in 5 of 8 rats. These data suggest that activation of descending systems by i.c.v. morphine produces an increase in the release of adenosine from the spinal cord which may contribute to antinociception. (Supported by MRC of Canada).

152.6

Spinal opioid effects are enhanced in a model of unilateral inflammation. J.L.K.Hylden, G.M.Herrera* and R. Dubner. Neuorbiology & Anesthesiology Branch, NIDR, NIH, Bethesda, MD. 20892.

The present work addresses the possibility that unilateral carrageenan (CARRA)-induced inflammation/hyperalgesia, and its concomitant alterations in spinal cord opioid peptides, is associated with an increase in the analgesic efficacy of opioid agonists at the spinal level. Opioid agonists were administered via intrathecal (i.t.) catheters to rats at 3 or 24 h after CARRA (2-6 mg in 0.2 ml saline, s.c. into the plantar surface). Rats were tested for cutaneous hyperalgesia or analgesia by measuring the paw withdrawal latency to a thermal stimulus. The dose-response curves of opioid agonists with mu- and/or delta-receptor selectivity (DADL, 0.3-3 μg; DAGO, 3.3-100 ng; DPDPE, 5-50 μg) were shifted to the left in rats with inflamed hindpaws, when compared to control. This enhanced analgesic effect was only observed in inflamed paws at the time of maximal inflammation (3 h) and was not apparent after the inflammation had resolved (24 h). The kappa agonist U50,488H (10-100 μg) was not analgesic in this assay. The opioid antagonist naloxone (10 μg, i.t.) failed to alter paw withdrawal latencies of either inflamed or non-inflamed paws. The long-term systemic antagonist naltrexone (5-10 mg/kg, s.c.) likewise had no effect on the hyperalgesic response of inflamed paws, but significantly decreased the withdrawal latencies of contralateral, uninflamed paws. The data show that mu- and delta-opioid agonists have enhanced analgesic efficacy at the spinal level during conditions of inflammation.

152.7

OPIOID BINDING IN RAT SPINAL CORD: EFFECTS OF EXCITATORY AMINO ACIDS. L. Lichtblau, S. Lei and G. L. Wilcox, Department of Pharmacology, University of Minnesota, Minneapolis, MN 55455.

Opioid agonists act within the spinal cord to produce analgesic effects, some of which involve selective interactions with excitatory amino acids. The properties of opioid binding sites in membranes from rat spinal cord were examined at physiological temperature and ion concentrations, and the susceptibility of this binding evaluated for modulation by excitatory amino acid agonists.

Adult male Sprague-Dawley rats were decapitated and their spinal cords removed to ice. The tissue was homogenized with a Dounce Homogenizer and centrifuged at 1000 x g for 10 min at 4°C. The supernatant was then centrifuged at 100,000 x g for 60 min at 4°C. The resulting membrane pellet was resuspended in 10 volumes of buffer and stored frozen at -70°C. Each assay tube (1 ml) contained approximately 500 μg membrane protein. [^{3}H]-naltrexone (NTX; 13.66 Ci/mmol) binding was carried out in a Krebs-HEPES buffer (pH 7.4 at 37°C) for 25 min. Saturation and competition data were analyzed by computer.

Under the above conditions, the apparent dissociation constant (K_d) of [^{3}H]-NTX was 0.77nM and the number of binding sites (B_{max}) was 52 fmol/mg protein. These data are comparable to other studies in mouse brain and spinal cord, but the binding conditions allow manipulations of agonist binding by other ligands. Competition for binding by various ligands (see table below) suggest that [^{3}H]-NTX binding in rat spinal cord under physiological conditions occurs with greatest affinity to μ receptors.

Ligand	Receptor	K_i (nM)
Naloxone	μ,κ,δ	1.4
[D-Ala^2, N-Me-Phe^4, Gly^5-ol]-enk (DAMGO)	μ	22.9
U50,488	κ	3007
[D-Pen^2, D-Pen^5]-enkephalin (DPDPE)	δ	5500

Further analysis suggests that DAMGO displaces NTX with two distinct affinities. NMDA but not AMPA increased the proportion of binding sites in the high affinity state.

(Supported by USPHS grants DA-01933 and DA-04274).

152.8

OPIOID INHIBITION OF EXCITATORY AMINO ACID (EAA) AGONIST-INDUCED EXCITATION OF RAT SPINAL PROJECTION NEURONS. S. Lei and G. L. Wilcox, Dept Pharmacology, Univ Minnesota, Minneapolis, MN 55455.

We have previously observed excitation of spinal nociceptive neurons by N-methyl-D-aspartate (NMDA) and the quisqualate receptor agonist, (RS)-alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid HBr, (AMPA), using electrophysiological techniques. The μ opioid agonist, [D-Ala^2, N-Me-Phe^4, Gly^5-ol]-enk (DAMGO) more uniformly inhibited NMDA-induced excitation than did morphine. The present study tested DAMGO for inhibition of EAA-induced excitation and the effects of EAA agonists on responses to natural peripheral stimulation.

Extracellular recordings were conducted *in situ* in the spinal cord of urethane-anesthetized male rats. 1.5 MΩ tungsten microelectrodes glued to seven barreled borosilicate glass microelectrodes were used to record from single spinal neurons and to apply various drugs iontophoretically. Thirteen of 22 cells tested dose-dependently responded to iontophoretically administered NMDA. DAMGO currents, superimposed on repeated NMDA pulses, inhibited NMDA-induced excitation in 8 of 11 neurons tested. Eleven of 18 cells tested showed increasing firing rate upon the ejection of AMPA, but AMPA-elicited excitation was reduced by DAMGO in relatively few cells (2 of 6). Four cells (all WDR) which responded to both NMDA and AMPA, were tested with DAMGO. Although DAMGO altered NMDA-elicited firing in all the cells, none of the cells' responses to AMPA was inhibited by DAMGO.

Subthreshold currents of NMDA increased peripheral stimulation-induced firing in six of eleven neurons tested. Subthreshold currents of AMPA increased stimulation-induced firing in 3 of 5 neurons tested. Subthreshold currents of iontophoretically applied NMDA greatly increased noxious thermal stimulation-evoked firing (50°C).

This evidence is in agreement with previous behavioral studies DAMGO to inhibit NMDA-elicited effects more selectively than AMPA-elicited effects. Thus, μ opioid agonists may selectively block NMDA receptor-mediated responses. That both NMDA and AMPA at subthreshold doses can increase responses to noxious peripheral stimulation supports the hypothesis that EAAs act as nociceptive neurotransmitters in the mammalian spinal cord and that NMDA and AMPA receptors have an important role in mediating spinal nociceptive transmission.

(Supported by USPHS grants DA-01933 and DA-04274).

152.9

Differential tolerance between the spinal mu opioid agonists morphine and sufentanil, correlation with drug efficacy. *Sosnowski, M., Yaksh, T.L. (SPON P. Wilson). Dept of Anesthesiol, Univ. Calif. San Diego, La Jolla, CA 92093.

Chronic exposure of spinal opioid receptors to an intrathecal (IT) agonist results in a progressive loss of effect (tolerance). Speculatively, the degree of shift of the agonist dose response curve should be proportional to the magnitude of receptor down regulation and this in turn inversely related to the size of the spare receptor population. In previous work with the non-competitive mu receptor ligand, βFNA, we showed that for spare receptors: Suf > Mor (Soc Neurosci Abst: 14: 32, 1988). Rats pre-pared with IT catheters and osmotic infusion minipumps were infused with saline (Sal) or equiactive concentrations of Mor: 20 nmol/ul/h or Suf: 0.6 nmol/ul/h. After 7 days of IT infusion, IT dose response curves on the HP test for Mor or Suf were carried out in Sal, Mor and Suf infused animals. Each animal was used for one dose of one drug. After continuous infusion of Mor or Suf, the greatest shift relative to Sal infused animals (Tol Ratio) was observed in Mor infused animals and least in Suf animals.

Test drug	Sal Infused	Mor Infused	Suf Infused
Mor ED50 (nmol):	0.65	30.0	6.10
Tol Ratio		46.1	9.5
Suf ED50 (nmol):	0.07	0.68	0.21
Tol Ratio:		9.7	3.0

These observations are consistent with work indicating that Mor 1) possesses few spare receptors as compared to Suf and 2) down regulates more of its receptor population than Suf in producing an equivalent magnitude of analgesia. (DA02110).

152.10

DELTA RECEPTOR INVOLVEMENT IN MORPHINE SUPPRESSION OF NOXIOUSLY EVOKED ACTIVITY OF SPINAL WDR NEURONS IN CATS

K. Omote,* L.M. Kitahata, J.G. Collins, K. Nakatani,* Dept. of Anesth., Yale Sch. of Med., New Haven, CT 06510

Complex pharmacology associated with modulation of sensory information at the level of the spinal cord provides an opportunity for the development of more effective ways to block the processing of pain information. The purpose of this study was to evaluate the relative role of mu and delta opiate receptors in morphine suppression of noxiously evoked activity of spinal WDR neurons.

This protocol was approved by the Yale Animal Care and Use Committee. Extracellular single WDR neuronal activity evoked by noxious radiant heat was recorded in decerebrate spinally transected cats. All drugs were administered spinally. Pretreatment with B-FNA an irreversible, selective mu antagonist, antagonized the suppressive effects of the selective mu agonist DAGO, but not the selective delta agonist DPDPE. As expected, pretreatment with B-FNA significantly antagonized the effects of spinally administered morphine on noxiously evoked activity of WDR neurons. In addition, however, co-administration with morphine of ICI 174, 864, a selective delta antagonist, in the B-FNA pretreated animals produced an even greater antagonism of morphine's ability to suppress noxiously evoked activity than observed in the absence of the delta antagonist.

The results of this study support the hypothesis that morphine is capable of suppressing noxiously evoked activity at the level of the spinal dorsal horn, not only through an interaction with mu receptors, but also as a result of interaction with systems that are antagonized by ICI 174, 864 assumably delta receptors.

Supported by NIH NS09871

152.11

YOHIMBINE ADMINISTERED INTRATHECALLY (i.th.) REVERSES BOTH OPIOID AND NONOPIOID CONDITIONAL ANTINOCICEPTION (CA) IN RATS. A. H. Lichtman and M. S. Fanselow. Dept. Psych, Dartmouth College, Hanover, NH 03755, and Dept. Psych, UCLA, LA, CA 90024.

The present study examined whether the relative amount of training is a factor in activating opioid and nonopioid mediated CA. During the acquisition phase, a daily tail flick latency was assessed 2 min prior to 3 electric grids shocks (2 mA, .75 s duration, 20 s iti). Control animals were given a daily tail flick test followed by an equivalent period of time in the chamber. When a rat's tail flick latency exceeded 10% of the maximal possible analgesic effect it was tested either the next day or after 2 more acquisition days. On the test day, half the subjects in each group were administered naltrexone (7 mg/kg, ip) and the rest received saline. The efficacy of naltrexone in attenuating CA was inversely related to the amount of training that they had received during acquisition.

In Experiment 2, we examined the neurochemical mechanisms underlying CA at the spinal level. Both forms of CA were completely prevented by administration of the noradrenergic antagonist yohimbine (100 µg, i.th.). In contrast, neither quaternary naltrexone (10 µg, i.th.) nor the 5-HT antagonist methysergide (50 µg, i.th.) had consistent effects across the entire test session. These results indicate that a critical noradrenergic synapse, at the spinal level, is in the final common pathway of both opioid and nonopioid mediated CA.

152.12

INTRATHECAL OPIOIDS IN THE RAT MECHANICAL VISCERAL PAIN MODEL R.W. Colburn*, J.A. DeLeo, D.W. Coombs,C.J. Winfree*
Anesthesia Research Laboratory, Department of Anesthesiology, Dartmouth-Hitchcock Medical Center, Hanover, NH 03756

Pharmacologic profiles of the opiate receptor systems suggest a differential association of mu, delta, and kappa receptors with visceral chemical and cutaneous thermal stimuli in the rat. The current study attempts to profile the association of these opioid receptors with a visceral mechanical stimulus(duodenal distention).

Each 250g Holtzman rat was implanted with a duodenal balloon catheter and a PE-10 intrathecal(i.t.) catheter. Five to thirty minutes prior to testing rats were infused i.t. with 10 µl of drug or vehicle followed by 10 µl of saline flush. Testing consisted of 5 intraduodenal balloon pulses (0.5-0.85 ml) followed by one minute held inflation. Writhing response was graded on a 0-4 behavioral scale and reported as percent of control response(%CR) for each treatment group (n≥6rats/group). Agents studied were mu-agonists: morphine and [Tyr-d-Ala-Gly-(Me)Phe-Gly-ol] (DAGO), delta-agonist: [D-Pen^2, D-Pen^5] enkephalin (DPDPE), and kappa-agonists: U50-488H and beta-Funaltrexamine(b-FNA). Rank order of potency and ED_{60}(ug) were DAGO(.19)>> Morphine(.55)>b-FNA(1.53)>U50-488H(3.09) >DPDPE(5.41). At high doses(10-50ug) the antinociceptive effect of both kappa agonists plateaued (@50-60% CR), while both mu agonists continued with linear responses to the highest dose tested (@20-26% CR). These findings suggest even further differential association of the opioid receptor systems relative to the mechanical (mu mediated) or chemical (mu and kappa mediated) visceral stimulation. Supported by PHS Grant CA 33865 and Hitchcock Foundation Grant HF103.

152.13

EFFECTS OF DYNORPHIN ON THE RESPONSES OF NEURONS IN THE DORSAL HORN OF THE MEDULLA. S.S. Mokha. Dept. of Physiology Meharry Medical College, Nashville, TN 37208.

It has previously been reported that mu and delta opioid receptors are involved in modulating nociceptive input in the dorsal horn of the medulla (Mokha,S.S.,J.Physiol.,398: 85,1988). The present study investigated the effects of dynorphin A(1-13) on the responses of neurons in the dorsal horn of the medulla (trigeminal nucleus caudalis). Extracellular single unit recordings were made in rats anaesthetized with halothane. Nociceptive neurons were activated by thermal stimuli. The effects of intravenously administered dynorphin-(1-13) (0.4-1.6 mg/kg) were tested on the responses of 22 nociceptive and cold receptive neurons. Dynorphin reduced the responses of 8/8 selectively nocireceptive neurons in the superficial laminae of the dorsal horn of the medulla. It reduced the responses in 3/8 multireceptive neurons in the deeper laminae, enhanced the responses in 2/8 and produced a biphasic effect in 2/8 neurons. Dynorphin reduced the activity of 3/7 cold receptive neurons in the superficial laminae, enhanced the activity of 2/7 and produced a biphasic effect in 1/7 neurones. It is concluded that kappa opioid receptors exert a widespread influence on somatosensory informatiom from the face.

Supported by: NIH RR03032

152.14

KAPPA AND DELTA-OPIOID AGONISTS SYNERGIZE TO PRODUCE POTENT ANALGESIA. C. Miaskowski*, Y.O. Taiwo* and J.D. Levine* (SPON: A.J. Miller), Schools of Nursing, Dentistry and Medicine, University of California, San Francisco CA 94143

A recent study from our laboratory suggests that the analgesic synergy produced by low-dose naloxone and pentazocine is the result of agonists action at delta and kappa opioid receptors, respectively. This study demonstrates that the simultaneous intrathecal administration of a selective kappa (U50,488H) and a selective delta ([D-Pen^{2,5}]-enkephalin, DPDPE) opioid agonist can produce analgesic synergy. The interaction of the analgesic effects of these two agents was studied, in the rat, using the Randall-Selitto paw-withdrawal test. Intrathecal administration of both U50,488H and DPDPE, as single agents, produced dose-dependent increases in mechanical nociceptive threshold. However, when the dose response curves for both U50,488H and DPDPE in the presence of a fixed low-analgesic dose of the other agent were compared with the dose response curves for the respective agonist administered alone, the curves for the combination regimens were shifted to the left. A statistically significant deviation from parallelism between the dose response curves of the single versus the combined agents demonstrates that the simultaneous administration of opioid agonists, that act at kappa and delta opioid-receptor sites, can produce analgesic synergy.

DIFFERENTIATION, MORPHOGENESIS AND DEVELOPMENT: NEUROMUSCULAR DEVELOPMENT

153.1

A QUANTITATIVE DEVELOPMENTAL ANALYSIS OF THE MORPHOLOGICAL ORIENTATION OF MOTONEURONS IN THE CERVICAL REGION OF THE SPINAL CORD. J.B. Taylor and W.J. Anderson. Indiana University School of Medicine, Terre Haute Center for Medical Education, Terre Haute, Indiana, 47809.

Previous investigations in our laboratory have morphologically described compact dendrite bundles in the lumbar and cervical spinal cord of the rat. The cervical dendrite bundle group is composed of three motoneuron dendrite columns including the medial dendrite bundle (MDB), the central dendrite bundle (CDB) and the lateral dendrite bundle (LDB).

A developmental study has been performed using Long Evans Hooded rats, the animals were sacrificed at days 1, 3, 5, 8, 10, 15, 20 and 30. Paraffin tissue was prepared at each of the above ages and stained with H&E. Golgi impregnation according to the method of Anderson and Felton was performed on each of the given ages while epon thick tissue was prepared for ages 5 day and 30 day. This study offers a complete quantitative analysis of the developmental orientation of the motoneurons in each of the three dendrite bundles using the Scholl Analysis technique on the Golgi-impregnated tissue. Results indicate motoneurons initially migrate to their destinations within small clusters of cells. the CDB has been identified at levels C3-C5 as the phrenic nucleus. The CDB cells at day 1 show extensive longitudinal interactions between the various cell clusters. The MDB cells present transverse fiber orientation early and then develop more extensive longitudinal interconnections. The LDB cells present few fibers at day 1, increased transverse projections and later increased longitudinal projections.

153.2

IMMOBILIZATION EFFECTS ON CHOLINERGIC ENZYME ACTIVITY IN EMBRYONIC CHICK LUMBAR SPINAL CORD. L.J. Haverkamp, J.L. McManaman, and S.H. Appel, Dept. of Neurology, Baylor College of Medicine, Houston, TX 77030.

Neuromuscular blockade has been demonstrated to prevent naturally occurring motoneuron death, alter patterns of muscle innervation, delay synapse elimination at poly-innervated junctions, and result in sprouting of stable junctions. We report here that such immobilization during embryonic development also increases choline acetyltransferase (CAT) activity in the lumbar spinal cord.

Daily injection of 2mg curare from E6-9, with sacrifice on E10, resulted in a mean increase of lumbar enlargement (LE) CAT activity to 171% of that of saline injected controls. Continuing the immobilization begun E6 on through E11 resulted in an increase of LE CAT to 240% of control, while immobilization from E10-13 resulted in an increase of LE CAT to 155% of control.

We interpret the increase in CAT seen after E6-9 curare treatment to be primarily the result of an increase in number of motoneurons, due to inhibition of naturally occurring cell death. However, since loss of motoneurons is largely complete by E10, we interpret the additional 70% increase in activity obtained by prolonging immobilization to E11, as well as the increase seen with treatment from E10-13, to be an increase in levels of CAT/motoneuron resulting from immobilization. Experiments are under way to document this possibility.

153.3

BRANCHING PATTERNS OF THE PHRENIC NERVE DURING DEVELOPMENT AND REINNERVATION. A.S. Norton*, P.K. Berger* and M.B. Laskowski. Dept. of Biological Sciences and WAMI Medical Program, Univ. of Idaho, Moscow, ID 83843.

Our previous studies have shown that the phrenic motor column projects topographically onto the surface of the diaphragm. This orderly map is present already at birth and is partially reestablished upon reinnervation. One potential mechanism for establishing topography lies in the choices that growing axons must make at branch points. In this study we asked whether nerves follow stereotypical patterns of branching early in development and upon reinnervation as a partial explanation for topographic specificity. Embryonic phrenic nerve-diaphragm preparations (E-15 to E-21) were aldehyde-fixed and stained with the silver/cholinesterase technique. Invariably even at the earliest stages, the phrenic nerve bifurcates into a rostral and caudal branch, and from the latter emerges a branch to the crus. After this primary bifurcation, however, further branching is asymmetric and variable between right and left sides of the diaphragm and between muscles of siblings. Within this asymmetry, we observed consistent patterns: 1) the complexity of branching increases dramatically with development, 2) higher-ordered branching occurs on the costal side of the primary branch, 3) the midcostal right side is far more arborized than the left. Three weeks after crush, the phrenic nerve remains bifurcated into rostral and caudal primary branches. However, secondary and higher ordered branching follows a markedly different pattern than normal. We conclude that both developing and regenerating phrenic nerves display consistent primary branching, but higher ordered patterns are highly variable. Thus axons in the phrenic nerve whether developing or regenerating are consistently offered a binary choice at the primary branch point. The selection to enter the rostral or caudal branch is not random and may be in response to positional cues.
(Supported by PHS #NS-27024).

153.4

FIBER-TYPE EXPRESSION IN RAT AND MOUSE SOLEUS DURING POSTNATAL DEVELOPMENT. D.J. Wigston, K.A. Shirley*, G. Schwartz*, and A.W. English. Departments of Physiology and Anatomy & Cell Biology, Emory University School of Medicine, Atlanta, GA 30322.

We analyzed the fiber-type composition of a mixed fiber-type muscle, soleus, in both rat and mouse, to determine whether the adult pattern of myosin expression is fixed soon after birth or whether it changes during maturation. We identified fast- and slow-twitch myofibers in muscles from animals varying in age from 1 week to 1 year, using monoclonal antibodies specific for fast and slow myosin isoforms. In cross-sections containing profiles of all myofibers in the muscle, we counted the fibers positive for fast myosin, and in adjacent sections, those positive for slow myosin. We also counted the total number of fibers in each section. Mouse soleus contained about 1,000 myofibers and rat 2,300 at all ages studied, suggesting that myogenesis ceases in soleus by 1 week after birth. In mouse, the relative proportions of fibers positive for fast and slow myosin were the same at all ages studied, 60% being fast and 40% slow. In rat, however, the proportions of fast and slow fibers changed dramatically. At 1 week after birth, 40% of rat soleus fibers were positive for fast myosin, whereas between 1 and 2 months, this value fell to 20% or fewer. The reason why 50% of the fast fibers present in rat soleus at 1 week are apparently converted to slow, whereas in mouse soleus fiber-type expression is constant, is unclear but may be related to differences in the amount of body growth between these two species.

153.5

MUSCLE FIBER TYPE DIFFERENTIATION IN THE OPOSSUM DURING POST-NATAL DEVELOPMENT. S. H. Astrow* and W. J. Thompson. Dept. of Zoology, University of Texas, Austin, TX, 78712.

Like other newborn marsupials, the anatomical structures of the opossum, Monodelphis domestica, are highly underdeveloped relative to other mammals such as the rat. Since much of the development of the hindlimb in the opossum takes place ex utero, it is a favorable preparation for studying the role of neuronal innervation in muscle development. In this study we used immunohistochemistry to examine the timetable of expression and distribution of muscle fiber types in the hindlimb of Monodelphis from birth until 2 weeks of age. Three myosin antibodies specific for embryonic myosin, slow myosin, and adult type IIa and neonatal myosin (fast/neonatal) were applied to cryostat sections. By postnatal day 3 (d3) muscle cleavage is largely complete and individual muscles can be identified. Prior to d7, all myotubes express slow as well as embryonic myosin. Fiber type heterogeneity is first detected at d7, when some of the primary fibers along the peripheral regions of muscles begin to express fast/neonatal myosin. By d9, fibers in the peripheral regions of tibialis anterior (TA) and extensor digitorum longus (EDL) no longer express slow myosin; labeling with the fast/neonatal and slow myosin antibodies appears to be complimentary in these regions. At d14 fast/neonatal myosin expression is also visible in the deep portions of TA and EDL. Small, presumably secondary myotubes expressing fast/neonatal myosin arise late in the second week of postnatal life. Thus, fiber type differentiation in the opossum is similar to, albeit more protracted than, that seen that in the rat. Myotubes assume a fiber type partially on the basis of birthdate: primary myotubes give rise to slow muscle fibers and secondary myotubes are largely determined to express fast myosin. In addition, some primary myotubes undergo a transformation of fiber type from slow to fast. It has been suggested previously that intramuscular position may determine which fibers undego this transformation, since the primary fibers which convert to fast are regionalized. However, in several muscles in the opossum, transformed fibers are intermixed with those which have not transformed, indicating that position alone can not account for the pattern of fiber types seen in the adult.

153.6

TIMING AND DIRECTION OF MUSCLE CLEAVAGE IN THE CHICK THIGH. S. Schroeter and K. W. Tosney. Department of Biology, University of Michigan, Ann Arbor, MI 48109-1048.

Individual muscles of the vertebrate limb are derived from two condensations of muscle cells, the dorsal and ventral muscle masses. In transverse sections at the light microscopic level, muscles appear to become separated from one another by "cleavage planes," regions of decreased cell density. These planes progress in a stereotyped pattern that was first recognized and broadly outlined by Romer (*J. Morphol. Physiol.*,43:347,1927).

We are re-examining the cleavage process in the thigh to more completely detail the temporal-spatial progression of cleavage during stages 27 to 32. We find that cleavage does not proceed in the same direction for all muscles. For instance, while the cleavage plane between the iliotibialis cranialis and iliotibialis lateralis progresses proximally, the cleavage plane between the adjacent ambiens and the iliotibialis cranialis progresses distally. We also find that cleavage is not necessarily uniform along the proximodistal axis. For instance, cleavage between the iliotibialis lateralis and iliofibularis is complete anteriorly while the muscles are still uncleaved posteriorly. We also detect cleavage slightly sooner than previously reported: muscles begin to cleave as growth cones begin to ramify within them. Our more detailed documentation reveals more complex separation patterns between muscles than previously described.

Supported by NIH grant NS-21308.

153.7

EFFECTS OF NEUROMUSCULAR BLOCKADE ON MYOTUBE DEVELOPMENT. B.J. Nelson and L.T. Landmesser. Dept. Physiology and Neurobiology, Univ. of Connecticut, Storrs, CT 06269.

Innervation has been proposed as necessary for development of the predominantly fast secondary myotubes, but not for primary myotube formation or their expression of fast and slow markers. The effect of curare-induced neuromuscular blockade on the development of myotube type was studied in chick iliofibularis muscle, which in adult has a fast posterior region and a mixed anterior region. Frozen sections of hindlimb were immunocytochemically stained with antibodies to slow muscle myosin (S58) and fast muscle Ca^{2+}-ATPase (5D2). From St 29 to 37, the antibodies recognized mutually exclusive myotube populations. At St 29-33, when only primary myotubes are present, 5D2 staining was restricted to the posterior (fast) region, whereas the anterior region was only labeled by S58. After St 33, small 5D2-positive cells appeared in the anterior IFIB, corresponding to secondary myoblast differentiation. The ratio of 5D2- to S58-positive cells increased tenfold between St 33 and 40. Chronic curare treatment dramatically decreased the number of both 5D2- and S58-labeled cells, but did not alter the fast/slow staining pattern, nor the normal increase in the ratio of 5D2- to S58-positive cells. This indicates that in IFIB, both slow primary and fast secondary myotubes are equally affected by activity blockade, thus leaving the spatial distribution of fiber types unaffected.

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153.8

DELAYED SYMPATHETIC DENERVATION OF EMBRYONIC HEART MATURING IN OCULO COMPROMISES GROWTH. T.C. Love* and D.C. Tucker. Psychology Dept,Univ Alabama Birmingham,Birmingham,AL 35294.

Embryonic heart tissue grows less well in a sympathetically denervated eye chamber than in an intact eye chamber (Tucker & Gist, Circ. Res. 1986). By morphological criteria, hearts cultured in oculo resemble mature hearts after 4-5 wks in oculo. We tested whether sympathetic denervation of a mature graft would compromise growth in oculo.

Embryonic hearts grafted into intact eye chambers of adult male rats (Innervated, N=22) were compared to (a) grafts sympathetically denervated after 4 wks in oculo (Denervated, N=10) and (b) to grafts in eye chambers denervated before grafting (Noninnervated, N=14). Size and beating rate measurements were taken biweekly. Chronic recording electrodes were implanted at 8 wks to estimate autonomic controls of graft beating rate in unanesthetized hosts.

Noninnervated grafts grew less well than Innervated grafts (1.86 ± .33 vs 4.15 ± .61 mm^3). Growth was equally compromised in Denervated and Noninnervated grafts after 8 wks in oculo (1.73 ± .21 vs 5.08 ± .69 mm^3). Intrinsic beating rate, estimated after combined β-adrenergic and muscarinic blockade, was lower in Innervated grafts than in Noninnervated grafts (141 ± 28 vs 206 ± 24 bpm). Intrinsic beating rate did not differ between Denervated and Noninnervated grafts (214 ± 22 bpm). These results suggest that sympathetic innervation affects growth and intrinsic beating rate during the mature phase of growth rather than during early cardiac development.

154.1

ANATOMY OF THE NASAL CAVITY AND ITS FOREBRAIN PROJECTIONS IN THE AXOLOTL. H.L. Eisthen*, D.R. Sengelaub, and J.R. Alberts* (SPON: A. Strickholm). Dept. of Psychology and Program in Neural Science, Indiana University, Bloomington, IN 47405.

The axolotl (Ambystoma mexicanum) is an neotenic, aquatic salamander whose feeding and social behavior are influenced by chemical stimuli. To understand the sensory bases of these behaviors, we are conducting anatomical studies of the nasal cavity. Reconstructions reveal that the nasal cavity, which extends from the external naris on the dorsal snout to the internal naris in the roof of the mouth, is essentially an elongated tube with a lateral pouch. In cresyl violet-stained sections, we observe spatially separate and morphologically distinct receptor populations in the nasal cavity. The medial wall appears to contain ciliated olfactory receptors, and the lateral pouch contains a non-ciliated sensory epithelium, corresponding to the loci of olfactory and vomeronasal epithelia in other salamanders.

We examined the forebrain projections of these receptor populations using HRP (50% Sigma type VI, 5% DMSO), placed either by injection (0.3 µl) or implantation in crystal form into the appropriate region of the nasal cavity and visualized with TMB. After HRP placement into the medial nasal cavity, densely-fasciculated fibers could be seen throughout the olfactory nerve, terminating in discrete glomeruli in the main olfactory bulb. In contrast, labeling after lateral pouch placements was restricted to fibers on the lateral edge of the olfactory nerve. These fibers terminated in a small region lateral and caudal to the main olfactory glomerular field, corresponding to the accessory olfactory bulb described by Herrick (1921) for the tiger salamander (Ambystoma tigrinum), a closely-related species.

The existence of a vomeronasal system in the axolotl, an aquatic organism, is particularly striking because this system appears to have been lost in wholly-aquatic mammals and reptiles.

154.2

AN HRP STUDY OF PATHWAYS LINKING THE PREOPTIC AREA TO AUDITORY NUCLEI IN HYLA CINEREA. **J. D. Allison and W. Wilczynski. Dept. of Psychology, University of Texas, Austin, TX 78712.**

Cells in the ventral hypothalamus (VH) and preoptic area (POA) respond to acoustic signals, and pathways linking the VH to three auditory nuclei, the anterior (AN) and central thalamic nuclei (CN) and the secondary isthmal nucleus (SIN), have been described (see Neary, in Evolution of the Amphibian Auditory System, Fritzsch, et al., eds., Wiley, 1988). In the present study HRP was iontophoretically injected into the POA of the green treefrog to describe its auditory inputs. After a 4 day survival, the injected animals were sacrificed under anesthesia and the brains extracted, fixed, cut at 40µ, mounted, and reacted with standard techniques using TMB (Wilczynski & Allison, Brain Behav. Evol., in press). Heavy retrograde labeling in the ipsilateral AN and a few lightly labeled cells in the ipsilateral SIN were found. No connections with the CN were found. Anterogradely labeled fibers were seen bilaterally in the AN. In addition, we found bilateral reciprocal connections with the medial pallium and septal nuclei, unilateral reciprocal connections with the dorsal and lateral hypothalamus, afferents from the ventral hypothalamus, and bilateral efferents to the dorsal and ventral habenula. Compared with the VH, the POA has much stronger connections with the AN, much lighter connections with the SIN, and no connections with the CN. (Supported by NSF grant BNS 8606289 and a Univ. of Texas BRSG.)

154.3

ONTOGENY OF AMPHIBIAN LHRH SYSTEMS. L.E. Muske and F.L. Moore. Dept. Zoology, Oregon State Univ., Corvallis, OR 97331.

Immunocytochemical (ICC) studies were conducted on three anuran amphibians (genus Rana) during development from early tadpole to juvenile frog, using antisera to three different forms of LHRH (luteinizing hormone-releasing hormone). Antisera to chicken II and mammalian LHRH's revealed two anatomically and developmentally distinct LHRH systems. The forebrain-spinal cord system projects from the POA through the ventromedial brainstem into the spinal cord, and may be the source of LHRH in frog sympathetic ganglia. Intensity of labeling was robust in premetamorphic tadpoles, but decreased with age. Immunolabeling in the hypothalamic-hypophyseal (H-H) LHRH tract was first detected in late prometamorphosis and increased with age. Development of irLHRH in the H-H tract coincided with first appearance of irLHRH in the nervus terminalis (NT) in one species, suggesting species differences. Analyses of ICC developmental changes with different LHRH antisera support the conclusions that the forebrain-spinal cord system, hitherto undescribed in larval or neonatal vertebrates, develops first and synthesizes chicken II-like LHRH, and that the H-H system develops later and synthesizes mammalian LHRH. We suggest that LHRHir neurons of the forebrain-spinal cord and H-H systems are chemically, functionally, and embryonically distinct.

Supported by NIH 1 FS32 NS07921-02 and R012508.

154.4

HISTAMINERGIC SYSTEM IN XENOPUS BRAIN: A COMPARATIVE AND DEVELOPEMENTAL STUDY. **M.S. Airaksinen and P. Panula. Dept. Anatomy, Univ. Helsinki, 00170 Helsinki, Finland.**

To study the evolution of the histaminergic system in vertebrates, we compared its distribution in adult and developing brain of Xenopus laevis, using an antiserum to carbodiimide-fixed histamine.

The histamine-containing cell bodies were located in the dorsal posterolateral hypothalamus. This area may be homologous to the tuberomammillary nucleus in mammals. Each cell had a thick process that terminated in the ventricle lumen. Analysis of adjacent sections indicated that the cells were not immunoreactive for GABA or serotonin.

The distribution of histaminergic fibers in Xenopus showed many similarities to mammals. The highest density was found in the medial amygdala and septum. In the primordial piriform cortex and striatum very few fibers were seen. There was a distinct, dense network of fibers around the cell bodies of nucleus accumbens. In diencephalon, highest fiber densities were found in the anterior and ventral thalamus, and preoptic and posterolateral hypothalamus. In hindbrain, the fiber density was highest in the central gray and the raphe area, as in most mammals.

Histamine-immunoreactive neurons appeared in the hypothalamus of Xenopus on the fourth embryonal day.

The results suggest that there is a widespread histaminergic system in the Xenopus brain. Besides, histamine may play a role in early developement of the nervous system.

154.5

DISTRIBUTION OF PIGMENTED CELLS AND PROCESSES IN THE BRAIN OF DEVELOPING TADPOLES. **N.J. Uray and P.S. Sexton. Dept. of Anatomy, Kirksville College of Osteopathic Medicine, Kirksville, MO 63501.**

In examining slides of brains of developing Rana catesbeiana tadpoles, we found pigmented neurons and processes with a specific distribution pattern from the caudal medulla to the telencephalon. In the medulla oblongata the pigmented cells comprise the ventrolateral cells of the motor column and cells in the octavolateral region. A different column of pigmented cells forms the ventralmost cells of the thalamic region, isolated cells in the dorsal infundibular nucleus and the lateral cell of the septum. Two kinds of pigment bearing processes are also present. One type appears to be the fine processes of the pigmented cells which ramify in the grey matter, the dorsal island of Kingsbury, the infundibulum of the hypothalamus and the neuropil of the habenula and epithalamus. A second course fiber type which is heavily pigmented is associated exclusively with the ventricular system terminating among ependymal cells and apparently contacting the CSF of the fourth ventricle. An anatomical "map" of these cells and processes may have significance in the interpretation of data that is generated in immunocytochemical procedures that utilize brown-black pigment granules. **Supported by NIAA Grant R01 AA07537-01.**

154.6

CARNOSINE-LIKE IMMUNOREACTIVITY IN THE RETINA OF AMPHIBIA. A.Fasolo&*, B. Mulatero&*, S.Biffo §&*, C. Artero&*, D. Cantinoδ* and F.L. Margolis§ (SPON: European Neuroscience Association). &Dip. Biologia Animale, δDip. Anatomia Fisiologia Umana, Universita' TORINO (I); §Roche Inst. Molec. Biol. Nutley, N.J. (U.S.A.).

The retina of frogs is rich in carnosine, an aminoacyl-histidine dipeptide (Margolis and Grillo, Neurochem. Int., 6:207-209, 1984). A polyclonal antibody against carnosine has been developed and used for localization of carnosine-like immunoreactivity (IR) in rodent and avian central nervous system (Biffo et al., in press). We used immunocytochemical methods to study the distribution of carnosine-IR in the retina of different amphibian species (Rana esculenta; Xenopus laevis; Ambystoma tigrinum; Triturus cristatus). In the retina of all these species an important carnosine immunopositive pattern was observed. Photoreceptor cell bodies were often stained as well as some bipolar elements. Moreover, an extensive network of fine cell processes was seen in the inner plexiform layer. These data suggest that the amphibian retina is a useful system in which to study the neural function of carnosine. (Supported, in part, by the Italian MPI.60-40%; NATO grant n.86/332; and CEE grant n.ST2J.0468).

154.7

MORPHOLOGY AND SYNAPTIC PHYSIOLOGY OF HIPPOCAMPAL NEURONS IN THE POND TURTLE STUDIED WITH WHOLE-CELL RECORDING. P. H. Desan, J. J. Lo Turco, A. R. Kriegstein. Dept. of Neurology, Stanford Med. Ctr., Stanford, CA, 94305.

The anatomy and synaptic currents of pyramidal cells in areas DM and M of the cerebral cortex of the pond turtle were studied with whole-cell recording and dye injection. These areas are analogous to the mammalian hippocampus. The medial wall of the cerebral hemisphere containing areas DM and M was dissected from adult pond turtles (Pseudemys scripta elegans), anchored in a petri dish with a plasma-thrombin clot and perfused with turtle physiological buffer. Whole-cell recording in both voltage clamp and current clamp modes were made as described elsewhere at this meeting (Kriegstein, Blanton and Lo Turco, 1989). Recording pipettes contained 120 mM CsF, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 11 mM EGTA, 10 mM HEPES, 5 mM NaCl, and a saturating concentration of Lucifer Yellow CH. Intrahippocampal connections were activated with bipolar stimulation caudal to the recording site.

In successfully filled pyramidal cells, infrequently branching, spiny apical dendrites formed either a wide or narrow symmetrical arborization originating from 3-6 primary dendrites. The number of spiny basal dendrites was variable. In some cells profuse recurrent ascending axon collaterals were observed, and often one branch could be followed to the fornix. Frequent spontaneous synaptic current events were present: such events were uniformly inward at potentials more negative than -70 mV, either inward or outward at potentials near -40 mV, and uniformly outward at potentials more positive than 0 mV. Stimulation of intrahippocampal projections evoked a complex conductance, consisting of components reversing near either the chloride equilibrium potential or the nonspecific cation equilibrium potential.

154.8

REPTILIAN THALAMIC RETICULAR NUCLEUS. M.B. Pritz and M.E. Stritzel*. Div. Neurol. Surg., Univ. California Irvine Medical Center, Orange, CA 92613-4091.

All mammals possess a thalamic reticular nucleus. While its anatomy and physiology vary depending on the species investigated, the thalamic reticular nucleus shares at least two properties in all mammals so far examined. It projects to nuclei of the dorsal thalamus and its neurons are immunoreactive for GABA/GAD. We investigated whether such a nucleus is present in other amniotes.

Injections of horseradish peroxidase (HRP) were placed in a number of dorsal thalamic nuclei in reptiles, Caiman crocodilus. Tissue was processed using standard neurohistochemical methodology employing tetramethylbenzidine as the chromogen. Although not all dorsal thalamic nuclei have been injected with HRP, we have identified retrogradely labeled neurons in the nucleus of the dorsal peduncle of the lateral forebrain bundle (dpLFB) which are embedded within, for the most part, the telencephalic projecting thalamus axons. Injections of different parts of the dorsal thalamus retrogradely label different parts of the dpLFB. Dendritic visualization of such retrogradely labeled cells indicates that their processes are primarily distributed parallel to the entry zone of ascending dorsal thalamic axons. Additional immunocytochemical experiments using a polyclonal anti-GAD antibody and standard immunocytochemical methodology employing the avidin-biotin complex technique indicates that while some GAD (+) cells are present in the dpLFB, they are a population separate from those that project to the dorsal thalamus.

These results indicate that a thalamic reticular nucleus may be common to all amniotes and thus be a feature that has arisen early in evolution. Furthermore, the characteristics of these two cell populations in this reptilian thalamic reticular nucleus suggest that the intrinsic organization of the thalamus in Caiman may be organized quite differently from that of mammals.

154.9

DISTRIBUTION OF GABA-LIKE IMMUNOREACTIVITY IN TURTLE CEREBELLUM. D. Vyas, M. T. Clarke* and J. C. Houk. Dept. of Physiology, Northwestern University Medical School, Chicago, IL 60611.

It has been recognized from morphological and hodological studies that reptilian cerebellum is similar to that of mammals. The organization of GABA-ergic inhibitory neurons in mammalian cerebellum is well understood. However, the distribution of GABA in the cerebellum of lower vertebrates has not been studied.

We examined GABA-like immunoreactivity (IR) in 40 μm thick sections of turtle cerebellum using peroxidase-antiperoxidase method. The turtle was chosen because of our long range goal of correlating the neuroanatomy of the cerebellorubrospinal system with *in vitro* electrophysiological studies of motor pattern generation (Neurosci. Lett., 97:123,1989).

Our results demonstrate that all three layers of the cerebellum contained IR elements for GABA. In the molecular layer numerous bouton-like structures, somata and the processes of stellate cells contained dark reaction product. Parallel fibers did not display any IR for GABA. The perikarya of Purkinje cells showed weak GABA-IR. In the granular cell layer, Golgi cells and glomeruli were immunoreactive while granule cells were immunonegative. Both, medial and lateral cerebellar nuclei contained darkly stained terminals of Purkinje cell axons, and a few lightly stained small-sized neurons.

We conclude that stellate, Purkinje and Golgi cells are GABA-ergic neurons in the turtle cerebellum. The pattern of IR of these GABA-ergic neurons in the turtle cerebellum is comparable to that described in higher vertebrates including mammals.

154.10

MARGINAL NUCLEI IN THE SPINAL CORD OF THE BOA CONSTRICTOR. D. M. Schroeder, Medical Sciences Program, Indiana University School of Medicine, Bloomington, IN 47405.

Marginal nuclei are prominent in the spinal cord of reptiles, especially snakes, and recent evidence suggests that marginal neurons function as intrinsic mechanoreceptors. These neurons are located segmentally along the length of the spinal cord and are adjacent to the denticulate ligament. The ultrastructure of this nucleus has been described for snakes representing Viperidae and Colubridae families but not Boidae. The latter snakes represent an evolutionary older family. In the boa constrictor, each marginal nucleus extended about 800 μm rostrocaudally. In this area the collagen within the denticulate ligament, which is usually quite prominent, disappears for a distance of 100 μm. The nucleus consisted of a medial and lateral neuropil. The lateral neuropil, adjacent to the denticulate ligament, contained large neurons and many had short, stubby dendrites that extended towards the edge of the spinal cord; some neurons were immediately adjacent to the glia limitans. Within the neuropil fingerlike processes, originating from the stubby dendrites, were surrounded by glial processes and accompanied by an axonlike structure. Many dendritic profiles were present in the neuropil, as well as numerous boutons containing primarily round clear vesicles. Few myelinated fibers were present but unmyelinated axons were present throughout. The medial neuropil had fewer and smaller neurons and very few fingerlike processes. In general, the marginal nucleus of the boa had only minor differences compared to that of the other species. Supported by PHS S07RR7031J.

154.11

THE PARATYMPANIC ORGAN: MORPHOLOGY, INNERVATION, AND FUNCTION. C.S. Von Bartheld and E.W Rubel. Hearing Development Laboratories RL-30, Univ. of Washington, Seattle, WA 98195.

The paratympanic organ (Vitali organ) is a small sensory organ in the avian middle ear. The medial side of its lumen contains mechanoreceptors with afferent and efferent nerve fibers. We investigated the central connections of this organ by injections of fluorescent carbocyanine tracers (DiI, DiO) into the paratympanic organ of chick embryos and hatchlings. Approximately 40-50 ganglion cells in the facial ganglia give rise to paratympanic nerve fibers that enter the brainstem with the facial nerve, but project to the lateral, superior and descending vestibular nuclei. Approximately 40 efferent neurons are labeled ipsilaterally, adjacent to the superior olivary nucleus and the facial motor nucleus.

Two elastic ligaments attach to the paratympanic organ in the middle ear. The columellar-squamosal ("Platner's") ligament inserts at its caudal pole; the superior drum-tubal ligament attaches to the rostral tip of the organ. To study the effect of pressure and movement of the extracolumella on the configuration of the paratympanic organ, we perfusion-fixed embryonic and posthatch chickens while exerting positive pressure to the ear canal on one side and negative pressure on the other side. Serial sections were cut and stained with aldehyde fuchsin and neutral red. Parts of the lumen of the paratympanic organ expand differentially as a function of the tension of the ligaments and the position of the extracolumella; the displacement of fluid in the lumen may stimulate the paratympanic hair cells. This mechanism would explain the avian sensitivity to changes of atmospheric pressure and supports the hypothesis that this organ functions as a barometer and altimeter.

Supported by NIH grants NS07246, NS08578 and NS24522.

154.12

CHARACTERIZATION AND SPECIES DISTRIBUTION OF NEURONAL NADPH-DIAPHORASE. B.T. Hope and S.R. Vincent. Kinsmen Laboratory of Neurological Research, Department of Psychiatry, University of British Columbia, Vancouver, Canada.

NADPH-diaphorase is a histochemically defined enzyme found throughout the nervous system. It is found within somatostatin and neuropeptide Y neurons of the striatum and within cholinergic neurons of the mesopontine tegmentum of rats. Previous studies have found NADPH-diaphorase in several mammalian brains. We now report the presence of this enzyme activity in the striatum and mesopontine tegmentum, in the rat, cat, baboon, human, cow, pig, guinea pig, chicken, turtle, and frog. We have failed to detect any neuronal NADPH-diaphorase activity in the brains of any species of fish examined. Thus, NADPH-diaphorase appears early in the phylogenetic tree (ie. Amphibia) and its expression in particular neuronal populations is subsequently strongly conserved. The function of the enzyme is unknown. Histochemical characterization has demonstrated that the reaction is catalyzed by an enzyme which can utilize β-NADPH, deamino-NADPH, α-NADPH, but not β-NADH or 3'-NADPH. Electron microscopy indicates that the enzyme is associated with the membranes of the endoplasmic reticulum and nuclear envelope. Neither superoxides nor hydrogen peroxide are generated in the reaction. Under anaerobic conditions, all tetrazoliums with redox potentials more positive than NADPH can be reduced. The enzyme does not appear to be a metalloprotein, but may possess thiol groups which participate in the reaction mechanism. This novel enzyme appears to be distinct from any previously characterized NADPH-dependent oxidoreductases.

154.13

THE DISTRIBUTION OF NADPH-DIAPHORASE IN THE PERIPHERAL NERVOUS SYSTEM. S.R. Vincent, Y. Aimi* and H. Kimura*. Kinsmen Lab. of Neurological Research, Dept. of Psychiat., The Univ. of British Columbia, Vancouver, Canada, & Div. of Molecular Neuroanatomy, Shiga Univ. of Medical Sciences, Japan.

NADPH-diaphorase histochemistry has proven to be a useful tool for neuroanatomical studies of the central nervous system, where it selectively stains particular populations of neurons. We have now examined the distribution of this enzyme activity in the peripheral nervous system of the rat and guinea pig. In the enteric nervous system, many intensely staining neurons were found throughout the myenteric plexus, and many fibers were present in the circular muscle. A few stained cells were also found in the submucous plexus. NADPH-diaphorase positive neurons were not detected in the sympathetic ganglia, however, a dense network of stained fibers surrounded many of the principal cells in the prevertebral ganglia. Although the intermediolateral horn of the spinal cord did contain many strongly staining neurons, sectioning of the splanchnic nerve had no observable effect on the fiber staining in the guinea pig celiac-superior mesenteric ganglion complex. In contrast, interruption of the fibers from the gut drastically reduced the fiber staining in this ganglion. This indicates the presence of an NADPH-diaphorase containing projection from the myenteric plexus to the prevertebral ganglia. In the sensory system, a few positive neurons were seen in the dorsal root ganglia at cervical and lumbar levels. In contrast, the thoracic ganglia contained many positive neurons. NADPH-diaphorase histochemistry is an important technique for anatomical investigations of the peripheral nervous system.

154.14

CGRP IS AN EVOLUTIONARILY CONSERVED MARKER OF THALAMIC AUDITORY RELAY NEURONS. A. Reiner and S.E. Brauth, Dept. Anat. & Neurobiol., UT-Memphis, Memphis, TN; Dept. Psychol., Univ. of Maryland, College Park, MD

The distribution of calcitonin gene-related peptide (CGRP) in the auditory thalamotelencephalic pathways of turtles, caiman, pigeons, budgerigars and rats was examined using immunohistochemical techniques. In colchicine- treated turtles and pigeons, numerous CGRP+ perikarya were observed in the thalamic auditory relay nucleus, particularly in the periphery of the nucleus. In turtles, caiman, and pigeons, CGRP+ fibers were observed within the portions of the dorsal ventricular ridge (DVR) of the telencephalon previously reported to receive input from the auditory thalamus. In colchicine-treated rats, CGRP+ perikarya were abundant along the lower and medial edges of the medial geniculate nucleus (MGN) but sparse within the MGN itself. Fluorogold injections into auditory cortex retrogradely labeled some of these CGRP+ neurons, thus suggesting that at least some of these CGRP+ neurons are part of the rat auditory thalamus .

The present results are consistent with previous studies suggesting that the thalamic auditory relay neurons of reptiles and birds are comparable to parts of the MGN of mammals and suggest that these cell groups have been inherited at least in part by modern amniotes from their reptile common ancestor. Studies are needed to determine if CGRP is present in thalamic auditory relay neurons in modern amphibians, and on this basis may have been characteristic of the earliest land vertebrates. Supported by NS-19620 (A.R.) and MH-40698 (S.E.B.).

154.15

THE AVIAN LOBUS PAROLFACTORIUS: AN INTEGRATIVE BRAIN AREA PERHAPS INVOLVED IN STRESS. W.J. Kuenzel and S. Blaehser*. Poultry Sci. Dept. Univ. of Maryland, C.P.,MD 20742 and Anat. & Cytobiol.Inst.,Univ. of Giessen , Giessen, FRG.

Antibodies (ab) to corticoliberin (CRF), vasotocin (AVT), substance P(SP), neurophysin I and II(NP II) were used with immunocytochemistry to map the distribution of immunoreactive (i.r.) perikarya and fibers throughout the chick brain. It was found that the medial parolfactory lobe (LPO) from its rostral to caudal extent contained i.r. perikarya to CRF. When an ab to NP II was used, the entire LPO showed intensely stained i.r. perikarya. The entire LPO also showed i.r. fibers to SP. Since CRF is a well known neuropeptide that stimulates the release of pituitary ACTH and NP II is a carrier protein for AVT that likewise releases ACTH suggests that the LPO may have a neuroendocrine function. SP is involved in nociception as well as implicated in enhancing inflammatory reactions. Anatomically the LPO is a proposed component of the basal ganglia and an area involved with vocalization (area X). Hence the LPO can receive sensory as well as motor information, contains neural elements that would be regarded as neuroendocrine and adrenal-related and has direct neural connections to limbic structures. Perhaps the LPO is an integrative brain area involved in stress or emotional behaviour.

154.16

SEVEN MORE BRAIN TRAITS WHOSE VARIANTS ELUCIDATE MAMMALIAN PHYLOGENY. J.I. Johnson, Anat. Dept., Mich. State U., East Lansing, MI 48824; R.L. Reep, Dept. Neurosci., U. Fla., Gainesville, FL 32610; R.C. Switzer III, Depts. Pathol., Med. Biol., U. Tenn., Knoxville, TN 37920; J.A.W. Kirsch, Dept. Zool., and W.I. Welker, Dept. Neurophysiol., U. Wis., Madison, WI 53706.

We present seven additional traits which provide new evidence for assessing mammalian relationships. Four distinguish restricted groups from all other mammals: 1) Mirroring of the complete SI body representation (monkeys); 2) Rindenkerne, clumps of cell bodies in layer 6 of cerebral cortex (sirenians: manatees and dugongs); 3) Posteriorly-pointing digits in the SI body representation (bats, mega- and micro-); 4) Tectopetal connections from both, vs. contralateral, retinas (primates and megabats). Complex sortings are seen in : 5) Loss of lamination in dorsal cochlear nuclei, convergent in several lines where some species leave the ground (bats, monkeys, seals, dolphins and manatees); 6) Separation of claustrum from cortex is related to gyrencephaly but occurs in lissencephalic primates and marsupials, and 7) Lack of a distinct external cuneate nucleus in monotremes and sirenians.(Supported by NSF grant BSR 85-03687.)

154.17

THE INFLUENCE OF NODAL ANATOMY ON CONDUCTION VELOCITY IN MYELINATED NERVE FIBERS: A MODELING STUDY, J. A. Halter and J. W. Clark, Jr.[1], Division of Restorative Neurology and Human Neurobiology, Baylor College of Medicine, Houston, Texas, 77030 and [1]Rice University, Houston, Texas

A mathematical model of the myelinated nerve fiber was developed, representing the myelinated nerve fiber as a multi-axial electrical equivalent circuit including separate intra-axonal, peri-axonal and extra-axonal longitudinal conductive pathways with independent representations of the myelin sheath versus the underlying axolemmal membrane. The node is represented as a separate segment with an adjacent myelin sheath attachment region and a non-cylindrical fluted section transitioning to the stereotypical internodal region. The peri-axonal space extends to the nodal compartment, the nodal gap is represented and Na and K channels are included in the paranodal and internodal axolemmal membrane. Both amphibian and mammalian nerve fibers were modeled.

Results indicate that the peri-axonal conductive pathway and the axon radius present in the myelin sheath attachment region are sensitive parameters affecting conduction velocity. As the radius was decreased from that seen in the internode, the conduction velocity increased until a relative radius of 20% was attained after which the conduction velocity rapidly declined. This provides modeling evidence for the role of the nodal constriction in promoting higher conduction velocities.

155.1

PHARMACOLOGIC INFLUENCES ON SEXUAL BEHAVIOR IN MALE RATS. D. Yells*, S. Hendricks, B. Graber, and D. Fitzpatrick*. Depart. of Psychiatry and Psychology, Univ. of Neb., Omaha, NE 68182.

Previous research has suggested that manipulations of catecholamine and opiate metabolism can facilitate sexual behavior in male rats. The effects of these drugs are likelihood of copulating, e.g., sexually naive or castrated. Facilitation of sexual behavior in male rats has been reported to result from treatment with a number of drugs including Yohimbine (an alpha-2 antagonist), Naloxone (an opiate block), and Lisuride (a dopamine agonist). The present study evaluated the long-term effects of continual treatment with these drugs on post-castration sexual behavior of male rats. We also examined the effects of a combined injection of Yohimbine and Naloxone in this paradigm. Various components of male rat copulatory behavior were modified by drug treatments. The drug-induced facilitation became more obvious with increasing times post-castration.

155.2

DIFFERENCES IN PENILE REFLEX LEVELS AND SYNAPTIC COVERAGE OF SNB MOTONEURONS. M.G. Leedy, J.C. Bresnahan, and M.S. Beattie Depts. of Anatomy and Surgery, Ohio State Univ., Columbus, OH 43210.

Two groups of male rats were chosen based on their responses in three tests for penile reflexes: those exhibiting no reflexes in any of the tests (No Reflex group), and those six animals exhibiting the greatest number of reflexes (High Reflex group). Testosterone levels did not differ between these two groups. All animals, while gonadally intact, received bilateral injection of cholera toxin conjugated to HRP into the bulbocavernosus muscle, thereby labeling the motoneurons within the spinal nucleus of the bulbocavernosus (SNB). Analysis at the light microscopic level of these motoneurones showed no differences between the two groups, for dendritic lengths, somatic area, nuclear or nucleolar area. At the ultrastructural level, ANOVAs indicated that the High Reflex animals had significantly less synaptic coverage, measured as fewer terminals per 100 um membrane length, less synaptic apposition, less active site apposition, and more glial coverage, compared to the No Reflex group. (NS-10165)

155.3

EFFECT OF PERINEAL MUSCLE DENERVATION ON PENILE REFLEXES IN RATS. Edward P. Monaghan & S. Marc Breedlove. Psychology Dept., Univ. Calif., Berkeley, Berkeley, CA 94720.

The striated perineal muscles, bulbocavernosus (BC) and levator ani (LA) are involved in male reproductive function in rats, and, along with their innervating motoneurons in the spinal nucleus of the bulbocavernosus (SNB), are sexually dimorphic. Other researchers have performed muscle excision and electromyographic recordings to implicate the BC muscle in penile reflexes. As our initial step in investigating the SNB-BC system, we looked at the effect of BC/LA denervation on penile reflexes. By transecting the pudendal nerve just proximal to the BC/LA muscles we removed SNB input while leaving these muscles intact.

For behavior tests rats were held in a plexiglass cylinder with their ventral surface exposed. After five minutes the penile sheath is rolled back to expose the penis and the occurrence of erections, cups, and flips are recorded for a period of twenty minutes. Animals received at least two behavior tests prior to denervation and one final test two to three days after surgery. Denervation essentially eliminated the cups, reduced the number of erections, and had no effect on flips, thus supporting the findings of Hart & Melese-D'Hospital (1983) and Sachs (1982).

Supported by NSF BNS 8451367.

155.4

LOCALIZATION OF MOTOR NEURONS OF A SEXUALLY DIMORPHIC MUSCLE IN GUINEA PIGS. Louise M. Freeman* & S. Marc Breedlove, Psychology Department, University of California, Berkeley CA 94720.

The retractor penis (RP) muscle attaches between the penis and the pubis in adult male guinea pigs. An analogous but smaller muscle, the retractor clitoris (RC), is present in females. To determine the anatomical location of motor neurons innervating these muscles we used the retrograde tracer horseradish peroxidase (HRP).

Under ketamine anesthesia, adult guinea pigs were bilaterally injected (6.0-10.0 μL) with HRP (Sigma VI, 30% solution) in either the RP or RC. After three days, we perfused the animals and frozen-sectioned the spinal cords at 50 μm. Clusters of HRP-labeled motor neurons were found in the L5 to S1 levels of both sexes. Labeled motor neurons occupied the central region of the ventral horn. We will examine RP/RC motor neurons to determine whether they are sexually dimorphic in dendritic extent or number.

Supported by a NSF Graduate Fellowship and the March of Dimes.

155.5

UNILATERAL ADMINISTRATION OF ANDROGEN TO BULBOCAVERNOSUS AND SUBSEQUENT EFFECTS ON SNB DENDRITIC LENGTH. Mark N. Rand & S. Marc Breedlove, Dept. Psychology, U.C. Berkeley, CA 94720.

Systemic administration of androgen has been shown to increase the length of dendrites in the sexually dimorphic spinal nucleus of the bulbocavernosus (SNB) in rats (Kurtz, Sengelaub & Arnold, Science 232:395, 1986) and in white-footed mice (Forger & Breedlove, J. Neurobiol. 18:155, 1986). We previously reported that local, lateralized administration of androgen to the SNB target muscles bulbocavernosus and levator ani (BC/LA) results in a lateralized anabolic effect mediated at or near the muscle (Rand & Breedlove, Soc. Neurosci. Abstr. 13:54, 1987). The present study tested the idea that such peripheral, unilateral administration of androgen to target musculature could increase dendritic length of SNB motoneurons in the ipsilateral spinal cord.

Adult male rats 90-120 days of age were castrated and implanted with two capsules, one containing testosterone (Sigma) and the other an anti-androgen (SCH16423, Schering Corp.). The capsules had a single diffusable surface and were sutured one on each side of the BC/LA so that the diffusable surface faced the muscle. After 30 days the capsules were removed and the medial BC on each side was injected with 1 μL of cholera toxin-HRP conjugate (CT-HRP). Four days later the animals were sacrificed and the BC/LA and spinal cord were dissected out. The lumbar spinal cord was frozen-sectioned at 50 μm and reacted with TMB to visualize the CT-HRP filled motoneurons and their processes. These were then traced without knowledge of steroid treatment, and lengths measured with a digitizing tablet and microcomputer. Dendritic length of motoneurons ipsilateral to BC/LA treated with androgen were compared with lengths ipsilateral to peripheral anti-androgen treatment. We are currently completing the measurement and analysis of SNB dendrites, and will present these data as well as muscle-half weights resulting from the peripheral, lateralized administration of androgen to the SNB system.

Supported by NSF #BNS 8451367

155.6

NO SEX DIFFERENCE IN SIZE OF MOTOR NEURONS WHICH INNERVATE M. SPHINCTER CLOACAE IN JAPANESE QUAIL. C.M. Seiwert and E. Adkins-Regan, Department of Psychology, Cornell University, Ithaca, NY 14853.

The foam gland complex of the Japanese quail (Coturnix japonica) is a sexually dimorphic structure located directly beneath the sphincter cloacae muscle (mSC) in the dorsal wall of the cloaca. The gland secretes a fluid that is whipped into a stiff foam in the male, possibly by the undulation of mSC (Klemm, R.D., J. Morph., 141:171, 1973). Motor neurons innervating mSC are located in lateral area IX of synsacral spinal segments 8 through 10. Foam production, gland size, and muscle size are androgen-dependent (Nagra et al., Anat. Rec., 133:415, 1959). Although present in both sexes, the gland and muscle are markedly reduced in females and no foam is produced. This research investigates whether the sex difference in musculature is reflected in the CNS.

Somal area of mSC motor neurons in males and females was measured with a computerized system following injection of .2% cholera-toxin conjugated horse radish peroxidase into the muscle. Labelled cells were visualized with a modification of the TMB technique. No sex difference was found in mean cell size [n=3 males, x+SEM=551.4μ+94.7μ, 12-45 cells per male; n=3 females, x+SEM=527.8μ+13.1μ, 15-31 cells per female]. There was, however, a sex difference in cell size variance: male cell size was more variable regardless of whether the bird or the cell was the unit of analysis ($F_{2,2}=52.6$, $p<.025$ or $F_{84,64}=2.1$, $p<.001$, respectively). The greater range of cell sizes in males raises the possibility of a sex difference in the number of cell classes. Cell number may also vary with sex. This possibility is currently under investigation. (Supported by NSF #BNS 88-09441).

155.7

RAT ANAL SPHINCTER MOTONEURON SIZE IS NOT TESTOSTERONE DEPENDENT. W.F. Collins, III, A.W. Seymour and S.P. Wargo*. Dept. of Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook, NY 11794.

The spinal dorsomedial nucleus [DMN or SNB] innervates the sexually dimorphic bulbospongiosus [BS] and the non-sexually dimorphic anal sphincter [AS] muscles (McKenna and Nadelhaft, '86). In adult males, somal cross sectional area and dendritic length of BS MNs is reduced in castrated animals compared to rats that are gonadally intact or castrated and given testosterone (Kurtz, et al., '86). In the present study, we have examined the effect of castration on AS MN size in adult male rats (Sprague-Dawley). Ketamine/Xylazine (90mg/10mg per Kg IM) anesthesia was used for all surgical procedures. Twenty-four 60 day old rats were separated into three treatment groups; (1) castration with testosterone propionate [C+TP] pellet implant, (2) castration with placebo [C+P] pellet implant and (3) sham castration. Thirty days following castration, the AS or BS muscles were injected with horseradish peroxidase (0.5-2.0 ul, 50%). After 2 days survival, rats were transcardially perfused with saline (27° C) followed by cold (4° C) perfusate (paraformaldehyde (4%) and glutaraldehyde (1.25%) in PBS). Frozen longitudinal sections (50 um) of spinal cord were cut and reacted using tetramethylbenzidine. Labeled MN profiles with nuclei were traced using a drawing tube attachment and cross sectional area measured using a digitizing tablet and computer. Measurements within treatment groups were pooled for comparisons. No significant difference in body weight was observed across treatment groups. The penis and attached muscles (weight) and the BS MNs (area) were significantly smaller in the C+P group compared to either the C+TP or sham groups. The C+TP and sham groups were statistically the same. In the case of AS MNs, no difference was observed in cross sectional area between treatment groups. Thus, in contrast to the sexually dimorphic BS motor system, AS MN size is not testosterone dependent in adult male rats. Supported by NS24206 (WFC) and NS14899 & NS16996 to L.M. Mendell.

155.8

INHIBITION OF TESTOSTERONE-INDUCED COPULATION IN QUAIL BY A NOVEL SPECIFIC AROMATASE INHIBITOR, R76713

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In Japanese quail, as in other higher vertebrates, the central aromatization of testosterone (T) seems to play a critical role in the activation of copulatory behavior. The strongest evidence supporting this idea comes from experiments showing that the aromatase inhibitor, androstatrienedione (ATD) blocks the T-induced behavior. However recent studies have questioned the specificity of ATD. It was shown that this compound inhibits the 5α-reduction of testosterone and also decreases the binding of androgens to their receptor. This might potentially explain the behavioral effects of ATD. A new triazole derivative, R76713 (Janssen , Beerse, B) was recently shown to inhibit selectively the aromatase without affecting other steroid-metabolizing enzymes and without interacting with the estrogen-, progestin- or androgen-receptors. This compound was tested here for its capacity to interfere with the induction by T of copulatory behavior. In a first experiment, R76713 inhibited in a dose-dependent manner (range 0.01 to 1 mg/kg) the activation by T silastic implants of sexual behavior and of the hypothalamic aromatase activity in castrated male quail. The 5α- and 5β-reductases were not systematically affected. Stereotaxic implantation of R76713 in the medial preoptic area similarly blocked the behavior activated by a systemic treatment with T demonstrating that the central aromatization of T is implicated in the activation of behavior. Finally the behavioral inhibition produced by R76713 could be reversed by the simultaneous treatment with a dose of estradiol which is not behaviorally effective by itself suggesting that the behavioral deficit induced by the inhibitor is specifically due to the suppression of estrogen production. These data confirm the critical role of the preoptic aromatase in the activation of reproductive behavior and demonstrate that R76713 is an ideal tool for the *in vivo* study of estrogen-dependent processes

155.9

VOLUNTARY EXERCISE MAY INCREASE CLEARANCE RATE FOR TESTOSTERONE. David Pieper, Providence Hospital, Department of Physiology, Southfield, MI 48037.

Voluntary exercise (EX) inhibits short photoperiod (SP) induced testicular regression in golden hamsters which is due, in part, to hyper-responsiveness to the negative feedback of testosterone on gonadotropin secretion. The following experiment was initially designed to determine whether exercise alters the responsiveness to testosterone (T) feedback. 40 hamsters were castrated, and placed on 14L:10D (LP) or 10L:14D (SP). One half of each group was given access to an exercise wheel while the other half was maintained in similar cages without wheels (SED). 8 w later, they were implanted with a 20 mm silastic capsule of T or an empty capsule. At 4 w after T capsule implant, T was much higher in the LP SED (5.62±0.33 ng/ml) and SP SED (6.62±1.06) groups than the LP EX (3.80±0.25) or SP EX (2.22±0.58) groups ($p<0.01$). The post-castration increase in serum LH was less pronounced in the EX (572±117 LP; 388±25 SP) than SED (1361±345 LP; 1487±353 SP) groups at 4 w ($p<0.05$). Similar results were obtained for serum FSH. T implants resulted in very low or non-detectable LH and FSH levels in all hamsters on SP. The LP EX animals with T generally had higher FSH and LH levels than the LP SED group. We conclude that exercise may inhibit the effect of SP by an independent influence of EX to increase the rate of T metabolism or clearance.

155.10

SEX DIFFERENCES IN RESPONSIVENESS OF RAT BRAIN TO ANDROGENS CORRELATE WITH REGIONAL DIFFERENCES IN ANDROGEN RECEPTOR CONTENT. C.E.Roselli and T.A.Fasasi*. Physiology Dept., Oregon Health Sciences Univ., Portland, OR 97201.

Males are generally more responsive than females to the behavioral and neuroendocrine actions of androgen. To determine whether this difference is associated with sex differences in the concentration and/or distribution of androgen receptors within brain, we measured cytosolic (ARc) and nuclear (ARn) androgen receptors in microdissected brain regions. We also measured microsomal aromatase activity (AA) as an index of tissue responsiveness to androgen. In the bed nucleus (n.) of the stria terminalis (BNST), periventricular preoptic area (PVPOA), medial preoptic n. (MPN) and ventromedial n. (VMN) testosterone induced greater amounts of AA in males than in females ($p<.01$). Except for MPN, this difference was correlated with significantly greater ARn levels in males compared to females ($p<.05$); no associated sex differences in ARc levels were observed. When we compared brain ARc levels in gonadectomized rats, we found that BNST from males contained significantly greater ARc levels than from females ($p<.05$). These results suggest that sex differences in androgen responsiveness may be due in part to sex differences in androgen-binding capacity in specific hypothalamic nuclei. Supported by HD 23293.

155.11

RESTORATION OF AGE-RELATED DEFICITS IN BRAIN ANDROGEN METABOLISM AND BINDING IN MALE RATS BY TESTOSTERONE TREATMENT. K. C. Chambers, J. E. Thornton, and C. E. Roselli. Dept. of Psychology, USC, Los Angeles, CA 90089 and Dept. of Physiology, OHSU, Portland, OR 97201.

Age-related deficits in male rat sexual behavior are accompanied by decreases in brain aromatase activity (AA), nuclear androgen receptors (ARn) and testosterone (T) levels. To determine if the deficits in brain androgen metabolism and binding in aged males are due to low T, we measured AA, ARn, and cytosolic AR (ARc) in the preoptic area, basal hypothalamus, and amygdala of old and young gonadectomized T-treated Fischer 344 males (GX-T). Untreated gonadectomized rats (GX) and intact young males (YI) also were studied for comparison. T levels in young and old GX-T were comparable and were higher than in YI males. In sexual behavior tests, all of the young but only 42% of the old GX-T males ejaculated. T-treatment stimulated AA in all brain areas to levels equivalent to YI levels. Although ARn levels in old GX-T were lower than in young GX-T, they were comparable to YI levels. No age or treatment related differences in ARc were observed. These data suggest that there is an association between decreased T levels in old males and decreased brain ARn and AA levels but no association between decreased T and decreased sexual behavior. Thus the age-related deficits in behavior are probably not due to changes in AA and ARn. HD 20970 and HD 23293.

155.12

SEX DIFFERENCES IN ANDROGEN RECEPTOR BINDING IN MICROPUNCHED BRAIN NUCLEI. M.Y. McGinnis and S.E. Katz*(SPON: J.E. Shriver). Dept. of Anatomy, Mount Sinai Sch. Med., CUNY, New York, NY 10029.

The biological basis of sex differences in male sexual behavior has been examined by measuring androgen receptors using various autoradiographic and biochemical methods. The results of these studies have been equivocal. To determine whether a more precise, localized measurement would reveal sex differences in androgen receptors, we examined cytosol androgen receptor binding in micropunched brain nuclei of male and female rats gonadectomized 1-3 weeks previously. Brains were sectioned in a cryostat at 300 u. Punches 500 u in diameter were obtained from MPOA, LPOA, BNST, ant HYPO, VMN, DMN, ARCUATE n., cort AMYG n., med AMYG n., parietal CTX, dorsal HIPPO, and lat SEPT. Samples were processed for cytosol androgen receptor binding using [^{3}H]R1881 as ligand. Results indicate that androgen receptor binding is significantly higher in the MPOA ($p\leq0.001$) and the ARCUATE n. ($p\leq0.05$) of males relative to females. Results suggest that sex differences in male sex behavior may be mediated, at least in part, by sex differences in androgen receptors. Supported by NSF grant BNS-8616996.

155.13

Androgen-binding in estrogen-receptor containing cells in the brain of the canary (Serinus canaria).
Manfred Gahr, University of Kaiserslautern, 3049, 6750 Kaiserslautern, FRG (Spon.: Maria Kaltwasser).

Even though there are many indications for interactions between estrogen-sensitive and androgen-sensitive neural circuits for the behavioural control the sites of interaction are unknown. This report combined in-vivo autoradiography (ARG) with the androgen 5a-dihydrotestosterone (DHT) and immunocytochemistry with the monoclonal estrogen-receptor antibody H222Spy to study the cellular basis for connections between estrogenic and androgenic circuits. Six adult male canaries were anesthetized and castrated. Birds 1 and 2 were implanted with estradiol benzoate (measured 17b-estradiol: 8ng/ml). Each animal was injected with 5µg (3µCi)/g body weight ^{3}H-DHT (SA=170 mM/Ci) 48hr after castration. In addition bird no.6 was injected with a 100x amount of untritiated DHT. ARG, immunostaining, and analysis (5x background criteria) were done as described in PNAS,85:7380,1988. Besides areas containing either tritium- or antibody-labeled cells or both,each animal (except no.6) contained antibody-labeled DHT-binding cells primarily in the nucleus ICo, Tn, OM, and the dorsal preoptic area. DHT is not convertable into estrogens. This fact, the double-labeling in animals with high estrogen levels, and the low affinity of estrogen receptors to DHT indicate a coexistence of androgen- and estrogen-receptors in the same neurons. Supported by DFG Gu/148-7,8.

155.14

CENTRALLY IMPLANTED TESTOSTERONE ACTIVATES SEXUAL BEHAVIOR IN CASTRATED MALE FERRETS. Y.P. Tang and C.L. Sisk, Neuroscience Program and Dept. of Psychology, Michigan State University, East Lansing, MI 48824.

This experiment assessed the ability of centrally implanted testosterone proprionate (TP) to reinstate sexual behavior in castrated male ferrets. The loss of sexual behavior after castration was confirmed in 30-min tests with receptive females in which the total amount of time spent engaged in three components of male sexual behavior (neck grips, mounts, thrusts) was quantified. Bilateral cannulae containing approximately 2 mg TP in cocoa butter were then stereotaxically aimed at the preoptic area (POA). Tests for sexual behavior were conducted on days 3, 7, 14, and 21 post-implantation. Based on histological evaluation of cannulae placement, ferrets were classified as either (1) Miss (n=3; implants not in POA), (2) Unilateral implant in POA (n=3), and (3) Bilateral implant (n=6). The amount of time spent neck gripping, mounting, and thrusting significantly increased between pre- and post-TP tests in the Bilateral group, but not in the Miss and Unilateral groups. Luteinizing hormone concentrations in blood samples collected on each day of behavioral testing were within the normal range of castrated ferrets, and did not significantly decline after TP implantation. These results demonstrate that central administration of TP into POA activates the neural substrates for sexual behavior in castrated male ferrets. Supported by the Whitehall Foundation.

155.15

EFFECT OF TESTOSTERONE REDUCTION ON MALE SEIZURE SUSCEPTIBILITY. J. Thomas and J.H. McLean, Dept. of Psychology, Univ. of New Orleans, New Orleans, LA 70148.

The following study was conducted as part of research efforts to define more clearly the relationship between sex-associated gonad hormones and seizure susceptibility. On the basis of existing findings two hypotheses seem plausible in regard to the effects of testosterone in males: 1) that TT does not significantly modulate mechanisms underlying seizure susceptibility in males; or 2) that TT has a pro-convulsant effect in males. Naive, male Long-Evans hooded rats received either sham operations or orchiectomies under anesthesia (Nembutal, 65 mg/kg). After a 3 week recovery period, independent groups in each surgical condition were injected (sc) with picrotoxin (PIC) (3.0 or 7.0 mg/kg) and observed for behavioral signs of seizures. Castrated males were found to have significantly lower thresholds than sham- operates to all types of seizures. These results indicate that endogenous TT has anti- convulsant effects on PIC-induced seizures at the dose levels tested. This result contrasts with the effects of gonad hormones in females, where estrogen reduction was found to have different effects on susceptibility at threshold and supra-threshold levels of stimulation.

155.16

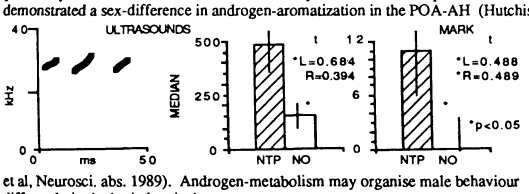
EXPRESSION OF THE EJACULATORY REFLEX IN THE CASTRATED MALE B6D2F1 HYBRID HOUSE MOUSE (Mus musculus) IS REDUCED BY THE AROMATASE INHIBITOR ATD. K. Sinchak* and L.G. Clemens. (SPON: B. Wee) Department of Zoology, Michigan State University, East Lansing, MI 48824.

This experiment investigated the hypothesis that retention of the ejaculatory reflex in B6D2F1 males following castration may be dependent on endogenous nongonadal steroid hormones. It has been shown some male B6D2F1 mice retain the ejaculatory reflex long after adrenalectomy and castration (Thompson An.Beh. 24:519, 1976), indicating expression of the ejaculatory reflex is independent of gonadal and adrenal hormones. Recent evidence with rats suggests dehydroepiandrosterone (which can be reduced or aromatized) may be synthesized in the brain (Corpechot Proc.Nat.Aca.Sci. 78:4704, 1981). To test whether such non-gonadal steroids may play a role in the retention of the ejaculatory reflex of B6D2F1 male mice, castrated males that continued to exhibit the ejaculatory reflex were administered 1,4,6-androstatriene-3,17-dione (ATD), an aromatase inhibitor that blocks the enzymatic conversion of androgens to estrogens via silastic capsule implanted subcutaneously in the nape of the neck. ATD implants significantly reduced the frequency of the ejaculatory reflex in the castrated B6D2F1 males. Copulatory behavior that results in an ejaculatory reflex, appears to be maintained by endogenous estrogen in castrated B6D2F1 males. The origin of this estrogen is unknown.

155.17

IS ASYMMETRIC BRAIN DEVELOPMENT RELATED TO SEXUAL BEHAVIOUR? S.D. HOLMAN, R. HUTCHISON,* A. WOZNIAK* and J. HUTCHISON. MRC Neuroendocrine Development and Behaviour Group, IAPGR, Cambridge, CB2 4AT, UK.

Sexual dimorphism of the brain is influenced by androgen and oestrogen metabolites formed as a product of aromatization in brain cells. Courtship ultrasonic calling is masculinized in females by implanting androgen into the neonatal preoptic-anterior hypothalamic (POA-AH) region (Holman and Hutchison, 107:355, 1985). Neonatal androgenization also masculinizes the Sexually Dimorphic Area pars compacta cells (SDA-PC) in the POA-AH (Yahr, Behav. Neur. Biol. 49: 119, 1988). This study relates the volume of the PC and androgen-metabolism in the POA-AH to sexually differentiated ultrasonics in Mongolian gerbils. Ultrasounds and courtship interactions were measured using MICRO. Females received either a 100µg testosterone propionate (NTP) or a vehicle (NO) injection neonatally, and TP in adulthood. Ultrasounds and scent-marking were masculinized by neonatal TP treatment. Marking shown by NTP and NO females was positively correlated with the PC volume on both sides of the brain. However, ultrasound emission was positively correlated with the PC volume only in the left hemisphere. Data also demonstrated a sex-difference in androgen-aromatization in the POA-AH (Hutchison



et al, Neurosci. abs. 1989). Androgen-metabolism may organise male behaviour differently in the brain hemispheres.

155.18

BRAIN AREAS FORMING BEHAVIORALLY EFFECTIVE ESTROGENS BY AROMATASE ACTIVITY CONTAIN IMMUNO-REACTIVE ESTROGEN RECEPTORS. J.B. Hutchison, M. Gahr*, R. Hutchison*, A. Wozniak* and S.D. Holman. MRC Neuroendocrine Development and Behaviour Group, Institute of Animal Physiology, Babraham, Cambridge, CB2 4AT, UK.

Estrogens as metabolites of androgens act on the preoptic area (POA) to influence specific components of behavior. POA aromatase activity is influenced by changes in circulating testosterone (T) and environmental stimuli. Formation of estrogens in POA cells depends on an active aromatase system which has both high affinity (K_m<10nM) for T as substrate and high activity (V_{max} >100 fmol.h^{-1}.mg tissue^{-1}). Aromatase activity (AA) in the gerbil POA is less active (Holman et al., Neurosci. abs. 1989). The distribution of AA in the dove and gerbil is compared. Estrogen-receptor containing cells (ERC) of the male dove were immuno-stained with a monoclonal antibody (H222Spy) which binds estrogen receptors in the avian brain (Gahr, B.Res. 402, 173, 1987). Aromatase assay of individual samples and ERC staining were carried out on the same coronal sections. High AA and ERC density overlapped in the POA and anterior hypothalamic areas of behaviorally active male doves. ERC density was also high in the tuberal complex and nucleus intercollicularis which showed low estrogen formation. Castration of doves reduced behavior and aromatase activity (>75%) in the POA, but ERC distribution underwent little or no change.T aromatized to estrogens in the POA could act on neurones via estrogen receptors. Local formation of estrogens, but not of estrogen receptors in the POA, appears to account for the effects of T on behavior in male doves. The aromatase system in the male gerbil is likely to be under a different type of control.

155.19

NEUROENDOCRINE RESPONSE TO, AND RECOVERY FROM, AGONISTIC ENCOUNTERS IN MALE GOLDEN HAMSTERS.
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The purpose of the present study was to characterize the neuroendocrine response to, and recovery from, an agonistic encounter in male golden hamsters. In Exp 1, we measured plasma adrenocorticotropin (ACTH), cortisol, beta-endorphin (B-EP), and beta-lipotropin (B-LPH) in pairs of hamsters sacrificed immediately after either a 5, 15, or 30 min encounter in which one of the hamsters established dominance. There were no differences in the three time points so the groups were pooled. In submissive hamsters, plasma ACTH & B-EP were significantly higher than in dominants or controls. Plasma cortisol & B-LPH were higher in submissives than in controls. Hormonal levels in dominants were not significantly elevated in any group. These results support the hypothesis that "losing" is more stressful than is "winning" in terms of pituitary peptide release.

In Exp 2, blood was collected immediately, 1 hr, or 3 hrs after a 30 min agonistic encounter. Plasma cortisol and ACTH returned to baseline levels in both dominant and submissive hamsters at 1 and 3 hrs. Further characterization of the neuroendocrine responses of dominant and submissive hamsters to both acute and chronic agonistic encounters is ongoing.

NEURAL-IMMUNE INTERACTIONS II

156.1

EFFECTS OF INTERLEUKIN-1-BETA ON IN VITRO RELEASE OF CATECHOLAMINES FROM THE HYPOTHALAMUS.
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Interleukin-1-beta (IL-1B) has been shown to alter the release of hypothalamic and pituitary hormones. This experiment was done to determine if IL-1B affects the release of norepinephrine (NE) from the hypothalamus since NE influences the release of hypothalamic and, in turn, pituitary hormones. Individual hypothalami from 5-6 mo. old male rats were incubated at 37°C in Krebs-Ringer Henseleit (KRH) medium in an atmosphere of 95%O_2-5%CO_2. The released NE was analyzed by high performance liquid chromatography after alumina extraction. Basal release was determined by incubating the hypothalami for 1 hr in KRH. This was followed by incubation for 1 hr in KRH (control), or in KRH containing 100 ng or 50 ng of IL-1B. There were no differences in the basal release rates among the three groups (21.0-25.8 pg/mg/hr). The presence of 100 ng of IL-1B in KRH increased the rate of NE release by 57±12%, whereas in its absence the release rate decreased by 13±10%. This difference was significant ($p<0.005$). The presence of 50 ng of IL-1B in KRH produced an increase of 18%, indicating a dose response. During the next hr, the hypothalami from the three groups were incubated in KRH alone. Under these conditions, the release rates in the experimental groups were the same as in the control group. The proof that the hypothalami remained viable during the entire procedure was obtained by challanging the hypothalami in the end with K^+ (60mM), which produced increases of >500% in all three groups. These results indicate that IL-1B stimulates the release of NE from the hypothalamus, providing further evidence of a neuroimmunoendocrine connection.

156.2

INTERLEUKIN-1 ENHANCES THE ACCUMULATION OF EPINEPHRINE AND VASOACTIVE-INTESTINAL POLYPEPTIDE IN CULTURED ADRENAL CHROMAFFIN CELLS.
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Recently, neural cells have been shown to be targets of the action of cytokines, including interluekin (IL)-1 alpha and beta and IL-6. 24-hr treatment of bovine adrenal chromaffin cells with 10^{-10} or 10^{-9} M recombinant human (rh) IL-1 alpha resulted in a dose-related increase in medium levels of vasoactive-intestinal polypeptide (VIP) and epinephrine (Epi). IL-1 alpha (10^{-9} M) increased the medium concentration of Epi (0.88+ 0.04; Mean + SE, N=8) and VIP (55.6 + 1.65) to 1.8 + 0.13 nMoles/well/24 hr and 117.1 + 11.02 pg/well/24 hr, respectively. Identical treatment of chromaffin cells with rh IL-1 beta (10^{-9} M) also resulted in a significant increase in the accumulation of VIP and Epi in the medium. In contrast to the stimulatory effects of IL-1, exposure of chromaffin cells to rh IL-6 (10^{-10} M) for 24 hr did not alter the accumulation of VIP or Epi, as compared to untreated cells. Continued exposure to rh IL-1 for an additional 48 hrs maintained elevated medium levels of VIP. Intracellular VIP levels were also elevated, indicating modulation of both synthesis and secretion of VIP by IL-1.

156.3

EFFECT OF INTERLEUKIN 1 ON PROLACTIN SECRETION, ADENYLATE CYCLASE ACTIVTY AND CALCIUM FLUXES IN RAT PITUITARY CELLS.
T.Florio*, O.Meucci*, E.Landolfi*, M.Grimaldi*, A.Marino* and G.Schettini. Inst. of Pharmacology, II School of Medicine, Univ. of Naples Via S. Pansini 5, 80131 Naples ITALY

Recent findings indicate that interleukin 1 (IL1) beta modulates neuroendocrine functions in a classical hormone manner. In this study we report IL1 modulation of prolactin secretion, evaluated by using reverse hemolytic plaque assay, of calcium fluxes and adenylate cyclase (AC) activity in rat normal pituitary cells. IL1 (10^{-14} to 10^{-8} M) reduced both basal and VIP-stimulated prolactin secretion. Similarly IL1 inhibited AC activty both in basal and VIP-stimulated conditions. Higher IL1 concentrations (10^{-10} to 10^{-8} M) restored the enzymatic activity to control value. IL1 also showed a biphasic effect on free intracellular calcium rise induced by maitotoxin (MTX). IL1 from 10^{-9} to 10^{-7} M potentiated MTX-induced increase of cytosolic calcium levels whereas lower concentration of IL1 (10^{-14} to 10^{-12} M) reduced MTX effect. Both the effects of IL1 on AC actvity and calcium fluxes were reverted by means of polyclonal anti-IL1 antibody, thus supporting the specificity of these findings. In conclusion our data show that IL1 modulates prolactin secretion through a direct action on pituitary cells, likely interacting with both AC activity and calcium flux.

156.4

MODULATION OF MAJOR HISTOCOMPATIBILITY COMPLEX (MHC) ANTIGEN EXPRESSION IN HUMAN NEURAL CELLS.
B. A. Boyer Kollas* and B. Wigdahl. Department of Microbiology and Immunology, The Pennsylvania State University College of Medicine, Hershey, PA 17033.

Previous studies by other investigators utilizing immunohistochemical techniques have demonstrated a low level of MHC class I and II expression in the human nervous system. However, in vivo animal model and in vitro studies have indicated that treatment of neural cell populations with (i) auto-antigens, (ii) interferon (IFN), or (iii) infection with neurotropic viruses induces the expression of MHC class I and II antigens. Expression of MHC proteins on cells of the human nervous system would potentially allow these cells to participate in immune response reactions. We examined human fetal dorsal root ganglia (DRG) isolated from aborted human fetuses ranging from 12 to 16 weeks gestation for their surface expression of MHC class I and II antigen by indirect immunofluorescence in conjunction with fluorescence-activated flow cytometric (FACS) analysis using monoclonal antibodies directed against the (i) heavy chain HLA- A, B, and C (class I), and (ii) HLA-DR region-associated, Ia-like bimolecular complex (class II). FACS analysis demonstrated that between 59 and 87% of human fetal DRG neural cells expressed detectable levels of MHC class I antigen while between 13 and 26% expressed class II antigen. Treatment of human fetal DRG neural cell populations for 48 hr with gamma-IFN (γ-IFN; 100 U/ml) enhanced the level of MHC class II expression by greater than 40%. These results were similar to those obtained in γ-IFN-treated U-373 MG human glioma cells. Current studies are examining the accumulation of MHC class I and II RNA in γ-IFN-treated human fetal DRG cells by RNA blot hybridization. These studies suggest that γ-IFN or other cytokines may alter the ability of specific human fetal neural populations to participate in immune reactions by modulation of MHC class I and II cell surface glycoprotein expression.

156.5

DUAL BINDING OF SEROTONIN AND N-ACETYL MURAMYL DIPEPTIDE TO SEROTONIN RECEPTORS. F.C. Westall. Institute for Disease Research, P.O. Box 1293, Alta Loma, CA 91701.

There are (Brain Res. Bull.,12: 485, 1984) structurally similar serotonin binding sites on the tryptophan peptide (phe-ser-trp-gly-ala-glu-gly-gln-arg) of the myelin basic protein, LHRH and MSH-ACTH 4-10. Furthermore, the adjuvant peptide, muramyl dipeptide (N-acetyl muramyl-L-ala-D-isogln) also binds to these sites (J.Inf.Ded.Biol.,2: 1,1986). Upon performing competition experiments using both muramyl dipeptide and serotonin with either LHRH or tryptophan peptide, we observed by nuclear magnetic resonance techniques that both compounds can simultaneously bind to either receptor. The serotonin is held predominantly through pi bonds to the aromatic rings, while the muramyl dipeptide binds to the more hydrophilic portions of the site. Our work helps to explain the contradictory effects of muramyl dipeptide and serotonin on neurological and immunological systems. Also, since both tryptophan peptide and LHRH are also autoimmune antigens, the results of the competition studies provide a model for how antigens are bound by the macrophage without interfering lymphocyte recognition.

156.6

INTERLEUKIN-1 ANTISERA DECREASES NEURONAL SURVIVAL IN DEVELOPING SPINAL CORD CULTURES. D.E. Brenneman, M. Schultzberg and I. Gozes. Lab. of Developmental Neurobiol., Clinical Neuroscience Branch, and Lab. of Molecular Genetics, NICHD and NIMH, Bethesda, MD 20892.

Interleukin-1 (IL-1), a protein that regulates the growth and activity of many cells including immune system cells, has been shown to occur in peripheral nerves and adrenal chromaffin cells in rodents, and in the human brain. In vitro studies have demonstrated the production of IL-1 by amoeboid microglia. Previous work has shown that nonneuronal cells stimulated by vasoactive intestinal polypeptide (VIP) secrete neuronal survival factor(s). We now investigate IL-1 as a possible mediator for these effects on neuronal survival in dissociated spinal cord cultures derived from fetal mice. After nine days treatment with antisera (1:2500 dilution) to holo murine interleukin-1 alpha, and to a synthetic peptide corresponding to amino acids 201-215 of the murine IL-1 alpha, neuronal cell counts were decreased to 40% of control cultures. Normal rabbit serum at the same dilutions had no effect on spinal cord neuron survival. Addition of VIP prevented the neuronal cell death associated with the antisera to IL-1. Immunoprecipitations utilizing the IL-1 antisera, followed by SDS polyacrylamide gel electrophoresis of the ^{35}S-methionine labelled proteins secreted by nonneuronal cell cultures in the presence of VIP, are now in progress to identify the endogenous peptide. In summary, the data imply that this cytokine or another substance that shares structural homology to IL-1, may have a role in the determination of neuronal survival during development. (MS is from Karolinska Inst., IG is on sabbatical from Weizmann Inst.)

156.7

ELECTROPHYSIOLOGICAL ANALYSIS OF THE ACTIONS OF PLATELET ACTIVATING FACTOR ON RAT MYENTERIC NEURONS IN CELL CULTURE. D.J. Fickbohm*[1], J.T. Wachsman*[2], D.W. Powell*[2,4] & A.L. Willard[1,3] (SPON: J. Dean). Curriculum in Neurobiology[1], Depts. of Medicine[2] and Physiology[3], and Core Center in Diarrheal Diseases[4], Univ. of North Carolina, Chapel Hill, NC 27599-7545.

Platelet Activating Factor (PAF), a lipid mediator of inflammation, stimulates Cl^- secretion by the intestinal mucosa by a process sensitive to tetrodotoxin and hexamethonium (Bern et al, *J. Clin. Invest. in press*, 1989). These observations suggest that PAF may trigger release of neurotransmitters from enteric neurons during inflammatory responses in the gut. We have used whole cell patch clamp methods to test directly the hypothesis that PAF excites rat myenteric neurons.

Myenteric neurons were dissociated from small intestines of newborn rats and grown in cell culture for up to 25 days. PAF was applied either by whole bath superfusion or by ejecting it from a micropipet. At concentrations ranging from 1 to 20 μM, PAF caused a reversible increase in membrane conductance and depolarizations as large as 30 mV. In addition, PAF often caused dramatic increases in nicotinic cholinergic synaptic activity (i.e. increased frequency of EPSPs).

The effects of PAF were not prevented by extracellular solutions that contained 0 (i.e no added) Ca, 2 mM Mg and 500 μM Cd (a concentration that completely blocks voltage-gated Ca currents in these cells). This suggests that influx of extracellular Ca is unnecessary for the actions of PAF. In a wide variety of other cell types (e.g. Kornecki & Erhlich, *Science* 240:1792, 1988), PAF causes elevation of intracellular Ca. We hypothesize that PAF causes release of intracellular stores of Ca in myenteric neurons and that the elevated Ca causes release of acetylcholine and activation of Ca-dependent cation channels. Supported by NIH grants to DWP (DK15350; DK34987) and ALW (NS24362).

156.8

METABOLIC EFFECTS OF GP120 IN BRAIN REGIONS RICH IN VIP RECEPTORS. A.S. Kimes, G. Szabo*, E.D. London, L. Raymon* and B. Tabakoff, Addiction Res. Ctr., NIDA, Baltimore, MD 21224 and Unit for Special Projects, IRP, NIAAA, Bethesda, MD 20892.

The glycoprotein (GP120) on the coat of the Human Immunodeficiency Virus (HIV) is toxic to neuronal cells in culture (Brenneman, D., et al., Nature 335:639, 1988), and binds to sites in the brain which have a distribution similar to VIP receptors (Hill, J., et al., Psychopharm. Bull. 22:690, 1986). In the present study, the effect of 1 pmole and 10 pmole GP120 administered intracerebroventricularly on regional metabolic rate(s) for glucose (rCMRglc) was measured using the 2-deoxy-D-[1-^{14}C]glucose method (Sokoloff, L., et al., J. Neurochem. 28:897, 1977) in rat brains. GP120 significantly decreased rCMRglc in the suprachiasmatic nucleus (1 pmole dose) and in the lateral habenula (10 pmole dose) by 19% and 12%, respectively, compared with controls receiving cerebrospinal fluid. The effects are consistent with the hypothesis that GP120 interacts with VIP receptors, as these regions have high densities of binding sites for VIP (Shafer, M.M. & Moody, T.W., Peptides 7:283, 1986). As the toxic effect of GP120 in cell culture had an inverted U-shaped dose response curve, we extended our studies to include lower doses of GP120. Preliminary results (N = 2/dose) indicate that 1, 10 and 100 fmole GP120 decrease rCMRglc in the above-mentioned regions and in several additional areas. The GP120-induced reduction in brain metabolism may be related to functional deficits in AIDS dementia.

GP120 was a gift from the National Cancer Institute.

156.9

SIGMA RECEPTORS IN LYMPHOCYTES: QUANTIFICATION ON T AND B CELLS AND DISTRIBUTION IN LYMPHOID TISSUES. S.A. Wolfe, Jr. and E.B. De Souza. Neurobiology Laboratory, Neuroscience Branch, NIDA/ARC, Baltimore, MD 21224.

The psychotomimetic drug phencyclidine (PCP) and several of its analogs suppress activity in *in vitro* immunologic functional assays. In addition, ^{3}H-PCP binds specifically to human peripheral blood leukocytes (HPBL). Since PCP binds to both PCP and σ receptors in brain, we previously surveyed rat spleen and HPBL, and found no evidence of PCP receptors, however we detected σ receptors in these tissues in densities equal to or exceeding that of brain. In the present study, we used fractionated lymphocyte populations and characterized cell lines to identify and quantify σ receptors on rat, human and mouse T_H, $T_{eff/suppr}$ and B cells. The highest density of σ receptors occurred on B cells, but T cells also displayed σ binding sites. In autoradiographic studies using the ligand d-3-(3-hydroxyphenyl)N-(1-propyl-2,3[^{3}H]piperidine) (^{3}H-3-PPP) and 3,4-[^{3}H]-(N)-[1(2-thienyl) cyclohexyl]-piperidine (^{3}H-TCP) to specifically label σ and PCP receptors, respectively, we confirmed the absence of PCP receptors, but detected σ binding sites in rat lymphoid tissues. Spleen contained a higher density of σ receptors than did thymus, mesenteric lymph nodes, or Peyers patches. In spleen, σ receptors were found in the white pulp areas, and were maximal in the outer (B cell) zones of the follicles. The presence of high densities of this brain/nerve receptor on lymphoid cells suggests a physiological role for endogenous σ ligands in modulating immune responses and in the integration of the CNS-endocrine-immune axis.

156.10

HUMAN NATURAL KILLER CELLS EXPRESS Na^+ CHANNELS. R.N. Mandler, L. Seamer*, A. Bankhurst*, M. Lennon*, E. Rosenberg* and D. Whitling*. Depts. of Neurology, Medicine and The Cancer Center, The Univ. New Mexico Sch. Med., Albuquerque, NM 87111.

We studied voltage-gated excitability of human purified natural killer (NK) cells using flow cytometry and the voltage-sensitive dye oxonol. Cells were obtained from normal volunteers after Ficol-Hypaque density gradient centrifugation, plastic adherence for macrophage removal, nylon-wool columns for B-cell removal and negative selection with monoclonal antibodies for T cells removal. The Na^+ channel agonists Batrachotoxin (BTX) (1-4 uM) and Veratridine (Ver) (100-400 uM) depolarized a population of highly purified (99%) human NK cells as determined by Leu 11-Leu 19 flow cytometric assay. BTX and Ver responses were concentration, time, temperature, and Na^+ dependent. The Na^+ channel antagonist tetrodotoxin (TTX) (1 uM) blocked BTX and Ver responses. Ver (100 uM) produced significant inhibition of cytotoxicity when purified NK cells were incubated with K562 tumor target cells in a 4 hr Cr^{51}-release cytotoxicity assay. These results strongly suggest presence of functional Na^+ channels in NK cells. Activation of voltage-dependent Na^+ channels depolarizes cells and reduces their cytotoxic function.

156.11

MODULATION OF CHANNEL ACTIVITY AND SECRETION BY NEUROTRANSMITTERS IN LACRIMAL GLAND LYMPHOCYTES. B.Walcott, E.J.Roemer* and P.R.Brink. Dept. of Anat. Sci., State University of New York, Stony Brook, NY 11794.

Anatomical studies have shown close physical proximity between AChE and catecholamine positive autonomic fibers and large numbers of plasma cells in the medulla of avian Harderian glands, suggesting a functional relationship. To test this, we have mechanically isolated plasma cells from the glands, suspended them in RPMI medium and treated them for 20 min. with 0.5 mg/ml collagenase. Plasma cells were identified by their morphology when compared to identical preparations stained with specific antibodies. Whole cell and cell-attached patch recordings were made within several hours of the isolation of the cells. Patch recordings revealed a maxi-K channel of 230 pS and, rarely, a small K channel of 40 pS. The maxi-K channel was Ca^{++} dependent. Carbachol ($1X10^{-5}$ M) caused a reversible increase in membrane current due to an increase in maxi-K channel open time. Norepinepherine ($2X10^{-7}$ M) caused a dramatic increase in cell current indicative of massive channel opening. In addition, carbachol ($1X10^{-5}$ M) caused pooled cells (approx. 10^5/ml) to transiently release 2 times the baseline amount of IgG into the medium. Thus, it appears that neurotransmitters have a direct effect on secretory functions of plasma cells.

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156.12

DISTINCT SUBSETS OF LEWIS RAT T-HELPER LYMPHOCYTES RECOGNIZE THE 68-86 ENCEPHALITOGENIC REGION OF MYELIN BASIC PROTEIN. M. D. Mannie, P. Y. Paterson, D. W. Thomas, and R. Nairn (SPON: R. Holz). Department of Microbiology and Immunology, University of Michigan School of Medicine, Ann Arbor, MI 48109-0620.

T-helper lymphocytes which mediate experimental autoimmune encephalomyelitis (EAE) in Lewis rats recognize the 68-86 region of myelin basic protein (MBP). However, it is not known whether these T cells represent a homogenous population or a heterogenous population consisting of distinct subsets of T-helper cells. To address this question, we used six T cell hybridomas reactive with clonotypically unique epitopes in the 68-86 region of MBP to determine whether these hybrids required the same or different accessory signals for activation. When cultured with accessory cells (Lewis rat splenocytes) and MBP, all six hybrids were induced to secrete IL-2. Two observations indicated that these hybrids represented either of two exclusive subsets of T helper cells (designated THYB-1 and THYB-2). First, during a four hour preincubation of accessory cells with MBP, signals required for the activation of THYB-1 hybrids remained stable whereas those required by THYB-2 hybrids decayed rapidly. Second, radioresistant adherent cells were both necessary and sufficient for MBP-induced activation of THYB-1 hybrids. In contrast, both radioresistant adherent cells and radiosensitive nonadherent cells were required for MBP-induced activation of THYB-2 hybrids. These data indicate that at least two distinct subsets of MBP(68-86) reactive T helper cells can be defined by their distinct requirements for accessory cell signals. (Supported by a Postdoctoral Fellowship from the National Multiple Sclerosis Society, NIH grant AI-19273, and the Mulvihill Family Foundation.)

156.13

^{3}H-SPIPERONE LABELS SIGMA RECEPTORS IN LYMPHOCYTES. T. Coccini* and L.G. Costa. Department of Environmental Health, University of Washington, Seattle, WA

^{3}H-spiperone binds to dopamine D2 receptors in striatum and under the assumption that it labels the same receptors in lymphocytes, this binding site has been suggested as a biological marker for schizophrenia. We confirmed the presence of a specific, saturable and reversible binding of ^{3}H-spiperone to rat and human lymphocytes. However, although some dopaminergic drugs such as haloperidol, spiperone and pimozide were potent inhibitors of ^{3}H-spiperone binding, other specific dopaminergic compounds such as dopamine, apomorphine, sulpiride, eticlopride had minimal effects. The stereospecificity of butaclamol was the opposite of that expected for dopamine receptors. Serotoninergic drugs were weak inhibitors of ^{3}H-spiperone binding. On the other hand, drugs specific for the sigma receptors, such as pentazocine, SKF 10,047 and DTG were potent inhibitors of ^{3}H-spiperone binding in lymphocytes. Sigma receptors in lymphocytes were also identified with ^{3}H-DTG and ^{3}H-haloperidol: their binding characteristics are comparable to those of sigma receptors in brain tissue (cerebellum). Sigma receptor density in lymphocytes was similar with the three ligands (values of Bmax=77-95 fmole/10^6 cells), and a good correlation was found in a group of volunteers between the binding of ^{3}H-spiperone and ^{3}H-DTG. These findings indicate that sigma receptors are present in lymphocytes and that ^{3}H-spiperone binding in these cells occurs to these sites and not to dopamine D2 sites. (Supp. in part by grants from NIEHS, ES-04696, and Fondazione Clinica del Lavoro, Pavia).

156.14

AUTORADIOGRAPHIC LOCALIZATION OF BETA-2-ADRENERGIC RECEPTORS IN THE RAT THYMUS: ONTOGENESIS AND HORMONAL CONTROL. *B. Marchetti, *M.C. Morale, *U. Scapagnini, ▲G. Pelletier (SPON: M.A. Kling). Dept. of Pharmacology, University of Catania, 95125 Italy and ▲Molecular Endocrinology, CHUL, Quebec, G1V 4G2, Canada.

A substantial body of evidence suggests a feedback between the thymus and the brain, accomplished via direct innervation, thymic peptide secretion and endocrine-like pathways. We have used the potent beta adrenergic antagonist,iodocyanopindolol, to characterize and localize specific beta adrenergic receptors (ADR-R), of the beta-2 subtype, during thymus development and maturation of cell-mediated immune response. The density of beta ADR-R increases progressively between birth and 70 days of age, reaching half maximal levels at 17 days and a plateau at 40 days after birth. Castration sharply reduced the density of ADR-R, this effect being counteracted by estradiol alone or in combination with progesterone. T-cell blastogenic response to isoproterenol varied as a function of sexual maturity. Present data indicate that thymic ADR-R are involved in a regulatory system and suggest a clear interdependence between the development of neurendocrine and immune systems.

156.15

EFFECT OF PERINATAL THYMECTOMY ON ADULT SEXUAL BEHAVIOR. D.F. Bloom*, G.J. Bloch*, and R.A. Gorski (SPON: C. Duchala). Dept. Psychol., Dept. Anat./Cell Biol.; Lab. of Neuroendo., Brain Research Institute, UCLA, L.A., CA 90024.

The presence of the thymus appears necessary for the normal development of the reproductive axis, as perinatal thymectomy (thyX) of the female rodent results in ovarian dysgenesis and premature cessation of ovulation. The critical period for thyX coincides, perhaps significantly, with the critical period for brain sexual differentiation that results in sex-appropriate gonadotropin release and behavior. Thus far, no attention has been given to the effect of thyX on behavioral aspects of reproduction. Eight Long-Evans rat pups were thymectomized on day 3 of life; 11 (controls) were sham-thymectomized. All animals were ovariectomized in adulthood, and 2 wks later each received (s.c.) 2 ug of estradiol benzoate (EB) on day 0 and day 1 (day of test 1); 5ug EB was injected on day 2, and 2 ug EB was injected on day 3 (day of test 2). Test 3 was performed on day 4. On day 5, all rats were injected with 250ug of progesterone 5 hrs before test 4. For each test, a lordosis quotient (LQ, # responses per 20 male mounts X 100) was computed. On test 1, only 1 thyX rat (LQ=10%) and 2 control rats (LQ=20% & 40%) performed. With experience and repeated estrogen administration (test 2), behavior increased, and thyX animals revealed significantly lower ($p=.018$) LQ's than did controls (28.8±7.9% vs 64.5±11.1%). This trend continued (20±5.3% vs 65.5±9.2%, $p=.002$) on test 3. On test 4, all rats, except for 2 thyX animals (LQ's=10% & 90%) showed LQ's of 100%, revealing the physical capability of thyX females to perform high levels of lordosis. The apparently lowered estrogen sensitivity may result from the effect of thyX on the development of neural substrates of behavior.

156.16

SYNERGY OF cAMP PRODUCTION IN LYMPHOCYTES WITH DUAL BETA-ADRENERGIC AND MITOGENIC STIMULATION. S.L. Carlson, K. Trauth* and T.L. Roszman*, Dept. of Microbiology and Immunology, Univ. of Kentucky Medical Center, Lexington, KY 40536

Many reports have shown that manipulation of the autonomic nervous system can alter immune function via stimulation of the ß-adrenergic receptor (ßAR) on lymphocytes. To examine the mechanism of adrenergic effects on lymphocytes, we have studied the generation of second messenger signals within human T cells as a result of ßAR and mitogenic stimuli. The ßAR signals via the cAMP second messenger system whereas mitogens signal via the phosphotidylinositol pathway. We have found that stimulation of lymphocytes with both a ß-adrenergic agonist (isoproterenol, ISO) and mitogen (phytohemagglutinin or anti-T3 monoclonal antibody) results in a synergistic rise in cAMP compared to ISO alone. This effect is observed whether the mitogen is added several minutes before or after the ISO. In addition, the synergy follows a dose response depending on the concentration of mitogen added. Not only is cAMP accumulated more rapidly in T cells exposed to mitogen, but the levels of cAMP remain elevated for several hours compared to ISO stimulation alone. Both $T8^+$ and $T4^+$ T cells show a cAMP synergy with mitogen, however the absolute level of cAMP is higher in $T8^+$ cells, consistent with the higher number of ßAR expressed on these cells. Current studies are focused on determining the mechanism of the synergy. Stimulation of the ßAR results in a rapid desensitization of the ßAR. Receptor binding studies indicate that T cells incubated in the presence of the mitogen and ISO have less ßAR desensitization than cells incubated with ISO alone, which may be involved in the mechanism of the cAMP synergy. Other experiments have also suggested that increased intracellular calcium as a result of the mitogenic stimulus may be involved in the observed synergy.

(Supported by NS-17423 and 1 F32-NS08591)

156.17

THE CHARACTERIZATION OF NEURORECEPTORS DURING DYNAMIC PHASES OF IMMUNOCYTE DEVELOPMENT. K. Bulloch[1], T.I. Bonner[*2], R. Cassin[*3], D. Darko[*1], T. Rodojcic[*1] and M. Roman[*1]. [1]University of California, San Diego, La Jolla, CA 92093 [2]NIMH, Bethesda, MD 20982, [3]Helicon Foundation, San Diego, CA 92109.

The innervation of lymphoid tissues and organs implies that there are neuroreceptors on immunocytes that are capable of transducing signals from the nervous system to the immune system. We have focused our research on characterizing the types and function of these receptors at the cellular level as a first step in understanding the mechanisms of neuroimmune interactions. In order to gain a better understanding of the expression of beta adrenergic receptors (BAR) during the life cycle of a specific class of immunocyte, we have employed in vitro assays mimicing the in vivo phases of cell proliferation and differentiation. In an assay where Con A was used to stimulate thymocyte and splenic T-cell proliferation, BAR receptors were up regulated without changes in the Kd. PMA plus Ca^{2+} induced maturation of the monocyte cell line U937 down regulates their BAR receptors (Bmax) without affecting their affinity (Kd). Agonist induced levels of c AMP are proportionally reduced in PMA treated cells. Similar changes in BAR's Bmax are observed with PMA treatment of the thymocyte cell line, BW5147, as well as fresh thymocytes. However, no changes were observed in agonist stimulated c AMP levels. In characterizing the lymphoid muscarinic acetylcholine receptor (M AChR) on a B cell lymphoma, we have now demonstrated that carbachol specifically inhibits division. This phenomenon is not due to agonist toxicity. Using Northern Blot analysis our data indicates that the m-2 and m-5 M AChR probes hybridize to 1.9 kb and 3.0 kb mRNA isolated from this cell line. The results of these experiments advance our knowledge of the structure and function of the innervation and receptor fields of the immune system in dynamic models of normal physiological states as well as providing avenues for the development of new cancer therapies. Supported by a gift from the Kettering Foundation and grant #N00014-89-J-1256 of the ONR.

156.18

MODULATION OF ALVEOLAR MACROPHAGE FUNCTION BY NEUROPEPTIDES. I.Lemaire, M. St-Jean and S. Ouellet. (SPON: S. Lemaire). Lab. of Immunopharmacology, Dept. of Pharmacology, Fac. Health Sci., Univ. of Ottawa, Ottawa, Ont. Canada K1H 8M5.

Accumulating evidence suggest bidirectional communication between the nervous and the immune system. Alveolar macrophages (AM) present in the lung play a major role in immune reactions and inflammation. Since mediators of the nervous system are also present in the lung, we investigated the effects of neuropeptides on AM functions. Neurotensin (NT) (10^{-10}-10^{-6}M) as well as bombesin (BN) (10^{-9} -10^{-6} M) caused a significant enhancement of interleukin-1 (IL-1) production by activated AM whereas vasointestinal peptide (VIP) (10^{-9} -10^{-6} M) inhibited LPS-induced IL-1 production. NT also enhanced over the same range of concentrations tumor necrosis factor-α (TNF-α) production by activated AM. Furthermore, NT caused an increase in the proportion of large and mature macrophages as evidenced by AM morphology and sedimentation on Percoll density gradient. Our data demonstrate that neuropeptides present in the lung can exert dual regulation of AM functions associated with inflammatory response. (Supported by MRC).

SUBCORTICAL SOMATOSENSORY PATHWAYS I

157.1

BRAINSTEM PROJECTIONS TO SOMATOSENSORY THALAMUS IN THE MONKEY. H.J. Ralston and D.D. Ralston Department of Anatomy, University of California, San Francisco, CA 94143.

The somatosensory thalamus of the monkey receives projections from the dorsal column-lemniscal system (DCN), the spinothalamic tract and the trigeminal system (V), all of which interact with thalamocortical relay cells (TCR) as well as the GABAergic interneuronal population. These afferents are involved in both simple and complex synaptic arrays, exciting and modulating the output of the TCR cells. In addition, there are presumed to be afferent inputs from several other neuronal groups in brainstem which utilize various transmitters, including norepinephrine (NE), acetylcholine (ACh) and serotonin (5HT) to regulate the transfer of afferent information through the thalamus. These brainstem afferent projections to the thalamus have been shown in several mammalian species, but have been little studied in the primate.

In this study the somatosensory thalamus of the monkey (*Macaca fascicularis*) was physiologically characterized and injected with 5% wheatgerm agglutinin-horseradish peroxidase (WGA-HRP) or WGA-apoHRP-gold under sterile neurosurgical conditions. The animals were cared for using N.I.H. and U.C.S.F. Animal Care Committee guidelines. Subsequently, brainstem sections were processed to demonstrate retrogradely labeled neurons and some specimens processed for immunocytochemistry. In addition to labeling in DCN,V and nucleus solitarius, there was extensive labeling found in mesencephalic, pontine and medullary reticular formation. In particular, there were numerous labeled neurons in NE cell groups (locus coeruleus), cholinergic nuclei (nucleus cuneiformis) and neuronal groups containing 5HT (dorsal raphé). Thus, the primate somatosensory thalamus receives projections from brainstem neuronal nuclei which contain neurotransmitters shown in other species to have profound effects on the firing properties of thalamic neurons, influencing the transfer of information by thalamic neurons to the cortex.
Supported by NS21445 and NS23347 from N.I.H.

157.2

ORIGINS OF CALCITONIN GENE-RELATED PEPTIDE (CGRP) IMMUNOREACTIVITY IN THE RAT SOMATOSENSORY THALAMUS. S.C. Ahlgren*, P.T. Ohara and A.M. Williamson. Dept of Anatomy, University of California, San Francisco, CA 94143.

Small injections of WGAapoHRP-Au (Basbaum A.I. and Menetrey,D., J.Comp.Neurol., 261:306, 1987) were made in lateral or medial thalamic nuclei. After 2 days survival the animals were perfused and reacted to visualize the WGAapoHRP-Au, then processed for immunocytochemistry using a primary antibody to CGRP (Sternini,C. and Brecha, N.C., Gastroent., 90:1155.). A second series of injections using PHA-L were made into various brainstem sites known to contain CGRP-positive cell bodies to determine thalamic projections from these sites.

As previously shown, CGRP immunoreactivity is largely confined to medial thalamic nuclei with nucleus submedius being heavily labelled. Following WGAapoHRP-Au injections into medial thalamic nuclei double-labelled cells are found in the medial and ventral portions of the parabrachial nucleus and in laminae I and II of the trigeminal nucleus caudalis. Injections into lateral thalamic nuclei resulted in some double labelled cells in the nucleus caudalis.

These results show that medial thalamic nuclei implicated in affective aspects of nociception receive CGRP innervation from several brainstem sites.

Supported by NS21445 and NS23347 from NIH

157.3

Distribution of GABA terminals on identified thalamocortical neurons in the VPL nucleus of the cat. X.-B. Liu*, C.N. Honda and E.G. Jones Department of Anatomy and Neurobiology, University of California, Irvine, California 92717. Spon. E. Davis, Jr.

We have used immunocytochemistry on vibratome sections containing thalamo-cortical relay cells, identified by intracellular recording and injection of HRP, to map the distribution of GABA synapses on the relay cells, after re-embedding and thin sectioning for electron microscopy.

Dense DAB reaction product in injected cell profiles contrasted with the less densely stained immunocytochemically defined GABA terminals. Serial thin section analysis confirmed the separate origins of the two types of profile. GABA terminals with features of presynaptic dendrites and presumed to derive from intrinsic neurons made symmetrical type synapses mainly on middle and distal segments of relay cell dendrites. More proximal dendrites and the cell soma received GABA positive terminals, not identifiable as presynaptic dendrites. These may derive from axons of reticular nucleus cells and of intrinsic neurons.

Supported by NIH grant NS22317.

157.4

ELECTROPHYSIOLOGICAL STUDY OF SPINO-THALAMIC INPUTS TO VL/VA. C.-T. Yen and E.G. Jones. Dept. of Zoology, National Taiwan University, Taipei, Taiwan, ROC and Dept. of Anatomy and Neurobiology, University of California, Irvine, CA 92717, USA.

The ventrolateral and ventroanterior nuclei of the thalamus (VL/VA) are known to receive direct inputs from spinothalamic tract fibers. This connection was studied by intra- and extracellular physiological techniques in cats. The animals were anesthetized with ketamine and barbiturates, and paralyzed with gallamine triethiodide. Their body functions were maintained within physiological ranges. Glass micropipettes filled with a buffered HRP solution were used to record from VL/VA neurons. Tungsten microelectrodes were used to activate the ipsilateral and contralateral spinothalamic pathways at the level of C2/3. Fifty-nine VL/VA neurons were recorded, 13 responded to spinal cord stimulation. Of these, 7 were inhibited and the other 6 had short latency excitatory responses. Their initial latencies varied from 3 to 10 msec (mode 6 msec). In experiments in which stimulation electrodes were also placed on the primary motor cortex, thalamocortical (TC) cells were identified. A small number of these cells responded to cord stimulation with 3 msec latency. This response could follow repetition rate higher than 20 Hz, indicating a monosynaptic connection between the spinothalamic tract fibers and some TC cells.

Supported by NIH grants TW03905 and NS22317.

157.5

THE RACCOON SPINOCERVICAL AND SPINOTHALAMIC TRACTS: AN HRP STUDY. Benjamin H. Pubols Jr., and John H. Haring. Good Samaritan Hospital & Medical Center, Portland, OR 97209, and St. Louis University, St. Louis, MO 63104.

In different raccoons, either the lateral cervical nucleus or the thalamic ventrobasal complex was injected with 1-2% solutions of WGA-HRP. Following 24 h or 4 d, respectively, the animals were sacrificed and both injection and target sites (spinal cord segments C_6-T_2) were processed using the TMB method. All labelled cells were counted in every fifth 50 μm section.

All labelled spinocervical tract (SCT) cells were located ipsilateral to the injection sites. Most were in laminae III and IV, with a few located more deeply or in lamina I. 50% of labelled cells were located in the medial 1/3 of the dorsal horn, the region of representation of the glabrous surfaces of the forepaw (Pubols, Hirata, and West-Johnsrud, 1989). The mean number of labelled SCT cells/section was 4.63.

75% of spinothalamic tract (STT) cells were located contralateral to the injection sites. Of these, 43% were in lamina I, the remaining 57% in laminae III-VI. Only 20-25% were located in the medial 1/3 of the dorsal horn. The mean number of labelled STT cells/section was < 0.50.

We conclude that the SCT plays a more critical role than does the STT in relaying tactile information from the glabrous surfaces of the forepaw to higher centers of the raccoon somatosensory system. [Support: NS-19486, USPHS.]

157.6

PRETECTAL NEURONS RELAY SPINAL INPUT TO CAT MOTOR THALAMUS. R.Mackel,H.Asanuma,A.Iriki* and E.Jorum*. The Rockefeller University, New York, NY 10021.

Lesioning the pretectal area (PT) abolishes spinal input to thalamic VL neurons (1). To confirm that PT neurons convey the spinal input, extracellular recordings were made from PT neurons identified as projecting to VL.

Experiments were performed on 10 cats anesthetized with chloralose. For antidromic stimulation of PT neurons, an array of stimulating electrodes was placed in VL. Spinal input was studied by stimulating the dorsal column(DC) and spinothalamic tract(ST) in the cervical spinal cord. Stimulation and recording sites were marked by lesion.

A total of 117 PT neurons were antidromically identified by their fixed latency, all-or-none responses and ability to follow high frequency trains. 62/86 PT cells (72%) tested for spinal input were activated by stimulation of DC and/or ST afferents. Of these neurons 56% received input from both spinal sources, 36% from DC only and 8% from ST only. The range of orthodromic latencies was wide (3-20ms). The PT neurons were located caudo-laterally in PT and projected to more caudal parts of VL.

These data indicate that PT neurons which project to motor thalamus process a considerable amount of somatosensory information which could play an important role during movement execution. (1) Brain Res (1989),47, 135-139.
The study was supported by NS-10705 and NS-26288.

157.7

GLUTAMATE IMMUNOREACTIVITY IN IDENTIFIED TERMINALS IN THE VENTRO POSTERO LATERAL NUCLEUS OF THE RAT THALAMUS. R. Spreafico*, A. Amadeo* and S. De Biasi. Dip. Fisiologia Ist.Neurologico "C.Besta" and Dip. Fisiologia e Biochimica generali, Sez.Istologia e Anatomia umana, Milano, Italy.

Although the synaptic organization of the ventro postero lateral (VPL) nucleus of the rat thalamus has been extensively studied, little is known about the neurochemical content of its terminals. In the present work experiments were carried out in rats a) to investigate whether the medial lemniscal pathway, the spinothalamic tract and terminals from brainstem nuclei may use glutamate (Glu) as neurotransmitter; and b) to study the relationship of the identified profiles with GABA immunoreactive terminals. Ultrastructural identification of synaptic terminals was performed by injecting WGA-HRP in the dorsal column nuclei, in the dorsal spinal cord and in the brainstem of deeply anesthetized rats. Single and double immunogold staining was then performed on thin sections of VPL from the injected rats, using polyclonal anti-Glu and anti-GABA sera. All the anterogradely labeled terminals are large and mainly contact proximal dendrites of VPL neurons. Most of these HRP-labeled terminals are stained by the anti-Glu serum. Glu-immunoreactivity is also in some small terminals not labeled by HRP, that contact distal dendrites. None of the HRP-labeled or Glu-positive terminals is immunoreactive for the anti-GABA serum, which stains medium sized boutons synapsing on proximal and distal dendrites.

157.8

MISMATCH BETWEEN NEURONAL RECEPTIVE FIELDS AND PROJECTED FIELDS EVOKED BY MICROSTIMULATION IN THE SENSORY THALAMUS OF PATIENTS WITH SPINAL CORD INJURY. F.A. Lenz, J.O. Dostrovsky, L. Lee', R.R. Tasker'. Dept. of Neurosurg., Johns Hopkins Hosp., Baltimore, MD. 21205 Dept. of Physiol. & Neurosurg., U of Toronto, Canada.

The sensory thalamus was explored in patients with central pain following spinal cord injury to determine the optimal site for implantation of deep brain stimulating electrodes for the treatment of pain. The same procedure was carried out in a control population of patients operated for treatment of dyskinesia. Receptive fields of thalamic single neurons were determined by standard methods. At the same sites where neurons were recorded, microstimulation was carried out to determine the part of the body where microstimulation (<25uA) evoked sensations (projected field). Receptive fields and projected fields were determined at 38 sites in 3 patients with spinal cord injury. In these patients, regions of the thalamus that represented parts of the body distant on the thalamic homunculus from the area of sensory loss were defined as 'unaffected' regions. Regions of thalamus that represented parts of the body adjacent to the area of sensory loss often exhibited increased representations of those parts of the body and were defined as 'affected' regions of thalamus. In control patients and in unaffected regions of thalamus in patients with spinal-cord injury there was usually a good match between receptive and projected fields. However, in affected regions of thalamus there was frequently a mismatch between receptive and projected fields. Projected fields were often referred to parts of the body where the patient experienced anesthesia as a result of the spinal injury. The activity of cells located affected regions of thalamus may be responsible for sensations referred to the anesthetic part of the body.

157.9

RELATIONSHIP OF THALAMIC NEURONAL RESPONSES AND MICROSTIMULATION EVOKED SENSATIONS ELICITED AT THE SAME SITES IN MAN. L. Lee*, J.O. Dostrovsky, R.R. Tasker* and F.A. Lenz (SPON: M.D. McClean). Dept. Physiology and Div. Neurosurg., Univ. of Toronto, Canada, and Dept. Neurosurg., Johns Hopkins Univ., Baltimore, MD, USA.

The use of both microstimulation and microrecording during stereotactic thalamotomy procedures provides a unique opportunity for correlating evoked thalamic neuronal responses with sensations evoked by electrical stimulation at the same site. In the present study thalamic ventrobasal complex neurons responding to sensory stimulation were recorded at 518 different sites in a total of 80 different electrode trajectories in 22 patients undergoing stereotactic thalamotomy for motor disorders. Most of the neurons had small cutaneous receptive fields (RFs) on the digits, lips and face. There were fewer neurons with RFs on other body regions such as leg, arm and torso and their RFs were larger. Stimulation at all of these recording sites (1 second, 300Hz, trains of 0.1ms pulses, <25uA) induced sensations (usually tingling) referred to a part of the body (projected field). In the well represented hand and face regions microstimulation usually resulted in identical or overlapping projected and receptive fields. However when the receptive fields were on less well represented regions of the body such as torso or leg the projected field was frequently different from the receptive fields of the neurons recorded at that site. The current thresholds necessary to evoke sensation were also usually higher at those sites where there was a large mismatch between the receptive and projected field. These findings indicate that the receptive and projected field sizes are inversely related to the degree of representation of that body region and that there is usually an excellent correspondence between the neuronal RFs and the projected fields in well represented regions.
(Supported by the Canadian MRC and Parkinson's Foundation)

157.10

BROADLY DISTRIBUTED INHIBITION IN VENTROPOSTERO-LATERAL NUCLEUS OF RAT. W.A. Roberts* and J. Wells. (SPON: W. W. Pendlebury) Department of Anatomy and Neurobiology, University of Vermont, Burlington, VT 05405.

We have studied the extracellular evoked response of single ventroposterolateral nucleus neurons using stimulation of brachial plexus and sciatic nerve. Thirteen percent of VPL projection neurons were responsive to both forelimb and hindlimb inputs. We also demonstrated the existence of mutually inhibitory interactions between inputs from forelimb and hindlimb in 45% of VPL units. The map of VPL we have obtained using direct nerve stimulation differs from that obtained with modality specific inputs in that a somatopically overlapping distribution was obtained. By appropriately timing the delivery of stimuli, forelimb inputs caused the inhibition of responses to hindlimb stimulation, and similarly, hindlimb inputs inhibited responses to forelimb stimulation. The inhibition had a duration that was variable and may reflect a combination of mechanisms including recurrent inhibitory collateral input from the thalamic reticular nucleus or an intrinsic hyperpolarizing inhibitory after potential of the VPL neuron. The responsiveness of VPL neurons to both forelimb and hindlimb inputs and an inhibitory correlate to this overlapping of inputs may explain the shifting of VPL maps following lesions of peripheral nerve, spinal cord or DCN.

157.11

LESIONING THALAMIC POSTERIOR GROUP (PARS MEDIALIS) IMPAIRS FOCUSED ATTENTION AND ONE OF ITS ECOG INDEX IN CAT M.H.Canu*, C.Durand*, M.F.Montaron*, J.J.Bouyer, A.Rougeul* and P.Buser*. *Institut des Neurosciences, Département de Neurophysiologie comparée, CNRS-Université P. & M. Curie, 9 quai Saint Bernard, F-75005 Paris.*

In the cat as in the monkey, motionless behavioural states of focused attention upon a target are accompanied by short volleys of fast electrocortical (ECoG) rhythms (ca 40 Hz) designated as "beta rhythms". Fronto-parietal beta rhythms mainly develop in two foci, one on the motor cortex, the other in the associative posterior parietal cortex (area 5a). The latter focus depends upon a thalamic rhythmic center located within the posterior thalamic nuclear group (pars medialis, POm). This issue was as yet mainly based upon simultaneous electrophysiological recordings from the thalamus and the cortex, including computing correlation and coherence functions between global rhythms and also comparing cortical waves and thalamic spikes using a "wave triggered" recording. We have now considered the effects of kainic acid lesions of the thalamic site. In the present study, kainic acid was injected bilaterally into the POm. The lesions have as yet been partial; however, very clear changes were observed concerning both ECoG and behaviour. The lesioned animals displayed for their behaviour an unusual distractibility : instead of remaining motionless during at least 60 mn with attention focused on the target (a mouse in a transparent box placed in front of them), their immobility was interrupted by numerous episodes of motor activity. Simultaneously, the beta rhythms, which appear only during attentive immobility, became much less frequent and their amplitude was reduced. These results support our previous findings and our hypothesis that integrity of the POm neurons (assuming that kainic acid mainly destroys cells and not passing fibres) both generate beta rhythms and are in some way governing attentive immobility. Other data from our group have indicated that the POm-parietal 5a cortex system is under a gating influence from the mesencephalic ventral tegmental area.

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157.12

A GOLGI AND WGA-HRP STUDY OF THE RETICULAR THALAMIC NUCLEUS (RTN) OF THE RAT.

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Aim of the present study is to characterize the morphology of different cells types of RTN and to relate these data with those obtained by the axonal transport of WGA-HRP. Twenty rats' brains were used for silver impregnation methods by means of a Golgi-Kopsch technique, and cut in both coronal and horizontal 100 µm thick sections. In ten other animals WGA-HRP was injected in different areas of the somatosensory cortex and in the thalamus. Three morphologically different neurons were found in RTN: round-shaped neurons (R), with multidirectional dendritic arborization, large fusiform neurons (F), with dendrites arborizing in the horizontal plane; small fusiform neurons (f), mainly located in the medial and lateral border of RTN, with dendrites arborizing in the vertical plane. All cellular types were labeled after thalamic injections of WGA-HRP. Only labeled terminals were found in RTN after S_1 injections of the enzyme; they predominate in the most medial and lateral borders of the nucleus. It seems reasonable to conclude that axon collaterals of cortico-thalamic neurons synapse preferencially on the vertical dendritic arborization of "f" neurons. These data suggest a peculiar and precise intrinsic organization of RTN, subserving a more complex functional arrangement between the thalamus and the cortex in the rat.

157.13

A HIERARCHICAL NETWORK SIMULATION FOR MODELING INTERACTIONS IN THE SOMATOSENSORY PATHWAY. J.P. Utz and J.K. Chapin. Dept. of Physiol. & Biophys., Hahnemann Univ., Phila., PA 19102-1192.

A neuronal network simulation program has been developed as an aid for investigation of the mechanisms of information processing in the mammalian somatosensory system. Computation was done in C under Unix System V/68 on a 16 MHz MC68020 microprocessor. Initial studies have examined feedforward propagation of "sensory" volleys through a 5 layer serial network. Each layer contains a 12x12 array of neurons (720 total), each of which receives convergent excitatory input from 25 radially arranged neurons in the previous layer. The neurons in the first layer receive pseudorandom excitatory input, providing a background "noise". Neuronal characteristics were modeled using difference equations with initialized constants (e.g. membrane time constant, threshold and conductance decay constants) to calculate state variables (e.g. membrane potential, ion conductances, threshold, spike accommodation). Output of the network was recorded as membrane potential, and spike and synaptic events occurring at each neuron every cycle (1 ms). Parametric studies of this simplified network have shown that forward propagation through the network layers require sufficient: 1) background excitation of neurons, 2) synaptic convergence on target neurons, and 3) synaptic efficacy. Propagation of input volleys through the layers cannot occur in the absence of these factors, even if single input neurons fire rapidly. On the other hand, increasing these factors results in an amplification of the signal as it propagates forward through the layers. These results emphasize the importance of these factors in determining the threshold of transmission of sensory information, and also its pattern and amplification through successive layers. *Supported by PHS grants NS26722, AA06965, and AA00089.*

157.14

SOMATOSENSORY TRANSMISSION THROUGH THE LEMNISCAL SYSTEM DURING MOVEMENT IS FACILITATED AND THEN SUPPRESSED: VELOCITY DEPENDENCE . H.-C. Shin, B.K. Jin and J.K. Chapin, Dept of Physiology/Biophysics, Hahnemann U., Phila., PA 19102

We have previously shown that cutaneous sensory transmission from the forepaw to single neurons in the rat somatosensory (SI) cortex is strongly suppressed during treadmill locomotion. To determine the level in the lemniscal somatosensory system at which this modulation may occur, we have here simultaneously recorded single neurons in the forepaw areas of the cuneate nucleus (nC), ventroposterolateral (VPL) thalamus, and the SI cortex in awake rats. Modulation of sensory transmission to these cells was tested by stimulating through electrodes chronically implanted under the skin of the palmar forepaw. Evoked unit responses to this stimulation were measured during resting behavior as a control, and during three standardized speeds of locomotory movement: Slow (1.0 steps/s), Medium (1.5 steps/s), and Fast (2.0 steps/s). These studies confirmed the strong suppression of neural responses in the SI cortex to such stimuli during movement. However, neural responses in the nC were facilitated, and in the VPL were similar to control. Furthermore, at all levels, greater speeds of movement produced increased sensory suppression. Thus, responses of neurons in the nC were facilitated by $+59.4 \pm 29\%$, $+54.9 \pm 7\%$, and $+23.6 \pm 38\%$ during slow, medium, and fast running speeds, respectively. Responses of neurons in the VPL were facilitated by $3.9 \pm 11\%$ during slow running, but were suppressed by $-7.3 \pm 6\%$ during fast running. By contrast, in the SI cortex neuronal responses were suppressed by $-44.2 \pm 3\%$, $-55.9 \pm 3\%$, and $-55.8 \pm 5\%$ during slow, medium, and fast running respectively. These results suggest that afferent feedback ascending through the lemniscal somatosensory system during movement may be first facilitatated in the dorsal column nuclei before being suppressed at thalamic and then cortical levels. *Supported by PHS grants NS26722, AA06965, and AA00089.*

SUBCORTICAL SOMATOSENSORY PATHWAYS II

158.1

RAT TRIGEMINAL GANGLION NEURON RESPONSES TO WHISKER MOVEMENTS IN DIFFERENT DIRECTIONS. S. Lichtenstein*, G.E. Carvell*, and D.J. Simons. Depts. of Physiology and Physical Therapy, University of Pittsburgh, Pittsburgh, PA 15261.

Extracellular recording electrodes were used to study the response properties of 123 trigeminal ganglion cells in barbiturate anesthetized rats. All units displayed single-whisker receptive fields, determined manually. When the distal end of the whisker was displayed 5.7 deg (1 mm) from its neutral position using controlled stimuli, 81% of cells responsed with statistically more spikes/stimulus to movements in 1-3 of 8 cardinal directions than to the others. The more directionally selective the cell, the more vigorous was its response. Seventy-five percent of the cells were slowly adapting, 25% rapidly adapting. A number of quantitative analyses indicated that the former were more directionally selective than the latter. This difference is consistent with the hypotheses of Rice et al. (JCN: 252, 154-174, 1986) concerning structure-function relations in the vibrissal follicle. Specifically, lanceolate endings, which are associated with the ringwulst and mesenchymal sheath, are proposed to mediate less directionally selective, rapidly adapting responses; Merkel cell endings, which are interposed between the rigid glassy membrane and the hair shaft, are proposed to mediate more directionally selective, slowly adapting responses.

Quantitative comparisons between the response properties of peripheral and central neurons in the whisker/barrel system indicate that the afferent signal is progressively and substantially transformed by mechanisms that function to integrate information arising from different peripheral receptors and from different, individual vibrissae. Supported by NS-19950.

158.2

TERMINATIONS OF CERVICAL PRIMARY AFFERENTS ON SPINOTHALAMIC, PROPRIOSPINAL AND SPINOCEREBELLAR NEURONS. P.S. Bolton* and D.J. Tracey, School of Anatomy, University of New South Wales, Kensington NSW 2033, Australia.

The possibility of direct contact between primary afferents and neurons in the upper cervical spinal cord (C1-C4) was investigated in the rat. Primary afferent fibers and their terminals were diffusely filled with horseradish peroxidase (HRP). Neurons in the upper cervical spinal cord projecting to the thalamus, cerebellum and thoracolumbar cord were labelled by retrograde transport of HRP, and putative contacts between primary afferents and the soma or proximal dendrites were identified under the light microscope.

Spinocerebellar neurons were found in the central cervical nucleus (CCN) and in laminae 5-7. Approximately 90% of cells in CCN received apparent synaptic contacts from primary afferents, while 9-16% of spinocerebellar cells in lamina 5-7 received such contacts. This underlines the difference between the spinocerebellar pathways involving CCN and other neurons in the cervical spinal cord.

Propriospinal neurons descending to thoracolumbar levels of the cord (T9-L3) were located in laminae 7-8. Approximately 20-40% of these long descending proprospinal neurons received apparent synaptic contacts from primary afferents. This connection is probably an important component of the pathway mediating neck reflexes.

Spinothalamic neurons were found in three main groups in lamina 1, laminae 4-6, and laminae 7-8. Approximately 8-10% of spinothalamic neurons in laminae 4-6 received apparent synaptic contacts on the soma or proximal dendrites, suggesting that a significant proportion of these neurons are monosynaptically activated by primary afferents.

158.3

MAPPING OF PRIMARY AFFERENTS FROM FORELIMB DIGITS TO THE CERVICAL SPINAL CORD IN THE RAT. S. Maslany*, D.P. Crockett, J. Zhang* and M.D. Egger. Dept. of Anatomy, UMDNJ-Robert Wood Johnson Med. Sch., Piscataway, NJ 08854-5635.

A mixture of 25% free HRP and 2.5% WGA-HRP was injected intracutaneously into individual forelimb digits in 13 adult rats. After 3 days, the rats were perfused transcardially; transverse sections (60-μm thick) were cut through the cervical spinal cord. The tissue was reacted for HRP using the TMB-method.

The labeled cutaneous primary afferents projected to Rexed's laminae I - III, with the densest label in lamina II. The label was distributed in fusiform columns which, at their greatest width, extended from the medial border of the dorsal horn to approximately 50-60% of the distance across it. Projections from digit 1 extended rostocaudally from caudal C4 to caudal C6; projections from digit 5 extended from caudal C6 to rostral C8. Comparisons of the projection maps from each of the five digits indicated that, in the cervical spinal cord, there is extensive overlapping among the cutaneous primary afferent fibers from these digits. Such overlapping projections contrasted sharply with our earlier findings of discrete projections to the cuneate nucleus from the cutaneous primary afferent fibers from each digit.

We also examined projections to the cervical spinal cord and to the cuneate nucleus in adult rats in which some digits had been deafferented neonatally (PD0, n=3; PD2, n=1) or in adulthood (n=2). Labeling from the intact digits in the deafferented rats was similar to that found in the normal adults.

158.4

LOCALIZATION OF INTRINSIC NEUROTRANSMITTERS IN THE SUPERFICIAL FELINE MEDULLARY DORSAL HORN. M.A. Henry[1,2,3], L.E. Westrum[1,2,3], N.A. Nousek-Goebl*[1], and L.R. Johnson[4]. Depts. of Neurological Surg.[1], Biol. Struct.[2], and Restor. Dent.[3], Univ. of Wash., Seattle, WA 98195; and Depts. of Surg. Dent. and Cell and Struct. Biol., Univ. of Colo.[4], Denver, CO 80262.

The localization of neurotransmitters intrinsic to the medullary dorsal horn (MDH) was done as part of a study examining the organization of the trigeminal system. Sections from the caudal medulla were incubated with primary antibodies to neurotensin (NT), met-enkephalin (ENK), serotonin (5-HT), tyrosine hydroxylase (TH) and glutamic acid decarboxylase (GAD), the synthesizing enzyme for gamma-aminobutyric acid. Axons and terminal puncta are labeled by each antibody in specific patterns and densities, whereas NT, ENK, and GAD also show cell bodies, mainly in laminae III and IV. Lamina I and outer II (IIo) show the greatest amount of label in each case, with a reduction in inner II (IIi) and occasional axons or puncta deeper (III-IV). TH and NT label is modest as compared to very dense label for GAD, ENK, and 5-HT. These findings emphasize the importance of the superficial laminae (I and IIo) as specific regions for internal neurochemical modulation of nociceptive stimuli and suggest particular systems (TH and NT) as optimum candidates for studies of deafferentation remodeling by intrinsic pathways. (Supported by NIH grants DE04942 and DE00219. LEW is a research affiliate of the CDMRC.)

158.5

TOPOGRAPHIC ORGANIZATION OF THE DORSAL COLUMN NUCLEI PROJECTION TO THE INFERIOR OLIVE IN MONKEY AND RAT. K.E. Schultze*, H.H. Molinari, and N.L. Strominger. Department of Anatomy, Albany Medical College, Albany, NY 12208

It is known that the dorsal column nuclei (DCN) project to precerebellar structures, such as the inferior olive and pontine nuclei. Studies of the pontine projection have demonstrated distinct species differences in DCN input. A study by Kalil (1979) in primates suggested that the projection to the inferior olive might likewise differ across species. The present study investigated this possibility by examining the topography of the projection from DCN to the inferior olive in cynomolgous monkeys and albino rats.

Wheat germ agglutinin conjugated with horseradish peroxidase was injected into either the gracile or cuneate nucleus. The antero-gradely transported tracer was visualized with tetramethylbenzidine. The projection to the rostral dorsal accessory olive (DAO), where the topographic organization of the DCN input is most distinct, was analyzed in detail and compared with published data from cats.

In contrast to Kalil's results, both the cuneate and gracile nuclei in monkeys were found to project to DAO. The cuneate projection, revealed with injections in the caudal part of the nucleus, terminated primarily in medial DAO. The gracile projection in both monkeys and rats terminated most heavily in lateral DAO. These data parallel findings in cat and suggest that the organization of the DCN projection to the inferior olive does not show significant interspecies variability.

Supported by NSF grant BNS-8809840.

158.6

SYNAPTIC RELATIONSHIPS BETWEEN GABAERGIC TERMINALS AND CUNEOTHALAMIC NEURONS IN THE RAT CUNEATE NUCLEUS. C.Y. WEN, J.Y. SHIEH* and K.N. CHEN*. Dept. of Anat., Col. of Med., Nat'l. Taiwan Univ., Taipei, Taiwan 10018.

Cuneate nucleus comprises several types of neurons in mammals. The major cells of this nucleus were the neurons projecting to the contralateral ventrobasal thalamic nuclei. Synaptic activities are very complicated within the nucleus. It is believed that the inhibitory activity might be mediated by the GABA. Some GABAergic terminals may be intrinsic in the cuneate nucleus. It seems that the intranuclear inhibitory interneurons release GABA as a hyperpolarizing inhibitory neurotransmitter upon cuneate relay neurons.

Young adult albino rats were used for the present study. The cuneothalamic neurons (CTN) were labelled with HRP and GABAergic terminals were identified by postembedding immuno-gold protocal. Retrograde axon transport of HRP study showed that some of the postsynaptic neurons were the CTN, which were large and were rich in cytoplasmic organells. GABAergic terminals and CTN were found forming direct axo-somatic synapse. It may be morphologically a postsynaptic inhibitory basis. [Supported by NSC78-0412-B002-140, ROC].

158.7

LONG ASCENDING UNMYELINATED PRIMARY AFFERENT SYNAPSE IN THE NUCLEUS GRACILIS. K. Chung, J.T. Patterson*, W.T. Lee* and R.E. Coggeshall, Marine Biomed. Inst. and Dept. Anat. & Neurosci., Univ. Texas Medical Branch, Galveston, TX 77550

The dorsal funiculus of the mammalian spinal cord is thought to consist of large primary afferent fibers, but recent studies have shown large numbers of unmyelinated fibers in this path. This study dertermines the presently unknown destination of these fibers. To eliminate all primary afferent fibers in the fasciculus gracilis (FG), anesthetized rats were unilaterally deafferented from the midthoracic cord caudally. One week later, the numbers of unmyelinated fibers in the FG at the 2nd cervical level of the cord were counted in eletron micrographs and compared to normal counts. The numbers of CGRP immunostained (primary afferent marker) unmyelinated axons in the C2 FG and terminals in the nucleus gracilis (NG) also estimated in normal and deafferented rats. The data indicate that 80% of the unmyelinated axons in the C2 FG are removed following dorsal rhizotomy, and thus are presumably ascending primary afferent. Approximately 10% of the unmyelinated fibers were CGRP immunostained and 90% of these were eliminated after deafferentation. The majority of CGRP immunostained terminals in the NG were also eliminated after deafferentation. These data suggest that large numbers of unmyelinated fibers in the FG in rat are long ascending primary afferent fibers and they make synapse in the NG. Thus there is a possible noxious input to the nucleus gracilis. (Supported by NIH NS11255 & NS10161)

158.8

INGROWTH AND DEVELOPMENT OF SOMATOSENSORY AFFERENTS IN THE RAT DORSAL COLUMN NUCLEI. J.R. Norris*, H. Dickinson-Anson*, S. Catalano*, & H.P. Killackey Dept. of Psychobiology, Univ. of California, Irvine, CA 92717

The recently developed carbocyanine, fluorescent tracers (e.g., DiI), which can be used for tracing neural connections in *fixed* tissue, provide a number of advantages for studying the developmental anatomy of neural systems. In the present study, DiI was used to study the ingrowth and development of ascending somatosensory afferents to the dorsal column nuclei (DCN) of rat.

Pre- and postnatal rats were perfused with phosphate buffered, 4% paraformaldehyde. The brainstem and cervical spinal cord were exposed and small DiI crystals were implanted in the dorsal fasciculus of the spinal cord. Following transport times of 1-6 weeks, the brains were removed and sectioned at 75 um with a vibratome. The sections were mounted in buffer, sealed under a coverslip, and examined with an epifluorescent microscope.

Approximately 3-4 days prior to birth, fibers, generally organized into small fascicles of 2-5, exit the dorsal fasciculus at right angles and enter the DCN. Initially, the fibers are relatively straight, simple, and unbranched. Between E19 and the day of birth, few qualitative changes occur, and the fibers are still relatively simple and unbranched in structure. During the first postnatal week, the fasciculation of the fibers becomes less apparent, the course of the fibers becomes more irregular, and the fibers become branched. These results indicate that a significant amount of development of the ascending somatosensory afferents in the rat DCN occurs postnatally. (NSF grant BNS 87-19311)

158.9

DISTRIBUTION AND ORIGIN OF CGRP-IMMUNOREACTIVITY IN THE CAT DORSAL COLUMN NUCLEI. M. Fabri and F. Conti. Institute of Human Physiology, University of Ancona, Ancona, Italy.

Adult cats were used to study the distribution of calcitonin gene-related peptide (CGRP) immunoreactivity in the dorsal column nuclei (DCN). Animals were perfused with 4% paraformaldehyde, and 25 μm-thick vibratome sections were reacted with an antiCGRP serum (Sternini et al., Gastroenterology, 1987).

CGRP-positive fibers and terminals were present in the DCN (mostly in the cuneate nucleus; CN) of all animals: they were denser in the middle than in the caudal and rostral CN. In the middle CN, CGRP-positive fibers were mostly concentrated in the dorsal and ventrolateral portions, sparing the ventral CN. In colchicine-treated animals, CGRP-positive neurons were present in the CN (1.1%) and in the external CN (14.3%), but not in the gracile nucleus. In three additional animals, WGAapoHRP-Au was injected (2 ul) in the CN and dorsal root ganglia (DRG) were embedded in wax, cut in 7 μm-thick sections and reacted first for the retrograde tracer and then for CGRP. DRG neurons were observed which contained both black grains of WGAapoHRP-Au and CGRP immunoproduct.

158.10

SPECIALIZATION OF THE PARATRIGEMINAL NUCLEUS IN THE MUSKRAT MAY RELATE TO THE DIVING RESPONSE.
S. M. DeLisa* and S. O. E. Ebbesson (SPON: D. Feist) Institute of Marine Science, University of Alaska Fairbanks, Alaska 99775,USA.

The apnea, bradycardia and peripheral vasoconstriction of the diving response are elicited upon immersion of the nose. In a preliminary search for primary afferent connections of the pathway mediating the diving response, we traced projections from the rhinarium in muskrats and compared them with projections in rats. Horseradish peroxidase (HRP) and wheatgerm agglutinin-HRP were injected into the skin lateral to the naris. After 48 h survival, perfused brains were removed and frozen, sectioned and reacted with tetramethylbenzidine. The muskrat differed from the rat with respect to one structure, the paratrigeminal nucleus (Pa5). The Pa5 is massive in muskrats and receives a much greater input from the rhinarium in muskrats than in rats. We also found terminal labeling of Pa5 in muskrats following HRP injections in the soft palate and larynx. Stimulation of these structures produces a response similar to the diving response. The intensity of projections to Pa5 from the rhinarium, the overlapping of trajectories from the rhinarium, soft palate and larynx, and the prominence of Pa5 in muskrats, suggest that this structure may play a role in mediating the diving response.

Our findings, combined with the description of the Pa5 as an extension of the marginal zone of the caudal part of the spinal trigeminal nucleus (Gobel and Hockfield 1977), suggest a thermoafferent function for the Pa5 in the production of the diving response. Kilduff *et al.* (1983, 1987) proposed a similar function for the Pa5 in ground squirrels during hibernation, when it is the only structure to increase its relative 2-deoxyglucose uptake. Specialization of the Pa5 for thermoafferent function in these situations may be comparable to specialization of a similar nucleus, the lateral descending trigeminal nucleus, for infrared reception in pit vipers and boids (Molenaar 1974, 1978; Schroeder and Loop 1976).

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158.11

VIBRISSA PRIMARY AFFERENT ARBORS HAVE DIFFERENT PROPERTIES IN ROSTRAL AND CAUDAL PRINCIPALIS AND ROSTRAL ORALIS. J.W. Moran*, W.M. Panneton & M.F. Jacquin (SPON: E.R. Shuter). Dept. of Anatomy & Neurobiology, St. Louis University School of Medicine, St. Louis, MO 63104.

Vibrissae are represented throughout the rat trigeminal (V) brainstem complex, yet metabolic staining and receptive field patterns vary along its rostrocaudal axis. Such intersubnuclear variations may reflect differences in the way single primary afferent axons arborize in different brainstem regions. 4 vibrissa-sensitive primary afferents were stained with HRP. Heavily labeled collaterals in rostral (N=6) and caudal (N=21) principalis (PrV) and rostral oralis (N=6) were compared quantitatively (means ± SD's).

	Rost. PrV	Caud. PrV	Rost. Oralis
# end swellings	6.8±6.6	25.3±16.5	13.7±8.2
# fiber swellings	6.8±4.9	31.1±26.3	9.2±5.3
Arbor area (u^2)	3308±1914	7889±3365	17015±12947
Arbor diameter (u)	62.8±16.2	97.8±21.8	138.9±48.5
Arbor volume (u^3)	41472±44916	156420±130354	272192±226296
Arbor limits in X	57.2±7.5	120.7±39.2	171.7±77.1
Arbor limits in Y	96.5±39.2	118.8±42.4	133.0±32.8

Thus, collaterals from single axons have different properties along the rostrocaudal axis of the rostral V brainstem complex. These data suggest that peripheral and central target factors dictate arbor structure in the V brainstem complex, and that physiological distinctions between rostral V brainstem regions may reflect differing primary afferent arbor morphologies. NIH DE07662, DE07734, HL38471.

158.12

TRIGEMINAL PROJECTIONS TO THE CONTRALATERAL DORSAL HORN ARISE FROM SUPRAORBITAL AND MENTAL NERVES. L. Pavaloti*, W.M. Panneton & M.F. Jacquin (SPON: J. Gibbons). Dept. of Anatomy & Neurobiology, St. Louis University School of Medicine, St. Louis, MO 63104.

Primary afferent fibers in the ophthalmic and mandibular divisions of the trigeminal nerve project into lamina III-V of the contralateral medullary and rostral cervical dorsal horns (Barcia et al., Neurosci. Abst. 13:187, '87). We have previously shown that these crossing fibers do not innervate cornea, dura or nasal mucosa. Retrograde double labeling methods were used to determine if these fibers originate in midline hairy skin. Multiple injections of diamidino yellow (DY) were made into the rat medullary and cervical dorsal horns on the right and a mixture of HRP solutions was injected into the supraorbital nerve on the left (N=2). Up to 94 ganglion cells in a single case were labeled with DY in the ophthalmic portion of the left trigeminal ganglion. 79% (N=105) of these ganglion cells also contained HRP. Their size did not differ from non-crossing single labeled cells (mean diameter of 23.6μ vs 24.8μ). Similar experiments were done to test if fibers in the mental nerve contribute to the crossed primary afferent projection (N=2). Significantly fewer cells were labeled with DY in the mandibular ganglion (N=9) but some (N=3) were double labeled with HRP. These data indicate that a substantial portion of the trigeminal primary afferents that project to contralateral dorsal horn innervate midline hairy skin via the supraorbital nerve. Support: NIH DE07762, DE07734, HL38471.

158.13

PATTERNS OF CONTRALATERAL DEGENERATION AFTER UNILATERAL PRIMARY DEAFFERENTATION OF TRIGEMINAL NUCLEI. L.E. Westrum and M.A. Henry. Depts. Neurosurg., Bio. Struct., and Restor. Dent., Univ. of Washington, Seattle, WA 98195.

As a part of a larger study on anatomical remodeling in the trigeminal nuclei (TN) after different types of trigeminal nerve lesions, we present here observations following complete primary deafferentation with emphasis on contralateral and possible transneuronal changes in the caudal subnuclei. Total unilateral retrogasserian rhizotomy in adult felines was followed by survival times of 3-20 days and the TN was studied by LM silver stains or conventional EM. All survival times showed massive ipsilateral degeneration and in each case contralateral degeneration localized to the partes interpolaris and caudalis. EM preparations revealed axonal and synaptic terminal degeneration in the same sites. Shorter survivals (3-5 days) had degenerated round-vesicle (R) terminals with type I contacts onto dendrites whereas longer survivals showed degenerated flat-vesicle (F) terminals at type II sites. Dendrites showed minimal alterations suggestive of transynaptic degeneration. The findings confirm contralateral terminations which might be initially primary afferents (R terminals) and subsequently second-order afferents (F terminals). This may represent a new form of selective transynaptic change affecting mainly axons with minimal dendritic alterations.
(Supported by NIH Grants DE04942 and DE00219. LEW is an affiliate of the CDMRC.)

158.14

PRIMARY AFFERENT NEUROTRANSMITTER LOCALIZATION IN THE FELINE MEDULLARY DORSAL HORN. L.R. Johnson[1], L.E. Westrum[2], M.A. Henry[2] and N.A. Nousek-Goebl[1,2]. [1]University of Colorado, Denver, CO 80262, [2]University of Washington, Seattle, WA 98195

As a part of studies of primary afferents within the trigeminal nuclei, neurotransmitter localization in the medullary dorsal horn (MDH) is being analyzed. Sections from the caudal medulla of adult cats were incubated with primary antibodies to calcitonin gene-related peptide (CGRP), somatostatin (SOM) and substance P (SP). The isolectin Griffonia simplifolia (I-B4) that co-localizes with FRAP was also used. CGRP and SP labeling is similar although SP labeling shows more discrete terminal puncta while CGRP reveals varicosing axonal arrays. Label is heaviest in the islets of Cajal, laminae I and outer II (IIo). Labeled fibers leave IIo to enter inner lamina II (IIi) and III. Scattered fibers and puncta occur in laminae IV and V. SOM labeling of axons and terminals is heaviest in IIi, reduced in laminae I and IIo, and sparse in III and IV. Labeled cell bodies are present in laminae III-VI. I-B4 labeling of axons and terminals is greatest in lamina IIo and less in IIi, with some axons in laminae I, IV and V. The observed variations in the distribution and character of the axonal and terminal labeling suggest a distinct sublaminar organization of these neurochemically defined primary afferents. (Supported by NIH grants DE04942 and DE00219. LEW is affiliated with CDMRC.)

158.15

CORTICOTRIGEMINAL AXONS AND THEIR ROLE IN INTERPOLARIS RECEPTIVE FIELDS. M. Wiegand*, W.E. Renehan, S. Stansel* & M.F. Jacquin. Anatomy & Neurobiology, Univ. of Louisville Sch. Med., KY 40292 & St. Louis Univ. Sch. Med., MO 63104.

PHA-L and WGA-HRP anterograde tracing methods were used to study corticotrigeminal axons in 22 adult rats. Deposits restricted to SI barrel cortex labeled crossing fibers in the pyramidal decussation that terminated heavily in caudal medullary and rostral cervical dorsal horns laminae III-V, and the caudal half of interpolaris. Moderately dense label occurred in rostral portions of caudalis and interpolaris. A small number of terminals were labeled in the substantia gelatinosa. Pontine terminals arose largely from other fibers that crossed in the pons and provided few terminals in oralis and moderate numbers through all of principalis. A small number of axons did not decussate and ended in all ipsilateral trigeminal subnuclei. Cortical projections were topographic; connections were heaviest between matching whisker representations. Single axon arbors had longitudinal orientations and stringy shapes, with terminal and en passant boutons. Many axons ascended through caudal trigeminal subnuclei and gave rise to multiple collaterals.

The receptive fields of 190 interpolaris cells contralateral to acute ablation of SmI cortex were also studied with single unit recording methods. As with chronic cortical lesions (Neurosci. Abstr. 13:145, '87), most cells had normal response properties. However, a small % of local circuit and projection neurons expressed unusual convergence of tactile inputs. Support: NIH DE07662, DE07734.

158.16

ULTRASTRUCTURE OF CGRP IMMUNOREACTIVITY IN TRIGEMINAL NUCLEUS. N.A. Nousek-Goebl*,[1] M.A. Henry,[1,2,3] L.E. Westrum,[1,2,3] and L.R. Johnson.[4] (Spon: R. Haschke) Depts. Neurosurg.,[1] Biol. Struct.[2] and Restor. Dentistry,[3] Univ. of Wash. Seattle, WA 98195, and Depts. of Surg. Dent. and Cell and Struct. Biol, Univ. of Colo.,[4] Denver, CO 80262

Calcitonin gene-related peptide (CGRP) has been shown to be an important neuropeptide in peripheral sensory systems in general and in dental-trigeminal pathways in particular. We are studying the light microscopic (LM) distribution and fine structural (EM) localization of this neurotransmitter in the brainstem trigeminal nuclei. Special emphasis is on patterns of label in the laminated pars caudalis (PC) and medullary dorsal horn (MDH). LM shows heavy labeling of fibers and especially axonal varicosities with some isolated terminal puncta. Reactivity is greatest in outer layer II, less in I and inner II, and sporadic in lamina III. By EM, label occurs in thinly myelinated and unmyelinated axons, often in clusters. Terminals are frequently labeled and have round synaptic vesicles with type I contacts onto small dendrites. They are sometimes associated with flat-vesicle terminals in a complex. The EM findings confirm an extensive CGRP-positive axonal plexus in specific PC/MDH laminae and identify a class of CGRP synapses consistent with primary afferent terminals. (Supported in part by NIH grants DE04942 and DE00219. LEW is an affiliate of the CDMRC.)

158.17

SOMATOSENSORY NEURONS IN THE INTERCOLLICULAR NUCLEUS OF THE CAT. A.Blomqvist, I.Danielsson* and U.Norrsell*, Dept. Cell Biol., Univ. Linköping, and Dept. Physiol., Univ. Göteborg, Sweden.

The intercollicular region of the midbrain contains neurons which respond exclusively to somatosensory stimuli. The location of these neurons appears to correspond to the nucleus intercollicularis (INC), which receives dense projections from somatosensory nuclei of the brain stem and spinal cord. The purpose of the present investigation was to characterize the response properties and determine the location in the intercollicular region of somatosensory-responsive neurons.

Extracellular sampling of unitary activity was made with tungsten electrodes in the intercollicular region of cats anesthetized with chloralose. Single units were characterized with adequate somatosensory, visual and acoustic stimuli, as well as electrical nerve stimulation by forelimb and hindlimb electrodes, and their stereotaxic coordinates noted. With the aid of the coordinates the units' positions were determined by means of unbiased, cytoarchitectonic identification of the structures which contained the electrode tracks.

Out of 494 units, 138 were activated exclusively by somatosensory stimuli. INC was found to be the only structure in the intercollicular region that exclusively contained neurons solely responsive to somatosensory stimuli. The somatosensory-responsive units in INC had shorter response latencies than somatosensory units outside INC and could follow higher stimulation frequencies.

The findings suggest that INC constitutes a specific mesencephalic structure for somatosensory function.

158.18

CONVERGENCE PATTERNS ON SOMATOSENSORY NEURONS IN CAT SUPERIOR COLLICULUS. H. R. Clemo and B.E. Stein. Department of Physiology, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298-0551.

Somatosensory information reaches the superior colliculus (SC) from a variety of sources. However, the specific convergence patterns that give rise to SC cell receptive field properties are not yet known. To begin examining these convergence patterns, stimulating electrodes were implanted in the contralateral dorsal column (DCN) and trigeminal (nV) nuclei and ipsilateral somatosensory cortex (SIV) in nembutal-anesthetized animals. The receptive fields of SC neurons antidromically activated from any one or combination of these sources were studied.

The majority (57/97; 59%) of somatosensory SC neurons studied received converging inputs from multiple sources. Most (39/57; 68%) of these were activated from both ascending (DCN and/or nV) and descending sources (SIV), but a substantial proportion (18/57; 32%) were activated from the two ascending sources. Regardless of the specific source or number of different inputs a given SC neuron received, in the majority of cases there was always a close correspondence between the afferent receptive fields and that of the target neuron on the SC: afferent receptive fields either matched or were included within the SC receptive field. However, in 26% of the SC neurons studied, afferents that could be demonstrated electrically were not apparent when natural stimuli were used (i.e., SC neurons with facial receptive fields were sometimes activated from the DCN).

Supported by grants BNS 879234 and EY 05554.

158.19

RESPONSE PROPERTIES AND SOMATOTOPY OF VIBRISSA-ACTIVATED NEURONS IN RAT SUPERIOR COLLICULUS. C.-Q. Kao*, J.G. McHaffie & B.E. Stein. Dept. Physiol., Med. Col. Va., Richmond, VA 23298.

Receptive fields (RF's) of vibrissa-activated neurons (n=59) in the superior colliculus (SC) always consisted of multiple (2-12) contiguous contralateral mystacial vibrissae; small rapid displacements of any single vibrissa was sufficient for activation. All were rapidly-adapting, and the majority (91%) required high velocity stimuli. Few exhibited direction selectivity (10%) or spatial summation (32%), and none showed spatial inhibition. Their distribution revealed only a crude somatotopy: upper rows were generally located rostromedial and lower rows caudolateral. Even less topographic fidelity was evident in the columns. Multiple-vibrissa RF's and crude somatotopy are in contrast to the precise vibrissa representation reported elsewhere in the CNS. The data indicate that while these SC neurons are poorly suited for feature detection, they can signal the occurrence and general location of transient stimuli.

Supported by NIH grant EY 05554.

158.20

DISSOCIATION OF TACTILE AND ACOUSTIC COMPONENTS IN AIR-PUFF STARTLE. SEPARATION OF MOTOR AND CARDIOVASCULAR RESPONSES. B.K. Taylor*, R. Casto* and M.P. Printz. Dept. Pharmacology M-036, Univ. Ca. San Diego, La Jolla, CA. 92093

Complex cardiovascular and behavioral (motor) responses result from tactile (air-puff) or acoustic startle stimulation. Motor, pressor, tachycardic and bradycardic responses in conscious, unrestrained, chronically-catheterized rats arise from tactile stimuli (12.5 psi, 100 msec, 30 trials). An acoustic component of this stimulus was characterized (range 84-94 dB between 1-31.5 KHz). To evaluate this component, the tympanic membranes of adult, male, Sprague-Dawley rats were ruptured under halothane anesthesia using standard procedures for stereotaxic placement. Treated rats exhibited minimal response to acoustic startle (116 dB). Importantly, the motor response to air-puff startle was nearly abolished (groups stat. sig. with $F(9,297)=2.75$, $p<.004$; 1st trial control = 66+6.1 units, punctured = 18+6 units). In contrast, there was only minor, non-significant attenuation of the cardiovascular components from control values. Control vs punctured, trial 1: BP = +32.2±2.4 vs +25.0±2.9 mmHg; bradycardia = -18±4 vs -20±6 bpm; tachycardia = 9±3 bpm vs +11±4 bpm.

These results indicate that air-puff startle may contain both tactile and acoustic components. Further, we conclude that the motor and cardiovascular responses may be separated through discriminatory afferent stimuli.

159.1

NEUROANATOMICAL INTERCONNECTIONS OF AVIAN BRAINSTEM LOCOMOTOR REGIONS. D.M.S. Webster, G.N. Sholomenko and J.D. Steeves. Depts of Zoology and Anatomy, U.B.C. Vancouver, Canada, V6T 2A9.

Previous studies have shown that electrical or neurochemical stimulation of localized regions in the avian mid- and hindbrain will evoke locomotion in acute decerebrate geese and ducks. These locomotor sites are situated within the ventromedial medullary and pontine reticular formation, dorsolateral pontobulbar locomotor strip (PLS), medial mesencephalic reticular formation and dorsolateral midbrain reticular formation. The purpose of this study was to examine, using a variety of neuroanatomical retrograde tracers, afferent projections to the locomotor regions of the ventromedial pontobulbar reticular formation and PLS. The major findings were: 1) that the ventromedial pontobulbar reticular formation receives the majority of its afferent input from other regions of the reticular formation; 2) that the PLS projects medially into the ventromedial pontobulbar reticular formation; 3) that the most prominent input from the mesencephalon is from the dorsolateral mesencephalic reticular formation which projects to both the ventromedial pontobulbar reticular formation and the PLS. These results demonstrate that, similar to other vertebrates, rostral brainstem locomotor regions have the appropriate connectivity to exert control over more caudal locomotor sites which, in turn, give rise to direct reticulospinal pathways that are important for the initiation and ongoing control of voluntary locomotion. Supported by NSERC of Canada and the Rick Hansen Man in Motion Foundation.

159.2

SPINAL CORD AND CRANIAL NERVE PROJECTIONS TO LAMPREY BRAINSTEM NUCLEI REVEALED BY USING ANATOMICAL TRACERS IN VITRO. R. Dubuc, Y. Ohta* and S. Grillner. The Nobel Institute for Neurophysiology, Karolinska Institutet, Box 60400, S-104 01, Stockholm, Sweden

The lamprey nervous system is a useful model to study the cellular bases of vertebrate locomotion and other patterns of behaviour. Not only physiological, but also anatomical techniques are important to establish interactions between different cells or cell groups. To trace the inputs to the brainstem from the spinal cord and cranial nerves, anterograde and retrograde tracers (cobalt-lysine, HRP and dextran-amines) were used under *in vitro* conditions. This has the important advantage of providing better control for local application of the tracers. Cobalt-lysine was applied for periods ranging between 18 to 60 hours and axonal transport of the tracer occurred, on an average, at a rate 1.25 cm/day. Dorsal column (DC) fibres were anterogradely labelled within the alar plate. Terminal branches were observed in a longitudinal cell group in the medial margin of the alar plate, extending from just caudal to the obex to the nucleus octavolateralis dorsalis. When cobalt was applied iontophoretically within the posterior rhombencephalic reticular nucleus, cells within the area of termination of DC fibres were retrogradely labelled, as well as cells within the nuclei octavomotorius ventralis and posterior. Cobalt-lysine is a useful anterograde and retrograde tracer in the lamprey nervous system, filling extensively terminals, cell bodies and dendritic arborization, somewhat better than HRP and dextran-amines. (R.D. receives a Centennial fellowship from the Canadian MRC)

159.3

EFFECTS OF 5-HT ON RETICULOSPINAL NEURONS AND DISTRIBUTION OF 5-HT IMMUNOREACTIVE FIBERS IN THE LAMPREY BRAINSTEM. G. Viana Di Prisco, R. Dubuc, P. Wallén* and S. Grillner. The Nobel Institute for Neurophysiology, Karolinska Institutet, Box 60400, S-104 01 Stockholm, Sweden

5-HT affects the locomotor pattern by decreasing the late after-hyperpolarization (AHP) due to Ca^{2+} dependent K^+ channels in spinal neurons in the lamprey (Wallén *et al.*,1989, *J. Neurophysiol.* 61). Immunohistochemical studies have shown the presence of a rich 5-HT innervation both in the spinal cord (Van Dongen *et al.*,1985, *J. Comp. Neurol.* 234:501) and the brain including reticulospinal (RS) neurons (Brodin *et al.*,1986, *Neurosci. Lett.* 67:53). This study investigates the possible role of the brainstem innervation by local extracellular application of 5-HT onto presumed glutamatergic RS neurons recorded intracellularly. 5-HT elicited, in most cases, a small membrane hyperpolarization. Moreover, a specific reduction of the late AHP was observed in some cells without a concomitant hyperpolarization, as in spinal neurons. Cells were subsequently filled intracellularly with Lucifer Yellow and the brainstem was processed for 5-HT immunohistochemistry. Close appositions between 5-HT immunoreactive fibers and 5-HT responsive RS cell bodies and dendrites were observed. Interestingly however, the distribution of labeling revealed the most dense 5-HT innervation within the alar plate and also 5-HT fibers in several cranial nerve trunks.

R.D. receives a Centennial fellowship of MRC Canada.

159.4

RUBROFACIAL PROJECTIONS IN THE CAT. AN ANTEROGRADE L.M. AND E.M. STUDY UTILIZING WGA-HRP AS A TRACER. Gert Holstege and Diane Daly Ralston, Dept. Anatomy, Univ. Calif. San Francisco CA 94143

It has recently been shown that the red nucleus (RN) not only projects indirectly, but also directly to motoneurons innervating distal limb muscles (Holstege 1987 in cat, Holstege et al. 1988; Ralston et al. 1988 in monkey). In the brainstem RN projects also directly to the intermediate facial subnucleus containing orbicularis oculi muscle motoneurons and to a lesser extent to the dorsal portion of the medial facial subnucleus, containing motoneurons innervating ear muscles. There is no agreement whether RN projections terminate in the lateral facial subnuclei, innervating peri-oral muscles. Therefore we investigated RN projections to the intermediate and lateral facial subnuclei at LM and EM levels. In two cats relatively large injections of WGA-HRP were made in RN and dorsally adjacent tegmentum. At the LM level many labeled fibers were observed in the intermediate and dorsal part of the dorsomedial facial subnuclei, but the impression was gained that in the lateral facial subnuclei only fibers of passage were present. At the E.M. level many labeled myelinated axons were found in the lateral facial subnuclei, but only a very small number of labeled terminals. They contained rounded synaptic vesicles and formed asymmetric contacts upon proximal and distal dendrites. On the other hand, the intermediate facial subnucleus contained a very dense population of terminals, at least 200 times as many as the in lateral facial subnuclei. Most terminals were large in diameter, filled with primarily rounded synaptic vesicles and forming asymmetric contacts with the somata and proximal dendrites of the motoneurons. Only occasionally, contacts were seen upon small diameter dendrites. The results indicate that RN has a strong excitatory influence upon orbicularis oculi motoneurons in the same way as its projections to the distal limb inter- and motoneurons. The direct RN influence on peri-oral muscle motoneurons is negligible.

159.5

GABAERGIC SYNAPSES IN THE MACAQUE RED NUCLEUS REVEALED BY IMMUNOGOLD ELECTRON MICROSCOPIC TECHNIQUES. D.D. Ralston and A. M. Milroy. Department of Anatomy, University of California, San Francisco, CA 94143.

The red nucleus of the primate is known to be composed of two divisions, a parvicellular or rostral two thirds of the nucleus and a magnocellular or caudal one third. Both divisions of the nucleus contain a population of small neurons (10-20 µm in diameter) considered to be interneurons, many of which may use gamma aminobutyric acid (GABA) as the neurotransmitter. Although the rat and the opossum contain a population of small neurons within the nucleus, these have not been identified as GABAergic interneurons. However, immunohistochemical analyses in the cat have demonstrated the presence of glutamic acid decarboxylase (GAD) in the red nucleus at a light and ultrastructural level.

In this study we are demonstrating the results of an immunohistochemical analysis of antibodies to GABA conjugated to 10 nm gold particles in the macaque monkey. The animals were cared for following N.I.H. and University of California Animal Care Facility guidelines. Electron microscopic analysis has revealed the presence of small labeled neurons as well as labeled synaptic profiles upon rubral neurons in both divisions of the normal nucleus in the macaque monkey. The majority of the profiles are small to moderate size, contain flattened to pleomorphic vesicles and form symmetric contacts with all regions of the dendritic arbor. Many of these profiles have been shown to be dendritic in origin due to the presence of ribosomes. Occasional GABAergic profiles contain dense cored vesicles. Labeled myelinated axons have also been observed, suggesting the presence of two sources of GABAergic interaction within the nucleus: one from axons of GABA positive neurons; the other from the dendrites of these neurons which form presynaptic dendrites (PSD's).

These results demonstrate the presence of GABAergic interneurons that interact with rubral neurons by means of axonal and dendritic synaptic input.
Supported by NS23347 from N.I.H.

159.6

PROJECTIONS OF THE PARVOCELLULAR RETICULAR FORMATION TO NUCLEI OF THE MEDULLA OBLONGATA. G.J. Ter Horst*, J.C.V.M. Copray*, S.B.R. Liem* and J.D. Van Willigen* (Spon: European Neuroscience Association). Dept. Neurobiology, Univ. of Groningen, Groningen, NL9712KZ The Netherlands.

Anatomical investigations gave evidence that the parvocellular reticular formation (PCRt) is an intermediate area in nervous ciruitry for limbic control of food-intake. In previous studies globally the connections of PCRt with brainstem nuclei for autonomic and orofacial motor control were identified with retrograde tract tracing techniques. Now, we would like to know all projections of PCRt and their precise sites of termination in the nuclei of the brainstem. Therefore, we studied the projections of PCRt in the rat with Phaseolus vulgaris leuco-agglutinin (PHA-L) as a tracer. In this presentation we describe the efferent connections of the rostral PCRt to nuclei of the brainstem. Projections of PCRt were traced to motor neurons of the jaw closing muscles, the mesencephalic trigeminal nucleus, and to the dorsal parts of the principal, oral, interpolaris and caudal spinal trigeminal nuclei. Terminal boutons of PCRt were also identified in the parabrachial, facial, solitary and hypoglossal nuclei, and in the dorsal gigantocellular nucleus, the caudal PCRt dorsal to nucleus ambiguus and in nucleus linearis. Efferent connections of PCRt to the dorsal motor nucleus of the vagus and nucleus ambiguus were not found. These data show that neurons in the rostral PCRt may participate in neural networks that underlie orofacial motor activity such as jaw and tongue movement. Anatomical evidence for involvement of the rostral PCRt in regulation of the autonomic nervous system was not found.

159.7

BRAINSTEM RETICULAR NUCLEI WHICH PROJECT TO THE CEREBELLUM-A QUANITTATIVE STUDY IN THE RAT. D.B. Newman and C.Y. Ginsberg* Department of Anatomy, USUHS, Bethesda, MD 20814-4799

The nuclear origins of projections from the brainstem reticular formation (RF) to the cerebellum were determined in rats using retrograde transport of 1% WGA-HRP, 10% HRP, or 2.5% Fluoro-Gold. Counts of retrogradely labeled cells were done on a large number of select cases whose injection sites comprised a wide sampling of vermal, hemispheric, and nuclear regions. The strongest reticulocerebellar projections arose from the classic precerebellar lateral reticular, paramedian and reticulotegmental nuclei. However, strong cerebellar projections also arose from catecholamine cell groups such as A6 (locus ceruleus), A5, and C1, from the serotinergic raphe nuclei (particularly raphe pontis and obscurus), and from the cholinergic pedunculopontine nucleus. Labeled cells were also seen in several non-aminergic isodendritic reticular nuclei involved in visuomotor activity (e.g. paragigantocellularis dorsalis, raphe interpositus, and the paramedian pontine RF), as well an RF nucleus associated with the trigeminal system (reticularis dorsalis). Injections into the deep nuclei produced more labeled cells in skeletomotor RF nuclei such as gigantocellularis, magnocellularis, and pontis caudalis. Supported by USUHS-DOD Grant RO7059 to D.B.N.

159.8

LESIONS OF MEDULLARY RAPHE NUCLEI IN TWO ANIMAL MODELS OF MOVEMENT DISORDERS. S. Wieland, M.S. Kreider, and I. Lucki. Dept. of Psychiatry, Univ. of Pennsylvania , Philadelphia, PA 19104.

Administration of the neurotoxin 3-acetylpyridine (3-AP) produces spontaneous involuntary motor dysfunctions in rats. We previously reported that rats treated with 3-AP demonstrated increased sensitivity to the 5-HT_{1A} agonist 8-OH-DPAT and the 5-HT_2 agonist DOB without concurrent changes in 5-HT content or receptor densities (Soc. Neurosci. Abstr. #404.11, 1988). We further studied the effects of 3-AP on the morphology of the serotonergic (5-HT) system by visualizing the 5-HT-containing neurons using immunocytochemistry. There was a large reduction in the number of 5-HT immunoreactive neurons in the raphe obscurus of 3-AP-treated rats as compared to saline-treated rats. In contrast, 3-AP treatment did not affect any other 5-HT-containing nucleus in the brain stem.

We also studied the role of 5-HT in the genetically dystonic rat (*dt*). The *dt* rat displays slow, twisting movements of the limbs and trunk, similar to that seen in rats treated acutely with 3-AP. The *dt* rat was more sensitive to 8-OH-DPAT as seen by a 5-fold shift to the left of the dose-response curve in 8-OH-DPAT's ability to produce the 5-HT behavioral syndrome. The *dt* rats showed a significant decrease in the number of head shakes produced by the 5-HT_2 agonist DOB. Immunocytochemical analysis of 5-HT-containing neurons revealed a large decrease in the number of 5-HT immunoreactive neurons in the raphe magnus of the *dt* rat as compared to the phenotypical control littermate. No other 5-HT-containing cell group in the brain stem was visibly different in the *dt* rat as compared to the control group.

These results suggest that the 5-HT system is altered in these two animal models of movement disorders.

This research has been supported by the Dystonia Medical Research Foundation and MH 36262, MH 14654, and GM 34781.

159.9

NATURALLY AND ELECTRICALLY EVOKED BURST ACTIVITY IN THE RED NUCLEUS OF ANESTHETIZED TURTLES. *R. Sarrafizadeh*, J. Keifer, and J.C. Houk.* (Spon: K. McKenna) Department of Physiology, Northwestern University Medical School, Chicago, IL 60611.

Bursts of red nucleus discharge are associated with commands that specify movement velocity in the awake monkey (Gibson et al, J. Physiol. 358: 551, 1985). Recently we developed an *in vitro* turtle hindbrain preparation to study mechanisms that may underlie burst generation in the cerebellorubrospinal system (Keifer & Houk, Soc. Neurosci. Abs., 1989). To explore the relation between bursts recorded from the *in vitro* turtle brain and motor commands recorded during movement, we studied naturally and electrically elicited responses of red nucleus cells in anesthetized turtles.

Single rubral units were recorded with tungsten microelectrodes in turtles (*Chrysemys picta)* anesthetized with sodium pentobarbitol (10mg/kg). A total of 61 cells responded to single pulse stimulation of the spinal cord with latencies characteristic of antidromic (3-4msec, 19% of sampled cells) or synaptic (6-24msec, 81% of sampled cells) activation. This is similar to our *in vitro* results except that a larger percentage of units was activated at longer synaptic latencies (14-24msec, 45% *in vivo* vs 4% *in vitro).*

Bursts of action potentials were obtained in response to single pulse stimulation of the spinal cord in 58% of the excited cells, approximately twice the percentage found in *in vitro* studies. Brief trains delivered to the cerebellum, natural somatosensory stimulation, and execution of a scratch reflex also evoked bursts of activity in these cells. A second population of units was inhibited by spinal cord, cerebellum, and somatosensory stimulation, and also during the scratch reflex. Short latency responses to spinal cord stimulation could not be obtained from the inhibited units.

In conclusion, burst activity of red nucleus neurons recorded *in vivo* to natural and electrical stimuli is similar to that evoked electrically *in vitro*. This suggests that the *in vitro* preparation will be useful in studies of the mechanisms that may underlie the generation of motor programs in the cerebellorubrospinal system.

159.10

BURST GENERATION IN RED NUCLEUS IS BLOCKED BY EXCITATORY AMINO ACID RECEPTOR ANTAGONISTS IN THE *IN VITRO* TURTLE BRAINSTEM. J. Keifer and J.C. Houk. Dept. of Physiology, Northwestern University Medical School, 303 E. Chicago Ave., Chicago, IL 60611.

In vivo studies in mammals have suggested that the cerebellorubrospinal circuit functions as a recurrent excitatory loop that generates motor commands and transmits them to the spinal cord via the rubrospinal pathway. Bursts of activity recorded from magnocellular red nucleus (RN) neurons in the monkey are associated with commands that specify movement velocity (Gibson et al., J. Physiol. 358: 551, 1985). We have developed an *in vitro* preparation from the turtle that exhibits functional synaptic connections between the cerebellum, brainstem and spinal cord in which to study burst generation in the RN (Keifer & Houk, Neurosci. Lett., 97: 123, 1989).

Bursts of activity lasting several seconds recorded extracellulary from single units in the RN were triggered by single pulse stimulation of the contralateral spinal cord, or brief train stimuli to the cerebellar cortex. Bath application of specific excitatory amino acid receptor antagonists APV (DL-2-amino-5-phosphonovaleric acid, 100μM, 9/9 cells), a specific NMDA (N-methyl-D-aspartate) receptor antagonist, CNQX (6-cyano-7-nitroquinoxaline-2,3-dione, 10μM, 3/3), a specific non-NMDA receptor antagonist, and AP4 (DL-2-amino-4-phosphonobutyric acid, 100μM, 6/6), an agonist of a presynaptic receptor, blocked burst activity in the RN. Using a multibarreled pipette for ejection of drugs and recording, iontophoresis of APV (12/16) or CNQX (7/7) into the RN blocked bursting while AP4 (5/5) did not.

These data suggest that turtle RN neurons have both NMDA and non-NMDA receptors and that excitation mediated by these receptors has an important role in the generation of burst activity in the RN. The site of action of the AP4 sensitive receptor appears to be elsewhere in the cerebellorubrospinal circuit. The possibility that bursts are produced by a combination of bistable membrane properties induced by NMDA channels and positive feedback will be explored.

159.11

ALTERATIONS OF RUBRAL SINGLE UNIT ACTIVITY AND MOVEMENT PARAMETERS AFTER INJECTIONS OF GABA OR SEROTONIN IN CAT RED NUCLEUS AREA.
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UPS CNRS UA 1121, Neurobiol. Bat 440 91405 ORSAY, FRANCE.

The motor impairments and the neuronal inactivations previously observed with injections of muscimol in the Red Nucleus (RN) area support the inhibitory GABAergic control of rubral activity through GABA A receptors. However, injections of bicuculline, intended to block the action of endogenous GABA, depressed most of the rubral neurons, with a dysruption of the motor performance. These unexpected results might be explained by the release of a second inhibitory rubral input, possibly serotoninergic. The presence of a dual inhibitory control was tested by comparing the neuronal and motor effects of GABA or 5HT, injected into the RN area of cats performing a Reaction Time (RT) task. GABA injections (0.1-1 ug) produced inactivations of 6/7 neurons, followed (1ug) by an increase of RTs, with a slowed down movement velocity, as observed with the muscimol. 5HT injections (0.4 ug) produced also an increase of RTs, with a delayed movement initiation, as observed with bicuculline. 5HT injections (0.2-0.4 ug) had depressing (4/7), activating (2/7) or mixed (1/7) effects on the neuronal activity. Experiments are in progress to further differentiate the GABAergic and serotoninergic inhibitory control of rubral neuronal discharges in relation to the movement parameters.
(SPON: J. Requin)

159.12

SENSORY PROPERTIES OF RUBROMOTONEURONAL (RM) CELLS. K. Mewes* and P.D. Cheney (SPON: T.J. Imig). Dept. of Physiol., KU Med. Ctr., K.C., KS 66103.

RM cells were identified by their postspike facilitation (PSF) of target muscle emg activity in rhesus monkeys trained to make alternating wrist movements. The sensory properties of 19 RM cells were studied using passive flexion and extension movements of the wrist. In addition, 16 of these cells were tested in relation to flexion and extension torque pulse perturbations applied during active movement. Eighteen of 19 RM cells showed brisk (mean depth of modulation, DOM: 62±28 Hz) responses to passive movement at short latency. Sixteen cells had bidirectional and two unidirectional responses, while one cell was unresponsive. Most RM cells also responded to torque pulses (15 of 16). Cell discharge was even more brisk for torque pulses (mean DOM of 161±53 Hz) and showed a similar predominance of bidirectional patterns (13 of 16). The mean onset latencies for responses to torque perturbations was 31±11 ms. Target muscle stretch in association with either torque perturbations or passive movements was not consistently the most effective stimulus direction. This contrasts with corticomotoneuronal cells which respond either exclusively or preferentially to target muscle stretch (Cheney and Fetz, J. Physiol. , 349:249, 1984). Only 2 of 13 RM cells had cutaneous receptive fields. Supported by NSF grant BSN-8216608 and NIH Grant NS 25646.

159.13

LOSS OF MUSCLE TONE AFTER ELECTRICAL OR CHEMICAL STIMULATION OF THE MEDIAL MEDULLA IN THE INTACT CAT. E. Schenkel*, Y.Y. Lai and J. M. Siegel. Sepulveda VAMC, UCLA Sch. Med., Sepulveda CA 91343

Electrical and chemical stimulation of the medial medulla have been found to produce suppression of muscle tone in decerebrated cats. However, several reports have indicated that medial medullary stimulation in intact cats does not produce such suppression. We now report success in inducing suppression of muscle tone with both electrical and chemical stimulation of the medial medulla of the intact cat. Guide cannulas were implanted as previously described (E. Shenkel and J. M. Siegel, Neurosci. Lett. 98:159-165, 1989). Concentric bipolar stimulating electrodes or 29 g cannulas were inserted through the guides to targets at P 9-15, H 5-8, L 0-1.5. Electrical stimulation (500 msec to 5 sec trains at 100-150 Hz and 80-150 μA) caused suppression of muscle tone at effective sites, often followed by excitation. Microinjection of acetylcholine (0.2-0.5 μl @ 200 μg/μl) caused similar but longer lasting effects. When administered in a waking, standing cat, the animal would rapidly collapse to the floor, in association with reduction but not complete abolition, of nuchal muscle tone. Suppression of muscle tone was not associated with PGO spiking. We hypothesize that we are activating a medullary relay in the pontomedullary descending pathway responsible for suppression of muscle tone in REM sleep. (Supported by the Veterans Administration and PHS grants MH43811 and HL41370.)

159.14

MUSCLE TONE SUPPRESSION ELICITED BY CORTICOTROPIN-RELEASING FACTOR MICROINJECTED IN MEDIAL MEDULLARY RETICULAR FORMATION. Y.Y. Lai and J.M. Siegel, UCLA, Sch. Med. and VAMC, Sepulveda, CA 91343.

The medial medullary reticular formation (MMRF) is thought to mediate muscle atonia in REM sleep and in cataplexy. Previous studies in our laboratory (J. Neurosci., 8:4790, 1988) have found that muscle atonia could be elicited by L-glutamic acid and cholinergic agonists microinjected in rostral and caudal MMRF, respectively. In the present experiment, we found that corticotropin-releasing factor (CRF) microinjected in rostral MMRF also produced decreases of muscle tone or atonia. Experiments were performed on unanesthetized, decerebrated cats. Neck muscles were dissected and implanted with bipolar EMG electrodes. Injection sites were identified by electrical stimulation (500 ms trains with 100 Hz, 0.2 ms, and 20-70 μA rectangular cathodal pulses). Sites which produced inhibition of muscle tone were microinjected with 0.5 μl of a range of concentrations of CRF. Injections with concentrations as low as 0.1 nM were found to produce muscle inhibition. The magnitude of the muscle inhibition was dose dependent. The latency and duration of muscle suppression were 18.8 ± 16.9 sec (n = 12) and 4.4 ± 3.1 min, respectively. This finding suggests that CRF may play a role in MMRF mediated suppression of muscle tone in REM sleep and in other states. (Supported by the Veterans Administration and PHS grants HL41370 and MH43811.)

159.15

PONTO-GENICULO-OCCIPITAL WAVES FACILITATE THE STARTLE REFLEX: EFFECTS OF PCPA. M.-F. Wu and J. M. Siegel. Neurobiol. Res., VAMC, Sepulveda, CA 91343 and Dept. Psychiat., UCLA Med. Ctr., Los Angeles, CA 90024.

PGO waves have been hypothesized to be a correlate of a central alerting process. Such alerting would be expected to modulate the sensorimotor processing associated with the startle response. We tested this hypothesis by presenting startle-eliciting stimuli following spontaneous PGO waves during waking and non-REM sleep, after the animal has been treated with *p*-chlorophenylalanine (PCPA), which releases PGO waves during waking and non-REM sleep.

Cats were given PCPA methyl ester HCl (125 mg/kg/day, ip) for 6 days. Tests were done between Day 4 and Day 6 when spontaneous waking PGO waves occurred. Startle-eliciting stimuli (115 db, 20 msec noise pulse) were presented between 0 and 400 msec after the peak of the PGO wave, or randomly without association with the PGO wave.

Startle amplitude was increased by 50-200% following the PGO wave, an effect most evident within 200 msec of the peak. Baseline startle response was also enhanced by PCPA as compared to pre-drug level. The present results support the hypothesis that the PGO wave facilitates the sensorimotor response to external stimuli. (Supported by PHS Grants MH43811 and NS14610 and the Veterans Administration.)

159.16

ELECTRICALLY EVOKED STARTLE-LIKE REFLEXES IN RATS: COLLISION EFFECTS BETWEEN BILATERAL RETICULAR FORMATION SITES. C.M.E.Hempel* and J.S.Yeomans (SPON:N.Wiener).Dept. Psychol., U. Toronto, Canada M5S 1A1.

Startle-like responses were elicited by stimulation of pontine reticular formation (RPC) with single, 0.1 ms duration pulses of 400-1000 uA. A second (T) pulse delivered to the same site always reduced current thresholds as interpulse (C-T) intervals increased from 0.3-0.6 ms, suggesting that short-refractory-period reticulospinal neurons mediate this response. When the first (C) pulse was delivered to one RPC site and the second (T) pulse to the contralateral RPC, current thresholds decreased sharply at C-T intervals of 0.4-1.0 ms, suggesting axonal connectivity between startle-mediating RPC sites on either side of the brainstem. The difference between unilateral and bilateral stimulation conditions suggests conduction times of 0.15-0.55 ms between the two sides, or conduction velocities of 5-18 m/s assuming straight-line conduction. These conduction velocities are much slower than large reticulospinal axons, so smaller axon collaterals passing through both stimulation fields may be responsible for the observed collision. The size of the collision was greatest (almost 100%) when both electrodes were in the center of the RPC. This connection may allow bilateral coordination of reflexes evoked from one RPC. (Supported by NSERCC grant to J.Y.)

159.17

NMDA-INDUCED LOCOMOTION IN THE RAT AND CAT. Y. Ishikawa*, Y. Atsuta*, R.D. Skinner and E. Garcia-Rill, Dept. of Anatomy, University of Arkansas for Medical Sciences, Little Rock, AR 72205

Previous studies from this lab showed that controlled locomotion can be induced by electrical and chemical stimulation of the mesencepahlic locomotor region (MLR) and the medioventral medulla (MED) in the rat and cat. In the present study, we used the precollicular transected preparation and assessed forelimb and hindlimb locomotion using EMGs. A cannula/electrode ensemble was used to establish the locations of the locomotor regions by electrical stimulation before injecting any agents. We found that localized injections of the excitatory amino acid agonist, N-methyl-D-aspartic acid (NMDA) induced locomotion when injected into either the MLR or MED. Low concentrations of NMDA (lmM or less) failed to induce locomotion. Four-limb locomotion was induced in 50% of cases by 5mM , in 63% of cases by 10mM and in 78% of cases by 20mM NMDA. Higher concentrations of NMDA had long lasting effects, inducing locomotion for more than 2 min. (longest 24 min.) in most cases. Similar effects were obtained after injection of NMDA into the MLR and MED of the rat and cat. NMDA-induced locomotion could be blocked by injection of similar concentrations of the NMDA antagonist APV5. These results suggest that NMDA receptors play a role in the control of locomotion by the brainstem. Preliminary results suggest that injection into the MLR of the rat and cat of the cholinergic agonist, carbachol, can block NMDA- and electrically-induced locomotion. This effect can be reversed by atropine, pointing to the presence of an inhibitory cholinergic input to the MLR in both species. Supported by USPHS grants NS21981 and NS20246.

159.18

ENTRAINMENT OF WINGBEAT AND RESPIRATION DURING FREE-FLIGHT, "FICTIVE" FLIGHT AND PASSIVE WING FLAPPING IN GEESE. G.D.Funk, W.K.Milsom & J.D.Steeves. Dept. Zool., U.B.C., Van. B.C. V6T 2A9.

To describe the relationship between wingbeat and respiratory pattern, wingbeat frequency (f_w) and breathing frequency (f_v) were recorded during free-flight in 5, 4-5 month old, trained Canada geese. The two patterns were predominantly coupled at 3 wingbeats per breath. To assess whether afferent activity stimulated through wing flapping could account for the coupling of the two systems, we examined the relationship between f_v and f_w during passive wing flapping. Results indicate that; (1) passive flapping will entrain respiratory rhythm, and (2) removal of wing afferent activity through dorsal root section of the brachial plexus has no effect on this entrainment, implicating a role of chest wall and/or lung afferents in this coupling.

We were also interested in whether the afferent activity associated with wing flapping, although sufficient to entrain respiration and wingbeat, was necessary for this coupling. Flapping was induced in decerebrate birds through electrical stimulation of the dorso- and ventrolateral medullary reticular formation and the medial mesencephalic reticular formation. The birds were then paralyzed and pectoralis nerve (main wing depressor nerve) and respiratory nerve (internal and external intercostal) discharges were recorded during "fictive" flight. A very tight coupling between pectoralis and intercostal nerve discharge was observed. These results suggest that, although afferent activity will entrain respiration to locomotion, there is a central program synchronizing the outputs of the two systems. Supported by the NSERC of Canada.

159.19

CLASSIFICATION OF JAW-MUSCLE SPINDLE AFFERENTS
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The responses of jaw-muscle spindle afferents were studied in rats (300-350g) deeply anesthetized with sodium pentobarbital supplemented with chlorpromazine. Ninety-three afferents (47 masseter, 24 temporalis, 8 other) were studied in the mesencephalic nucleus of the trigeminal nerve during ramp muscle stretches prior to and following the infusion of the depolarizing drug suxamethonium, and during constant muscle stretch. Thirty-one percent of the afferents had initial dynamic indices <10 imp/sec, showed no increase in dynamic responsiveness following suxamethonium, had low coefficients of interspike-interval variation and therefore were classified as secondary afferents. Seventeen percent of the afferents showed dynamic indices >100 imp/sec following suxamethonium, higher coefficients of interspike-interval variation and therefore were considered to be clear primary afferents. Fifty-two percent of the afferents showed intermediate responses. This large percentage of intermediate-type responses may be due to the considerable morphological variability of afferent terminations on the intrafusal fibers.

159.20

MANY SPINAL NEURONS HAVE DIFFERENT FIRING PATTERNS DURING LOCOMOTION EVOKED FROM THE MIDBRAIN VS. THE MEDULLA.
N.A. Bernau and R.B. Leonard. Marine Biomed. Inst., Univ. of Tex. Med. Branch Galveston, Tx 77550.

We are using the Atlantic stingray, Dasyatis sabina, to study spinal cord cells involved in generating locomotion. Swimming can be evoked by electrical stimulation in both the medulla and the midbrain. Previously we reported data showing that half of the cells whose activity was modulated during swimming, fired tonically during swimming evoked from the medulla. These tonic cells received a shorter latency input from the medullary locomotor region than phasic cells. Consequently, we thought that the tonic cells may be following the tonic stimulation used to evoke locomotion. We used extracellular recording in paralyzed, decerebrated animals to show that 60% of the tonic cells became phasic during midbrain-evoked or spontaneous locomotion. Conversely, 50% of the cells that fired phasically during spontaneous or midbrain-evoked locomotion, fire tonically during locomotion evoked from the medulla (phasic-tonic). The other 50% fired phasically during locomotion, regardless of how it was evoked (phasic-phasic). The proportion of phasic-tonic cells that were phase transition cells vs. those that fired in phase with the motor nerves was similar to that of the phasic-phasic cells. Some cells continued to fire tonically during locomotion, regardless of how it was evoked. These cells may not be involved in generating locomotion. Supported by NS 11255.

CONTROL OF POSTURE AND MOVEMENT IV

160.1

DYNAMIC POSTURAL RESPONSES IN THE CAT INVOLVE INDEPENDENT CONTROL OF LIMB LENGTH AND ORIENTATION.
C. Maioli* and R. Poppele (SPON: F. Lacquaniti). Istituto di Fisiologia dei Centri Nervosi, CNR, 20131 Milan, Italy.

Tilt in the sagital plane induces active postural adjustments involving reciprocal changes in fore- and hindlimb lengths and a maintenance of limb axes nearly vertical. When animals are subject to tilt of the visual environment alone or together with the base of support, their responses suggest that the two geometric components, limb length and limb orientation are controlled separately.

Tilt of the visual environment alone evokes postural adjustments in limb orientation only. Limb lengths remain constant while the orientation of the limbs deviates in the direction of the stimulus. In contrast, simultaneous tilting of the platform with the visual environment (a conflict stimulus) evokes changes in both limb length and orientation. The changes in limb length are indistinguishable from responses to platform tilt alone. However, the changes in limb orientation differ from those evoked by platform tilt alone, consistent with the conflicting visual stimulus.
Supported by CNR and NIH (NS 21143).

160.2

BILATERAL LABYRINTHECTOMY DOES NOT IMPAIR QUIET STANCE IN THE CAT.
D. Thomson*, J.M. Macpherson, J.T. Inglis and R.H. Schor†. Dept. of Anatomy, Queen's Univ., Kingston, Ont. K7L3N6 and †Dept. of Otolaryngol., Univ. of Pittsburg Med. Sch., Pittsburg PA 15261

The vestibular system is thought to play an important role in the maintenance of stance posture. We compared stance in the quadrupedal cat before and after bilateral labyrinthectomy. Analysis was limited to periods of quiet stance, with head stationary and facing forward. Data included ground reaction forces (3D) and the tonic activity of selected limb and trunk muscles. Cats were labyrinthectomized by drilling into the canals and vestibule on both sides. As soon as the animals could stand (1-2 days post-op), they were tested on the force platform. After the lesion, there was no change in stability of quiet stance. The distribution of vertical forces did not vary; excursions of the centre of pressure (body sway) did not increase. Horizontal plane forces became more laterally directed and increased in amplitude, transiently. Surprisingly, EMG activity of limb and trunk extensors increased transiently. Stance only became unstable during movements, such as head turning. Supported by the MRC.

160.3

DESCENDING PROJECTIONS OF THE DORSOLATERAL PONTINE CHOLINERIGIC NEURONS TO THE PARAMEDIAN RETICULAR NUCLEUS IN THE CAT
P. Shiromani, Y.Y. Lai and J. M. Siegel. Dept Psychiatry, UCSD, Sepulveda VAMC and UCLA.

Microinjections of acetylcholine into the *paramedian reticular nucleus* (PRN) of the medulla produces loss of muscle tone in the decerebrate cat. Activation of this pathway may also produce the atonia during physiologic REM sleep. In order to determine the source of the cholinergic innervation to the PRN we injected *WGA-HRP* (1%, 0.05 ul) into the PRN of three anesthetized cats. The tissue was processed for HRP using TMB as the chromagen and the sections were processed for ChAT immunohistochemistry. Counts were made of HRP-positive, ChAT positive or HRP+ChAT cells.

HRP labeled cells were found in the *pedunculopontine tegmental* (PPT) cholinergic field and in the *lateral dorsal tegmental* (LDT) nucleus. Of the HRP labeled cells in the PPT, 10-25% were also ChAT positive on the ipsilateral side while contralaterally 10-24% were ChAT positive. In the LDT, 8-16% were ChAT positive ipsilaterally while contralaterally 5-21% were ChAT positive. Of the ChAT cells, only about 0.9-1.5% of ChAT PPT cells, and 0.5%-1.4% of ChAT LDT cells project to the PRN. Thus, even though the LDT and PPT do not heavily innervate the PRN, of those cells that do project, about 15% are cholinergic. This further elucidates the networking subserving REM sleep and muscle tone.

160.4

HINDLIMB MUSCLE SYNERGIES DURING BACKWARD WALKING IN THE CAT.
J. A. Buford, J. L. Smith, and R. F. Zernicke. Laboratory of Neuromotor Control, UCLA, Los Angeles, CA 90024.

Last year we described kinematics of backward (BWD) walking, and here we describe synergies for flexor-extensor pairs at the hip (iliopsoas, IP; anterior biceps femoris, aBF), knee (semitendinosus, ST; vastus vateralis, VL), and ankle joints (tibialis anterior, TA; lateral gastrocnemius, LG). For BWD and forward (FWD) treadmill walking at 0.6 m/s, step cycles began and ended at paw off and swing occupied 35% of the cycle. Cycle periods were shorter for BWD (582 ± 43 ms) than for FWD (663 ± 75 ms) walking. EMG was digitized (1000 Hz) and muscle onset and offset latencies were calculated from paw off times determined from high-speed (100 fr/s) ciné film.

Grillner's (1981) prediction of coactivation between hip flexors and knee extensors during BWD stance was not supported by our data. For BWD and FWD walking, extensor muscles (aBF, VL, LG) at the three joints were coactive during stance. Swing phase recruitment was different as LG and aBF onsets came at mid swing during BWD walking but near paw contact during FWD walking. VL onsets just before paw contact and offsets just before paw off were similar for both directions, but average VL burst amplitude was greater during BWD walking.

For BWD and FWD walking, onsets of the three flexors (IP, ST, TA) preceded paw off by 5-10% of the cycle period. Offset of IP came early in BWD swing but late in FWD swing. A second IP burst sometimes appeared at the end of BWD swing. TA offset came just after paw contact for BWD walking but just before paw contact for FWD walking. ST had one burst throughout BWD swing compared to the two-burst ST pattern during FWD swing.

The locomotor synergy of extensor activity during stance and flexor activty during swing dominated both BWD and FWD walking, suggesting similar motor control for both directions. The change in central pattern generator coupling proposed by Grillner does not appear necessary for BWD walking. The differences in BWD and FWD synergies occurred mostly during swing, when muscle torques interact with joint torques related to gravity and segment inertias. Feedback may monitor these torque-related factors and help control swing phase recruitment by interacting with a common central pattern generator that is used for both BWD and FWD walking. Research supported by NIH NS19864.

160.5

RHYTHMIC FLUCTUATIONS OF MEMBRANE POTENTIAL AND ANTIDROMIC DISCHARGES OF IDENTIFIED MUSCLE PRIMARY AFFERENTS DURING FICTIVE LOCOMOTION IN THE CAT. J.-P. Gossard, J.-M. Cabelguen* and S. Rossignol, Centre de Recherche en Sciences Neurologiques, Université de Montréal, Québec, Canada.

Our previous experiments with intra-axonal recordings revealed that the membrane of cutaneous primary afferents was depolarized twice during the fictive step cycle and that phasic antidromic discharges were rare. The present study focusses on presynaptic mechanisms with similar intra-axonal recordings of identified muscle primary afferents of the hindlimb during fictive locomotion spontaneously occurring in decorticated cats or induced by Clonidine (chronic spinal cats) or Nialamide and DOPA (acute spinal cats). On the basis of conduction velocities and stimulation threshold, 62% of recorded units were classified as group I afferents (33/53). Muscle stretches and twitch contractions indicated that 75% of all units were presumably spindle afferents (40/53). Results showed that the membrane potential of all muscle afferents fluctuated (amplitude of up to 1.5 mV) at the rhythm of the fictive locomotion. All units had a flexor-related depolarization and 79% had an additional, but usually smaller, extensor-related depolarization. Phasic discharges, related to the parent locomotor efferent activity, were seen in 9 units despite additonal large doses of Flaxedil. Those discharges, considered as antidromic because of the absence of phasic peripheral input, were found in afferents innervating flexor (5/18) and bifunctional (4/16) muscles but were never encountered in extensor afferents (0/19). Similar results were obtained in decorticated and spinal preparations. The results thus indicate that patterns of presynaptic depolarization during fictive locomotion are similar in all muscle afferents. However, the absence of antidromic discharges in extensor afferents suggest that presynaptic mechanisms and efficacy of transmission may be controlled differently in specific afferent pathways by the spinal locomotor network. (Supported by the MRC and the FRSQ).

160.6

INFLUENCE OF PHASIC PROPRIOCEPTIVE INPUTS ON FICTIVE FORELIMB LOCOMOTION IN THE CAT. P. Saltiel and S. Rossignol, CRSN, Université de Montréal, Québec, Canada.

After decortication or decerebration and spinalization at T13, locomotor-like activity can be recorded after paralysis using cuff electrodes placed around the nerves of the right (R) and left (L) long heads of Triceps (Tri) in the forelimbs. This study examines the changes in the fictive pattern when the R forelimb was suddenly protracted or retracted at different moments of the R Tri burst. These shoulder movements, in the order of 10-15 degrees, were produced with the scapula and the elbow fixed and were monitored with a potentiometer.

Retraction early in the R Tri burst, before L Tri has ended,markedly shortened R Tri while frequently prolonging the L Tri until the next R Tri burst as if to assume weight support which the R forelimb is unable to accept. Retraction during the R Tri, after termination of the L Tri, shortened the R Tri and prematurely activated the L Tri. This response would normally speed up weight transfer to the L forelimb, before the R forelimb reaches the end of its stance and is no longer able to support weight. The L Tri silence almost never decreased below 50% of its normal duration. This might ensure that the L forelimb has swung forward enough to assume weight support upon contacting ground.

Protraction prolonged the R Tri burst when occurring during the first two-thirds of the burst and delayed activation of L Tri. This prolongation could imply that landing on the L forelimb is delayed until the R forelimb is ready to transfer weight. No significant changes occurred when the R forelimb was protracted in the last third of the R Tri burst. Such a protraction would more or less mimick the normal sequence of events whereby the R forelimb is protracted during swing when the L forelimb can accept the body weight.

These results suggest that, even in fictive locomotion, proprioceptive perturbations that could threaten the equilibrium in real locomotion may evoke strong bilateral responses which seem to be best understood in terms of providing adequate weight support or weight transfer. (Supported by the MRC).

160.7

ACTIVITY OF RETICULOSPINAL NEURONES DURING FICTIVE LOCOMOTION IN THE DECEBRATED CAT. M.C. Perreault, T. Drew and S. Rossignol, Centre de Recherche en Sciences Neurologiques, Univ. de Montréal, Montréal, Québec, Canada.

Neurones of the medial medullary reticular formation (mMRF) have been shown to discharge rhythmically during locomotion in both intact and decerebrated preparations (see Drew et al., J. of Neurophysiol., 55 375-401, 1986). However, as the respective contribution of rhythmical peripheral feedback and central drive to such a modulation is difficult to establish when movements are present, the aim of the present work was to study the discharge of reticulospinal neurones (RSNs) in paralysed cats.

RSNs recorded from the mMRF with glass-tungsten electrodes (AP:-5 to -15, L:0.8 to 2.5), were antidromically identified by stimulation of their axon through wire electrodes inserted in the spinal cord at the level of L1-2 in six decerebrated and curarized cats. The fictive locomotor rhythm, either occurring spontaneously or evoked by stimulation of the mesencephalic locomotor region, was monitored with cuff electrodes placed around the proximal part of sectioned nerves supplying extensor and flexor muscles of the four limbs.

Of a total of 38 identified RSNs, 79% (30/38) were active while 21% (8/38) were silent during fictive locomotion. 67% (20/30) of the active RSNs showed phasic increases in the discharge frequency which could be related to the rhythmical activity of nerves from the anterior (11/20) or posterior (9/20) limbs whereas the activity of the remaining 10 RSNs was found to be unrelated to the locomotor rhythm. Strong relationships were observed with both the onset and the duration of the activity of nerves to either extensors or flexors lying both ipsilaterally and contralaterally to the recording site.

Thus, even in the absence of phasic peripheral afferent input, RSNs display rhythmic patterns of activity which are similar to those observed during locomotion in unparalysed animals. This suggests that the modulation of RSNs is in part of central origin and probably occurs, directly or indirectly, through the spinal central pattern generator. (Supported by the FRSQ and MRC).

160.8

ADAPTATION TO TREADMILL SPEED OF CHRONICALLY IMPLANTED CATS BEFORE AND AFTER SPINALISATION. M. Bélanger, T. Drew and S. Rossignol, CRSN, Univ. de Montréal, Montréal, Québec, CANADA.

An increase in walking speed can be achieved by altering the step length and/or the step duration. Whereas intact cats can easily reach speeds of upwards of 2.5 m/s on a treadmill, spinal cats are generally incapable of following speeds above 0.8 m/s. The purpose of this study was to examine and compare, in the same chronically implanted adult cats (N=5), the changes in kinematics and EMG activity before and after spinalisation during changes in treadmill speed. Before spinalisation (BS), an increase in treadmill speed from 0.2 to 0.8 m/s resulted in a decrease of the step cycle duration together with a parallel reduction in the duration of the extensor activity (VL, LG). Correspondingly, there was an initial increase in the stance length which reached a plateau at a speed of 0.6 m/s. In addition, there was a progressive and steep increase in the amplitude of the extensor muscle activity. The amplitude of the flexor muscle activity (St, TA) slightly increased whereas its duration remained constant throughout. After spinalisation (AS), although the step cycle and the extensor activity, for any given speed, were generally of shorter duration than BS, there was a similar, although less pronounced, reduction in their durations as treadmill speed increased. However, both reached a plateau at approximately 0.7 m/s. The stance length, although comparatively shorter at slower speeds, increased to BS values at 0.8 m/s. Furthermore, in contrast to BS, the amplitude of the extensor muscle activity, which was generally smaller AS, remained relatively constant as the speed of locomotion increased. Changes in the amplitude of flexor muscle activity and duration were similar to those seen BS. In conclusion, the spinal cat adapts to changes in treadmill speed (in the range of 0.2 to 0.8 m/s) by increasing its stance length up to the BS value attained at 0.8 m/s and by decreasing its cycle duration. However, the spinal cat cannot walk much faster than 0.8 m/s because it appears to lack the capability of increasing the amplitude of its extensor output which would allow it to shorten its stance duration further as seen BS. (Supported by MRC and FRSQ).

160.9

EFFECTS OF NORADRENERGIC, SEROTONERGIC AND DOPAMINERGIC DRUGS ON THE INITIATION OF LOCOMOTION IN THE ADULT SPINAL CAT. H. Barbeau and S. Rossignol. Centre de recherche en Sciences Neurologiques, Univ. de Montréal, and School of Physical & Occupational Therapy, McGill Univ.

The effects of noradrenergic, serotonergic, and dopaminergic precursors and agonists on the initiation of locomotion were investigated within the first week after complete spinalization at Th13 in adult cats. The electromyographic (EMG) activity of Vastus Lateralis (VL) and Semitendinosus (St) was recorded bilaterally through percutaneously implanted copper wires in 6 spinal cats for a total of 13 experiments. The movement of the hindlimbs on the treadmill was also simultaneously video-recorded before and after the injection of drugs.

Without drug injection, strong and continuous perineal or abdominal stimulation did not induce any episodes of coordinated stepping on the treadmill during the first week after spinalization. St was continuously active as opposed to VL in which minimal or no activity was present. Injection of apomorphine (0.3 to 0.5 mg/kg, n=3), a dopaminergic agonist, failed to induce locomotion in such an early stage after spinalization. After injection of DL-5-HTP (50 mg/kg, n=2), a serotonergic precursor, a marked tonic increase in both St and VL amplitude was induced by the movement of the treadmill belt without triggering the locomotor pattern. In contrast, injection of either L-Dopa (50-60 mg/kg, n=1), a noradrenergic precursor, or clonidine (150 µg/kg, n=2), a noradrenergic agonist, induced locomotion on the treadmill. The animal demonstrated a bilateral foot placement on the sole and a complete weight support of the hindquarters. The spinal cat could follow the treadmill speed up to 0.80 $m.s.^{-1}$. The triggering effect of L-Dopa (n=2) or clonidine (n=3) was also observed when combined with 5-HTP or apomorphine.

The present results confirm previous studies showing that the locomotor pattern cannot be expressed spontaneously in the acute stage after spinalization. We further demonstrate that L-Dopa or clonidine, but not apomorphine nor 5-HTP, are capable of inducing locomotion in the first week after spinalization. This supports the importance of the noradrenergic system for the initiation of locomotion. (Supported by a group grant from the MRC. H.B. received a scholarship from the FRSQ.)

160.10

FICTIVE MOTOR PATTERNS IN CHRONIC SPINAL CATS. K.G.Pearson and S.Rossignol. CRSN, Universite de Montreal, Quebec, Canada.

Following transection of the spinal cord at T12, adult cats have the capacity to step with their hindlegs on a treadmill. We have recorded the fictive motor patterns in hindleg motor nerves following decerebration and immobilization at different times after spinalization. In cats in which hindleg stepping on the treadmill had recovered (between 30 and 40 days post-spinalization) fictive rhythmic motor patterns could be generated in response to stimulation of the perineum in the absence of drugs. The pattern consisted of short duration flexor bursts alternating with longer extensor bursts. Usually the durations of the bursts in different flexor nerves was not identical. Gentle stimulation of a paw sometimes gave high frequency bursts (up to 10/s) in ipsilateral motor nerves. This activity appears to be related to paw shaking. Squeezing of the paw gave rise to relative long duration bursts in ipsilateral flexor nerves. In animals spinalized between 1 to 10 days prior to recording, vigorous bilateral rhythmic activity was elicited by perineal stimulation following the administration of Clonidine. This activity was characterized by synchronous bursts in ipsilateral flexor motor nerves alternating with synchronous activity in extensor motor nerves. The administration of DOPA following Clonidine resulted in a spontaneous and more complex pattern. In all animals leg flexion decreased the duration of flexor bursts and reduced the frequency of the rhythmic motor activity. We conclude that the complexity and basic features of the fictive motor pattern in chronic spinal cats depends on numerous factors such as time after spinalization, drug treatment, site of stimulation, and leg position. (Supported by the MRC of Canada).

160.11

HINDLIMB KINEMATICS AND MUSCLE SYNERGIES DURING SCRATCHING. P. Carlson Kuhta, J.L. Smith, and R.F. Zernicke. Neuromotor Control Lab., Department of Kinesiology, UCLA, Los Angeles, CA 90024-1568.

Although the hindlimb kinematics of 'air' scratching in decerebrated cats are described (Deliagina et al., *Brain Res.* 100:297, 1975), nothing is known about interlimb coordination during a scratch response in which the pinna is contacted by the hind paw. To determine the hindlimb kinematics of scratching in normal cat, we filmed the response at 100 fr/s, and EMG from selected flexor and extensor muscles at the hip, knee, and ankle joints was synchronized with the film.

During a scratch response, the cat stood with the thigh elevated and held nearly horizontal to the floor. The paw was held above the ankle and generally made elliptical excursions about the pinna, usually making contact during each cycle. The range of motion was: ankle, 55 deg; knee, 45 deg; hip, 10 deg. The ankle and knee joints changed from flexion to extension simultaneously, followed by the hip joint about 20 ms later. Reversal from extension to flexion was sequential; the knee joint led, followed by the ankle and hip joints.

The kinematic cycle period (CP), defined as the time from one peak ankle flexion to the next, averaged 188±32 ms (5-7 cycles/s). At the ankle joint, time from peak flexion to peak extension was 62±8% of CP. Paw contact (PC) at the pinna normally occurred 20-40 ms after peak ankle flexion, and paw-off (PO) usually coincided with peak ankle joint extension. On average, PC duration was 48% of the CP.

Extensor EMG was largely coactive and reciprocal with flexor EMG. Usually extensor EMG occurred prior to or near peak flexion, while flexor EMG occurred prior to peak extension. At the ankle, extensor activity occurred prior to PC and remained active for most of PC, while flexor usually occurred about 20 ms prior to PO. Ankle extensor EMG was variable and related to PC duration. These results are consistent with our hypothesis that the duration of extensor muscle recruitment is modulated by motion-dependent feedback during PC. During 'air' scratching, in contrast, the absence of this modulation may account for the brief extensor bursts and prolonged flexor bursts in the decerebrated cat. Funded by NIH NS19864.

160.12

KINETIC AND KINEMATIC ANALYSIS OF LOCOMOTOR CAPABILITIES OF POSTURALLY TRAINED ADULT SPINAL CATS. K.L. Perell,* R.J. Gregor, E.G. Fowler, J.A. Hodgson,* and R.R. Roy. BRI and Kinesiology Dept., UCLA, LA, CA 90024-1568.

We have demonstrated that cats spinalized as adults (T12-T13) and subjected to standing postural therapy (Sp-S)(30 min/day, 5 days/ wk) for 6 months have pronounced locomotor output deficiencies when compared to nonexercised (Sp) spinal cats (Soc. Neurosci. Abstr., 14:64, 1988). In order to describe these deficits in detail, force transducers (J. Biomech, 14:489, 1982) were implanted on the distal tendons and EMG electrodes in the muscle bellies of the soleus (SOL) and medial gastrocnemius (MG). Bone pins were implanted as muscle endpoint markers for muscle length and joint kinematic measures (J. Biomech, 17:685, 1984). Additionally, the tibialis anterior (TA) muscle was instrumented for EMG. Peak forces in SOL and MG were similar in Sp-S and Sp cats, while the MG force impulse was significantly shorter in the Sp-S. Timing of TA EMG was more variable in the Sp-S cats with shorter burst durations than in Sp cats. In general, deficits in locomotor output in the cats trained in postural therapy were manifested in two ways. While the SOL maintained its output, the MG did not contribute to increased locomotor speed. This extensor deficit coupled with premature onset of TA EMG, i.e., occurring much sooner relative to the end of stance in the Sp-S than Sp cats, resulted in poor propulsive capabilities in the Sp-S cats and the inability to walk at speeds above 0.2 $m \cdot s^{-1}$.

Supported by NIH Grant NS 16333.

160.13

DIFFERENTIAL MODULATION OF SHORT-LATENCY EPSPS FROM SUPERFICIAL PERONEAL AND MEDIAL PLANTAR NERVES IN SPINAL MOTONEURONS DURING FICTIVE STEPPING IN THE DECEREBRATE CAT. G. N. Sholomenko, A. K. Moschovakis and R. E. Burke, Lab of Neural Control, NINDS, NIH, Bethesda, MD 20892.

Short-latency (< 3 ms) EPSPs produced in flexor digitorum longus (FDL) motoneurons by electrical stimulation of low threshold (<2.5xT) afferents in the superficial peroneal (SP) nerve are systematically increased in amplitude during the early flexion phase of fictive stepping in unanesthetized, decerebrate cats (Schmidt et al., *Exp. Brain Res.* 71:568, 1988). We have now shown that EPSPs with similar latencies and thresholds, produced by stimulation of the medial plantar nerve (PLANT; innervating the central plantar pad and the ventral surface of the toes), are modulated in a completely different manner during fictive stepping. When recording intracellularly from FDL motoneurons in precollicular decerebrate cats, short-latency PLANT EPSPs are markedly reduced or completely suppressed throughout the flexion phase of fictive stepping, at the same time that SP EPSPs are increased in amplitude. This indicates that the two species of EPSPs are produced by separate interneuronal pathways that are subject to differential control by the central pattern generator for locomotion. It seems possible that excitatory interneurons in the PLANT reflex pathway may play a role in the irregular "facultative" action of FDL during the stance phase of normal walking, which apparently acts to help stabilize the animal during perturbed step cycles in intact cats (O'Donovan et al., *J. Neurophysiol.* 47:1126, 1982).).

160.14

EFFECT OF PERTURBATION PROFILE ON VISCOELASTIC AND REFLEX PROPERTIES OF CAT HINDLIMB MUSCLES. R.F. Kirsch and W.Z. Rymer Northwestern University Medical School, Chicago, IL. 60611.

Stochastic inputs are frequently used to characterize the properties of muscle and reflex systems, but the impact of perturbation bandwidth on these properties has not been directly addressed. We have examined the force and EMG responses of isolated soleus and m. gastroc. muscles of the decerebrate cat to controlled stochastic length perturbations of three amplitudes (±0.4, ±0.8, and ±1.6 mm) and bandwidths (0-10, 0-30, and 0-100 Hz). These responses were collected for a variety of background force levels modulated by the crossed-extension reflex, both before and after deafferentation (L5-S2). Viscoelastic properties were summarized by fitting the data to a model of the form $f(t) = f0 + K*x(t) + B*v(t)$, where f(t)=total force, f0=mean force, x(t)=muscle length, v(t)=velocity, K=elastic stiffness, and B=viscous stiffness. Velocity was calculated numerically from the length record and K and B were estimated using standard multiple regression techniques.

Although this simple model fit the data of individual trials surprisingly well (85-98% variance accounted for), the values of the viscoelastic parameters K and B were found to depend on the form of the perturbation. For matched mean force levels, K decreased with increasing amplitude, while B decreased modestly. Increasing bandwidth caused a slight increase in K, but a large decrease in B, especially between the lowest and middle bandwidths. The elastic component dominated the response to low bandwidth perturbations; the viscous component accounted for <5% of the variance in this case. Deafferentation caused a decrease in K and an increase in B, but this effect was dependent on bandwidth as well, being rather significant (30% decrease in K) for the lowest bandwidth but essentially imperceptible for the widest bandwidth. Increasing the bandwidth of the perturbation also had adverse effects on force production in the muscle; for example, mean force dropped by more than 50% upon application of the widest bandwidth and largest amplitude perturbation, even though the EMG was most strongly modulated by this perturbation.

The above results suggest that (1) reflexes actively enhance muscle stiffness for relatively restricted bandwidth (<15Hz) perturbations and (2) the viscoelastic and contractile properties of muscle itself are highly dependent not only on mean force but also on the nature of the applied perturbation.

160.15

Motor cortex activity in the hindlimb region of the cat during a task which requires a voluntary modification of locomotion. Witold WIDAJEWICZ* and Trevor DREW. Centre de recherche en sciences neurologiques, Université de Montréal, Québec, CANADA. H3C 3J7

We have recently shown (Brain Res. 457 181-187, 1988) that identified pyramidal tract neurones (PTNs) recorded from the forelimb region of the cat motor cortex may markedly increase their discharge when the cat has to voluntarily make a modification of its gait in order to step over obstacles attached to a moving treadmill belt. The present study was designed to determine whether the motor cortex plays a similar role in controlling the trajectory of the hindlimbs which have to make changes in trajectory similar to those of the forelimb but without the benefit of direct visual control.

Recordings were made from the hindlimb region of the motor cortex of two chronically implanted, unrestrained, intact cats using conventional glass-insulated micro-electrodes. PTNs were identified by stimulation of their axon in the pyramidal tract at P7 with microwires while EMGs were recorded from flexor and extensor muscles acting around the hip, knee and ankle.

51 PTNs with receptive fields on the contralateral hindlimb were recorded while the cat stepped over the obstacles on the moving treadmill belt. 13/51 (26%) showed a marked increase in their discharge frequency immediately prior to, and/or during, the changes in EMG activity associated with the modification of the hindlimb gait. Temporal relationships between cell discharge and muscle activity were made on the basis of raster displays and showed that all 13 neurones were best related to flexor muscle activity of the contralateral hindlimb. Microstimulation in the region of the recorded neurones normally caused EMG activity in the contralateral hindlimb at currents of 25μA or less.

These preliminary results, taken together with the observation that stimulation within the hindlimb areas of the cortex may modify EMG activity during locomotion, suggest that the motor cortex may play a direct role in controlling hindlimb activity in circumstances which require a voluntary modification of gait. (Supported by the MRC and the FRSQ).

161.1

THE INFLUENCE OF HEAD POSITION AND TASK SPEED ON STANCE DURING VOLUNTARY ARM MOVEMENT. C.L. Kaiser* and S.S. Palmer. Dept. of Kinesiology, University of Illinois at Urbana-Champaign, Urbana, IL 61801

This study investigated stance during shoulder flexion in response to a tone stimulus using a force platform computerized system. A ratio of integrated torque during initial upward and forward movement over subsequent downward and backward movement was called the ratio of anticipatory torque to volitional torque. Twelve subjects performed 60 trials, with three head positions, and at both self-paced and ballistic speeds. A univariate and multivariate repeated measures analysis determined significance of torque ratios and response times. A linear correlation coefficient between the two variables was -0.693. The mean torque ratio for self-paced trials was 1.29, for ballistic 1.64 (p=.06). In head-neutral and head-extended trials, ratios were significantly greater for ballistic versus self-paced trials. Mean response time for all head-extended trials was 1008 ms, faster than that of other head positions (1090 ms).

EMG was recorded from leg, shoulder and neck muscles. In all conditions, hamstrings was activated before deltoid, trapezius and sternocleidomastoid. In ballistic trials, a second burst of EMG in deltoid occurred simulatneously with that of hamstrings and neck muscles (neck flexor and extensors were coactivated). The relative timing of torques and EMG patterns supports the theory that a simple motor task during stance utilizes adaptive and integrative strategies.

161.2

THE PRE-MOVEMENT SILENT PERIOD IN AN ANTAGONIST MUSCLE. R. Agostino,* M. Hallett and J. N. Sanes, Human Motor Control Section, Medical Neurology Branch, NINDS, NIH, Bethesda, MD 20892.

During a rapid voluntary movement, a silent period (SP) can be recorded in tonically activated antagonist muscles prior to any detectable change in limb position. The present study investigated the effects of changing the peak velocity, movement size and pre-load on the SP in antagonist muscles.

Subjects performed visually guided wrist flexion movements by moving a handle attached to a torque motor. A pre-load assisting flexion was used so that the extensor muscles had to be contracted tonically to maintain an initial position of 20° of extension. The surface electromyogram was recorded from wrist flexor and extensor muscles. Thirty movements were collected for each experimental condition, each after some practice.

In one series of experiments, subjects performed a 40° movement at three different peak velocities (300°, 450° and 600°/sec). The SP was longer and larger during the slower movements. The SP size correlated positively with movement duration (MT) and timing of the antagonist (ANT) burst, relative to the onset of voluntary movement. The SP size correlated negatively with the sizes of the first agonist (AG1) and ANT bursts. In a second series of experiments, subjects performed movements of different sizes (20°, 40° and 60°), each with a peak velocity of 450°/sec. The SP was longer and larger during the larger movements. The SP size correlated positively with MT and the ANT burst timing. The SP size correlated negatively with ANT burst size. In a third experimental series, subjects performed 40° movements at a peak velocity of 450°/sec, while three different pre-loads opposed extension (0.23, 0.42 and 0.63 Nm). The SP was larger with heavier pre-loads. The SP size correlated negatively with AG1.

These results suggest that subjects initiate movement by a combination of agonist activation and ANT inhibition, which is not simply reciprocal inhibition. These relationships are predicable to a certain extent by the intended peak velocity, movement size and initial activation of the muscle. Furthermore, the timing and size of ANT excitation and the intended MT strongly influenced SP.

161.3

STIMULATION TRIGGERED ENHANCEMENT OF "YIELD TASK" INHIBITION OF M2 LATENCY PERTURBATION EVOKED EMG ACTIVITY J.S. Thomas, Department of Physiology, Meharry Medical College, Nashville, TN 37208.

Subjects instructed to either "YIELD to" or "ASSIST" handle movement produced by a step increase in the torque load applied to elbow prime movers, are typically able to effect a marked reduction in the amplitude of the "M3" latency (75-100 ms) component of the EMG activity evoked to the load change, as compared with that of a "RESIST" task. YIELD task reductions in "M2" latency (50-75 ms) activity are far more modest and variable, unless preceded by a visual, auditory, or cutaneous conditioning signal 20-50 ms prior to the test loading torque step. Since there is no change in EMG activity to the conditioning signal alone (at the same latency as the reduction in M2 amplitude), it is argued that the effect of the conditioning pulse on M2 transmission must occur prior to the motoneuron, possibly at a relay site in a "long loop" pathway of M2 latency excitatory transmission. On this interpretation, it may be possible to use this technique to asses the relative amplitudes of "long" and "short" loop contributions to M2 latency activity in specific subjects and disease processes.

161.4

KINESTHETIC THRESHOLD IN THE THUMB INTERPHALANGEAL JOINT. M.A.Sabbahi, D. Judy. School of Physical Therapy, TWU, 1130 MD Anderson Blvd., Houston, TX 77030.

Kinesthetic sensation has been tested primarily in large joints, despite its importance in rehabilitation of movements after injury. This study investigated passive kinesthetic sensation in the thumb interphalangeal (IP) joint.

Thirty-five normal subjects aged 8-75 years were tested. Subjects' thumbs were positioned in a kinesthesiometer (K) with the distal phalanx moulded in putty. Subjects were blindfolded and audio white noise was applied. The IP joint was passively moved 2^{o} into flexion or extension from neutral position using a variable speed electric motor. Forty random flexion and extension movements were recorded in each subject on a chart recorder and the amplitude and output velocity were calculated. Kinesthetic threshold (KT) was calculated as the speed at which the subject was 70% correct in identifying movement direction. Flexion, extension and combined thresholds were calculated for different age groups.

KT was directly correlated with age (r=.71) for subjects over 23 years. A Kruskal-Wallis ANOVA demonstrated significantly lower KTs for the 23-40 age group ($\bar{X}=10^{o}$/min) as compared to either the 15 ($\bar{X}=27^{o}$/min) or 40 ($\bar{X}=22^{o}$/min) age groups. A Wilcoxon Signed Ranks test showed significantly lower flexion KTs within the 23-40 age group (.01 level. These results indicate that thumb flexion kinesthesia is more sensitive during movement. These kinesthetic sensation vary directly with age.

161.5

EFFECTS OF STATIC LIMB POSITION ON EMG EVOKED BY CERVICAL AND CORTICAL MAGNETIC STIMULATION IN MAN B. Day*, D. Cros*, K.L. Kerman*,B.T. Shahani and J.P. Donoghue, Dept. of Neurology, Mass. General Hospital, Boston, MA 02114 and Center for Neural Science, Brown University, Providence RI 02912

The effect of motor cortex output upon the motor apparatus may be shaped by state of the motor neuron pool. In the present study we used magnetic stimulation to evaluate the effect of different limb positions on the electromyogram (EMG) evoked by cortical stimulation and to compare this effect with that evoked by cervical stimulation. Cortical stimuli were delivered with a magnetic coil centered at the vertex in awake human subjects . Surface electrodes were placed over the flexor and extensor carpi radialis muscles (FCR and ECR). Single magnetic stimuli approximately 1.2x threshold were delivered with the wrist fixed in each of three positions: 30^{o} flexion, neutral, 20^{o} extension with the subjects seated and relaxed. Responses were measured as the integral of 10 trial averages of rectified EMG. Cervical stimulation was used to estimate the effect of peripheral factors upon EMG amplitude.

Position effects were evident after either cortical or cervical stimulation. The cervical-evoked EMG in FCR decreased with wrist extension to 33-77% compared to flexed position, without significant effect in ECR EMG. In ECR, cortical stimulation (after correction for peripheral effects) with wrist extended resulted in 150-180% larger activity than in flexed position; FCR showed inconsistent results in different subjects, but did vary with position. Thus, cortical stimulation evokes larger activity with the muscle at a shorter length for the wrist extensor. The studies suggest that motor cortex output may have different effects on neuron pools depending on initial limb position and that this effect may be muscle specific. These results also show that limb position must be controlled when examining stimulation effects. (Supported by NS 25074)

161.6

RESPONSES OF WRIST MUSCLES ELICITED BY PERTURBATION ABOUT THE ELBOW JOINT. G.F. Koshland, L. Gerilovsky* and Z. Hasan Dept. Of Physiology, Univ. of Arizona, Tucson, AZ 85724.

It has been shown that long latency (70 ms) responses occur in elbow muscles even when these muscles are not stretched by perturbations of the upper or lower arm segments (Gielen, CCAM et al. *J Physiol.* 407:275, 1988; Lacquanti, F & Soechting, JF *Exp. Brain Res.* 61:482, 1986). To test the generality of this phenomenon for other inertially coupled joints, we examined the response of wrist flexor and extensor muscles, elicited by a perturbation that extended the elbow.

The subject's forearm was affixed to a frame connected to a torque motor and was free to move in a vertical plane. Surface EMG recordings were obtained from biceps and triceps brachii, brachioradialis, and flexor and extensor carpi ulnaris muscles. With the wrist free to move and the forearm in a supinated position, the elbow perturbation (extension) caused the wrist to flex. A long latency response occurred in the wrist flexor, which was not evoked by stretch of the wrist flexor and occurred too late to cause wrist flexion. Moreover, its latency did not vary with increased amplitude of the perturbation or with increased initial activity of the wrist flexor. When the forearm was repositioned in pronation, the same elbow perturbation caused the wrist to extend, and a long latency response occurred in the wrist extensor.

Similar responses were obtained for perturbations after the wrist and fingers were immobilized in a contoured splint. Taken together, the results support the notion that long latency responses depend on initial limb configuration, but with unchanged configuration, the responses are obligatory, even when the muscles cannot have a mechanical effect as in the case of immobilization. Supported by U.S. grants INT-8520863 (NSF), 2R01-NS19407 & 5T32-NS07309 (NIH).

161.7

WRIST-AIRJET SYSTEM FOR IDENTIFICATION OF JOINT MECHANICAL PROPERTIES OF THE UNCONSTRAINED HUMAN ARM. Y. Xu*, D.J. Bennett, J.M. Hollerbach, and I.W. Hunter. Depts. Aero. & Astro. Engrg. and Brain & Cognitive Sciences, MIT, Cambridge, MA, and Biomedical Engrg. Unit, McGill U., Montreal, PQ.

We describe a wrist-mounted airjet system for determining the human arm's joint mechanical properties that is simple and unencumbering. The airjet creates thrusts in opposite directions alternated at high frequencies, and consists of a nozzle, a fluidic flip-flop, two light-weight solenoids, a force sensor and a wrist cuff. Based on the Coanda effect, the nozzle and fluidic flip-flop are specifically designed to generate large thrusts under high flow rates, pressures and frequencies. The solenoids control the flip-flop, which operates under bistable modes to generate pseudorandom binary force sequences. The thrust frequency response is a flat 4 N up to 75 Hz, and useable thrust exists beyond 100 Hz. The rise time in a step response is between 1-2 msec. The static pressure at the nozzle inlet is about 80 psi, generated by air cylinders. These specifications were carefully selected for efficient and robust system identification of the arm's joint mechanical properties. Individual forearm/wrist casts are created for each subject, and the airjet system is rigidly clamped to a cast. Arm displacements are measured very accurately by an Optotrak System (Northern Digital Corp.). Calibration tests with a high-performance linear motor and LVDT have verified the performance of the system. This research is supported by a Whitaker Foundation Biomedical Engineering Grant and NIH Grant AM26710.

161.8

IDENTIFYING THE MECHANICAL IMPEDANCE OF THE ELBOW JOINT DURING POSTURE AND MOVEMENT. D.J. Bennett, Y. Xu*, J.M. Hollerbach, and I.W. Hunter. Depts. Brain & Cognitive Sciences and Aero. & Astro. Engrg., MIT, Cambridge, MA, and Biomedical Engrg. Unit, McGill U., Montreal, PQ.

Our goal is to characterize the human arm's joint mechanical properties during unconstrained posture and movement. As described in an accompanying poster, we have developed a wrist-mounted airjet system for this purpose. As the first step in these studies, we present preliminary results on elbow joint mechanical impedance. For these studies, the airjet system is configured for a uni-axial thrust of 4 N, switched at rates of up to 100 Hz as a pseudorandom binary sequence. We record force, position, acceleration and EMG during the perturbation. Data are analyzed with linear system identification techniques to identify the impedance transfer function between force and position. Two basic experiments were performed, one studying posture and the other movement.

The posture experiment investigates the changes in the mechanical impedance while controlling (1) the subject's instructions ("resist," "resist lightly," or "do not resist"), (2) the size of the perturbations, and (3) a constant bias force (10 to 40 N) against which the arm operates. The second experiment characterizes the time course of the impedance changes during elbow flexion and extension. To this end, repeated trials are ensemble averaged, and a linear perturbation model is identified at each instant in time. This research is supported by a Whitaker Foundation Biomedical Engineering Grant and NIH Grant AM26710.

161.9

THE FORCE:FATIGABILITY RELATIONSHIP IN SINGLE MOTOR UNITS:1. A SYSTEMS APPROACH TO THE STUDY OF MUSCLE FATIGUE. D.G. Stuart, Y. Laouris* and L. Bevan. (SPON: Z. Hasan). Department of Physiology, University of Arizona, Tucson, AZ 85724.

Most research on muscle fatigue has focused on the study of mechanisms of action at single failure sites within the CNS and peripheral neuromuscular system. However, a systems approach (cf. Bigland-Ritchie et al., J. Physiol. 379:451, 1986) is also required because, under normal circumstances, fatigue results from failures at several sites, as determined by the task at hand. A systems approach of potential impact is to determine the circumstances under which there is, or is not, a clear association between the magnitude of muscle/motor-unit force output and fatigability, across and within different fatigue-inducing tasks. For whole muscles, this association is close during voluntary and imposed activations of varying strength and duty cycle at varying muscle length, and even in muscles subjected to imposed chronic stimulation. For single motor units, the force: fatigability relationship is also strong at varying levels of force output and activation rate, and when comparing the two parameters' values across a muscle's unit population. However, the association is not evident when comparing values for units of similar type (particularly S). In one disuse paradigm, the association breaks down, when comparing force/fatigability across unit types (normal vs reduced-use). A force-optimization paradigm also alters the force:fatigability relationship within a single unit, when fatigued by regular vs force-optimized activation trains. Similarly, the literature on associations between single-fiber histochemistry/ biochemistry, fiber size and muscle/ motor unit force/ fatigability indirectly reveals examples both in support and against a close association between force and fatigability. In summary, the force:fatigability relationship provides a rubric for further systems studies on muscle fatigue. (Supported in part by USPHS grants NS 25077, HL 07249, NS 07309 and RR05675).

161.10

THE FORCE:FATIGABILITY RELATIONSHIP IN SINGLE MOTOR UNITS:2. A NEW EXPERIMENTAL PARADIGM SUPPORTED BY SIMULATIONS. Y. Laouris*, L. Bevan, R.M. Reinking* and D.G. Stuart (SPON:D. Blask). Department of Physiology, University of Arizona, Tucson, AZ 85724.

A method was developed to compare the effects of different activation patterns on the force production and fatigability of single motor units. Preliminary experiments revealed that delivering two different activation patterns to the same unit sequentially was impractical, because the depression in force output following the first test lasted several (4-6) hours (cf Jami et al, J.Physiol., London, 340:129, 1983). As an alternative, we used a random alternation of two activation patterns delivered to the same motor unit, and decomposed the force profile to assess the contribution of each pattern to the overall force record (cf Cooper et al, J.Physiol.,London, 397:585, 1988). Before proceeding, it was necessary to consider the potentially complicating effects of history-dependent behavior brought on by the temporal effects of one activation pattern on the other. Modeling and simulation were used to: 1) show that short-term, history-dependent effects could be minimized by randomly alternating triple- rather than single trains of the two test patterns; and 2) predict the time course of experimentally derived, decomposed force profiles. With this strategy, it was possible to proceed with the experimental paradigm. (Supported in part by USPHS grants NS 25077, HL 07249, NS 07309 and RR05675).

161.11

THE FORCE:FATIGABILITY RELATIONSHIP IN SINGLE MOTOR UNITS: 3. SIGNIFICANCE OF THE UNIT-ACTIVATION PATTERN. L. Bevan, Y. Laouris*, R.M. Reinking*, S.J. Garland*, N. Radicheva*, and D. G. Stuart. Department of Physiology, University of Arizona, Tucson, AZ 85724.

The goal of the present work was to compare the effects of activation patterning on force production during fatigue in single motor units of the cat hind limb. The stimulus trains used to produce force were relatively similar, i.e., a constant number of stimuli/train (10-20), and train duration (500 ms) and rate (1/s), but subtle changes in the activation pattern produced different force. Two activation patterns (one regular, one optimized) were delivered in random alternation to axons of functionally-isolated motor units, restricted to the type FF category. The force produced by each activation pattern was extracted from the overall force record, so that profiles of fatigability for the two activation patterns could be compared (cf J.Physiol., London, 397:585,1988). It was shown that the optimized activation pattern, which significantly augmented force production initially, maintained the ability to enhance force production throughout the fatiguing procedure. This result suggests that a subtle change in activation patterning is a potential mechanism by which the nervous system can adjust force during fatigue. Furthermore, use of such a strategy would imply that a unit's force:fatigibility relationship is task dependent.(Supported in part by USPHS grants NS 25077, HL 07249, NS 07309 , NSF Int 8520863, RR005675 and the Ontario Ministry of Health).

161.12

BEHAVIOR OF MOTOR UNITS IN HUMAN BICEPS BRACHII MUSCLE DURING FATIGUE. S.J. Garland*, L.P. Serrano*, G.A. Robinson, and R.M. Enoka. Departments of Exercise & Sport Sciences and Physiology, University of Arizona, Tucson, AZ 85721.

Motor units maintain force differently over time (i.e., fatigability varies), so we examined the influence of fatigue on the behavior of 12 motor units from 8 human biceps brachii muscles with a subcutaneous branched-bipolar electrode. We measured: (1) recruitment and derecruitment threshold forces and discharge rate during a ramp-and-hold task; (2) discharge rate during a fatigue-inducing, sustained, submaximal voluntary contraction. During fatigue, 11/12 motor units could be followed. Of these, 6 showed an initial increase in discharge rate that subsequently declined. The other 5 motor units showed steady decreases in discharge rate. These behaviors were evenly distributed between high threshold (>25% of a maximum voluntary contraction (MVC)) and low threshold (<25% MVC) motor units. During the fatiguing contraction, 1 low threshold and 3 high threshold motor units failed to discharge tonically, 2 low threshold motor units maintained their rates, and the remainder showed decreased rates. After fatigue, recruitment forces decreased 11-72% from prefatigue forces in all but 2 motor units; both failed to be recruited. Thresholds for derecruitment decreased 20-70% in 7 motor units, increased 18% in 1 motor unit, and did not change in the others. During the ramp-and-hold task, postfatigue recruitment and derecruitment forces changed irrespective of threshold force while discharge rate during holding declined more in high threshold motor units (44-87%) than low threshold motor units (15-48%). Thus, high threshold motor units were more likely to drop out or to reduce discharge rate during a fatiguing contraction. Supported by the Ontario Ministry of Health, NS 07309, HL 07249, RR 05675 and NS 20544.

161.13

CHARACTERIZATION OF MOVEMENT RELATED VARIABLES OBTAINED FROM SCALP-RECORDED CEREBRAL ACTIVITY IN MAN. D.H. York, D. Fousek*, T. Spagnolia* and M. Schafe*. Dept. of Physiology, School of Medicine, University of Missouri, Columbia, MO 65212.

This study was aimed at characterizing specific components of scalp recorded activity associated with a movement, relative to specific movement variables. Subjects performed a ballistic thumb flexion movement over a fixed displacement, against a known load. Cerebral activity was segregated based on different movement times for comparisons across load and displacement. Five major waveform components were defined across 7 subjects, which are related to the movement task. The amplitude of an initial N1-P1 component was correlated with acceleration. Its onset latency was correlated with the load. The latency of a third component, termed N2 correlated with peak velocity of flexion. The completion of movement was correlated with a fourth component, termed P2, following which, a fifth component termed N3 was consistently defined. In experiments involving passive thumb flexion, performed over the same displacement, load and movement time, only P2 and N3 were defined, suggesting that these two components may represent sensory feedback. (supported by NIH NS24960)

161.14

TASK INFLUENCE ON TIMING AND GRASP PATTERNS IN HUMAN PREHENSION. T. Iberall, M.J. Preti* and R. Zemke*, Departments of Computer Science, Neurobiology, and Occupational Therapy, University of Southern California, Los Angeles, Calif 90089.

Examining task effects on prehension has raised important questions about the underlying neural mechanisms. While Fitts' law suggests an important constraint that relates task index of difficulty to movement time (MT), Marteniuk et al, CJP, 1987, show the influence of intent and task context, arguing that increased MT is due to an extended deceleration time. In addition, Newell et al, in press, show the effect of object size on the number of fingers used to grasp the object.

Subjects were instructed to either place (easy task) an upright cylinder on a platform or balance it on two horizontal dowels (difficult task). Four light weight cylinders of different heights were used (3-9 cm). MT was analyzed from the start of the movement to the time that the cylinder was grasped. Kinematic analysis using a WATSMART system revealed a significant difference in this MT for the easy vs difficult task. More time was spent in the deceleration phase (time from wrist peak resultant velocity to time of grasp) during the difficult task than the easy task. More fingers were used as cylinder height increased. These data support the results cited above, suggesting the powerful influence of task and object properties.

To model the neural controller, we simulated the grasping component with an adaptive neural net. Using these experimental data as a training set, the network learned to use a different number of fingers depending on task and object properties.

161.15

EFFECT OF LOAD MAGNITUDE PREDICTABILITY ON AUTOMATIC COMPENSATORY GRIP FORCE ADJUSTMENTS.
C.J. Winstein, F.B. Horak, and J.H. Abbs. Speech and Motor Control Laboratories, Waisman Center, Univ. Wisconsin, Madison, WI 53705. R.S. Dow Neurological Sciences Institute of Good Samaritan Hospital, Portland, OR 97209.

Effective object grasp requires precise modulation of neuromuscular outputs by cutaneous and muscle mechanoreceptors. Automatic precision grip force responses have been observed within 60-90 ms in response to natural object slips (Westling & Johansson, 1984), and to slips induced by object load changes (Cole & Abbs, 1988; Winstein et al., 1988). In this study, the effect of predictability on these grip force adjustments was evaluated in eight subjects. Different magnitude load perturbations were delivered to an object held between the thumb and index finger. Two load orders (random, blocked) were combined with two pre-load grip conditions (unconstrained, constrained). With random order, the load magnitudes were unpredictable, whereas, in blocked conditions, the same load was presented consecutively ten times allowing load magnitude to be predicted. In constrained conditions, initial grip forces were maintained near the natural minimum level necessary to prevent pre-loaded object slippage, while no initial grip force restrictions were imposed in unconstrained conditions.

Generally, compensatory grip force responses were scaled to load magnitude in all four conditions. However, the initial rate of these grip force responses were more closely related to load magnitude in the predictable than in the unpredictable conditions (r = 0.65 vs 0.48), and most closely related to load magnitude when initial grip forces were maintained near the minimum natural level. These results suggest that anticipatory processes pertaining to load magnitude permit the response gain of these rapid grip force adjustments to be set, at least partially, prior to perturbation onset. Moreover, this response gain setting appears to be independent of the prevailing level of motoneuron pool excitability. (Supported by NIH grants NS-13274, HD-03352, AG-06457, NS-01094.)

161.16

SINGLE MOTOR UNIT ACTIVITY PATTERNS OF HUMAN INTRINSIC LARYNGEAL MUSCLES DURING RESPIRATION. C.M. Chanaud and C.L. Ludlow. Speech and Voice Unit, NIDCD, NIH, Bethesda, MD 20892.

Multiple patterns of activation have been recorded from laryngeal muscles during respiration, suggesting that these muscles may be functionally complex. The purpose of the current study was to determine whether different discharge patterns occur across single motor units within these muscles.

Bipolar concentric EMG needles were percutaneously inserted into the left and right thyroarytenoids (TA) and cricothyroids (CT). EMG and respiration signals were recorded in normal volunteers. Single motor units were identified in records containing a few, distinct units and repeat firings were selected by waveform shape and peak amplitudes.

During respiration, the patterns and rates of discharge of TA and CT units were modulated relative to the phase of the respiratory cycle (inspiratory vs. expiratory). Multiple discharge patterns were found across TA and CT units: of the TA units, 45% had inspiratory bursts, 45% had inspiratory and expiratory bursts and 10% had an expiratory burst; of the CT units, 40% had inspiratory only and 60% had inspiratory and expiratory bursts. Discharge rates ranged 5-30 Hz, usually varying between 10-25 Hz, depending on the respiratory phase.

Multiple activity patterns were found across motor units within the human TA and CT laryngeal muscles. These two muscles may contribute to multiple mechanical or kinematic functions of the human larynx.

161.17

ANTICIPATED AND PERIPHERALLY INDUCED EMG ACTIVITY IN ACTIVITY IN SIMULATED CHEWING. F. Bosman, A. van der Bilt*, M.A. Koks*, and H.W. van der Glas. Dept. of Oral Pathophysiology, Univ. of Utrecht, Padualaan 14, 3584 CH Utrecht, The Netherlands.

Additional elevator muscle activity (AMA) is necessary to overcome the resistance of the food during the closing phases in chewing movements. Preceding research on chewing an artificial testfood suggested that peripheral stimuli play an important role in the origin of the AMA.

Eleven subjects participated in the experiments. The AMA was evoked by an external force supplied by a solenoid in a magnetic field. The solenoid was attached to the mandible of the subjects who performed open-close movements at a natural rate controlled by a metronome. At a preset level of jaw opening a force in downard direction was applied. Jaw movement and EMG activity of masseter, temporal and digastric muscles were recorded.

In the cycles wherein a force was applied the EMG of the closing muscles increased about 100 ms before application of the force. About 60 ms after the onset of the force the EMG activity showed a much larger increase. In the first cycle without force the EMG activity started at the same degree of jaw-opening as in the previous cycles. However, the second EMG burst did not occur.

The results suggest that in chewing the texture of the food is anticipated for, whereas the main part of the AMA is induced by peripheral stimuli.

161.18

KINESTHETIC THRESHOLDS DURING ACTIVE PRODUCTION OF OROFACIAL AND FINGER MOVEMENTS. L.F. De Nil and J.H. Abbs (SPON: M.A. McCall), Speech & Motor Control Laboratories, Waisman Center, Univ. Wisconsin, Madison, WI 53706.

Previous studies of finger and orofacial kinesthesia have typically been done using a paradigm in which positional changes around a joint were externally imposed. In real life, however, organisms normally have active control over their movements. Such movements may be characterized by enhanced kinesthesia, as is suggested by the observation that increased muscular tension and velocity result in higher kinesthetic acuity.

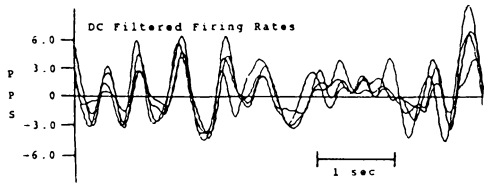
In the present study, 12 subjects were instructed to make the smallest possible vertical movement with their jaw, lower lip, and tongue. Preliminary data were collected also for the right index finger. To separate the motor from the sensory component, the movement task was performed both with and without visual feedback. The Just-Noticeable-Difference (JND) was significantly larger without visual feedback, indicating that performance in this condition was mainly limited by sensory processes. In the nonvisual condition, the JND for the jaw was more than 2 times smaller (.699 mm) than previously found for passive positioning. The JND's for the lip and tongue were .698 mm and 1.411 mm, respectively. A directional difference was found for the finger, flexion yielding a significantly smaller JND (.814 mm) than extension (1.298 mm). Similarly, a directional trend was apparent for the lip with downward movements (.590 mm) yielding smaller JND's than upward ones (.806 mm).

The data indicate that, at least for orofacial systems, kinesthetic acuity is notably higher during active movements than during more passive positioning. As a result, data from passive positioning tasks should be interpreted cautiously in terms of the sensory information available to the organism during actively controlled movements. Also of interest is the fact that the magnitude of the JND was independent of whether a motor system was endowed with joint receptors or muscles spindles. (Supported by NIH grants NS-13274 and HD-03352.)

161.19

THE COMMON DRIVE OF MOTOR UNITS IN HUMAN ORBICULARIS ORIS MUSCLE. G. Kamen, N. Paul*, C.J. De Luca. NeuroMuscular Research Center, Boston Univ., Boston, MA 02215.

We have previously identified a phenomenon of motor control termed "common drive", in which human motor unit firing rates are positively correlated when a subject attempts a constant-force isometric contraction. In the present study, we endeavoured to identify the role of spindle afferents in this common drive behavior by recording from the orbicularis oris, a muscle which is reportedly devoid of muscle spindles. Recordings were made using a specially-designed needle electrode while the subject maintained a contraction of the perioral sphincter muscle. The firing occurrences of individual units were identified using our motor unit decomposition technique. The figure shows the common modulation observed among DC-filtered motor unit firing rates, and suggests that common drive behavior can occur in muscles devoid of muscle spindles.



161.20

LINKAGE BETWEEN ORBICULARIS OCULI ACTIVITY AND VOLUNTARY MOVEMENTS. I.C. Bruce and P.W.P. Poon*, Department of Physiology, University of Hong Kong, Hong Kong.

As a prelude to a study of eye-hand co-ordination, activity of the eye-closer, orbicularis oculi (OO), was examined during simple movements.

EMG was recorded from OO and wrist flexors during a simple reaction task. Subjects made rapid wrist flexions in response to a low-intensity tone while fixating on a stationary target. Sessions were repeated with cued responses from axial or ankle muscles. Rectified EMGs were averaged with respect to the cue.

In all subjects, EMG activity in OO was linked to that of the muscle involved in the reaction task. In a given subject, the same EMG pattern was found in OO related to wrist, axial or ankle activity, although the pattern varied between individuals. In the simple excitatory pattern shown by most subjects, OO activity showed a single peak in parallel with that of the responding muscle. The onset latencies of the OO patterns were shortest for wrist, preceding the onset of voluntary EMG by up to 70 ms, intermediate for axial, and longest for ankle, following voluntary EMG onset by at least 55 ms. Control experiments, in which the subject did not respond to the cue, showed no "startle" effect of the auditory cue on OO activity. Processing of the video image of the eye during some sessions indicated that OO activity was not associated with eye movements.

The findings suggest that motor "programmes" for simple reaction tasks include a component affecting eye-closure.

LONG-TERM POTENTIATION III

162.1

NMDA RESPONSES OF SMALL NEURONAL NETWORKS IN CULTURE M.B.Gordon*, S.P.Fracek and G.W.Gross, Dept. of Biological Sciences and Center for Network Neuroscience, University of North Texas, P.O. Box 5218, Denton, Tx, 76203

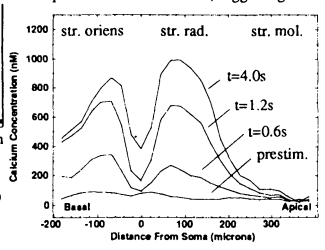
The NMDA (n-Methyl-D-Aspartate) receptor is known to participate in long term potentiation in hippocampal slices, and could play an important role in information storage. This study was undertaken to ascertain the effects of NMDA on small neuronal networks obtained from embryonic mouse spinal cord (SC), olfactory bulb [OB], cerebellum [CB], and hippocampus [HC]. The dissociated tissues were grown as monolayer minicultures (1mm diam., 500-800 neurons) on multimicroelectrode plates which make possible simultaneous spike sampling from up to 64 extracellular electrodes photoetched onto a glass plate. This allows the long term monitoring of spatio-temporal patterns across the monolayer culture.

Our initial studies, (n=20), show that responses to NMDA concentrations ranging from 20 to 150 µM are best described in terms of 3 distinct phases. Phase 1 represents an abrupt and immediate replacement of pre-stimulus burst patterns by intense tonic spiking on all electrodes. Phase 2 is characterized by an initial inhibition of Phase 1 spiking, gradual increase in spiking, emergence of spike clusters (i.e. bursts), and gradual increase of burst frequencies. Phase 3 starts when bursts fuse to yield intense tonic spiking. In SC and OB cultures, this phase lasted until washout, however, in the HC and CB we observed cycling through Phases 2 and 3 until washout. Variability within each phase was seen among the different tissues tested and among channels, particularly in SC and CB. In the SC, entrained (i.e. directly coupled) pre-stimulus bursting among channels ranged from 70 to 95%, but was reduced to 30 to 60% in Phase 2. Initiation of alternating activity between channels was also observed in SC subsequent to NMDA washout. The tissue-specific differences consisted primarily of the variability in Phase 2, the appearance of cycling through Phases 2 and 3, and the degree of entrainment between channels. Both classes of NMDA antagonists, represented by ketamine and 2-aminophosphonovalerate (2-APV), were effective in these cultures in concentrations ranging from 20 to 90 uM. Above 90 µM, all activity was reversibly inhibited. Supported by a grant from W.W. Caruth (Dallas) via the Communities Foundation of Texas (to GWG) & NSF grant BNS-8719319 (to SPF).

162.2

OPTICAL IMAGING OF ION CONCENTRATION DYNAMICS IN PYRAMIDAL CELLS IN HIPPOCAMPAL BRAIN SLICE. D.W. Tank & W.G. Regehr*, AT&T Bell Laboratories, Murray Hill, NJ 07974.

CA1 neurons in guinea pig hippocampal slices were iontophoretically microinjected with the fluorescent calcium indicator fura-2 or the fluorescent sodium indicator SBFI. Low magnification images acquired at two excitation wavelengths with a large dynamic range CCD camera were used to construct maps of ion concentration in soma, apical dendrites and basal dendrites. Fura-2 measurements demonstrate that stimulation of Schaffer collateral afferents produces widespread dendritic accumulation to micromolar levels in the proximal apical and basal dendrites, corresponding to stratum radiatum and stratum oriens, respectively (See Fig.). Accumulation in distal apical dendritic regions located in stratum moleculare is greatly reduced. Most of the response appears to result from activation of voltage-dependent calcium channels (Regehr & Tank, accompanying abstract). Similar experiments with SBFI-filled neurons demonstrate stimulation-induced increases in the ratios of fluorescence excited at 340nm and 380nm in basal and apical dendrites and soma, suggesting mM changes in monovalent cation (Na^+ and K^+) concentration. We are attempting to determine the contribution of synaptic and voltage-dependent conductances to these changes.



Increasing calcium accumulation in a CA1 hippocampal cell during stimulation (20 Hz;10 sec) in stratum radiatum versus distance to soma along representative dendrites. Stimulation begins at t=0s.

162.3

CALCIUM IN HIPPOCAMPAL PYRAMIDAL CELLS DURING TETANIC STIMULATION: IMPLICATIONS FOR LTP. W.G. Regehr* & D.W. Tank, (SPON: R. Cholewiak) AT&T Bell Labs, Murray Hill, NJ 07974.

Popular models for the induction of long-term potentiation (LTP) presume a calcium influx through postsynaptic NMDA receptor channels. Only localized postsynaptic sites that have the necessary conjunction of presynaptic transmitter release and sufficient postsynaptic depolarization are envisaged to accumulate calcium to levels necessary for induction, thus predicting that well localized calcium accumulations only occur near activated afferents. Using microfluorometric imaging techniques and the calcium indicator fura-2, we have measured intracellular free calcium levels in CA1 hippocampal pyramidal cells during (a) intracellular microelectrode stimulation, and during tetanic afferent stimulation of fiber tracts in (b) stratum moleculare, (c) stratum radiatum, and (d) stratum oriens. Similar widespread calcium accumulations in proximal dendrites (described in preceding abstract) with peak calcium levels in the micromolar range were observed for (a)-(d), both in normal saline and in the presence of the NMDA-receptor antagonist APV. Our measurements suggest that calcium accumulations are primarily due to influx through voltage-dependent non-NMDA calcium channels. The lack of appreciable accumulation in distal apical dendrites suggests a different learning or computational role for afferent inputs in stratum moleculare. While the role of influx through voltage-dependent calcium channels in the induction of LTP remains unclear, the resulting accumulations should be taken into consideration in models of LTP induction.

162.4

OPTICAL MONITORING OF LTP AND RELATED [Ca2+]i CHANGES WITH A 256CH PHOTODIODE ARRAY SYSTEM. T.Iijima*, M.Ichikawa and G.Matsumoto (SPON:T.Shiida). Electrotechnical Laboraory, Tsukuba, Ibaraki 305, Japan

We developed a compact optical recording system of 256 ch photodiode array to study neuro-plasticity. We applied this system to a hippocampal slice to observe the neuronal activity change in LTP. A stimulation of the Shaffer-commissural path evoked double-peak optical signals in the dendritic portion of CA1 pyramidal cell; the first peak originated from the activity of input fibers and the second one from the electrotonic potential change of somatic excitation. A tetanic stimulation (100Hz, 1s) increased the both peaks (150-200%) to be sustained for over 3 hrs. An application of phorbor ester also changed the optical signal. However a large increase was detected, along with the increased second peak, only in the dendritic portion within 90 um from the soma. This may indicate the activity change in presynaptic terminal in the definite region. Propagation characteristics of electrical signal from mossy fiber - CA3 region - CA1 region was also studied. The LTP induced in CA3 region largely affected the signal transmission. The [Ca2+]i change concerned with LTP was investigated in a single cell level with this system.

162.5

TWO DIMENSIONAL ANALYSIS OF THE CHANGES IN $[Ca^{2+}]i$ IN THE RAT HIPPOCAMPAL SLICE DURING LONG TERM POTENTIATION. Y. Kudo*, E. Ito* and A. Ogura* (Spon: H. Akagi). Department of Neuroscience, Mitsubishi Kasei Institute of Life Sciences, 11 Minamiooya, Machida, Tokyo 194, Japan

The cytosolic concentration of Ca^{2+} ($[Ca^{2+}]i$) is suggested to play a key role in the establishment of the long term potentiation (LTP) of synaptic transmission in the hippocampus. Here we applied a microscopic image analysis system to the rat hippocampal slices loaded with fura-2 to examine the spatial and temporal changes of $[Ca^{2+}]i$ in the CA1 and CA3 regions during tetanic stimulations. The effects of glutamate receptor agonists were examined in addition to know which subtype(s) of glutamate receptor is(are) responsible for the $[Ca^{2+}]i$ increase. An LTP-inducible tetanization (50 Hz, 5 sec) of the Schaffer collateral caused a small but significant increase in the $[Ca^{2+}]i$ of the st. radiatum of CA1, while those of stt. oriens and pyramidale remained unaltered. A tetanic stimulation to the mossy fiber also resulted in the $[Ca^{2+}]i$ elevation of the CA3 region. The application of any of the agonists (50 μM), NMDA, quisqualate and kainate, caused the increase of $[Ca^{2+}]i$ in TTX-treated preparations. The increase was most remarkable in the layer of st. radiatum in both of the CA1 and CA3 regions. The response to quisqualate and kainate was observed after the treatment of APV (10 - 50 μM). These results further support the requirement of Ca^{2+} for the establishment of LTP and suggest that all subtypes of glutamate receptor participate in the $[Ca^{2+}]i$ elevation.

162.6

ELECTRICAL TEST STIMULI WERE REQUIRED FOR CALCIUM-INDUCED LTP. K.Uruno*, K.Kato*, K.Saito* and H.Kato* (SPON: M.Sato). 2nd Dept. of Physiol., Yamagata Univ. Sch. of Med., Yamagata, 990-23, Japan.

To examine properties of calcium-induced long-term potentiation (LTP), Guinea pig hipocampal slices (400um) were perfused with high calcium (4mM) and low magnesium (0.1mM) solution for 10-20 min. Test stimuli were delivered every 20 sec to Schaffer collateral /commissural pathway. Population spike and field EPSP were monitored in pyramidal cell layer and in radiatum of CA1, respectively. 10 min perfusion of high calcium and low magnesium solution produced LTP of both pop.spike and EPSP. Perfusion for 20 min without test stimuli resulted in only transient potentiation, which was sometimes succeeded by depression of population spike. These results suggest that synaptic transmission and subsequent events are required for calcium-induced LTP. Further, since MK-801 blocks calcium-induced LTP, calcium influx through receptor mediated channels may play a key role as in post-tetanic LTP.

162.7

DIFFERENT SUSCEPTIBILITIES TO DRUGS OF LTPS IN THREE INPUT SYSTEMS TO HIPPOCAMPAL CA3 REGION. M. Satoh, S. Kaneko*, K. Ishihara*, H. Katsuki* and M. Sugimura*. Dept. of Pharmacology, Fac. of Pharm. Sci., Kyoto University, Kyoto 606, Japan.

We have demonstrated *in vitro* that long term potentiation (LTP) in the mossy fiber(M)-CA3 system was augmented by anti-amnesic drugs and inhibited by pro-amnesic drugs. On the other hand, it is well known that the hippocampal CA3 region receives inputs from the fimbria (F) and commissural/associational fiber (C/A) besides F. Thus, we compared effects of several drugs on LTPs in the M-, F- and C/A-CA3 systems. Slices of the hippocampus, 400-500μm thick, were prepared from adult guinea-pigs. A single slice was perfused in a recording chamber with artificial CSF (35° C). Test and tetanic stimui were given at 0.2Hz and 33Hz, respectively. D-APV (a NMDA-antagonist: 10μM) suppressed LTPs in F- and C/A-CA3 systems, but did not affect LTP in M-CA3. However, naloxone (an opioid antagonist:1μM) and scopolamine (a muscarinic cholinergic blocker: 10μM) depressed LTP in M-CA3 system but did not LTPs in F- and C/A-CA3 systems. These findings indicate that mechanisms of production of LTP in M-CA3 system are different from those in F- and C/A-CA3 systems.

162.8

Hippocampal Mossy Fibers Induce LTP of Select Heterosynaptic Afferents. ***J. E. Bradler and G. Barrionuevo*, Depts. of Behavioral Neuroscience & Psychiatry, University of Pittsburgh, Pittsburgh, PA 15260**

We have previously reported that tetanization of the mossy fibers reliably induces heterosynaptic LTP of non-mossy fiber afferents, but tetanization of non-mossy fiber afferents induces a heterosynaptic depression of mossy fiber responses (1). Tetanization of a subset of mossy fibers has been reported to heterosynaptically potentiate a separate, nontetanized subset of mossy fibers (2,3). More recently, however, an absence of heterosynaptic LTP in a nontetanized subset of mossy fibers following tetanization of a separate set of mossy fibers has been demonstrated (4). The resolution of this discrepancy is essential for determining if heterosynaptic LTP represents a non-specific enhancement of all heterosynaptic afferents, or whether it is a potentiation of only select heterosynaptic afferents. Three stimulation sites were used to evoke EPSCs in hippocampal CA3 neurons maintained in an *in vitro* slice preparation. Two sets of separate mossy fibers were stimulated: one electrode was placed in the ventral blade of the dentate granule cell layer, a second was placed in the dorsal blade of the granule cell layer. A third electrode was placed in the fimbria, to stimulate non-mossy fiber afferents. Other methods were similar to those used in our previous report (1). In all cells tested (n=12), tetanization of the mossy fibers induced homosynaptic mossy fiber LTP (mean 85±18%), heterosynaptic fimbrial LTP (91±17%), and heterosynaptic depression of the nontetanized mossy fiber response (38±17%). Supported by NS01196 and NS24288.

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2. Yamamoto & Chujo (1978) Exp. Neurol. 58, 242-250.
3. Yamamoto et. al. (1980) Exp. Brain Res. 38, 469-477
4. Higashima & Yamamoto (1985) Exp. Neurol. 90, 529-535

162.9

VOLTAGE-DEPENDENCE OF LTP AT THE HIPPOCAMPAL MOSSY FIBER SYNAPSE. *D.B. Jaffe* and D. Johnston*, Div. of Neurosci., Baylor College of Medicine., Houston, TX 77030.

Recent work in our laboratory suggests that postsynaptic, voltage-gated Ca^{2+} channels may modulate the induction of mossy fiber LTP. We tested the hypothesis that the induction of mossy fiber LTP is dependent upon the activation of postsynaptic Ca^{2+} channels, and therefore dependent upon the postsynaptic membrane potential during high-frequency stimulation (HFS). Intracellular recordings were made from CA3 pyramidal cells in the hippocampal slice preparation. An artificial CSF bath containing 10 μM picrotoxin was used to reduce synaptic inhibition and 20 μM APV to block any NMDA evoked potentiation. HFS was applied as 8 trains (50 msec duration) of 100 Hz stimulation at 0.2 Hz. LTP was induced in 5 of 10 control cells (>20% increase in synaptic response 15 minutes post HFS) with a mean increase in EPSP amplitude of 15 ± 8%. Voltage-clamping of CA3 pyramidal cells (holding potential -80 mV) during HFS blocked the induction of mossy fiber LTP in 17 of 18 cells. HFS delivered under current-clamp paired with depolarizing current pulses (3 nA, 50 msec, square pulse) induced LTP in 8 of 10 cells (mean increase in EPSP amplitude 47 ± 14%), which was significantly greater than control cells (unpaired t-test, $p < 0.05$). Postsynaptic depolarization (8-20 square pulses, 3-6 nA injected current, 0.2 Hz) alone or paired with single EPSPs had no effect on synaptic responses. These data suggest that mossy fiber LTP has a Hebbian-like mechanism which is not dependent upon NMDA-receptor activation. A rise in $[Ca^{2+}]_i$, possibly through voltage-dependent Ca^{2+} channels, may be the initial trigger for the expression of LTP at the mossy fiber synapse (Williams & Johnston, this volume). However, a second factor, dependent upon high-frequency synaptic stimulation, may also be required for LTP induction. (NIH grants NS11535 & HL31164, and AFOSR 88-0142)

162.10

HIPPOCAMPAL CA3 MOSSY FIBER LTP IS BLOCKED BY POST-SYNAPTIC CALCIUM CHELATORS. *S.H. Williams & D. Johnston*, Division of Neuroscience, Baylor College of Medicine, Houston, Tx 77030.

Long-term potentiation (LTP) of the hippocampal CA1 Schaffer collateral synapse requires postsynaptic Ca^{2+} influx, which is mediated by glutamate gated, voltage-sensitive, Ca^{2+} permeant, NMDA channels. At the CA3 mossy fiber (MF) synapse, however, the NMDA antagonist APV does not prevent LTP. While NMDA receptor activation is not required, a postsynaptic Ca^{2+} increase may still be necessary. We have tested this hypothesis using postsynaptic injections of the Ca^{2+} chelator BAPTA.

Single electrode current- and voltage-clamp techniques were used to study single CA3 cells in hippocampal slices. Picrotoxin was routinely added to the saline to reduce inhibition. Tetanic stimulation of MF (3 episodes, 100 Hz, 1 sec) induced LTP (> 20% increase 15 min after tetanus) in 6/6 control cells. In a further 4/5 cells, LTP was seen in the presence of 50μM D-APV. In contrast, cells that were iontophoretically loaded with BAPTA (10 mM in the recording pipette) showed LTP in only 2/9 cases. The magnitude of LTP in control cells (EPSP +61 ± 12%, EPSC +58 ± 13%) did not significantly differ from APV treated cells (EPSP +39 ± 4%, EPSC +45 ± 16%), but both control and APV groups showed significantly greater LTP (ANOVA) than BAPTA loaded cells (EPSP +4±9%, EPSC 0±14%). BAPTA did not affect resting potential, input resistance, and membrane time constant. The EPSP was stable with time in control and BAPTA treated cells. Quin-2, a Ca^{2+} chelator similar to BAPTA, blocked LTP in 75% of cells ($n = 4$).

A postsynaptic rise in Ca^{2+} appears necessary for MF LTP. The Ca^{2+} source could be intracellular stores or influx through voltage gated ion channels. The second possibility appears the most likely (Jaffe & Johnston, this volume).

(NIH grants HL31164 & NS11535, and AFSOR 88-0142).

162.11

THE NMDA RECEPTOR IS NOT INVOLVED IN Ca^{2+}-INDUCED LTP IN THE RAT HIPPOCAMPAL CA1 REGION. A. Obenaus, J.A. Shacklock, K.G. Baimbridge, Dept. of Physiology, Univ. of British Columbia, Vancouver, B.C., Canada V6T 1W5.

The effect of D-(-)-2-amino-5-phosphonovalerate (D-APV) on Ca^{2+}-induced long-term potentiation (LTP) has been examined in the in vitro rat hippocampal slice. Additionally, the effect of a transient increase in $[Ca^{2+}]_o$ on $[Ca^{2+}]_i$, has been determined using hippocampal pyramidal cell cultures loaded with the Ca^{2+} sensitive fluorescent probe, Fura II.

D-APV (50uM) affected neither the baseline population spike and EPSP amplitudes, nor did it prevent the induction of LTP by a transient (10min) perfusion with ACSF containing 4mM Ca^{2+}. In cultured hippocampal neurons perfused with a medium containing 2mM Ca^{2+}, the resting $[Ca^{2+}]_i$ was 105nM. Perfusion of 4mM Ca^{2+} for 10min doubled the $[Ca^{2+}]_i$ to 314nM. Neurons observed during the transition to high Ca^{2+} revealed a rapid and sustained increase in $[Ca^{2+}]_i$ which persisted after the return to 2mM $[Ca^{2+}]_o$. D-APV (50uM) when applied prior and during the high $[Ca^{2+}]_o$, did not effect the changes in $[Ca^{2+}]_i$.

Our results suggest that NMDA receptors are not involved in the underlying mechanism(s) seen in Ca^{2+}-induced LTP in the CA1 region. Rather a sustained increase in $[Ca^{2+}]_i$ may be the critical feature.
(Supported by the MRC to KGB).

162.12

POSITIVE MODULATION OF LONG-TERM POTENTIATION BY GLYCINE.
D. L. Tauck and G. A. Ashbeck*. Department of Biology, Santa Clara University, Santa Clara, CA 95053.

Long-term potentiation (LTP) is the increased synaptic efficacy induced by a brief burst of high frequency afferent stimulation. Accumulated evidence suggests that calcium influx through the NMDA receptor/channel is required for the development of LTP. In micromolar concentrations, glycine potentiates numerous responses of the NMDA receptor including calcium flow through the channel. The present study documents the role of glycine as a positive modulator of LTP.

Slices of rat hippocampus were prepared and maintained in vitro by standard techniques. A stimulating electrode positioned in stratum radiatum evoked field potentials in stratum pyramidale of area CA1. Baseline responses were recorded for 20 minutes at a frequency of 0.05 Hz and a stimulus intensity which elicited a 1.5 mV population spike. To induce LTP, the stimulus was applied at a frequency of 100 Hz for 1 second. Twenty minutes after the burst, the average control response had increased to 4.0 mV while the response in 1 micromolar glycine had increased to 8.9 mV. This modulation of LTP by glycine was insensitive to strychnine.
(Supported by Santa Clara University Presidential Research Grant.)

162.13

WITHDRAWN

162.14

CALCIUM-INDUCED CHANGES IN SYNAPTIC EFFICACY IN THE RAT VISUAL CORTICAL SLICE Borroni, A. & Teyler, T. NEOUCOM, Rootstown, Ohio 44272

Long-term potentiation has been studied as a cellular mechanism underlying learning and memory. In the hippocampal slice, LTP can be induced by transient exposure to an extracellular medium containing higher than normal levels of Ca^{2+} (4mM) and K^+ (6.25mM). This result, along with other studies, suggests that the activation of voltage gated Ca^{2+} channels may be required for the induction of LTP in some areas of the hippocampus. In the present experiment, elevated levels of calcium (4mM) and potassium (6.25mM) were used to investigate the similarities between neocortical and hippocampal LTP induction.

White matter stimulation, 1 mm lateral to a recording tract through Oc1 of the rat neocortical slice (400um thick), was sampled every 2 minutes. The slice was exposed to elevated levels of extracellular calcium and potassium for 20 minutes at a rate of .5ml/min. This manipulation produced increases in the evoked response which lasted at least twenty minutes (duration of experiment) after returning to normal medium. Current source density analysis will be used to elucidate the laminar distribution of the Ca^{2+}-K^+ induced changes in the evoked response.
(supported by grants ONR & EPA)

HIPPOCAMPUS AND AMYGDALA I

163.1

SYNAPTOLOGY OF NEUROPEPTIDE Y (NPY) IMMUNOREACTIVE NEURONS IN THE HILAR AREA OF THE RAT HIPPOCAMPUS.
T.Deller*[1] and C.Leranth[1,2]. (SPON: R. J. Austin-LaFrance) Dept of OB/Gyn[1] and Section of Neuroanat[2], Yale Med Sch, New Haven, CT 06510

Synaptology and morphology of NPY-immunoreactive neurons in the fascia dentata were studied using an antiserum against NPY. Transection of the perforant pathway and fimbria fornix transection were used to study extrinsic afferents. Retrograde tracing technique was applied to determine the possible commissural projections of NPY-cells. Results: The majority of NPY terminals were found in the outer molecular layer of the dentate gyrus where they established exclusively symmetric synaptic contacts on dendritic shafts and occasionally on spines of the granule cells. A moderate number of NPY synapses were also found on dendrites in the inner molecular layer and on the cell body of granule cells. Numerous symmetric NPY synapses were also found in the hilar area, some of them on the cell body of neurons which project to the contralateral hippocampus. At least three types of NPY-neurons could be distinguished: Type 1: pyramidal shaped cells in the subgranular and granular layer with long apical dendrites reaching into the outer molecular layer of the dentate gyrus. After transection of the perforant pathway degenerated boutons were found on distal dendritic shafts of the type 1 NPY-neurons. Type 2: multipolar cells located in the polymorph layer of the hilus. They receive mossy axon collaterals. Type 3: Fusiform neurons, some of which could be retrogradely labeled with HRP-injected into the contralateral hippocampus. This study provides a basis for further electrophysiological studies on the controversial effect of NPY on cells in the dentate hilar area. Supported by NIH grant NS26068 (to C.L.) and Cusanuswerk Fellowship (to T.D.)

163.2

HIPPOCAMPAL AFFERENTS, VASOPRESSIN-, AND MONOAMINE-IMMUNOREACTIVE FIBERS TERMINATE ON THE SAME "SOMATO-SPINY" TYPE OF LATERAL SEPTAL AREA NEURONS.
R.L. Jakab*[1] and C. Leranth[1,2]. (SPON: S. Diaz-Cintra) Yale Univ., Med Sch. Dept. Ob/Gyn[1] and Section of Neuroanatomy[2], New Haven, CT. 06510 U.S.A.

A population of lateral septal area (LSA) neurons is believed to be a pacemaker for hippocampal theta rhythm generation. Vasopressin (VP) can inhibit the depression of spontaneous rhythmic activity of these LSA neurons evoked by monoamines, and enhance their excitatory responses to glutamate (M. Joels and J.A. Urban, 1985). In order to elucidate the synaptological relationships of neuronal elements responsible for the aforementioned physiological phenomena, the combination of EM immunostaining and acute axonal degeneration techniques were used. The fimbria-fornix in adult male Sprague-Dawley rats was transected and after a 36 hours survival period vibratome sections of the ipsilateral septal area were immunostained for either VP or the monoamine marker tyrosine hydroxylase (TH). Distribution of degenerated axon terminals (representatives of hippocampo-septal excitatory amino acid containing afferents) and VP- or TH-immunoreactive boutons was examined by electron microscopy. A population of LSA neurons which established asymmetric synapses with degenerated hippocampal afferents were observed to have characteristic spines on their soma, and the same neurons in most cases were also found to form symmetric synapses with VP- or TH-immunoreactive boutons. These results suggest that the VP, monoamine, and hippocampal excitatory amino acid containing fibers terminate on the same "somato-spiny" LSA neurons. Therefore, VP may act monosynaptically as a modulator in the responsiveness of these neurons to monoamines and glutamate. Supported by NIH grant NS 26068 (to C.L) and Soros Foundation Fellowship (to R.L.J).

163.3

POSSIBLE ANATOMICAL BASIS OF THE HYPERSENSITIVITY OF FASCIA DENTATA SOMATOSTATIN NEURONS IN EXPERIMENTALLY INDUCED- AND TEMPORAL LOBE EPILEPSY.

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A selective loss of somatostatin (SS) immunoreactive neurons has been reported in the fascia dentata of patients with temporal lobe epilepsy (R. Robbins et al., 1987). Experimentally-induced epilepsy in rats also dramatically decreases the number of SS neurons in the same hippocampal area (R.S. Sloviter, 1987). The aim of the present study was to determine whether SS neurons of the fascia dentata are involved in a specific excitatory circuitry which results in their selective damage by non-physiological hyperstimulation. Perforant pathways were transected in anesthetized adult Sprague Dawley rats and after a 28-32 h surviving time, animals were sacrificed. Ipsilateral fascia dentata Vibratome sections were immunostained for SS using a monoclonal antibody (SS_8). Correlated light- and electron microscopic analysis demonstrated that both granule cell dendrites and SS immunoreactive dendrites which extend up to the outer third of the molecular layer are densely covered with degenerated perforant pathway axon terminals. Furthermore, other deep hilar area dendrites of the same SS neurons were found in postsynaptic position to granule cell axon collaterals. These observations suggest that a single perforant pathway stimulus can doubly excite SS neurons. First by a direct excitatory synapse on the outer molecular layer dendrites of SS neurons, and secondly by an indirect pathway involving the mossy axon collaterals of granule cells receiving perforant pathway stimulation. This study was supported by an NIH grant (NS 26068) of C.L.

163.4

CHANGES IN SOMATOSTATIN-IMMUNOREACTIVITY OF NON-PYRAMIDAL CELLS IN THE KAINIC ACID LESIONED RAT HIPPOCAMPUS.(SPON: BRA UK)

R.C. Bruce* and H.V. Wheal. Department of Neurophysiology, Univ. of Southampton SO9 3TU. UK.

The CA1 pyramidal cell region in the kainic acid (ICV) lesioned hippocampus exhibits epileptiform activity that is associated with a loss of synaptic inhibition and GABA mediated IPSPs(Ashwood et al.Exp Brain Res.62:189-198,1986). In the normal rat hippocampus neurones exist in which GABA and somatostatin(SOM) are colocalised (Kosaka et alExp Brain Res.71:388-398,1988). We have investigated the effects of the chronic KA lesion on the numbers of SOM-immunoreactive(SOM -I) neurones in the hippocampus at 7 and 28 days post lesion. A monoclonal antibody to SOM-10 was used(Vincent et alJ Comp Neurol. 238:169-186,1985).

When the ipsilateral and contralateral hippocampi were compared at 7 days post lesion, there was a highly significant difference in the number of SOM-I non-pyramidal cells found in the stratum oriens of CA1(n=8). This reduction in the number of SOM-I cells in the lesioned hippocampus was still apparent at 28 days post lesion(n=9). No changes in the numbers of SOM-I cells in the stratum oriens of the contralateral hippocampus were observed.

In contrast, no difference was observed in the SOM-I of cells in the hilar regions 7 days post lesion(n=5). However by 28 days a highly significant reduction in the immunoreactivity of the hilar region in the lesioned hippocampus had developed(n=7). There was also a progressive loss of activity in the hilus of the contralateral hippocampus.

This project was funded by the Wellcome Trust.

163.5

ULTRASTRUCTURAL LOCALIZATION OF SOMATOSTATIN-LIKE IMMUNOREACTIVITY IN THE RAT DENTATE GYRUS.

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Antisera to somatostatin (SOM) were localized ultrastructurally in the hilus (H) and molecular layer (ML) of the rat dentate gyrus by the peroxidase-antiperoxidase (PAP) method. Two types of perikarya with SOM-like immunoreactivity (SOM-LI) were in the hilus: (1) ovoid (6-10μm) and labeled cytoplasm with an accumulation of PAP-reaction product in some Golgi apparatus; and (2) larger (11-16 μm) and a more abundant cytoplasm than the first type, but lacking labeled Golgi. The majority (96% of 374) of synapses on perikarya and dendrites with SOM-LI were from unlabeled terminals forming both asymmetric and symmetric junctions. The remaining pre-synaptic terminals contained SOM-LI and made primarily symmetric synapses. In both the H and ML, terminals with SOM-LI were associated: (1) with single unlabeled perikarya and dendrites (49% of 215 H; 76% of 326 ML); (2) with two unlabeled perikarya and dendrites simultaneously (5% H; 4% ML); and (3) with SOM-containing perikarya and dendrites (6% H; 3% ML). In all three cases, the majority of terminals with SOM-LI formed symmetric synapses on the shafts of small dendrites. The remaining SOM-labeled terminals (40% H; 17% ML) were without any detectable synaptic relations. However, a few of these terminals were in direct apposition to other terminals; some also contained SOM-LI. We conclude that in the dentate gyrus, SOM: (1) may be synthesized and/or packaged in the Golgi of only type of neuron; and (2) most likely inhibits (symmetric synapses) SOM-containing neurons and non-SOM-containing interneurons and granule cells. (Supported by NIMH MH42834 and NIH 18974.)

163.6

MEMBRANE PROPERTIES OF INTERNEURONS IN STRATUM ORIENS/ALVEUS OF THE CA1 REGION OF RAT HIPPOCAMPUS.

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Despite their profound influence on hippocampal pyramidal cells, little is known of the intrinsic properties of interneurons. Intracellular recordings were obtained from interneurons (n=19), located at the border of stratum oriens and the alveus (O/A) in rat hippocampal slices, to analyze their membrane properties. Results (n=14/19) indicate that RMP ranged between -52 and -73 mV, R_{in} between 29-83 MΩ and action potential (AP) amplitude between 48-87 mV. 2 types of interneurons with short (<1.0 ms; n=10) or long-duration (>1.2 ms; n=4) APs were encountered. Interneurons displayed single spike after hyperpolarizations (AHP) (amplitude -8 mV, duration 32 ms) and burst AHPs (amplitude -4 mV, duration 700 ms). V-I relationship in response to 200 ms hyperpolarizing pulses showed time-dependent inward rectification and anodal break excitation (8/11 neurons).

In 5 interneurons, exponential curve fitting and peeling was used to estimate passive cable properties from the charging function of averaged responses to small hyperpolarizing pulses. The membrane time constant t_0 ranged between 6.18-12.0 ms, and the equalizing time constant t_1 between 1.15-2.33 ms. Estimates of ρ, the dendrite to soma conductance ratio, varied between 0.44-4.40 and of L, the electrotonic length, between 1.06-1.31. In 5 interneurons, repetitive discharge during long (700 ms) depolarizing pulses was examined. Spike frequency adaptation was present in each cell and the ratio of final to initial interspike frequency varied between 0.32-0.45.

O/A interneurons have distinctly non-pyramidal membrane properties. They are electrically compact and display spike frequency adaptation. Physiologically more than one type of interneuron may be found in O/A region.

Supported by FRSQ and the Sloan, Banting and Savoy Foundations.

163.7

MORPHOLOGY AND SYNAPTIC ORGANIZATION OF PHYSIOLOGICALLY IDENTIFIED INTERNEURONS IN SLICE CULTURES OF RAT HIPPOCAMPUS.

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Hippocampal slices retain their basic structural and connective organization when cultured by the roller tube technique. Previous physiological and morphological studies have shown that slice cultured hippocampal pyramidal cells exhibit typical pyramidal cell characteristics. Information on the intrinsic interneuron populations and the overall synaptic organization of hippocampal organotypic cultures is generally lacking.

Interneurons were first identified physiologically in CA1 stratum oriens, and then injected intracellularly with biocytin, and examined at the electron microscopic level. Numerous fusiform and multipolar-shaped interneurons were found in stratum oriens and at the stratum oriens/stratum pyramidale border. Both types of cells possessed an extensive multipolar dendritic tree extending approximately 200 μm from the soma. Dendrites were slightly varicose, aspiny, and had numerous synaptic contacts (both asymmetric and symmetric) along their length. The axon and its collaterals projected towards CA1 pyramidal cells and made numerous synaptic contacts. Synaptic contacts from these interneurons appeared to be of the symmetric type. Synaptic contacts were also observed making contact with other interneurons in this region. Studies are on-going to examine the immunoreactive nature of these cells, and the overall synaptic organization of organotypic hippocampal cultures.

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163.8

PARVALBUMIN IMMUNOREACTIVITY IS LOCALIZED TO ALL FIVE TYPES OF BASKET CELL IN THE RAT DENTATE GYRUS.

C. E. Ribak, L. Seress*, S. F. Bullock* and K. G. Baimbridge. Dept. of Anatomy and Neurobiology, Univ. of Calif., Irvine, CA 92717 and Dept. of Physiology, Univ. of British Columbia, Vancouver, BC, V6T 1W5.

Previous studies have indicated that many GABAergic neurons in the hippocampal formation contain immunoreactivity for the calcium-binding protein parvalbumin (PAR-ir), including two types of basket cell in the dentate gyrus. These studies did not determine whether all five types of basket cell that are GABAergic contain PAR-ir and what the ultrastructural features of PAR-ir basket cells are. Light and electron microscopic immunocytochemical studies were made to resolve these issues. In light microscopic preparations, PAR-ir was localized to many pyramidal and fusiform basket cells as previously described. In addition, horizontal, inverted and molecular layer basket cells were less frequently observed to contain PAR-ir. Electron microscopic preparations revealed that cells containing PAR-ir in the granule cell layer displayed the characteristic features of basket cells, such as Nissl bodies, intranuclear rods, nuclear infoldings, asymmetric and symmetric axosomatic synapses and aspinous dendrites. A variety of axon terminals that form synapses with the PAR-ir dendrites was observed. These results indicate that all five types of basket cell have PAR-ir. (Supported by NSF Grant BNS 86-15579)

163.9

CONDITIONING PULSES REDUCE PAIRED PULSE INTERACTIONS IN THE RAT DENTATE GYRUS. D.D. Mott* and D.V. Lewis. Depts. of Pharmacology, Neurobiology and Pediatrics (Neurology), Duke University Medical Center, Durham, NC 27710.

Heterosynaptic afferent input has been shown to alter the response of dentate granule cells to perforant path excitation. We used paired perforant path stimuli to investigate this phenomenon.

Paired pulse effects were studied in hippocampal slices from adult, male Sprague-Dawley rats. Perforant path stimulation evoked an EPSP and a population spike in the dentate gyrus granule cell layer. Responses to paired perforant path stimuli exhibited an early (10 msec) inhibition, an intermediate (50 msec) facilitation and a late (400 msec) inhibition. Paired pulse effects were quantified as the percent change in the amplitude of the perforant path-evoked population spike.

Conditioning pulses (1-4 pulses, 50 Hz) delivered to the alveus or stratum lucidum of CA3 had marked effects on the responses of granule cells to paired PP stimulation. Conditioning pulses reduced early (10 msec) inhibition, converting the response to one showing facilitation. In addition, they reduced the intermediate (50 msec) facilitation but had no effects on the late (400 msec) inhibition. The effect of conditioning pulses lasted for up to 1000 msec with a maximal effect at 200 msec. Phaclofen (1 mM, bath-applied) but not naloxone (10 uM, bath-applied) antagonized the effects of the conditioning pulses. In the presence of phaclofen, conditioning pulses only slightly reduced the early inhibition and caused no change in paired pulse facilitation. Phaclofen reduced the amplitude of the perforant path-evoked population spike but had no consistent effect on paired pulse interactions. All drug effects were reversible.

These results suggest that heterosynaptic input can affect granule cell responsiveness through a $GABA_B$ receptor dependent mechanism.

Supported by NIH grant NS 22170 and the Chemical Industries Institute of Toxicology Predoctoral Fellowship.

163.10

RELATIVE CONTRIBUTIONS OF FEEDBACK AND FEEDFORWARD INHIBITION IN THE DENTATE GYRUS OF THE RABBIT HIPPOCAMPAL SLICE. T.P. Harty, T.W. Berger and G. Barrionuevo. *Departments of Behavioral Neuroscience and Psychiatry, University of Pittsburgh, Pittsburgh, PA, 15260.*

We evaluated the relative contributions of feedback and feedforward GABAergic inhibition to paired-impulse activation of population spikes evoked by perforant path stimulation of granule cells of the dentate gyrus. Using a rabbit hippocampal slice preparation, antidromic (activates feedback elements only) and orthodromic (activates feed-forward and feedback elements) conditioning impulses were paired with orthodromic and antidromic test impulses. These testing procedures were performed using short interstimulus intervals (10-50 ms) in the presence of alphaxalone (an allosteric GABA agonist) and bicuculline (a GABA antagonist). In the presence of alphaxalone, antidromic and orthodromic conditioning impulses reduced orthodromic test responses by 34% and 83%, respectively, relative to comparable test responses in the presence of bicuculline. This suggests that feedforward inhibition can account for as much as 50% of orthodromically activated inhibition. However, neither orthodromic nor antidromic conditioning impulses significantly reduced antidromic responses. One possible interpretation of these findings is that feedforward and feedback interneurons selectively project to a population of granule cells that are activated orthodromically but not antidromically. The population of granule cells activated antidromically excite feedback interneurons but are not inhibited by them. It remains possible that GABAergic feedback inhibition is unable to reduce antidromically activated population spikes. Intracellular studies will be necessary to clarify this issue. These studies were supported by grants from the ONR, AFOSR, NIH and NIMH.

163.11

COMPARISON OF DIRECT AND INDIRECT INPUT TO THE HIPPOCAMPAL REGIO INFERIOR FROM THE ENTORHINAL CORTEX USING TRANSSYNAPTIC TRANSPORT OF WGA-HRP. T.W. Berger and P.M. Rice*, *Departments of Behavioral Neuroscience and Psychiatry, University of Pittsburgh, Pittsburgh, PA 15260.*

Transsynaptic transport of WGA-HRP injected into the entorhinal cortex (Van Hoesen et al., Brain Research, 398, 1986) of adult New Zealand white rabbits was used to compare the distributions of direct (presumed monosynaptic) and indirect (presumed disynaptic, mediated by entorhinal monosynaptic afferents to the dentate gyrus) entorhinal input to the CA3 pyramidal cell region of the hippocampus. While animals were maintained on halothane anesthesia, WGA-HRP (2-3%) was pressure injected into the entorhinal cortex. Animals were allowed 3-5 days of survival, after which time they were perfused and the tissue prepared, sliced, and reacted with tetramethyl benzidine, as described by Gibson et al. (Brain Research, 298, 1984).

Densely labeled processes indicative of axonal and terminal labeling were localized within the outer two-thirds of the molecular layer of the dentate gyrus and in the stratum lacunosum-moleculare of CA3. In addition, labeling was observed within somata of the granule cell layer, and not within the inner one-third of the molecular layer, indicating transsynaptic transport of WGA-HRP from entorhinal terminations in the dentate. Less dense, but clearly detectable labeling also was localized in the stratum lucidum of CA3, consistent with transsynaptic transport of WGA-HRP along granule cell mossy fiber axons. Particularly notable was the lack of correspondence between the dense monosynaptic innervation of CA3 and the disynaptic input indicative of those CA3 cells receiving indirect input as part of the trisynaptic pathway.
Supported by NSF, NIMH, ONR, and AFOSR.

163.12

LONG-TERM POTENTIATION OF MONOSYNAPTIC ENTORHINAL CORTICAL INPUT TO CA3 PYRAMDIAL NEURONS OF THE HIPPOCAMPUS. M.F. Yeckel and T.W. Berger, *Depts. of Behavioral Neuroscience and Psychiatry, Univ. of Pittsburgh, Pittsburgh, PA 15260.*

The CA3 and CA1 pyramidal cell regions of the hippocampus receive both a direct, monosynaptic input from the entorhinal cortex (EC) and an indirect, di- and/or tri-synaptic input via EC projections to the dentate gyrus (DG). We recently have demonstrated that for any given subpopulation of pyramidal neurons, EC excitation through monosynaptic afferents predominates over the di- or tri-synaptic input (Yeckel et al., Soc. Neurosci. Abstr., 14, 1988). Additionally, activity in both CA3 and CA1 can be evoked when DG cell activity was inhibited, indicating that EC-induced excitation of pyramidal cells can occur independently of DG excitation. We have investigated further the functional characteristics of the direct EC-to-CA3 projection, and specifically, its ability to express long-term potentiation (LTP).

Ten trains of 10 impulses (400 Hz) were delivered to the angular bundle of halothane anesthetized rabbits while recording from the cell body layers of DG, CA3, and CA1. Recordings from CA3 revealed that short-latency (4-6 ms) monosynaptic population spike responses to angular bundle stimulation were potentiated for at least 1 hr following high frequency stimulation. Comparison of input/output functions for EC-to-DG and EC-to-CA3 projections revealed a greater magnitude increase in CA3 spike amplitudes in response to low intensities, and a greater magnitude increase in DG spike amplitudes in response to high intensities. Increases in both di- (EC-CA3-CA1) and tri-synaptic (EC-DG-CA3-CA1) activation of CA1 also were seen post-LTP. These observations support the conception that excitation of EC afferents initiates a two-phase feedforward excitation of hippocampal pyramidal cells, and demonstrate that these feedforward pathways also are modifiable by high frequency stimulation. Supported by NSF, ONR, AFOSR, and NIMH.

163.13

QUANTITATIVE ESTIMATION OF THE NUMBER AND DISTRIBUTION OF ACTIVE DENTATE GRANULE CELLS IN THE HIPPOCAMPUS AS A FUNCTION OF IMPULSE ACTIVITY OF ENTORHINAL AFFERENTS. G. Chauvet*, G. Barrionuevo, and T.W. Berger (SPON: G. Barrionuevo). *Lab. of Theoretical Biology, School of Medicine, Angers, France, and Depts. of Behavioral Neuroscience and Psychiatry, Univ. of Pittsburgh, Pittsburgh, PA.*

A mathematical model based on a two-level field theory[+] is described that relates the number and distribution of active neurons in a volume to the extracellular current density they generate during action potential discharge. The anatomical structure includes a density-connectivity function, $\pi(s,r";r)$, that describes the density of synapses at s with density ρ_0 in postsynaptic neurons localized in r and which are connected to presynaptic neurons with density ρ_1 in r". The physiological problem is the determination of active spaces and density functions, as dependent on stimulus intensity.

The specific geometrical model considered is of entorhinal perforant path innervation of the hippocampal dentate gyrus. The model describes the interaction between a set of active afferents, assumed to be a cylinder with a diameter dependent on stimulation intensity, and conically shaped dendritic trees of granule cells. It is assumed that perforant path afferents provide topographically organized, excitatory input through en passage synapses.

It is shown that information from specifically defined experiments allows an estimation of the minimum (space element) and maximum active volumes, active density-connectivity and granule cell density functions as a function of the number of active perforant path fibers. Full experimental implementation of this model should allow extracellular field potentials recorded from the dentate gyrus to be interpreted in terms of the number of granule cells activated and the strength of the synaptic input responsible for the activation. [+]Chauvet, G., Correlation principle and physiological interpretation of synaptic efficacy, in Systems with Learning and Memory Abilities, J. Delacour and J.C.S. Levy, Elsevier (1988). Supported by AFOSR, ONR, NH09116 and MH00343.

163.14

RESPONSE PREDICTION FOR THE HIPPOCAMPUS OF THE RABBIT. *J. M. Solomon, D.N. Krieger, T.W. Berger, and R. J. Sclabassi. Departments of Neurosurgery, Electrical Engineering, and Behavioral Neuroscience, University of Pittsburgh, Pittsburgh, PA 15213. (Spon: M. Bennett). We have been characterizing the nonlinear functional properties of the hippocampal formation in the rabbit utilizing the kernels from an orthogonalized functional power series. Using both *in-vivo* and *in-vitro* preparations, we have experimentally estimated the first four kernels (h_0, h_1, h_2, h_3), by cross-correlating the evoked population spike activity with a random point process input. The resulting functions are interpreted both as generalized recovery functions and as n^{th} order impulse responses. These kernels are then utilized in N^{th} order convolution integrals to model the system response to alternative inputs. This paper reports on experiments from both classes of preparations where, besides the random trains, the perforant path was stimulated with doublets and triplets over a range of interstimulus intervals from 10 ms to 1000 ms, and the observed responses were predicted using an input/output model based on the previously measured kernels. Both the twin pulse and triplet experiments had prediction mean square errors of less than 5%, when modeled utilizing kernels through the third order for both the *in-vivo* and *in-vitro* experiments.

163.15

FREQUENCY DOMAIN PROPERTIES OF SECOND ORDER KERNELS FROM THE HIPPOCAMPUS OF THE RABBIT. R. J. Sclabassi, D. N. Krieger, T. Biedka,* and T.W. Berger Departments of Neurosurgery, Electrical Engineering, and Behavioral Neuroscience, University of Pittsburgh, Pittsburgh, PA 15213.

This paper examines the frequency domain characteristics of second order kernels of a functional power series expansion. Experimental data was obtained by stimulating the medial and lateral bundles of the perforant path, both *in-vivo* and *in-vitro*, using random stimulus trains, and recording the evoked population spike in the granuale cell layer. Second order kernels were obtained by cross-correlation. The second-order kernels were transformed into the frequency domain using a two dimensional dFT The results are presented in the ω_Δ and ω_τ plane, with the maximum frequency component in either axis being 500 Hz. All three classes of preparations studied demonstrated significant activity in the frequency domain. Medial perforant path data demonstrated a 1/f fall-off in the ω_Δ dimension, terminating in a notch at $\omega_\Delta = \omega_\tau$. Lateral perforant path data demonstrated a similar fall-off, again terminating in a notch at $\omega_\Delta = \omega_\tau$, with a parallel notch occurring at $\omega_\Delta + 50Hz. = \omega_\tau$. In contradistinction, the slice data was nearly constant in the τ but demonstrated significant periodicities in the Δ dimension.

163.16

NONLINEAR RESPONSE PROPERTIES OF HIPPOCAMPAL DENTATE GRANULE CELLS ARE NOT DEPENDENT ON MEAN FREQUENCY AFTER LONG-TERM POTENTIATION OF ENTORHINAL CORTICAL INPUT. J.R. Balzer*, R.J. Sclabassi, and T.W. Berger (Spon: S. Wolff). *Departments of Behavioral Neuroscience, Neurological Surgery, and Psychiatry, University of Pittsburgh, Pittsburgh, PA 15260.*

In previous experiments (Robinson et al., Soc. Neurosci. Abstr., 11, 1985), we have used nonlinear systems analysis to characterize the transformational properties of the hippocampal perforant path-dentate projection (PP-DG). Perforant path fibers were stimulated with trains of impulses having a Poisson distribution of inter-impulse intervals; different distributions having mean frequencies (λ) of 1.0, 2.0, 3.3, 5.0, and 10.0 Hz were used. First, second, and third order kernels were computed by cross-correlating intervals of the stimulus train with amplitude of the granule cell population spike. Results showed that first order kernels, which reflect the average population spike amplitude per impulse, were not altered by changes in λ. In contrast, second and third order kernels, which reflect the nonlinear response of granule cells as a function of intervals within the train, varied substantially depending on λ.

We examined the effect of LTP on the relationship between granule cell nonlinearity and λ. Results showed that after the induction of LTP, nonlinear response characteristics were much less dependent on λ. Together with our previous findings that LTP decreases the dependence of nonlinear characteristics on the intensity of random train stimulation, these data show that the dentate component of the hippocampus provides much less transformation of entorhinal input after induction of LTP, and that the transformation that does occur is the same for most levels of synchrony and mean firing rate of entorhinal neurons. Supported by NSF, ONR, AFOSR, and NIMH.

163.17

THE USE OF WAVEFORM RELAXATION IN COMPARTMENTAL MODELLING OF HIPPOCAMPAL CA1 PYRAMIDAL NEURONES H.V. Wheal and E.W Stockley*. Department of Neurophysiology, University of Southampton. SO9 3TU, UK.

The interpretation of electrophysiological data related to synaptic function in the CA1 region of the hippocampus is limited by a lack of knowledge of the spatial distribution and efficacy of synapses. In order to overcome this limitation we have developed a compartmental model based on the 3-dimensional morphology of pyramidal cells and their dendrites.

Intracellular electrophysiological data was obtained from CA1 pyramidal cells that were subsequently filled with HRP. After histological processing the cells were optically sectioned, digitised and reconstructed in 3-dimensions. This database provided the structure for the generation of a multi-compartmental model. We have written a circuit simulator based on waveform relaxation (WR) specifically to solve the resulting large (>2000 nodes) equivalent circuits. The program was first tested by simulating idealised structures with analytic solutions. Our simulations showed that waveform relaxation converged slowly when applied to the tightly coupled compartments of a typical cell structure. However, significant improvements in solution time were obtained using succesive over relaxation and by combining WR and sparse matrix gaussian elimination into a hybrid simulator.

Comparison of simulated and measured input resistances and time constants has enabled us iterate towards a consistent set of passive model parameters. We are now adding active membrane conductances to the modelling scheme based on modified Hodgkin/Huxley dynamics and data obtained from dissociated pyramidal cells.

163.18

SIMULATIONS OF SYNAPTIC POTENTIALS USING REALISTIC MODELS OF HIPPOCAMPAL PYRAMIDAL NEURONS. J. Wathey, W. Lytton, J. Jester and T. Sejnowski, Salk Institute, La Jolla, CA.

Different portions of a pyramidal cell may operate independently along one physiological dimension while interacting along another. Parameters that may be involved include voltage changes, second messengers, ion fluxes, morphological and metabolic changes. To assess the relative contribution of these influences, we have modeled an anatomically realistic CA1 pyramidal cell at different levels of physiological detail. We used the simulation program "CABLE" (M. Hines and J. Moore) with morphological data provided by D. Amaral.

Initially, we determined whether passive properties could produce asymmetrical voltage responses in apical and basilar dendritic fields to synaptic input on the opposite side. With low membrane resistance, a basilar tetanus showed 30% less influence on the apical region than a normalized apical tetanus produced in the basilar region. Even with maximal synaptic input, however, the resulting depolarization in the other compartment was only 5-10 mV with these parameters, too low to be significant. With passive somatic and dendritic membranes, the addition of a spike-generating axon had virtually no effect on the membrane potential elsewhere in the cell. Soma-generated spiking was reflected throughout the neuron and transformed a low frequency signal in the apical dendrites into passively conducted spikes in the basilar dendrites. Slower Ca^{++}-mediated spiking in the apical dendrites smoothed the signals received via the soma from the basilar dendrites to produce more consistent depolarization in the apical dendrites. These possibilities can be tested by studying interactions between different parts of pyramidal cells in slice preparations.

163.19

COMPUTER SIMULATION OF HIPPOCAMPAL PLACE CELLS. Patricia E. Sharp, SUNY Health Sciences Center, Brooklyn, N.Y.

It has been suggested that the hippocampus is involved in configural learning and that deficits seen on spatial and other tasks after hippocampal damage are due to a configural component of these tasks (Sutherland & Rudy, *Psychobiol.*,1989). Hippocampal pyramidal cells show location-specific firing thought to be crucial in the hippocampus' role in spatial learning. It has been suggested that this firing results from the 'local view' available in a cell's field (McNaughton, In *Neural Connections, Mental Computation*, Nadel *et al.*, 1989).

Here, these ideas are combined in a model in which pyramidal cells are the output layer of a competitive learning pattern classification device (Rumelhart & Zipser, In *Parallel Distributed Processing*, Rumelhart *et al.*, 1986). The inputs are 'configurations' of environmental stimuli as viewed from various locations. These patterns are 'classified' on the basis of their similarity. Since views available from contiguous regions of space are similar, single cells come to fire in a circumscribed region (place field).

In these simulations, computerized rats run 'sessions' of continuous locomotion in a 'cylinder' with discrete 'stimuli' on its walls. For each input cycle (at 8 Hz): 1) the distance and angle of each stimulus to the rat's current head position is calculated 2) these results establish activity in 'neocortical' units which have a .16 probability of responding to a given stimulus when that stimulus is in its receptive field 3) these units establish activity in 'entorhinal cells' to which they project and 4) these, in turn, project to 'hippocampal cells'. Hebb-like changes in synaptic weights occur for active cells. Firing-rate maps for these theoretical units show place fields remarkably similar to those of actual cells (Muller, *et al., J. Neurosci.*, 1987; Quirk *et al., Soc. Neurosci. Abstr.*, 1988).

163.20

BACK-PROPAGATION CALCULATIONS OF HIPPOCAMPAL PLACE CELL FIRING FIELDS. R.U.Muller*, G.J.Quirk and J.L.Kubie. (Spon. M. Halpern) Dept. of Physiol. SUNY Health Sciences Center, Brooklyn, N.Y. 11203

The spatial firing patterns of hippocampal (HP) place cells and entorhinal cortex (EC) cells were recorded under identical conditions. The firing patterns of HP cells were crisper than those of EC cells. This is not unexpected, since EC is likely the route by which processed sensory information reaches HP.

We used back-propagation of error (Rumelhart et al, 1986) to calculate spatial firing patterns of place cells with EC cells as inputs. With 40 each of EC, "hidden" and place cells, the total error sum of squares (TSS) decreased along an exponential-like path. After 500 iterations, the patterns calculated for place cells strongly resembled the "teaching" patterns. Another run with place cells as inputs gave similar results, except that TSS was lower at each iteration than with EC inputs. In both cases, the decay of TSS was associated with large variations of TSS around the running average, an indication of "chaotic" behavior.

A third calculation was done with randomized versions of EC firing patterns as inputs. The decay of TSS was slower than with the original EC patterns, but the form of the decay was the same, and the computed patterns again strongly resembled the teaching patterns. Thus, back-propagation does not require a spatial signal in the inputs to yield close facsimiles of place cell spatial firing patterns, and may therefore be too powerful (due to the teaching input?) to serve as a method for investigating the origins of place cell properties. In contrast, it may be possible to generate testable connectivity schemes between EC, the dentate gyrus and the hippocampus by minimizing differences between the computed properties of hidden cells and the measured spatial firing properties of dentate cells.

163.21

THE FIRING OF ENTORHINAL PLACE CELLS IS MORE SENSORY-BOUND THAN THAT OF HIPPOCAMPAL PLACE CELLS.
G.J. Quirk, and R.U. Muller*, SUNY Health Sciences Center, Brooklyn, N.Y.

The entorhinal cortex (EC) is the main path for conveying sensory information to the hippocampus (HP), and cells there show location-specific firing when examined in freely-moving rats (Quirk and Ranck '86, *Neurosc. Abstr.* 12:1524). Place cells in both structures fire most rapidly in a single region of the apparatus; this region rotates with the controlling cues. To further understand environmental control over the firing of cells in the two structures, recordings were made in differently shaped chambers. Previous work showed that changing apparatus shape dramatically alters the firing patterns of HP cells, i.e. the firing pattern in a cylinder does not predict the firing pattern in a square (Muller and Kubie '87, *J. Neurosc.* 7:1951-1968).

28 EC and 19 HP cells were examined in a cylinder (76 cm diam) and a square (69 cm on a side) that were uniformly gray save for a white cue-card attached to the wall in each chamber. Mean firing rate and 3 measures of location-specificity (spatial autocorrelation, field size, no. of high-rate patches) were compared in the cylinder and square for the EC and HP cells. In all measures, the firing patterns of EC cells were significantly more similar in the two apparatuses than were HP cells. Unlike HP cells, the location of EC firing-fields in the two apparatuses often occupied a similar position relative to the cue-card.

These data suggest that the firing of EC cells is more tightly controlled by the cue-card than HP cells, and that the extraordinary sensitivity of HP cells to the shape of the chamber is a property that arises after the EC.

Supported by NS 14497 and NS 07117.

163.22

WITHDRAWN

CEREBELLUM II

164.1

CEREBELLAR LUGARO CELLS ARE MOLECULARLY DISTINCT FROM GOLGI AND PURKINJE CELLS.
M. Sahin* and S. Hockfield, Sect. of Neuroanatomy, Yale University School of Medicine, New Haven, CT 06510.

The cerebellar cortex contains five major cell types. These cell types have been differentiated from one another by their location, size and shape and in some cases by molecular characteristics. Another less numerous cell type, the Lugaro cell, has been identified by its shape and location. Lugaro cells are long, horizontally-oriented, fusiform cells that are located in or slightly below the Purkinje cell layer. Their principle dendrites emerge from opposite poles of the cell body and extend long distances in the horizontal plane. Despite their distinct morphology, Lugaro cells have not been differentiated from the Golgi cells of the granule layer by many authors, who classify all the large neurons of the granular layer as Golgi cells.

In the present study, we sought to differentiate the Lugaro cell by its molecular characteristics. We used cell-type specific antibodies against the three large cell types located at the junction of molecular and granular layers: Cat-301 and Cat-304 for Lugaro cells; Rat-303 for Golgi cells; and anti-calbindin for Purkinje cells. Double-label immunocytochemistry on cat cerebellar sections with subclass- or species-specific secondary antibodies showed that each class of neuron was identified by only one cell-type specific antibody. These results demonstrate that Lugaro cells are molecularly distinct from Golgi and Purkinje cells with respect to their surface and intracellular antigens. This molecular characterization of Lugaro cells resolves the question of whether they make up a distinct cell type and should permit further studies to determine their role in cerebellar circuitry and information processing. Supported by BNS8812163 (S.H.)

164.2

MORPHOMETRIC ANALYSIS OF FUSIFORM CELLS IN THE GRANULAR LAYER OF THE RAT CEREBELLAR CORTEX.
J. LAINE*, B. BERTHIE* & H. AXELRAD* (SPON: W.R. WEBSTER) Lab. de Physiol.-CHU Pitié-Paris 13- FRANCE

The fusiform neurons, present in the granular layer, form the less known cell type of the mammalian cerebellar cortex. Since their description by Lugaro (1894) only qualitative studies on their morphology have been published, the exact target of their occasionaly impregnated axon is still under debate and there has been no functional recording of these interneurons. In view of a complete morphofunctional study of these cells- necessary to apprehend the full dynamics of the cerebellar network- we present here some quantitative data obtained from 40 Golgi impregnated neurons of the rat vermis, using the modification of Gabbott & Somogyi (J. Neurosci. Methods., 11:221;1984). 1-Cell localization: 16 had their soma at the level of the Purkinje Cell layer(PCl),21 were located about 4 µm beneath the PCl, intermingled between the basket cell's terminal "pinceaux", and 3 were more deeply situated in the depth of the granular layer. 2- The cell soma has a great axis parallel to the PCl with a mean length of 16.6µm and mean small axis of 9.7µm (mean ratio:1.8; range: 1.18-2.57). 3- The mean number of dendrites is 4.2(range 2-8) with 89% of them arising at either pole,thus giving the cell it's specific bearing. 4- Due to the fact that 84% of the 167 dendrites were cut the total spatial span of these is difficult to assert, nevertheless the longest ones had their termination farther than 320µm from the soma. 4-Of the 21 complete polar dendrites 14 branched dichotomously with a mean of 3 branches.No clear correlation for the branching point distances was found. 5-In our series , as for other groups, unfortunately no axon was impregnated for more than 20-30µm.These cells appear therefore as a relatively homogeneous type.

164.3

PROJECTIONS FROM THE BRAINSTEM NUCLEI TO VENTRAL PARAFLOCCULUS (vPFL), DORSAL PARAFLOCCULUS (dPFL) AND FLOCCULUS (FL).
S.A. Azizi, A.J. Painchaud* and D.J. Woodward, *Dept Cell. Bio. U. T. Southwestern Medical Center, Dallas, TX 75235*

This study was undertaken to demonstrate the organization of detailed projections from brain stem nuclei to visual-vestibular areas of the lateral cerebellum. Small quantities (.01 to .2µl) of HRP-WGA were pressure/iontophoretically-injected into discrete areas of the cerebellar cortex in 45 rats. The sensitive chromogen, tetramethyl benzidine, was used to visualize labelled neurons. Three-dimensional images of the PFL and FL were obtained with the aid of a computer. Results of this investigation demonstrated that with regard to olivary projections, the FL receives almost exclusively from the dorsal cap, a portion of the vertical lamella. The vPFL receives from the rostral lamella of the medial accessory olive and the ventral lamella of the principal olive, while dPFL receives mainly from the principal olive (for nomenclature see Azizi and Woodward, JCN 263:467, 1987). With regard to mossy fiber afferents, FL receives mainly from the vestibular nuclei and lateral zones of the basilar pons. The vPFL receives from lateral zones of the rostral basilar pons, while dPFL receives from the medial zone of the rostral basilar pons, among other minor projections, but not any vestibular input.

These data suggest that, although FL, vPFL and dPFL receive climbing fiber input from distinctly separate regions of the olive, these same structures receive mossy fiber input from similar pontine regions. We hypothesize that the pontine projections to these structures may serve to transmit common gain control/gating signals from the cerebral cortex concurrently to both FL and pFL. *(Supported by DA 2338, NIAAA 2901 and Biological Humanics Foundation).*

164.4

PURKINJE CELL ANTIGENIC COMPARTMENTATION AND THE TOPOGRAPHY OF SPINOCEREBELLAR TERMINAL FIELDS.
C. GRAVEL, Lab. Neurobiology, Laval U., Quebec, and R. HAWKES, Dept. Anatomy, U. Calgary, Alberta, Canada.

WGA-HRP injections into the lower thoracic-higher lumbar (LTHL) region of the rat spinal cord reveals parasagittal bands of mossy fiber terminals in the cerebellar cortex. The LTHL terminal fields and the Purkinje cell compartments were compared by immunoperoxidase staining of adjacent sections with anti-zebrin I (mabQ113) and 3D reconstruction. The comparison reveals a complex relationship, including:

- PC compartments subdivided longitudinally by LTHL terminal fields
- some PC compartment boundaries strictly obeyed by the LTHL terminal fields, others not
- differences in terminal field position between the dorsal and ventral surfaces of the same lobule.

One result of the subdivision of PC zones is that anatomical compartment widths are now compatible with the microzones and patches identified electrophysiologically.

164.5

AFFERENT ORGANIZATION OF THE LATERAL RETICULAR NUCLEUS OF THE RAT: AN ANTEROGRADE TRACING STUDY. N. Rajakumar*, A.W. Hrycyshyn and B.A. Flumerfelt. (SPON: J.A. Kiernan) Dept. of Anatomy, The University of Western Ontario, London, Canada, N6A 5C1.

The organization of afferent fibres within the lateral reticular nucleus (LRN) of the rat was investigated following placement of HRP-WGA into the red nucleus, fastigial nucleus, various levels of the spinal cord or the sensorimotor area of the cerebral cortex. The pattern of distribution of anterogradely labelled profiles visualised with TMB histochemistry revealed that the caudal three-fourths of the LRN received a large, topographically organized projection from the entire length of the contralateral spinal cord, the lateral part of the rostral half of the LRN received a smaller projection from the contralateral red nucleus, the dorsal part of the middle third of the LRN received a smaller projection from the contralateral fastigial nucleus, and the extreme rostromedial part of the nucleus received a sparse projection from the cerebral cortex. The distribution of afferents from different levels of the spinal cord, red nucleus, and fastigial nucleus overlapped in the middle third of the LRN, whereas the cerebral cortical receiving area of the nucleus was devoid of the other inputs. These data suggest that the middle third of the LRN integrates spinal and supraspinal impulses to the cerebellum, and is involved in a cerebello-rubro-LRN reverberating circuit, while the rostral part provides an additional cerebral cortico-cerebellar pathway. (Supported by M.R.C. Canada).

164.6

Development ot Cerebellar Cortical Efferents: An In-vitro Anterograde Tracing Study in Rat Brain Slices. **Leonard M. Eisenman, M.P.A. Schalekamp* and Jan Voogd*. Department of Anatomy, Jefferson Medical College, Philadelphia, PA. 19107, USA., and *Department of Anatomy, Erasmus University, Rotterdam, The Netherlands.**

The anatomical and presumably functional organization of the cerebellum has been demonstrated to be based on a parasagittal schema of afferent and efferent connectivities and biochemical heterogeneities. The manner is which this organization is established during development still eludes us. We were interested in using an in-vitro slice technique to examine the efferents from Purkinje cells to the deep cerebellar and vestibular nuclei to determine their developmental timetable. We iontophoretically injected the nascent cerebellar cortex in embryonic day 18 (E-18) through postnatal day one rats with a 5% solution of horseradish peroxidase (HRP). After six to seven hours of survival in an in-vitro chamber, the slices were removed, placed in fixative and processed histochemically. The results demonstrate that transport from the Purkinje cells can be seen and followed to the region of the cerebellar or vestibular nuclei. In E-18 slices, labeled axons with growth cones could be observed coursing through the white matter and into the cerebellar or vestibular nuclear region. In sections from E-19 through E-20 the labeled axons could be followed into the nuclear regions and seen to ramify among the cell bodies. Terminal swellings, however, were not observed. In E-21 sections the labeled axons could be seen to form terminal swellings over the somas of vestibular neurons. These results demonstrate that the cortico-nuclear and cortico-vestibular projections are forming in late embryonic life and that they appear to begin forming synaptic contacts with their target cells at the latest by day E-21. These findings suggest the possibility that the corticonuclear and corticovestibular projections may be present early enough to serve as the organizing system of cerebellar topography. (Supported by NIH grant NS16531)

164.7

RE-DEFINING RAT RED NUCLEUS: CYTOARCHITECTURE AND CONNECTIVITYC.L.Tucker*, S.A.Lee* & P.R.Kennedy, Bioengin. Center, Georgia Tech, Atlanta, GA 30332.

Rat red nucleus (RN) is considered to be limited rostrally by the pre-rubral area. We have studied RN cell types and number, distributions and locations in thionin-stained and fluorescently stained sections. Diamidino Yellow (DY) was placed in the dorsolateral funiculus of the spinal cord that contains the rubrospinal tract (RST); Fluoro-Gold (FG) was placed near the inferior olivary nucleus (ION).

Cells corresponded to parvo and magno cell descriptions (Parvo: large central round nucleus, prominent nucleolus, small cytoplasmic/nuclear ratio, rounded outline; Magno: relatively small oval nucleus, eccentrically placed, large cytoplasmic/nuclear ratio, with rectangular or pyramidal cytoplasmic outline.) Parvos were large (30-45u), medium (15-30u) or small (10-15u). Magnos were larger or smaller than 25u. Counts of nucleus-containing cells revealed 3,008 magnos that were situated mainly caudally, as expected, but also extended into and beyond the pre-rubral area. 19,904 parvos extended from the caudal pole through the pre-rubral area and surrounded the fasciculus retroflexus. The magnos tended to be located ventrally, whereas the parvos were located throughout the nucleus.

Labelling the transected RST at C3 with DY in 6 rats produced labelled magnos and parvos. Surprisingly, only 24% of these labelled cells were magnos and 76% were parvos (combined result from 2 rats analyzed in detail). Even though unlabelled (thionin stained) magnos were seen extending into and beyond the pre-rubral area, DY-labelled magnos were only seen caudal to it. DY-labelled large and small parvos were similarly distributed. DY-labelled medium parvos, however, extended from the caudal pole through the pre-rubral area into the para-fascicular region.

In these two rats accurate injections of FG were made in ION producing extensive labelling of RN. 63% of cells labelled with DY from the spinal cord were also labelled with FG from ION. This confirms a similar report of doubly labelled cells using FG and Fast (or True) Blue (Kennedy PR, Neurosci. Abstr., 13(2):852, 1987). 85% of DY-labelled magnos and 55% of DY-labelled parvos also contained FG.

These results suggest that rat RN is more extensive and complex than previously believed. (Support by grant NIH-RO1NS24602-01A2).

164.8

ULTRASTRUCTURAL STUDY OF NORMAL AND HYPERTROPHIC CAT INFERIOR OLIVE. C.I.de Zeeuw*, T.J.H.Ruigrok* J.C.Holstege*, M.P.A.Schalekamp* and J.Voogd* (SPON:S.Gielen). Dept. of Anat., Erasmus Univ. Rotterdam, 3000 DR Rotterdam, The Netherlands.

Several months after cerebellectomy, neurons in the inferior olive (IO) can become hypertrophic. The normal and hypertrophic IO were studied after intracellular labeling with HRP, GABA-immunogold labeling and injection of WGA-HRP in the mesodiencephalon. Compared with the normal, the hypertrophic IO showed 1) larger cellbodies, 2) more and longer somatic spines, which were more often linked by gap junctions, 3) longer distal dendrites, 4) axon collaterals bearing varicosities filled with vesicles, 5) less GABAergic boutons in the neuropil, but an equal percentage apposed to somata, and 6) relatively more mesodiencephalic terminals. The present data show that 1) hypertrophic olivary cells are affected by trophic factors not only at the cellbody but also at the level of somatic spines, dendrites, and axon, 2) the ratio of excitatory to inhibitory terminals is increased in the hypertrophic neuropil, and 3) the electrotonic coupling between hypertrophic olivary cells may be enhanced (Ruigrok et al.,'89, Suppl.Eur.J.Neurosci.) due to a high number of gap junctions between somatic spines.

164.9

TEMPORAL EXPRESSION OF CRF IN THE DEVELOPING CEREBELLUM. **S.L. Cummings, J.S. King, and W.S. Young III. Dept. of Anatomy and Neuroscience Program. The Ohio State Univ., Columbus., OH and Lab. Cell Biology, NIMH, Bethesda, MD**

Immunohistochemistry and *in situ* hybridization histochemistry were employed to analyze the ontogeny of corticotropin releasing factor (CRF) in the developing opossum cerebellum. CRF-IR beaded fibers are present by postnatal day (PD) 3. At this time, CRF mRNA is transcribed in the inferior olivary complex, but is not evident in precerebellar nuclei that are sources of CRF-IR mossy fibers in the adult. By PD 18, CRF-IR puncta are within and subjacent to the immature Purkinje cell layer, and paramedian aggregates of CRF-IR puncta which likely are precursors of sagittal bands reported in the adult (Cummings et al., JCN 280:501) are evident in the vermis. By PD 40, CRF-IR climbing fibers are in the mid stage of development, and by PD 76 mature CRF-IR climbing fiber arbors are present in the molecular layer. CRF mRNA hybridization signal is present by PD 18 in brainstem nuclei which are potential mossy fiber sources. CRF-IR terminals with the phenotype of mossy fibers can be differentiated within the internal granule cell layer by PD 40. These data demonstrate that CRF is present in early arriving cerebellar afferents, and suggest that CRF is expressed first in climbing fibers. The role of this peptide in cerebellar development remains to be determined. (NS 08798)

164.10

CORTICOTROPIN RELEASING FACTOR (CRF) POTENTIATES THE ACTIONS OF ASPARTATE AND GLUTAMATE IN THE CAT'S CEREBELLUM. Georgia A. Bishop. Dept. of Anatomy and Neuroscience Program. The Ohio State Univ., Columbus, Ohio 43210

Immunohistochemical studies (Cummings, '89,JCN, In Press) have shown that CRF is present throughout the cat's cerebellum in climbing and mossy fibers as well as a beaded plexus of fibers. In this study, single units in lobules V and VI were isolated extracellularly to determine the effects of CRF, aspartate (ASP) and glutamate (GLU) on the firing frequency of the cell. ASP and GLU increased the firing rate of the unit. When applied alone, CRF had no effect on the firing rate of the cell. However, this peptide enhanced both sub- and suprathreshold effects of ASP in all cases and of GLU in most cases. ASP occasionally induced a rhythmic firing in isolated units. Simultaneous application of CRF abolished this rhythmic pattern of firing. These data suggest that CRF does not function as a classic neurotransmitter in the cerebellar cortex. Rather, it acts as a neuromodulator which potentiates the effects of both ASP and GLU. (Supported by NS 18028).

164.11

SUPPRESSION OF PURKINJE CELL ACTIVITY BY CHEMICALLY CODED EXTRINSIC AFFERENTS. Christopher W. Kerr and Georgia A. Bishop. Department of Anatomy and Neuroscience Program.

Studies from this laboratory have shown that serotonin (5HT) is present in a unique afferent system that is morphologically distinct from classically defined cerebellar afferents (climbing and mossy fibers). 5-HT immunoreactive fibers (IR) and varicosities form a dense plexus within the granule and Purkinje cell layers throughout the cerebellar cortex. A double-label paradigm was used to determine the source of this afferent system. The hemisphere receives 5HT afferents from cells located in the peri-olivary reticular formation. In contrast the anterior vermis receieves its 5HT projection from cells located in the paramedian and lateral reticular nuclei. Serotoninergic fibers in the paramedian lobule and posterior vermis arise from cells located in the lateral reticular nucleus. Although climbing and mossy fibers are excitatory to their target neurons, 5HT appears to suppress activity. Thus the present study provides experimental evidence that an extrinsic afferent system suppresses Purkinje cell activity. (Supported by NS 18028).

164.12

SYNAPTIC DISTRIBUTION OF IDENTIFIED PURKINJE CELL COLLATERALS: ZONE X Yi Fei Chen*, Georgia A. Bishop and James S. King. Dept. of Anatomy and Neuroscience Program, The Ohio State University, Columbus, Ohio

Studies from this laboratory have shown that all Purkinje cells give rise to recurrent collaterals that form a dense plexus within the vicinity of the cell of origin. However, the role of these collaterals in controlling cerebellar output has not been included in most theories of cerebellar function as little is known about their postsynaptic targets. To define the circuits in the area of collateral distribution we have cut serial thin sections through the local axonal plexus of an HRP filled Purkinje cell located in zone x. Targets of the 81 boutons within the ganglionic plexus include the cell bodies and proximal dendrites of basket cells and dendrites of Purkinje cells. Eight boutons contact a single basket cell. Two other basket cells were approximated by single axosomatic boutons. Six boutons contact the proximal dendrites of a single Purkinje cell. Synapses also are formed with the dendrites of interneurons. Thus, recurrent collaterals likely have a differential role in cerebellar function. They may increase Purkinje cell activity via their input to basket cells. They also may decrease cortical output by directly suppressing adjacent Purkinje cells. It is unknown at present if these direct and indirect circuits interact with the same Purkinje cell(s).(NSF 8505971)

164.13

REDUCED CELL SIZE IN THE DEEP CEREBELLAR NUCLEI (DCN) OF GENETICALLY DYSTONIC (*dt*) RATS. J. Lutes & J.F. Lorden. Dept. Psych., UAB, Birmingham, AL 35294.

The movement disorder of the *dt* rat is established by postnatal day 12. Although there are no signs of lesions or degeneration at the light micorscopic level, within 2 wk of the appearance of the movement disorder, increased glucose utilization, decreased muscimol binding, and increased glutamate decarboxylase (GAD) activity can be detected in the DCN of the mutants in comparisons with unaffected littermate controls. Thus, we have looked for morphological effects on the cells of these nuclei. Cross-sectional areas of the somas of 300-600 cells in each nucleus were measured in normal (n=4) and *dt* (n=3) rats at 20 days of age. Cell size in the mutant rats was significantly reduced in the dentate and fastigial nuclei ($p<.005$), but not the interpositus nucleus, the site at which increased GAD activity was detected earliest. Reductions in cell size in the DCN have also been reported in mutant mice in which the Purkinje cells are reduced in number (Roffler-Tarlov & Herrup, 1981). In the *dt* rat, the Purkinje cells are not lost but are reduced in size and show some functional abnormalities. The primary gene defect in the *dt* rat is unknown; however, the alterations in cell size in the DCN may be secondary to changes in the Purkinje cells that terminate in the DCN. (Supported by NS18062 and the Dystonia Med. Res. Fdn.).

164.14

THE GENETICALLY DYSTONIC (*dt*) RAT IS BEHAVIORALLY SENSITIVE TO QUIPAZINE. S.E. Stratton & J.F. Lorden. Dept. Psych., Univ. of Alabama at Birmingham, Birmingham, AL 35294.

The *dt* rat is behaviorally insensitive to harmaline, a drug known to act on the olivo-cerebellar system. In rabbits, quipazine has been shown to have behavioral and electrophysiological effects similar to those of harmaline (Barragan et al., 1985). Quipazine's effects, however, are present at birth while harmaline is ineffective in the rat until 9-10 days of age, the time at which the movement disorder of the *dt* rat first appears. Because quipazine may act on some of the same pathways as harmaline, the behavioral sensitivity of the *dt* rat to quipazine was examined before and after the onset of the motor syndrome. Normal and *dt* rats, 8 and 16-19 days of age, were given quipazine (20, 40 or 60 mg/kg, i.p.). Hyperactivity, forelimb padding, and hindlimb ataxia were seen in normal rats following quipazine. At all ages studied, the effect in *dt* rats was equal to or greater than that in normal rats. In the *dt* rat, harmaline rhythmically activates the inferior olive, but fails to produce rhythmic activation of the cerebellum or a motor tremor. The effectiveness of quipazine suggests that the defect in the CNS of the *dt* rat is independent of the pathway through which quipazine's motor effects are mediated. (Supported by 1FMH09735, NS18062 and the Dystonia Med. Res. Fdn.)

164.15

GLUTAMIC ACID DECARBOXYLASE ACTIVITY IN LATERAL VESTIBULAR AND DEEP CEREBELLAR NUCLEI IN RATS WITH CLIMBING FIBER LESIONS. J.Litwak*, C.Rizzo*, M.Beales*, G.A.Oltmans and J.F.Lorden. Dept. of Pharm. and Mol. Biol., Chicago Med. Sch., N.Chicago, IL 60064 and Dept. of Psych., Univ. of Alabama, Birmingham, AL 35294.

The neurotoxin 3-acetylpyridine (3-AP) selectively destroys climbing fiber afferents to the cerebellar cortex. Following 3-AP administration Purkinje cell activity increases, producing a strong inhibition of the firing rate of neurons in the lateral vestibular (LVN) and the deep cerebellar nuclei (DCN). In parallel with the increased Purkinje cell activity, glutamic acid decarboxylase activity (GAD) in the DCN is increased. The changes in single cell activity of both the Purkinje cells and the LVN and DCN cells changes during the postlesion period. In the current study GAD activity in the specific divisions of the DCN and the LVN was measured at several postlesion intervals to compare biochemical and electrophysiological conditions.

Adult male rats were injected with 75 mg/kg of 3-AP or saline. At 7 and 14 days postlesion the LVN and specific divisions of the DCN were removed using the punch procedure, and GAD activity determined. In the LVN GAD activity was increased greater than 30% at 7 days post-lesion, but then decreased to 15% above control at 14 days postlesion. In contrast, in the DCN GAD activity was increased 12, 24, and 29% (medial, interpositus, lateral divisions, respectively) at 7 days, and then increased to 33, 30, and 40% above control at 14 days. Thus, these results suggest that climbing fiber lesions may have a differential effect on Purkinje cell influences in specific target areas at different postlesion intervals. (Supported in part by the Dystonia Medical Research Found.)

164.16

CHOLINE ACETYLTRANSFERASE ACTIVITY IN CEREBELLAR PROJECTION SITES OF RATS WITH CLIMBING FIBER LESIONS. M.W. O'Brien*, M. Beales*, G.A. Oltmans, and J.F. Lorden (SPON: S. Hoff). Dept. of Pharm. and Mol. Biol., Chicago Med. Sch., N. Chicago, IL 60064 and Dept. of Psych., Univ. of Alabama, Birmingham, AL 35294.

The neurotoxin 3-acetylpyridine (3-AP) has been used to selectively lesion the inferior olive-climbing fiber (CF) input to the cerebellum. This lesion produces increased cerebellar Purkinje cell activity, increased glutamic acid decarboxylase activity in the deep cerebellar nuclei (DCN), and decreased single cell activity in the intrinsic cells of the DCN. To examine the lesion's transsynaptic effects, choline acetyltransferase (ChAT) activity was measured in several motor nuclei including known DCN projection sites.

Adult and juvenile rats were injected with 75 mg/kg 3-AP or saline. At selected post-lesion periods the specific brain nuclei were removed using a micropunch dissection technique, and ChAT activity determined. In young animals there was a significant decrease in ChAT activity in the ventropostero-medial thalamus at 7 days post-lesion. In other areas examined (red nucleus, pontine nucleus, ventral thalamus) no differences were found between lesion and control animals at any of the post-lesion intervals studied (3,7,14 days). (Supported in part by the Dystonia Medical Research Found.)

164.17

AUTORADIOGRAPHIC LOCALIZATION OF AMINO ACID NEUROTRANSMITTER RECEPTORS IN THE CEREBELLAR CORTEX OF PIGEON (COLUMBA LIVIA) AND TURTLE (PSEUDEMYS SCRIPTA). RL Albin, EK Richfield, A Reiner, AB Young, JB Penney. Dept. of Neurology, University of Michigan, Ann Arbor, MI 48109; and Dept. of Anatomy, University of Tennessee, Memphis, TN 38163.

Glutamate and GABA are the main neurotransmitters of cerebellar cortex. We used receptor autoradiography to determine the comparative distribution of GABA and glutamate receptors in the cerebellar cortex of rats, pigeons (Columba livia), and turtles (Pseudemys scripta). Benzodiazepine receptors are found in both granule cell and molecular layers of all three species. GABA-A receptors are densest in the granule cell layer with GABA-B receptors densest in the molecular layer of all three species. In rats and turtles, NMDA receptors are densest in the granule cell layer and quisqualate receptors densest in the molecular layer. In pigeons, quisqualate receptors are densest in the molecular layer with NMDA receptors were densest in the molecular layer. Our results indicate overall conservation of receptor distribution but the NMDA receptor distribution in pigeons suggests divergent organization of the avian cerebellum. Supported by NS19620, NS19613, NS 010300.

164.18

TRANSECTION OF THE OLIVO-CEREBELLAR TRACT CAUSES LARGE DECREASES IN N-ACETYLASPARTYLGLUTAMATE, BUT NOT ASPARTATE OR GLUTAMATE IN SELECTED AREAS IN THE RAT CEREBELLUM. M.A.A.Namboodiri, L.Williamson, J.R.Moffett, D.Garrison, #A.C.Murphy, J.H.Neale and +M.Palkovits. Dept. Biology, Georgetown University, Washington, D.C. 20057, #Lab. Cell Biology, NHLBI, NIH, and +Lab. Cell Biology, NIMH, NIH, Bethesda, MD 20892.

The levels of NAAG, aspartate and glutamate were measured in five areas of the cerebellar cortex, the cerebellar nuclei and in the inferior olive of rats ten days after unilateral transection of the olivo-cerebellar tract (OCT). Brain regions were microdissected by the punch technique and NAAG levels were measured using a radioimmunoassay with high specificity (IC50 NAAG = 2.5 nM, NAA = 100 uM) and sensitivity (smallest detectable amount = 1-2 pg/assay), while aspartate and glutamate levels were determined using a Beckman amino acid analyzer. Transections of the OCT immediately lateral to the inferior olive caused substantial decreases in NAAG levels in the ipsilateral cerebellum, as compared to NAAG levels on the contralateral side. Depletions in NAAG levels correlated well with numbers of transected olivo-cerebellar fibers: NAAG levels in the ipsilateral paramedian lobe and vermis (lobules VIII-IX) decreased by 72-94% in rats with total transections of fibers running to these cerebellar areas, while smaller depletions (vermis VIII-IX: 22-24%, vermis IV-V: 44-46%, paraflocculus: 26-44%, paramedian lobe: 31-42%) were measured in rats with partial transections of the OCT. No alterations were observed in the cerebellar nuclei, and a slight elevation in the contralateral inferior olive. Also, no significant alterations in the levels of aspartate or glutamate were detected in any of the above areas. These results indicate that NAAG is present in the olivo-cerebellar tract where it, or glutamate derived from it, may serve as a neurotransmitter. (Supported by NIH Grant DK 37024 MAAN and NIDA grant DA 02297 to JHN).

ACETYLCHOLINE II

165.1

ACETYLCHOLINE-IMMUNOREACTIVE FIBERS IN CEREBRAL AND PERIPHERAL BLOOD VESSELS. T.J-F. Lee, F.J-P. Miao*, T. Okuno*, and K. Kawashima* (SPON: G.N. Pandey). Dept. of Pharmacol. Southern Il. Univ. Sch. of Med. Springfield, IL, and Kyoritsu College of Pharmacy, Tokyo, Japan.

It has been established that cholinergic vasomotor control exists in some vascular beds. The presence of cholinergic vasomotor nerves has been supported by the presence of small agranular vesicle-containing nerve terminals, acetylcholinesterase- and choline acetyltransferase (ChAT)-containing fibers, high ChAT activity and high affinity choline uptake system. ACh-containig fibers in vascular wall, however, has not been demonstrated. In this study, the presence of ACh-immunoreactive (ACh-I) fibers in cerebral and peripheral blood vessels was examined using avidin-biotin complex method. Tissues were fixed in situ by a mixture of allyl alcohol and glutaraldehyde. The results indicate that ACh-I fibers exist in cerebral arteries, pulmonary and mesenteric arteries and veins, and aortae. The distribution patterns of ACh-I fibers are similar to those of ChAT-I fibers. These results, for the first time, provide the most direct evidence for the presence of cholinergic innervation in the vascular wall and further suggest a functional significance of cholinergic innervation in vasomotor control of local blood flow. (Supported by NIH HL 27763, 24683, and SIU School of Medicine)

165.2

NICOTINIC MODULATION OF NEURONAL RESPONSES TO PERIPHERAL STIMULATION IN RAT SOMATIC SENSORY (SI) CORTEX K.M. Jacobs, D. Werner*, and J.P. Donoghue, Center for Neural Science, Brown University, Providence, RI 02912

Iontophoretic application of acetylcholine (ACh) to SI neurons typically enhances discharge evoked by natural sensory stimulation within the cell's receptive field. Less frequently response suppression is observed. The different laminar distributions and suspected locations of muscarinic and nicotinic receptors in SI (Sahin et al, '88) suggests that these receptor subtypes may mediate different effects of ACh.

In the present study we tested the hypothesis that input suppression is mediated by nicotinic receptors and that nicotinic effects are most frequently obtained in mid-cortical layers, where these receptors are most abundant. The sensory responses of 53 single neurons in rat SI cortex were recorded during urethane anesthesia (1.5 g/kg) while the receptive field was stimulated by air puffs or a mechanical stimulator. Responses were recorded before, during, and after the application of cholinergic agonists or antagonists applied iontophoretically through multibarrel glass pipettes. Clear effects of nicotine (NIC) application (H-Tartrate, 0.5M, 10-100 nA) were observed in 39 cells (74%). In 25 cells (64%) the sensory evoked discharge was enhanced by NIC, while it was suppressed in 10 cells (26%). Enhanced cells either returned towards baseline or remained enhanced for minutes after NIC application ended. Enhancement produced during NIC application could be blocked by concurrent application of mecamylamine (MEC). In 4 of 8 cells ACh induced enhancement could not be blocked by MEC. None of the NIC effects showed a preference for the midcortical layers, and similar percentages of enhanced and suppressed cells were found in middle and infragranular layers. All of the NIC-modulated cells located in the supragranular layers (4 of 7) showed enhancement. These results demonstrate that NIC effects can be elicited in all cortical layers and that nicotinic receptor activation can either enhance or suppress sensory responses in SI cortex. Since some cholinergic effects cannot be blocked by NIC antagonists, similar forms of modulation may be produced via muscarinic receptors as well. (Supported by NIH NS22517 and March Of Dimes 5-562)

165.3

GALANIN mRNA IN THE PRIMATE NUCLEUS BASALIS OF MEYNERT. L.C. Walker, D.L. Price and W.S. Young III. Neuropathology Lab., The Johns Hopkins Univ. Sch. Med., Baltimore, MD 21205; Lab Cell Biology, NIMH, Bethesda, MD 20892

Immunocytochemical studies of the nucleus basalis of Meynert (nbM) in nonhuman primates indicate that, in addition to a population of smaller galaninergic cells, most large, cholinergic projection neurons also contain the peptide galanin [Melander and Staines, *Neurosci. Lett.* 68:17-22, 1986; Walker et al., *J. Comp. Neurol.* 280: 272-282, 1989]. Studies in humans suggest that galanin occurs in small- or medium-sized neurons (some of which are also cholinergic) but not in the large cholinergic neurons characteristic of the nbM [Chan-Palay, *J. Comp. Neurol.* 273:543-557, 1988]. We used hybridization histochemistry to detect galanin mRNA in the basal forebrain of baboons (*Papio*). Hybridization with a [^{35}S]-labeled synthetic DNA probe to bases 228-271 of rat galanin cDNA [Kaplan et al., *Proc. Natl. Acad. Sci. USA* 85:1065-1069, 1988] disclosed a population of intensely labeled, small-to-medium-sized neurons. These neurons may correspond to the smaller cells showing galanin-like immunoreactivity in the human nbM. Most large, hyperchromic neurons were lightly labeled, and some large cells appeared to be unlabeled. Low levels of galanin mRNA in many large neurons may explain the difficulty in detecting galanin-like immunoreactivity in these cells.

165.4

EFFECTS OF GROWTH FACTORS ON REGENERATION OF CHOLINERGIC PROJECTIONS FROM BASAL FOREBRAIN TO MEDIAL CORTEX. T.W. Farris and L.L. Butcher, Lab. of Chemical Neuroanatomy, Dept. of Psychology, UCLA, L.A., CA 90024-1563.

To determine the regenerative effects on cholinergic projection neurons of three putative growth-promoting factors, we administered ganglioside GM1 (30 mg/kg,IP), thyroxine (T4, 2.5 mg/kg, IP) and estradiol benzoate (EB, 10 ug/kg, SC) daily for 14 days to 8 week-old rats with unilateral axotomies of the medial cholinergic pathway arising from the basal nuclear complex and projecting to cingulate, medial and occipital cortices. Brain tissue was processed for acetylcholinesterase (AChE) histochemistry and choline acetyltransferase (CAT) and dopamine beta-hydroxylase (DBH) immunohistochemistry and was analyzed via light microscopy. All measures in all groups demonstrated a build-up of stain proximal to the cortical cut. This effect was greatest for AChE staining and was greatest in GM1-treated rats and least in EB-treated rats. Loss of AChE staining intensity distal to the cut was least in T4-treated rats and was unchanged for GM1 or EB. In all conditions, AChE-positive and DBH-positive fibers immediately adjacent to the cut, which run parallel to the midline on the side contralateral to the cut, turned toward the cut changing course up to 80 degrees to reach the cut. This effect was most pronounced in the GM1-treated rats. Collectively, these data support findings that (1) cholinergic basal nuclear complex (CBNC) neurons are sensitive to thyroid hormones (Gould et al, 1989); (2) GM1 augments the regeneration of injured CBNC neurons; and (3) suggest that medial pathway axotomy is a good model system for studying *in vivo* growth-promoting factors known to exert neuronotrophic effects on cholinergic morphologic and biochemical measures *in vitro*.

165.5

CORTICAL CHOLINERGIC FUNCTION AFTER BASAL FOREBRAIN LESION. M. Downen, K. Sugaya*, S.P. Arneric and E. Giacobini. (SPON: R. Becker). Dept. Pharmacology, Southern Ill. Univ. Sch. Med., Springfield, IL 62794

Changes in presynaptic cholinergic function in the cerebral cortex were determined following ibotenic acid lesion of the basal forebrain. K^+-evoked (50 mM) acetylcholine (ACh) release was measured at 2, 4 and 6 weeks following unilateral ibotenic acid injection into the ventromedial portion of the globus pallidus. Release was determined following preincubation with ^{3}H-choline chloride (NEN) in micropunches taken from the fronto-parietal cortex of each hemisphere. Ipsilateral to the lesion, K^+-stimulated release was expressed as percent change from spontaneous release following lesion and compared to evoked release from the contralateral control hemisphere. Potassium-evoked release was significantly decreased in the lesioned hemisphere in all cortical regions sampled at 2 and 4 weeks postlesion ($p < .01$). There was no difference in percent release across cortical regions in both intact and lesioned hemispheres at 2 and 4 weeks after lesion. At six weeks, release was not significantly changed in the lesioned hemisphere as compared to the intact hemisphere. A recovery toward control levels was seen in the rostral punches. However, there was an unexpected reduction of release in the caudal punches from the intact hemisphere. Significant regional differences were seen in percent K^+-evoked release at 6 weeks ($p < .01$). A rostral caudal gradient was observed with the rostrally located punches showing greater release in both hemispheres. Basal forebrain lesion did not alter spontaneous release and there was no regional difference in baseline release at any time point examined. Choline acetyltransferase (ChAT) activity depletion following lesion showed a rostral-caudal gradient. The greatest depletions were seen in the frontal and lateral micropunches. Lesion-induced release effects may be a more sensitive measure of neuronal function than alterations in enzyme activity.

165.6

BUNGAROTOXIN BINDING FOLLOWING NUCLEUS BASALIS LESION. K. Sugaya*, M. Downen and E. Giacobini (SPON: D.M. Caspary). Dept. of Pharmacology, Southern IL Univ. School of Medicine, Springfield, IL, 62794-9230

The nucleus basalis magnocellularis (NBM) represents the major extrinsic source of cortical cholinergic innervation. ^{125}I-alpha-bungarotoxin (alpha-BTX) and ^{125}I-kappa-bungarotoxin (kappa-BTX) specific binding was studied in rat brain at 2, 4, 6 and 12 weeks after ibotenic lesion of the basal forebrain. A unilateral injection of ibotenic acid was made into the NBM. The BTXs specific binding in coronal sections of rat brain was detected by computer-assisted autoradiographic image analysis. There was a significant decrease (32%) of alpha-BTX binding at 2 weeks in sections taken from frontal cortex at 1.5-2.0 mm anterior to Bregma (layers 1 and 2) in the lesioned hemisphere as compared to the contralateral intact hemisphere. The nucleus basalis lesion resulted in a loss of projections from the NBM to the frontal cortex. Our results suggest that BTX binding sites are located on the presynaptic projection from the NBM.

165.7

NEUROGENESIS OF THE MAGNOCELLULAR BASAL NUCLEI IN RHESUS MONKEYS. I. Rackauskas*[1], P. Rakic[2], and J.H. Kordower[1], (SPON: M.Fiandaca)[1]Dept. of Anatomy and Cell Biology, Univ. Illinois Sch. Med., Chicago Ill. 60302.[2] Sect. of Neuroanatomy, Yale Sch. Med. New Haven Ct. 06510

The neurogenesis of the magnocellular basal forebrain in Rhesus monkeys was assessed via tritiated thymidine autoradiography. Thirteen neonatal Rhesus monkeys whose mothers were injected with tritiated thymidine between E27 and E50 of gestation served as subjects. Coronal sections from the level of the subcallosal gyrus through the lateral geniculate nucleus were obtained from the archival collection of Rakic (e.g. Rakic, P., *J. Comp. Neurol.* **147:** 523, 1973). These sections were previously processed for autoradiography via standard procedures and counterstained with toluidine blue. The analysis was encompassed the magnocellular neurons within vertical limb of the diagonal band (Ch2) and the four subsectors of the nucleus basalis (Ch4). No autoradiographically labeled neurons were observed at E27 and all neurogenesis within the magnocellular basal nuclei in Rhesus monkeys was complete by E48 Within this developmental window, a bimodal pattern of neurogenesis was observed within the magnocellular basal forebrain. An initial "burst" of neurogenesis was observed at E30 throughout the basal forebrain's rostrocaudal extent. A short quiescent period soon followed that merged into a second wave a neurogenesis which occurred along a general caudal to rostral gradient. The anterior basal forebrain (Ch2, Ch4am, and Ch4al) was generated simultaneously between E36-E45 with peak neurogenesis observed between E40-E43. The intermediate division of Ch4 began its second wave of development at about the same time, but both peaked (E36-E40) and completed its neurogenesis earlier (E43). The posterior division of Ch4 was generated first, generally completing its cell genesis between E33-E36. No neurogenic gradient was observed in a radial direction. These data demonstrate that the magnocellular basal forebrain is generated early in gestation in Rhesus monkeys. This places these neurons in a strategic position to influence their target cells, most of which are generated within the cerebral cortex at a later time. (Support: NS25655 and The American Health Assistance Foundation (JHK), NS22807 (PR)).

165.8

RESPONSES OF CENTRAL CHOLINERGIC NEURONS TO AXONAL INJURY IN NONHUMAN PRIMATES. V.E. Koliatsos, W.C. Mobley*, H.J.W. Nauta and D.L. Price. Neuropathology Lab., The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

In monkeys (*Macaca mulatta* and *fascicularis*), we examined responses of basal forebrain cholinergic neurons to axotomy. Under general anesthesia and sterile conditions, animals had complete unilateral transections of the fornix *ca.* 5 mm caudal to commissural decussation. Two weeks later, monkeys were deeply anesthetized with pentobarbital and perfused with paraformaldehyde. Transverse sections through the septum were processed for immunohistochemistry (i.e., choline acetyltransferase [ChAT] and epitopes of phosphorylated neurofilaments) and Nissl stains. Ipsilateral to the lesion, there was a severe reduction in the number of ChAT-immunoreactive medial septal/diagonal band neurons. In Nissl stains, these cells showed reductions in basophilia and cell size. Some loss of neurons was apparent. These results indicate that primate central cholinergic neurons respond to axonal injury in a way similar to rodent cholinergic neurons [Koliatsos et al., *Brain Res.* 482:205-218, 1989]. More recently, we have begun to analyze the effectiveness of nerve growth factor (mouse) delivered intraventricularly in preventing degeneration and in restoring the properties of these basal forebrain cholinergic neurons.

165.9

EXTENDED SURVIVAL OF MEDIAL SEPTAL CHOLINERGIC NEURONS FOLLOWING LESION OF THE FIMBRIA-FORNIX. M.D. Applegate, V.E. Koliatsos and D.L. Price. Neuropathology Lab., The Johns Hopkins Univ. Sch. Med., Baltimore, MD 21205.

It is established that transection of fimbria-fornix axons reduces the number of choline acetyltransferase (ChAT)-immunoreactive neurons in the medial septum. However, it is not clear whether reductions in immunoreactive cells are due to decreased levels of cholinergic markers in viable cells or to degeneration of septal neurons. To clarify this issue, neurons in medial septum were retrogradely labeled by injecting Fluoro-Gold bilaterally into dorsal hippocampus in rats. Seven days later, the dorsal fornix, the rostral tip of the dorsal hippocampus, and the antero-dorsal fimbria were aspirated unilaterally. At 1, 7, and 14 days postlesion, rats were perfused, and the septal region was processed for ChAT-Texas Red immunofluorescence. At days 1 and 7, ChAT immunoreactivity was decreased ipsilateral to the lesion, but there was little change in the number of retrogradely labeled neurons. By day 14, ChAT immunoreactivity was virtually absent, and these cells appeared atrophic; however, the number of retrogradely labeled neurons was only moderately decreased. Further studies will be needed to determine how long these atrophic neurons persist following the lesion and whether NGF treatment can induce them to regain normal size and cholinergic properties.

165.10

NUCLEUS BASALIS LESIONING IN THE AGED RAT: NEUROCHEMICAL AND BEHAVIORAL CONSEQUENCES. G.W. Arendash[1], G.J. Sengstock[1]*, K.B. Johnson[1]* and E.M. Meyer[2]. Dept. of Biology, Univ. of South Florida, Tampa, FL 33620[1] and Dept. of Pharmacology, Univ. of Florida, Gainesville, FL 32610[2].

A number of researchers have attempted to mimic the degeneration of nucleus basalis (NB) cholinergic neurons in Alzheimer's Disease (AD) by neurotoxically destroying the analogous NB region in young adult rats. Since the loss of these neurons probably doesn't occur until later in the life of AD patients, NB lesioning in aged rats may approximate more closely the impact that a degeneration of NB cholinergic neurons is having in Alzheimer's-diseased patients. Therefore, 20-21 month old Sprague-Dawley rats were given bilateral NB lesions using ibotenic acid and sacrificed 2.5 or 5 months later. Beginning one month after lesions, animals were tested for cognitive abilities in passive avoidance, active avoidance, and/or Lashley III maze tasks. Compared to sham-lesioned controls, lesioned rats were significantly deficient in the retention of passive avoidance behavior, as well as in the acquisition of 2-way active avoidance behavior. However, no deleterious lesion effect was observed in Lashley III spatial maze learning. A substantial cholinergic hypofunction was found in the neocortex of lesioned animals at both sacrifice time points. These results warrant a continued characterization of the aged, NB-lesioned rat as a useful animal model for the cholinergic hypofunction of AD.

165.11

TOPOGRAPHY OF PEDUNCULOPONTINE TEGMENTAL NUCLEUS (PPT) INTERCONNECTIONS WITH THE LATERAL GENICULATE NUCLEUS (LGN) AND N. PREPOSITUS HYPOGLOSSI (PH) IN THE CAT. S. Higo, K. Ito, D. Fuchs, D. Rye, B. Wainer, and R. W. McCarley, Laboratory of Neuroscience, Department of Psychiatry, Harvard Medical School/VAMC, Brockton MA 02401.

Cholinergic PPT neurons modulate excitability of many thalamic and brainstem nuclei, and, in REM sleep, PPT neurons transmit eye movement-gated PGO waves to the dorsal LGN (LGNd). However possible sources of eye-movement related input to PPT, such as PH, have not been investigated.

WGA-HRP injections were made in the rostral, middle and caudal PPT zones, and in each case the resulting anterograde labeling confirmed as cholinergic by subsequent retrograde double labeling studies using WGA-HRP and ChAT immunohistochemistry. LGNd showed an ipsilateral dominance of anterograde labeling from PPT, but no clear topographic differentiation; terminal labeling was dense in layers A, A1, and C, and sparse in layers C1-3 and in the medial interlaminar nucleus. LGNd did not project to PPT. Ventral LGN (LGNv) also had ipsilaterally dominant anterograde labeling from PPT but, in contrast, labeling was topographically organized, with caudal PPT projecting to lateral LGNv and rostral PPT to medial LGNv. Anterograde labeling from all zones of PPT was present bilaterally in medial PH. PH projected to all PPT zones, with main sites of origin ipsilaterally in rostral PH and contralaterally in caudal PH.

These data indicate PPT interconnections with the visual/oculomotor system and suggest a possible anatomical substrate for transfer of eye movement-related information from PH to PPT, and thence to LGNd/v.

165.12

DO CHOLINERGIC DIAGONAL BAND NEURONS HAVE INHIBITORY AUTORECEPTORS? R. T. Matthews, Dept. of Anatomy, Texas A&M University, College Station, TX 77843.

Cholinergic neurons of the medial septum/diagonal band area have been electrophysiologically characterized in vitro (Griffith & Matthews, Neurosci. Lett. 71:169, 1986). Extracellular recordings from slices of guinea pig forebrain in vitro can distinguish at least a subpopulation of these cholinergic neurons (Matthews, Neurosci. Abs. 14: 923, 1988). The hypothesis was tested that diagonal band of Broca (DBB) cholinergic neurons have inhibitory autoreceptors which distinguish them from non-cholinergic neurons. Spontaneously active or glutamate-driven DBB neurons were recorded with 5-barrel pipettes and the effects of iontophoresed acetylcholine (ACh), carbachol (CARB) or vehicle were measured. ACh at iontophoretic currents of 2-40 nA (0.2 M, pH 4.0) had variable effects on the firing rates of both presumed cholinergic and non-cholinergic neurons with 17 & 29% excited, 33 & 41% inhibited and 50 & 30% unchanged, respectively. CARB at 1-20 nA (0.2 M, pH 4.0) had variable effects on firing rates of presumed cholinergic neurons with 40% increased, 40% decreased and 20% unchanged, while most non-cholinergic neurons (90%) were potently excited. These data do not support the hypothesis that all cholinergic neurons have an inhibitory autoreceptor which can be used to rapidly distinguish neuron types within the DBB in vitro. (Supported in part by Bristol-Myers Co.)

165.13

ACTIONS OF MUSCARINIC AGONISTS ON CELL TYPES WITHIN GUINEA PIG BASAL FOREBRAIN NUCLEI IN VITRO. W.H.Griffith and J.A.Sim*. Department of Med. Pharmacol. and Toxicol. College of Med. Texas A&M University, College Station, TX 77843.

The present study examined muscarinic agonist-induced changes in membrane potential, input resistance and after-hyperpolarization (AHP) in fast AHP (FAHP), slow AHP (SAHP) and burst-firing neurons in the vertical and horizontal limbs of the nucleus of the diagonal band of Broca (nDBB). Intracellular and single-electrode voltage-clamp techniques were used in an in vitro brain slice preparation from guinea pig. Bath applied muscarine (10uM) or bethanechol (10-30uM) induced small and variable effects across all cell types, which included membrane depolarization (1-5mV) in 49% (n=16), hyperpolarization (1-8mV) in 33% (n=11) or no change in 18% (n=6). Changes in input resistance were not correlated with the direction of membrane response. In only 4 of 16 cells was a membrane depolarization associated with a marked increase in cell firing. The most consistent action of these agonists was a reduction in a long duration AHP (2-3s) that occurred in some cells following a train of action potentials (n=7). Inhibition of the AHP-current was also recorded under voltage-clamp in the presence of TTX. Two other AHP's (<50ms and 200-700ms duration) that followed single spikes in FAHP and SAHP cells, respectively, were not effected (n=5). These results suggest that muscarinic agonists have subtle, yet specific actions on some nDBB neurons. (Supported NS22456, AG07805)

165.14

MUSCARINIC RESPONSES IN CULTURED CORTICOCALLOSAL NEURONS MEDIATED BY M_2 RECEPTORS. K. A. Jones and R. W. Baughman, Dept Neurobiology, Harvard Med School, Boston MA 02115.

In the neocortex acetylcholine (ACh), acting through muscarinic receptors, enhances excitability by at least 2 different mechanisms: 1) a slowly activating membrane depolarization, and 2) inhibition of an after hyperpolarization (AHP) that follows repetitive firing. We are interested in the cellular events that are responsible for muscarinic receptor channel coupling in pyramidal neurons, and as a first step have developed a culture preparation to study the receptor pharmacology of cholinergic excitation. Fluorescent latex microspheres were injected into the 1° and 2° visual cortex of 4-5 day-old rat pups. The contralateral visual cortex was enzymatically dissociated 18-24 h later and grown in culture for at least 3 weeks before recording. Whole-cell recordings of membrane potentials or currents were obtained from labelled corticocallosal cells with patch electrodes. In some cases 500 nM TTX, or 5 mM kynurenic acid plus 4 μM bicuculline was added to the medium to inhibit synaptic activity. Following a train of 20-50 spikes most cells displayed a slow component (lasting 3-6 sec) of the AHP, although a few cells produced an after depolarizing potential (ADP) with a similar time course. Currents underlying these potentials were voltage insensitive and reversed near E_K. Brief (1-2 sec) applications of 10-100 μM ACh either abolished the slow AHP or enhanced the ADP, and produced a slow (~1 min) depolarization that resulted from a voltage dependent inward current and a decreased conductance. Atropine (100 nM) abolished these effects. In contrast, the M_1 receptor blocker pirenzepine at low doses (100-500 nM) had either no effect or only slightly blocked the action of ACh. At higher doses (2-10 μM) the block was nearly complete. These results are consistent with the idea that the slow depolarization, slow AHP, and ADP are mediated by M_2 muscarinic receptors. (AFAR and EY03502)

165.15

CHOLINERGIC MARKERS IN HUMAN PUTAMEN. A QUANTITATIVE AUTORADIOGRAPHIC STUDY. **C.A. Levesque*, H.R. Brashear, and G.F. Wooten (SPON: C.A. Leslie). Dept. of Neurology, University of Virginia Health Sciences Center, Charlottesville, VA. 22908.**

Investigations of cholinergic neuronal markers in adult human postmortem tissue may add to our understanding of the pathophysiology of neurodegenerative diseases, particularly Alzheimer's Disease; but careful re-validation of assay conditions developed in other species is necessary. We have characterized the binding of [^{3}H]hemicholinium-3 ([^{3}H]HC-3), a presynaptic cholinergic marker by virtue of its selective binding to the sodium-dependent, high affinity, choline uptake site, and [^{3}H]quinuclidinylbenzilate ([^{3}H]QNB), a muscarinic cholinergic receptor ligand and postsynaptic marker. Blocks of human putamen were chosen for these studies because of their high concentration of cholinergic neurons. [^{3}H]QNB association and saturation data revealed that optimal binding occurred with incubation in 1.0 nM [^{3}H]QNB at 24° C for 30 minutes (K_d 0.3 nM and B_{max} 378 fmol/mg tissue wet weight). Higher temperatures and longer incubation times caused tissue degradation. [^{3}H]HC-3 binding was done at 24° C with 20 minute incubation at a concentration of 10 nM (K_d 1-5 nM range determined by an Eadie-Hofstee plot). This concentration provided full saturation with maximal separation between total and nonspecific binding. With both [^{3}H]QNB and [^{3}H]HC-3 binding the human putamen appears homogeneous without evidence of a patch and matrix pattern.

165.16

M1 AND M2 MUSCARINIC RECEPTORS MEDIATE ACETYLCHOLINE (ACh) MODULATION OF AUDITORY CORTICAL NEURONS. R. Metherate, J.H. Ashe and N.M. Weinberger Center for the Neurobiology of Learning and Memory, Dept. Psychobiology, University of California, Irvine 92717; Dept. Psychology, University of California, Riverside, CA 92521.

ACh modifies the activity of auditory cortical neurons via muscarinic receptors (Synapse 2:54, 1988). We now report the involvement of M1 and M2 muscarinic receptor subtypes in the effects of ACh.

In barbiturate-anesthetized guinea pigs, iontophoretically-applied ACh modified intensity functions (IFs) at best frequency by facilitating or depressing tone-evoked activity. Facilitation of evoked responses could decrease IF thresholds by over 10 dB. To examine mechanisms underlying multiple ACh effects, we applied the muscarinic receptor antagonists pirenzepine and gallamine to 14 cells whose single tone responses were modified by ACh. ACh-induced facilitation of spontaneous or evoked activity was antagonized more effectively by pirenzepine than by gallamine. However, gallamine effectively blocked ACh depression of activity.

As pirenzepine and gallamine are selective antagonists at M1 and M2 muscarinic receptors, respectively, these initial findings suggest that the modulatory effects of ACh in auditory cortex involve both receptor subtypes.

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165.17

CHOLINE ACETYLTRANSFERASE CONTAINING PROJECTIONS FROM THE FELINE PONTOMESENCEPHALON. Konrad Talbot, Nancy J. Woolf, Jean B. Harrison, Jennifer S. Buchwald, and Larry L. Butcher. Laboratory of Chemical Neuroanatomy, Depts. of Psychology and Physiology, University of California, Los Angeles, CA 90024.

Immunoreactivity for choline acetyltransferase (ChAT) was analyzed in the brains of unoperated cats and in cats in which stereotaxic lesions were made in the pedunculopontine and laterodorsal tegmental nuclei. Moderate to dense ChAT-immunoreactive fiber plexes were observed throughout the forebrain, especially in the lateral geniculate, lateroposterior, intralaminar and midline thalamic nuclei. Relatively discrete lateral and medial cholinergic pathways were traced. Lateral ChAT pathways largely originated in the pedunculopontine nucleus, ascended through the central tegmental fields, compact part of the substantia nigra, zona incerta, intralaminar, reticular and lateroposterior nuclei of the thalamus, and provided afferents to the diencephalon and basal forebrain. Medial cholinergic pathways were traced from ChAT-containing cells in the laterodorsal tegmental nucleus to the periaqueductal gray, dorsal raphe, ventral tegmental area, midline thalamus, hypothalamus, and lateral septal nucleus. ChAT-stained fibers throughout the diencephalon and basal forebrain were markedly reduced in cats with lesions in the pontomesencephalon, whereas ChAT fibers in the cerebral cortex were not altered. [Support: USPHS grants NS 25400 to J.S.B. and NS 10928 to L.L.B.]

165.18

CHOLINERGIC INNERVATION OF MONKEY THALAMUS: A CHOLINE ACETYLTRANSFERASE (ChAT)-IMMUNOCYTOCHEMICAL STUDY. C.A. Kitt, A.I. Levey, D.P. Friedman, M.L. Voytko, H.L. Fedor* and D.L. Price. Neuropathology Lab., The Johns Hopkins Univ. Sch. Med., Baltimore, MD 21205.

Distributions of cholinergic elements in monkey thalamus were investigated using immunocytochemistry and a monoclonal ChAT antibody. Two rhesus monkeys were anesthetized, perfused transcardially with 2or3% paraformaldehyde, and tissues sectioned in coronal or sagittal planes. The density of ChAT-immunoreactive processes varied across and within thalamic nuclei. ChAT-positive fibers were conspicuous in the dorsolateral anteroventral nucleus, parvocellular portion of mediodorsal nucleus (lateral region), and the reticular nucleus. Intralaminar nuclei also showed high levels of ChAT-positive fibers. Low levels of ChAT-immunoreactive elements were seen in midline nuclei. A patchy distribution of ChAT-immunoreactive processes was seen in anteromedial and lateral dorsal nuclei as well as within the ventral tier. Medial habenular and anterodorsal nuclei received sparse cholinergic inputs. All layers of the lateral geniculate nucleus contained ChAT fibers with a higher density in layers 1, 5, and 6. Dorsolateral and ventral aspects of the pulvinar complex contain dense levels of ChAT fibers. These results are compared to distributions of muscarinic and nicotinic cholinergic receptors and to patterns of acetylcholinesterase staining in thalamus.

165.19

REDUCTION OF [3H]HEMICHOLINIUM BINDING AND IN VIVO ACETYLCHOLINE RELEASE IN RAT STRIATUM BY CORTICAL DEAFFERENTATION: RESTORATION STUDIES WITH CHOLINE AND OXIRACETAM. G. Forloni, D. Amoroso*, N. Angeretti*, A.J.G. Ruiz* and S. Consolo* Dept.Cholinergic Neuropharmacology , "Mario Negri" Institute, 20157 Milan, Italy.

Frontal deafferentation of the rat striatum reduces the tone of striatal cholinergic neurons. This has been established by the measurement of several cholinergic parameters including in vivo acetylcholine (ACh) release by the microdialysis technique and [3H]hemicholinium-3 ([3H]HCh-3) binding to sodium-dependent high-affinity choline uptake (SDHACU) sites by biochemical and autoradiographic techniques. After two weeks, the lesion resulted in a 30-40 % reduction of ACh release and of [3H]HCh-3 binding. Acute i.p. injections of oxiracetam (OXI), 100 mg/kg i.p., induced time-dependent recovery of ACh output from the striata of decorticated rats. Starting 30 min after drug injection, ACh release was already significantly higher in the OXI-treated group than in the saline-treated group and the rate of ACh release continued to rise gradually, with complete recovery by 80 min after drug injection. The full effect lasted at least 100 min longer. A similar effect but with a different time-course was observed with choline chloride (100 mg/kg, i.p.).OXI also normalized SDHACU activity of decorticated rats 2 h after its administration but had no effect in sham-operated rats. The repletion is most likely associated with enhanced ACh formation. These results indicate that the striatum of decorticated rats could constitute a useful model for studying means to restore the deficit in cholinergic neurotransmission. US Air Force grant AFOR 87-0399

165.20

A CHOLINERGIC COMPONENT OF 4-BETA-PHORBOL 12,13-DIBUTYRATE INDUCED DEPOLARIZATION IN THE RAT SUPERIOR CERVICAL GANGLION. C.G. Acosta and J.H.Ashe, Dept. of Psychology, University of California, Riverside, CA 92521.

The sucrose-gap technique was used to record the effect of phorbol esters on the ganglionic potential of the rat superior cervical ganglion. Exposure of ganglia to 4-beta-phorbol 12,13-dibutyrate (ß-PDBu) resulted in ganglionic depolarization (DP) that could be repeatedly elicited by subsequent exposures. Phorbol esters that are ineffective activators of protein kinase C did not produce a ganglionic DP. The amplitude of the ß-PDBu-induced DP was concentration dependent over the range of 0.25 to 2.50nmol. ß-PDBu-induced DP was significantly reduced, but not eliminated, when elicited in the presence of quinuclinidyl benzilate (80 to 150nM), and was increased in amplitude and/or duration when elicited in the presence of eserine (1.5mM).

Transection of the preganglionic nerve, 12 to 14 days before the ganglion was isolated for recording, significantly reduced the amplitude of the ß-PDBu-induced DP relative to contralateral surgical control or non-surgical control ganglia. Depolarizations produced by methacholine (0.6µmol) were not significantly reduced in comparison to responses obtained from intact ganglia.

These results indicate that ß-PDBu-induced ganglionic DP consists of more than one component. The effect of ß-PDBu may involve presynaptic terminals with a subsequent release of acetylcholine, and possibly other neurotransmitters, as well as postsynaptic mechanisms.

MONOAMINES AND BEHAVIOR I

166.1

UK-14304 IMPROVES DELAYED RESPONSE PERFORMANCE IN AGED MONKEYS: FURTHER EVIDENCE FOR THE ROLE OF α-2 ADRENERGIC RECEPTORS IN MEMORY ENHANCEMENT. A.F.T. Arnsten and P.S. Goldman-Rakic. Section of Neuroanatomy, Yale Medical School, New Haven, CT 06510.

The α-2 adrenergic agonists clonidine, B-HT920 and guanfacine (GFC) have all been shown to improve the delayed response performance of aged monkeys, and their ability to improve memory has been related to their affinity for the proposed Ri α-2 receptor subtype (J. Neurosci. 8: 4287, 1988). Low doses of the Ri-selective agonist GFC can improve memory without sedative or hypotensive side effects, and a single dose of GFC can have very longlasting, beneficial effects on memory, suggestive of actions through a second messenger system (ibid). In the present study, another α-2 agonist, UK-14304 (UK), which has been shown to enhance adenylate cyclase activity in rodent neocortex (JPET 237:725, 1986), was tested ior effects on memory, blood pressure and sedation in 6 aged monkeys. As with GFC, low doses of UK were able to improve memory without hypotensive or sedative side effects, producing an average of 18.3% improvement with optimal dosage. Longlasting beneficial effects could also be seen after low dose UK administration.The beneficial effects of UK were blocked bythe α-2 antagonist, idazoxan, indicating drug actions at α-2 adrenergic receptors. The separation between UK doses that improved memory (0.000001-0.001 mg/kg) and those that produced hypotension and sedation (0.01-0.05 mg/kg) was not as great as for GFC, consistent with GFC's greater selectivity for the Ri site.

Supported by NIA grant AGO6036

166.2

INDUCTION OF ANXIETY-LIKE BEHAVIORS IN RATS BY OPIATE AND ALPHA-2 ADRENERGIC BLOCK. H. Hsieh*, S. Hendricks, D. Yells*, and B. Graber. Dept. Psychiatry, Creighton Univ. and Dept. Psychology, Univ. of Neb., Omaha, NE 68182.

Naloxone, and opiate receptor blocker and Yohimbine, an alpha-2 noradrenergic receptor blocker, have been shown to induce anxiety and/or panic states in human subjects. Further, these two drugs apparently act synergistically with respect to anxiety induction. The purpose of the present research was to establish an animal model for these effects by determining the behavioral responsiveness of rats to these drugs in several paradigms known to be sensitive to anxiolytic and anxiety inducing manipulations. These behaviors included general activity, open field, and conflict behavior. Rats were tested in these paradigms after administration of varying doses of Yohimbine and Naloxone given separately and in combination. Behavioral effects of the two drugs given separately were in some instances parallel and in other instances, and at specific doses, in opposite directions. When given together these drugs had synergistic effects on some behavior measures. In addition to the behaviors measured it was observed that rats would on occasion suddenly become agitated and difficult to handle following drug treatments. These reactions could possibly be construed as panic. Our findings indicate that rats treated with these drugs may represent a useful model for studying some aspects of the neurochemical substrates of panic and anxiety.

166.3

Alpha-2 Noradrenergic Antagonist (Idazoxan) Decreases Progestin Receptors in the Ventromedial Hypothalamic Nucleus and in the Arcuate Nucleus-Median Eminence. P. A. Vincent and H. H. Feder. Institute of Animal Behavior, Rutgers Univ. Newark, NJ 07102.

Steroid-dependent lordosis behavior in ovariectomized (OVX) guinea pigs is attenuated by alpha-1 and/or alpha-2 noradrenergic (NE) receptor antagonists. Correlated with the decrease in lordosis after alpha-1 NE receptor blockade by prazosin is a decrease in "cytosol" progestin receptors in the ventromedial hypothalamic nucleus (VMN). We examined whether alpha-2 NE receptor blockade with idazoxan (IDA) affects progestin receptors differently than alpha-1 receptor blockade. Ovx female guinea pigs were treated with estradiol benzoate (10ug) at hr 0 and IDA (5 mg/kg; sc) or saline vehicle (VEH) at hr 40 (one hr before assay). A decrease in "cytosol" progestin receptors was found in the arcuate nucleus-median eminence (VEH; 221.9 $\pm$ 17.3 vs. IDA; 161.6 $\pm$ 16.2 fmole/mg protein; $p < 0.05$) and in the VMN (VEH; 40.1 $\pm$ 9.3 vs IDA; 19.3 $\pm$ 3.6 fmole/mg protein; $p = 0.05$). Thus, blockade of either alpha-1 or alpha-2 NE receptor subtype is sufficient to inhibit lordosis and decrease "cytosol" progestin receptors in the VMN. In contrast, blockade of alpha-2 receptor but not of alpha-1 receptor, decreases "cytosol" progestin receptors in the arcuate nucleus-median eminence.

166.4

ANTAGONISM OF DOPAMINE D-2, BUT NOT D-1/D-2, MEDIATED STEREOTYPY BY THE ALPHA-1 ADRENERGIC ANTAGONIST, PRAZOSIN. L.T. Meltzer, T.G. Heffner and J.N. Wiley*. Department of Pharmacology, Parke-Davis Pharmaceutical Research Division, Warner-Lambert Co., Ann Arbor, MI 48105.

Due to the structural similarity of dopamine (DA) and noradrenaline (NA), single compounds can interact with receptors for both systems. We have observed that compounds may have DA agonist as well as NA alpha-1 antagonist actions. To determine the behavioral consequences of this combined activity, we assessed the ability of the NA alpha-1 antagonist, prazosin, to modify the stereotyped behavior in rats produced by either the selective DA D-2 agonist, quinpirole, or the combination of quinpirole plus the selective DA D-1 agonist, SKF 38393.

Prazosin (1, 3 & 10 mg/kg IP) produced a dose-related inhibition of the stereotyped sniffing and licking induced by quinpirole (0.3 & 3.0 mg/kg SC). In contrast, prazosin (10 mg/kg IP) did not antagonize the stereotypy produced by the combination of quinpirole (0.3-3 mg/kg SC) plus SKF 38393 (10 mg/kg SC). These data indicate that compounds that have both DA agonist as well as alpha-1 antagonist actions, may appear selective for the DA autoreceptor.

166.5

CHANGES IN BRAIN MONOAMINES COINCIDENT WITH DEPRESSED BEHAVIOR IN RATS. S. Caldecott-Hazard, K. Moradzadeh*, B. Nguyen*, K. Tachiki*. LBES, UCLA, and Dept. Psychiatry, VAMC, Sepulveda, CA. 91343.

We previously found that a rat model of depressed behavior, withdrawal from chronic amphetamine (AmpWD), produced changes in brain glucose utilization in the dorsal medial prefrontal cortex (DMFC) and habenula (HAB) as compared to controls. In order to further understand the role of these brain areas in depression, levels of dopamine (DA), norepinephrine (NE), serotonin (5HT) and metabolites were assayed during AmpWD. For comparison with previous reports, levels were also assayed in the caudate nucleus (CD). Rats were implanted (s.c.) with osmotic mini pumps containing either amphetamine, 15mg/kg/day, or saline. After 7 days, the pumps were removed and the rats sacrificed 24 hours later. Brains were frozen and brain areas isolated by punch biopsy. Tissue was homogenized, centrifuged, and the supernatant was analysed using a High Performance Liquid Chromatograph. Similar to prior reports (Ellison 1983), DA was decreased and HVA was increased in the CD of AmpWD rats. Also, DA, NE, 5HT, DOPAC, and 5HIAA were decreased in the DMFC of AmpWD rats. Small changes occurred in the HAB, but these data need to be verified. Changes in transmitter levels in DMFC may be indicative of their decreased synthesis and release. Decreased transmitter release may relate to decreased glucose utilization in the DMFC during depression.

166.6

CORRELATION OF NEUROTRANSMITTER RELEASE WITH SEXUAL ACTIVITY IN MALE RATS. E.T. Pleim*, J.A. Matochik, S. Chiang*, J. Mazzone*, R.J. Barfield and S.B. Auerbach. Dept. of Biol. Sci., Rutgers Univ., New Brunswick, NJ, 08903.

In vivo microdialysis of brain tissue allows more direct testing of hypotheses that implicate neurotransmitters in the control of various classes of behavior. The hypothesis that "rewarding" behaviors are accompanied by increases in dopamine release in the nucleus accumbens (N. Acc.) has been supported by studies of ingestive behavior, self administration of drugs, and brain stimulation. We have attempted to extend the DA reward hypothesis to male sexual behavior, using the method of microdialysis of N. Acc. in rats.

Sexually experienced adult Long-Evans male rats were stereotaxically implanted with guide tubes aimed at the posterior nucleus accumbens. On the morning of a test day, a concentric -type microdialysis probe was lowered to the N. Acc. and dialysate samples were collected at 30 minute intervals. Samples were analyzed for DA, DOPAC, HVA, and 5HIAA using an ESA HPLC-EC. After a stable baseline was established, a sexually receptive female was introduced to the test animal's enclosure and measures of male sexual behavior were recorded. After behavioral testing, DA peaks in the dialysate were confirmed using systemic apomorphine administration.

With these methods we are able to detect extracellular DA and metabolites in the N. Acc. of male rats during active copulatory behavior. No significant increase in DA release in the N. Acc. could be detected during or following copulatory activity. Therefore, the current experiment does not provide evidence that male sexual activity is an example of a rewarding behavior that is mediated by an increase in N. Acc. dopamine release.

Supported by NIH grant HD 04484, and NSF grant BNS-8708014.

166.7

TIME SERIES ANALYSIS IN BRAIN DIALYSIS EXPERIMENTS: DETECTING CYCLICITY AND LEAD–LAG RELATIONSHIPS IN BEHAVIOR AND NEUROCHEMISTRY. C.P. Lawler, P.M. Martin, L.L. Devaud, Q.D. Walker*, R.B. Mailman, and M.H. Lewis. Biological Sciences Research Center, Departments of Psychiatry and Pharmacology, Toxicology and Neurobiology Curricula, University of North Carolina, Chapel Hill, NC 27599–7250.

Intracerebral microdialysis allows investigators to examine concurrently, in a single animal, changes in behavioral and neurochemical parameters over time. Time series analysis is a powerful statistical method that can be applied to behavioral/neurochemical temporal patterns and interactions. Time series analysis conducted in the frequency domain (spectral analysis) allows for the detection of periodicities. Parallel neurochemical and behavioral time series can thus be compared to determine the presence of shared cycles, and the lead–lag relationships between the two series. To illustrate the utility of this approach, dialysis probes were implanted in rat caudate nucleus and nucleus accumbens. Dopamine and its acidic metabolites were measured by HPLC–EC every 15 min over a 24 hr period. Concurrent with neurochemical measurements, feeding, drinking, and other behaviors were recorded using a computer–supported observational method. A number of interesting preliminary findings emerged. In addition to a circadian rhythm, ultradian rhythms of 2.5 and 0.75 hr were found for water licking, and for DOPAC in the nucleus accumbens. These periodicities showed considerable shared variance and were phase locked; such coupling was less clear in the caudate nucleus. (Supported by training grants ES07126, HD38970, and PHS grants ES01104, MH37404, MH42938, MH33127, and HD03110)

166.8

MONOAMINE AND METABOLITE CONTENT IN THE RAT BRAIN FOLLOWING DEFEAT. W. W. Woodmansee, M. Fleshner*, and S. F. Maier. Psych. Dept, Univ. Colo., Boulder, CO 80309.

Inescapable electric shock produces a subsequent shuttlebox escape learning deficit. Monoaminergic alterations, particularly forebrain & hypothalamic norepinephrine (NE) depletion, have been proposed to be responsible for this deficit. Defeat in territorial aggression produces similar behavioral effects (unpublished data). We thus examined the effects of exposure to established aggressive colonies on brain monoamines & metabolites in male rats. Intruders were presented to different colonies for 5 successive 10 minute intervals. Behavioral measurements were obtained for each colony-intruder interaction. Defeated rats were sacrificed immediately following the 5th exposure to a territorially defensive colony & HPLC coupled with serial oxidative-reductive electrochemical detection was employed to measure NE, DA, & 5HT & their respective metabolites (free DHPG, free MHPG, DOPAC, HVA & 5HIAA) in anterior cortex, hypothalamus, & hippocampus. All comparisons were made to two control groups: home cage controls & defeat controls (placed in colonies but separated from residents by a clear Plexiglas barrier). Defeat was not found to alter NE, DA (except hippocampal DA) or 5HT levels in any brain region examined. However, both the defeat & defeat control rats showed significant activation of all three transmitter systems in each brain area. Moreover, NE & DA metabolite levels in cortex & hippocampus were generally more elevated in defeated rats than in defeat controls. Higher MHPG levels in the hippocampus were associated with a rapid & continuous display of submissive postures. 5HIAA was equally elevated in both groups in each brain region examined. These results indicate that NE depletion is thus not a necessary condition for display of the shuttlebox escape learning deficit. Supported by NSF grant BNS 8809527.

166.9

ISOLATION AND in vivo ACTIVITY OF NEUROTRANSMITTERS IN THE AMYGDALA. K.H. Tachiki*, A. Kling, R. Lloyd, K. Moradzadeh,* E. Mirzabeigi* F. Houriani*, O. Ricci* (SPON: R. Ritzmann). Psychiatry Service, UCLA/VA Med. Center, Sepulveda, CA 91343.

The amygdaloid nuclei plays a critical role in the regulation of emotion & social relationships. This diversity of function is paralleled by the presence of steroid receptors and terminals of virtually all neurotransmitters and neuromodulators. We report here on the effects of social isolation in Cebus monkeys on the in vivo levels of various neurotransmitters and/or their metabolites in the amygdala, employing the technique of perfusion by micro-dialysis. There was a marked difference in the basal levels of the biogenic amines and their metabolites from animal to animal (5-HIAA: 15.5 VS 44.0; DOPAC: 2.12 vs 5.06). Following six days of isolation, there was a marked increase in perfusate levels of NE (200%) and 5-HIAA (140%). The DA metabolites, DOPAC & HVA, showed no significant changes with isolation. Effects of isolation appear associated with increased release of NE and serotonin in the amygdala. Research was supported by the Joan B. Kroc Foundation and the Veterans Administration.

166.10

BULBECTOMY ATTENUATES COCAINE-INDUCED INCREASES IN LOCOMOTOR ACTIVITY IN MICE. M.G. Hadfield[1], A.R. Lumia[2*], N.A. Darmani[3*], B.R. Martin[3*], D.I. Schiff[4*] and J.H. Porter[1*]. Depts. [1]Path., [3]Pharmacol. and [4]Psychol., Med. Coll VA/VA Commonwealth Univ., Richmond, VA 23298 and Dept. [2]Psychol., Skidmore College, Saratoga Springs, NY 12866.

Several studies have shown that cocaine increases motor activity in mice. The present study was designed to see if bulbectomy alters this increase.

Adult male albino ICR mice (N=32) were either bulbectomized or sham-operated. One week later, they received 20mg/kg cocaine or saline, i.p. (N=8/group). 20 min. later, they were placed in an Omnitech Digiscan Activity Monitor. This measured horizontal activity (including distance and movements), vertical activity, stereotypies and revolutions.

Cocaine significantly increased every motor measurement except vertical activity. On the other hand, bulbectomy attenuated the cocaine response by decreasing horizontal movements, the distance traveled, the number of stereotypic episodes and the movement time. Rest time was increased. In virtually every instance, bulbectomy normalized the values so they were returned to control levels.

These data indicate that an intact olfactory system is required for cocaine to produce its clinical effects. Severance of monoamine pathways related to prominent limbic structures may be responsible for this effect. Further investigations using this model may yield important information concering cocaine's mechanisms of action. (Supported by NIDA grant #DA-02396)

166.11

HYPERSENSITIVITY TO REWARD-RELATED STIMULI AND TO INTRA-ACCUMBENS D-AMPHETAMINE FOLLOWING SOCIAL DEPRIVATION IN RATS. G.H.Jones*, C.A.Marsden[+] and T.W.Robbins. (SPON: Brain Research Association). Department of Experimental. Psychology, University of Cambridge, Cambridge, CB2 3EB, U.K. and [+] Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham, NG7 2UH, U.K.

Socially deprived rats develop many neurochemical and behavioural alterations consistent with dysfunction of the nucleus accumbens (NACC). For example, isolates show impairments in the acquisition of schedule-induced polydipsia and exhibit a greater anticipatory response to stimuli predictive of reward, both of which are dependent on normal regulation of mesolimbic dopamine.

To investigate the effects of social-isolation on the processing of reward-related stimuli, we used a two lever (CR and no-CR), new response procedure with conditioned reinforcement (CR). This procedure involves the pairing of a neutral stimulus (a light/noise compound) with sucrose reward, which thereby gains reinforcing properties itself (CR). Levers were absent during pairing. Lever pressing for CR (with sucrose no longer available) was tested, both undrugged and following a series of intra-accumbens infusions of d-amphetamine (AMPH; 3,10,20 ug). As previously, (Taylor and Robbins, *Psychopharm.* 84 1984), these infusions selectively enhanced responding on the CR lever, however, this increase was significantly greater in isolated rats when compared to socially-reared controls. Isolates also showed an apparent shift in the dose-response curve to the locomotor stimulating effects of intra-accumbens AMPH (3,10 ug). These results are further evidence for the involvement of dopamine-dependent mechanisms of the NACC in the processing of reward-related stimuli and also suggests disruption of the neural substrates underlying these processes following social isolation.

166.12

TESTS OF THE MONOAMINE HYPOTHESIS OF BEHAVIORAL DEPRESSION BY PRECURSOR LOADING. A.L. Beggs, W.B. Daigle*, S. Hotard*, and T. Hebert*. Dept. of Psychology, Univ. of Southwestern La., Lafayette, LA 70504-3131.

The monoamine hypothesis of depression is supported by reports that the symptoms of depression, such as decreased behavioral output, are alleviated by monoamine agonists while monoamine antagonists bring about, or exacerbate the symptoms. Three experiments were conducted. In the first, learned helplessness (LH) was established and rats were given ip injections of L-phenylalanine, L-tyrosine, L-tryptophan, or saline followed by active avoidance training and passive avoidance trials. Active avoidance data showed that L-phenylalanine rats required fewer trials to criterion. Passive avoidance data indicated L-tryptophan facilititates passive responding, while L-phenylalanine and L-tyrosine impair it. In another experiment, L-trytophan treated rats had longer latencies than other groups. An analysis of trials to criterion revealed differences between all groups with L-tyrosine requiring more trials, followed by L-phenylalanine, saline, then L-tryptophan. The results of these experiments generally support the contention that catecholamine precursor loading produces behavioral activation while serotonergic precursor loading is inhibitory to behavioral output.

166.13

BEHAVIORAL EFFECTS OF ACUTE TRYPTOPHAN DEPLETION IN PSYCHIATRIC PATIENTS AND HEALTHY SUBJECTS. P.L. Delgado, D.S. Charney, L.H. Price, W.K. Goodman, G.K. Aghajanian, G.R. Heninger, Dept. of Psychiatry, Yale University School of Medicine, New Haven, CT 06508

Brain serotonin (5-HT) content is dependent on plasma levels of the essential amino acid, tryptophan (TRP). This study investigates the behavioral effects of acute TRP depletion (ATD) in humans. METHOD: 12 obsessive compulsive (OCD), 43 depressed, and 7 schizophrenic patients (DSM-III-R), and 8 healthy subjects received a 24-hr., 160 mg/day, low-TRP diet followed the next morning by a 16-amino acid drink, in a double-blind, placebo-controlled (ATD and control testing), crossover fashion. On one test the diet and drink were supplemented with L-TRP (control) and on the other test neither the diet nor drink were supplemented (ATD). Behavioral ratings (Hamilton Depression Scale (HDRS), Yale-Brown OCD scale, or Brief Psychiatric Rating Scale) and plasma (for TRP levels) were obtained prior to, during and after testing. RESULTS: Total and free TRP decreased 80 to 90% 5 hrs. after the TRP-free drink (TFD). Drug-free, symptomatic OCD and medicated schizophrenic patients and healthy subjects were behaviorally unchanged by ATD or control testing. OCD symptoms in 5 antidepressant-remitted OCD patients were unchanged by ATD but the 3 OCD patients with previous depression became more depressed (mean 95% increase in HDRS score; 19 ± 7 pre-diet, 34 ± 4 7 hours after the TRP-free drink). 60% of 22 symptomatic, drug-free depressed patients were unchanged during ATD, but became clinically less depressed (40% decrease in HDRS) the day after the ATD while 68% of 21 antidepressant-remitted depressed patients relapsed (300% HDRS increase) during the ATD, with return to remitted state the day after. Implications: Preliminary results indicate that ATD may reverse antidepressant but not anti-OCD or antipsychotic responses in psychiatric patients suggesting that the antidepressant effects may be more dependent on 5HT availability. Behavioral effects of ATD appear restricted to depressed patients.

166.14

THE EFFECTS OF INBRED LEARNED HELPLESSNESS ON BEHAVIOR AND MONOAMINERGIC UPTAKE IN RATS. L.J. Molino, J.M. Caruso,* F.A. Henn and P.M. Whitaker-Azmitia. Dept. of Psychiatry, SUNY Stony Brook, Stony Brook, NY, 11794.

The learned helplessness model established by Maier and Seligman(1975) parallels the human condition of depression Henn et al(unpublished data) have been able to inbreed learned helpless(LH) behavior into rats. We investigated the uptake mechanisms of these inbred LH rats via a developmental model. Rats were trained with random inescapable shock for 40 min. at 0.8mA. 24 hrs. after training, animals were tested for avoidance/escape behavior. Failure to terminate shock within 20 sec. was recorded as a failure. Animals scoring high were grouped into the LH group. Animals scoring low were grouped into the non-learned helpless(NLH) group. Full-sib pairing occurred within each group. On postnatal day 15(D15) and D30, animals were tested for spontaneous alternation and vacillatory behavior. Rats were then sacrificed at D1, D15 and D30 after behavioral testing. Whole brains were removed and homogenized for Mazindol and Paroxetine assay. LH animals displayed significant vacillatory behavior and deficits in spontaneous alternation than NLH animals and controls. Biochemical analysis revealed no changes in uptake for D1. At D15 and D30 there are changes in uptake in LH and NLH animals in comparison to controls. We believe that the behavioral deficits displayed may be related to the changes in the uptake mechanisms.

166.15

STRAIN DIFFERENCES IN CENTRAL AMINES INDUCED BY ACUTE AND CHRONIC STRESSORS. N. Shanks, S. Zalcman*, and H. Anisman. Dept. Psychology, Carleton University, Ottawa, Ontario K1S 5B6, Canada.

A single session of uncontrollable footshock engendered region-specific alterations in the utilization and concentrations of brain norepinephrine (NE), dopamine (DA) and serotonin (5-HT). The magnitude of the amine alterations varied appreciably across six strains of mice, such that reduction of a particular amine was readily engendered in some strains, while in others the stressor was without effect. With a chronic footshock regimen applied over 14 consecutive days, utilization and synthesis was altered such that the amine reductions ordinarily associated with acute shock were absent, and in some cases amine levels exceeded control values. This adaptation, which stemmed from a compensatory increase of synthesis and/or moderation of utilization, was more readily apparent in some brain regions and also varied with the strain of mouse. These data may be relevant to strain-specific behavioral differences associated with acute and chronic stressor regimens.

166.16

CHRONIC STRESS INCREASES BOTH TYROSINE HYDROXYLASE ACTIVITY AND EVOKED NOREPINEPHRINE RELEASE IN LOCUS COERULEUS NEURONS. L.K. Nisenbaum, S.L. Castro, M.J. Zigmond, and E.D. Abercrombie *Department of Behavioral Neuroscience and Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260*

Norepinephrine (NE) turnover is elevated in the central nervous system during stress. Changes in tyrosine hydroxylase (TH) activity have been implicated in this response. We have examined the effects of stress on TH activity in locus coeruleus (LC) neurons and NE release from a terminal projection of the LC, the hippocampus. Three days of chronic cold (4°C) exposure resulted in a 99% increase in TH activity in the LC. After 7 days of cold, TH activity declined to 50% above control in the LC, and was no longer elevated after 14 days of cold exposure. In contrast, enzyme activity in the hippocampus remained unchanged after 3 days of cold exposure. However, 7 days after the onset of cold, TH activity in this structure increased 25% above control and remained elevated for the duration of the experiment (21 days). In order to test the functional significance of the increased TH activity resulting from chronic stress, NE efflux in the hippocampus was measured in awake, behaving rats using in vivo microdialysis. In rats cold-stressed for 21 days, baseline NE efflux did not differ from that of control animals. In contrast, these rats responded to another, acute stressor (30 minutes of intermittent tailshock) with a greater elevation of NE (89% above baseline, n=4) than did rats that had not been stressed previously (41% above baseline, n=5 $p<.01$). Thus, elevated TH activity resulting from chronic stress may contribute to an enhanced noradrenergic response to a subsequent, novel stressor. (Supported by USPHS grants MH43947, MH-18273, and MH-00058 and the National Alliance for Research on Schizophrenia and Depression)

166.17

Correlations Between Peripheral Calcium Parameters and CSF Amine Metabolite Levels in Depression and Mania. C.L. Bowden and M.A. Javors*. Depts. of Psychiatry and Pharmacology, Univ. Texas Health Science Center, San Antonio, TX 78284-7792.

Recent research has suggested that alterations in central serotonergic neuronal function may be associated with behavioral abnormalities like depression, suicide, and alcoholism. The purpose of this study was to examine the relationship of peripheral calcium parameters such as plasma [Ca++], platelet total Ca++ content, platelet membrane CaMgATPase activity, and platelet dense tubular system Ca++ uptake to CSF levels of 5HIAA, HVA, and MHPG, the respective major CNS metabolites of the biogenic amines serotonin, dopamine, and norepinephrine. Positive correlations were found between plasma [Ca++] and CSF 5HIAA in unipolar, bipolar depressed and manic patients. For unipolar patients (n=22), plasma [Ca++] was positively correlated with 5HIAA and HVA, platelet CaMgATPase with MHPG, and platelet Ca++ with 5HIAA. In bipolar depressed patients (n=11), plasma [Ca++] was positively correlated with 5HIAA and MHPG, platelet Ca++ with 5HIAA, and platelet CaMgATPase was inversely correlated with 5HIAA and HVA. In the manic subjects (n=12), plasma [Ca++] correlated positively with 5HIAA and HVA. The most consistent and strong relationships in these three subject groups were observed between Ca++ and 5HIAA. This Ca++-5HIAA relationship warrants further investigation of central Ca++ mechanisms and serotonergic function as potentially important mechanisms in affective disorder, especially bipolar disorder.

166.18

EFFECT OF OLFACTORY BULBECTOMY ON 'OPEN FIELD' BEHAVIOR AND BRAIN CATECHOLAMINES IN RATS. L.J. Petterborg*, P.K. Rudeen and D.B. Bylund. Depts of Anatomy & Pharmacology, Univ Missouri Med Sch, Columbia, MO 65212. (SPON: R.C. McClure)

Rats were either olfactory bulbectomized (OBX) or subjected to sham surgery (SHM). At two wks postop ambulatory and defecation scores were recorded for all rats during 3 min 'open field' behavior tests. At 3 and 6 wks following surgery alpha-2 adrenergic receptor (A2AR) densities were determined in the hypothalamus (MBH), amygdala (AMY) and cortex (CTX). At 4 wks after surgery, CA levels and turnover rates (K) were estimated by HPLC/EC. The OBX group (n=24) had signif-icantly greater mean ambulatory (77 vs 63) and defecation (1.50 vs 0.75) scores than did the SHM group (n=24). A2AR binding was significantly increased in the MBH at 3 wks (7.9 vs 7.0) and AMY at 6 wks (13.1 vs 12.0) in OBX rats. MBH NE (165 vs 207) and EPI (26 vs 38) were lower while DA (144 vs 69) was higher in OBX rats. AMY EPI K (231 vs 16) was increased while DA levels (407 vs 711) and K (-84 vs. 694) were significantly reduced in the OBX animals. These results suggest that alterations in behavior following olfactory bulbectomy may be related to site specific changes in CA metabolism and receptor dynamics.

166.19

PSEUDOCONDITIONING OF AVERSIVE-INDUCED YAWNING FREQUENCY DECREASE IN HY RATS. J. Valencia*, A. Moyaho* and B. Holmgren* (SPON: E. Soto) Dept. de Ciencias Fisiológicas, Instituto de Ciencias, Univ. Autónoma de Puebla, Apartado Postal 406, Puebla, MEXICO.

Conditioning of some inborn motor patterns, such as yawning, scratching and anal licking has been attempted in the past with dubious effects (Konorski, 1967). In a Sprague Dawley subline of high yawning rats (HY), we have tested whether it is possible to condition the yawn-reducing effect of aversive stimulation to the noise of a loud buzzer (80 dB, 5 sec) preceding by 4.5 painful electrical stimulation of the rats' paws (60 Hz, 0.5 sec, 0.6-1.3 mA).

In three initial 30' sessions (one daily) to habituate the animals (n=8) to the experimental setup (transparent glass cages with electrifiable floor), a significant increase in yawning was observed during the 3rd session. Application of the CS-UCS immediately after each yawn during 5 sessions determined a significant reduction (65%) in yawning (Wilcoxon-Wilcox paired test, p 0.02). This decrease did not persist during obervation sessions without any stimulation; on the contrary yawning increased. Retraining during 4 additional sessions showed persistence of the fall in yawning during the 2 initial extinction sessions (CS alone, at fixed intervals). From the 3rd to the 6th extinction sessions yawning increased significantly (p 0.02).

Since in a control experiment (n=7 rats), with randomized application of the buzzer noise/footshock pairing, quite similar results were obtained, we suggest our results are due to sensitization or pseudocondi tioning rather than to true conditioned reduction in yawning.

167.1

CHRONIC DIAZEPAM ADMINISTRATION PRODUCES LONG-LASTING REDUCTIONS IN CORTICAL 5-HT RELEASE
M J Neal, P K Hitchcott* & S E File, Division of Pharmacology, UMDS, St Thomas's and Guy's Hospitals, University of London, London, SE1 7EH, UK

Buspirone, a 5-HT_{1a} agonist, is ineffective as an anxiolytic in patients previously treated with benzodiazepines. We therefore examined whether chronic diazepam treatment produced changes in 5-HT release that could account for this. K-evoked (20mM) release of ^{3}H-5-HT from superfused frontal cortical slices from male hooded Lister rats was measured 30 min after i.p. injection of vehicle or diazepam (4mg/Kg), as appropriate. Compared with controls, there were no significant effects of acute diazepam, but after chronic (5 or 21 days) treatment release was significantly reduced. In rats tested 30h or 7 days after the last of 21 diazepam injections the release was still significantly decreased.

167.2

DIAZEPAM WITHDRAWAL: DECREASED SEIZURE THREASHOLD AND INCREASED ANXIETY REVERSED BY FG 7142 AND FLUMAZENIL.
P.K.Hitchcott*, S.E.File, H.J.Little† and D.J.Nutt†.
(SPON: R.D. Brown)
Psychopharm. Res. Unit., UMDS, Div. Pharmacol., Guy's Hospital, London SE1 9RT & Dept. Pharmacol., University of Bristol, U.K.

Diazepam was administered (4mg/kg/day for 20 days; i.p.) and 36h after the last dose male hooded Lister rats were tested in the social interaction (SI) test of anxiety and then bicuculline infused into the tail vein until the point of tonic convulsion (forepaw extension). Compared with controls (vehicle injections 20 days) the diazepam withdrawal group had decreased SI and had significantly lower seizure threasholds. FG 7142 (5 & 20mg/kg) injected 30 min before testing or flumazenil (4mg/kg; 20min) significantly reversed the decreases in SI and seizure threashold seen during withdrawal. The results from the SI test confirm previous reports with chlordiazepoxide withdrawal (Baldwin & File Brain.Res.Bull.(1987) 20:603-6) and suggest that similar changes in the GABA/benzodiazepine receptor complex also underlie the reduction in seizure threashold to bicuculline.

167.3

LONG-TERM ANXIOLYTIC EFFECTS OF CHRONIC ETHANOL OR CHLORDIAZEPOXIDE TREATMENT IN THE RAT.
S.E. File, P.S. Mabbutt† and P.K. Hitchcott†
(SPON: J.J. Stewart)
Psychopharm. Res. Unit, UMDS, Div. Pharmacol., Guy's Hospital, London SE1 9RT, U.K.

Male hooded Lister rats were used in 2 separate experiments. In experiment 1, rats were fed a liquid diet containing 10% ethanol or control for 5 weeks (mean ethanol intake 12g/kg/day). The liquid diet was then withdrawn and replaced by standard rat feed. In experiment 2, rats were chronically treated with chlordiazepoxide (CDP: 10mg/kg/day i.p.) or control for 4 weeks. Six weeks after withdrawal from ethanol or CDP the rats were tested undrugged in the elevated plus-maze test of anxiety.

Rats that had received chronic ethanol treatment spent a significantly ($p<0.05$) greater percentage of time on the open arms of the plus-maze compared with their controls. The rats previously treated with CDP also spent a significantly ($p<0.05$) greater percentage of time on the open arms of the plus-maze compared with their controls. There were no diferences in total arm entries. Thus there is evidence for anxiolytic effects lasting for considerable time after a period of chronic ethanol or CDP treatment.

167.4

BENZODIAZEPINE RECEPTOR ANTAGONIST ZK 93426, ETHANOL AND SOCIAL BEHAVIOR IN SQUIRREL MONKEYS.
E.M. Weerts* and K.A. Miczek. Dept. Psychology, Tufts University, Medford, MA 02155.

Blockade of the benzodiazepine-GABA-chloride ionophore receptor complex may modify some behavioral and physiological effects of ethanol (ETOH). In dyadic confrontations, animals are prompted to exhibit high levels of agonistic behavior, and this heightened level of activity may be relevant to ETOH effects. Socially housed dominant male squirrel monkeys confronted each other in a specially designed test locale with full visual, olfactory, and auditory access. Dominant males treated with 0.1, 0.3 g/kg ETOH increased aggressive threats and vocalizations, while 1.0, 1.5 g/kg ETOH reduced these behaviors. ZK 93426 (3.0 mg/kg) effectively antagonized the enhancing effects of ETOH, but not the aggression-decreasing effects; the latter effects were actually potentiated by ZK 93426. However, frequency of ETOH-induced staggering was significantly reduced when these animals were pretreated with ZK 93426. ZK 93426 may possess some agonistic properties as is evident by increased duration of sitting. When ZK 93426 was administered with a low dose of ETOH (0.1 g/kg), feeding duration increased 5-fold in the social colony immediately following the dyadic test. In mice tested for sleep-time following ETOH administration, pretreatment with ZK 93426 (10.0 mg/kg) did not fully protect against ETOH's depressant effects, although sleeptime was reduced. These results indicate that under narrow dose and time parameters ZK 93426 does attenuate incoordinating, depressant and aggression-enhancing effects of ETOH, but may also potentiate the aggression-decreasing effects.

167.5

DISCRIMINATION OF THE STIMULUS PROPERTIES OF FLUMAZENIL (Ro 15-1788), A BENZODIAZEPINE ANTAGONIST.
G.A. Rowan and I. Lucki. Department of Psychiatry & Pharmacology, University of Pennsylvania, Philadelphia, PA 19104.

Rats were trained to discriminate the stimulus properties of the benzodiazepine receptor antagonist flumazenil using a conditioned taste aversion procedure. Fluid-restricted rats were injected with flumazenil (33 mg/kg) or saline and then given access to a 0.25% saccharin solution for 30 minutes. When rats (N=15) received a drug trial, saccharin consumption was followed by an injection of LiCl (1.8 mEq/kg i.p.), while on saline trials saccharin consumption was followed by a second saline injection.

Acquisition of the discriminated taste aversion, as measured by the differential effects on saccharin drinking between drug and saline trials, developed after only five pairings of the flumazenil with the LiCl injections. Unconditioned controls (N=9), that received an identical sequence of drug and saline trials, but never received LiCl, did not show differences in saccharin intake. The discrimination was also verified using two-bottle preference measures between saccharin and water.

Flumazenil demonstrated dose-dependent generalization upon varying the training dose. Two other benzodiazepine antagonists of different classes, CGS-8216 and ZK 93426, also demonstrated complete substitution for the flumazenil stimulus. Partial generalization was exhibited with the inverse agonist FG 7142, while PTZ failed to generalize to the flumazenil stimulus. Chlordiazepoxide-dependent rats were also trained to discriminate the flumazenil stimulus using this procedure.

This research was supported by DA05367 and DA05186.

167.6

DIAZEPAM, PENTOBARBITAL, METHAQUALONE AND ETHANOL EFFECTS ON SEVERAL BEHAVIORS AND ANTAGONISM BY RO 15-1788.
R. Shukla*, D.J. Mokler and R.H. Rech. Dept. of Pharmacol./Toxicol., Michigan State Univ., East Lansing, MI 48824.

The effects of the benzodiazepine receptor antagonist on a conflict paradigm (condition suppression of drinking, CSD), rotarod performance (RR), motor activity (MA) and body temperature (BT) alone and in combination with diazepam (DZ), pentobarbital sodium (PB), methaqualone (MQ) and ethanol (ET) were studied in rats. DZ, PB, and MQ caused a significant increase in punished responding (shocks) and decrease in unpunished responding (water intake). RO 15-1788 caused a dose-dependent attenuation of the effects of DZ and did not reduce the effect of PB and MQ on either punished or unpunished responding. However, the unpunished responding of PB was significantly potentiated by RO 15-1788. All three agents (DZ, PB and MQ) caused a significant disruption of rotarod performance and motor activity. RO 15-1788 reversed the disruption caused by DZ only; disruption caused by PB and MQ was not modified. DZ, PB, MQ and ET caused a dose-dependent hypothermia. RO 15-1788 significantly blocked the hypothermic effect of DZ and ET, whereas the hypothermia induced by PB and MQ was not altered. It appears that RO 15-1788, being a specific benzodiazepine receptor antagonist, attenuated DZ effects on CSD, RR, MA and BT. ET-induced hypothermia may be reduced by a partial inverse agonistic effect of RO 15-1788. (Supported by grants from the 3M Foundation and a Pradhan Foundation Fellowship [R.S.].)

167.7

SEXUALLY DIMORPHIC ASPECTS OF SOCIAL INTERACTION IN ADULT RATS ARE ALTERED BY PRENATAL EXPOSURE TO DIAZEPAM. Renee Primus and Carol K. Kellogg, Dept. of Psychology, University of Rochester, Rochester, N.Y. 14620.

Environment-related social interaction (SI) in the rat was shown to be sexually dimorphic, differentially modulated by diazepam (DZ) as a function of pubertal age, and altered by prenatal exposure to DZ. In male rats, SI was decreased in the unfamiliar environment relative to the familiar environment at 35 and 60 days, whereas in female rats at 60 days, SI was equivalent in the two environments. Acute administration of DZ (0.5 and 1.0 mg/kg) prevented the decrease in SI in the unfamiliar environment in male rats at 60 days only. Prepubertal castration at 19 days completely abolished the differential impact of the two environments on SI and altered the effect of DZ on SI in male rats at 60 days. These effects of prepubertal castration were completely prevented by exposure to testosterone (T), via implanted silastic tubing, over days 30 through 60. Thus, T secretions in male rats influenced the development of environment-related SI and the effects of DZ thereon. Furthermore, induction of precocial puberty in male rats by early chronic exposure to T (days14-35) advanced the development of the anxiolytic action of DZ on SI.

Prenatal exposure to DZ (2.5 mg/kg) during the last week of gestation altered the development of environment-related SI in male and female rats, suggesting that perinatal as well as pubertal influences affect the development of this behavior. Adult male rats prenatally exposed to DZ exhibited an equivalent amount of SI in the two environments, a response similar to SI measured in unmanipulated female rats at 60 days. In contrast, adult female rats prenatally exposed to DZ demonstrated decreased SI in the unfamiliar environment relative to SI in the familiar environment, the typical response measured in unmanipulated adult male rats. Thus, the organization of this sexually dimorphic behavior was altered by prenatal exposure to DZ. Perinatal and pubertal influences appear to interact, perhaps via the BZD-GABA receptor complex, to organize environment-related SI. Sponsored by Grant MH31850.

167.8

A RAPID SCREENING METHOD FOR THE ASSESSMENT OF BENZODIAZEPINE RECEPTOR RELATED PHYSICAL DEPENDENCE IN MICE. R.A.Lewis and P.F.VonVoigtlander. The Upjohn Company, Kalamazoo, Michigan 49001.

CF-1 mice were injected subcutaneously with the test compound on a fixed schedule (0800 and 1600 for 3 days, the PM dose = AM dose x 2). If tolerated, a starting dose of 150 mg/kg/day was generally used initially and the dose lowered to 15 and 1.5 mg/kg/day in subsequent assays. Twenty-four hours after the last dose, the mice received an intravenous injection of flumazenil (2.5 mg/kg) and 5 minutes later they were tested for electroshock seizure thresholds by an up-down titration method. Flumazenil precipitated withdrawal was manifested by a lowering of the mA seizure threshold. We have found that compounds with benzodiazepine agonist properties significantly lower these thresholds in a dose related fashion. For example, the following compounds (lowest effective mg/kg/day dose) were active in this regard, chlordiazepoxide (150), diazepam (15), flurazepam (15), alprazolam (15), triazolam (15), midazolam (15), zopiclone (150), Ro 16-6028 (150) and Ro 17-1812 (150). In contrast, zolpidem (150), tracazolate (15) and CL 218872 (15) did not cause physical dependence by this criterion. This rapid and simple screening test may be readily used to predict the physical dependence inducing properties of compounds that act at the benzodiazepine receptor.

167.9

INCREASED PERIPHERAL BENZODIAZEPINE BINDING SITES FOLLOWING PORTACAVAL ANASTOMOSIS IN THE RAT. J-F. Giguere[1], E. Hamel[2] and R.F. Butterworth[1], [1]Andre-Viallet Clin. Res. Ctr, Hop. St-Luc (Univ. of Montreal), [2]Montreal Neurological Inst., Montreal, Que., H2X 3J4 CANADA

Recent reports suggest a role for benzodiazepine receptors in the pathogenesis of hepatic encephalopathy. In order to assess this possibility, four weeks after an end-to-side portacaval anastomosis in the rat, central and peripheral-type benzodiazepine receptors were measured using ^{3}H-Ro15-1788 and ^{3}H-PK11195 respectively as specific radioligands. Portacaval anastomosis (PCA) resulted in sustained hyperammonemia, astrocytic alterations and subtle neurological signs of encephalopathy. Affinities and densities of ^{3}H-Ro15-1788 binding sites were unaltered in rats with PCA. On the other hand, ^{3}H-PK11195 binding sites were increased 2 to 3 fold without concomitant changes of binding affinities. Quantitative autoradiographic studies using ^{3}H-PK11195 revealed widespread generalized increases of binding in all brain structures. Increased binding of ^{3}H-PK11195 in these brains probably results from astrocytic abnormalities rather than loss of neuronal structures since activities of neuronal marker enzymes (GAD and CAT) were unchanged following PCA. [Funded by MRC Canada and FRSQ Quebec]

167.10

INCREASED DENSITIES OF PERIPHERAL-TYPE BENZODIAZEPINE RECEPTORS IN AUTOPSIED BRAIN TISSUE FROM ALCOHOLIC CIRRHOTIC PATIENTS WITH HEPATIC ENCEPHALOPATHY. J. Lavoie, G. Pomier Layrargues and R.F. Butterworth, Lab. Neurochem. and Liver Unit, Andre-Viallet Clin. Res. Ctr, Hop. St-Luc (Univ. of Montreal), Montreal, Que. H2X 3J4 CANADA.

It has been proposed that benzodiazepine receptors may be modified in hepatic encephalopathy. In order to evaluate this hypothesis, the integrity of benzodiazepine receptors was evaluated in brain membrane preparations obtained at autopsy from nine alcoholic cirrhotic patients who died in hepatic coma. Histopathological studies revealed the presence of Alzheimer Type II astrocytosis in all cirrhotic brains. Binding to central and peripheral benzodiazepine receptors was evaluated using ^{3}H-Ro15-1788 and ^{3}H-PK11195 respectively. Data from saturation binding assays was analyzed by Scatchard analyses showed no modifications of either affinities (Kd) or densities (Bmax) of central benzodiazepine binding sites. In contrast, densities of ^{3}H-PK11195 binding sites were increased by 64% ($p<0.01$) in frontal cortex and by 31% ($p<0.02$) in caudate nuclei of patients with hepatic encephalopathy. Such changes could play an important role in the pathogenesis of hepatic encephalopathy resulting from chronic liver disease. [Supported by MRC (Canada) and FRSQ (Quebec)]

167.11

THE EFFECTS OF ETHANOL SUPERFUSED IN VIVO ON ISOLATED NORADRENERGIC CIRCUITS FROM LOCUS COERULEUS GRAFTS TO CO-GRAFTED BRAIN TISSUE IN OCULO. M.R. Palmer[1] and A-C. Granholm[2]. [1]Dept. of Pharmacology, Univ. of Colorado HSC, Denver, CO, 80262 USA and [2]Dept. of Cell Biology, University of Linköping, S-581 85 Linköping, Sweden

Fetal locus coeruleus (LC) was sequentially grafted with hippocampus CA1 or cortex cerebelli into the anterior eye chambers of adult rats. These brain grafts were allowed to mature and to innervate each other over a period of 4-6 months. Histological, electrophysiological, in vivo voltametric and pharmacological studies indicate that the LC neurons survive and functionally innervate the hippocampal co-grafts. Ethanol, when superfused over the co-grafts in urethane anesthetized animals, caused excitations of hippocampal neuronal activity at doses between 1-30 mM, while applications of 30 mM ethanol and higher depressed the activity of these same neurons. Similar results were observed from cerebellar neurons in LC-cerebellum double grafts except that the cerebellar neurons were more sensitive to the depressant effects of ethanol than were those recorded from hippocampus. The excitations caused by lower ethanol doses in double grafts could be prevented by the co-superfusion of 0.5 - 1.0 μM clonidine with the ethanol. Furthermore, the low-dose excitations were not observed in single grafts of hippocampus in oculo. Our data suggest that the excitations are mediated by changes in the catecholaminergic innervation from the LC grafts. This does not appear to be due to a postsynaptic change in norepinephrine (NE) sensitivity, however, since ethanol did not alter the NE dose-response curves in hippocampal grafts. Thus, the ethanol-induced excitations were probably disinhibitions mediated by an ethanol-induced depression of the inhibitory NE input to target grafts from the LC co-grafts. Indeed, the doses of ethanol which caused excitations in the hippocampal or cerebellar grafts also caused depression of neuronal firing rates in the LC grafts, and the doses of clonidine which prohibited the excitations also inhibited the firing rates in the LC grafts while causing an excitation in the co-grafts. (Supported by USPHS grants AA 05915 and AA 00102, and by the Magnus Bergvall Foundation in Sweden. Dr. Palmer is supported by an ADAMHA Research Scientist Development Award.)

167.12

ACUTE AND CHRONIC ETHANOL ALTER INHIBITION OF HIPPOCAMPAL CA3 NEURONS. L.P. Gonzalez, Department of Psychiatry and Behavioral Sciences University of Oklahoma Health Sciences Center, Oklahoma City, OK 73190-3000.

Acute ethanol administration inhibits the spontaneous *in vivo* release of ACh at several sites within the central nervous system. Additional alterations in cholinergic function are observed after chronic ethanol exposure. The study reported here examined ethanol effects on the response of hippocampal CA3 neurons to electrical stimulation of the cholinergic septo-hippocampal projection. Extracellular recordings of hippocampal unit activity were obtained using glass microelectrodes with tip diameters of 1 to 5 microns. Most of these neurons were observed to have firing rates of two to four Hz. Stimulation of the medial septum resulted in a brief increase in single-unit activity, followed by a longer period of reduced activity. Acute ethanol (1.5 g/kg, i.p.) had no effect on the period of increased activity, but it significantly increased the length of the period of inhibition. In rats chronically treated with ethanol, and tested ten hours after withdrawal, ethanol did not alter the period of inhibition. These results show a significant effect of ethanol on the hippocampal response to septal stimulation, and the development of tolerance to this effect with chronic ethanol exposure.

This work was supported in part by NIAAA grant AA07578.

167.13

ETHANOL REDUCES THE M-CURRENT IN CA1 HIPPOCAMPAL PYRAMIDAL NEURONS (HPNs) IN VITRO. S.D. Moore*, S.G. Madamba* and G.R. Siggins. (SPON: L. Koda). Research Institute of Scripps Clinic, La Jolla, California 92037

Previous in vivo studies showed that systemic ethanol enhanced HPN responses to iontophoretic acetylcholine (ACh) and somatostatin (SS), with little or no effect on responses to GABA, norepinephrine or 5-HT (Mancillas et al., Science 231:161, 1986). We (Moore et al., Science 239:278, 1988) previously reported that in HPNs SS and ACh in vitro reciprocally regulate the voltage-dependent K^+ current termed the M-current (I_M). Therefore, we tested ethanol superfusion on this current in rat CA1 HPNs in a slice preparation, using single electrode voltage-clamp. At rest, ethanol had little effect on membrane currents. However, at -40 mV holding potentials, ethanol in low concentrations (22 - 44 mM) significantly reduced I_M amplitude in 36 HPNs, by 30 to 42%. Atropine in doses (1 µM) that block muscarinic reduction of I_M did not alter ethanol-induced I_M reduction. Thus, the site of ethanol action is not on ACh release or receptor binding. Neither the anomalous rectifier (Q-current), the A-current, nor the AHP current were reproducibly altered by ethanol. Ethanol 44 mM, but not 22 mM, antagonized I_M augmentation by SS. Ethanol reduction of the M-current may account for the potentiation of ACh responses seen in vivo and provides a mechanism for the excitatory effects of ethanol on some central neurons that possess M-channels. Supported by AA-06420, AA-07456, and MH-44341.

167.14

N-METHYL-D-ASPARTATE (NMDA) STIMULATED NEUROTRANSMITTER RELEASE FROM BRAIN SLICES IS INHIBITED BY ETHANOL. R. A. Gonzales and J. J. Woodward. Div. of Pharmacology, Univ. of Texas, Austin, TX 78712.

The effect of ethanol in vitro on the release of [^{3}H]norepinephrine (NE) from rat cortical slices or endogenous dopamine from striatal slices stimulated by NMDA was studied. Cross-chopped (350 µm) slices were prepared from each region and washed for 40 min in oxygenated Krebs-Ringer buffer without magnesium. Cortical slices were loaded with [^{3}H]NE for 30 min followed by 1 hr of additional washing. Tissue was transferred to baskets and transferred (every 1 or 2 min) through a series of vials containing fresh oxygenated buffer with various concentrations of NMDA. NMDA (50 µM-1 mM) stimulated [^{3}H]NE release from cortical slices (up to 5.5% of total [^{3}H] taken up) or DA release from striatal slices (up to 3.1% of total) and this release was completely blocked by 1.2 mM Mg^{2+}. Tetrodotoxin (1.0 µM) attenuated NMDA-stimulated [^{3}H]NE release by >90%. Ethanol significantly reduced 100 µM NMDA-stimulated [^{3}H]NE release from cortical slices in a concentration-dependent manner in a range from 60-200 mM (32-53% inhibition). A similar inhibitory effect (38-70% inhibition) of ethanol (10-200 mM) was observed for 500 µM NMDA-stimulated endogenous DA release from striatal slices. The inhibition of NMDA-stimulated [^{3}H]NE release by 100 mM ethanol was not overcome with increasing concentrations of NMDA up to 1 mM indicating a non-competitive type of inhibition. The effect of 100 mM ethanol could be reversed after a 13 min wash period. Preincubation of the cortical slices with ethanol for 3 min enhanced the attenuating effect of ethanol on NMDA-stimulated [^{3}H]NE release compared to simultaneous addition of NMDA and ethanol. Changes in NMDA receptor function by ethanol may contribute to some of the intoxicating or anesthetic effects of acute ethanol administration. (Supported by NIAAA grants AA07297 and AA08089).

167.15

EFFECTS OF CHRONIC ALCOHOL ADMINISTRATION ON VASOPRESSIN- AND OXYTOCIN-CONTAINING NEURONS. JH deSchweinitz* and GP Kozlowski. Department of Physiology, Univiversity of Texas Southwestern Medical Center, Dallas TX, 75235-9040.

Learning and memory is enhanced by vasopressin (VP) and attenuated by oxytocin (OT). Since learning and memory is diminished in alcoholics, we investigated the effects of chronic ethanol (ETOH) administration on these neuropeptides. We previously reported reduced VP neuronal numbers after 5 & 15 da (Soc Neurosci Abstr, 14:196,1988) of ETOH. Here, we extended the treatment period to 30 & 60 da. Animals were administered either: 1. Lab Chow and water ad lib (CCTRL=Chow control), 2. control liquid diet (CTRL) or 3. ethanol liquid diet (ETOH). Male, Long-Evans rats were perfuse-fixed and immunocytochemistry was performed on their serial-sectioned (40 µm) brains using rabbit anti-VP 710 and anti-OT 729. All VP & OT neurons were categorized into major nuclear gps and counted. Means of total numbers of neurons/brain/treatment group for VP (n=5/gp) were: 48 da CCTRL, 3023±180; 30 da CTRL, 2502±214; 30 da ETOH, 1548±87; 60 da CTRL, 2323±125 and 60 da ETOH, 1606±197; whereas, for OT (n=3/gp) means were: 48 da CCTRL, 4754±274; 30 da CTRL, 4044±446 and 30 da ETOH, 4256±231. For VP, significant decreases ($p<.001$) occurred in all nuclear areas except for the anterior commissural nuc. and ant. hypothalamic area (AHA). For OT, there were no significant differences in total neuronal numbers amongst treatment groups but numbers of AHA neurons decreased while those of the paraventricular nucleus increased. Supported by NIAAA AA-06014.

167.16

DOES ETHANOL ENHANCE PRE/POSTSYNAPTIC EFFECTS OF BACLOFEN IN VITRO IN RAT HIPPOCAMPAL SLICES? G.D. Frye, L. Taylor*, W.H. Griffith Dept. Medical Pharmacol. & Toxicol., Texas A&M Univ. Col. of Med., College Station, TX 77483

The hypothesis that the CNS actions of ethanol involve changes in GABAergic transmission has recently received support. Ethanol enhances $GABA_A$-gated Cl^- uptake into brain vesicles, an action that is lost after chronic ethanol exposure. Whether acute or chronic ethanol treatment also changes the relative activity of $GABA_B$-mediated events in the CNS was examined in the present studies. The presynaptic effects of the $GABA_B$ agonist, baclofen (BAC), were tested on the CA1 dendritic field excitatory postsynaptic potential (EPSP) in hippocampal slices of rat brain. Bath-applied (-)BAC (1uM) alone reduced the EPSP by 20-30%. However, no further inhibition occurred when (-)BAC was applied with ethanol (10-60mM). Dose-related inhibition of EPSPs by (-)BAC (0.1-10uM) in slices from ethanol-naive or ethanol-dependent rats was not different. During intracellular recording from CA1 cells, the postsynaptic action of BAC was studied using the membrane hyperpolarization (5-10mV) induced by pressure ejection of (±)BAC (100uM). Bath applied ethanol 30mM did not enhance the amplitude of the postsynaptic response to BAC (n = 5). Based on these findings, ethanol does not appear to enhance either pre- or postsynaptic $GABA_B$-mediated inhibition in the hippocampus. (Supported by AA06322, AA00101, NS22456 & AG07805)

167.17

URETHANE POTENTIATES THE DEPRESSANT EFFECTS OF ETHANOL ON RAT BRAIN SLICES. W.R. Proctor and T.V. Dunwiddie, Veterans Administration Medical Services and University of Colorado Health Science Center, Denver, CO 80262

Electrophysiological analyses have shown that ethanol application has a generally depressant effect on neuronal activity. Superfusion of *in vitro* hippocampal slices with 80 mM ethanol *increases* the amplitude of the evoked population spike by 15-30 percent during the first 3-6 minutes, followed by a 30-40 percent amplitude decrease. Concurrent intracellular recordings from CA1 pyramidal neurons show minimal changes in a number of measured parameters: 80 mM ethanol usually produced small hyper-polarizations of the resting membrane potential (0.8±0.5 mV, n=18, $p<0.01$) and a small reduction in the calcium-activated potassium conductance (10±3%, n=18, $p<0.01$). However, no significant changes were observed in the input impedance, suprathreshold and subthreshold epsp amplitudes, $GABA_a$ and $GABA_b$ responses or firing thresholds of the sodium spike action potential.

To more closely mimic the *in vivo* recording conditions under which depressions of activity are commonly observed, simultaneous extracellular and intracellular recordings were made from hippocampal slices in 20 mM urethane. The depressant effect on the population spike amplitude was enhanced by 20 to 50 percent compared to the effects of ethanol alone. No additional effects of ethanol on ionic conductances were oberved. Although the mechanisms underlying these interactions are unclear, the present study suggests that at least one common anesthetic may significantly modify the effects of ethanol on neuronal activity. Supported by AA03527 & VA Med. Res. Service.

167.18

HIPPOCAMPAL SLICE ELECTROPHYSIOLOGY IS ALTERED BY PRENATAL ALCOHOL EXPOSURE. S.E. Tan*, E.L. Abel, C.S. Zajac, R.M. Yager* & R.F. Berman. Fetal Alcohol Res. Ctr., Depts. Ob/Gyn & Psychology, Wayne State Univ., Detroit, MI 48202.

The effects of prenatal alcohol exposure in rats on hippocampal slice electrophysiology, histology and learning were examined. Pregnant Sprague-Dawley rats consumed ethanol containing liquid diets containing 0%, 17.5% or 35% ethanol derived calories (EDC) from gestation day 8 until parturition. A fourth group was fed standard rat chow as an ad libitum control. Animals prenatally exposed to alcohol had lower birth weights and impaired passive avoidance learning at 17 days of age. At 90 days of age synaptic potentials in area CA1 of the hippocampus were characterized electrophysiologically in in vitro hippocampal slices. Slices from alcohol exposed rats had significantly less paired pulse inhibition compared to 0% EDC and controls. Hippocampal slices from 35% EDC rats also showed less long-term potentiation compared to controls. Litter mates were found to be impaired in radial-arm maze learning. Histological examination of brains from litter mates did not indicate altered number, density or nuclear volumes in area CA1. These data indicate that prenatal ethanol exposure can result in abnormal hippocampal synaptic physiology and suggest that these changes may contribute to the learning impairments observed in rats following such exposure.
(Supported by N.I.H. Grant No. P50 AA07606-02)

167.19

Effect of ethanol on neurons in somatosensory (S1) cortex and VPL thalamus in awake, behaving rats. Kosobud, A., Shin, H.-C., and Chapin, J.K. Dept. of Physiology/ Biophysics, Hahnemann University, Philadelphia, PA 19102.

By recording from arrays of chronically implanted microwire electrodes, it is possible to monitor multiple neurons simultaneously in awake, behaving animals. We have used this technique to study the effect of ethanol (EtOH) on higher order sensorimotor processing in the CNS. Microwire electrodes were implanted in S1 cortex and VPL thalamus of 4 adult female rats. In all, recordings from 16 cortical and 3 thalamic cells (4-5 total per rat) were obtained. First, the receptive field properties of each cell were determined. Some cells were selectively sensitive to touch stimuli on the forepaw pads, while others were most responsive to movement of finger or wrist joints. Continuous recordings were obtained from rats locomoting on a timed treadmill (cycle: 10 sec on, 10 sec off) over a 10 minute initial control period, and for 60 minutes following ethanol administration. Drug- and behavior-induced modulation of sensory transmission to these cells was continually tested by repeated mild stimulation (2/sec) through electrodes chronically implanted in the forepaw. In addition, the treadmill included patches of novel surface materials (one very soft, one very irregular). Finally, spike-triggered cross-correlate histograms were constructed to measure direct functional connections between the recorded neurons. A videocamera was used to record motor behavior throughout the experimental session. Overall, EtOH tended to inhibit the sensory responses and activity of cells in both cortex and thalamus, and blunted the distinctions between cell activity under the various conditions. However, selective effects were observed, including a stronger suppression of long versus short latency sensory responses, and a reduction in the inhibitory sensory gating normally observed during movement. Also, the neurons in the forepaw area of S1 cortex showed highly individual activity patterns correlated with movement and sensory stimuli. Responses of individual cells to ethanol were quite variable, with some being virtually unaffected, while neighboring cells showed alterations throughout the recording period. These results are consistent with the notion that EtOH selectively disrupts higher cortical sensorimotor processing. *Supported by PHS grants AA06965, AA00089, and NS26722.*

167.20

MULTIPLE WITHDRAWALS FROM CHRONIC ETHANOL TREATMENT "KINDLES" INFERIOR COLLICULAR SEIZURE ACTIVITY. Thomas J. McCown and George R. Breese, University of North Carolina School of Medicine, Chapel Hill, NC 27599-7250

Ballenger and Post proposed that multiple detoxifications from chronic ethanol abuse kindles withdrawal seizures (Brit. J. Psychiat. 133:1,1978). To test this hypothesis in rats, we used a seizure paradigm where seizure activity is electrically elicited from the inferior collicular cortex(IC)(McCown Exp. Neurol. 84:526, 1984). Rats received ethanol liquid diet (ELD) for 5 days and then were withdrawn for 1 day. Seven days after 6 cycles of this regimen, the number of IC stimulations needed to cause seizure generalization was significantly reduced compared to liquid diet controls(8±1 vs. 12±1). After 10 cycles the number of needed IC stimulations was further reduced(6±1), while the same period of continuous ELD reduced the number of IC stimulations to 9±1. Conversely, multiple withdrawals from chronic ELD significantly attenuated amygdala kindling. These findings show that withdrawals from chronic ELD "kindle" the IC, thus increasing ethanol withdrawal seizure sensitivity. (supported by NS-76596)

SEROTONIN III

168.1

MDMA-INDUCED RELEASE OF SEROTONIN (5-HT) AND INHIBITION OF NEURONAL FIRING IN DORSAL RAPHE (DR) BRAIN SLICES: POTENTIATION BY L-TRYPTOPHAN (TRP). J.S. Sprouse, C.W. Bradberry, R.H. Roth and G.K. Aghajanian, Depts. of Psychiatry and Pharmacology, Yale Univ. Sch. of Med., New Haven, CT

Previous work from this laboratory demonstrated that the novel psychedelic MDMA (3,4-methylenedioxymethamphetamine) inhibits the firing of 5-HT-containing DR neurons in midbrain slices. The change in DR neuronal firing is correlated inversely with efflux of 5-HT, suggesting that these inhibitory effects on unit activity could be due to the known 5-HT-releasing properties of MDMA. The present study investigated whether TRP, a 5-HT precursor, affects MDMA-induced changes in DR neuronal firing and 5-HT release.

Extracellular single unit recordings of DR neurons were made in the in vitro rat brain slice; microdialysis probes resting on the slice surface provided a means to estimate 5-HT release from the DR. TRP at 100 μM enhanced both MDMA-induced inhibition of unit activity and 5-HT release; TRP at 500 μM enhanced MDMA-induced 5-HT release and increased basal release to detectable levels. These data suggest that MDMA acts indirectly at the somatodendritic autoreceptor of DR neurons via release of endogenous 5-HT. The question is raised as to what effect TRP may have on the behavioral and neurotoxic actions of MDMA.

168.2

EFFECT OF MDMA ON DOPA ACCUMULATION IN THE STRIATUM AND NUCLEUS ACCUMBENS. J.F. Nash, H.Y. Meltzer and G.A. Gudelsky. Dept. of Psychiatry, Case West. Res. Univ., Cleveland, OH 44106.

The effect of MDMA on the synthesis of DA in the terminals of nigrostriatal and mesolimbic neurons was estimated by measuring the accumulation of DOPA in the striatum and nucleus accumbens 30 min following the administration of the decarboxylase inhibitor, NSD 1015. MDMA produced a dose- and time-dependent increase in DOPA accumulation in the striatum but not in the nucleus accumbens. Although the concentrations of 5-HT and 5-HIAA in both the striatum and nucleus accumbens were reduced 3 hr following an injection of MDMA (20 mg/kg, s.c.), 5-HT and 5-HIAA concentrations were significantly reduced only in the striatum 7 days after administration of MDMA. Pretreatment with the 5-HT_2 antagonist, ketanserin, significantly attenuated the reduction in 5-HT concentration in the striatum 3 hr following MDMA administration and completely blocked 5-HT depletion at 7 days post-administration. Moreover, ketanserin completely blocked MDMA-induced DOPA accumulation in the striatum. These data are supportive of the hypothesis that DA plays a role in MDMA-induced 5-HT depletion.

168.3

MAJOR METABOLITES OF MDA DO NOT MEDIATE ITS TOXIC EFFECT ON SEROTONIN NEURONS IN THE RAT BRAIN. U. D. McCann* and G. A. Ricaurte (SPON: L. Mamounas). Dept. of Behav. Biol., Walter Reed Army Inst. of Res., Bethesda, MD 20307 and Dept. of Neurol., Johns Hopkins Sch. of Med., Baltimore, MD 21224.

3,4-Methylenedioxyamphetamine (MDA) is toxic to serotonin (5HT) neurons in the rat brain (Ricaurte et al., Science 222:986-988, 1985). The major metabolites of MDA in the rat are alpha-methyldopamine (α-MeDA) and 3-O-methyl-α-methyldopamine (3-O-Me-α-MeDA) (Marquardt et al., Biochem. Pharm. 27:1503-1505, 1977). The purpose of this study was to determine if either of these metabolites mediates the toxic effect of MDA on 5HT neurons in the rat brain.

Potential neurotoxic activity of α-MeDA and 3-O-Me-α-MeDA was evaluated in two ways: (1) By systemically administering repeated high doses of their precursors, α-MeDOPA and 3-O-Me-α-MeDOPA, both of which readily cross the blood brain barrier, and (2) By intraventricularly administering various doses of the parent compounds themselves. Two weeks after drug treatment, rats were killed and their brains were analyzed for regional 5HT content. Neither the precursors given systemically nor the parent compounds (α-MeDA and 3-O-Me-α-MeDA) given intraventricularly produced a lasting depletion of serotonin in the rat brain. Furthermore, combined administration of α-MeDOPA and 3-O-Me-α-MeDOPA was also without effect.

These results suggest that neither α-MeDA nor 3-O-Me-α-MeDA, which comprise over 95% of MDA's metabolites (Marquardt et al., 1977), mediate the 5HT toxic effect of MDA, and suggest that either a minor metabolite is involved or that another mechanism is at work.

168.4

REINNERVATION OF CEREBRAL CORTEX BY 5-HT AXONS AFTER DENERVATION BY PSYCHOTROPIC AMPHETAMINE DERIVATIVES. M.E. Molliver, L.A. Mamounas and P. Carr*. *Depts. of Neuroscience and Neurology, The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205*

The drugs 3,4-methylenedioxyamphetamine (MDA) and p-chloroamphetamine (PCA) cause degeneration of 5-HT projections to forebrain, persisting for months. Fine axon terminals in cortex are highly vulnerable, while beaded axons and raphe cell bodies are spared. The long-term cytotoxic effects of these drugs in the rat were analyzed with 5-HT immunocytochemistry. An increased density of 5-HT axons is first detected in frontal cortex, 2 to 4 months after drug treatment. Progressive re-innervation follows a fronto-occipital gradient; partial recovery extends to occipital cortex at 8 months. Straight, longitudinally oriented 5-HT axons extend from rostral to caudal, within layers I and VI, before axon terminals appear in middle cortical layers; this bilaminar pattern of ingrowth simulates perinatal development of 5-HT innervation. The re-innervation reflects sprouting of fine axons, while the beaded axons, which survive drug treatment, do not expand their terminal arborizations. Thus, after drug-induced neurotoxicity, there is regeneration of 5-HT innervation, with sprouting of the axon type that was initially lost. There is no evidence for aberrant re-innervation by beaded axons, yet further studies are needed to determine whether the normal pattern of 5-HT projections is re-established. [Support: DA04431, NS15199].

168.5

MDMA, COCAINE, AND FENFLURAMINE BUT NOT METHAMPHETAMINE PRODUCE NEUROPATHOLOGY OF CULTURED 5-HT NEURONS. E.C. Azmitia, X.P. Hou, P.M. Whitaker-Azmitia, S.A. Hochberg, and R.M. Murphy. Dept. Biology, Chemistry, New York Univ., New York, NY and Dept. Psychiatry, SUNY-Stony Brook, NY.

A variety of drugs of abuse have similar actions on 5-HT neurons -- inhibit ^{3}H-paroxetine binding, inhibit uptake of ^{3}H-5HT and promote release of ^{3}H-5HT. MDMA, cocaine and fenfluramine share these properties while methamphetamine does not. These four drugs were studied for direct effects on fetal serotonergic neurons in cultures. 14 dg rat fetuses were dissected and the raphe area dissociated mechanically. The cells were plated on 96-well plates coated with polylysine. Cells were incubated 24 hours for morphology and three to five days for uptake studies. Drugs were tested from 10^{-3}M to 10^{-10}M applied once, and cultured cells tested for ^{3}H-5HT uptake or after staining with an anti-5HT antibody. MDMA and cocaine produce similar changes in morphology (decrease in the density of 5-HT neurons, decreased fiber length) and MDMA, cocaine, and fenfluramine cause a decrease in uptake development after a single application. Multiple applications of these three drugs significantly potentiated the inhibitory properties. Methamphetamine, at concentrations up to 10^{-4}M, produce no inhibition of 5-HT neurons in culture. Our results indicate fenfluramine > S-MDMA = cocaine > R-MDMA > methamphetamine in neuropathology to 5-HT neurons. Research supported by NIDA 271-87-8144.

168.6

THE EFFECTS OF DRUGS OF ABUSE AND SELECTED SEROTONIN AGONISTS ON THE HIGH-AFFINITY SEROTONIN TRANSPORTER; DISPLACEMENT OF [^{3}H]-PAROXETINE. J.C. Poblete, P.M. Whitaker-Azmitia, and E.C. Azmitia. (SPON: J. Martin) Dept. Biology, New York University, Washington Square, New York, NY 10003.

Recently, paroxetine has been shown to be a potent inhibitor of the high affinity 5HT uptake carrier. Using ^{3}H-paroxetine, competitive binding assays were performed to assess the possible interactions of drugs of abuse and selected 5HT receptor agonists. ^{3}H-paroxetine was used at 0.2nM on rat brain membrane preparation, and the incubation time was one hour at room temperature. The drugs of abuse tested were S- and R-MDMA, S-methamphetamine, fenfluramine and cocaine; all of which have been shown to interact with the serotonergic system. The 5HT receptor agonists tested were ipsapirone for the $5HT_{1a}$, TFMPP for the $5HT_{1b}$, and DOI for the $5HT_2$. In addition to these drugs, we also examined the displacement of ^{3}H-paroxetine by fluoxetine, reserpine, Ca^{++} and 5HT. All of the drugs of abuse tested showed displacement of ^{3}H-paroxetine. The rank potency order was fluoxetine (10^{-8}M) > fenfluramine = cocaine = S-MDMA (10^{-6}M) > R-MDMA (10^{-5}M) > S-methamphetamine (10^{-4}M). Interestingly, the specific 5HT receptor ligands tested were also found to displace ^{3}H-paroxetine. The rank potency order of these agonists was fluoxetine (10^{-8}M) > 5HT (10^{-6}M) > TFMPP (high 10^{-6}M) > DOI (10^{-5}M) ipsapirone (10^{-4}M). Reserpine and Ca^{++} do not affect ^{3}H-paroxetine binding. A number of drugs below the concentration producing displacement potentiated ^{3}H-paroxetine binding. (NIDA 271-87-8144)

168.7

SEROTONIN NEURONS MAY BE A MAJOR TARGET FOR THE ACTION OF COCAINE: EVIDENCE FROM IN VITRO AND TISSUE CULTURE STUDIES. G.DeGeorge, S.A.Hochberg, R.Murphy, and E.C.Azmitia. Departments of Chem. and Biology, New York University, NY, NY, 10003

It is known that the drug of abuse cocaine exerts its neurochemical effects on dopamine neurons, causing both uptake blockade and release of DA. We have found that cocaine also inhibits the specific high affinity uptake of serotonin in rat brain synaptosomes. Synaptosomes were preincubated with cocaine (1mM-1pM) for 0-20 min, then incubated for 5 min with 50nM ^{3}H-5HT, resulting in a 50% inhibition at 1uM, with maximal at 100uM. This inhibition increased with longer preincubation times. This effect is similiar to that of MDMA on the 5-HT system. Cocaine also promoted the release of 5-HT in preloaded synaptosomes, exhibiting another neurochemical effect of MDMA. Morphometric analysis of cocaine in culture revealed significant neuropathology, with decreases in neuronal density, neurite length, and somal area. Studies in our lab also show cocaine effectively blocks paroxetine binding to the 5-HT transporter at 1uM, Thus it appears that 5-HT neurons are a primary target of cocaine. (Funded in part by NIDA 271-87-8144)

168.8

PSYCHOTROPIC AMPHETAMINES CAUSE A PREFERENTIAL ACUTE RELEASE OF NON-VESICULAR 5-HT. Xi Gu and E.C. Azmitia (SPON: J.F. Hyde) Dept. Biology, New York University, Washington Square East, New York, NY 10003.

Transmitter release can be either vesicular (Ca^{++}-dependent) or non-vesicular (Ca^{++}-independent). It has been previously reported that the MDMA-induced release of ^{3}H-5HT is calcium independent (Nichols et al, 1982; Schmidt, 1987). We have developed a release assay to study the differential effects of fluoxetine on K^+ and MDMA. In addition, we have looked at the dose response of cocaine, MDMA, and fenfluramine on 5-HT release. The release assay used was to preload the synaptosomes with ^{3}H-5HT (5 x 10^{-7}M) at 37°C for 20 min., incubate it with the drugs at 37°C for 10 min., and treat the washed synaptosomes with alcohol which extracted the unreleased ^{3}H-5HT for scintillation counting. Our results provided evidence for two pools of 5-HT for release: an amphetamine induced (non-vesicular and was inhibited by fluoxetine 10^{-7}M) and a K^+(50mM)-induced. In studies of a series of tissue concentration, we noted that fluoxetine can increase the amount of ^{3}H-5HT release in both control and K^+-stimulated systems. This is probably due to its inhibition of reuptake of the released ^{3}H-5HT. In contrast, the release with MDMA (10^{-5}M) was markedly inhibited by fluoxetine by as much as 70%, and this may be due to competitive binding effect of MDMA and fluoxetine for the same 5-HT transporter. Cocaine (10^{-6}M) and fenfluramine (10^{-6}M) were found to be compatible in release potential as MDMA (10^{-5}M). Research supported by NIDA 271-87-8144.

168.9

d-METHAMPHETAMINE AND OTHER SUBSTITUTED AMPHETAMINES PRODUCE A SIMILAR PATTERN OF SEROTONERGIC AXON DEGENERATION. Karen J. Axt and Mark E. Molliver. Dept. of Neuroscience, The Johns Hopkins University School of Medicine, Baltimore, MD 21205

It has previously been demonstrated that methylenedioxyamphetamine (MDA), methylenedioxymethamphetamine (MDMA), p-chloroamphetamine (PCA), and fenfluramine (FEN) cause selective ablation of a distinct class of serotonergic (5-HT) axon terminals in the rat brain; fine axons, characterized by small pleomorphic varicosities, are ablated, whereas beaded axons, with large spherical varicosities, are spared. In the present immunocytochemical study, the morphologic pattern of neurotoxicity was examined following administration of d-methamphetamine (d-MA) at doses previously demonstrated to produce neurochemical deficits. Two weeks after various dose regimens of d-MA (including 50 mg/kg b.i.d. x 4 days or 100 mg/kg x 1), selective loss of 5-HT axons was observed throughout the forebrain. Specifically, regions which in control rats are densely innervated by fine axons (e.g., parietal cortex, CA1 of hippocampus, striatum) were relatively devoid of 5-HT-immunoreactive fibers in d-MA-treated rats. Conversely, clusters of beaded fibers (e.g., along the borders of the granule cell layer in the dentate gyrus, layer 3 of lateral entorhinal cortex, and in the glomerular layer of olfactory bulb) exhibited normal 5-HT immunoreactivity. The *acute* effects of 100 mg/kg d-MA were also assessed. Four hours after d-MA, fine fibers were undetected and beaded fibers were intensely stained, suggesting that, like the other substituted amphetamines, d-MA acutely depletes 5-HT from fine axons. Lastly, d-MA was shown to be more neurotoxic than racemic MA. Administration of up to 100 mg/kg (x 4 days) of dl-MA resulted in no long-term change in 5-HT-immunoreactivity. In conclusion, although much larger doses of d-MA are required, this drug produces a pattern of 5-HT axon ablation similar to that reported after PCA, MDA, MDMA and FEN. [Support: DA04431, NS15199, and Pharmaceutical Manufacturers Association Foundation.]

168.10

FINE STRUCTURAL EFFECTS OF AMPHETAMINE AND ITS ANALOGS ON CULTURED NEUROBLASTOMA-GLIOMA CELLS (NG108-15). J. E. Johnson Jr., E. B. De Souza and A. D. Weissman. Neurobiology Laboratory, Neuroscience Branch, NIDA Addiction Research Center, Baltimore, MD 21224.

Amphetamine and several of its analogs, most notably 3,4-methylenedioxyamphetamine (MDA), and 3,4-methylenedioxymethamphetamine (MDMA), have been shown to produce a neurotoxic effect on brain serotonin neurons *in vivo*. To date, very few studies are available describing the effects of these drugs at the fine structural level. Therefore, in the present study, the serotonin-containing neuroblastoma cell line NG108-15 was exposed to 10^{-9} to 10^{-3} M amphetamine, methamphetamine, MDA, MDMA, MDE, and fenfluramine for 3-7 days in culture. Cells were processed according to Johnson and Weissman, 1988 (Brain Research Bulletin, 20, 39-47). By light microscopy, all of the above compounds produced decreases in the number of viable cells at 10^{-3} M, but had little effect on viability or cell morphology at lower doses. However, electron microscopy revealed cell degeneration and ultrastructural changes at pharmacologically relevant drug levels (10^{-6} M). Thus, the present study suggests a neurotoxic effect of the amphetamine class of drugs which goes beyond simple loss of cell monoamine content.

168.11

EFFECTS OF HIGH-DOSE FENFLURAMINE TREATMENT ON STRUCTURAL INTEGRITY OF RAT BRAIN 5HT NEURONS: ASSESSMENT USING QUANTITATIVE AUTORADIOGRAPHY OF ^{3}H-PAROXETINE-LABELED 5HT UPTAKE SITES. N.M. Appel, Wm.M. Mitchell*, J.F. Contrera and E.B. De Souza. NIDA/ARC, Baltimore, MD 21224 and FDA, Rockville, MD 20857.

High-dose fenfluramine treatment in rats causes region-specific decreases in density of serotonin (5HT)-like immunostained axons in brain, appearance of 5HT-like immunostained fibers with morphology characteristic of degenerating axons and long-lasting decreases in brain 5HT, 5-HIAA and 5HT uptake sites. In this study, we used quantitative autoradiography of ^{3}H-paroxetine-labeled 5HT uptake sites, an index of structural integrity of 5HT neurons, to asses regional effects of high-dose fenfluramine treatment on rat brain 5HT neurons. Subacute d,l-fenfluramine HCl treatment (24 mg/kg, s.c., b.i.d., 4 days) in rats significantly decreased the densities of 5HT uptake sites throughout brain at 18 h after cessation of drug treatment. The regional pattern of reduced ^{3}H-paroxetine binding in brain paralleled decreased 5HT-like immunostaining noted following an identical fenfluramine treatment regimen. Specifically, cerebral cortex, striatum, hippocampus, thalamus, and medial hypothalamus were most affected by this treatment; lateral hypothalamus, septum and amygdala were affected to a lesser extent. Preliminary studies suggest that catecholamine uptake sites, assessed by ^{3}H-mazindol autoradiography, were unaffected by this drug treatment. These data suggest that subacute, high-dose fenfluramine treatment causes selective degeneration of 5HT axons in rat brain. Since pharmacokinetic studies show that this dosing regimen exposes rat brain to drug concentrations greater than 600 times those resulting from the therapeutic oral dose, caution must be exercised in extrapolating these data to humans.

168.12

d-FENFLURAMINE PRODUCES LONG-TERM EFFECTS ON CENTRAL SEROTONIN NEURONS IN NONHUMAN PRIMATES. G.A. Ricaurte, M.E. Molliver, J.M. Witkin*, D.C. Molliver, M.A. Wilson, J.L. Katz*. Depts. of Neurol. and Neurosci., Johns Hopkins Univ. Sch. of Med. and NIDA Addiction Research Center, Baltimore, MD 21224.

d-Fenfluramine is prescribed clinically for the treatment of obesity. Recent studies with 3,4-methylenedioxymethamphetamine (MDMA), a congener of *d*-fenfluramine, have renewed concern that fenfluramine may be neurotoxic in humans. As little information is presently available on the neurotoxic potential of *d*-fenfluramine in primates, the present studies assessed the long-term effects of *d*-fenfluramine on serotonin (5HT)-containing neurons in the monkey CNS.

In the first experiment, *d*-fenfluramine was given to 4 monkeys (Saimiri sciureus) subcutaneously at a dose of 5 mg/kg twice daily for 4 days, with 4 control monkeys receiving only saline. Two weeks later, the animals were sacrificed and brains were analyzed neurochemically and histologically. *d*-Fenfluramine produced an 80-90% depletion of 5HT and 5HIAA in all brain regions examined. The depletion was associated with a profound reduction in the density of 5HT-immunoreactive axons in the cortex, and with the presence of markedly swollen axons in more proximal subcortical areas. In a second experiment, monkeys (N=3) received a similar regimen of *d*-fenfluramine at a dose of 1.25 mg/kg, a dose that more closely approximates that used clinically. Regional brain 5HT concentrations in these animals were decreased by 25-35%.

These results show that *d*-fenfluramine produces lasting effects on 5HT neurons in the primate CNS at doses approaching those prescribed to humans. Potential neurotoxicity of *d*-fenfluramine in humans needs to be investigated. [Supported by USPHS Grants DA05707 and DA04431].

168.13

SEROTONIN INFLUENCES BARREL FORMATION IN DEVELOPING SOMATOSENSORY CORTEX OF THE RAT. M.E.Blue and M.E. Molliver, Department of Neuroscience, The Johns Hopkins University School of Medicine and The Kennedy Institute, Baltimore, MD 21205

Using serotonin (5-HT) immunocytochemistry, we have previously shown that in early development, 5-HT axons densely innervate sensory areas in cerebral cortex of the rat (D'Amato et al.). Dense patches of 5-HT axons are especially prominent in a part of the somatosensory cortex (SI) containing the cortical representation of the whisker pads, the posteromedial barrel subfield (PMBSF). The present study examines the developmental role of serotonin in barrel formation. Tangential sections through cortex were examined at postnatal days P0, P1, P2, P4, P6 and P30. Dense patches of 5-HT staining are evident in cerebral cortex as early as P0, and by P4 5-HT axons are organized into discrete patches in the PMBSF that form a pattern closely resembling barrels. However, the characteristic cellular configuration of the barrel fields is not evident in adjacent Nissl stained sections until P6. These findings show that 5-HT axons form a barrel-like pattern in SI before there is cytologic evidence of barrel formation. To examine whether serotonin may regulate barrel formation, 5-HT axons were lesioned by administering the selective 5-HT neurotoxin, p-chloroamphetamine (PCA) to neonatal rats. Within the first few days after PCA treatment the 5-HT staining in the barrels diminishes and by one week post-treatment the 5-HT patches are absent. At P6 (3 days after PCA), barrels are not evident in Nissl stained sections of PCA treated rats but are present in controls. By P30, 4 weeks after PCA treatment, 5-HT axons are uniformly distributed in the PMBSF, 5-HT axon density approaches that of controls and the cellular configuration of barrels is evident in adjacent Nissl stained sections. These results indicate that the process of barrel formation is delayed in cortex following neonatal depletion of serotonin. Thus, the 5-HT projection to neocortex may play a trophic role in the development of cortical structures. (Support: HD19920)

168.14

SEROTONIN INNERVATION IN VISUAL AREAS OF CEREBRAL CORTEX IN MACAQUE MONKEYS: LAMINAR DISTRIBUTION OF FINE AND BEADED AXONS. M.A. Wilson and M.E. Molliver. *Dept. of Neuroscience, The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205*

Serotonin (5HT) neurons of the midbrain raphe nuclei project widely upon the cerebral cortex in primates, however, there is considerable heterogeneity and specificity in this projection. We have utilized immunohistochemical methods to examine the density, laminar distribution and morphology of 5HT axons in selected visual areas of cerebral cortex. Two species of macaque monkeys were studied, rhesus (*macaca mulatta*) and cynomolgus (*macaca fascicularis*); these species exhibit similar patterns of 5HT innervation.

The density and laminar distribution of 5HT axons vary among different cytoarchitectonic and functional areas of macaque cerebral cortex. In most areas of parietal and temporal neocortex, there is a moderate density of beaded fibers in layer I, and a moderately high density of fine fibers in layers II-VI; few beaded fibers are found below layer II. Primary visual cortex (V1) exhibits a laminar distribution of 5HT axons that is strikingly different. In V1 there is a low density of 5HT axons in layers I-II and a prominent, high density band of both fine and beaded 5HT axons in layer IV. Certain parietal and temporal visual association areas, such as Area 7a and posterior inferior temporal cortex, also have a marked increase in 5HT axon density in layer IV. Thus, in contrast with other areas, V1 and visual association areas are characterized by a prominent density of 5HT axons in layer IV. However, unlike V1, visual association areas exhibit a moderate density of beaded 5HT axons in layer I. These laminar patterns of 5HT innervation may reflect differences in the postsynaptic targets of 5HT axons in functionally distinct areas of primate cerebral cortex. Preliminary retrograde transport experiments indicate that there may also be differences in the location of the raphe neurons which provide 5HT input to visual vs. somatomotor areas. (Support: NIH NS21011, DA04431.)

168.15

EFFECTS OF NEONATAL 5,7-DIHYDROXYTRYPTAMINE LESIONS ON SPINAL 5-HT$_1$ RECEPTORS. V. Hlibczuk* and M. R. Pranzatelli (SPON: S. DiMauro). Departments of Neurology and Pediatrics, Columbia University, NY 10032.

To compare effects and routes of injection on recovery in the spinal cord and to delineate the involvement of 5-HT$_{1A}$ receptors in supersensitivity following 5,7-DHT, we injected rat pups intracisternally or intraperitoneally on postnatal day 2 and 5 with 5,7-DHT (100 mg ip, 100 µg ic) or vehicle. Fourteen weeks later, 5-HT$_1$ receptors were measured in saturation radioligand binding assays using [^{3}H]DPAT, [^{3}H]5-HT (with DPAT and mianserin), and [^{3}H]mesulergine to identify 5-HT$_{1A}$, 5-HT$_{1B}$, and 5-HT$_{1C}$ sites and unlabelled 5-HT (10 µM) to define nonspecific binding. Rats with 5,7-DHT lesions exhibited a small reduction (-13%) in B_{max} of spinal 5-HT$_{1A}$ sites and a significant increase (+33%) in 5-HT$_{1C}$ sites ($P=0.02$), with no changes in 5-HT$_{1B}$ sites or in K_ds compared to controls. Route of 5,7-DHT injection did not significantly alter spinal 5-HT$_1$ sites or spinal 5-HT content, which was reduced 81-89%. These data describe novel changes in 5-HT$_{1C}$ receptors in rats with neonatal 5,7-DHT lesions. (Supported by NIH grant NS01158 (CIDA), the Myoclonus Research Fund, the United Cerebral Palsy Research and Education Foundation (R381-88), and the William Randolph Hearst Foundation.)

168.16

DEGENERATION IN THE SUPRAEPENDYMAL NEURONAL COMPLEX OF THE THIRD VENTRICLE IN THE HAMSTER FOLLOWING ELECTROLYTIC LESIONING OF THE RAPHE NUCLEI. R. D. Fessler* and J. A. Mitchell. Departments of Anatomy & Cell Biology and Neurosurgery, Wayne State University School of Medicine, Detroit, MI 48201.

Electrolytic lesions of the raphe nuclei (RN) were performed in the golden hamster (Mesocricetus auratus) to ascertain whether the supraependymal neuronal complex (SENC) in the third ventricle receives input from the RN. Golden hamsters (5 males, 10 females, 70-105g) were lesioned to ablate the raphe nuclei. Controls were prepared by stereotaxic placement of the lesioning electrode without passing current. One to five days following RN lesions, animals were anesthetized and perfused via intracardiac puncture with Karnovsky's aldehyde fixative. The third ventricles were prepared for examination by scanning electron microscopy (SEM). The brainstems were sectioned and the extent of RN ablation determined. SEM 24 to 36 hours following RN lesions revealed a marked decline in the number of SENC processes spreading over the tanycytic ependyma of the median eminence. Degeneration of small caliber SENC processes occurred over 24-48 hours. Specimens were nearly devoid of processes and fascicles three to five days after RN ablation. Controls did not exhibit loss of SENC fascicles or individual processes. The results suggest that many of the intraventricular processes associated with the SENC originate in the raphe nuclei.

168.17

SEROTONERGIC INNERVATION OF RAT LOCUS COERULEUS DERIVES FROM NON-RAPHE BRAIN AREAS. V. A. Pieribone, E. J. Van Bockstaele, M.T. Shipley[1] and G. Aston-Jones. Div Behav Neurobiol, Dept Mental Hlth Sci, Hahnemann Univ, Phila., PA 19102; [1]Dept Cell Biol Anat, U Cincinnati, OH 45267

Using standard immunocytochemical methods we examined the morphology and degree of serotonin immunoreactive (5-HT-IR) fibers and terminals in the rat locus coeruleus (LC) and surrounding areas. As previously reported, 5-HT innervation of LC is dense; however, there is no 5-HT-IR fiber pathway from which the innervation appears to derive. Using tract tracing, double labeling and lesions, we have sought to determine the afferent source(s) of 5-HT innervation of LC.

Retrograde transport studies from LC by this laboratory (Science 234:734; Neurosci. Lett. 85:297) revealed no LC afferents in midline raphe groups. Major inputs to LC were found only in the dorsomedial (prepositus hypoglossi; PrH) and ventrolateral medulla (paragigantocellularis; PGi). Interestingly, a lateral division of the B3 5-HT cell group resides in rostromedial PGi. Using iontophoretic injections of the fluorescent retrograde tracer Fluoro-Gold in LC combined with 5-HT immunofluorescence, we have identified 5-HT-IR LC afferents in PGi (B3 cells) and as well as a few in PrH.

Previous studies by others had indicated that dorsal raphe may be a major source of 5-HT in LC. However, in each of three animals 5-HT innervation in LC remained dense despite extensive lesions of dorsal raphe. In addition, injections of the tract tracers WGA-HRP or PHA-L into dorsal raphe failed to produce significant anterograde labeling in LC even though adjacent structures contained dense fiber labeling. These results support those of our previous retrograde experiments indicating that dorsal raphe does not substantially innervate LC.

As described by others, we identified a small collection of 5-HT-IR neurons just near the ventromedial rostral border of LC. Combined tyrosine hydroxylase and 5-HT immunofluorescence revealed 5-HT-IR processes from these neurons that ramify within LC. Experiments using discrete cytotoxic lesions of this area are in progress to determine if these neurons provide substantial 5-HT innervation of LC. Support: PHS grant NS24698 & ONR/AFOSR Contract N00014-86-K-0493.

168.18

INPUT TO SEROTONERGIC NEURONS IN THE DORSAL RAPHE OF THE RAT; COMBINED LABELING OF PRE- AND POSTSYNAPTIC ELEMENTS. M.R. Park and A. Abi-Dargham*, Dept. Anatomy & Neurobiology, Univ. Tennessee, Memphis, The Health Science Center, Memphis, TN 38163.

A major projection to the dorsal raphe nucleus is from an axis of structures lying along the medial forebrain bundle, including lateral hypothalamic (LHA) and ventral tegmental areas (VTA). Confirming physiological findings of excitatory responses to LHA stimulation in putative serotonergic neurons, we present light microscopic evidence that the targets of the LHA and VTA afferents to the dorsal raphe nucleus are neurons that are immunoreactive to serotonin. This is been demonstrated through the use of double labeling protocols that combine anterograde axonal labeling using phaseolus vulgaris leucoagglutinin (PHA-L) and immunohistochemical labeling for serotonin. Varicose PHA-L labeled axons are seen to course along proximal dendrites of serotonin labeled neurons in the dorsal raphe nucleus. The observation of close appositions between possible pre- and postsynaptic elements constitute data that go one step beyond the classical criterion for establishing a pathway by identifying terminal axonal arborizations at the light microscopic level.

Double chromogens to differentiate PHA-L and serotonin labeling were developed in two ways. In one series, diaminobenzidine (DAB) and nickel intensified DAB (brown and blue-black reaction products, respectfully) were used in a modified avidin-biotin complex (ABC) procedure in which the primary and secondary antibody reactions were done sequentially. The second set of procedures used combinations of fluorescent labeled avidin or secondary antibodies and primary antibodies raised in separate species.

Light microscopic observations such as these are useful for observing the pattern of possible connections but do require verification at the ultrastructural level.

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168.19

QUANTIFIED DISTRIBUTION OF THE SEROTONIN INNERVATION IN ADULT RAT HIPPOCAMPUS. S. Oleskevich and L. Descarries, Centre de recherche en sciences neurologiques (Département de physiologie), Université de Montréal, Montréal, Québec, Canada H3C 3J7.

Serotonin (5-HT) axon terminals (varicosities) were uptake-labeled for light microscope radioautography in horizontal slices of adult rat hippocampus incubated with 1 μM [^{3}H]5-HT. The labeled varicosities, detected as small aggregates of silver grains, were counted with the aid of an image analysis system, in representative sectors of subiculum (SUB), Ammon's horn (CA1, CA3a, CA3b) and dentate gyrus (DG-medial blade, crest and lateral blade) (N rats = 6). After double correction for duration of exposure and section thickness, and measurement of the mean diameter of hippocampal 5-HT varicosities in electron microscope radioautographs, the results were expressed in number of terminals per mm^3 of tissue. The overall density of hippocampal 5-HT innervation was thus evaluated at 2.7 x 10^6 varicosities per mm^3, but appeared significantly higher in SUB (3.6 x 10^6) and Ammon's horn (3.1 x 10^6) than DG (2.2 x 10^6). SUB and DG-crest (2.0 x 10^6) had the highest and lowest regional densities. There were also significant differences in terms of laminar distribution in each region. The stratum moleculare of SUB and CA1, and the stratum oriens of CA3 (5.2 x 10^6 varicosities in CA3-a), had high values in sharp contrast with the low density in the pyramidal cell layer (0.7 x 10^6). Similarly, the granular layer of DG had a much lower density (1.1 x 10^6) than adjacent layers. These quantitative data further document the heterogeneous regional and laminar distribution of the hippocampal 5-HT innervation. They also demonstrate a greater average density of the serotonin than the noradrenaline input to rat hippocampus (Oleskevich et al., *J. Neurosci.*, 1989, in press) and important differences in the regional and laminar distribution of these two monoamine innervations. (Supported by MRC and FRSQ).

168.20

SEROTONIN-LIKE IMMUNOREACTIVITY IN THE EARLY EMBRYO OF THE TOBACCO HORNWORM, MANDUCA SEXTA. W.A. Radwan*, N.A. Granger and J.M. Lauder. Dept. of Cell Biology and Anatomy, Univ. of North Carolina, Chapel Hill, NC 27599

The presence of serotonin (5-HT) in the early Manduca embryo was studied immunocytochemically in paraffin sections with a specific 5-HT antiserum. At stage 2 (10% development), when the germ band has formed and separated from the serosa, 5-HT-like immunoreactivity (IR) was detected as fine granules in both the serosa and germ band. Intense 5-HT IR was seen adjacent to these same structures and was also evident as caps on scattered clusters of yolk granules. By stage 4 (20% development), when gastrulation has been completed, 5-HT IR was located in the cells of the dorsal organ (an invagination of serosal cells that may be involved in molting of the first serosal cuticle). Intense 5-HT IR was observed in the first and second serosal cuticles, especially over the dorsal organ. Light staining was seen in the cells of the ectoderm and presumptive endoderm. By stage 7 (35% development), when dorsal fusion of the amniotic folds occurs, several embryonic structures exhibited 5-HT IR, including the ectoderm of the dorsal head region and of the posterior abdominal segments, the developing mouthparts, and the lining of the hindgut. The existence of 5-HT IR in the embryo during major developmental events prior to neurogenesis suggests that 5-HT might act as a morphogenetic stimulus.

SEROTONIN RECEPTORS II

169.1

THREE-DIMENSIONAL COMPUTER MODELING OF THE 5-HT$_3$ RECEPTOR PHARMACOPHORE. A.W. SCHMIDT and S.J. PEROUTKA. Department of Neurology, Stanford University Medical Center, Stanford, CA 94305.

Three-dimensional computer-based steric molecular modeling was used to define a "pharmacophore" of the 5-HT$_3$ receptor binding site. Examination of published radioligand binding data revealed 19 different chemical structures with K_i values of less than 10 nM for this site. Of the 19 potent 5-HT$_3$ agents, 10 compounds are derivatives of a common "core" structure (e.g., ICS 205-930, LY 278458, granisetron, DAU 6215). By overlapping the common features of these 19 agents, the following rules were developed:

1. An aromatic ring must be present with a nitrogen atom positioned approximately 6.5 angstroms from the center of the aromatic ring.
2. The nitrogen is embedded in a ring structure whose plane can be placed perpendicular to that of the aromatic ring.
3. No substitutions are present on the side chain to hinder the folding of the non-aromatic ring portion of the molecule back over the aromatic ring.
4. The atom adjacent to the aromatic ring in the molecule along the most direct path to the nitrogen is always trigonal.

This model accurately predicts nanomolar affinity for all known potent 5-HT$_3$ agents and inactivity for drugs such as ergots and 5-HT$_2$ antagonists. This modeling technique also allows for computer-based screening to identify previously unrecognized pharmacological agents that display affinity for the 5-HT$_3$ receptor binding site. Finally, the 5-HT$_3$ "pharmacophore" will be compared to the pharmacophore proposed for the 5-HT$_{1A}$ receptor (Sleight and Peroutka, 1989; this volume).

169.2

[^{3}H]5-METHYL-URAPIDIL BINDING TO SEROTONIN-1A RECEPTORS IN RAT BRAIN. Pingyu Zhong*, Yiwang Chen*, and K.J. Kellar. (SPON: R. L. Verrier). Department of Pharmacology, Georgetown University School of Medicine, Washington, D.C. 20007.

5-Methyl-urapidil (5-MeU) is a derivative of urapidil, a centrally acting antihypertensive drug that lowers blood pressure by an agonist action at 5-HT-1A receptors on the ventral surface of the medulla (Gillis et al., Drugs 35:20, 1988). 5-MeU is 150 times more potent than urapidil in competing for 5-HT-1A binding sites labeled by [^{3}H]8-OH-DPAT. Furthermore, 5-MeU appears to be a full agonist at 5-HT-1A receptors because it and 8-OH-DPAT lower blood pressure in cats to the same extent (Mandal et al., FASEB J. 3:A1013, 1989) and raise prolactin levels equally in rats (Giblin et al., these abstracts). We have measured the binding of [^{3}H]5-MeU to rat brain membranes. [^{3}H]5-MeU (54 Ci/mmol; Byk Gulden, FRG) binds with a K_d of 1 nM. The IC_{50} values of 5-HT (1nM), 8-OH-DPAT (4nM), spiperone (50nM), WB4101 (4nM), and isobim (1nM) in competition studies indicate that [^{3}H]5-MeU binding sites in the cortex represent predominantly 5-HT-1A receptors, but that approximately 10% of the binding sites are alpha-1-adrenoceptors. The number of sites bound appears to be slightly less than that labeled by [^{3}H]8-OH-DPAT; and 5MeU competes for [^{3}H]8-OH-DPAT binding sites with a Hill slope less than 1. Thus, [^{3}H]8-OH-DPAT may label a site in addition to 5-HT-1A receptors also. These data indicate that 5-MeU is a high affinity agonist at 5-HT-1A receptors, and should prove to be useful in studies of these receptors.

169.3

[^{3}H]KETANSERIN BINDS TO A NON-5HT$_2$ SITE IN RABBIT CEREBRAL CORTEX AND NEOSTRIATUM. T.A. Reader, L. Lima* and K.M. Dewar CRSN, Département de physiologie, Université de Montréal, Montréal, (Qué.) Canada and IVIC, Caracas, Venezuela.

A characterization of [^{3}H]ketanserin ([^{3}H]KTS) binding in the rabbit frontal cortex (fCTX) and neostriatum (CPU) was carried out. The association and dissociation kinetics in fCTX could be fitted to two-site models, suggesting that [^{3}H]KTS labels two cortical sites. The saturation curves revealed a single high-affinity site when mianserin was used to define non-specific binding. The curves could be fitted to a two-site model when unlabelled KTS was used for nonspecific counts and the parameters of the high-affinity site were similar to that obtained for one-site in the presence of mianserin. 5-HT$_2$ antagonists inhibited [^{3}H]KTS binding in fCTX at nM concentrations; however, the curves were best fitted to two-site models. In contrast, [^{3}H]KTS binding to CPU membranes could only be inhibited at high (micromolar) concentrations. Micromolar concentrations of monoamine uptake blockers inhibited [^{3}H]KTS binding in both fCTX and CPU. This study demonstrates that [^{3}H]KTS labels a non-serotoninergic recognition site in the rabbit fCTX and CPU that is similar to that found in the neostriatum of the rat, i.e.: probably a monoamine transport site.

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169.4

SOLUBILIZATION AND CHARACTERIZATION OF MELATONIN RECEPTOR SITES FROM CHICKEN BRAIN. K.C.Chung* and M.L. Dubocovich. Dept. Pharmacol., Northwestern Univ. Med. Sch., Chicago, IL 60611.

The pharmacological characteristics of 2-[^{125}I]-iodomelatonin binding sites was determined in solubilized fractions from whole chicken brain. P_2 membrane fractions were homogenized in 50 mM Tris.HCL buffer (20% glycerol, 1 mM EDTA, 1 mM PMSF, 1mM mercaptoethanol, pH 7.4) and solu-bilized with digitonin (0.5%). Aliquots of the soluble fraction were incubated at 25°C with 2-[^{125}I]-iodomelatonin, and drugs or vehicle. Binding of 2-[^{125}I]-iodomelatonin was time dependent, saturable and increased linearly with protein concentration. Specific binding defined with 10 μM 6-chloromelatonin represented 95% of total binding. Scatchard analysis of 2-[^{125}I]-iodomelatonin (0.025-2.5 nM) reveal binding to two sites with affinities of K_1=0.073 $\pm$ 0.036 nM and K_2=2.17 $\pm$ 1.06 nM (n=3), and $Bmax_1$=4.54 $\pm$ 0.74 fmol/mg protein and $Bmax_2$=40.1 $\pm$ 12.2 fmol/mg protein (n=3), respectively. The relative order of affinities of melatonin analogues for 2-[^{125}I]-iodomelatonin binding sites was: 2-iodomelatonin $\geq$ 6-chloromelatonin > melatonin > N-acetyl-5-hydroxy-tryptamine > N-acetyltryptamine >> 5-methoxy-tryptamine $\geq$ 5-hydroxytryptamine $\geq$ methysergide. We conclude that the solubilized melatonin receptor site retained the binding properties and pharmacological characteristics of the functional ML-1 melatonin receptor (Dubocovich, M.L., FASEB J. 2: 2765, 1988). Supported by grant MH-42922.

169.5

REGION-DEPENDENT HETEROGENEITY OF THE 5-HT$_{1A}$ RECEPTOR SYSTEM IN RAT AND COW BRAIN. Frank Yocca[1], Lawrence Iben[1]*, and Saul Maayani[2], [1]CNS Biology, Bristol-Myers Co., Wallingford, CT 06492-7660 and [2]Dept. of Anesthesiology, Mount Sinai Sch. Med., New York, NY 10029.

Apparent neuronal receptor heterogenity across regions or species may reflect differences in the coupling between the same receptor subtype and different membrane components such as G proteins, resulting in receptor system heterogeneity. To test this hypothesis, the 5-HT$_{1A}$ receptor and its related binding sites were assayed in membrane preparation from the hippocampus (H), cortex (C) and the dorsal raphe (DR) of rat and cow. Functional (inhibition of forskolin stimulated adenylyl cyclase; FSAC) and occupancy (agonist binding) studies were done in parallel. Modulation of occupancy was assessed by 10μM GPPNHP. High level of agonist occupancy (^{3}H-8-OH-DPAT binding) was observed in the DR, H and C of both species, and a marked regional dependence of the IC_{50} values of the GPPNHP effect was found across the brain regions tested (μM) cow: DR (0.8) < H (6.6); rat: H (3.1) < C (5.3). No FSAC could be detected in the DR of both species while the pharmacology of the binding sites was indistinguishable across all regions (See Iben & Yocca). These characteristics reflect differences in regional 5-HT$_{1A}$ receptor systems (i.e. coupling to G protein) and may explain reported differences in relative 5-HT$_{1A}$ agonist efficacy in rat DR and H as measured by electrophysiological methods (Sprouse and Aghajanian, 1987). (Supported in part by USPH GM 34852).

169.6

COMPARISON OF ^{3}H-MESULERGERINE (^{3}H-MES) BINDING TO 5HT$_{1c}$ RECEPTORS IN RAT CORTEX (RC) AND BOVINE CHOROID PLEXUS (BCP). F.P.Bymaster*, L.R.Reid*, R.D.Marsh* and D.T.Wong. Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285

A unique serotonin (5HT) binding site designated 5HT$_{1c}$ was found in mammals. This study was to determine if ^{3}H-MES, a 5HT$_{1c}$ antagonist, bound to 5HT$_{1c}$ sites in RC and BCP. The incubation mixture for RC contained 30 nM spiperone to mask 5HT$_2$ sites. The inhibition of ^{3}H-MES binding by several drugs is shown in the table. Mianserin, a 5HT$_{1c}$ antagonist, was equipotent as an inhibitor of ^{3}H-MES binding in the brain regions, and the aryl piperazines TFMPP and mCPP exhibit high affinity for the 5HT$_{1c}$ recognition sites in the 2 tissues. Other analogs (LY165163, MK212 and quipazine) were of intermediate activity. Compounds with affinity for other 5HT binding sites were weak inhibitors of ^{3}H-MES binding. We conclude that ^{3}H-MES binding to BCP and RC appears to be the same recognition site and exhibits the characteristics of 5HT$_{1c}$ binding.

Compound	IC_{50}, nM RC	BCP	Compound	IC_{50}, nM RC	BCP
5HT	49	7	8-OH-DPAT	>1000	16000
Ketanserin	89	195	Spiperone	950	1790
Mianserin	6	6	LY165163	866	850
TFMPP	27	63	mCPP	75	78
LY278584	>10000	>10000	RU-24969	64	170
Quipazine	1033	475	MK212	798	363

169.7

DOSE RELATED EFFECTS OF THE 5HT$_{1A}$ AGONIST 8-OH-DPAT ON GLUCOSE UTILIZATION IN RAT BRAIN STUDIED WITH 2-DG AUTORADIOGRAPHY. P.M. Grasby*, T Sharp*, P. Kelly* and D.G. Grahame-Smith* (SPON : European Neuroscience Association). M.R.C. Unit and University Department of Clinical Pharmacology, Radcliffe Infirmary, Oxford OX2 6HE . UK..

Selective 5HT$_{1A}$ agonists produce diverse behavioral, neuroendocrine and electrophysiological effects in rats due to stimulation of central pre- or postsynaptic 5HT$_{1A}$ receptors. There is evidence that low doses of 5HT$_{1A}$ agonists, such as 8-OH-DPAT, preferentially stimulate presynaptic 5HT$_{1A}$ somatodendritic autoreceptors, leading to reduced 5HT neurotransmission while higher doses also stimulate postsynaptic 5HT$_{1A}$ receptors leading to an overall increase in 5HT neurotransmission. Here we studied the effect of a low dose (0.05mg kg^{-1} s.c.) and two higher doses (0.25 + 1.0 mg kg^{-1} s.c.) of 8-OH-DPAT in discrete areas of the rat brain, using the 2-DG autoradiographic technique to measure cerebral glucose utilization as an index of functional brain activity. Groups of five male adult rats (250-350 g) received 8-OH-DPAT or saline 5 min before the injection of 40 μCi i.v. of ^{14}C 2-DG. 8-OH-DPAT induced dose dependant reductions (5 - 36%) in glucose utilization in selected brain regions, notably in limbic structures. The highest dose of 8-OH-DPAT (1.0 mg kg^{-1}) increased glucose utilization by 30% in a columnar pattern in the sensorimotor cortex. Our data suggest that the expected decrease or increase in 5HT neurotransmission (caused by a low or higher doses of 8-OH-DPAT, respectively) is not associated with diametrically opposite effects on regional glucose utilization.

169.8

QUANTITATIVE AUTORADIOGRAPHY OF 5-HT-1A SEROTONIN RECEPTORS IN IMINODIPROPIONITRILE (IDPN)-INDUCED DYSKINETIC RATS. S. Przedborski*, M. Wright*, S. Fahn and J.L. Cadet. Columbia University, Department of Neurology, New York, NY 10032.

Injection of IDPN to rats causes persistent motor symptoms such as lateral and vertical twitches and random circling. Increases of 5-HT level in the caudate and in the nucleus accumbens (Cadet J.L. et al., 1988) and changes in 5-HT-2 receptor density in discrete brain regions (Cadet J.L. et al., 1987) have been found in IDPN-treated rats. Activation of 5-HT-1A receptors causes similar behaviors in rats. In the present study, we use in vitro 8-OH[3H]DPAT binding autoradiography to evaluate the effect of IDPN on 5-HT-1A receptor in rat brain.

IDPN-treated rats displayed increases of 8-OH[3H]DPAT binding in the frontal cortex and in the caudate-putamen. In contrast, there were significant decreases in the interpeduncular nucleus, in the pyramidal layer of the CA3 field of hippocampus, in the superior colliculus, and in the pars reticulata of the substantia nigra.

These results provide further evidence for an involvement of the 5-HT system in the development of the IDPN-induced dyskinetic syndrome.

169.9

AUTORADIOGRAPHIC LOCALIZATION OF [^{3}H]BMY 7378, A SEROTONIN$_{1A}$ PARTIAL AGONIST, IS COMPARED TO THE PATTERN OF LABELING OF [^{3}H]8-OH-DPAT IN RAT BRAIN. Sandra L. Moon, Frank Yocca, Raymond C. Lamy*, and Lawrence Iben*. CNS Biology, Bristol-Myers Co., Wallingford, CT 06492.

BMY 7378, a buspirone analog, binds in a uniform manner with high affinity and displays low intrinsic activity at serotonin$_{1A}$ (5HT$_{1A}$) receptors; whereas 8-OH-DPAT, a 5HT$_{1A}$ agonist, apparently binds in a heterogeneous fashion to 5HT$_{1A}$ sites (Yocca et al., Soc. Neurosci. Abstr., 14,#1,551, 1988). In an attempt to investigate whether BMY 7378 and 8-OH-DPAT might have differences in regional localization, their autoradiographic distributions were compared under similar incubation conditions in rat brain.

[^{3}H]BMY 7378 was custom synthesized by New England Nuclear (85Ci/mmol), and an incubation condition was devised that yielded appropriate biochemistry and pharmacology. With the use of this incubation procedure on tissue sections 92% specific binding was achieved.

The binding patterns of the two ligands were virtually identical. Regions of highest binding were the dentate gyrus, hippocampus (CA$_{1\&4}$), lateral septum, entorhinal cortex, interpeduncular nucleus, and dorsal raphe. Labeling of the median raphe and thalamus was sparse. Moderately labeled were the intermediate through deep layers of cortex, nuclei of the hypothalamus and amygdala. Moreover, unlabeled BMY 7378 (0.3nM-0.1μM) added to the [^{3}H]8-OH-DPAT incubation uniformly decreased binding in all brain regions measured.

169.10

AGONIST AND ANTAGONIST PROPERTIES OF BMY 7378, A 5-HT-1A PARTIAL AGONIST, IN 8-OH-DPAT DISCRIMINATION IN PIGEONS. J.A.Stanley*, C.P.VanderMaelen and S.L.Moon (SPON:T.L. Martin). CNS Biology,Bristol-Myers Co.,Wallingford, CT 06492.

BMY 7378 was examined for possible agonist and antagonist activity as indicated by the key chosen during substitution or combination tests in pigeons trained to discriminate the 5-HT-1A agonist 8-OH-DPAT (DPAT) from saline. During training, six pigeons (at 85% weight) were allowed access to food following every 30th peck (FR30) made on the key associated with the pre-session injection. Following discrimination acquisition, test sessions were run during which BMY 7378 was either substituted for or given 15 min prior to the training dose of DPAT. When BMY 7378 (1.0 and 3.0 mg/kg) was substituted for DPAT, 4/6 birds selected the DPAT key suggesting common interoceptive stimulus properties. When these same birds were pretreated with these same doses of BMY 7378 prior to DPAT administration, 3/6 selected the saline key, suggesting an antagonism of the DPAT cue. The three birds who did not demonstrate this apparent antagonism had previously shown nearly complete generalization of the BMY 7378 cue to the DPAT cue, indicating that under either test condition the agonist aspect of BMY 7378 can prevail. These results are consistent with previous data indicating that the partial 5-HT-1A agonist, BMY 7378, can exhibit both agonist and antagonist properties in model systems sensitive to 5-HT-1A agents.

169.11

STRUCTURE-AFFINITY STUDIES: PRELUDE TO THE DESIGN OF 5-HT1D LIGANDS. *R A Glennon* A Ismaiel* M E Pierson* C Chaurasia* Virginia Commonwealth University, Richmond, VA 23298 and K Davis* M Titeler, Albany Medical College, Albany, NY 12208.*

Structure-affinity relationships (SAFIRs) have been developed for the binding of serotonergic and related agents for 5-HT1D sites. Groups of agents examined in search of appropriate structural templates include aminotetralins, phenalkylamines, arylpiperazines, and tryptamines. Most derivatives, with the exception of the tryptamines, display low affinity for 5-HT1D sites. Detailed SAFIR data for 5-HT derivatives reveals that both aromatic rings are necessary for binding, and that O-methylation has little effect on affinity. The following changes decrease affinity: N,N-dimethylation (3- to 5-fold; larger alkyl groups decrease further), α-methylation (50-fold), 2-methylation (300- to 500-fold), removal of 5-OH (10-fold), cyclization of sidechain to β-carboline (> 100-fold). 7-Alkylsubstitution is not well tolerated. 5-Substitution alters affinity.

Using the SAFIR generated with the tryptamines, we were able to increase the affinity of 1-phenylpiperazine (Ki > 1 μM) by more than 5000-fold; e.g. MEP-179, 1-(7-methoxy-1-naphthyl)piperazine, Ki = 2 nM). Continued use of these SAFIR data should allow for the eventual design and synthesis of useful, high-affinity site-selective 5-HT1D ligands. (Supported in part by PHS grant NS23523.)

169.12

^{3}H-NAN-190 AND ^{3}H-BMY-7378: ANTAGONIST RADIOLIGAND PROBES OF THE BRAIN 5HT$_{1A}$ RECEPTOR. L. Fitzgerald* and M. Titeler (1), R.A. Glennon*(2), and F. Yocca (3) (SPON: H. Tedeschi) (1)Dept. Pharmacol.& Toxicol., Albany Med. Coll., Albany, N.Y. 12208; (2) Dept. Med. Chem., Virginia Commonwealth Univ, Richmond, Va. 23298; (3) Div. of Drug Develop., Bristol-Myers, Inc., Wallingford, Ct. 06482

Brain 5HT$_{1A}$ receptors have been extensively studied with agonist radioligands such as ^{3}H-8-OH-DPAT. BMY-7378, a putative partial agonist, and NAN-190, a putative antagonist, were radiolabelled by DU PONT NEN in order to further probe the drug-5HT$_{1A}$ receptor interaction.

The two novel radioligands appear to label a similar site (5HT$_{1A}$ receptor) in homogenates of rat hippocampus and cortex as does ^{3}H-8-OH-DPAT. In rat striatal homogenates only ^{3}H-BMY 7378 and ^{3}H-NAN-190 produced a significant radioligand signal. Drug affinities were different in competition assays using striatal or hippocampal tissues. 5HT demonstrated a K_I values of 10^{-7}M and other 5HT$_{1A}$ drugs demonstrated significantly lower affinities for the striatal binding site compared to the hippocampal and cortical binding site (5HT$_{1A}$ receptors).

The preliminary data indicate that the receptor binding properties of ^{3}H-BMY 7378 and ^{3}H-NAN 190 are significantly different than the receptor binding properties of ^{3}H-8-OH-DPAT. We will present data further exploring the possibility that a novel state of the 5HT$_{1A}$ receptor or a novel 5HT receptor subtype has been labelled with these radioligands. Supported by PHS MH-40716.

169.13

^{125}I-DOI: AN HALLUCINOGEN RADIOLIGAND PROBE FOR HUMAN BRAIN 5HT$_2$ RECEPTORS. S.Leonhardt*, M. Titeler (SPON: D.Poulos). Dept. Pharmacol. & Toxicol., Albany Med. Coll., Albany, N.Y. 12208

The phenylisopropylamine hallucinogens appear to exert their effects through stimulation of brain 5HT$_2$ receptors (1). We decided to monitor the hallucinogen-brain interaction with a radioiodonated phenylisopropylamine hallucinogen, ^{125}I-DOI and human cortical tissue homogenates.

Detailed studies in human frontal cortical homogenates indicate that ^{125}I-DOI labels a guanyl nucleotide sensitive state of human brain 5HT$_2$ receptors. The specific binding (defined by 1uM cinanserin) is saturable, with a B_{max} of 0.5 pmol/g wt weight tissue, and a K_D of 0.5nM. The binding site displays the pharmacological properties of a 5HT$_2$ receptor with high affinities for prototypical 5HT$_2$ antagonists. Potent 5HT$_2$ agonists displayed nM affinities for the site. The binding site appears to be identical to the site labelled with ^{3}H-DOB in rat cortical tissue homogenates (2). These data support the hypothesis that hallucinogens act through stimulation of brain 5HT$_2$ receptors. Supported by PHS MH-40716.

1. M. Titeler, R.A. Lyon, and R.A. Glennon, Psychopharmacol., *94*, 213-216 (1988)
2. R.A. Lyon, K.H. Davis, and M. Titeler, Mol. Pharmacol., *31*, 194-199 (1987)

169.14

CELLULAR LOCALIZATION OF NEOCORTICAL SEROTONIN 1a AND 1b SITES. P.B.Crino, B.A.Vogt, L.Volicer, and R.G.Wiley; Boston Univ. Sch. Med., Boston, MA, 02118; V.A. Hosp., Bedford, MA, 01730, and Nashville, TN, 37203.

Experimental ablations followed by autoradiography with 8-OH-DPAT and ICYP were used to characterize 5-HT1a and 5-HT1b receptor distributions in rat posterior cingulate cortex. Ablations included cortical undercutting, destruction of raphe afferents, cortical ibotenic acid injections, and thalamic injections of the immunotoxin OX7-ss-saporin. Peak 8-OH-DPAT binding was in layer Vb and was not affected by undercut ablations while cortical ibotenic acid injections reduced binding by greater than 50%. Thalamic OX7-ss-saporin injections decreased layer VI binding by 45%. Peak ICYP binding was in layer I. Undercutting decreased grain density by 54% while raphe lesions reduced it by 57%. Ibotenic acid reduced grain density in all layers by 28-47%.

Thus, 5-HT1a sites are located postsynaptically on cortical neurons including cortico-thalamic projection neurons while 5-HT1b sites are located presynaptically on raphe afferents and postsynaptically on cortical neurons. Supported by NIA Grant AG06419 and NINDS 18745.

169.15

CELLULAR LOCALIZATION OF THE SEROTONIN AUTORECEPTOR USING CONCURRENT IMMUNOCYTOCHEMISTRY AND AUTORADIOGRAPHY. H.M. Akbari , P.M. Whitaker-Azmitia, and E.C. Azmitia (SPON: S.M. Feldman) Dept. Biology, New York University, Washington Square East, New York, NY 10003.

The presence of presynaptic autoreceptors on serotonergic neurons has been hypothesized based on electrophysiological and autoradiographic studies. We directly examined the presence or absence of 5-HT_{1a} and 5-HT_{1b} receptors on 5-HT neurons of the midbrain raphe using a combined transmitter immunocytochemistry/receptor autoradiography method. ^{3}H-8-OH-DPAT was used as a selective ligand for the 5-HT_{1a} receptor and ^{125}I-CYP for the 5-HT_{1b} receptor. The effects of fixation on the binding kinetics were assessed on both sections and homogenized tissue. Although the B_{max} was decreased, the K_d was virtually unchanged. Thus, the specificity of the ligands for the receptor was not significantly altered by fixation. Specific binding of 8-OH-DPAT and lack of I-CYP binding on serotonergic neurons indicated that the 5-HT_{1a} receptor and not the 5-HT_{1b} functions as the autoreceptor on the cell bodies of 5-HT neurons. Differential binding densities of 8-OH-DPAT were observed with highest densities in the B8 and B9 cell groups and lowest in B7. We are currently investigating the functional significance of this finding. This work is supported by NSF BNS-8812892 and NINDS NS25391.

169.16

CHARACTERIZATION AND USE OF ANTI-IDIOTYPIC ANTIBODIES FOR THE LOCALIZATION OF SEROTONIN RECEPTORS. H. Tamir, K.P. Liu*, P.Y. Yu*, M.D. Gershon, and A.L. Kirchgessner. Div. Neuroscience, N.Y. State Psychiatric Inst. and Dept. Anatomy and Cell Biol. Columbia Univ. P&S N.Y., N.Y. 10032.

In order to locate receptors and other proteins that physiologically bind serotonin (5-HT), an anti-idiotype serum was prepared from affinity purified polyclonal antibodies to 5-HT. The immune serum was purified by affinity chromatography on anti-5-HT-Sepharose or a β-carboline affinity column was used to remove antibodies to 5-HT. Punctate surface immunostaining was demonstrable on fibroblasts transformed with either a cDNA clone encoding the 5-HT_{1C} receptor or a cDNA clone encoding the 5-HT_2 receptor, but not on control fibroblasts that had not been transformed. The anti-idiotypic antibodies also inhibited the binding of ^{3}H-5-HT to membranes obtained from the rat cortex, striatum, and raphe area, but had little effect on the binding of ^{3}H-5-HT to membranes from the cerebellum or hippocampus. In vibratome sections of rat CNS the anti-idiotypic antibodies immunolabeled cells and/or fibers in: the raphe nuclei, substantia nigra, globus pallidus, entopeduncular nucleus, thalamic nuclei, dentate gyrus, cerebral cortex, subfornical organ, supraependymal plexus, lateral hypothalamus, choroid plexus, and anterior horn cells. Simultaneous demonstration of 5-HT immunoreactivity revealed that a subset of neurons in the dorsal raphe nucleus were doubly labeled. The distribution of immunolabeling and regions in which the anti-idiotypic antibodies inhibit the binding of ^{3}H-5-HT are compatible with the conclusion that the antibodies recognize 5-HT_{1C} and 5-HT_2, but not 5-HT_{1A} receptors. Immunolabeled structures in the periphery included neurons in the bowel, Purkinje fibers in the heart, and a subset of cells in the adenohypophysis. Supported by grants MH 37575, NS07062, and NS12969.

169.17

DETERMINATION OF 8-HYDROXY-2-(DI-n-PROPYL-AMINO)TETRALIN (8-OH-DPAT) IN RAT BRAIN BY LIQUID CHROMATOGRAPHY WITH ELECTROCHEMICAL DETECTION. K.W. Perry* and R.W. Fuller. Lilly Research Laboratories, Eli Lilly and Co., Lilly Corporate Center, Indianapolis, IN 46285.

Tissue was homogenized in 0.1 M TCA, 8-OH-DPAT was adsorbed by a solid phase extraction column then eluted and injected onto a CN HPLC column and detected by electrochemical oxidation. The method is sensitive to ~5 ng/g. At 30 min. after a 1 mg/kg s.c. dose the concentration in whole brain was ~1 μg/g with no differences observed in six different brain regions. The half-life of 8-OH-DPAT in whole brain was 26 min. with similar half-lives in the hypothalamus, midbrain and hippocampus. Concentrations in whole brain were about 10-fold higher after s.c. than after i.p. doses, consistent with earlier data on 5-HIAA lowering showing 8-OH-DPAT to be more potent s.c. than i.p. Pretreatment with proadifen (SKF 525A), an inhibitor of microsomal drug metabolism, slightly increased brain levels of 8-OH-DPAT. Pindolol, which antagonized the elevation of serum corticosterone caused by 8-OH-DPAT, did not change brain levels of 8-OH-DPAT. This analytical method should be useful in correlating brain levels of 8-OH-DPAT with various neurochemical, behavioral or other functional effects described for this compound.

CATECHOLAMINE RECEPTORS: DOPAMINERGIC (D2)

170.1

VANADATE INHIBITS AGONIST BINDING TO THE D_2 DOPAMINE RECEPTOR. Z. Elazar*, L. Levi* and S. Fuchs. Dept. of Chemical Immunology, The Weizmann Institute of Science, Rehovot 76100, Israel.

Vanadate ions are known to act via different mechanisms; e.g. vanadate is a powerful inhibitor of phosphatases, it inhibits Na^{+}-K^{+}-ATPase, it is known to activate adenylate cyclase coupled to Gs proteins and was recently shown to inhibit the GTPase activity of transducin. We have shown that orthovanadate (in the micromolar range) inhibits the high affinity binding of the D_2 dopamine receptor to specific agonists (apomorphine and N-propylnorapomorphine), while it does not affect the binding to D_2 antagonists (spiperone and haloperidol). These effects of vanadate are similar to those observed with guanine nucleotides or their analogs. However, the effect of vanadate on agonist binding was shown to be additive to that of guanine nucleotides, suggesting that vanadate and guanine nucleotides may exert their effect on the D_2 dopamine receptor via different mechanisms. Indeed, in contradistinction with guanine nucleotides, vanadate did not affect GTPγS binding in striatal membranes, nor did it induce the dissociation of the complex between the D_2 dopamine receptor and its related G proteins. The observed effects of vanadate on the D_2 dopamine receptor system may be explained by either direct modification of the receptor molecule, or alternatively by an indirect effect resulting from inhibition of phosphatase activity.

170.2

LIGAND BINDING CHARACTERISTICS OF THE CLONED RAT DOPAMINE D2 RECEPTOR. J.B. Fischer*[1], K.B. Howie*, J.R. Bunzow*[1], A.C. Server and O. Civelli[1]. Cambridge Neurosci. Res. Inc., Cambridge, MA 02139, and [1]Vollum Inst., OHSU, Portland, OR 97201.

The dopamine D2 receptor was recently cloned from rat brain and expressed in the Ltk⁻ mouse fibroblast cell line (L-RGB2 cells; Bunzow, J.R. et al. Nature 336:783, 1988) where it exhibited the appropriate ligand binding profile. This cloned D2 receptor also is fully functional, i.e., able to mediate the lowering of cAMP levels by dopaminergic agonists (Albert, P.R. et al., Abstr. 71st Ann. Mtg. Endocrine Soc., June 1989). Here we report further ligand binding properties of the cloned D2 receptor and compare them with the rat striatal D2 receptor using a more complete set of ligands. Using [^{3}H]domperidone we confirmed the L-RGB2 receptor density (≅1 pmol/mg protein) previously reported using [^{3}H]spiperone. A single binding site (Kd for [^{3}H]domperidone of 0.4 nM) was found in both L-RGB2 and striatal membranes; identical ligand dissociation rates were also found. A large series of neuroleptic drugs showed very similar IC_{50} values in the L-RGB2 and striatal membranes (correlation r = 0.97). None of the drugs tested suggested that the cloned D2 receptor was a subtype with a unique pharmacological profile. We conclude that this cloned D2 receptor represents the rat brain D2 receptor as measured in striatal tissue.

170.3

CLONING OF THE BOVINE D-2 DOPAMINE RECEPTOR. C.L. Chio*, G.G. Martin* and R.M. Huff* (SPON: I. Abraham). Dept. of Cell Biology, The Upjohn Company, Kalamazoo, MI 49001.

Dopamine D-2 receptors are implicated in the etiology of schizophrenia. Structural analysis and expression of a dopamine D-2 receptor clone will be useful in the search for more effective antipsychotic compounds.

A clone for the rat D-2 receptor was obtained using the polymerase chain reaction technique, with rat whole brain mRNA as the substrate. Oligonucleotides used as primers were based on the published sequence for the rat D-2 dopamine receptor (Bunzow *et al.*, Nature 336:783-787, 1988). The 1479 bp clone, containing the entire coding region (100% identical to the published sequence) was used to screen a bovine caudate cDNA library constructed in Lambda ZapII. One clone of 5 x 10^5 clones screened hybridized with the rat probe at high stringency. The insert cDNA is 2350 bp. Partial sequence analysis of this bovine clone reveals 96% amino acid homology with the rat D-2 clone. cRNA made from this clone is being expressed in *Xenopus laevis* oocytes and cos cells for characterization of the synthesized protein, binding sites and physiological responses.

170.4

IDENTIFICATION OF A D2 RECEPTOR SUBTYPE WHICH ELEVATES INTRACELLULAR CALCIUM. K.L. O'Malley, K.R. Stone* and R.D. Todd. Departments of Anatomy & Psychiatry, Washington University School of Medicine, St. Louis, MO 63110.

Using gene transfer techniques we have isolated a mouse fibroblast cell line (DA10-4) which expresses a novel D2 receptor subtype (Todd et al., Soc. Neurosci. Abst. 14, 411). The expressed receptor has lower affinities for (+) butaclamol, ketanserin and SCH23390 than the D2 site described by Bunzow et al. (Nature 336:783-787). Using the calcium sensitive dye Indo-1 and flow cytometry, we have found that receptor stimulation with D2 selective agonists increases intracellular calcium levels. The changes in calcium levels require extracellular calcium and can be blocked by cobalt or lanthanum. Receptor occupancy is required to maintain elevation of calcium levels and can be reversed by D2 antagonists. No desensitization was observed over 15 minutes.

The DA10-4 D2 sites are the product of a novel D2 gene. Using oligonucleotide primers from the Bunzow et al. D2 sequence (RGB-2) in combination with polymerase chain reaction (PCR) amplification of DNA, we have found that RGB-2 sequences have not been transfected into DA10-4 cells. PCR analysis of reverse transcribed mRNA from DA10-4 cells and rat striatum demonstrates that the homologous mouse RGB-2 gene has not been activated via transfection in DA10-4 cells. Therefore we have identified a novel D2 receptor subtype.

170.5

Behavioral and Neurochemical Effects of Chronic Administration of SKF-38393 in Reserpinized Rats. **J. L. Neisewander, I. Lucki, and P. McGonigle. Departments of Pharmacology and Psychiatry, Univ. of Pennsylvania School of Medicine, Philadelphia, PA.**

We have reported previously that chronic administration of the selective D1 partial agonist SKF-38393 fails to decrease D1 receptor density in the caudate-putamen (CPu), nucleus accumbens (NAc), and substantia nigra (SN). To determine whether intrinsic dopaminergic tone masks SKF-38393 induced down-regulation of receptors, rats were treated with the agonist following depletion of endogenous dopamine by reserpine. Rats were given either reserpine (1 mg/kg, SC; daily for the first 4 days, then every other day) or vehicle on days 1-28. Half of each treatment group were given SKF-38393 (5 mg/kg, SC; twice daily) while the other half received vehicle on days 14-28. Behavior was assessed on day 14 and 28. Rats were sacrificed 24 h after their last treatment and D1 receptor density was measured using quantitative autoradiography of ^{3}H-SCH23390 binding. In control rats, chronic SKF-38393 did not significantly decrease D1 receptor density in the CPu, NAc, or SN. In reserpinized rats, however, chronic SKF-38393 produced a decrease in D1 receptor density in the NAc, but not in the CPu or SN. In control rats, acute administration of SKF-38393 increased grooming and tongue protrusions. Chronic administration of SKF-38393 sensitized the tongue protrusion response but not grooming. In contrast, acute administration of SKF-38393 in reserpinized rats produced stereotyped behavior that did not change following chronic administration of the agonist. Reserpinized rats given vehicle exhibited a spontaneous increase in tongue protrusions that could be blocked by the D2 antagonist spiroperidol. (Supported by USPHS grant GM 34781, MH 43821 and MH 14654)

170.6

HUMAN PITUITARY DOPAMINE D_2 RECEPTORS ARE CODED FOR BY ALTERNATIVELY SPLICED mRNAs. D.K.Grandy, M.M. Marchionni*, H.P.Makam*, L.Reed*, J.R.Bunzow*, M. Litt*, O.Civelli. Vollum Institute, Or. Hlth. Sci. Univ., Portland, OR. 97201.

The receptors which mediate the effects of dopamine are divided into two types, D_1 and D_2, on the basis of their ligand selectivities and coupling with G proteins. The dopamine D_2 receptors have been further differentiated into two subtypes, the post-synaptic and somatodendritic autoreceptors. The nature of this diversity at the molecular level was studied in the human pituitary. A cDNA library was screened using the rat dopamine D_2 receptor cDNA (Bunzow et al., Nature, 336:577, 1988) as probe. The nucleotide sequence of a full-length human clone revealed a strong identity with the rat sequence. One notable exception, however, was the presence of an 87-basepair insertion in the human pituitary RNA. The source of this extra sequence was investigated at the level of the gene. Localized to chromosome 11q22-23, the human gene contains an exon which corresponds to the 87 basepair insertion found in pituitary D_2 receptor mRNA. Currently, studies are underway to demonstrate the function of this extra sequence.

170.7

[^{125}I]EPIDEPRIDE, A HIGH AFFINITY DOPAMINE D2 RECEPTOR LIGAND. A. Janowsky*, R.A. Henningsen*, P.R. Albert*, T. DePaulis*, R. Kessler* and K.A. Neve (SPON: J. Janowsky) VA Medical Ctr. and Depts. of Psychiatry and Pharmacology, Oregon Health Sciences Univ., Portland OR 97207.

We have characterized the in vitro binding of a new radio-iodinated substituted benzamide to DA D2 receptors. At the range of concentrations used in these studies, the binding of (S)-(-)-N-[(1-Ethyl-2-pyrrolidinyl)methyl]-5-iodo-2,3-dimethoxybenzamide approached equilibrium within 1 hr at 30°C. At a ligand concentration of 100 pM, non-specific binding (2 uM (+)-butaclamol) was 5% of total binding to membranes from rat striatum and from GH_4 cells transfected with a D2 receptor cDNA, and 20% of total binding to cortical membranes. The density of binding sites was approximately 1000 fmol/mg protein in striatum and transfected cells, and 20 fmol/mg protein in membranes prepared from medial prefrontal cortex. The K_d values were 15 pM to 30 pM in transfected cells, rat striatum, and cortex. K_d values were constant between 20 and 100 mM NaCl, but increased at lower concentrations to a maximum of approximately 300 pM in the absence of added NaCl. Epidepride inhibited the specific binding of [^{3}H]spiroperidol in GH_4 cells with K_i values of 18 pM and 500 pM in the presence and absence, respectively, of 50 mM NaCl. Because of its high potency, selectivity, and specific radioactivity, epidepride will be useful for in vivo and in vitro studies of dopamine D2 receptors.

170.8

FUNCTIONAL CHARACTERIZATION OF A RAT DOPAMINE D-2 RECEPTOR cDNA EXPRESSED IN A MAMMALIAN CELL LINE. K.A. Neve, R.A. Henningsen*, T.A. Schock*, J.R. Bunzow*, and O. Civelli*. VAMC; Vollum Institute and Depts. of Psychiat. and Pharmacol., Oregon Health Sci. Univ., Portland, OR 97207.

The recent cloning of a complementary DNA for the rat dopamine (DA) D-2 receptor makes it possible to create cell lines expressing this receptor. LZR1 cells (previously described as L-RGB2Zem-1 cells; *Nature* **336**:783-787) were created by transfecting the D-2 cDNA (RGB-2) into mouse fibroblast Ltk$^-$ cells. LZR1 cells express a high density of D-2 receptors, whereas the wild-type cells do not. A number of agonists competitively and stereoselectively inhibited the binding of [^{3}H]spiroperidol to the expressed D-2 receptors. DA was a more potent inhibitor of radioligand binding in the presence of $MgCl_2$ than in the presence of GTP, NaCl, and $MgCl_2$. DA reduced forskolin-stimulated adenylate cyclase activity by 27% in membranes prepared from LZR1 cells. Inhibition of adenylate cyclase by DA was blocked by (+)-butaclamol or prior treatment of intact cells with pertussis toxin. Pertussis toxin-treatment also abolished the high-affinity binding of DA observed in the absence of GTP/NaCl. Treatment of intact cells with 100 μM DA caused a time-dependent loss of [^{3}H]spiroperidol binding, reducing the density of receptors from 1017 fmol/mg protein to approximately 750 fmol/mg after 4-6 hr. These data indicate that the RGB-2 cDNA directs the expression of a DA D-2 receptor capable of interacting with guanine nucleotide-binding proteins and inhibiting adenylate cyclase activity. Furthermore, the density of receptors on LZR1 cells can be regulated by treatment with DA.

170.9

EFFECTS OF CHOLECYSTOKININ OCTAPEPTIDE ON STRIATAL DOPAMINE RECEPTOR BINDING IN THE RAT. M. Nakashima*, S. Kito and R. Miyoshi(SPON:Y.Yamamura). Third Department of Internal Medicine, Hiroshima University School of Medicine, Hiroshima 734, Japan.

The effects of cholecystokinin octapeptide(CCK-8) on dopamine(DA) receptor binding were examined in vitro. Wistar strain male rats were used. P_2 fractions of the striata were prepared and used as the receptor preparation. From DA/^{3}H-SCH23390 competitive binding experiments, competition curves were best fitted to a two-site model. Preincubation of the receptor preparation with CCK-8 caused a partial conversion of high affinity binding sites into low affinity ones. When SK&F 38393, a selective D_1 agonist, was used as a competitor, competition curves were monophasic and a single high affinity state was observed. By preincubating with CCK-8, in addition to high affinity binding sites, low affinity sites appeared. In presence of 120mM NaCl, dopamine binding sites were not altered by CCK-8. Addition of 50μM GTP resulted in a partial conversion of the high affinity sites into low affinity ones which was further advanced by CCK-8. In contrast, CCK-8 had no effect on antagonist binding. Likewise, CCK-8 caused a significant reduction of dopamine D_2 high affinity sites when observed by using ^{3}H-spiperone as a ligand. These results suggest that CCK receptor-stimulation modulates the binding characteristics of dopamine receptors.

170.10

POST-SYNAPTIC DOPAMINE (DA) D-2 RECEPTOR DEPOLARIZES SUPRAOPTIC NEURONES (SON) IN RAT HYPOTHALAMIC EXPLANT. C.R. Yang, C.W. Bourque, L. Renaud. Centre for Research in Neuroscience, Montreal General Hospital and McGill Univ., 1650 Cedar Ave., Montreal, P.Q. Canada H3G 1A4.

A prominent dopaminergic innervation of SON neurosecretory cells has been described (Buijs et al., Brain Res. 326:65-72, 1984) but the cellular mechanisms of DA action are unknown. Current- & voltage-clamp techniques were used to study the actions of DA on SON neurons in superfused rat hypothalamic explants.

Dopamine, administered by bolus injection (final concentration 100-250μM) or bath-application (1-10μM), induced slow depolarization of 3-7mV in 47 of 70 SON cells. This depolarization persisted in the presence of tetrodotoxin (2μM). Furthermore, the action of DA was mimicked by D2 agonist quinpirole (LY171555, 50μM), but not the D1 agonist SKF38393. Under voltage-clamp (V_h= -60mV), DA evoked an inward current (50-100pA, n=5), accompanied by 10-35% increase of steady-state input conductance. The current activated by DA was voltage-independent below resting potential and reversed between -18 and -35mV. The amplitude of the DA response or its reversal potential were not affected by intracellular injection of Cl^-. Thus, post-synaptic DA D2 receptors depolarize SON neurons, presumably via activation of non-selective cationic channels.
(Supported by FCAR, FRSQ, MRC).

170.11

EFFECTS OF ASCORBIC ACID, ISOASCORBIC ACID AND GLUCOASCORBIC ACID ON THE BINDING OF THE D_2 AGONIST ^{3}H-N-0437. L.C. Tolbert, J.J. Spollen*, M.L. Smitherman* and P.E. Morris*. Neuropsychiat. Res. Prog. and Dept. Psychiatry, Univ. of AL at B'ham., B'ham., AL 25294.

Ascorbic acid (AA) is a water-soluble vitamin with multiple known functions in the body. A number of investigators, using in vitro and in vivo techniques, have explored the possibility that AA may have a neuromodulatory role in CNS dopaminergic function. For example, it has been reported to modulate DA agonist binding and cyclic AMP stimulation as well as antagonizing the behavioral and physiological effets of amphetamine and potentiating the effects of haloperidol. The purpose of the present study was to evaluate the specificity of the AA effect on dopaminergic function. Binding studies were utilized as a system uncomplicated by questions of transport etc. [^{3}H] N-0437, (2-(N[2,3n)-^{3}H] propyl-N-(2-thiofuranyl)-2'-ethylamino)-5-hydroxy-1,2,3,4-tetrahydronaphathalene), was chosen as ligand for selectivity, D_2 agonist, and stability. The effects of AA, d-ascorbic acid (isoascorbic acid), and d-glucoascorbic acid on the binding of ^{3}H N-0437 to rat striatal membranes were evaluated. D-glucoascorbic acid was synthesized from published methods. The rationale for the choice of these three compounds was that they have relatively similar redox properties but if the AA effect was a specific receptor mediated phenomena the compounds should have differences in potency. Utilizing 2.5 nM ^{3}H N-0437 and increasing concentrations of the 3 compounds, in micromolor ranges, we confirmed substantial differences in their inhibition curves. The IC_{50}'s calculated from the linear transformed curves were: AA 93.3; isoasc 199.5, and glucoasc 9,549.9 μM. However, utilizing low concentrations, ≤30 nM AA, a significant enhancement in ^{3}H-N0437 binding was observed. In a similar manner, linear transformants of dissociation curves of ^{3}H-N-0437 in the presence of low concentrations, ≤30 nM, AA were inconsistent with effects on a single non-interacting binding site.

170.12

LOCALIZATION OF THE mRNA FOR DOPAMINE D2 RECEPTOR IN THE RAT BRAIN BY in situ HYBRIDIZATION HISTOCHEMISTRY. G. Mengod*, M.I. Martinez-Mir*, M.T. Vilaró*, and J.M. Palacios
Preclinical Research, Sandoz Ltd, Basel 4002, Switzerland

[^{32}P]-labelled oligonucleotides derived from the coding region of the rat dopamine D_2 receptor cDNA have been used as probes to localize in the rat brain the cells containing the mRNA coding for this receptor by using in situ hybridization histochemistry. The highest level of hybridization was found in the intermediate lobe of the pituitary gland. High mRNA content was observed in the anterior lobe of the pituitary gland, nucleus caudate-putamen and accumbens, and the olfactory tubercle. Lower levels were seen in the substantia nigra pars compacta, and ventral tegmental area as well as in the lateral mammilary body. In these areas the distribution was comparable to that of the dopamine D_2 receptor binding sites visualized by autoradiography using [^{3}H]SDZ 205-502 as a ligand. However, in some areas like the olfactory bulb, neocortex, hippocampus, superior collicullus, and cerebellum, D_2 receptors have been visualized but no significant hybridization signal could be observed. The mRNA coding for these receptors in these areas could be contained in cells outside of the brain areas, be different from the one recognized by our probes or be present at levels bellow the detection limits of our procedure. The possibility to visualize and quantify at the microscopic level the mRNA coding for the dopamine D_2 receptors will allow to gain more information about the in vivo regulation of the synthesis of these receptors and their alteration after selective lesions or drug treatments.

170.13

DOPAMINE D_2 RECEPTORS IN THE CEREBRAL CORTEX: DISTRIBUTION AND PHARMACOLOGICAL CHARACTERIZATION WITH [^{3}H]RACLOPRIDE. M.S. Lidow, P.S. Goldman-Rakic, P. Rakic and R.B. Innis, *Section of Neuroanatomy, Yale University, School of Medicine, New Haven, CT 06510*

An involvement of dopamine in the regulation of cognitive functions as well as a widespread dopaminergic innervation of the neocortex have focused attention on the identity of cortical dopamine receptors. However, while the presence and distribution of dopamine D_1 receptors in the neocortex have been well documented a comparable information on cortical D_2 sites is lacking. We report here the results of binding studies in the neocortex and neostriatum of rat and monkey using the D_2 selective antagonist, [^{3}H]raclopride. Tissue homogenates were incubated for 30 min at room temperature with 0.1-6.0 nM of the radioligand in 50 mM Tris-HCl (pH 7.4) containing 120 mM NaCl and 0.1 % ascorbic acid. Nonspecific binding was determined in the presence of 1.0 μM (+)butaclomol. A variety of ligands were used to characterize [^{3}H]raclopride binding in competition studies. In both, neocortex and neostriatum, [^{3}H]raclopride bound in a sodium dependent and saturable manner to a single population of sites with pharmacological profiles of dopamine D_2 receptors with K_d = 2.3±0.3 nM in rat and 1.1±0.3 nM in monkey tissue. D_2 sites were present in all regions of the neocortex, although their density was 50-75 times (rat) and 75-300 times (monkey) lower than in the neostriatum. The density of these sites in monkey and to a lesser extent in rat neocortex displayed a rostral-caudal gradient (B_{max} = 4.3±0.4 fmol/mg protein in rat prefrontal and 2.4±0.46 fmol/mg protein in rat occipital cortex; and B_{max} = 2.4±0.2 fmol/mg protein in monkey prefrontal and 0.6±0.1 fmol/mg protein in monkey occipital cortex). This gradient corresponds to gradient in dopamine concentration within the cortex (Brown, R.M. *et al.*, Brain Res., 168: 133, 1979). Thus, the present study establishes the presence and widespread distribution of dopamine D_2 receptors in the neocortex.

170.14

THE DOPAMINERGIC INNERVATION OF THE PRIMATE FRONTAL CORTEX. S.M. Williams*, M. Geffard and P.S. Goldman-Rakic, (SPON: W.B. Stewart) Sec. of Neuroanatomy, Yale Univ. Sch. of Med., New Haven, CT 06510 and Institut de Biochemie Cellular et Neurochemie du CNRS, Bordeaux, France.

Previous biochemical, radioautographic and tyrosine hydroxylase immunohistochemical studies have indicated that the primate cerebral cortex receives a robust and regionally heterogeneous dopaminergic innervation. So far, however, the dopamine-containing axons of the primate cerebral cortex have not been directly visualized immunohistochemically due to the lack of a dopamine-specific monoclonal antibody. Using such an antibody, we have examined the distribution of dopamine (DA) afferents in several regions of the frontal cortex of the rhesus monkey.

All regions of the frontal cortex that were examined demonstrated an elaborate dopaminergic innervation; however, regional differences were evident in their density and laminar distribution. The density of dopamine-immunoreactive (DA-IR) fibers followed a medial to lateral gradient, with the medial cortical areas receiving the most dense DA innervation. Thus, the medial prefrontal cortex (Walker's areas 25 and medial 9), the anterior cingulate cortex (AC [area 24]) and the supplementary moter cortex (SMA [area 6]) contained a higher concentration of DA-IR axons than the dorsolateral prefrontal cortex (areas 46 and 12) and the lateral primary motor cortex (area 4). In all prefrontal areas examined, analysis revealed a bilaminar distribution pattern of DA-IR processes. Labeled fibers were concentrated in the superficial layers I, II, and IIIa, intermediate in density in the infragranular layers V and VI and lowest in layers IIIb and IV. In SMA, AC and motor cortex, the superficial layers were also densely innervated but the characteristic bilaminar pattern was less prominent. These results corroborate and extend the previous findings indicating the regional and laminar specificity of the DA innervation of the non-human primate frontal cortex. Moreover, the DA innervation observed with this dopamine-specific antibody is consistent with the distribution of DA receptors in frontal cortical areas as demonstrated by *in vitro* receptor autoradiography. Electron microscopic studies are currently in progress to determine the postsynaptic targets of these DA fibers. Supported by MH44866.

170.15

PRE AND POSTSYNAPTIC MECHANISMS IN THE DISCRIMINATIVE-STIMULUS EFFECTS OF GBR 12909. K.F. Melia* and R.D. Spealman*. (SPON: P.B. Dews). Harvard Medical School, NERPRC, Southborough, MA 01772

The discriminative-stimulus effects of the dopamine transport inhibitor GBR 12909 (GBR) were studied in squirrel monkeys trained under a two-lever drug discrimination procedure. To investigate presynaptic mechanisms, several inhibitors of monoamine uptake were studied in substitution tests for GBR. Drugs with prominent effects on dopamine transport including cocaine, WIN 35,428, mazindol, bupropion, and methylphenidate occasioned 89-100% GBR-appropriate responding. In contrast, the selective norepinephrine uptake inhibitors desipramine and talsupram and the selective serotonin uptake inhibitor citalopram occasioned no more than 20% GBR-appropriate responding. To investigate postsynaptic mechanisms, D_1 and D_2 dopamine receptor agonists were tested. The D_2 agonists (+)-PHNO and quinpirole occasioned 75 - 85% GBR-appropriate responding, whereas the D_1 partial agonists R-SKF 38393 and SKF 75670 engendered no more than 10% GBR-appropriate responding. The results suggest that the discriminative-stimulus effects of GBR are mediated presynaptically by inhibition of dopamine uptake and postsynaptically by activation of D_2 dopamine receptors. A facilitating role for D_1 receptors cannot be excluded. (Supported by DA00499, DA02658, DA00088, and RR00168).

170.16

CHARACTERIZATION AND REGULATION OF D_2 DOPAMINE (DA) RECEPTORS IN RAT ANTERIOR PITUITARY TUMORS. J.Y. Lew and M. Goldstein. Neurochem. Res. Labs. N.Y. Univ. Med. Ctr., New York, NY 10016.

The binding subunits of the D_2 DA receptor in the rat anterior pituitary tumor (7315a) were identified using a photoaffinity probe N-(p-azido-m [^{125}I]iodophenethyl)spiperone (^{125}I-NAPS). The photoaffinity labeled membrane proteins were subjected to SDS-gel electrophoresis and subsequently the autoradiograms were developed. One major peptide with M.W of 32-34 KDa and two minor peptides with M.W of 87-89 KDa and 153-155 KDa were obtained. The labeling of all three peptides was prevented by incubation of membranes with non-radioactive spiperone (Spi) (10 μM) prior to the addition of ^{125}I-NAPS. The low M.W peptide might represent the deglycosylated receptor binding subunit or a proteolytic fragment of the larger peptides.

The effects of chronic treatment with haloperidol (1 mg/kg, s.c. for 4 wks) on the DA receptor density of ^{3}H-Spi binding in the tumor and in the striatum were investigated. Scatchard analysis of the data shows an increase from 94.6 to 351.7 fmole/mg protein in the tumors and an increase from 1185 to 1422 fmole/mg protein in the striata of the haloperidol-treated animals. The treatment with haloperidol did not significantly change the K_d for ^{3}H-Spi binding neither in the tumor no in the striatum. The greater increase of B_{max} for ^{3}H-Spi in the tumor as compared to the striatum might be due to the increased regulation of the D_2 DA receptor in the peripheral tissue or to the absence of D_1 receptors in the tumor. Supported by NIMH 02717 and NINCDS 06801.

170.17

POLYMERASE CHAIN REACTION CLONING OF D2 RECEPTOR SUBTYPES. L.A. Snyder*, J. Brosius , J.L. Roberts and S.C. Sealfon*. Fishberg Research Center in Neurobiology, Mount Sinai Medical Center, New York, N.Y. 10029

The dopamine D2 receptor is an important component of brain circuitry and has been implicated in the pathophysiology of Parkinson's disease and schizophrenia. A rat brain D2 receptor has been cloned by Civelli et al. (Nature336:783). Based on the published sequence, we designed oligonucleotides for PCR cloning.

Oligo(dT) primed cDNA was synthesized from weanling rat brain RNA. When used as a PCR template, with oligos spanning the coding region, at least 2 distinct bands were observed on agarose gels. One band was the expected size of ~1250 bp. An unexpected second band, ~100 bp larger, was also seen. Both bands hybridized to an internal oligonucleotide probe. When estrogen-induced rat prolactinoma cDNA was used as a PCR template for this reaction, a single band was observed corresponding in size to the larger brain product.

Using a second set of oligonucleotides spaced 330 bp apart and spanning the known intron site of the published clone, only one PCR product was found with both brain and prolactinoma cDNA. This suggests that the unexpected larger product is not due to differences in the region immediately surrounding the intron.

Our data suggests that there is more than one D2 receptor mRNA, with tissue specific expression. There may be multiple D2 receptor genes or alternative processing of hnRNA.

170.18

CELLULAR LOCALIZATION OF D-2 DOPAMINE RECEPTOR USING CROSSLINKABLE AND PHOTOAFFINITY SPIPERONE ANALOGS, DC Chugani* and ME Phelps. UCLA School of Medicine, Los Angeles, CA 90024

We have proposed that the D-2 receptor ligand spiperone is accumulated in striatum by agonist-mediated receptor internalization (Chugani et al., (1988)J. Cereb. Blood Flow & Metab., 8:291) in order to account for the in vivo kinetics of spiperone and it analogs. This model has been tested by measurement of subcellular distribution of spiperone analogs by subcellular fractionation and immunocytochemistry. Bovine striatal slices (200 μm) were incubated for up to 24h in oxygenated Yamamoto's artificial CSF with the photoaffinity label ^{3}H-azido-methyl-spiperone (AMS) in the presence and absence of (+)butaclamol. As has been found with spiperone in vivo, the uptake of AMS in slices is slow reaching equilibrium at 8-10h. Incubation in the presence of the metabolic inhibitor sodium azide (0.2%) resulted in a large decrease in uptake, indicating an ATP-requiring process, not simply binding, was necessary for uptake. Subcellular fractionation of slices following AMS uptake revealed specific enrichment in both synaptosome and endosome fractions. Polyclonal antibodies have been raised against carboxy-oxime-spiperone (COSP)conjugated to hemocyanin. Rats were administered COSP (1 mg/kg, i.v.) and perfused 4h later with carbodiimide (1%) and glutaraldehyde (0.25%). The brain was postfixed in 4% paraformaldehyde, and vibratome sections (100 μm) were cut. Immunofluorescence revealed numerous labeled processes in striatum, and this labeling was eliminated by pretreatment of the rats with (+)butaclamol. EM studies to determine the subcellular distribution of this labeling are in progress.

170.19

EFFICACY AND POTENCY COMPARISONS AMONG APORPHINE ENANTIOMERS: EFFECTS ON SUBSTANTIA NIGRA (SN) DOPAMINE (DA) CELL FIRING. L. Martin*, R.F. Cox and B.L. Waszczak (SPON: R.A. Schatz). Pharmacol. Sect., Northeastern Univ., Boston, MA.

Extracellular single unit recording studies were carried out on male rats to determine responses of SN DA neurons to iv administration of the enantiomers of the aporphine congeners: apomorphine (APO), N-n-propylnorapomorphine (NPA), and 11-hydroxy-N-n-propylnorapomorphine (11-OH NPa). The R-(-)-configuration was found to be the most critical determinant of agonist efficacy and potency. All (-)-aporphines were full agonists able to completely inhibit DA cell firing. The order of their potencies, defined by their ID_{50}'s, was: (-)NPA, 2.0±0.4 nmol/kg>(-)11-OH NPa, 4.7±0.7 nmol/kg> (-)APO, 18.0±4.0 nmol/kg. Thus, potency was improved about 9-fold by replacing the 6N methyl of APO with an n-propyl (ie. NPA). Conversely, the 10-hydroxy was not essential for agonist activity (ie. 11-OH NPa) but could improve potency.

In the (+)-series responses varied. (+)NPA exhibited agonist properties and could fully inhibit DA cells, but its potency was low (ID_{50} 1550 nmol/kg). (+)APO produced only slight but significant decreases in firing at high (8434 nmol/kg) doses, and (+)11-OH NPa was devoid of efficacy in that it caused no significant changes in firing (n=10-17). While efficacy and potency were dramatically reduced or lost, the (+)-enantiomers apparently did retain affinity for DA receptors since they could act as antagonists if given before dose-response curves to (-)APO or NPA.

170.20

N-(p-ISOTHIOCYANATOPHENYL)SPIPERONE, A SELECTIVE IRREVERSIBLE ANTAGONIST OF D_2 DOPAMINERGIC RECEPTORS. S.X. Xu,Y. Hatada[1] Ian Creese & D.R. Sibley[1]. Rutgers' Center for Molecular & Behavioral Neuroscience, Newark, NJ 07102, [1]Experimental Therapeutics Branch, NINDS/NIH, Bethesda, MD 20892.

N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ), an irreversible protein-modifying reagent, has been used extensively in studies involving inactivation of dopaminergic receptor subtypes. This agent is nonselective, however, as it inactivates both the D_1 and D_2 subtypes as well as many other neurotransmitter receptors. Here, we present N-(p-isothiocyanatophenyl)spiperone (NIPS), a novel, highly selective irreversible inactivator of D_2 dopaminergic receptors. In *in vitro* studies, NIPS was found to exhibit a K_i of approximately 20 nM for [^{3}H]methylspiperone binding to D_2 receptors in rat striatal membranes. The affinity of NIPS was dependent on the time of assay, however, as its potency increased with increasing times of incubation. Preincubation of the membranes with NIPS followed by extensive washing resulted in a major reduction of the D_2 receptor maximum binding capacity (B_{max}) as well as a slight decrease in affinity. In *in vivo* administration studies, using [^{3}H]SCH 23390 and [^{3}H]spiperone to assay D_1 and D_2 receptors *in vitro*, we found that 24 hr after injection (s.c) with 5-40 mg/kg of NIPS the D_2 receptor B_{max} was decreased by 58-76%, with no change in D_2 receptor affinity. In contrast, there was no effect on the D_1 receptor B_{max} or affinity. In addition to the reduced D_2 binding capacity, at 20 mg/kg, there was also a small (24%) reduction in frontal cortex 5-HT_2 receptors. However, there was no effect on alpha$_1$, alpha$_2$ adrenergic or muscarinic cholinergic receptors in the frontal cortex, nor on 5-HT_{1A} receptors in the hippocampus. Interestingly, following a single dose (s.c) of either NIPS (20 mg/kg) or EEDQ (10 mg/kg) the D_2 receptor recovery rate was slower following NIPS ($t_{1/2}$ = 169 hr) than following EEDQ ($t_{1/2}$ = 76.7hr). These results suggest that NIPS is a highly selective and irreversible inactivator of D_2 dopaminergic receptors and may prove useful in *in vitro* and *in vivo* functional studies of this receptor subtype. Supported by NIMH 00316 and NIDA 04612.

170.21

Interactions of Dopaminergic Metabolites at CNS Dopamine D2 Receptors. J.N. Cammack*, E. Michaelis, and R.N. Adams (SPON: A.Oke). Pharmacology Dept., Univ. of Kansas, Lawrence, KS 66046.

In-vitro binding experiments were performed on rat striatal membrane preparations using (^{3}H)spiperone, the potent butyrophenone D2 antagonist. Varying concentrations of the dopamine metabolites 3,4-dihydroxyphenyl acetic acid (DOPAC), homovanillic acid (HVA), and 3-methoxy-tyramine (3-MT) were incubated with the receptor membranes and log-dose inhibition curves were generated. These binding parameters can be compared to the inhibition of spiperone by DA. Several metabolites were as potent, or more so than DA, at blocking specific spiperone binding to this D2 site. The functional implications of these results are far ranging, especially with regard to several neuropathological states known to have a malfunctioning dopaminergic component as possible etiological factors i.e. , Parkinson's disease and schizophrenia. Continuing work in this area should provide answers to the physiological significance of DA metabolites interacting at this D2 locus (for example, whether this interaction is antagonistic or agonistic in nature).

170.22

BIPHASIC DOSE-DEPENDENT EFFECTS OF INTRASTRIATAL QUINPIROLE ON LOCOMOTOR ACTIVITY. C. Van Hartesveldt and G. A. Cottrell. Psychology Department, University of Florida, Gainesville, FL 32611.

Eilam and Szechtman (1988) reported that the D2 agonist quinpirole produced inhibition of forward progression at low doses, and sequential inhibition and excitation at high doses in rats.

We asked whether quinpirole might affect locomotion by acting directly on the dorsal striatum. Castrated male rats received 2 mg/kg or 0.02 mg/kg quinpirole sc, or saline, 20 ug or 40 ug/side quinpirole in 0.5 ul in the dorsal striatum. All rats were placed in activity monitors where activity was recorded for 2 hr.

The low dose of sc and intrastriatal quinpirole significantly decreased activity during the first 15 min but had no effect later. The high dose of sc and intrastriatal quinpirole significantly decreased activity during the first 15 min but significantly increased activity later. Thus both the inhibitory and excitatory effects of quinpirole can be elicited from the dorsal striatum.

170.23

D2 DOPAMINE RECEPTORS MEDIATE THE SUPPRESSION OF CONDITIONED AVOIDANCE RESPONDING INDUCED BY HALOPERIDOL. J.F. McElroy*, J.J. Stimmel* and J.M. O'Donnell* (SPON: D.L. Hjeresen). Life Sciences Division, Univ. of California Los Alamos National Laboratory, Los Alamos, NM 87545.

The relative importance of D1 and D2 dopamine (DA) receptors in mediating the effects of the antipsychotic drug haloperidol (HAL) on conditioned avoidance responding (CAR) in rats has been investigated. Two-way discrete trial CAR was measured in a shuttlebox. Each of 40 daily trials consisted of a 10 sec conditioned stimulus (120 dB tone) followed by a 20 sec unconditioned stimulus (0.9 mA foot shock). Drugs were administered i.p. 30 min before testing to well-trained (> 80% CAR) rats. HAL suppressed CAR in a dose-dependent manner (ED_{50}=0.07 mg/kg), with baseline responding (91% CAR) completely suppressed by 0.1 mg/kg HAL (2% CAR). Acute treatment with the D2 selective agonist quinpirole antagonized the suppressive effect produced by 0.1 mg/kg HAL in a dose-dependent manner (ID_{50}=0.4 mg/kg), with 1.0 mg/kg quinpirole completely preventing the HAL effect (94% CAR). Pretreatment with the D1 selective agonist SKF-38393 (0.1-30 mg/kg) did not antagonize 0.1 mg/kg HAL (< 16% CAR). Apomorphine (0.003-3 mg/kg), an agonist at both DA receptors, partially antagonized HAL (44% CAR). These results demonstrate that D2 receptors predominantly mediate the suppressive effect of HAL on CAR behavior. Moreover, the results with apomorphine are consistent with the existence of a functional interaction between D1 and D2 receptors.

170.24

EFFECT OF I.V. N-n-PROPYLNORAPOMORPINE (NPA) ON SUBSTANTIA NIGRA (SN) DOPAMINE (DA) NEURONS AFTER INTRANIGRAL RECEPTOR INACTIVATION BY EEDQ. R.F. Cox and B.L. Waszczak. Pharmacol. Sect., Northeastern Univ., Boston, MA 02115.

These studies explored a new approach for determining the contribution of somatodendritic autoreceptors in mediating the inhibition of SN DA cell firing caused by i.v. DA agonists. One day before extracellular single unit recording studies, male rats were given unilateral SN injections of the receptor inactivator N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ; 6 or 12 ug/0.5 ul in 23% Molecusol vehicle, VEH). Neither EEDQ nor VEH injections significantly changed the number of active DA cells or their firing rates. However, in EEDQ-injected rats, dose response curves to i.v. NPA were shifted to the right and maximal responses were depressed relative to curves from VEH-treated rats. Interestingly both the 6 and 12 ug EEDQ injections produced similar 4-fold rightward shifts (ID_{50}'s 1.8 and 1.6 ug/kg, respectively, vs. 0.4 ug/kg in VEH-treated rats) and similar (20%) reductions in the maximal attainable response to NPA. Accordingly, Furchgott analysis showed that both doses of EEDQ inactivated 56% of the total receptors contributing to this response. The fact that both doses caused the same level of inactivation may suggest that SN DA receptors were fully inactivated with both treatments. If quantitative autoradiography (in progress) confirms this is true, it could be concluded that SN DA receptors contribute substantially (~60%), but not exclusively, to the inhibition of DA cell firing by i.v. NPA. (NS 23541)

170.25

INHIBITION OF TYROSINE HYDROXYLASE BY ENANTIOMERIC APORPHINES IN RAT STRIATAL MINCES. R.G. Booth*, N.S. Kula*, J.L. Neumeyer, and R.J. Baldessarini. Mailman Research Center, Harvard Medical School and Medicinal Chemistry Section, Northeastern University, Boston, Mass.

Effects of enantiomeric mono- and dihydroxyaporphines on rat brain tyrosine hydroxylase (TOH) were assessed by measuring formation of $^{14}CO_2$ during the synthesis of dopamine (DA) from ^{14}C-L-tyrosine in striatal minces. Stereoselectivity of presynaptic "autoregulation" of DA synthesis was probed by examining the ability of the R(-) (DA agonist) and S(+) isomers of the noncatechol apomorphine analog 11-hydroxy-N-*n*-propylnoraporphine (11-OH-NPa) and the corresponding catechol-containing congener, 10,11-dihydroxy-N-*n*-propyl-noraporphine (NPA) to inhibit TOH in the presence or absence of the DA receptor antagonist fluphenazine (FLU). R(-) and S(+)11-OH-NPa (EC_{50}=40 and 100 µM, respectively) were about 100-fold less potent than R(-) and S(+)NPA (EC_{50}=0.25 and 1.5 µM) at inhibiting TOH in striatal tissue from normal rats. Furthermore, most of the inhibition of TOH observed with the 11-OH-NPa isomers appeared to be *indirect* and DA-mediated since in reserpinized animals, >50% inhibition of the enzyme could not be achieved even at ≥100 µM aporphine. However, at least part of the inhibition could be attributed to autoreceptor mechanisms since the inhibition observed in tissue of DA-depleted rats was accompanied by an increase in stereoselectivity (2500-fold, R>S) that was fully blocked by FLU. In contrast, the dihydroxyaporphines appeared to inhibit TOH mainly via an autoreceptor mechanism since inhibition could be blocked by FLU in untreated as well as DA-depleted tissue. The stereoselectivity of the NPA enantiomers (6-fold, R>S) was similar in both DA-depleted and nondepleted animals and >90% inhibition of the enzyme was observed with 50 µM R(-)NPA in reserpinized animals (EC_{50}=1.5 µM). These studies demonstrate that synthesis of DA in its nerve terminals can be influenced stereoselectively by aporphine analogs of DA, probably via putative autoreceptor mechanisms. [Supported by NIMH grants MH-34006 and MH-47370 and the Bruce J. Anderson Foundation.]

171.1

IMMUNOCYTOCHEMICAL LOCALIZATION OF DARPP-32, A MARKER OF DOPAMINOCEPTIVE NEURONS, IN THE CEREBRAL CORTEX AND HIPPOCAMPUS OF RHESUS MONKEY. B. Berger, P. Greengard, P.S. Goldman-Rakic INSERM, Paris, France, Section of Neuroanatomy, Yale Univ. Sch. Med., New Haven, CT 06510, Rockefeller Univ., New York, NY.

DARPP-32 (DA-32), a dopamine (DA) and cyclic AMP-regulated neuronal phosphoprotein is specifically enriched in dopaminoceptive neurons containing D1-dopamine receptors. We have localized DA-32 like immunoreactive neurons (LIR) in the cerebral cortex of the neonatal and adult rhesus monkey. Staining was particularly good in the youngest monkeys in whom granular parietal, temporal, occipital and insula areas contained a single row of intensely stained pyramidal cells in layer Va (Vb in area 17). The cells had thick apical dendrites which branched diffusely in layer I. Smaller variably labeled neurons were present in layers IIIa and VI and/or Vb, but never in layer IV. In the prefrontal, motor, and posterior cingulate cortices, a large number of labeled neurons, including Betz cells, populated layers V and VI. In the hippocampal formation, the granule cells of the dentate gyrus and their processes (dendrites, mossy fibers and terminals, in the stratum lucidum of CA3) were intensely labeled. Deep polymorphic neurons were also positive in the subicular complex. In the one adult monkey studied so far, labeled neurons were observed mainly in the entorhinal, temporal, insular and some parietal areas. Labeling of glial cells was more prominent compared with the younger cases. The distribution of DARPP-32 neurons in the infragranular layers of the primate cortex is matched by a high concentration of D1 receptors and dopamine fibers in these layers. (supported by INSERM, Foundation Singer-Polignac and NIMH).

171.2

EFFECT OF SCH 39166, A NOVEL D1 RECEPTOR ANTAGONIST, ON CHOLINERGIC FUNCTION IN THE RAT STRIATUM. C. E. Tedford*, G. Crosby Jr.*, L. C. Iorio and R. E. Chipkin (SPON: A. Barnett).
Schering-Plough Research 60 Orange St., Bloomfield, NJ 07003.

Receptor binding studies in our laboratories have shown that a novel benzonaphthazepine (SCH 39166) displays marked selectivity toward the D1 receptor. To further characterize dopamine receptor selectivity, we utilized modulation of ^{3}H-ACh release in the rat striatum as an index of D2 receptor activity. We investigated the effects of SCH 39166 on electrically stimulated ^{3}H-ACh release from rat striatal slices. Furthermore, comparisons were made between SCH 39166 and either the selective D2 antagonist, S-sulpiride, the selective D1 antagonist, SCH 23390, or the nonselective agonist, apomorphine. Stereoselectivity was also investigated by comparing the effects of SCH 39166 and SCH 23390 on ^{3}H-ACh release with those of SCH 39165 and SCH 23388, their respective stereoisomers.

Results indicated that apomorphine inhibited ^{3}H-ACh release from striatal slices (I.C. $_{50}$ - 0.31 uM). The D2 receptor antagonist, S-sulpiride caused a slight increase in ^{3}H-ACh release alone, and completely reversed the inhibition of ^{3}H-ACh release seen with apomorphine. In contrast, SCH 39166, as well as, SCH 23390 did not reverse the inhibition of ^{3}H-ACh release induced by apomorphine. Interestingly, at high concentrations, both D1 antagonists (SCH 23390 and SCH 39166) inhibited ^{3}H-ACh release. This effect however was not stereoselective and thus probably not related to D1 receptor blockade.

In summary, these studies indicate that the benzonaphthazepine, SCH 39166, represents a novel and selective D1 receptor antagonist.

171.3

EFFECTS OF 6-HYDROXYDOPAMINE LESIONS ON DOPAMINE UPTAKE SITES AND ON DOPAMINERGIC AND MUSCARINIC RECEPTORS IN THE RAT NIGROSTRIATAL SYSTEM. D. Vine*, F.Filloux, J.K. Wamsley, Dept. Psychiat., Univ. Utah Sch. Med., SLC, UT 84132.

Male rats received unilateral 6-hydroxydopamine injections of the medial forebrain bundle or substantia nigra compacta (SNc). Three weeks later, lesion efficacy was demonstrated by circling in response to apomorphine and D-amphetamine. Rats were sacrificed 4 weeks after injection and slide-mounted coronal tissue sections (10μ) were labeled with [^{3}H]BTCP, [^{3}H]SCH 23390 and [^{3}H]QNB to identify dopamine (DA) uptake sites, D1 and muscarinic (AChM) receptors, respectively. There was a marked loss of DA terminals in the ipsilateral caudate-putamen (CPu) of lesioned vs. control rats as indicated by a marked decrease in [^{3}H]BTCP binding of 73% ($p<0.0005$). There were smaller decreases in binding (27-43%, $p<0.05$) in the ipsilateral SNc and substantia nigra reticulata (SNr), indicating partial DAergic denervation of the SNr. Despite this modest DAergic denervation, there was no change in [^{3}H]SCH 23390 (D1) binding in the SNr. This last result could be due to reported D1 "receptor reserve." [^{3}H]QNB binding in the CPu ipsilateral to the lesion was reduced by 12.5% ($p<0.05$). This could derive from loss of posited AChM heteroreceptors on DAergic terminals, or from down-regulation of post-synaptic AChM receptors on GABAergic neurons due to increased ACh release from striatal neurons disinhibited by loss of DAergic input.

171.4

PHARMACOLOGICAL PROFILE OF THE BINDING OF [^{125}I] SCH 23982 IN HUMAN BRAIN. M. Laruelle*, A. Sidhu, M. Casanova*, D.R. Weinberger and J.E. Kleinman. NIMH Neurosciences Center at St. Elizabeths, Washington, D.C. 20032.

The benzazepine [^{3}H]SCH23390 is commonly used to label the D_1 dopaminergic receptor. Recently an iodinated analogue, [^{125}I]-SCH23982, was introduced (Sidhu et al, Eur J Pharmac, 128:213,1986). We studied the binding of this ligand in the human caudate nucleus (CN) and mid frontal gyrus (MFG). In CN, we observed a single saturable site ($K_d = 4.8 \pm 0.92$nM (Means $\pm$ SEM, n = 3), $B_{max} = 276 \pm 22$ fm/mg Protein). Competitive experiments were in accordance with the D_1 pharmacology. In MFG, the K_d was 3.8 ± 0.95nM and the B_{max} was 352 ± 37 fm/mgP. The observed B_{max} in MFG was thus about 13% of the B_{max} in CN. This contrasts with the distribution of the dopaminergic innervation in the human brain but is compatible with an interaction of these ligands with the serotonergic $5HT_2$ receptor. The $5HT_2$ antagonist ketanserin displaced [^{125}I]SCH23982 in a monophasic manner ($K_i = 150$nM) in CN but in a biphasic manner in the MFG ($K_{i_1} = 1.4$nM, 89%, and $K_{i_2} = 656$nM, 11%). Adding ketanserin to the incubation medium (50nM final concentration) induced no changes in the B_{max} in CN but a 82% reduction in MFG. Competition studies with dopamine (DA) and serotonin (5HT) gave the following order of potency: in CN, DA > > 5HT; in MFG 5HT > > DA; in MFG with 50nM ketanserin, DA > 5HT for [^{3}H]SCH23390 and 5HT > DA for [^{125}I]SCH23982. It can be concluded that [^{125}I]SCH23982 labels the D_1 receptor in human CN and the $5HT_2$ receptor in human MFG. As this phenomenon is more marked than with the tritiated ligand, it can be suggested that the iodination decreases the relative D_1 to $5HT_2$ selectivity of this compound.

171.5

STRIATONIGRAL LESIONS AND INTRANIGRAL INJECTION OF RECEPTOR INACTIVATOR EEDQ PREVENT D-1 AGONIST EFFECTS ON SUBSTANTIA NIGRA PARS RETICULATA (SNpr) NEURONS. B.L. Waszczak and L. Martin*. Pharmacol. Sect., Northeastern Univ., Boston, MA.

Previous studies revealed that iontophoretic application of dopamine (DA) or the D-1 agonist SKF 38393 (SKF) causes current-related increases in firing of SNpr neurons. These studies were undertaken to assess whether the excitatory effect of SKF was attributable to activation of D-1 receptors on striatonigral terminals in the SNpr. Extracellular single unit recordings were made in anesthetized male rats which received either: 1) striatal injections of kainic acid (KA; 1 ug/0.5 ul at 2 sites) 1-2 wks prior, or 2) intranigral injections of the receptor inactivator N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ; 12.5 ug/0.5 ul 22.5% Molecusol) 24 hrs prior to recording. Both treatments prevented the excitatory effects of applied (+)SKF (0.05M) on SNpr cells. In KA-lesioned rats, cells which could not be inhibited by striatal stimulation (indicating their striatal inputs were destroyed) showed only modest (9±5%) increases in firing at 10 nA (+)SKF, whereas cells still sensitive to striatal stimulation (inputs intact) had increases to 73±14% above baseline (n=8-11), like unlesioned rats. After intranigral EEDQ, (+)SKF increased firing only 11±5% above baseline at 8 nA while controls exhibited 61±16% increases at 8 nA (n=11-13). These results suggest that the previously described excitatory effect of DA and SKF on SNpr cells is mediated by activation of D-1 receptors on impinging striatonigral terminals. (Support: NS 23541)

171.6

Synthesis of an aldehyde-containing analog of SCH-23390. T.Filtz*, S.Chumpradit*, H.Kung* and P.Molinoff. Departments of Pharmacology and Radiology, Univ. of PA, Philadelphia, PA 19104

SCH-23390 is a high affinity antagonist selective for D_1 dopamine receptors. This antagonist does not contain a functional group that can be conveniently coupled to commercially available resins for affinity chromatography or to photolabile compounds for photoaffinity labelling of receptors. To construct an affinity resin for purification of dopamine D_1 receptors an aldehyde analog of SCH-23390, (RS)-5-(4'-formyl phenyl)-8-chloro-2,3,4,5-tetrahydro-3-methyl-[1H]-3-benzazepin-7-ol (Aldehyde-SCH-23390), was synthesized. 7-Methoxy-4'-bromo-phenyl SCH-23390 was lithiated, formylated and then demethylated to form the aldehyde. NMR and IR analyses were performed to characterize the product. Aldehyde-SCH-23390 displaced ^{125}I-SCH-23982 binding from caudate membranes with a K_i value of 3 nM. The placement of an aldehyde group on the phenyl ring allows for easy coupling of the antagonist to primary amines. Aldehyde-SCH-23390 has been coupled to diaminodipropyl-amine agarose for affinity chromatography. The aldehyde has also been conjugated with biotin-hydrazide for fluorescent labelling of dopamine D_1 receptors. (USPHS NS18591 and NS24538)



Aldehyde-SCH-23390

171.7

^{3}H-SCH 39166, A NEW D-1 SPECIFIC RADIOLIGAND: IN VITRO AND IN VIVO BINDING PROPERTIES. R.D. McQuade, G.C.Crosby*, R.A.Duffy * & R.E.Chipkin, Schering-Plough Corp., Bloomfield, NJ 07003

A new radioligand, ^{3}H-SCH 39166 ([-]trans 6,7a,8,9,13b-hexahydro-3-chloro-2-hydroxy-N-(^{3}H)methyl-5H-benzo[d]naphtho-{2,1-b}-azepine, 81 Ci/mmole) has been characterized for its binding to rat striatal membranes. In vitro, ^{3}H-SCH 39166 labels a single class of saturable receptors, with a K_D of 1.7 nM. Competition studies with dopaminergic antagonists demonstrate that binding is D-1 selective and stereospecific. Dopamine yields a biphasic inhibition curve with K_I values of 0.2 and 2.6 uM. The high affinity sites are converted to low affinity by addition of 0.1 mM GPP(NH)P, with all sites exhibiting a single K_I of 2.4 uM.

In vivo, ^{3}H-SCH 39166 labels striatal membranes with optimal binding 1 h after subcutaneous injection. This labeling exhibits both pharmacological specificity (1 umole of SCH 23390 inhibits 90% of the striatal binding) and tissue selectivity (specific cerebellar levels are less than 5% of specific striatal levels).

These data demonstrate that ^{3}H-SCH 39166 is a selective D-1 radioligand, both in vitro and in vivo.

171.8

AGONIST-INDUCED DESENSITIZATION OF D_1 DOPAMINE RECEPTOR-COUPLED ADENYLYL CYCLASE ACTIVITY IN CULTURED NS20Y NEUROBLASTOMA CELLS. Anne C. Barton and David R. Sibley. Experimental Therapeutics Branch, NINDS, NIH, Bethesda, MD 20892.

NS20Y neuroblastoma cells, which express a homogeneous population of D_1-dopamine (DA) receptors, were used in the present study as a model system to demonstrate agonist-induced stimulation and desensitization of D_1 receptor-coupled adenylyl cyclase (AC) activity. Membranes prepared from NS20Y cells showed a dose-dependent increase in cAMP production in response to various dopaminergic agonists. DA exhibited an EC_{50} of 5 μM and a maximal response of 3-4 fold over basal at 100 μM DA which could be antagonized by the active stereoisomers of SCH-23390 and butaclamol. A 1 hr preincubation of NS20Y cells with 100 μM DA induced homologous desensitization of D_1 receptor-coupled AC activity, decreasing DA- but not PGE-, adenosine- or forskolin-stimulated cAMP production. Desensitization was both dose and time dependent. As early as 5 min after preincubation with 100 μM DA, cAMP production was decreased by 45-50% with maximal desensitization (85-90% reduction) occurring by 60 min. Desensitization of D_1 receptor-coupled AC activity decreased the maximal amount of cAMP produced with no decrease in potency for DA-stimulated AC activity. Observed EC_{50} values for control and partially desensitized NS20Y cell membranes were ≈ 5 μM. Examination of [^{3}H]-SCH-23390 binding in control and maximally desensitized NS20Y cell membranes revealed no change in K_D, however, a 65% decrease in receptor number was observed. This decrease in D_1 receptor number does not appear to correlate with the 85-90% decrease in DA-stimulated AC activity after a similar desensitization suggesting a possible functional uncoupling of the D_1 receptor from its effector in addition to receptor downregulation.

171.9

EXPRESSION OF RAT STRIATAL mRNA CODING FOR A D_1 DOPAMINE RECEPTOR COUPLED TO Ca^{2+} MOBILIZATION IN *XENOPUS* OOCYTES. F.J. Monsma, Jr.[1], R.M. Burch[2]*, D.R. Sibley[1], & L.C. Mahan*. Laboratory of Cell Biology, NIMH, [1]Experimental Therapeutics Branch, NINDS/NIH, Bethesda, MD 20892 & [2]Nova Pharmaceutical Corp, Baltimore, MD 21224.

D_1 dopamine (DA) receptors have been classically defined as being coupled to activation of adenylate cyclase activity. We have examined the possibility that additional transduction pathways exist for D_1 receptors by using functional expression assays in *Xenopus* oocytes that have been injected with rat striatal poly (A+) RNA (mRNA). Defolliculated oocytes were assayed electrophysiologically 72 hr after injection with 50 ng of striatal mRNA. Application of 0.1 mM DA induced an inward current (40-100 nA) that was consistent with the activation of the endogenous oocyte Ca^{2+}-dependent Cl^- channels. This current could also be elicited by the addition of the D_1-selective agonist, SKF-38393 but not by the selective D_2 agonist, quinpirole. Prior addition of the DA antagonist cis-piflutixol completely abolished the DA-induced current but had no effect on the serotonin (5HT)-induced current which is also expressed from striatal mRNA. 0.1 mM DA was also found to stimulate $^{45}Ca^{2+}$ efflux by 2-3 fold in striatal mRNA-injected oocytes. This response was mimicked by SKF-38393 and blocked by the D_1-selective antagonist, SCH-23390. No efflux was observed with 0.1 mM quinpirole and the DA response was not blocked with 0.1mM of domperidone, a D_2-selective antagonist. Assay of size-fractionated striatal mRNA using the oocyte/$^{45}Ca^{2+}$ efflux assay revealed a single peak of DA-stimulated activity corresponding to an mRNA size of 2.5-3.0 kb. Oocytes which were injected with the peak mRNA fractions then prelabeled with *myo*[^{3}H]inositol, additionally showed a 4-fold increase in IP_3 production in response to DA. These data suggest the existence of CNS D_1 receptors which are coupled to PI turnover and Ca^{2+} mobilization as well as D_1 receptors which activate adenylate cyclase activity.

171.10

THE D1 DOPAMINE (DA) RECEPTOR DOES NOT REGULATE DA SYNTHESIS IN STRIATAL SLICES: ANOMALIES WITH SKF 38393. R.J. Brooderson F.J. White and M.P. Galloway. Depts. Pharmacol. & Psychiat., CCN Program., Wayne St. Univ. Sch. Med., Lafayette Clinic, Detroit, MI 48207.

DA synthesis is controlled, in part, by nerve-terminal D2 autoreceptors. In view of a recent report that D1 receptors may also exist on DA terminals and control membrane excitability (Diana et al. Neuropharmacol. 28, 1989), we sought to determine the existence of synthesis modulating D1 autoreceptors. In a rat striatal slice preparation possessing functional D2 autoreceptors, the D1 selective agonist SKF 38393 produced a concentration dependent (0.3-10 μM) decrease of NSD 1015-induced DOPA accumulation which was neither stereoselective nor blocked by the D1 antagonist SCH 23390. In contrast to SKF, CY-208-243 (0.03-10 μM) a non-catecholic D1 agonist produced only a weak inhibition of DOPA accumulation. This effect was blocked by the D2 antagonist eticlopride (3 μM), but not SCH 23390, suggesting D2 receptor involvement. Since the effects of SKF on DA synthesis do not appear to be D1 receptor-mediated and since catechols, such as SKF, directly inhibit tyrosine hydroxylase (TH), SKF may inhibit TH directly after entry into DA cells. However, nomifensine (3 μM), which blocks the DA transporter, blocked the effects of only the lowest concentration of (-)SKF, suggesting that SKF also enters DA cells through membrane diffusion. Thus, the effects of SKF on DA synthesis in vitro do not appear to be mediated through D1 receptors on striatal DA terminals. Supported by DA-4120, MH-41227 (MPG).

171.11

D-1 DOPAMINE STIMULATION OF cAMP ACCUMULATION IN COS-1 AND MOUSE L CELLS. M.E. Steffey*, R.W. Barrett, S.J. Fink, R.R. Bhatt*, E. Gomez* and R.G. MacKenzie. Neuroscience Research Division, Pharmaceutical Discovery, Abbott Laboratories, Abbott Park, Il 60064 and Dept. Neurology, Massachusetts General Hosp., Boston, MA 02114.

We have screened a variety of cell lines for D-1 dopamine responsiveness and report here that the COS-1 and mouse L cell lines have endogenous functional D-1 dopamine receptors as measured by cAMP accumulation. Cells in 24-well plates were washed with EBSS and pre-incubated for 10 min in EBSS containing 0.5mM IBMX. Following the addition of agonist or agonist plus antagonist, the cells were incubated for 15 min and cAMP accumulation was terminated by the addition of 0.1N HCl. The cAMP released from the cells was acetylated and measured by radioimmunoassay. Dopamine stimulates cAMP accumulation 8-fold above basal with an EC_{50} of 0.6μM in COS-1 cells and 3-fold above basal with an EC_{50} of 0.5μM in L cells. Moreover, both cells exhibit a D-1 pharmacologic profile for the stereoisomers of the agonists (-)-apomorphine > (+)-apomorphine and (+)-SKF-38393 > (-)-SKF-38393 and antagonists (+)-SCH-23390 > (-)-SCH-23388 and cis(Z)-flupenthixol > trans(E)-flupenthixol. The COS-1 cell also exhibits a pronounced β-adrenergic stimulation of cAMP accumulation (10-fold above basal, isoproterenol EC_{50} = 12nM) but this is clearly dissociable from the dopamine response by: (1) selective antagonists and (2) desensitization studies in which pre-incubation with dopamine or isoproterenol results in homologous desensitization of the dopamine or β-adrenergic response respectively.

171.12

DOPAMINE RECEPTORS LINKED TO ADENYLATE CYCLASE IN CULTURED ENDOTHELIUM DERIVED FROM HUMAN CEREBRAL MICROVESSELS. F. Bacic*, R.M. McCarron* and M. Spatz, (Sponsor: E. Streicher) Lab. of Neuropathology and Neuroanatomical Sciences, NINDS, NIH, Bethesda, MD 20892

Dopamine as a vasoactive substance can affect both peripheral and cerebral arteries. The contractile activity of dopamine is mediated by α-adrenergic and serotoninergic mechanisms while dilatation of the vessels is associated with D_1-receptors. This report will demonstrate the presence of dopamine D_1-receptors linked to adenylate cyclase (AC) activity in cultured endothelium derived from cerebral microvessels of human brain. A modified technique of Gerhart et al., (Brain Res., 21: 785, 1988) was used for isolation, separation and cultivation of dissociated cerebral microvascular endothelium. The cultured endothelium derived either from capillaries or small (<100 μM) or large (>100 μM) arterioles and venules stained positively for Factor VIII-related antigen and was negative for GFAP. AC activity of endothelium derived from small microvessels exhibited a dose-dependent response to dopamine ($EC=6x10^{-7}M$). All other fractions demonstrated significant but less responses to dopamine ($10^{-5}M$). D_1- but not D_2- antagonists [SCH-23390, RC(+) and sulpiride (S-), respectively] inhibited the dopamine stimulated formation of cAMP in small microvessels. These findings indicate the existence of dopaminergic D_1-receptors linked to AC in human cerebrovascular endothelium.

171.13

PHARMACOLOGICAL CHARACTERIZATION OF THE TWO RECEPTORS LABELED BY ^{3}H-SCH-23390 and ^{3}H-MESULERGINE IN HUMAN CHOROID PLEXUS. S.J. Boyson and L. O'Keefe. Depts. of Neurology and Pharmacology, Univ. of Colorado Health Sciences Center, Denver, CO 80262.

^{3}H-SCH-23390 and ^{3}H-mesulergine each label two sites in the choroid plexus of several species. The pharmacological characteristics of these sites were investigated in choroid plexus and in caudate-putamen (as a reference tissue) collected at post-mortem within 36 hours after death. Membranes were prepared and kept at -70°C until use. No patient had had recent exposure to neuroleptics. Membranes were incubated in 2 nM ^{3}H-SCH-23390 with a competing drug for 100 min, washed and collected by filtration. Assays were carried out in triplicate three times. Displacement by virtually all ligands revealed two sites. Drugs which discriminated two sites included D_1/D_2 antagonists (α-flupenthixol, fluphenazine), D_1-selective (NO-756 & SKF-81297), D_1/5-HT_{1c} ((R)-SCH-23390, (S)-SCH-23388, (R)-SKF-83566), 5-HT_{1c} (mesulergine), 5-HT_{1c}/5-HT_2 (ketanserin), and ß-adrenergic (propranolol). Displacement curves obtained by nonlinear regression analysis (PROPHET) revealed that the split of sites with all discriminating drugs was approximately 1:2 in the choroid plexus. The latter site is the 5-HT_{1c} receptor. Binding in the caudate-putamen was also examined. Correlation of the IC_{50} values obtained for the second site in the choroid plexus and the D_1 receptor in the caudate-putamen revealed that the receptor in the choroid plexus was not identical to the D_1 receptor in the caudate-putamen (r^2 = 0.4).

Supported by USPHS NS01195 and AG04418-0451.

171.14

D_1 DOPAMINE AGONIST INDUCES C FOS PROTEIN IN THE STRIATUM OF 6-OHDA-LESIONED RATS. R.A. Mueller, L.M. Grimes, H. Criswell, L.S. Carter*, W.C. McGimsey*, W. Stumpf, and G.R. Breese. Depts. of Anesthesiology, Cell Biology and Anatomy and BSRC, UNC-Chapel Hill, NC 27599.

Induction of the proto-oncogene c fos has been correlated with neural activation in selected brain regions (Science, 1988, 240:1328). The purpose of the present study was to examine the pattern of D_1 agonist induced c fos expression in rats treated as neonates to destroy nigrostriatal dopminergic neurons. Nine rats (3 controls, 6 lesioned) were given injections of SKF-38393 (3 mg/kg). Two hours later, rats were perfused with 4% paraformaldehyde and 0.2% glutaraldehyde. Serial Vibratome sections taken throughout the brain were stained for c fos, and tyrosine hydroxylase (TH). Staining for TH in the mesencephalic dopaminergic system in the lesioned rats revealed that most TH positive cells were absent with only a few cells surviving in the ventral tegmental area (VTA) and dorsal substantia nigra (SN). Expression of c fos was absent in control rats given SKF-38393. In lesioned rats, strong labeling of c fos was noted in the caudate (CP) with a dorsolateral to ventral medial gradient. No expression of c fos was observed in any site with even minimal sparing of TH containing fibers. These results suggest a sensitivity of c fos expression to the D_1 agonist in CP, but other neural systems involved in the behavioral responses do not respond with changes in c fos protein accumulation. (NS 21345 and HD 23042.)

171.15

EFFECTS OF BILATERAL ANTEROMEDIAL PREFRONTAL CORTICAL LESIONS ON DOPAMINE AGONIST INDUCED BEHAVIORS IN THE RAT. A.R. Braun, G.E. Jaskiw, R.H. Sexton, K. Vladar* and D.R. Weinberger, CBDB, NIMH Neurosciences Center at St. Elizabeths, Washington, D.C. 20032

The behavioral effects of apomorphine (APO) and of the selective D1 and D2 dopamine receptor agonists SKF 38393 (SKF) and LY 171555 (LY) were evaluated in rats that had received bilateral ibotenic acid lesions of the antero-medial prefrontal cortex (AMPC) (5 g/ 1 AP +3.5, ML +0.7, VD -3.5mm) and sham lesioned controls after a miminum 6 week recovery period. Lesioned (LES) animals treated with SKF 32 mg/kg were more active, expressed a greater variety of behaviors and displayed significantly more vigorous grooming (a behavior specifically elicited by D1 receptor stimulation). LES animals treated with LY 3 mg/kg displayed less non-stereotypic sniffing and grooming and more compulsive sniffing and oral stereotypy. In LES animals treated with the combination of LY and SKF, the frequency of weaker sniffing stereotypies was significantly decreased. These animals expressed more intense compulsive oral stereotypies, as did LES animals treated with 1.25 mg/kg APO. The induction of typical unconditioned behaviors by dopamine agonists does not appear to require the presence of an intact AMPC, but this cortical area may significantly modify the behaviors expressed. Lesions of the AMPC may result in increased sensitivity of D1 receptor associated mechanisms in subcortical areas or other regions of cortex.

171.16

IN VITRO INTERACTION OF L-PROLYL-L-LEUCYL-GLYCINAMIDE WITH DOPAMINE D_1 RECEPTOR AND ITS MODULATION OF D_1 RECEPTOR. S.K. Gupta*, R.L. Johnson*+ and R.K. Mishra. Neuropharmacol. Lab., McMaster Univ., Health Sciences Centre, Hamilton, ON, Can., L8N 3Z5; +Dept. of Medicinal Chem., Univ. of Minnesota, Minneapolis, MN, USA, 55455.

Earlier reports have demonstrated the modulation of CNS dopamine D_2 receptor by PLG and its active analogues. In the present abstract, we report the interaction of PLG with brain dopamine receptor D_1 receptor in vitro. PLG caused an increase in specific binding of [^{3}H]SKF-38393 to bovine striatal membranes in a dose-dependent manner, the maximum effect was observed at 1 µM. Scatchard analysis of [^{3}H]SKF-38393 revealed that there was no change in K_d, however, there was an appreciable increase (B_{max}). The competition curves [^{3}H]SCH-23390/SKF-38393 in the presence of 1 µM PLG displayed an increase in the B_{max} of high-affinity binding sites without an apparent change in K_d. Also, 1 µM PLG protected the high-affinity site even in the presence of 100 µM 5'-guanylylimidodiphosphate. These results demonstrate, in vitro, regulation of the dopamine D_1 receptor by either exposing the unmasked sites of the receptor or by changing the conformation of the receptor (supported by OMHF and NIH).

171.17

CHARACTERISATION OF THE DOPAMINE RECEPTOR MEDIATING THE ELECTRICAL RESPONSE OF THE COCKROACH SALIVARY GLAND TO NERVE STIMULATION. A.M.Evans* and K.L.Green*. (SPON: Brain Research Association). Dept. of Pharmacology, Edinburgh University, Edinburgh, EH8 9JZ, U.K.

Stimulation of the nerve supplying the cockroach salivary gland *in vitro* induces secretion, with an associated membrane hyperpolarisation which can be measured using intracellular microelectrodes. Previous studies (House, C.R., *Biol. Rev.*, 55:417, 1980) have shown that dopamine is the transmitter at this synapse. We have used selective dopamine agonists and antagonists to characterise the post-synaptic receptor involved. The effect of nerve stimulation is mimicked by dopamine applied by perfusion (5×10^{-8}M) or locally by pressure ejection from a broken microelectrode. Perfusion with either the DA_1 agonist SK&F38393 ($>10^{-6}$M) or the DA_2 agonist quinpirole, LY171555 ($\geq 10^{-4}$M) also induced a hyperpolarisation. The responses to nerve stimulation and to locally applied dopamine, SK&F38393, and quinpirole were inhibited following perfusion with the DA_1 antagonist (±)SCH23390 (10^{-5} to 10^{-4}M). The DA_2 antagonist (±)sulpiride ($\leq 10^{-4}$M) had no effect on the responsiveness of the preparation. It is concluded that the dopamine receptor mediating the membrane hyperpolarisation of cockroach acinar cells is similar to the DA_1 sub-type found in vertebrates.

Supported by the S.E.R.C.

171.18

THE RESPONSES OF SUBSTANTIA NIGRA PARS RETICULATA NEURONS TO GABA AND SYSTEMIC SKF 38393 IN 6-HYDROXYDOPAMINE-LESIONED RATS ARE REDUCED BY INTERMITTENT BUT NOT CONTINUOUS LEVODOPA ADMINISTRATION. B.G. Weick, T.M. Engber, Z. Susel*, T.N. Chase and J.R Walters. NINDS, Bethesda, MD 20892

The *intermittence* of levodopa (LD) administration has been implicated in the development of motor fluctuations in parkinsonian patients. Behavioral studies have indicated that *intermittent* LD administration but not *continuous* infusion of LD (adjusted to give a similar average daily blood level) abolishes the rotational response to systemic D-1 agonist SKF 38393 administration in rats with 6-hydroxydopamine-induced nigrostriatal dopamine pathway lesions (NSL). Since systemic SKF 38393 administration reduces firing rates of neurons in the substantia nigra pars reticulata (SNpr), a basal ganglia output nucleus, in NSL rats but not in unlesioned rats, we have examined the effects of *continuous* and *intermittent* LD on this response and on the sensitivity of these neurons to iontophoresed GABA. As in the behavioral experiments, we found that *intermittent* but not *continuous* LD abolished the response of SNpr neurons to SKF 38393, 10 mg/kg i.v. (1 ± 14% increase and 54 ± 11% decrease, respectively; no-LD NSL rats, 39 ± 11% decrease, n = 9-11). *Intermittent* but not *continuous* LD also reduced GABA-mediated inhibition in SNpr (30 ± 11% and 62 ± 11%, respectively; no-LD NSL rats, 67 ± 8%; unlesioned controls, 31 ± 9%, n = 9-16). These data support the behavioral observations indicating that the intermittence of LD administration is important in determining the response to dopamine receptor stimulation in this animal model of parkinsonism. The results further suggest that LD treatment schedule affects the tonic activity of GABAergic striatonigral neurons, inducing changes in GABA receptor sensitivity in the SNpr which contribute to the SKF 38393-mediated effects

172.1

BIOCHEMICAL AND ULTRASTRUCTURAL APPROACH OF DOPAMINE RECEPTORS IN THE RAT BRAIN USING ANTI-IDIOTYPIC DOPAMINE ANTIBODIES. N. MONS, M. GEFFARD, P. DUBOURG* and A. CALAS. Neuroimmunology, IBCN-CNRS. and *Neurobiology URA 339 CNRS, Bordeaux, FRANCE.

Alternative immunizations with poly- and monoclonal anti-dopamine (DA) antibodies allowed us to induce a highly anti-idiotypic response in rabbits. In order to remove anti-isotypic and anti-allotypic antibodies, the anti-idiotypic antibodies (Ab2) were affinity-chromatographed on mouse and rabbit non-specific immunoglobulins linked to cyanogen-bromide activated ACA-22 .Using ELISA tests, the capacity of Ab2 to bind to poly- and monoclonal idiotypic sites was evaluated by competition experiments. The Ab2 specifically recognized both poly-clonal and monoclonal anti-DA antibodies. Their ability to recognize DA receptors was demonstrated (1) by (3H)DA displacement and (2) by competition experiments between the Ab2 and different conjugates for the binding to membranes enriched with DA receptors. Our results demonstrate that these Ab2 were the internal images of conjugated DA. The Ab2 were used for ultrastructural preembedding immunocyto-chemistry on vibratome sections with paraformaldehyde fixation and PAP technique. In the striatum, most of the immunoreactive structures were post-synaptic thickenings on dendritic spines and more rarely on dendrites. Presynaptic labellings were very rare. In some cases the same terminal was in contact with two post-synaptic thickenings, only one of them being labelled. These results show that our anti-idiotypic antibodies can specifically recognize DA receptors and offer new perspectives for the structural and biochemical characterization of these receptors.

172.2

SELECTIVE UNILATERAL INACTIVATION OF STRIATAL DOPAMINE (DA) D1 OR D2 RECEPTORS BY N-ETHOXY-CARBONYL-2-ETHOXY-1,2-DIHYDROQUINOLINE (EEDQ): A NOVEL ROTATIONAL MODEL. O. Giorgi and G. Biggio. Dept. of Exp. Biology, Univ. of Cagliari, Italy.

The unilateral intrastriatal infusion of the irreversible DA receptor blocker EEDQ induces a significant decrease in the density of D1 (-48%) and D2 (-45%) DA receptors; in this experimental conditions, the systemic administration to rats of the selective D2 agonist LY 171555 results in ipsilateral rotations (Giorgi and Biggio, Soc. Neurosci. Abs. 14, 453, 1988). The selective D1 agonist SKF 38393 (10 mg/kg i.p.) fails to induce rotations "per se" but potentiates the circling behavior elicited by LY 171555. LY 171555 is unable to induce rotations in EEDQ treated rats following DA depletion by α-methyl-p-tyrosine, whilst the combined administration of LY 171555 and SKF 38393 elicits intense circling behavior in DA depleted rats. Finally, a selective inactivation of striatal D1 or D2 DA receptors can be obtained by the administration of EEDQ plus raclopride or EEDQ plus SCH 23390, respectively. LY 171555 elicits rotations in rats with a selective blockade of D2 DA receptors, but has no effect in animals in which D1 DA receptors have been selectively inactivated. These results support the view that D1 DA receptors play a permissive role in the expression of the motor effects mediated by striatal D2 DA receptors.

172.3

MOLECULAR BIOLOGICAL STUDIES OF D1 AND D2 DOPAMINE RECEPTORS. A.J. MacLennan*, W. Xu*, M. Khrestchatisky*, M.B. Jackson and A.J. Tobin (SPON: C. Sternini). Dept. of Biology, UCLA, Los Angeles, CA 90024

Adult, male Wistar rats were treated with haloperidol for 3 weeks at increasing doses of 2 to 4 mg/kg/day. Poly A RNA was isolated from their frontal cortex and striatum and then injected into Xenopus oocytes. Oocytes injected with striatal RNA responded to 10 uM dopamine (DA) and the D1 DA receptor agonist SKF 38393 (10 uM) when tested with the voltage clamp technique 5 to 8 days after injection. These DA-induced inward currents were significantly reduced by the D1 DA receptor antagonist SCH 23390 (10uM) and significantly increased by the D2 receptor antagonist sulpiride (100 uM). Oocytes injected with RNA transcribed from a cDNA library display "D1" receptor responses. In addition, we have identified three cDNA clones that hybridize at medium stringency to oligonucleotide probes which correspond to a published D2 DA receptor sequence (Nature 336: 783-787, 1988). This work was supported by a grant to AJT from the Scottish Rite Schizophrenia Research Program. AJM was supported in part by a Canadian MRC Fellowship.

172.4

CHARACTERIZATION OF DOPAMINE RECEPTORS IN AN IMMORTALIZED DOPAMINERGIC CELL LINE. M. Wainwright*, B.D. Perry, H.K. Choi, A. Heller, and P.C. Hoffmann. (SPON: J.E.G. Williams) Depts. Psych, Pharmacol/Physiol, Univ Chicago, Chicago, IL 60637

Immortalized dopaminergic (DA) cells were prepared by the fusion of fetal (embryonic day 18) rostral mesencephalic tegmental (RMT) cells with N18 TG2 neuroblastoma cells (Choi et al, Neurosci Abstr. 14, 319, 1988). 125I-SCH 23982 (SCH:D1) and 125I- or 3H-Spiperone (SPIP:D2) were used to label DA receptor binding sites. SCH specific binding, defined by (+)Butaclamol was saturable and reversible. Saturation studies obtained a Kd of 0.5 nM and a Bmax of 35 fmol/mg prot. Drug competition at SCH sites was consistent with that of a D1 receptor with (+)butaclamol > chlorpromazine >> haldol > serotonin. SPIP saturation studies obtained a Kd of 0.35 nM and a Bmax of 3-7 fmol/mg prot. DA stimulated adenylate cyclase (AC) activity was demonstrated and was greater in the presence of haldol suggesting functional coupling of both D1 and D2 sites to AC. These immortalized cells may prove useful for examining aspects of dopamine receptor regulation.

Support: MH-28942 and Brain Research Foundation

172.5

STRIATAL cAMP RELEASE IN-VIVO: EFFECTS OF SKF38393 AND SCH23390 P.H. Hutson and N. Suman-Chauhan. (SPON: E. Wong) MSD, Neuroscience Research Centre, Terlings Park, Harlow, Essex, UK. The technique of intracerebral dialysis has been used to monitor the extracellular concentration of striatal cAMP in response to activation of dopamine receptors by the selective D1 agonist SKF38393. Rats (250-350g) were anaesthetised with chloral hydrate (400mg/kg ip) and implanted with a dialysis probe in the striatum. The probe was perfused continuously at 2µl/min with Krebs Ringer pH 7.4 containing IBMX (1mM) and after 1h, serial 20min samples were collected for determination of cAMP by RIA (Amersham UK) All drugs were dissolved in Ringer containing IBMX and administered via the probe for 20 min (agonists) or 40 min (antagonists). In-vitro recovery of cAMP was 22 ± 5.6% 2µl/min (mean ± SEM, n=4). Basal values for striatal extracellular cAMP concentration in the anaesthetised rat were 16 ± 1 fmol/40µl (mean ± SEM, n = 38) SKF38393 (5, 10, 20, 100µM) dose-dependently increased cAMP. Administration, via the probe, of the selective D1 antagonist SCH 23390 completely prevented the increase of cAMP by SKF38393.

SKF38393	(10µM)	163 ± 11.6
"	" + SCH23390(10µM)	106 ± 15.6
"	" (100µM)	99 ± 6.2

Values are cAMP (means ± SEM as a % of basal, n = 5.)

These data demonstrate the potential for intracerebral dialysis in monitoring the consequences of post-synaptic DA receptor activation in-vivo.

172.6

CHARACTERIZATION OF THE GUINEA PIG STRIATAL DOPAMINE AUTORECEPTOR. E.A. Johnson*, C.E. Tsai*, J. Lucci*, A.J. Azzaro. Depts. of Neurology, Pharmacology and Psychiatry, West Virginia University, Health Science Center, Morgantown, WV 26506.

Autoreceptor regulation of dopamine synthesis has been well characterized in rat but not in guinea pig (GP). To further investigate GP as a potential model for human dopaminergic neurofunction, we characterized the GP striatal dopamine (DA) autoreceptor by measurement of the effects of selective DA agonists (D_1, SkF 38393; D_2, RU 24926) and antagonists (D_1, Sch 23390; D_2, Sulpiride) on GP striatal synaptosomal and free tyrosine hydroxylase (TH) activity, and in rat striatal synaptosomes. The D_2 agonist produced a dose-dependent inhibition of TH in both GP (IC_{50}=79 nM) and rat (ED_{50}= 96 nM) synaptosomes. This effect was antagonized by sulpiride. In contrast, the D_1 agonist produced dose-dependent inhibition of TH in both rat (IC_{50}=199 nM) and GP (IC_{50}=99 nM) synaptosomes which could not be blocked by Sch 23390. Inhibition of TH in GP was not due to direct effects on the enzyme, since both agonists were much less potent inhibitors of the free enzyme (RUZ4926, IC_{50}=447 uM; SkF 38393, IC_{50}=36 uM). The potent D_2 agonist-induced/D_2 antagonist-reversed inhibition of TH in GP striatal synaptosomes indicate that the GP DA autoreceptor is of the D_2 receptor subtype. The D_1 agonist results raise a possibility of a 3rd DA receptor subtype.

172.7

REGULATION OF DOPAMINE (DA) SYNTHESIS IN VIVO AFTER ACUTE OR CHRONIC AMPHETAMINE (AMPH). C.B.Tyler, M.J. Keegan* and M.P. Galloway, NPRU, Lafayette Clinic, Cell. & Clin. Neurobiol., Wayne State Univ., Psychiatry, SCH. MED.,Detroit, MI 48207

To examine the effects of AMPH on mechanisms regulating DA synthesis, AMPH (3 mg/kg) was administered to male rats at either 45 or 90 min prior to sacrifice. Some animals also received the D1 antagonist, SCH 23390 (0.5 mg/kg), or one of the D2 antagonists sulpiride (20.4 mg/kg) or eticlopride (0.25-5.0 mg/kg). AMPH initially increased DA synthesis in the striatum (ST) and olfactory tubercles (OT) but not in the nucleus accumbens (NA) or prefrontal cortex (PFC). Prior administration of either D1 or D2 antagonists enhanced both the magnitude and duration of the AMPH-induced increase. In the NA, AMPH decreased DA synthesis, an effect which was sensitive to prior treatment with D2 but not D1 antagonists. Neither D1 or D2 antagonists altered AMPH's effects in the PFC. Potential changes in DA autoreceptor (AR) sensitivity after chronic AMPH were examined in female rats treated with escalating doses of AMPH (1-10 mg/kg) for 30 days. Eight days later, the reversal by quinpirole (3-300 ug/kg) of GBL-induced increases in DA synthesis was examined. No differences in basal DA synthesis indicated a lack of neurotoxicity with AMPH treatment. In the posterior ST and NA, but not the anterior ST and OT, the effect of a low dose of quinpirole was enhanced by prior exposure to AMPH, relative to saline controls. Support: DA4120, MH41227, State of Michigan DMH.

172.8

D_1 AND D_2 AGONIST ACTIONS ON STRIATAL ADENYLATE CYCLASE ACTIVITY IN ADULT 6-OHDA-LESIONED RATS. H. Jurevics*, H. Criswell, R.A. Mueller and G.R. Breese. Depts. of Anesthesiology, Psychiatry, Pharmacology and the BSRC UNC-Chapel Hill, NC 27599.

Adult rats treated intracisternally with 6-OHDA show greater hyperactivity after systemic administration of quinpirole than after SKF-38393 (JPET 234:447, 1985). The present study examined if striatal adenylate cyclase activity (ACA) to these dopamine agonists is altered in 6-OHDA lesioned rats. Initially, crude homogenates of striatal tissue from control and lesioned rats were incubated with SKF-38393 (100 μM) or quinpirole (100 μM). The ACA to SKF-38393 was not altered by the 6-OHDA treatment. In the presence of 10 μM GTP, quinpirole decreased basal ACA in tissue from control but not 6-OHDA lesioned rats. By combining SKF-38393 and quinpirole, the increase in ACA to SKF-38393 was enhanced (85 $\pm$ 13% from 35 $\pm$ 7%; $p < 0.05$) in unlesioned rats but not in the lesioned rats (42 $\pm$ 5% from 47 $\pm$ 10%: $p < 0.1$). SKF-38393, forskolin (0.5 - 100 μM), NaF (10 mM) and Gpp (NH)p (10 μM) were as effective in increasing ACA in striatum from 6-OHDA lesioned rats as in control rats indicating that adenylate cyclase and G protein coupling to the enzyme are not altered by the lesion. However, these data suggest that coupling of the D_2 receptor to the enzyme complex is impaired after 6-OHDA treatment. (Supported by NS 21345 and HD 23042.)

172.9

DOPAMINE (DA) RECEPTOR SUPERSENSITIVITY AFTER 6-OHDA: GENERATION OF A RECEPTOR RESERVE FOR REGULATION OF STRIATAL CHOLINERGIC ACTIVITY. A. Enz,* M. Goldstein and E. Meller. Preclinical Research, Sandoz, Ltd., Basel, Switzerland and Dept. of Psychiatry, NYU Medical Center, New York, NY 10016.

Unilateral denervation of the nigrostriatal DA pathway with 6-OHDA resulted in a supersensitive response for N-propylnorapomorphine (NPA)-induced elevation of ACh levels, seen as a 4-fold leftward shift in the dose-response curve (ED50: 8.8 and 2.2 ug/kg, intact and denervated sides, respectively), without a change in the maximum response. On the intact side, irreversible DA receptor inactivation with N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) depressed the maximal response (to 48% of control maximum), without altering the ED50, while it caused a smaller depression in maximal response (to 76% of corresponding vehicle control), as well as a small rightward shift in ED50, on the denervated side. Response was a linear function of receptor occupancy in the intact striatum, indicating the absence of receptor reserve, whereas it was nonlinear in the denervated striatum; a small receptor reserve of 25-30% was estimated to be present. 6-OHDA-induced supersensitivity appears to reflect the generation of a postsynaptic D2 receptor reserve, which may account for the observation that weak partial agonists elicit response at supersensitive DA receptors but not at normosensitive receptors devoid of spare receptors. The effects of 6-OHDA denervation on response elicited by weak partial DA agonists will also be presented. Supported by NS 23618, NS 08601 and MH 35976.

172.10

POSTMORTEM STABILITY OF MONOAMINES AND NEURORECEPTOR BINDING. M. Al-Tikriti, R. H. Roth, R. B. Innis. Depts. Psychiatry, Pharmacol., & Neuroanatomy, Yale Univ. Sch. of Med., New Haven, CT 06510.

Research on monoamine and receptor binding levels in postmortem human brain tissue depend upon detailed knowledge of the effects of the postmortem interval, tissue preparation, and storage on all outcome measures.

We have used a rat model of the autopsy process, in which rats are sacrificed and the heads cooled following the known temperature curve of the human brain after death. Six animals for each of 7 postmortem timepoints (up to 27 hr) were examined. Samples from multiple brain regions were frozen and later assayed for monoamines (DA, NE, and 5-HT) and neuroreceptors (D1, D2, 5-HT1A, 5-HT2, and CCK).

Preliminary analysis of the data show that both caudate DA and cortical NE levels decrease to approximately 50% within a 3 hr postmortem delay, then further decline to approximately 30% at 9 hr and maintain this concentration until the final 27 hr timepoint. If the frozen samples were thawed even for just a few minutes and then refrozen, DA levels were markedly depleted.

Homogenate receptor binding was performed with 3H-raclopride (D2), 3H-SCH23390 (D1), 3H-8-OH-DPAT (5-HT1A), 3H-ketanserin (5-HT2), and 3H-cholecystokinin (CCK). Except for CCK, receptor binding levels were showed significant postmortem stability, with typically less than 20% decrease at 27 hr postmortem. CCK receptor binding decreased to approximately 50% within 3 hr and maintained this level through the final 27 hr timepoint. The effects of both a single freezing and a thawing/refreezing were insignificant for all five receptors.

These studies support the feasibility of human postmortem neurochemical and receptor binding studies but demonstrate the need for careful tissue handling (not allowing thawing of samples for neurochemical measurements) and for controlling postmortem delay (especially for monoamine measurements and the CCK receptor).

Supported in part by the Yale Mental Health Clinical Research Grant MH-30929 and the Center for Neuroscience and Schizophrenia MH-44866.

CATECHOLAMINE RECEPTORS: ADRENERGIC

173.1

POSTNATAL DEVELOPMENT OF PRE- AND POSTSYNAPTIC ALPHA$_2$ RECEPTORS IN RAT SPINAL CORD. V.C. Gandhi and D.J. Jones (SPON: L. Bunegin). Dept. of Anesthesiology, UTHSC, San Antonio, TX 78284-7838

The establishment of norepinephrine (NE) - mediated events in synaptic transmission requires concomitant development and maturation of the pre- and postsynaptic elements of noradrenergic terminals. Previous studies have established that in spinal cord alpha$_2$ receptors regulate processes at both sites including the inhibition of NE release and inhibition of NE-stimulated cyclic AMP (cAMP) accumulation. The purpose of the present study was to determine if a correlation exists in the postnatal development of pre- vs postsynaptic alpha$_2$ receptors in rat spinal cord.

K^+-induced [^{3}H]-NE release and [^{3}H]-NE uptake were measured on PND 1 to 60. Significant NE release as well as uptake was observed at birth. NE inhibited [^{3}H]-NE release in a concentration-dependent manner PND 1-60. Maximum inhibition with 1 uM NE of [^{3}H]-NE release occurred at PND 7 (55%) with a subsequent decrease on PND 12 to adult levels (30%). This decrease in sensitivity may be due to a decrease in the number of functional noradrenergic terminals since [^{3}H]-NE uptake also decreased after maximal levels at PND 5.

cAMP accumulation stimulated by NE is a postsynaptic event which is inhibited by alpha$_2$ receptor agonists such as clonidine. Clonidine (1 uM) inhibited NE-stimulated cyclic AMP accumulation 30-40% up to PND 7 with a decrease to 20% at PND 12 and thereafter. The results suggest that the sensitivity of pre- and postsynaptic alpha$_2$ receptors appear to develop in spinal cord in parallel postnatally.

Supported by NINCDS 14546.

173.2

ALPHA 2-BINDING SITES IN THE MEDULLA OBLONGATA OF TREE SHREWS. G. Flügge, S. Schiller* and E. Fuchs. Dept. of Reproductive Biology, German Primate Center, Kellnerweg 4, 3400 Göttingen, FRG.

Alpha 2-adrenoceptors are supposed to be primarily located presynaptically on catecholaminergic fibers (Starke, K., Rev. Physiol. Pharmacol., 107:73-146, 1987) but in the rat brain, they could not be found in all areas revealing PNMT-immunopositive varicosities or cells. We investigated the distribution of ^{3}H-rauwolscine binding sites in the tree shrew medulla oblongata because the anatomy of the medullary adrenergic system of this species resembles that of primates (Mittendorf, A. J. Comp. Neurol., 274:178-189, 1988).

In contrast to the rat, not only the NTS and the nX but also the nXII, part of the n. reticularis parvocellularis, and the area of the adrenergic group C1 were distinctly labeled. Scatchard analysis revealed one type of high affinity binding sites in each nucleus (K_d 0.6 to 1.3 nM) although association/dissociation experiments indicated the existence of high and low affinity binding sites. Competition experiments demonstrated that ^{3}H-RAUW bound to α2-adrenoceptors. In the nXII and the area of C1, ^{3}H-RAUW obviously interacted with dopamine binding structures.

In summary, in the tree shrew medulla oblongata there are α2-receptors with higher affinity for ^{3}H-rauwolscine than in the rat. Furthermore, α2-adrenoceptors in the ventrolateral medulla can be quantified and pharmacologically characterized. In this region, α2-sites have been observed in the human medulla but not in the rat.

173.3

COMPARISON OF THE BINDING CHARACTERISTICS OF α_2-ADRENERGIC RECEPTORS IN RAT BRAIN AND KIDNEY. R. Dawson, Jr. and D.R. Wallace. Dept. of Pharmacodynamics, Univ. of Florida, Gainesville, FL 32610.

The aim of the present study was to examine the binding characteristics of renal versus cerebral cortical α_2-adrenoceptors in the rat. α_2-Adrenoceptor binding was evaluated using either [^{3}H]p-aminoclonidine (PAC), [^{3}H]idazoxan (IDZ) or [^{3}H]rauwolscine (RAU). Crude homogenates or subcellular fractions were used to assess binding in whole kidney or cerebral cortical homogenates from Sprague-Dawley rats. The ratio of specific kidney/brain binding is given below.

Ligand	Crude Homogenate	P_1	P_2
PAC (2nM)	0.32±0.03 (7)	2.0 (2)	0.31±0.06 (7)
IDZ (1nM)	0.30±0.02 (5)	2.0 (2)	0.36 (2)
RAU (2nM)	2.90±0.63 (5)	6.8 (2)	0.97±0.10 (5)

ratio = dpm/mg protein kidney÷dpm/mg protein brain. (n)

Prazosin (1.0μM) displaced 12% of the specific PAC (2nM) binding in brain but 83% of RAU binding. PAC (2nM) binding in kidney was inhibited 60% and RAU was 98% displaced by prazosin (1.0μM). Magnesium stimulation of PAC binding was similar in brain and kidney but GTP inhibition of PAC and IDZ binding was 1.6 times greater in kidney than brain. Amiloride inhibition (IC_{50}) of [^{3}H]RAU binding was comparable in brain (17±5μM) and kidney (10±1μM). Thus, differences exist in brain and renal α_2-adrenoceptors that may be explained by the high numbers of α_2-B subtype in the kidney, whereas the α_2-A subtype is numerous in rat cerebral cortex.

173.4

DEVELOPMENTAL CHARACTERIZATION AND TISSUE DISTRIBUTION OF A RAT ALPHA-2 ADRENERGIC RECEPTOR SUBTYPE. S.K. McCune, D.E. Brenneman and M.M. Voigt, Lab. of Developmental Neurobiology, NICHD and NINDS, NIH, Bethesda, MD 20892.

Alpha-2 adrenergic receptors are involved in numerous physiologic functions. In the brain, a role for norepinephrine has been implied in depression, Parkinson's disease and Alzheimer's disease. Using oligonucleotide probes directed against the third and fourth transmembrane regions of the human platelet α_2 adrenergic receptor, a rat genomic clone encoding an α_2 receptor was isolated and used to study the developmental expression of an α_2 adrenergic receptor subtype. Riboprobe complementary to sequence in the coding region was used to probe Northern blots. A 3.2kb species was present in brain and to a lesser degree in kidney. The message was barely detected in heart but not in liver, lung or pancreas. Using mRNA from embryonic day 15 (E15), embryonic day 18 (E18), newborn, postnatal day 8 and adult rat brains, it was shown that the 3.2kb message is developmentally regulated. It was also shown that a 1.8kb species which strongly cross-hybridizes to the probe was present only in prenatal life, while a 2.5kb species was expressed only postnatally. The cloning and characterization of the two smaller mRNAs are in progress.

173.5

AUTORADIOGRAPHIC, KINETIC, AND EQUILIBRIUM CHARACTERIZATION OF BRAIN ALPHA-2 ADRENERGIC RECEPTORS USING A NEW RADIOLABELED AGONIST, [^{125}I]P-IODOCLONIDINE. B.W. Siegel*, B.M. Baron, J.A. Miller. Merrell Dow Research Institute, Cincinnati Ohio, 45215.

[^{125}I]p-Iodoclonidine ([^{125}I]PIC, a novel ligand generously provided by Dr. R. Garlick of DuPont NEN) binding was measured in well-washed membranes obtained from rat hippocampus + cortex. [^{125}I]PIC bound to a single class of saturable binding sites with Kd = 0.55 nM and Bmax = 230 fmoles/mg protein. Binding was > 90 % specific at 0.6 nM [^{125}I]PIC. IC_{50} values for UK 14,304, epinephrine, BHT 920, phentolamine, and idazoxan were 3, 13, 17, 21, and 21 nM, respectively. [^{125}I]PIC binding was sensitive to guanine nucleotides with rank potency GTPγS > GDPβS > GppNHp ~ GTP ~ GDP > GMP >> ATP. Association was monophasic and complete by 90 min. at 25°C. Dissociation was biphasic with 30 % of the sites dissociating rapidly (K_1 = 0.32 min.$^{-1}$) and the remainder dissociating 50-fold slower (K_2 = 0.0061 min.$^{-1}$).

Quantitative autoradiography was performed in sagittal sections of rat brain and cross sections of spinal cord. The highest levels of binding were found in cortical, hippocampal, hypothalamic, and cerebellar regions. Low levels of binding were found in spinal cord gray matter where cervical, thoracic and lumbar regions demonstrated higher densities than sacral gray matter. Binding in all these regions was heterogeneous and ranged from 1.4 fmol/cm^2 (sacral gray) to 7.42 fmol/cm^2 (cortex).

173.6

MOLECULAR MODELLING OF RECEPTOR PROTEINS: G-PROTEIN COUPLED RECEPTORS. V.B.Cockcroft*, B.Brewster*, G.G.Lunt, Molecular Neuroscience Group, Biochemistry Dept., University of Bath, BATH BA2 7AY U.K.

We previously produced a detailed molecular model of the nAChR and proposed some features that may be common to members of the direct ligand-gated superfamily. We now extend our receptor modelling to members of the G-protein coupled superfamily. Aligned sequences in conjunction with published data, were used to construct a model of the human β-1 adrenergic receptor. Helices (TM1 to TM7) were arranged in the membrane with conserved and polar residues facing the binding pocket. It is proposed that receptor activation takes place through a charge relay mechanism: the docking of an agonist's positively charged amine group onto the conserved aspartate of TM2 causes rearrangement of existing through-protein charge interactions. These involve electrostatic interactions between the conserved aspartate of TM2, and a salt bridge between the conserved aspartate of TM3 and the conserved arginine of TM4. This model serves as a good starting point for modelling of other members of the superfamily.

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173.7

MEASUREMENT OF EXTRACELLULAR cAMP IN THE BRAIN BY MICRODIALYIS: EXTENSION TO UNANESTHETIZED RATS. E.A. Stone and S.M. John*, Dept. Psychiatry, New York University Sch. Med., New York, NY 10016.

We have previously shown that cAMP in the extracellular fluid of the rat brain can be detected by microdialysis sampling with RIA. All experiments so far have utilized anesthetized (urethane) rats only. In the present studies therefore we have applied the technique to nonanesthetized animals. Rats were implanted under pentobarbital anesthesia with 3.5 mm dialysis probes in the prefrontal cortex. Twenty-four hrs later the animals were tested for cAMP recovery at a perfusion rate of 1 ul/min. Baseline cAMP values in the awake animals were found to average 6.0 ± 2.2 (SD) fmol/20 min (n=15) and were relatively stable during 5 hr perfusions between 10-1500 hrs. Several animals showed higher values (15-20 fmol/20 min) which may have resulted from local inflammation near the probe. Inhibition of phosphodiesterase with rolipram produced a dose-dependent rise in cAMP levels. Infusion of norepinephrine (10^{-4} M) to stimulate brain beta adrenoceptors produced 3-10 fold increases in cAMP levels. The response of unanesthetized rats appeared greater than anesthetized animals. The results indicate that the microdialysis-cAMP method can be successfully applied to unanesthetized animals and may be a useful technique to study brain receptor function in vivo during behavioral states. Supported by MH44442 and Air Force grant 89-0208.

173.8

SEROTONERGIC NEURONS ARE NOT NECESSARY FOR AGONIST-INDUCED REGULATION OF β-ADRENOCEPTORS (BARs) WHEN ^{125}I-IODOPINDOLOL (^{125}I-IPIN) IS THE RADIOLIGAND. G.A.Ordway[1]*, C.Gambarana[1], J.Hensler[2], A.Frazer[2]. [1]Case Western Res. Univ, Cleveland, OH; [2]Univ Penn & VAMC, Phila, PA 19104.

Following lesion of serotonergic neurons with 5,7-dihydroxytryptamine (5,7-DHT), chronic administration of desipramine (DMI) to rats does not result in down-regulation of BARs as measured using ^{3}H-dihydroalprenolol (^{3}H-DHA). Our initial saturation experiments indicated that in cortical homogenates ^{3}H-DHA bound specifically to two sites whereas ^{125}I-IPIN bound to a single site. 5,7-DHT treatment did not alter the Bmax or K_d of ^{125}I-IPIN binding. The binding of ^{3}H-DHA was significantly increased in cortical homogenates from 5,7-DHT-treated rats. Chronic administration of DMI produced a significant decrease of comparable magnitude in the density of BARs in cortical homogenates from either sham or 5,7-DHT treated rats when ^{125}I-IPIN was the radioligand. Subtypes of BARs were measured by quantitative autoradiography using ^{125}I-IPIN in experiments done to determine the effect of isoproterenol (ISO; 15μg, icv) in sham and 5,7-DHT treated rats. 5,7-DHT did not significantly alter the ability of ISO to decrease binding of ^{125}I-IPIN to either β_1 or β_2 receptors in any brain region examined. From these data, it may be inferred that the absence of serotonergic neurons does not influence the regulation of BARs when ^{125}I-IPIN is the radioligand. **(Supported by Vet. Adm. & USPH grants MH29094 & MH14654.)**

173.9

DEVELOPMENT OF BETA-ADRENERGIC RECEPTORS: COMPARISON OF ADULT, NEONATAL AND FETAL RAT HEART. A. Torres*, L.E. Crawford*, K.E.J. Dickinson* and D.C. Tucker (SPON:E. Taub). Departments of Psychology and Gastroenterology, University of Alabama at Birmingham, Birmingham, AL 35294.

Beta-adrenergic receptors (β-AR) were studied in fetal, neonatal and adult rat heart. Preliminary studies with iodopindolol detected beta-adrenergic receptors at E-12 and E-13. Receptors were assayed in crude homogenates from ventricles of adult and neonatal rats (<24 hrs old) and from whole hearts of fetuses (E-17) with iodocyanopindolol as the radioligand and alprenolol as the nonspecific displacer. Scatchard analysis indicated similar affinities at all ages with receptor number ordered: newborn > adult > fetal (K_D= 38, 30, 45 pM; B_{max}= 51.1, 36.6, 29.4 fmol/mg protein for newborn, adult, E-17, respectively). Displacement studies with D- and L- propranolol verified that the assay was selective for β-AR. A slight but measureable shift in isoproteronol displacement with the addition of guanosine triphosphate (GTP) was demonstrated at all ages, indicating that the receptors were able to couple to G-proteins and, thus, are likely to be functional. These findings suggest that β-AR number is higher in newborn than in adult heart and that these receptors could respond to circulating catecholamines. How efficiently the receptors mediate changes in beating rate or force of contraction is a subject for future study.

173.10

MOLECULAR CLONING AND EXPRESSION OF THE RAT β_1-ADRENERGIC RECEPTOR GENE. C.A. Machida[1], J. Bunzow[2]*, R.P. Searles[1]*, H. Van Tol[2], P. Teal[1]*, K. Neve[3], and O. Civelli[2]. [1]Division of Neuroscience, Oregon Regional Primate Research Center, Beaverton, OR 97006, [2]Vollum Institute for Advanced Biomedical Research, Oregon Health Sciences University, Portland OR 97201, and [3]Veterans Administration Medical Center, Portland, OR 97201.

The β-adrenergic receptors (β_1- and β_2-ARs) regulate a wide variety of physiological processes through their interactions with catecholamines. To begin analyzing the differential expression of the β-AR subtypes, we cloned the rat β_1-AR gene and expressed these receptors by transfection into β-AR deficient mouse L cells. Nucleotide sequence analyses of the coding region of the rat β_1-AR gene show that it encodes a 463 amino acid protein, with 1 potential Asn-X-Ser(Thr) glycosylation site. The primary amino acid sequence of the rat β_1-AR shows a high degree of similarity with both the human β_1- (88%) and hamster β_2-ARs (43%). A potential 90 bp intron is observed in the 3' nontranslated region of the rat β_1-AR gene. Blot analyses of RNA show high concentrations of β_1-AR mRNA in pineal, heart, and lung, with lower levels in thalamus, amygdala, septum, and hippocampus. Analyses of the transfectant's genomic DNA, mRNA, and β-AR binding characteristics ([^{125}I]-iodocyanopindolol binding and rank order potency of adrenergic agonist displacement) clearly demonstrate expression of the β_1-AR gene and receptor subtype.

SECOND MESSENGERS II

174.1

LITHIUM-SELECTIVE ALTERATION OF BRAIN G_S PROTEIN FUNCTION. S. Avissar,* G. Schreiber,* C.S. Aulakh,* and D.L. Murphy* (SPON: P.A. Newhouse). LCS, National Institute of Mental Health, Bethesda, MD 20892.

We recently demonstrated that lithium inhibits the coupling of both muscarinic cholinergic receptors and ß-adrenergic receptors to pertussis toxin-sensitive and cholera toxin-sensitive G proteins, respectively, thus suggesting lithium alteration of G protein function as the single site for both the antimanic and antidepressant effects of this drug (Nature 331:440-442, 1988). One of the most puzzling aspects of the ability of lithium to ameliorate the manic-depressive condition is its relatively selective action upon the CNS.

In the present study, we show that lithium selectively attenuates the function of G_S proteins in the CNS assessed through isoproterenol-induced increases in GTP binding to these proteins. *In vitro* therapeutic lithium concentrations (1-1.5 mM) inhibit G_S protein function in rat cerebral cortex, while 4- to 6-fold higher concentrations of lithium are required to alter G_S protein function in rat cardiac ventricles. *In vivo* chronic lithium treatment (rat chow containing lithium carbonate) to rats totally abolished isoproterenol effect in the CNS but did not affect peripheral cardiac G_S protein function. Lithium-selective action of CNS G_S protein function may stem from the heterogeneity of the α_S subunit proteins: in the heart, the major species is a 45 kDa molecule, while in the brain, a 52 kDa molecular weight species predominates. The heterogeneity in α_S subunits may thus be the biochemical basis for lithium-selective action on the CNS and for the scarcity of peripheral side effects.

174.2

ANTIDEPRESSANT DRUGS ALTER ß-ADRENERGIC RECEPTOR COUPLING TO G_S PROTEIN G. Schreiber,* S. Avissar,* C.S. Aulakh,* and D.L. Murphy* (SPON: A.M. Mellow). LCS, National Institute of Mental Health, Bethesda, MD 20892.

Our recent findings on the alteration by lithium of brain G protein function coupled to both ß-adrenergic and cholinergic receptors suggest G proteins as a single site for both therapeutic effects of lithium (Nature 331: 440-442, 1988).

In the present study, the effects of chronic treatment with imipramine, clomipramine (tricyclic antidepressants), and clorgyline (a selective MAO type A inhibiting antidepressant) on the coupling of both muscarinic cholinergic receptors and ß-adrenergic receptors to pertussis toxin-sensitive and cholera toxin-sensitive G proteins was investigated. Imipramine (5 mg/kg/day), clomipramine (5 mg/kg/day), clorgyline (1 mg/kg/day), or saline was subcutaneously administered continuously by means of osmotic minipumps to male Wistar rats. Three-week treatment with these antidepressants resulted in ~50% inhibition of isoproterenol-induced increases in GTP binding to rat cerebral cortex G_S protein, without affecting muscarinic receptors coupling to G proteins. Studies are currently in progress to determine whether these findings on the uncoupling of the ß-adrenergic receptor from G_S protein and ß-adrenergic receptor desensitization both induced by antidepressant treatment are independent phenomena.

174.3

Quantitation of GTP-Binding(G-)Protein mRNA In Rat Brain Using InSitu Hybridization: Thomas Akompong*,J.A.Angulo & B.S.McEwen. (SPON.Maureen Gannon) Lab,Neuroendocrinology The Rockefeller University.New York,NY 10021

The G-proteins are universal intermediaries coupled to different receptors and effectors in several signal transduction systems. The distribution of two G-proteins G_i and G_s,were determined using ^{125}I oligonucleotide probes of the α-subunits of these G-proteins by in insitu hybridization. The specificity of the probes was established by competition with 30x excess cold probe. The cortex has more mRNA for G_i and G_s than the striatum. The dentate gyrus and the habenular nucleus have higher G_s and G_i mRNA compared with the cortex and pyramidal cells of CA1-CA3 of the hippocampus, which have equal mRNA for G_i and G_s as compared to the cortex. We also studied the in vivo modulation of the mRNA levels for these G-proteins by estradiol and progesterone in ovariectomized female rats. Preliminary results show that these hormones affect the levels of G_s and G_i in the striatum as well as the hippocampus.

174.4

DOPAMINERGIC AND NORADRENERGIC NEURONS EXPRESS MULTIPLE G PROTEIN mRNAs IN THE RAT BRAIN. S.L. Drinnan, B.T. Hope, P.B. Reiner and S.R. Vincent, Kinsmen Laboratory of Neurological Research, Dept. of Psychiatry, Univ. of British Columbia, Vancouver, Canada, V6T 1W5.

The guanine nucleotide binding family of proteins (G proteins) transduce extracellular signals into intracellular events. G_i, G_o and G_s are members of a group of heterotrimeric G proteins found in the brain which affect membrane conductances and second messenger systems. The expression of mRNAs coding for the alpha subunits of these three G proteins was examined in rat brain by *in situ* hybridization using radiolabelled synthetic oligonucleotide probes. Northern blot analysis indicated that the three probes hybridized specifically to mRNA species corresponding in size to those for the alpha subunits of G_i-2, G_o and G_s. The three probes showed widespread and unique distributions throughout the brain. Neurons in various regions known to contain aminergic neurons were labelled. Therefore, dopaminergic substantia nigra neurons and noradrenergic locus ceruleus neurons were identified immunohistochemically and subsequently processed for *in situ* hybridization. The results demonstrate that identified catecholamine neurons in both cell groups express mRNAs coding for all three G-protein alpha subunits investigated. This is consistent with physiological data indicating a role for G proteins in receptor and drug actions in SN and LC neurons.

174.5

DEVELOPMENTAL EXPRESSION OF Go-ALPHA IN PRIMARY NEURONAL CULTURES. J. G. Granneman and G. Kapatos. Laboratories of Biochemical Pharmacology and Neurochemistry, Center for Cell Biology Sinai Res. Inst. and Clin. Cell. Neuroscience Prog., Wayne State Univ., Detroit, MI 48235.

Guanine nucleotide binding proteins (G-proteins) play a central role in the transduction of receptor generated signals. Go is the most abundant G-protein in the central nervous system, where it has been localized in both neurons and in glia. We have characterized the developmental expression of the alpha subunit of Go in neurons by examining mesencephalic (MES) and hypothalamic (HYP) cultures that are essentially free of contaminating glia. Immunoblotting utilizing specific antisera against Go indicates that in neurons from both brain regions, membrane concentrations of Go increase dramatically during the first two weeks in vitro. Thereafter increases in the amount of Go per neuron kept pace with increasing process (axons and dendrites) formation. MES and HYP neurons maintained in culture for 4 days contained multiple molecular forms of Go whereas neurons maintained for two weeks appeared to contain only one form. Finally, increasing neuron density significantly increased membrane levels of Go in MES but not HYP cultures.

Supported by PHS grants DK37006, NS26081, and Sinai Research Inst.

174.6

GLUCOCORTICOID REGULATION OF ADP-RIBOSYLATION FACTOR (ARF) AND ENDOGENOUS ADP-RIBOSYLATION IN RAT CEREBRAL CORTEX. J.A. Clark, R.Z. Terwilliger*, E.J. Nestler, and R.S. Duman. Laboratory of Molecular Psychiatry, Depts. of Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06508.

ADP-ribosylation factor (ARF) is required for cholera toxin-catalyzed ADP-ribosylation of the stimulatory G-protein (Gsα) *in vitro*. While ARF constitutes up to 1% of brain protein, little is known about the regulation or function(s) of this factor *in vivo*. Previously, we reported that Gsα is under hormonal regulation in cerebral cortex (CTX), exhibiting a 30% increase in message and protein following one week exposure to corticosterone (CORT). These findings raised the possibility that ARF may be similarly regulated by hormonal treatment. ARF message in CTX was regulated by CORT, which produced a 50 to 100% increase in mRNA levels. Immunoblot studies are underway to determine the effect of CORT treatment on levels of the protein.

Given that Gsα and ARF are similarly regulated by CORT, we were prompted to look for a possible physiological function for ARF by studying endogenous ADP-ribosylation. ADP-ribosylation assays, performed on CTX homogenates in the absence of cholera toxin, revealed the endogenous ADP-ribosylation of two proteins corresponding to the predominant bands labeled in the presence of cholera toxin, presumably the 45 and 52kD forms of Gsα. This is the first demonstration of endogenous ADP-ribosylation in brain. Furthermore, this endogenous ADP-ribosylation was enhanced 40% by one week of CORT treatment. Together, these findings, in addition to preliminary studies demonstrating a differential regional distribution and developmental expression of this factor in brain, suggest that ARF may play a physiological role in the regulation of signal transduction.

174.7

THE GTP-BINDING PROTEIN, G_o, COUPLES MUSCARINIC RECEPTORS TO PHOSPHOLIPASE C IN *XENOPUS* OOCYTES. T.M. Moriarty, E. Padrell*, G. Omri, R. Iyengar* and E.M. Landau. Depts. of Psychiatry and Pharmacology, Mt. Sinai School of Medicine and Dept. of Psychiatry, Bronx V.A. Medical Center, New York, N.Y.

GTP-binding proteins (G-proteins) couple cell surface receptors to cellular effectors. Receptors activate the heterotrimeric G-protein and cause the dissociation of α from βγ subunits. Activated α subunits modulate effector function. Much evidence suggests that G-proteins serve to couple receptors to phospholipase C with resulting release of the second messengers IP_3 and DAG. In this study we sought to identify which of the known G-proteins might couple receptors to phospholipase C. *Xenopus* oocytes have a well characterized electrophysiological response to acetylcholine (ACh): receptor activation stimulates the production of IP_3, mobilization of Ca^{2+} and subsequent activation of Ca^{2+} dependent Cl^- channels. We have shown that the receptor evoked Cl^- current is sensitive to pertussis toxin (PTX) and is also modulated by the βγ subunits of G-proteins. We therefore tested the known PTX sensitive G-proteins, G_o, G_{i1}, G_{i2} and G_{i3}, for potential function in coupling receptors to the IP_3 mediated Cl^- current. G_o reconstituted the response to ACh in cells pretreated with PTX whereas G_{i3} was ineffective. G_o also augmented the response to ACh whereas G_{i1}, G_{i2} and G_{i3} did not. Preliminary experiments using GTPγS activated α subunits suggest that the α subunits of G_o, when directly injected into voltage-clamped oocytes, evoke a Cl^- current as does direct injection of IP_3. These results, in view of the report of a cDNA clone of α_o from *Xenopus* oocytes (Olate *et al.*, *FEBS Letts.* **244**:188-192, 1989), indicate that G_o couples muscarinic receptors to phospholipase C.

174.8

STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF TWO FORMS OF THE BOVINE BRAIN GTP-BINDING PROTEIN G_o. E. Padrell*, T.M. Moriarty, D.J. Carty*, E.M. Landau and R. Iyengar* (SPON:J. Goldfarb). Depts. of Pharmacology and Psychiatry, Mt. Sinai School of Medicine and Dept. of Psychiatry, Bronx V.A. Medical Center, New York, N.Y.

Heterotrimeric GTP-binding proteins (G-proteins) serve as coupling elements in many signal transduction systems. The most abundant brain G-protein, G_o, has an α subunit of Mr=39000 and is a pertussis toxin substrate. Sequence specific antiserum U-46 identifies recombinant α_o, but not α_{i1}, α_{i2} and α_{i3} protein. The antiserum U-46 recognized two distinct peaks of α_o eluting early and late on Mono Q columns using FPLC. The early peak was termed G_{o2} and the more abundant late peak was called G_{o1}. Both G_{o1} and G_{o2} were uniquely recognized by α_o-specific antiserum U-46 and not by α_i-specific antisera. Both α_{o1} and α_{o2} have similar molecular weights, co-purify with stoichiometric amounts of βγ-subunits and are pertussis toxin substrates. Both forms of α_o migrate distinctly on 9% SDS-polyacrylamide gels containing 4-8 M urea gradients. While α_{o1} and α_{o2} are recognized by an α_o C-terminus sequence specific antiserum, the N-terminus of α_{o2} appears to be different from that α_{o1} or the α_i proteins since the antiserum H-660, which interacts with recombinant α_o, α_{i1}, α_{i2} and α_{i3} does not recognize α_{o2}. Both forms of G_o appear to specifically augment the muscarinic stimulation of phospholipase C in *Xenopus* oocytes. These data indicate the presence of two distinct forms of G_o in bovine brain which couple receptor to phospholipase C.

174.9

STIMULATION OF PARTICULATE GUANYLATE CYCLASE (GC) BY BRAIN NATRIURETIC PEPTIDE IN INDIVIDUAL NUCLEI OF RAT BRAIN. A. Israel, M.R. Garrido* and Y. Mathison*. Universidad Central de Venezuela. Caracas-Venezuela.

Brain natriuretic peptide (BNP), a peptide isolated from porcine brain, is present in rat brain neurons. Binding sites for BNP are highly localized in several brain structures such as the subfornical organ (SFO), olfactory bulb (OB) and choroid plexus (CP). In various peripheral tissues BNP increases intracellular cyclic GMP (FEBS 232, 1988).

The presence of functional-coupled to GC binding sites for BNP was examined by measuring the BNP-induced formation of cGMP in several brain areas such as the paraventricular nucleous (PVN), median eminence (ME), pineal gland (PG), SFO, OB and CP. Male S-D rats (220-260 gr) were decapitated and their brains immediately removed. After removal, each area (pool of 10) was homogenized and centrifuged at 105.000 x g for 2 h The supernatant (soluble, S) and the pellet (particulate, P) were assayed for GC activity and cGMP was determined by a radioimmuno assay. In all areas examined, the relative distribution of basal GC activity was 75-80% GC in P whereas it was 20-25% in S fraction. P-GC activity was markedly stimulated by BNP and it had little effect on S-GC. The maximal increase in cGMP concentration by BNP was found to be similar in PVN, PG, CP and OB (97, 77, 115 and 147 %, respectively), while a lower response was observed in SFO and ME (43 and 48 % increase). Vmax values were (in pmol/5min/mg protein, at 500 nM): ME: 56.3 ± 4.7; SFO: 42.9 ± 18; CP: 104 ± 3; OB: 102.6 ± 2.5; PG: 154.1 ± 2; PVN: 208.4 ± 3. Some differences were observed when compared with ANP-stimulated GC activity.

Our results suggest that in the brain, activation of particulate GC and elevation of intracellular levels of cGMP constitute a common mechanism of action for both BNP and ANP (Grants CDCH F-07-10-1928-88).

174.10

EXCITATORY AMINO ACID RECEPTORS ACTIVATION INDUCES cGMP FORMATION IN RAT STRIATAL NEURONS IN PRIMARY CULTURE. U. di Porzio and A. Novelli. (SPON: M. Voigt). Lab. of Neurophysiology and Lab. of Mol. Biology, NINDS, NIH, Bethesda, MD 20892,USA.

The development of a responsiveness to excitatory amino acids (EAA) in striatal neurons is of interest for understanding the process of synaptogenesis in the striatum. Second messenger formation consequent to the activation of excitatory amino acid receptors (EAARs) is a reliable index of receptor function.

Here, we present data on the cGMP formation consequent to the activation of EAAR in striatal neurons in primary culture. Primary cultures of striatal neurons were prepared from 15-20 day-old rat embryos, and maintained for more than 20 days in culture (DIC). Intracellular cGMP levels after EAAR stimulation were measured at different DIC. Maximal stimulation (3-4 times over basal levels) was achieved at 13-17 DIC. N-methyl-D-aspartate (NMDA)(10μM) produced a maximal stimulation of cGMP formation in the absence of extracellular Mg^{++}. When Mg^{++}(1mM) was present, 1mM NMDA was required. Maximal stimulation of cGMP formation by glutamate was obtained at 50μM when Mg^{++} was absent, and at 1mM when Mg^{++} was present. In the presence of extracellular magnesium, kainate induced a dose-dependent elevation of cGMP levels, maximal at 100μM. Maximal stimulation of cGMP formation by quisqualate was limited to 1.5-2 times over basal levels and was achieved starting at 5μM. The time course of cGMP formation induced by EAAs reached a maximum from 30 sec. to 2 min., after addition of the agonist, and was dependent upon extracellular calcium. However, it was not mimicked by the calcium ionophore A23187 (10μM).

In conclusion, we have shown that in striatal neurons in primary culture, cGMP formation is coupled to all types of EAAR and develops with time in culture. Since other second messengers, such as arachidonic acid, have been reported to originate from the activation of a selective type of EAAR, cGMP appears to be an independent second messenger possibly mediating the intracellular action of EAA in striatal neurons in primary culture.

174.11

ANGIOTENSIN II DECREASES CYCLIC GMP IN NEURONAL CULTURES FROM RAT BRAIN. L.M. Myers* and C. Sumners. Dept. of Physiology, College of Medicine, Univ. Florida, Gainesville, FL 32610.

Angiotensin II (Ang II) has been shown to decrease atrial natriuretic factor-induced increases in cyclic GMP (cGMP) levels in cultured smooth muscle cells (Smith and Lincoln, Am. J. Physiol. 253:C147, 1987). The objective of this study was to determine the effect of Ang II on cGMP levels in neuronal cell cultures prepared from the hypothalamus and brain stem of 1-day-old rats. Experiments were conducted in serum-free medium in the presence of 1.0 mM 3-isobutyl-1-xanthine (IBMX) which was added 2 min prior to treatment. Treatment of neuronal cultures with 1 μM Ang II for 10 min resulted in approximately a 55% decrease in cGMP compared with control values (means ± SE, from 7 experiments, were 44.6 ± 7.7 and 100.7 ± 9.5 pmol cGMP/mg protein in Ang II treated and control cells, respectively). At 10 min, 1 and 10 nM Ang II tended to reduce cGMP levels while concentrations of 100 nM, 1 μM, and 10 μM resulted in percent decreases from control of 26%, 35%, and 46% respectively (control mean was 90.8 ± 10.1 pmol cGMP/mg protein, n=3 experiments). This Ang II effect was blocked by co-treatment with the specific Ang II receptor antagonist Sar^1 Ile^8Ang II (10 μM). In glial cell cultures (>95% astrocytes), cGMP was undetectable using the same radioimmunoassay procedures. Therefore, Ang II-induced decreases in the neuronal culture cGMP content could not be due to effects on the small percentage of glial cells present in neuronal cultures. In summary, we have determined that Ang II acts at specific receptors to decrease cGMP in cultured neurons (supported by PHS grant NS-19441).

174.12

THE EFFECTS OF HEPOXILIN A_3-GLUTATHIONE ADDUCT ON MEMBRANE PROPERTIES OF RAT HIPPOCAMPAL CA1 NEURONS. N. Gurevich[1], P.H. Wu*[2], C.R. Pace-Asciak*[2,3], E.J. Corey*[4], W-G. Su*[4] and P.L. Carlen[1]. Playfair Neuroscience Unit, Toronto Western Hosp. and Addiction Res. Fndt.[1]; Dept. Pharmacology[2], Research Institute, Hospital for Sick Children[3], U of Toronto, Canada; and Dept. Chemistry[4], Harvard Univ., Cambridge, MA, USA.

Hepoxilin A_3 (HxA_3), a metabolite of arachidonic acid formed through the 12-lipoxygenase pathway, hyperpolarizes the resting membrane potential (RMP), prolongs the post-spike train after-hyperpolarization (AHP), and enhances the inhibitory postsynaptic potential (IPSP) of rat hippocampal CA1 neurons. A new metabolite, hepoxilin A_3-glutathione (HxA_3-G), formed presumably through the action of glutathione S-transferase, was identified when HxA_3 was incubated with a liver homogenate. In CA1 neurons in vitro, HxA_3-G displayed similar effects to HxA_3 on the RMP, AHP and IPSP, but was more potent than HxA_3, increasing the IPSP up to 7-fold and causing hyperpolarization up to 9 mV at low μM concentrations. These data suggest that HxA_3-G may represent the product of neurobiological interest in the arachidonic acid metabolic pathway. (Support: OMH, MRC, CDA, NIH)

174.13

EFFECTS INDUCED BY ARACHIDONATE (AA) ON THE INTRINSIC AND SYNAPTIC PROPERTIES OF CA1 PYRAMIDAL NEURONS. C.Drapeau, L.Pellerin and M. Avoli. MNI, McGill Univ., Montreal, PQ, Canada.

The action exerted by AA, a 20-carbons polyunsaturated fatty acid, was studied in CA1 pyramidal neurons of the rat hippocampal slice maintained "in vitro". AA (50µM) was applied along with indomethacin (50µM), an inhibitor of the cyclo-oxygenase enzyme. The main results can be summarized as follows: (i) Perfusion of AA did not alter membrane resistance. (ii) Action potential amplitude and width increased by 5-8% and 8-14% respectively; the changes in width however did not reach significance. (iii) The threshold for action potential generation increased by 35% ($p<0.05$). (iv) There was a marked decrease in the number of action potentials induced by an intracellular depolarizing pulse. (v) In all neurons, AA induced at least a two-fold increase in the amplitude of the orthodromic EPSP. The increase in excitability was also observed when recording extracellularly. All these changes were not reversed after more than one hour of washing, presumably because of the AA's lipophilicity. Supported by MRC.

174.14

GLUTAMATE SPECIFICALLY INDUCES THE FORMATION OF 12-(S)-HYDROXYEICOSATETRAENOIC ACID IN RAT CEREBRAL CORTEX. L. Pellerin and L.S. Wolfe Experimental Neurochemistry, Montreal Neurological Institute, Montreal, Canada H3A 2B4

Arachidonic acid metabolites, especially those formed by lipoxygenases, have recently become implicated as possible second messengers. Piomelli et al. (PNAS 86: 1721, 1989) demonstrated that metabolites of the 12-lipoxygenase pathway are responsible for mediating the effect of histamine and FMRFamide on electrophysiological responses of Aplysia sensory neurons. As a first step toward a demonstration of a similar mechanism operating in vertebrates, we studied the formation of hydroxyeicosatetraenoic acids (HETEs) following application of various neurotransmitters. HETEs were separated by RP-HPLC and identified by GC-MS. Only glutamate and norepinephrine (100µM) were found to increase specifically 12-HETE by 100%. N-methyl-d-aspartate causes a similar increase while kainate was without effect. However 12-HETE can appear as two enantiomers,(S) or (R), that have quite different origin and properties. To determine which enantiomer(s) is(are) formed in cortex, peak corresponding to 12-HETE in previous experiments was submitted to HPLC chromatography on a DNBPG chiral phase column. It became clear that only the S form is present. These observations further suggest the presence of a specific 12-(S)-lipoxygenase in rat cerebral cortex which activity can be modulated by specific neurotransmitters. Supported by a grant from MRC. L.P is a NSERC scholar.

NEURONAL DEATH: MODELS AND MECHANISMS

175.1

UBIQUITIN MAY BE A CELL DEATH PROTEIN IN THE HAWKMOTH, Manduca sexta. L.M. Schwartz, M. Engelstein* and L.Kosz*. Zool. Dept., U. Mass. Amherst, Mass. 01003.

The death of the intersegmental muscles requires de novo transcription and translation. One of these regulated genes encodes for ubiquitin.

Manduca developmental Northern blots were probed with a Drosophila ubiquitin clone. mRNA levels were very low preceding the muscle's commitment to degenerate. Ubiquitin mRNA levels then greatly increased prior to emergence. When muscles were prevented from degenerating by injection of 20-hydroxyecdysone, ubiquitin mRNA remained at pre-commitment levels. Isolation and sequence analysis verifies that this transcript encodes for authentic Manduca ubiquitin.

Western blots demonstrate that ubiquitin is covalently linked to proteins at all stages. However, the number and intensity of ubiquitinated proteins increases dramatically after commitment.

These data suggest that ubiquitin may participate in the degeneration of the intersegmental muscles. To test this hypothesis, we are injecting ubiquitin anti-sense RNA into Manduca muscle to disrupt translation.

Supported by N.I.H. grant GM40458

175.2

MOTONEURON SURVIVAL IN VIVO FOLLOWING TREATMENT WITH EXTRACTS FROM ACTIVE AND INACTIVE MUSCLE. L. Houenou*, D. Prevette* and R. Oppenheim (SPON: T. Troost). Department of Anatomy, Wake Forest University School of Medicine, Winston-Salem, NC 27103.

In most vertebrates approximately 50% of spinal motoneurons die naturally during embryonic or fetal development. Previous studies have shown that chronic paralysis during the cell death period results in the survival of all motoneurons. We previously suggested that this rescue effect is mediated by the activity-dependent regulation of a target-derived trophic factor. Inactive muscle was postulated to produce more trophic factor than normally active muscle. We have now tested this notion and the results do not support our activity model. Motoneuron survival on day 9 following daily treatment of chick embryos in vivo with partially purified muscle extracts from either normally active or from genetically or pharmacologically paralyzed mouse and chick embryos did not differ. Extracts from both active and inactive muscles rescued 25-35% of the motoneurons that normally die. We conclude from these results that activity- blockade prevents motoneuron death in ovo by some means other than increased synthesis or production of a muscle-derived neurotrophic factor. Supported by NIH Grant NS20402 and by IBRO and Philippe Foundation Postdoctoral Fellowships to LH.

175.3

OLIVARY CELL COUNTS IN *NERVOUS* AND *LEANER* CEREBELLAR MUTANT MICE. H. Shojaeian, J. Mariani, N. Delhaye-Bouchaud, & K. Herrup. Institute de Neurosciences Univ. Pierre. & Marie Curie, Paris and E.K. Shriver Center, Waltham, MA.

A major factor controlling the extent of histogenic cell death is the size of the postsynaptic target. Quantitative studies suggest that one result of this neuron:target relationship is the numerical matching of pre- and postsynaptic elements. We and others have used neurological mutant animals as model systems as a means of deleting specific populations of neurons. Two such mutants lose Purkinje cells (PCs) postnatally: *leaner* (tg^{la}/tg^{la}) and *nervous* (*nr/nr*). These two mutations are of particular interest because both show regional variation in severity: *leaner* loses more Purkinje cells in anterior than posterior lobules while *nr/nr* loses more Purkinje cells in lateral than medial regions. In *nr/nr*, Purkinje cells are lost progressively from the third to the sixth postnatal week. Counts of inferior olive show that by P24, there is a 23% deficit of neurons and further losses (up to 40%) are observed by P66. In keeping with the pattern of PC loss, the medial accessory olive, which projects mostly to the hemisphere (where PC loss is the greatest), was significantly more affected than the other subnuclei. Unexpectedly, in *leaner* animals, there was no consistent loss of olivary neurons even in animals over one year old (by this age over 80% of the Purkinje cell targets have died. These results suggest either that there is a critical period of sensitivity after which olivary neurons are insensitive to target, or that there are unknown trophic factors in *leaner* that serve to stabilize the olivary cells.

Supported by NS20591 and the FYSSEN foundation.

175.4

QUANTITATIVE STUDY OF PROPORTIONS OF PURKINJE CELLS IN DEVELOPING CHECK CEREBELLUM. S. Zamenhof. Dept. of Microbiology and Immunology, and Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024-1747.

The proportions of Purkinje cells to granule cells in cerebella of newly hatched chicks (total 237) were determined using two methods: 1. Counting Purkinje and granule cells on histological sections, and 2. Disintegrating cerebella and counting stained cells in suspension in a hemacytometer. The reliability of the latter method was previously demonstrated also by determinations of cellular DNA (Zamenhof, S., *Wilhelm Roux's Arch.*, 180:1, 1976). The histological method gave proportion 0.3% ± 0.09%, and the suspension method, 0.28% ± 0.04%. Thus, the two methods give comparable results, but the histological method has higher standard deviation, probably because it is subject to regional differences in cerebellum; this makes the suspension method more reliable for the study of effects of extrinsic factors on these proportions. The factors were applied during egg incubation, only in the period of proliferation of Purkinje cells (5E - 7E days). The cerebella of surviving chicks were examined at hatching when these precocial birds already have final cerebellar cell numbers. Using such a method, statistically significant changes in proportions of Purkinje cells were produced by: Higher or lower incubation temperature (Zamenhof, S., *Brain Res.*, 109:392, 1976), excess glucose or excess oxygen (Zamenhof, S., and Klimuszko, D, *Brain Res.*, 128:385, 1977), and antifolate (Zamenhof, S., *Growth*, 49:28, 1985). It is concluded that despite high constancy, the final proportions of Purkinje cells can be changed by appropriate factors applied during an appropriate period of cerebellar development. The possible effect of such changes on functioning of cerebella remains to be studied.

175.5

CONTROL OF NEURONAL SURVIVAL BY ABNORMAL TARGETS IN THE DEVELOPING BRAIN. R. Linden, Instituto de Biofisica da UFRJ, Rio de Janeiro, Brazil.

Neuron death is largely dependent on the availability of targets for developing axon terminals. It has been suggested that mismatching specific motoneuron pools and muscles might further limit neuron survival in the PNS. We have shown that normal numbers of neurons survive in the middle division of the parabigeminal nucleus (PBm) after neonatal ablation of its major target, the contralateral superior colliculus (SC). Here we show that regulation in the PBm is due to its anomalous projection to the lateral posterior nucleus of the thalamus (LP). This projection was identified with the use of radioautographic tracing and histochemical detection of cholinesterase in adult rats that received unilateral SC ablations at birth. We then tested whether the anomalous PBm-LP pathway might sustain the PBm neurons. In rats receiving neonatal ablation of the SC, additional removal of LP prevented the survival of the PBm neurons, while removal of dLGN did not. Thus, interactions of PBm axons with anomalous targets may effectively replace the normal interactions with the SC with regard to the trophic requirements of those central neurons. (CNPq, FINEP, CEPG, FAPERJ)

175.6

BIRTHDATES OF INFRAORBITAL GANGLION CELLS AND EFFECTS OF NEONATAL NERVE DAMAGE UPON SURVIVAL OF NEURONS BORN ON DIFFERENT GESTATIONAL DAYS. H.L. Enfiejian, P. McCann, N.L. Chiaia, M.W. Miller, G.J. Macdonald and R.W. Rhoades (SPON: A.M. Golub). Depts. of Anatomy, Medical College of Ohio, Toledo, OH 43699 and Robert Wood Johnson Medical School, Piscataway, NJ 08854.

Distinct classes of ganglion cells may be differentially sensitive to neonatal axotomy. Several studies have shown that primary afferent neurons which survive such lesions have larger soma diameters than in normal animals. Since ganglion cell size is correlated with cell birthdate, this suggests that early born ganglion cells may be less sensitive to neonatal axotomy than later-born neurons. We have tested this directly in the V system by combining [^{3}H]-thymidine labelling with neonatal infraorbital nerve (ION) transection and retrograde HRP transport. Fetal rats were labelled with [^{3}H]-thymidine on embryonic days (E-) 9-14 (N ≥4 for each age). On the day of birth, the left ION was transected. Six weeks later, the ION on both sides was labelled with HRP. Tissue was then processed for visualization of both HRP and [^{3}H]-thymidine labelled cells. On the normal side, 5.8% of the ganglion cells that sent axons into the ION were born on E-9; the values for E-10 through E-14 were 21.6%, 16.6%, 21.9%, 30.4%, and 3.8%, respectively. Of the ganglion cells that sent axons into the regenerate ION, 4.9% were born on E-9 and the values for E-10 through E-14 were 17.6%, 15.7%, 27.1%, 28.7%, and 6.0%, respectively. There was a significant difference between the distributions of birthdates for ganglion cells that projected into the normal and regenerate ION's. In the normal ganglia, 56.1% of all ION ganglion cells were born on E-12 through E-14; in the ganglia ipsilateral to the damaged nerve, this value was 61.8% ($t=2.6$, $df=24$, $p<0.02$). Thus, later born V ganglion cells are slightly more likely to survive neonatal axotomy than early born neurons.

Supported by BNS 85 17537, DE 07734, and funds from the State of Ohio Research Challenge.

175.7

POPULATION DEATH CAUSES SURVIVING NEURONS TO PROPORTIONALLY INCREASE CATECHOLAMINE SYNTHESIS. **C.E. Greenwood, N. Seniuk*, W.G. Tatton and F. Biddle*. Departments of Physiology and Nutritional Sciences, University of Toronto, Toronto, Ontario M5S 1A8 and Department of Biochemistry, Univ. of Calgary, Calgary, Alberta, Canada T2N 1N4.**

The total number of substantia nigra compacta (SNc) dopaminergic and locus coeruleus (LC) noradrenergic neurons were determined using tyrosine hydroxylase (TH) immunocytochemistry and Nissl staining for alternate brainstem serial sections from aging (8 weeks-88 weeks) or MPTP-treated (37.5 - 300 mg/kg at 8 weeks of age) isogenic mice (C57BL/6Jbid). The total nuclear counts were combined with target tissue monoamine concentrations (measured by HPLC) for the striatum and cortex to determine mean (μ) levels of dopamine (DA), DOPAC and norepinephrine (NE) per surviving neuron. SNc and LC showed similar graded rates of neuronal death for both aging and MPTP treatment (e.g. μ 68% neuronal loss for 300 mg/kg MPTP and 80% loss at 88 weeks). With aging, μ cortical NE/neuron and μ striatal DOPAC/neuron increased exponentially in relation to cell loss reaching 3.5-5.0 times that at 8 weeks by 88 weeks of age. Striatal DA/neuron increased by only 30-60% for the same range of neuronal loss. At 20 days following MPTP treatment, μ striatal DA/neuron and DOPAC/neuron showed a biphasic relationship to neuronal loss (increasing to 1.5 fold up to 40% SNc neuronal loss and declining thereafter). We suggest that increases in the μ NE terminal content/neuron and μ DA terminal turnover/neuron in the aging animal represents attempts to compensate for the overall decrease in catecholamine innervation caused by the loss of adjacent neurons. The axonal damage caused by MPTP is known to decrease TH levels and thereby catecholamine synthesis. Hence the relative decrease in compensation by the MPTP-treated neurons may result from the additive effects of the response to the loss of adjacent neurons and a decreased TH synthetic response engendered by axonal damage. (Supported by MRC and NSERC of Canada).

175.8

TUMOR NECROSIS FACTOR-alpha (TNF-a)-INDUCED CYTOTOXICITY TO NCB20 AND NG-108 NEUROBLASTOMAS. D. Blake*, I. Baker, M. Connolly*, J. Ferkany, and P. Sweetnam. Nova Pharmaceutical Corporation, Baltimore, Maryland 21224-2788.

Tumor necrosis factor-alpha (TNF; cachectin) is believed to be an important mediator of inflammatory responses, cachexia, endotoxic shock, bone resorbtion, pyrogenesis and tumor necrosis. Possible roles for TNF in neurodegenerative disorders such as multiple sclerosis and AIDS have been proposed. The current study evaluated the response of various neuronal and non-neuronal cell lines to TNF.

Cells were grown in flasks, plated (2×10^4) on 6.4 mm wells, cultured for 24 hr. and exposed for an additional 24 hr. to various concentrations of TNF. Viability was determined using colorimetric bioassay (MTT). All cultures contained actinomycin D; control assays were performed in parallel in the absence of TNF or in the presence of the nonspecific cytotoxin A23187.

In sub-nanogram amount TNF induced a dose-dependent necrosis of NCB20, NG-108, and L929 but not IMR-32 cultures. The Ca^{++}-ionophore A23187 was lethal to all cultures. Heat inactivation of TNF eliminated the cytotoxic activity.

The data suggest that some neuronal cell lines are sensitive to the cyotoxic action of TNF. The mechanism by which TNF elicits this activity is being explored.

175.9

CELL DEATH DUE TO GDP-INDUCED CALCIUM INFLUX IN XENOPUS OOCYTES. B. Gillo*, S.C. Sealfon* (SPON: B. Cohen). Dept. of Neurology and Fishberg Center in Neurobiology. Mount Sinai Medical School. New York, N.Y. 10029

Xenopus oocytes are an excellent system for studying Ca stores and mobilization. Loading denuded oocytes with nominal 1-2 mM GDP-β-s (Gbs), an undegradable GDP analogue, leads to cell deterioration and death over several hours. We investigated the contribution of Ca influx to cell deterioration. Cell viability was evaluated by measure of membrane potential (Vm) and resistance (Rm). Following 1-2 hr incubation in Ca-containing medium, Vm and Rm were reduced 59% and 85% respectively in voltage-clamped, Gbs-injected cells compared to controls (n=14). In Ca-free medium, the Gbs induced reduction in Vm and Rm was significantly smaller, being 23% and 24%. The Ca-dependent Cl current was used as a monitor of changes in [Ca]i. After 2 hr in Ca-free media, Gbs-injected cells were exposed to 5mM Ca which evoked a current of 356±96nA(n=6); negligible current was elicited in controls (0-15nA, n=6). We suggest that elevations in [GDP]i lead to increased membrane permeability to Ca. This mechanism may contribute to the well known phenomenon of cell death caused by Ca influx.

175.10

NEURON SPECIFIC DIFFERENCES IN CALCIUM HOMEOSTATIC CAPACITY. L.R. Mills, P. Dou, and S.B. Kater, Program in Neuronal Growth and Development, and Dept. Anatomy & Neurobiology, Colorado State University, Ft. Collins, CO 80523

We have used the calcium ionophore A23187 to investigate the regulation of intracellular calcium in cultured *Helisoma* neurons. Two identified neurons (B5 and B19), were studied in two different states (growing and non-growing). Our findings demonstrate distinctive neuron-specific, as well as state-specific, responses to intracellular calcium loads. Thus different neurons, as well as the same neuron in different states, have very different calcium homeostatic capacities.

In these experiments, the behavior of neuronal growth cones, and the survival of neurons, were examined during exposure to a range of concentrations of A23187. In parallel experiments changes in intracellular calcium were measured using the calcium indicator fura-2. Under conditions where intracellular calcium levels rose only transiently (e.g., growing B5s, non-growing B5s, and non-growing B-19s), neurons survived and actively growing growth cones continued to elongate. At the other extreme, under conditions where intracellular calcium levels showed a sustained rise (e.g., in growing B19s), neurite degeneration and ultimately cell death occurred. These differences in calcium homeostatic capacity are due, at least in part, to differences in a sodium/calcium exchange mechanism. In the absence of extracellular sodium, neurons normally resistant to the effects of A23187 (e.g., growing B5s, non-growing B5s and non-growing B19s) showed sustained rises in intracellular calcium; these cells also died.

These studies indicate that calcium homeostatic capacities are under dynamic control. (1) They can be differentially expressed in different neurons. (2) They can change in the same neurons depending upon their growth status. Such differences can have profound consequences for the regulation of neurite outgrowth and the susceptibility of neurons to calcium-induced cell death.

175.11

ROLE OF CALCIUM CHANNELS IN THE ABILITY OF MEMBRANE DEPOLARIZATION TO PREVENT TROPHIC FACTOR DEPRIVATION INDUCED NEURONAL DEATH: EVIDENCE THAT LEVELS OF INTERNAL CALCIUM DETERMINE TROPHIC FACTOR DEPENDENCE. T.Koike, D.P.Martin and E.M.Johnson, Jr. Dept. of Pharmacology, Washington Univ. School of Medicine, St. Louis, MO 63110.

Sympathetic neurons depend on nerve growth factor (NGF) for their survival both in vivo and in vitro; these cells die upon acute deprivation of NGF. We studied the effects of agents that cause membrane depolarization on neuronal survival after NGF deprivation. High K^+ medium (≥33 mM) prevented cell death; the effect of K^+ was dose-dependent. The high K^+ saving was abolished either by withdrawal of extracellular Ca^{2+} or by preloading the cells with a Ca^{2+} chelator. The Ca^{2+} antagonists nimodipine and nifedipine effectively blocked the survival promoting effect of high K^+. Ca^{2+} agonists Bay K 8644 and (+)-(S) 202 791 did not by themselves save neurons from NGF deprivation, but strongly augmented the effect of high K^+.

Depolarizing agents, choline and carbamylcholine, acting through nicotinic cholinergic receptors, also rescued NGF-deprived neurons. The saving effect by nicotinic agonists was not blocked by withdrawal of extracellular Ca^{2+}, but was counteracted by a chelator of intracellular Ca^{2+}, suggesting the possible involvement of Ca^{2+} release from internal stores. Based on these findings we propose a "Ca^{2+} set point hypothesis" for the degree of trophic factor dependence of sympathetic neurons in vitro.

175.12

INVERSE REGULATION OF Ca^{++} CURRENT DENSITY AND SURVIVAL BY DEPOLARIZATION OF CULTURED RAT MYENTERIC NEURONS. J.L. Franklin* & A.L.Willard (SPON: R. Glasser). Physiology Dept., Univ. of N. Carolina, Chapel Hill, NC 27599.

Chronic depolarization enhances survival of myenteric neurons in culture. It also causes decreased Ca^{++} current density. We have tested the hypothesis that regulation of Ca^{++} currents influences survival.

Myenteric neurons from newborn rats were grown in culture medium containing 5 or 25 mM K^+. The latter concentration causes a sustained 25 mV depolarization. Whole cell patch clamp was performed with 20 mM Ba^{++} as charge carrier. Steps from -90 to 0 mV elicited Ba^{++} currents with transient and sustained components (τ_1=25-60 msec, τ_2>450 msec). By culture day 7, peak densities of transient currents in cells grown in 5 and 25 mM K^+ were 65±27 and 37±17 pA/pF, respectively (p<.007). Densities of sustained components (300 msec after pulses began) were 45±22 and 24±12 pA/pF (p<.01). Cells grown in 25 mM K^+ for 4 days and then switched to 5 mM for 1 day had current densities 60% greater than did cells grown for 5 days in 25 mM K^+. This recovery, to levels equal to 87% of the current density of cells grown 5 days in 5 mM K^+, was prevented by the transcriptional inhibitor DRB.

To test whether excess Ca^{++} influx might account for neuronal death in non-depolarized cultures, the Ca^{++} channel antagonist nifedipine was added to 5 mM K^+ medium. Dose-dependent enhancement of survival occurred; 25 nM nifedipine, which reduces Ca^{++} currents by ≈50% in these neurons, increased survival to 90%, the same as seen in 25 mM K^+. Survival in 5 mM K^+ controls was 61% (p<.01).

These results suggest that return of Ca^{++} current density to control levels after K^+ suppression requires transcription and that chronic depolarization rescues myenteric neurons by preventing excess Ca^{++} influx. Supported by NIH grant NS24362 to ALW.

175.13

Ca^{++}-DEPENDENT EFFECTS OF ACUTE K^+ DEPOLARIZATION ON SURVIVAL OF RAT MYENTERIC NEURONS IN CULTURE. J.C. Thigpen*, J.L. Franklin* & A.L. Willard (SPON: A. Stuart). Physiology Dept., University of North Carolina, Chapel Hill, NC 27599-7545.

Chronic depolarization with elevated K^+ increases survival in cell culture of many types of neurons, including myenteric neurons, whose survival increases 1.5 to 2 fold in medium containing 25 mM K^+ (control is 5 mM K^+). We have asked 2 questions about the effects of K^+: 1. Can acute (brief daily) treatments with elevated K^+ enhance survival? 2. Are the survival-enhancing effects of daily K^+ depolarizations Ca^{++}-dependent? The latter question was asked because depolarization can activate voltage-sensitive Ca^{++} channels in these neurons.

Myenteric neurons from small intestines of newborn rats were plated in medium supplemented with 25 mM K^+. On day 4, plating medium was replaced with fresh medium containing 5 mM K^+. Neurons were counted daily. Survival of neurons grown in 5 mM K^+ from days 4 to 10 increased from 55±9% to 88±5% when the cultures were switched to medium containing 50 mM K^+ for 60 minutes per day. Effects of daily hourly treatment with elevated K^+ were prevented if the high K^+ medium contained 0.2 instead of 2 mM Ca^{++} or if it contained nifedipine (1 μM), which reduces Ca^{++} currents in these neurons by >90%. It was also prevented by a 5 minute pretreatment with 3 μM TMB-8, which is reported to block release of *intracellular* Ca^{++}. To test whether other means of elevating intracellular Ca^{++} could enhance survival, we examined the effects of the Ca^{++} ionophore A23187. Daily 30 second treatments with 10 μM A23187 and 1 μM Ca^{++} increased survival in 5 mM K^+ from 56±12% to 86±11%; 5 minutes of A23187 was lethal.

These results suggest that brief daily elevations of intracellular Ca^{++} significantly enhance survival of myenteric neurons in cell culture, but that excess Ca^{++} influx is fatal. Support: NIH grant NS24362 to ALW.

175.14

INTERFERONS CAN PREVENT SYMPATHETIC NEURONAL DEATH INDUCED BY NGF DEPRIVATION. J.Y. Chang*, D.P. Martin and E.M. Johnson Jr. Dept. of Pharmacology, Washington University School of Medicine, St Louis, MO 63110.

We have examined the effects of interferons on survival of cultured rat superior cervical ganglion neurons deprived of nerve growth factor. These cells will die within 48 hours after treatment with antibodies against nerve growth factor. Addition of interferons (IFN-α/β or IFN-γ) concurrently with the anti-NGF antibodies can prevent the cell death in a dose-dependent manner during this period. IFN-γ is much more potent in this respect, with an EC_{50} of approximately 1 unit/ml. In contrast, the EC_{50} of IFN-α/β is approximately 1,000 units/ml. Sympathetic neurons have specific binding sites for IFN-γ on their cell bodies and neurites. Receptor crosslinking results in a major species of protein complex in the region of 100,000 daltons. Further experiments indicate that the IFN can save a majority of cells acutely deprived of NGF for a short period. It can slow down the overall dying process of these neuronal cells over an extended period of NGF-deprivation, but can not prevent the ultimate death. We speculate that IFN may have a role in preventing neuronal cell death during injury, thus minimizing the extent of damage resulting from trauma. (Supported partially by American Paralysis Association, No. JA2-8804-1)

176.1

THE KNEE JOINT OF THE RAT. ANATOMY AND NERVE SUPPLY. C. Hildebrand and E. Lideberg*. Dept. of Cell Biology, Fac. Hlth Sci., Univ. of Linköping, S-581 85 Linköping, Sweden.

Although the rat has been used in several recent papers on experimental arthritis, the gross morphology and nerve supply of the knee joint has not been described in this species. In the present study we examine the knee joint in adult rats. X-ray examination showed persisting tibial and femoral epiphyseal cartilages. The femoral component exhibited a conventional appearance. The proximal end of the tibia showed a deep intercondylar fossa where the cruciate ligaments attached. The thin fibula was fused to a prominent tibial bony shelf. Dissection revealed collateral, cruciate and patellar ligaments as well as menisci, with a conventional morphology. A large cartilaginous formation extended from the proximal border of the patella. Careful examination revealed the presence of a medial (MAN) and a posterior (PAN) articular nerve. The MAN courses as one or two branches from the saphenous nerve to the area surrounding the medial collateral ligament. It contains 10-30 myelinated and 30-100 unmyelinated axons. Behind the knee the PAN leaves the tibial nerve at the level of the gastroenemius muscular branches and courses through the popliteal fat to the capsule. Stimulation of the PAN did not elicit contraction of the popliteus muscle. The PAN is composed of 250-500 axons most of which (70-80 %) are unmyelinated. Neonatal administration of capsaicin has little effect on the proportion of unmyelinated axons in the PAN and MAN.

176.2

DIFFERENTIAL RESPONSIVENESS OF CUTANEOUS A-DELTA AND C-FIBER MECHANONOCICEPTORS TO PRURITOGENIC STIMULI IN RAT AND CAT HAIRY SKIN. H.A. Martin and R.P. Tuckett. Dept. Physiol., Univ. Utah Sch. Med., Salt Lake City, UT 84108

Cowhage is a classical itch-producing substance known to induce pruritus in the vast majority of human subjects. Since histamine is implicated as a mediator of pain, as well as itch, it was of interest to compare the responsiveness of cutaneous high threshold mechanonociceptors (HTMs) to these two classes of pruritogens. Saphenous sensory neurons were characterized according to conduction velocity and response to mechanical, thermal and chemical (spicules of cowhage, histamine iontophoresis [1%, 30 sec; 20 μA], hypertonic saline [3.6%, 3 μl, i.d.] and topical glacial acetic acid) stimuli. In rat, from a total of 7 Aδ and 10 C fiber HTMs, 2 C HTMs were activated by both cowhage (CH) and histamine (HI). Two Aδ and 1 C HMTs were activated only by HI. One Aδ HTM responded only to HI; the remainder were unresponsive. In cat, from a total of 5 Aδ and 5 C HTMs, 1 Aδ and 1 C HTM were activated by both CH and HI. One Aδ HTM responded only to HI; the remainder were unresponsive. HTMs that were responsive to HI and/or CH were responsive to heat and hence can be classified as polymodal nociceptors. The data suggest that only a subset of polymodal nonociceptors respond to pruritic stimuli. Hervé Martin is a N.A.T.O. grant recipient (Paris) and has been supported by Philippe Foundation, Inc. (New York). Supported by USPH grant NS15102.

176.3

PHYSIOLOGICALLY IDENTIFIED NEURONS IN CAT MEDULLA RESPOND TO IONTOPHORESIS OF ADENOSINE PRECURSORS. J.L. Henry and K.B. Austin*, Depts. Physiology & Psychiatry, McGill University, Montreal, Quebec, H3G 1Y6

Physiologically identified neurons located in the dorsomedial medulla at the level of the obex were recorded extracellularly and tested for their responses to iontophoretic application of ATP and AMP. Recording and iontophoresis was performed in cats which were either chloralose anesthetized (60 mg/Kg I.V.) or decerebrated at the intercollicular level and either spontaneously breathing or paralyzed with pancuronium bromide. Neurons were classified as either 1) somatosensory neurons which responded to tactile stimulation and were located in either the nucleus cuneatus or gracilis, 2) inspiratory neurons which fired in phase with phrenic nerve discharge or with respiration, responded to stimulation of the superior laryngeal and/or vagus nerves, and were located in the solitary tract nuclei. Most of the neurons tested responded to iontophoretic application of ATP. The response during application was an increase in firing rate. In about half the neurons tested a marked decrease in firing rate was observed after the application of ATP was terminated. The majority of cells that responded to AMP exhibited decreased firing rates. The responses to ATP and AMP were similar regardless of type of neuron or animal preparation. These effects of ATP and AMP on medullary neurons are similar to those previously reported for somatosensory nociceptive neurons recorded in the spinal cord (Salter and Henry, Neurosci. 15:815-825, 1985). In light of the fact that inspiratory neurons are a type of viscerosensory neuron, our data indicates that ATP and adenosine may be utilized synaptically in both visceral and somatic sensory systems.
[Supported by a research and development contract to JLH from the Defence Research Establishment Suffield (no. 01SG-97702-R-4-9456). KBA is a Fellow of the Research Institute of the Royal Victoria Hospital.]

176.4

A STUDY OF TYPE I CUTANEOUS MECHANORECEPTOR RESPONSIVENESS IN VITRO. R.P. Tuckett, J.Y. Wei, H.A. Martin, P. Tsai* and K.B. English. Dept. Physiol., Univ. Utah School of Medicine, Salt Lake City, UT 84108.

There is in vivo evidence that type I neurons display chemosensitivity. An in vitro preparation would help to rule out the possibility that such increased responsiveness is due to edema formation secondary to changes in vascular permeability. An in vitro skin preparation has been modified to obtain a higher yield of type I mechanoreceptive neurons than originally reported (P.W. Reeh, Neurosci. Letters, 66: 141-146, 1986). Modifications included dissection under hypothermic conditions and as moving the preparation to a saturated O_2 environment prior to placement in the perfusion system. O_2 loss was minimized by using gas-tight tubing and maintaining a flow of 100% O_2 gas above the surface of the perfusion chamber. Further improvements include placing the skin epidermis side up for precise Haarscheiben localization and improving perfusion underneath the preparation. Type I neurons appear to be relatively more sensitive to hypoxia than other cutaneous receptor populations, perhaps indicating that the Merkel cell/type I complex supports a transmitter mechanism of action potential generation. Supported by USPH grants NS07938 and NS15102.

176.5

MECHANICAL AND HEAT RECEPTIVE FIELDS OF CUTANEOUS C-FIBER NOCICEPTORS COINCIDE. **R.-D.Treede*, R.A.Meyer, and J.N.Campbell, Johns Hopkins Univ., Baltimore, MD 21205.**

The receptive fields (RFs) of polymodal nociceptors are usually mapped using mechanical stimuli. These RFs are then typically subjected to heat or chemical stimuli, assuming that the RFs are identical for all modalities. We tested this assumption by comparing the mechanical and heat RFs of 26 C-fiber nociceptors responsive to both mechanical and heat stimuli (CMHs). Standard teased-fiber techniques were used to record from single CMHs that innervated hairy skin of anesthetized monkey. Mechanical thresholds were determined with calibrated von Frey probes, and heat thresholds were determined with a laser thermal stimulator. The borders of the mechanical and heat RFs were mapped with 10 bar and 49°C stimuli. Mechanical thresholds (3.3 ± 1.6 bar, mean ± SD) and heat thresholds (41.1 ± 2.5 °C) of the CMHs were not correlated. Mechanical RF diameters (5.2 ± 1.7 mm) and heat RF diameters (4.3 ± 2.4 mm) were linearly correlated ($p \leq 0.01$) with a slope of one. The centers of the mechanical and heat RFs were within 0.4 ± 0.3 mm. The close match of both size and location of the mechanical and heat RFs suggests one of three possibilities: 1) the transducer for mechanical and heat stimuli is the same; 2) the transducers reside on the same branches; or 3) the distributions of mechanical and heat sensitive branches coincide. (Supported by DFG Tr236/1-1 and NIH NS-14447)

176.6

EVIDENCE THAT BRADYKININ INDUCED CUTANEOUS PAIN IS NOT MEDIATED BY HISTAMINE RELEASE. **S.L.Kozak*, R.A.Meyer, S.N.Raja, D.C. Manning and J.N.Campbell, The Johns Hopkins Univ. School of Medicine, Baltimore, MD 21205.**

Bradykinin (BK) is found in inflamed tissue and is known to cause both acute pain and hyperalgesia in humans. Since BK can degranulate mast cells and release histamine, we wished to determine if the cutaneous pain induced by BK is mediated by histamine. Trained subjects used the technique of magnitude estimation to rate the pain produced by intradermal injections to the volar forearm of BK or histamine phosphate (HIS). Four drug injections (10 nmole in 10 μl) at 10' intervals were administered at the same site. In a given session, one of four sequences was used: (1) BK-BK-BK-HIS; (2) HIS-HIS-HIS-BK; (3) BK-BK-BK-BK; (4) HIS-HIS-HIS-HIS. Pain ratings were recorded every 5s, and the area under the pain-time curve was determined. The pain produced by HIS and BK decreased significantly from 79 ± 20 (mean ± SEM) and 320 ± 26 on the first injection to 12 ± 6 ($p \leq 0.01$) and 10 ± 4 ($p \leq 0.001$) on the third injection, respectively. However, BK injected as the 4th injection after three HIS injections produced a similar level of pain (401 ± 80) as the first BK injection. These results indicate that there is pronounced tachyphylaxis for the pain produced by repeated injections of histamine, whereas there is no crossed tachyphylaxis for bradykinin. It is thus likely that the cutaneous pain produced by bradykinin is not mediated through release of histamine.

176.7

MECHANICALLY-INSENSITIVE NOCICEPTORS IN THE PRIMATE. K.D. Davis, R.A. Meyer, R.H. Cohen and J.N. Campbell, Department of Neurosurgery and Applied Physics Lab., The Johns Hopkins University, Baltimore, Md 21205.

Many psychophysical phenomena cannot be explained by the properties of "typical" mechanically-sensitive nociceptors. In order to study mechanically-insensitive afferents (MIAs), we have developed an electrocutaneous search technique to locate the cutaneous receptive fields of all A- and C-fiber afferents. In barbiturate-anesthetized monkeys, single fiber recordings were made from 34 Aδ- and 79 C-fibers. Cold and warm fibers were excluded from the study. A substantial proportion of the Aδ- (47%) and C- (19%) fibers had either extremely high mechanical thresholds, >10 bars (100 g/mm²), or were unresponsive to mechanical stimuli and were classified as MIAs. Many (13 of 20) of these MIAs did not even respond to intense forceps pinch. MIAs typically had coincident mechanical and electrical receptive fields encompassing a single punctate spot. Heat stimuli (49°C) excited less than half of the Aδ- and none of the C-MIAs. Some of the Aδ-MIAs had moderate heat thresholds (45°C-47°C) and their relatively short utilization times suggest a role in first pain sensation. Intradermal injection of capsaicin or histamine excited some of the Aδ- and C-MIAs not excited by noxious thermal stimuli. These data suggest that MIAs play an important role in pain. (Supported by NIH grant NS-14447 and the Canadian MRC)

176.8

RESINIFERATOXIN IS A POTENT CAPSAICIN-LIKE SENSORY NEUROTOXIN Janet Winter*, Andre Dray, John N.Wood and Stuart J.Bevan* The Sandoz Institute, 5 Gower Place, London WC1.

Resiniferatoxin (RTX), is a potent irritant found in plants of the Euphorbia family. This compound is a diterpene of the daphnane class with a 3-methoxy 4-hydroxy phenylacetic acid conjugated to the 20-OH position. The structurally-related tumour promoting phorbol esters have a free 20-OH function which plays an important functional role in the activation of kinase C. RTX is not a tumour promoter and much of its irritant activity resides in the 3-methoxy 4-hydroxy benzyl side chain which also occurs in the well-characterised sensory neurotoxin capsaicin. An analysis of the effects of RTX on sensory neurons either in culture, or in the isolated neonatal rat spinal cord/tail preparation has shown a potent capsaicin-like action, depolarising a subset of sensory neurons through the long-lasting activation of a specific cation conductance which is permeant to Na^+,Ca^{2+} and K^+. The species and cell type specificity of RTX are identical to capsaicin, but the EC_{50} for most actions is ≤1nM (cf capsaicin EC_{50} 0.1-0.3μM). RTX, like capsaicin, elevates cGMP but not cAMP in neonatal rat neurons from DRG which do not contain neurofilaments. Calcium uptake into mitochondria is dramatically enhanced by RTX . The ion fluxes (^{45}Ca,^{86}Rb and ^{14}C- guanidine) evoked by RTX in cultures of sensory neurons cross -desensitise with those evoked by capsaicin. In the tail cord preparation, application of pmolar RTX desensitised the response to subsequent capsaicin application, without affecting noxious heat or bradykinin-evoked ventral root depolarisations. RTX thus provides a novel potent tool for the investigation of sensory neuron function.

176.9

A PATCH-CLAMP ANALYSIS OF TETRODOTOXIN (TTX) -SENSITIVE AND -RESISTANT Na^+ CURRENTS IN NEURONS ISOLATED FROM SENSORY AND PARASYMPATHETIC GANGLIA OF THE ADULT RAT. **W.C. de Groat, F.F. Weight, G. White*. Section of Electrophysiology, NIAAA, Rockville, MD 20852.**

Using the whole-cell variant of the patch-clamp technique, Na+ currents were studied in neurons which were mechanically and enzymatically isolated from lumbar dorsal root ganglia (DRG) and the major pelvic ganglia (MPG) of adult rats. Under "normal" ionic conditions, neurons exhibited resting membrane potentials (-45 to -60 mV) and action potentials (80 to 110 mV) equivalent to those observed in intact ganglia. Na+ currents were studied under altered external (35 or 75 mM Na+, 0 Ca++, and 115 or 75 mM TEA) and internal (140 mM Cs+ replacing K+) ionic conditions. Both TTX (1μM) -sensitive (TTX_S) and TTX-resistant (TTX_R) Na+ currents were observed in DRG and MPG neurons. DRG neurons that exhibited only a TTX_R current were on the average smaller than neurons with only a TTX_S current (24 μm versus 36 μm). TTX_R currents activated and inactivated on average at more positive potentials than TTX_S currents (activation threshold ≈-43 vs ≈-50 mV and full inactivation ≈-35 vs ≈-46 mV, respectively) in DRG neurons. The existence of TTX_R Na+ currents in parasympathetic ganglia contrasts with the reported absence of such a current in sympathetic ganglia (Pflug. Arch. 411: 481, '88).

176.10

DIFFERENT EFFECTS OF CAPSAICIN ON THE MEMBRANE PROPERTIES OF CULTURED NEONATAL RAT DRG NEURONS M. Alreja*[1], D. Lo[2] and R.H. LaMotte[1] (SPON: L. Marks)
Department of Anesthesiology[1] and Section of Molecular Neurobiology[2], Yale Univ. Sch. of Med., New Haven, CT 06510.

Whole-cell voltage clamp and current clamp recordings were made from 1 day old rat DRG neurons maintained in culture and the membrane effectsof a brief exposure to capsaicin studied.

Under voltage clamp, at a holding potential of -70 mV, bath application of 1uM capsaicin (Sigma, Fluka) produced a transient, inward current accompanied by an apparent decrease in input conductance in 12 out of the 21 neurons tested. Capsaicin (Fluka) invariably produced a higher magnitude current than an equimolar dose of capsaicin (Sigma) irrespective of the order of administration. Preliminary data indicate a reversal potential between -70 and -100 mV for the inward current, suggestive of a potassium conductance since chloride containing electrodes were used. Similarly under current clamp, a transient depolarization associated with an increase in input resistance of the cell was observed.

A transient, inward current associated with an increase in input conductance was seen in 4 neurons. In 2 of the neurons the change in input conductance was prolonged. A reversal potential close to 0 mV has been reported for a similar current recorded by Bevan and Forbes (1988).

In 5 of the 21 neurons tested, 1uM capsaicin did not have any effect on the membrane properties of DRG neurons.

In conclusion, at least 3 populations of neonatal rat DRG neurons can be differentiated on the basis of their response to capsaicin.

REF: Bevan, S. and Forbes, C.A. - J. Physiol. 398:28p, 1988.

Supported by NIH grant NS14624

176.11

ELECTROPHYSIOLOGICAL PROPERTIES AND CHEMOSENSITIVITY OF CULTURED RABBIT TRIGEMINAL GANGLION NEURONS T.K. Baumann and R.H. LaMotte, Dept. of Anesthesiology, Yale University Medical School, New Haven, CT 06510

Adult rabbit trigeminal (primary afferent) neurons in dissociated culture display a variety of electrophysiological properties, e.g. various shapes of action potentials recorded at the soma, differences in accommodation, presence or absence of inward rectification (Baumann and LaMotte, Neurosci. Abstr. 14, 560), thus maintaining some of the differentiated properties of primary afferent neurons observed by others in vivo. Here we have examined the chemosensitivity of trigeminal neurons.

The effects of the irritant capsaicin on cultured newborn and adult rabbit trigeminal neurons were studied using the whole-cell patch-clamp recording technique. When capsaicin (1 or 10 μM) was applied for one minute to the cultures by superfusion (flow rate 1.5 ml/min.), some neurons (two thirds of the newborn, about one third of the adult) responded with an inward current of up to 350 pA. In some neurons the current was transient and faded when capsaicin was washed out of the bath, whereas in other neurons, the current did not return to control values 15 minutes after capsaicin was discontinued. When tested in the same neuron, the higher dose of capsaicin (10 μM) evoked larger transient currents than the lower dose (1 μM). In some neurons capsaicin caused the discharge of action potentials (recorded as currents). The total number of action potentials was greater at the higher dose of capsaicin. An attempt to establish a correlation between electrophysiological properties and chemosensitivity of cultured rabbit trigeminal neurons is in progress.

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176.12

SYMPATHETIC ACTIVATION INCREASES NOCICEPTOR RESPONSIVENESS AFTER NERVE INJURY. J.Sato* and E.R.Perl Dept. of Physiol., Univ. No. Carolina, Chapel Hill, NC 27599

To test whether nerve injury alters nociceptor reaction to sympathetic activation, under sterile conditions we damaged the great auricular nerve (GAN) of deeply-anesthesized domestic rabbits by stretching, by ligation or by partial division and allowed the animals to recover for 4 to 148 days. Post-operatively, none of the animals exhibited signs of discomfort or skin alterations. Terminally, under deep anesthesia, responses of single C-fiber polymodal nociceptors (CPM) to stereotyped heat stimuli were recorded from fine GAN filaments central to the site of damage and compared to CPM responses in control animals. In controls (n=12), sympathetic trunk stimulation (SS) neither caused CPMs to discharge nor altered heat-evoked activity, but suppressed the usual sensitization to repeated heat stimulation. In sharp contrast, SS substantially (1.5 to 3x) enhanced heat-produced sensitization in 1/2 to 2/3 of CPM units from injured nerves (n=44). SS enhancement of CPM sensitization relative to controls was significant for each type of nerve injury. For some injured nerve CPMs, SS was excitatory by itself and relative to controls increased initial responses to heat. We interpret these results as favoring altered sympathetic effects upon nociceptor terminals after nerve injury as an explanation for some causalgic-like pain syndromes. (Supported by grants NS 10231 and 14899 from NINCDS)

176.13

AN ABNORMALITY OF THE SYMPATHETIC VASOMOTOR INNERVATION IN RATS WITH A PAINFUL PERIPHERAL NEUROPATHY. S.Wakisaka*, K.C.Kajander and G.J.Bennett* (SPON: C. Pechura). NAB, NIDR, NIH, Bethesda, MD 20892.

Painful peripheral neuropathies in humans are sometimes accompanied by an abnormality of cutaneous temperature regulation that is presumed to indicate a dysfunction of sympathetic vasomotor control. The sympathetic dysfunction, in turn, is thought to be causally related to the neuropathic pain sensations. Rats with a painful neuropathy of the sciatic nerve (Pain 33:87-107, 1988) also display temperature abnormalities. As in humans, the incidence is variable, but about one-third of the rats have hind paws that are clearly abnormally hot or cold. As a first step in examining this phenomenon, we examined the effects of the neuropathy on the noradrenergic vasomotor innervation of the affected hind paw.

The plantar artery and vein were excised (from both the nerve-damaged and control sides) and prepared as whole mounts. The glyoxylic acid-induced histofluorescence method was used to visualize norepinephrine (NE). The temperature of the plantar hind paws was measured just prior to sacrifice.

On the control side, NE axons always formed a dense reticulum on the surface of the artery and a sparser web on the vein's surface. Beginning at 5 days post-injury, a few rats had a clear decrease in NE on the nerve damaged side. The NE decrease became increasingly severe and more common through days 5-18; at these times many rats had no detectable NE. By 30 days, NE could not be detected in any of the cases. NE began to reappear by 60 days and was close to normal by 180 days. The expected relationship between the status of the vasculature's NE and skin temperature was not always present. Thus, some abnormally cold paws had no detectable NE and some abnormally hot paws had normal NE.

The data indicate that the experimental neuropathy is associated with an abnormality of sympathetic efferents. The temperature abnormality, however, is not directly related to the sympathetic dysfunction.

176.14

DORSAL ROOT GANGLION NEURONS PROJECTING TO THE DORSAL COLUMN NUCLEI OF RATS. R. Giuffrida and A. Rustioni. Cell Biol. & Anat., Univ. N. Carolina, Chapel Hill, NC 27599.

This reinvestigation of dorsal root ganglion (DRG) cells at the origin of the primary afferents to the cuneate nucleus provides a background for a project aimed at identifying neurotransmitters in these neurons. Colloidal gold coupled to WGA-apo-HRP was injected into the cuneate nucleus of rats. After two to three days, rats were perfused with 4% paraformaldehyde and the ipsilateral cervical DRGs were postfixed and embedded in paraffin. The presence of the tracer was revealed by means of silver intensification of the gold particles. In 5 µm-thick sections counterstained with cresylviolet, neurons displaying the nucleolus were considered for quantitative estimates. The percentage of labeled neurons varied in different ganglia depending on the site and size of the injections. Highest percentages, up to 60 -65%, were found as a rule in C5 to C6; at more rostral levels, percentages were regularly lower, in the range of 15% to 25%. The greatest majority of retrogradely labeled neurons were of large size, but medium-size neurons were also labeled. These counts are, on the whole, significantly higher than those reported in previous publications.

176.15

AFFERENT PROJECTIONS AND EFFERENT ORIGINS OF THE VAGUS NERVE IN THE LAMB. J.M. Wild, B.M. Johnston* and P.D. Gluckman*. Depts. of Anatomy and Paediatrics, Univ. of Auckland Sch. of Med., Auckland, New Zealand.

The total sensory projections and motor origins of the vagus were defined in the lamb by injections of cholera toxin B subunit conjugated to horseradish peroxidase into the nodose ganglion (n=17), the cervical vagus (n=6), or the superior laryngeal nerve (n=2). The lambs were perfused after 3 days and brain and spinal cord sections were processed with TMB. Ganglionic injections showed a massive accumulation of reaction product in all ipsilateral and certain contralateral subnuclei of nucleus tractus solitarius (nTS). Other regions receiving descending sensory projections were the area postrema, the dorsal motor nucleus of X (DMNX), the nucleus ambiguus region caudal to the obex, the nucleus of the descending trigeminal tract, and lamina I of the cervical dorsal horn. TS descended to the level of C2 through the neck of the dorsal horn. Ascending projections from the level of entry of TS could be traced by labeled fiber fascicles to distinct dorsomedial and dorsal subnuclei of principal V, and, more rostrolaterally, to caudal regions of the lateral parabrachial nuclear complex. Retrogradely labeled cells were located in DMNX, in nucleus ambiguus, and in the dorsomedial ventral horn and lateral intermediate grey of the upper cervical cord. The last two cell groups had medial dendrites which crossed the midline.

176.16

ACTIVITY DEPENDENT LATENCY CHANGES IN SMALL DIAMETER CUTANEOUS AFFERENTS IN RAT SCIATIC NERVE. J.G. Thalhammer, S.A. Raymond, F. Popitz* and G.R. Strichartz. Anesthesia Research Lab., Brigham & Women's Hospital, Harvard Medical School, Boston, MA. 02115.

Each impulse in a series of electrically activated spikes in an axon shows a shift in conduction time (latency) that depends on previous discharges in the fiber (Raymond, J. Physiol. 290:273-303, 1979). Here we have compared these activity-dependent latency changes in functionally characterized cutaneous afferents recorded from rats fully anesthetized with Nembutal.

Forty-one Aβ, 15 Aδ and 10 C-fibers have been characterized according to their response to natural stimulation and studied with electrical stimulation of the nerve trunk. Generally, afferents with slower conduction velocity (CV) show more pronounced latency changes at any given rate, and some C-fibers (CV < 2m/s) show progressive slowing of conduction at stimulation rates below 0.5 Hz. However, among individual units there are large variations in the latency shifts at any one rate. Latencies may double or even triple for certain Aδ units (2< CV <30 m/s) stimulated for several minutes at 5 or 10 Hz, although those identified as cold fibers show no latency increase at 10 Hz, and increase less than 5% even after 20 sec stimulation at 20 Hz. In contrast, Aδ nociceptors with a resting CV (measured at 0.2 Hz) similar to cold fibers show increases as great as 150% in latency during repetitive discharge at 10 Hz, suggesting that the degree of latency shift during activity is correlated more tightly with modality than with diameter (CV).

176.17

SPATIAL FEATURES OF VIBROTACTILE MASKING EFFECTS ON AIRPUFF-ELICITED SENSATIONS IN THE HUMAN HAND A.Pertovaara, J.Kekoni*, I.Tikkala* & H.Hämäläinen Depts.Physiol.&Psychol.,Univ.Helsinki, Finland

The spatial features of vibrotactile masking on airpuff-elicited sensations were determined using a reaction time paradigm in man. The masking decreased monotonically with increasing distance, and identically to longitudinal and transversal directions in dorsal and palmar skin of the hand. The masking was stronger in the glabrous skin, especially in the fingers. In the glabrous skin the spread of high-frequency masking was less extensive than that of low-frequency masking. The mechanical spread of high-frequency vibration was, however, less extensive. A masker applied to the tip of the finger produced stronger masking than a masker in the base of the finger.

Mechanical spread of vibration can only partly explain the masking effects. The stronger afferent inhibition in the glabrous skin of the fingers may explain the superior spatial tactile discrimination properties of the glabrous skin. Peripheral innervation density/size of the cortical representation is of importance in determining the magnitude of masking.

176.18

Discharge properties of mechanoreceptive afferents in the cat anterior cruciate ligament. K.J. Cole, D. Pope and R.A. Brand. Depts. of Exercise Science and Orthopaedic Surgery, The University of Iowa, Iowa City, IA 52242.

Proprioceptive information from mechanoreceptors located in the knee's cruciate ligaments has been suggested to contribute to leg motor control. However, the discharge properties of identified cruciate afferents are virtually unknown. The present study was conducted to begin characterizing the discharge properties of cat anterior cruciate ligament (ACL) afferents to mechanical stimulation.

Activity of ACL afferents in the posterior articular nerve was recorded from each of 8 chloralose-anesthetized cats. A small block of the tibia containing the ACL insertions was removed and the leg was disarticulated at the knee. The preparation exposed the ACL and eliminated inadvertant stimulation of joint afferents due to relative tibial-femoral motion that occurs upon loading the ACL in the intact knee.

Afferents were activated upon probing the ACL with a 2 mm dowel using forces as small as 2 grams. Rapidly and slowly adapting afferents were activated. Many of these afferents responded to loads as small as 1.8 grams that stretched the ACL along its long axis; most adapted rapidly There appear to be functional subgroups of ACL afferents; some signal strain or changes in strain, whereas others may signal ACL impingement against the PCL or femoral notch. The correspondence of these signals to joint position or its derivatives must be examined in the intact knee.

176.19

THE THEORY OF TEMPORAL SUMMATION PRECLUDES CERTAIN CODES FOR SIGNALING THRESHOLD EVENTS IN THE PACINIAN PSYCHOPHYSICAL CHANNEL. S.J. Bolanowski, Jr. and C. M. Checkosky *. Institute for Sensory Research, Syracuse University, Syracuse, NY 13244 and Dept. Neurosurgery, University of Rochester Medical School, Rochester, NY 14642.

The theory of temporal summation refers to the psychophysical phenomenon in which threshold decreases with increasing stimulus duration. The effect is found in audition and is central. Vibrotactile thresholds mediated by the Pacinian-corpuscle (PC) population (the "P" channel) also exhibit temporal summation. Assuming that the tactile summator is also central, it may be possible to eliminate potential neural codes for signaling threshold in the P channel. Theoretically, temporal summation predicts a decrease in threshold at a rate of -3dB/doubling of duration from 10 to 100 msec. Thus by integrating the response from single fibers for various response criteria, those not having the potential for temporal summation can be rejected as possible coding schemes. PCs isolated from cat mesentery were stimulated with bursts of vibrations (300 Hz) having durations from 10 to 1000 msec, the intensity of the stimuli adjusted for fixed criterion responses of 1,2 and 4 impulses/burst and 1 impulse/cycle. Data were sampled over the entire burst duration, in effect analyzing the response with an ideal summator. The decreases in intensity for increases in duration from 10 to 100 msec were -0.9, -1.2, -2.3 and -0.2 dB/doubling, respectively, with neither the 1 impulse/burst nor the impulse/cycle criteria showing significant summation. The other two criteria show modest summation, indicating that they, but not the others, have the potential for being the neural code for threshold in the P channel. [Work supported by NS23933]

176.20

SYNAPTOLOGY OF PHARYNGEAL AFFERENTS IN THE PARATRIGEMINAL ISLANDS OF THE RAT. D.W. Saxon* and D.A. Hopkins. Dept. of Anatomy, Dalhousie University, Halifax, NS B3H 4H7.

The paratrigeminal islands (PTI) in the rat consist of a group of small neurons embedded in the dorsal aspect of the spinal tract of the trigeminal nerve (Chan-Palay, '78). Trigeminal, glossopharyngeal and vagal afferents, some of which derive from the pharynx, terminate in the PTI. WGA-HRP (5%, 3-5 ul) was injected into the pharyngeal musculature and mucosa of male Wistar rats to identify pharyngeal afferent terminations in the PTI. After 3-4 days the medulla oblongata was processed for light and electron microscopy with HRP histochemistry (Mesulam, '78) and ammonium molybdate stabilization (Marfurt et al, '86). Axon terminals in the PTI contained mainly round clear vesicles and formed asymmetric synaptic junctions with neuronal perikarya and dendrites. Terminals with pleomorphic clear vesicles and symmetric contacts were rare. Pharyngeal afferent terminal labeling was found exclusively in axon terminals containing round clear vesicles with occasional dense-cored vesicles. Labeled terminals formed asymmetric synaptic contacts with small diameter dendrites. Pharyngeal afferents projecting to the PTI have a similar synaptic organization to those projecting to the subnucleus interstitialis of the NTS.

Supported by MRC of Canada (Grant MT-7369).

176.21

CUTANEOUS AND PROPRIOCEPTIVE CONTRIBUTIONS TO TACTUAL DISCRIMINATION OF SOFTNESS. R.H. LaMotte and M. A. Srinivasan* Dept. of Anesthesiology, Yale Univ. Sch. of Med., New Haven, CT. 06510 and Research Lab. of Electronics, MIT, Cambridge, MA 02139.

The capacities of humans to discriminate between objects that varied in softness were measured under conditions that differed as to the relative contributions of cutaneous and proprioceptive cues available to the subject. A series of disks that differed in compliance (slope of displacement vs. force trace obtained by vertical indentations of each disk with a rigid probe) were cast using silicone rubber. Subjects made two-interval forced-choice discriminations using the distal pad of the middle finger. When the maximal force of indentation allowed was low (80g), optimal discrimination was achieved under active touch, wherein both proprioceptive and cutaneous information was available. Under passive touch, when only cutaneous cues were present, discrimination was much poorer and was confounded by alterations in indentation velocity. When the maximal force of indentation was higher (160g) under active touch, and cutaneous cues were eliminated by a local anesthetic, discrimination based on proprioceptive signals alone was not possible. However, without the anesthetic, optimal discrimination was possible under passive conditions based on cutaneous information alone, in spite of variations in indentation velocity. Responses of slowly adapting mechanoreceptive afferent fibers innervating the monkey fingerpad discriminated better the differences in the compliance of the rubber disks at higher forces of indentation. At lower forces, differences in responses to compliances were confounded by variations in the velocity of indentation in accordance with the corresponding psychophysical results. (PHS grant NS15888 and ONR contract N00014-88-K-0604).

NEURONAL DEATH: LESION INDUCED

177.1

PROTEIN KINASE C ACTIVITY AND PROTEIN LEVELS FOLLOWING RAT FORNIX-FIMBRIA TRANSECTION. K.L. Leach+ J.F. Tinklenberg,*+ and L.R. Williams. Cell Biology+ and CNS Diseases Research, The Upjohn Co., Kalamazoo, MI 49001.

Protein kinase C (PKC) is a calcium and phospholipid dependent kinase that has been implicated in a variety of cellular processes, including neurite outgrowth, and neurotransmitter release. In the present study, we examined the changes in PKC two weeks after fornix-fimbria (F-F) transection, an injury known to result in the loss of neurons in the medial septum and diagonal band (MS/DB) (PNAS 83:9231, 1986). Microdissections of the MS/DB and whole hippocampus from normal and transected rats were fractionated into cytosol (Cyt) and membrane (Mem) fractions. Western blot analysis using a sheep polyclonal antibody that cross-reacts with the α,β and γ PKC isozymes indicated no significant effect of F-F transection on the amounts of the 82 kDa PKC in either the Cyt or Mem fractions. PKC activity in control MS/DB Cyt and Mem fractions was 2994 and 3718 pmol/min/mg protein (n = 6), respectively. Following F-F transection, MS/DB Cyt and Mem PKC activities increased approximately 40%. Control hippocampal Cyt and Mem PKC activity was 6279 and 6644 pmol/min/mg protein, respectively; Cyt and Mem PKC activity decreased approximately 40% after F-F transection. Our results indicate that alterations in PKC specific activity occur within the affected brain regions, but without detectable changes in PKC protein levels.

177.2

DELAYED DEGENERATION IN SEPTAL CHOLINERGIC NEURONS AFTER PRIMARY ISCHEMIC OR IBOTENIC ACID HIPPOCAMPAL INJURY. B.T.Volpe, H. Baker, Dept. of Neurology, Cornell University School of Medicine, The Burke Rehabilitation Center, White Plains, New York 10605.

Animals exposed to transient forebrain ischemia by the method of four vessel occlusion were evaluated to determine whether delayed degeneration of cholinergic cells in the septum occurred. After several days, post ischemic animals reproducibly develop severe neuron loss in the CA1 region of the hippocampus. Animals were exposed to 30 minutes of ischemia or sham operation (n=6), and recovered for one week (n=3) or four months (n=4). Neurons, labelled with monoclonal antibody to choline acetyl transferase (ChAT), were counted in the medial septum, ventral limb and horizontal limb of the diagonal band (MS, VLDB, HLDB). One week after ischemia the number of ChAT positive cells in the MS, VLDB and HLDB was comparable to control values ($p>.05$). However, in animals who survived four months after ischemia, significant loss of ChAT positive cells occurred in the VLDB ($p<.05$). A comparable depression of ChAT positive cells in the septum (MS, VLDB) occurred in animals that survived 4 months after ibotenic acid lesions (n=3) of the anterior dorsal hippocampus (a region of cell loss similar to that in the ischemic animals). These preliminary data suggest that the loss of hippocampal CA1 neurons may provoke delayed degeneration of cholinergic cells in the septum. In view of the importance of the septohippocampal cholinergic system to spatial memory these results may have implications for the behavior of these animals. (Supported by the UPHS, MH40090)

177.3

LOSS OF ChAT LABEL PRECEDES CELL DEATH IN THE AXOTOMIZED RAT FOREBRAIN NUCLEI M.S. Grady and D. Maris* Dept of Neurosurgery University of Washington, Seattle, WA 98195.

The cholinergic neurons of the rat medial septal nucleus (MSN) and vertical diagonal band (VDB) are depleted following unilateral fimbria-fornix axotomy. A number of reports, using acetyl cholinesterase (AChE) or choline acetyl transferase (ChAT) histology suggest rapid neuron death. However, "resuscitation" of these neurons can be achieved using trophic factors even weeks after axotomy. This discrepancy can be resolved by hypothesizing a neuronal metabolic change preventing a positive AChE or ChAT reaction. If this hypothesis is true, then retrograde labelling of the MSN/VDB prior to axotomy should show a persistence of cells.

Sixteen adult rats were prepared with bilateral injections of .1ul of a 4% fluorogold (FG) solution in 2 locations of each hippocampus. Seven days later, a unilateral transection of the fimbria-fornix was performed stereotaxically. Animals were killed at 6, 10, 14, and 28 days after axotomy. Serial 35 micron sections through the MSN/VDB were alternately prepared for analysis using ChAT immunocytochemistry or coverslipped directly and examined for FG labelled cells. Morphometric measurements were performed with the Bioquant image analysis system, comparing the 2 hemispheres.

ChAT positive neurons showed a time dependent loss on the ipsilateral side. Likewise, area and longest dimension decreased slightly within 6 days. FG labelled cells showed a delayed time dependent loss compared to ChAT labelled cells. Nonetheless, significant cell loss and changes in area were seen at 14 and 28 days. These results confirm that neuron death follows fimbria-fornix axotomy, that disappearance of ChAT label precedes cell death, and that neurons shrink both in area and longest dimension.

177.4

LOSS OF BASAL FOREBRAIN CHOLINERGIC MARKERS PRECEDES CELL LOSS AFTER AXOTOMY. C.N. Svendsen*, T.S. O'Brien*, O.Isacson and M.V. Sofroniew. (SPON: H.G.J.M. Kuypers) Department of Anatomy, University of Cambridge, U.K.

Fimbria-fornix (FF) lesions result in an early loss of cholinergic enzyme staining followed by a gradual shrinkage and eventual disappearance of large Nissl-stained neurons from the medial septal nucleus in the basal forebrain. In order to better define the nature and time course of these events, the fluorescent tracer True Blue (T.B.) was injected into multiple sites in the rat hippocampus 7d prior to unilateral FF lesions. Animals were perfused 1-56d after FF-lesion, and medial septal neurons containing T.B. or immunohistochemically stained for choline acetyltransferase (ChAT) or nerve growth factor receptor (NGFr) were counted. Following FF lesions, the principal loss of both ChAT and NGFr staining from septal neurons occured between 6-8d, reaching a maximum by 9d. Prior to this, swollen and darkly stained, retrogradely degenerating, immunoreactive fibers descended along the FF towards the septum and appeared to reach the levels of their parent cell bodies coincidentally with the onset of staining loss. In contrast, no loss of T.B. labelled cells was seen until after 14d, and a loss comparable to that seen for ChAT and NGFr had occured by 28d. From14-28d, morphological changes included shrinkage of T.B. filled cells as well as the appearance of T.B. in the extracellular space and in glial cells, suggesting neuronal pathology, breakdown of membrane integrity or lysis and probable neuronal cell death. These findings suggest that proximal axotomy results in the delayed death of medial septal neurons, and that loss of ChAT and NGFr expression precede cell death. The onset of these events may be triggered by the arrival at the cell body of the retrogradely degenerating axon.

177.5

FATE OF RETROGRADELY LABELED SEPTOHIPPOCAMPAL NEURONS FOLLOWING FIMBRIA-FORNIX TRANSECTION: A TIME COURSE ANALYSIS. G. M. Peterson, G.W. Lanford and E. W. Powell. Dept Anatomy and Cell Biology, East Carolina Univ Sch Med, Greenville, NC 27858.

Marked neuronal loss has been demonstrated in the medial septum-diagonal band complex (MSDB) within two weeks of transection of the fimbria-fornix (FF). In this system, cell death has been documented by the disappearance of neurons stained for Nissl, acetylcholinesterase (AChE) and choline acetyltransferase (ChAT)-immunoreactivity (ir). There is now some evidence that these cells may not actually die following axotomy, but rather may become refractory to histological detection. To further study the effects of axotomy on septohippocampal neurons we have retrogradely labeled neurons in the MSDB prior to FF transection and then compared the number of labeled and histologically stained neurons in adjacent sections at three survival times. Eighteen adult female Sprague-Dawley rats received stereotaxic injections of Fluoro-Gold (FG; 2%, 50 nl) into the hilus of the dentate gyrus at 5 sites along its septotemporal axis. One week later the FF and overlying cortical tissue were bilaterally aspirated in 12 rats; the remaining animals served as time-matched, unlesioned controls. The animals were killed after an additional 3, 6, and 10 weeks and the brains processed for histological analysis. Frozen 40 μm thick sections were cut through the MSDB and hippocampus and 4 sets of adjacent sections were collected: one set was mounted without staining, and used for visualization of FG labeled neurons. The second set of sections was stained with thionin, the third set was histochemically stained for AChE and the fourth set was stained for ChAT-ir. Neurons were counted at three rostrocaudal levels of the MSDB. Compared to unlesioned controls, the number of Nissl-stained neurons and ChAT-ir neurons was greatly reduced at all survival times, as has been previously reported. Marked neuronal shrinkage was noted by 10 weeks, however the number of FG-labeled neurons in the lesioned brains did not differ from the control brains. These data indicate that the septohippocampal neurons do not die following axotomy, but apparently become histologically refractory. *(Supported by ADRDA IIRG-88-059)*

177.6

THE RESPONSE OF PGP 9.5-IMMUNOREACTIVE (IR) NEURONS IN THE RAT BASAL FOREBRAIN TO TRANSECTION OF THE FORNIX-FIMBRIA. M.R.Donald, M.D.Mills*, K.S.Jodelis, and L.R.Williams, CNS Diseases Research, The Upjohn Co., Kalamazoo, MI 49001.

Morphologic evaluation of Nissl-stained basal forebrain neurons indicates the majority of basal forebrain neurons are non-cholinergic neurons that are lost, i.e., uncounted, two weeks after fornix-fimbria (F-F) transection and spared by coincident treatment with NGF (PNAS 83:9231). Positive staining of neurons with a polyclonal antibody to protein gene product (PGP) 9.5, a neuron-specific protein (Brain Res. 278:224), has provided a more objective criteria for determining neuronal presence after F-F transection. The results show consistent, intense staining of neuronal cytoplasm and processes throughout the cortex, caudate-putamen, and basal forebrain. A 28% loss of IR neurons compared to the contralateral side is detected two weeks after a unilateral F-F aspiration in the medial septum of coronal sections representing the rostral third of the basal forebrain. Cholinergic neurons comprise only 10% of the total neuronal population in this region. Magnocellular neurons are lost and many of the surviving neurons on the side of lesion appear atrophied, dystrophic, and have significant reduction in the intensity of reaction product. These results provide greater resolution of the response of basal forebrain neurons to axotomy and indicate a large involvement of non-cholinergic neurons.

177.7

TRANSIENT ISCHEMIA-INDUCED CHANGES IN EXTRACELLULAR GLUTAMATE AND NEURONAL ACTIVITY IN THE IN VIVO MONGOLIAN GERBIL HIPPOCAMPUS. A.Mitani*, H.Imon[1]*, K.Iga[2]* and K.Kataoka* (SPON:N.Ogawa). Dept. of Physiology, [1]Anesthesiology and [2]Orthopedics, Ehime Univ. Sch. of Med., Ehime 791-02, Japan.

Glutamate (Glu) has been implicated as a mediator of ischemic neuronal death. We measured the extracellular concentration of Glu in halothane-anesthetized mongolian gerbil hippocampus by microdialysis technique and recorded spontaneous single neuronal activity (SSNA) in the hippocampal CA1 sector before, during, and for one hour after a 5-min period of transient ischemia which leads to delayed neuronal death in CA1 sector. The Glu was significantly increased during and for a few minutes after the ischemic period. The SSNA disappeared during the same period. It began to reappear about 15 min after recirculation and then returned to preischemic level. Thus, excitotoxicity of Glu was not observed in the above acute phases. We will also present ischemia-induced changes of the hippocampal extracellular glutamate and neuronal activity in chronic experiment.

177.8

THE EFFECTS OF LONG-TERM LOW-PROTEIN DIET ON THE HIPPOCAMPAL MOSSY FIBER SYNAPSES OF ADULT RAT. J.P.Andrade* and M.M.Paula-Barbosa, Dept. of Anatomy, Oporto Medical School, Oporto, Portugal.

We have previously demonstrated that adult rats fed for long periods with a low-protein diet displayed severe cell loss in the cerebellum and hippocampal formation (1).In the latter area the granule and CA3 pyramidal cells were found to be highly vulnerable to this condition with losses up to 40% after 18 months of experiment. Thus,we thought it would be of interest to study the synapse between these two cell types which form one of the major links of the hippocampal trisynaptic circuitry. The neuropil from the stratum lucidum of the undernourished animals presented unespecific axonal and dendritic degenerative changes. The synaptic contacts appeared with normal configuration albeit those formed over dendritic spines which seemed to be reduced. For quantitative purposes the disector method was applied to serial ultrathin sections. The number of synapses per unit volume was found to be significantly reduced after 6 months of treatment and its loss increased with the time of experiment.The fraction of the plasmalemma of mossy fiber endings occupied by active zones was reduced as well. We conclude that long-term undernourishment leads to a decrease in the MF/CA3 synapses. Conversely to what happened after long-term alcohol consumption where a similar synaptic loss was found, no signs of synaptic plasticity was detected. (1) Paula-Barbosa,M.M. et al., Exp.Neurol. 103: 186, 1989.

177.9

HEAVY METAL INDUCED ALTERATIONS IN THE NERVE GROWTH FACTOR LEVEL IN THE RAT BRAIN. L. Lärkfors*, A. Oskarsson*$, J. Sundberg*$ and T. Ebendal* (SPON: N.G. Carri). Dept. of Dev Biology, Uppsala Univ. and the Nat. Food Ad., Lab. of Tox.$ Uppsala, Sweden.

Nerve growth factor (NGF) has been found to play an important role for the cholinergic neurons in the basal forebrain during development and adulthood. Hypothetically decreasing levels of NGF could cause a degeneration of these cells. Heavy metals have also been suggested to cause neurodegeneration. To determine if heavy metals have any influence on the level of NGF during the first, sensitive postnatal period, we exposed pregnant Sprague Dawley rats with methyl mercury (Hg) in the food (4 mg/kg) or lead chloride (Pb) in the water (200 and 500 mg/l). The pups were also exposed during the first 25 or 50 postnatal days and the NGF levels were analyzed in the cortical areas and in the septum with a sensitive enzyme immunoassay. The pups exposed to Hg exhibited an increase in the hippocampus (60%) on P 25 and 50. In contrast, there was a decrease in the septum (30%) on P 25 and 50. The result from the Pb experiment is now being evaluated. The exact mechanism by which the low levels of heavy metals is affecting the NGF concentration in the developing brain is unknown. This finding might indicate a connection between exposure of heavy metals and neurodegeneration, such as that found in the basal forebrain in Alzheimers disease. Supported by the National Swedish Enviromental Protection Board (5324168-3).

177.10

EFFECTS OF NEONATAL AXOTOMY ON DRG NEURONS: AN IMMUNO- AND HISTOCHEMICAL ANALYSIS OF THREE NEURONAL POPULATIONS. B.T. Himes, C. Rogahn* and A.Tessler, Philadelphia VA Medical Center and The Medical College of Pennsylvania, Philadelphia, PA 19129.

Neonatal sciatic nerve lesion has been shown to cause 50% of L5 dorsal root ganglion (DRG) neurons to die. It is not clear if specific subpopulations of DRG neurons survive axotomy. Therefore the right sciatic nerve was cut and ligated in the lower half of the thigh in newborn rats. After a 60 day survival period the right and left L5 DRG were sectioned and every third section was processed for one of three techniques: Calcitonin Gene Related Peptide (CGRP) immunocytochemistry, Thiamine Monophosphatase (TMP) histochemistry or Carbonic Anhydrase (CA) histochemistry. These three markers identify distinct subpopulations of DRG neurons with little overlap. In control and unoperated L5 DRG both CGRP and TMP are found in approximately 40% of the neurons which are mostly small to medium sized. CA is localized in the largest DRG neurons and is present in approximately 15% of the total. Our results show that while the overall number of neurons in the operated L5 DRG is reduced 50% (75% of the axotomized neurons die), the relative proportions of each of the three different populations remains the same. These findings indicate that these subclasses of DRG neurons are equally susceptible to cell death following neonatal axotomy. Supported by the VA Medical Research Service, USAMRDC grant 51930002 and NIH grant 24707.

177.11

[$H]-THYMIDINE LABELLING OF DEGENERATING CELLS IN THE OLFACTORY EPITHELIUM OF BULBECTOMIZED RATS. V.McM. Carr and A.I. Farbman. Dept. Neurobiol. & Physiol., Northwestern Univ., Evanston, IL 60208.

Olfactory receptor neuronal cells(ORCs) of the olfactory epithelium(OE) undergo continual death and replacement throughout a vertebrate's life. Olfactory bulbectomy(OB-X) causes enhanced ORC death in the ipsilateral OE due, iniatially, to transection of ORC axons and, subsequently, to the absence of OB targets for the ORCs produced in reconstitutive processes.

To determine the time from ORC birth to death following OB-X, unilaterally OB-Xd rats were treated with [^{3}H]-TdR (2uCi/gm. b.wt., 3x/24 hrs) at 6 or 8 d. post op., a period of high mitotic activity in the affected OE (Monti Graziadei and Graziadei, 1979) and sacrificed 4-10 days later. Autoradiographs of the OE were examined for the presence of [^{3}H]-labelled (≥4 Ag grains) pycnotic cells. Results show that at 4-6 days post [^{3}H]-TdR exposure 10-20% of the pycnotic cells in the ipsilateral OE were labelled; at 10 days 5-10% were labelled. Most of the labelling occurred in the ORC region of the OE. Occasional labelled pycnotic cells also occurred in the control OE. These times appear insufficient for completion of proliferation, migration, and axonal outgrowth prior to degeneration.

177.12

REACTIVE ASTROGLIOSIS IN THE LATERAL GENICULATE NUCLEUS (LGN) OF THE CAT. R.E. Kalil, A.N. Lies and A.L. Shaikh. Center for Neuroscience, Univ. of WI, Madison, WI 53706.

Damage to visual cortex in the cat leads to the retrograde degeneration of neurons in the LGN. This neuronal degeneration is accompanied by the proliferation of glial cells. For example, astrocytes increase both in number and in size, and previous work with the electron microscope (Kalil and Behan, '87) suggests that these cells may sometimes behave abnormally by wrapping their processes around surviving neural elements in the degenerated LGN.

To collect further information on reactive astrogliosis in the LGN, visual cortex was ablated unilaterally in adult cats. Following survival periods that ranged from 1 day to 26 weeks, astrocytes in the LGN were stained with a monoclonal antibody against glial fibrillary acidic protein (GFAP) and prepared for light and electron microscopy. In comparison with normals, an increase in GFAP immunoreactivity (IR) is evident in the degenerating LGN as early as 3 days after a lesion of visual cortex. During the next 11 days GFAP-IR increases markedly, and then appears to decline slightly over the following 6 weeks. Counts of astrocytes in semi-thin sections show an elevation in cell density of more than 30% at 3 days survival and 75% at 2 weeks. These results demonstrate that damage to visual cortex in the adult cat triggers reactive gliosis in the LGN that occurs rapidly and persists for several weeks.

177.13

RETINAL GANGLION CELLS DO NOT SURVIVE TRANSECTION OF THE OPTIC NERVE IN ADULT RATS D.T. Ross and P.Tempesti*. Department of Neurosurgery, University of Pennsylvania, Philadelphia PA.

The identification of treatments which effectively rescue mammalian retinal ganglion cells from retrograde degeneration or enhance axonal regeneration following optic nerve injury has historically been complicated by difficulty in distinguishing different types of ganglion cells from populations of retinal interneurons in the ganglion cell layer. In order to overcome this problem for the present study retinal ganglion cells in adult male Long Evans rats were pre-labelled with the permanent fluorescent retrograde marker DiI (6.0 µl of a 1.5% solution in 100% ethanol) injected bilaterally at the coordinates of the lateral geniculate 2 days prior to unilateral intraorbital optic nerve transection. Fourteen days after optic nerve transection these animals were euthenized, perfused with a 4% paraformaldehyde solution and their retina prepared as wholemounts. Quadrants of these retina were reacted using florescence immunohistochemistry for tyrosine hydroxylase (TH), choline acetyltransferase (ChAT), and aspartate aminotransferase (AAT) with fluoroscein conjugated secondary antisera in order to identify populations of dopaminergic interplexiform cells, cholinergic, and GABAergic amacrine cells, respectively. Normal and experimental retina were examined for the presence of labelled ganglion cells, interneurons, and double labelled cells.

In normal retina extensive populations of large, medium and small neurons are intensely labelled, which appear to correspond to α, β and γ ganglion cells, respectively. Populations of Dopaminergic interplexiform cells, cholinergic and GABAergic amacrine cells are also identifiable in these retina but no DiI labelled cells are double labelled with either TH, ChAT, or AAT. Fourteen days following optic nerve transection no DiI labelled neurons persist in experimental retinae but populations of TH$^+$, ChAT$^+$, and AAT$^+$ neurons were present in normal densities. These results indicate that retinal ganglion cells undergo complete retrograde degeneration within 14 days after optic nerve transection and that the only neurons in the ganglion cell layer which survive are interneurons.

177.14

PRELABELING WITH TRUE BLUE IS SUPERIOR TO FLUORO-GOLD IN DEMONSTRATING THE EFFECT OF HINDLIMB AMPUTATION ON DORSAL ROOT GANGLION NEURONS (DRGs). W.T. Garrett*, R.L. McBride, J.K. Williams, Jr.* and E.R. Feringa (SPON: D.S. Feldman). VA Medical Center and Medical College of Georgia, Augusta, GA 30910.

Hindlimb amputation causes loss of dye-labeled neurons from the ipsilateral L-5 DRG. We compared DRGs prelabeled with Fluoro-Gold (FG) or True Blue (TB) to determine if the dyes contributed to neuron loss. We cut the right sciatic nerve 5 mm above the popliteal fossa in 42 adult rats, and soaked the proximal end in 2% FG or 1% TB for 1h. The ends were reapproximated. After 4 days, 10 rats were perfused and 16 rats had rt hindlimb amputation. At 20 weeks the remaining rats were perfused. Comparing 4 day and 20 week controls, no difference in the number of TB labeled cells was found; the number of FG labeled cells was decreased at 20 weeks without an offsetting increase in unlabeled neurons, suggesting FG caused cell death. Labeled, unlabeled and total cells decreased in amputated TB DRGs compared with controls. The FG DRGs showed no additional loss attributable to amputation. Thus FG's toxicity makes it inferior to TB in illustrating the effects of amputation on DRGs. Research supported by the VA.

177.15

20 WEEKS AFTER HINDLIMB AMPUTATION, BOTH THE SIZE AND NUMBER OF LOWER MOTOR NEURONS (LMNs) IS DECREASED. J.K. Williams, Jr.*, R.L. McBride, J.L. Hicks* and E. R. Feringa. VA Med Ctr and Medical College of Georgia, Augusta, GA 30910.

We studied the effects of hindlimb amputation on LMNs prelabeled with a retrogradely transported fluorescent dye. LMN somata in 14 rats, 7 weeks old, were labeled by soaking the proximal end of the right sciatic nerve, severed in the popliteal fossa, in 1% True Blue for 1 hour. The nerve was approximated with a sling stitch. Four days later, the right hindlimb was disarticulated at the hip in 7 of the rats. After 20 additional weeks, all rats were perfused with 10% formalin. Labeled neurons were counted on every fifth 30 µm frozen section of the lumbar cord. Control rats had 176±5 ($\bar{x}$±SEM) small (<30 µm greatest diameter), 347±17 large, and 523±16 total LMNs. Compared to controls, amputated rats had more small (230±22, $p \leq 0.05$), fewer large (135±19, $p \leq 0.001$), and fewer total neurons (366±40, $p \leq 0.01$). In control rats, axotomized LMNs had the potential to reinnervate target tissue. In amputated rats, where the target tissue had been removed, there was significant LMN loss and decrease in surviving neuron size. Supported by the VA Medical Research Service.

177.16

PRELABELED CLARKE'S COLUMN NEURONS ONE YEAR AFTER T-9 SPINAL CORD TRANSECTION. R.L. McBride and E.R. Feringa. VA Medical Center and Medical College of Georgia, Augusta, GA 30910.

The long-term effects of axotomy were studied in Clarke's column neurons labeled before spinal cord transection with a retrogradely transported fluorescent dye. Ten 8-week-old female rats were anesthetized with ketamine and xylazine and 4 µl of a 2% solution of Fluoro-Gold were injected into the cerebellum. Four days later, the spinal cord of five of the rats was tran-sected at T-9. Forty to 52 weeks after injection the rats were perfused with 10% formaldehyde. Labeled cells were analyzed on 30 µm frozen sections of the T-11 to L-1 lumbar cord. The mean number of labeled Clarke's column neurons per section was 7.5±1.4 ($\bar{x}$±SEM) in control rats and 0.2±0.0 in transected rats ($p<.001$). While only neurons were labeled in control rats, in transected rats unidentified labeled structures were also seen. These were similar to structures noted as early as five weeks after injury in other experiments and presumably are microglia, oligodendroglia or macrophages. If any of these labeled structures are shrunken neurons, even after one year they show no changes indicative of recovery. Supported by the VA Medical Research Service.

DIFFERENTIATION, MORPHOGENESIS AND DEVELOPMENT: FIBER GUIDANCE AND SYNAPTOGENESIS

178.1

INTERCELLULAR INTERACTIONS AMONG INSECT OLFACTORY CELLS IN CULTURE. L.P. Tolbert, L.A. Oland, and G. Or*. ARL Division of Neurobiology, U. of Arizona, Tucson, AZ 85721.

In the olfactory system of the moth, sensory axons induce the formation of large (ca. 60-um diameter) synaptic glomeruli, each surrounded by an envelope of glial cells. Previous observations (see Tolbert & Oland, TINS 12:70) have provided evidence that the development of glomeruli depends upon an interaction between the ingrowing sensory axons and the glial cells of the antennal lobe (AL). The current study is aimed at distinguishing between a passive role for glia and a more active one where glial cells might act as intermediaries in the morphogenetic influence of the afferent axons. In order to test directly whether AL glial cells pre-exposed to antennal cells can substitute for the antennal cells in inducing morphogenetic effects in AL neurons, we have turned to tissue culture (Stengl & Hildebrand, Hayashi & Hildebrand, Neurosci. Abstr. 14:379, 380), where we can readily control interactions between cell populations. Neurons and glial cells are dissociated from ALs at a stage before glomeruli form. In the first experiment, we have examined the effects of antennal cells on mixed cultures of AL neurons and glia and have found that a soluble factor(s) from dissociated antennal cells causes both neurons and glial cells to display morphological features different from control cells. In the second set of experiments, currently in progress, we are testing whether the AL glial cells can mediate these effects. We are exposing glia-enriched cultures to soluble factors from antennal cells and then co-culturing those glia with dissociated AL neurons. We will then determine if these neurons develop morphological features characteristic of the neurons in mixed cultures exposed directly to antennal cells. (Supported by NIH grant #NS20040 to LPT.)

178.2

THE FIRST EVENTS IN THE FORMATION OF INSECT OLFACTORY GLOMERULI INVOLVE SENSORY AXONS AND OLFACTORY-LOBE GLIA BUT NOT OLFACTORY-LOBE NEURONS. L.A. Oland, G. Or*, L.P. Tolbert. ARL Division of Neurobiology, U. of Arizona, Tucson, AZ 85721.

Our previous studies of intercellular interactions during development of the olfactory (antennal) lobe in the moth Manduca sexta have shown that formation of glomeruli requires both sensory axons from the antenna and neuropil-associated glial cells (see Tolbert and Oland, TINS 12:70). Arrival of sensory axons triggers changes in the morphology and distribution of glial cells that are followed by changes in the morphology of antennal-lobe (AL) neurons, suggesting that glial cells may mediate afferent-axon induced changes in AL neurons. To determine how glial cells and the neurons of the system interact during glomerulus formation, we have: 1. filled sensory axons with Lucifer yellow (LY), 2. stained AL neurons using the Golgi method, 3. filled individual AL neurons with LY, and 4. examined the distribution of synapses in regions of developing glomeruli. These studies have revealed that at the earliest stages of glomerulus development, when well defined spheroidal knots of neuropil first become visible, the knots comprise only sensory-axon terminals surrounded by glial cells. The arborizations of AL neurons at these stages are confined to a separate layer of neuropil deep to the axons; synapses are also restricted to this layer. Thus, the initial events in the construction of glomeruli are the creation of knots of terminal branches of sensory axons and the formation of glial envelopes around them. Processes of AL neurons must only secondarily invade the knots to form the synaptic neuropil characteristic of mature glomeruli. (Supported by NIH grant NS20040 to LPT.)

178.3

BRAIN MACROPHAGES TRANSIENTLY TARGET CERTAIN AXON TRACTS. C.E.Milligan*, T.J.Cunningham and P.Levitt. Dept. of Anatomy. Medical College of PA., Philadelphia, PA 19129.

The distribution of brain macrophages was mapped in the post-natal rat using immunohistochemical techniques employing a commercially available monoclonal antibody to cells of the rat monocyte/macrophage lineage. Throughout post-natal development the location, density and morphology of these cells change. From P0-P8 stained cells usually have a rounded morphology with no apparent processes and are located primarily within the cellular layers surrounding ventricles and corpus callosum, and lining and within the septum pellucidum. At P8, a substantial increase occurs in forebrain (sub-callosal white matter, internal capsule, fornix, anterior commisure) and cerebellar white matter; many of these cells have processes of varying lengths. From P12-18 the density of stained cells decreases rapidly. This transient tract associated distribution is specific, since many regions containing axon tracts have few or no macrophages throughout post-natal development. During early post-natal development cells with well developed processes also appear in the deep layers of the superior and within the inferior colliculi, where many degenerating profiles are seen. The association of macrophages with certain axon tracts and not simply with ventricles or the septum pellucidum, as well as, their appearance in areas of frank cellular degeneration, suggests that they play an important role in the modelling of these CNS regions during development. (Supported by grants 1-919 from March of Dimes and NS16487 from NINCDS.)

178.4

AFFERENTS FROM SUPERNUMERARY ANTENNAE INNERVATE OPTIC LOBES LACKING A RETINA. William J. Evans and Nicholas J. Strausfeld. ARL Div. Neurobiology, Univ. of Arizona, Tucson, AZ 85721.

Observations of developing antennal and optic lobes suggest that sensory afferents and glial cells play a vital role in determining the subunit organization of target neuropils [Tolbert LP, Oland LA (1989) Trends Neurosci 12:70-75; Fischbach K-F (1983) Dev Biol 95:1-18]. In the absence of ingrowing axons, antennal lobes are aglomerular and optic neuropils lack their normal arrangement of retinotopic columns. We have asked the following questions. 1) Will ectopic afferents invade a sensory neuropil? 2) If so, does cellular organization reflect either identity of the sensory afferents or neurons of the target neuropil? We have attempted to answer this in the moth *Manduca sexta* by obtaining eyeless adults after removing larval stemmata in late fourth or early fifth instar. Supernumerary antennae were grafted onto pupae destined to be eyeless adults. Control animals lacked compound eyes and laminas but possessed reduced optic lobes lacking retinotopic organization. After eclosion, animals having a third antenna in lieu of a compound eye were processed histologically. Bodian and Golgi preparations revealed that 1) in 20% of the experiments antennal afferents invaded glomerulus-like first-order neuropil; 2) projection neurons arising from this neuropil invaded a medulla having a columnar subunit organization; 3) projection neuron dendrites reflected the glomerulus-like organization of receptor endings whereas their terminals were similar to those of normal visual interneurons. The results suggest that antennal afferents can cause glomerular structures in a foreign target neuropil when this neuropil is denied its normal sensory input.

178.5

Substrata most suitable for rod photoreceptor cell attachment and process outgrowth in vitro I.J. Kljavin and T.A. Reh, Dept. Med. Physiol. Univ. Calgary, Alberta T2N 4N1 Canada.

In dissociated 3-day rat retinal cultures rods differentiate on top of retinal glial cells by process extension. To identify the molecules that might be involved in this glial-mediated process extension we cultured cells at low density on several ECM components (laminin, fibronectin, collagen I and Matragel). Rods, identified in vitro by a monoclonal antibody to opsin (from R. Molday, U.B.C.), adhered to these substrates but failed to extend processes. The glial mediated diffferentiation of rods appears to be specific to Müller cells in the retina: 90% of the rods on Müller cells showed process outgrowth, while only 23% of those on astrocytes, and less than 1% on 3T3 fibroblasts had processes greater than 2 body lengths. Therefore, Müller cells appear to have a unique capability of promoting differentiation and process outgrowth of rods in culture. Further studies are directed towards identifying the molecules responsible for this Müller rod interaction.

Support by MRC Canada, Retinitis Pigmentosa, T.A.R. is a Sloan Fellow.

178.6

VENTRAL CORD STIMULATION MAY ACTIVATE SYNAPSES IN THE WHITE MATTER OF DEVELOPING SPINAL CORD. L. Ziskind-Conhaim and F. Gigel* (SPON: W. Welker). Dept. of Physiol., and Ctr. for Neurosci., Univ. of Wisconsin, Madison, WI 53706.

Ventral root and cord stimulation via suction electrodes evoked antidromic action potentials that were recorded intracellularly in spinal motoneurons of rat embryos. These action potentials were followed by long-latency (10-150 ms) subthreshold potentials which disappeared when the cord was perfused with solutions that reduce synaptic activation (1 mM-Ca^{2+}/8 mM-Mg^{2+}; 1 mM-Ca^{2+}/0.5 mM Cd^{2+}). The subthreshold potentials could not be generated if 1) the stimulating electrode was located > 0.5 mm away from the cord, and 2) the white matter was destroyed. One possible explanation for these results is that electrical stimulation activates synapses located in white matter (marginal zone).

Electron microscopic observations revealed that at days 16-17 of gestation the number of synapses in the white matter (ventral and lateral funiculus) was at least 3-fold larger than in the intermediate and ventral gray horn. The number of synapses in the gray matter significantly increased by Days 19-20, while synapses in the white matter began to decline near birth, and were absent in the white matter of 2-week-old pups. We conclude that during embryonic development, ventral cord stimulation activates synapses in the white matter. Supported by Research Career Development Award and NIH grant NS 23808.

178.7

GENETIC SCREEN FOR MUTANTS AFFECTING NEURONAL CONNECTIVITY IN THE VISUAL SYSTEM OF DROSOPHILA. M.E. Grether*and H. Steller*(SPON: A.D. Lander). Depts. of Biology and Brain and Cognitive Sciences, MIT, Cambridge, MA 02139.

The overall objective of our research is to understand how genes control the assembly of a functional nervous system during embryonic development. To address this issue we use the larval visual system of Drosophila. The gene disconnected is required for the establishment of stable connections between the larval optic nerve and its synaptic partners during embryonic development. We have systematically screened for additional mutations that affect the development of the larval visual system by analysing the projection of the larval optic nerve in embryos homozygous for chromosomal deletions.

We find that a small number of deletions affect the formation of specific neuronal connections in this system. Ectopic position of the target cells due to defective morphogenetic movements does not necessarily prevent "normal" connectivity between the larval optic nerves and their targets. We have also observed axonal outgrowth from the larval photoreceptor cells in the absence of the target cells.

Two of the deficiency stocks, Df(2R)L+48 and Df(3L)w4, ru h e ca/TM6B contain phenotypes of interest to us. In each case, the larval optic nerve projects to an ectopic position in the brain of a basically normal embryo. We are mapping the loci responsible for these phenotypes.

178.8

ANTIGEN PRESENT IN B-PREGANGLIONIC NERVES APPEARS IN B-GANGLION CELLS FOLLOWING SYNAPTOGENESIS. S.L. Van Eyck, J.C. Thigpen* and L.M. Marshall. Dept. of Physiology, Univ. of North Carolina, Chapel Hill, NC 27599.

Lumbar sympathetic ganglia in frog have two main neuronal pathways formed by B- and C-type preganglionic axons which selectively innervate B- and C-type ganglion cells. We investigated the differentiation of the B pathway during development by examining the appearance of an antigenic marker present only in the B-type pre- and postganglionic neurons of adult bullfrogs.

Ninth and tenth paravertebral ganglia from Rana catesbeiana larval stages (XIV-XXV) were examined by immunocytochemistry for recognition by this monoclonal antibody raised against adult frog ganglia. Beginning at about stage XVI, heavily labeled preganglionic axons make synaptic contacts on the yet unlabeled ganglion cells. By the end of metamorphosis many of these innervated ganglion cells express this B-cell marker.

The results indicate that this particular phenotypic marker of the B pathway occurs subsequent to synaptogenesis. This raises the possibility that ganglion cell phenotype may be specified during development through innervation by a particular type of preganglionic axon.

BRAIN METABOLISM AND BLOOD FLOW I

179.1

IMPROVED AUTORADIOGRAPHIC RESOLUTION REVEALS SUBSTANTIAL UNDERESTIMATION BY THE IODOANTIPYRINE METHOD OF BLOOD FLOW IN SOME REGIONS OF RAT BRAIN. T.T. Soncrant, H.W. Holloway*, D.M. Larson*, N.H. Greig*, U. Freo* and S.I. Rapoport. Laboratory of Neurosciences, National Institute on Aging, NIH, 10/6C103, Bethesda, MD 20892.

The autoradiographic [14C]iodoantipyrine (IAP) method (Sakurada et al., Am. J. Physiol. 234:H59, 1978) has been used to measure regional cerebral blood flow (rCBF) in awake animals, yielding values of 40-250 ml/100g/min. The method uses a freely-diffusible tracer, IAP, that accumulates in brain in relation to blood flow during a 45-60 sec i.v. infusion. Regional brain radioactivity is determined by quantitative autoradiography, but resolution is poorer than in deoxyglucose studies. We have found that poor resolution is due in part to post-mortem (and probably pre-mortem) lateral diffusion of tracer in brain and can be improved by rapid freezing, so that distinct cortical layers are apparent. However, some brain regions then have IAP concentrations much higher than previously encountered. For those areas, rCBF calculations based on the IAP model are highly unstable, because failure to maintain a high blood-to-brain IAP concentration gradient leads to a markedly non-linear relation between brain radioactivity and blood flow. Preliminary results with alternate infusion schedules suggest that, in some regions, rCBF is much (possibly twofold) greater than previously recognized, and that coupling between blood flow and metabolism is maintained at a finer anatomic level than heretofore known. Alternate IAP infusion schedules and methods for rapid freezing of the brain that improve resolution and accuracy of rCBF measurements will be presented.

179.2

FREELY DISTRIBUTED IMAGE PROCESSING PACKAGES SIMPLIFY ANALYSIS AND DISPLAY OF FUNCTIONAL METABOLIC ACTIVITY DATA. A.J. Annala. Neural, Informational, and Behavioral Sciences Program, University of Southern California, Los Angeles, CA 90089.

Accurate interpretation of functional metabolic activity data from deoxyglucose autoradiography [2DG-QAR] experiments can be hampered by hardware and software limitations of low cost neurobiological image processing systems. Acquisition of high resolution cameras and development of custom programs for use of nonstandard analysis protocols are beyond the financial capability of all but a few large laboratories. Most of these limitations can be overcome by using image processing packages distributed without charge by investigators in university and government laboratories. These software tools (aips, alv, brlcad, ds, iraf, pbm, touchup, utah rle toolkit, and xim) are designed to be as computer, display device, and data format independent as possible. These packages offer a wide range of image processing functions including: grey scale and color image display, simple image arithmetic, linear and nonlinear filters, fast fourier transforms, geometric transformations, image registration and mosaicing, contour plotting, surface rendering, and volume visualization.

This poster presents a series of 2DG-QAR results generated by these tools including: a very high resolution image (2048H x 2048V pixels) constructed by overlapping frames (384H x 485V pixels) from a low cost video camera; registered pairs of grey scale histological and pseudocolor autoradiographic images of rat and rabbit brains employing a ratio correction for nonuniformities in the underlying light source; typical intensity histograms, surface plots, and automatically generated outlines of stained histological sections. Supported by ONR N00014-88-K-0112, NSF BNS-8718300 and McKnight Foundation grants to R. F. Thompson.

179.3

A SIMULATION MODEL FOR STUDYING INTERREGIONAL CORRELATIONS BETWEEN CEREBRAL METABOLIC RATES. Barry Horwitz, Lab. Neurosciences, National Institute on Aging, National Institutes of Health, Bethesda, MD 20892.

Correlation coefficients between pairs of regional metabolic rates have been used to study patterns of functional associations among brain regions in humans and animals (Horwitz et al., J. Cereb. Blood Flow & Metabol., 1984; Arch. Neurol., 1988). A computer simulation model is presented for the purpose of giving a partial vadidation for correlational analysis. The model generates a set of simulated metabolic data upon which correlational analysis is performed. The model consists of n regions, in which metabolism (or blood flow) in one region is a sigmoidal function of metabolism (or blood flow) in some or all of the other regions. The parameter linking metabolic rates between two regions is called a functional coupling constant. It is shown how the model parameters can be chosen so that simulated and experimental data have values similar to one another. Because the pattern of functional coupling constants in the model is known, these simulations demonstrate that the correlation coefficient between normalized metabolic rates is proportional to the strength of the functional coupling constant, and that correlational analysis yields information on regional involvement in neural systems not evident in the pattern of absolute metabolic values.

179.4

QUANTIFICATION OF THE GLYCOLYTIC AND OXIDATIVE COMPONENTS OF CEREBRAL GLUCOSE METABOLISM IN STIMULATED STRUCTURES. R. F. Ackermann and J.L. Lear*, Divison of Nuclear Medicine and Biophysics, UCLA School of Medicine, Los Angeles, CA 90024; and Division of Nuclear Medicine, U. of Colorado School of Medicine, Denver CO, 80262.

We have used double-label quantitative autoradiography to compare directly the local cerebral metabolic rate for glucose (LCMRG) derived from accumulation of [F-18] fluorodeoxyglucose (FDG), with that derived from the accumulation of [C-14] 6-glucose (GLC). In normal brain, at optimal experimental times, FDG-derived and GLC-derived LCMRG are similar. However, activating the hippocampus with kainic acid, or the optic tectum with flashing light, elevates FDG-derived LCMRG markedly, but leaves the GLC-derived LCMRG at near-normal values. Hypothesizing this discrepancy in stimulated structures to result from induction of aerobic glycolysis, we developed a kinetic model for quantifying the glycolytic and oxidative components of LCMRG. Analysis of the simultaneously obtained FDG and GLC data with this model reveals a significantly elevated glycolytic rate in stimulated structures; the oxidative rate remains relatively unaffected. It is known that Na^+-K^+ ATPase activity largely accounts for brain FDG accumulation, and that Na^+-K^+ ATPase activity can stimulate glycolysis. Thus, the transmembrane ion fluxes that accompany stimulation may trigger glycolysis in affected brain structures via the products of Na^+-K^+ ATPase activity.

179.5

PET MEASURED EVOKED CEREBRAL BLOOD FLOW RESPONSE IN AN AWAKE BEHAVING MONKEY. J.S. Perlmutter, L.L. Lich*, and S.Bucholtz*, Washington University School of Medicine, St. Louis, MO 63110.

We have developed a method to measure evoked CBF responses in an awake, behaving monkey using PET and $H_2{}^{15}O$. We trained an animal (operant conditioning, positive reinforcement only) to climb unassistedly into a modified primate chair that was then positioned in the scanner. A 20 gauge catheter was placed in a leg vein to permit $H_2{}^{15}O$ injection. A special headholder and acrylic skullcap permitted precise placement and accurate repositioning. We measured blood flow qualitatively with bolus injection of $H_2{}^{15}O$ and a 40 sec scan. Each session included scans at rest interposed with scans during vibration of a forepaw. Regional CBF responses were identified using subtraction image analysis. After global normalization, the resting image was subtracted on a pixel-by-pixel basis from a comparable image collected during vibration. The region of peak response occurred in the contralateral sensorimotor cortex with a mean magnitude of 10% (± 2.2%) of the gobal mean value for 6 separate experiments, significantly greater than the mean qualitative blood flow change (1.2% ± 2.5%; p=.002) in the same region for rest-rest pairs. This newly developed technique forms the basis for a wide variety of experiments including evaluation of the relationship between evoked blood flow and electrical responses in the brain.

179.6

THE EFFECT OF GENDER ON CEREBRAL METABOLIC RATES FOR GLUCOSE IN HEALTHY HUMANS MEASURED BY POSITRON EMISSION TOMOGRAPHY (PET). S. A. Miura*, B. Horwitz, M. B. Schapiro*, C. L. Grady*, A. Kumar*, E. Wagner*, and S. I. Rapoport, Lab. Neurosciences, National Institute on Aging, National Institutes of Health, Bethesda, MD 20892. (SPON: V. Murphy)

Gender differences have been observed in metabolic studies of the human brain. Using [18-F]-fluorodeoxyglucose and PET (Scanditronix PC1024 with transverse resolution=6mm, axial resolution=10mm) to measure the regional cerebral metabolic rate for glucose (rCMRglc), we studied young (20-40 years), healthy men (N=15) and women (N=9) to see if gender differences in rCMRglc could be detected. A 10 minute transmission scan was obtained prior to each emission scan to correct for attenuation.

Absolute values of the global grey matter metabolic rate for glucose did not differ between groups [men: mean ± SD = (8.67 ± 1.39) mg/100g/min; women: (8.54 ± 1.31) mg/100g/min; p>0.05]. Similarly, absolute values of rCMRglc for 76 individual regions of interest were not different between groups (p>0.05). Ratios of the 76 regional to global values also were compared and yielded gender differences in only 6 regional ratios, a result consistent with chance.

Thus, contrary to the findings of previous studies of resting cerebral metabolism, significant gender differences in absolute metabolic rates were not detected. Differences from previous studies may be due to errors introduced by their use of a calculated rather a transmission derived attenuation correction.

179.7

RESTING BRAIN GLUCOSE METABOLIC PATTERNS CORRELATE WITH SHORT-TERM MEMORY IN OPTIMALLY HEALTHY ELDERLY SUBJECTS. A.Berardi, J.V. Haxby* and S.I. Rapoport, Lab. of Neurosciences, National Institute on Aging, NIA, Bethesda, MD 20892.

Our laboratory has shown that in optimally healthy elderly subjects, right-left parietal brain metabolic asymmetry is correlated with discrepancy in visual-verbal memory performance (Berardi, A., Neurol., 37 (Suppl. 1):160, 1987). The present study attempted to reproduce this finding with independent subjects using a higher resolution scanner, the Scanditronix PC 1024-7B, and [18-F] fluorodeoxyglucose. 10 young (mean age: 30.7±5.4; range=22-39) and 14 elderly (mean age: 69.3±5.6; range 60-81) subjects were studied. All were right-handed and healthy. Visual and verbal short-term (STM) and long-term memory (LTM) measures were derived from continuous recognition memory tests matched for difficulty. Right-left parietal metabolic asymmetry and visual-verbal memory discrepancies were calculated as (R-L)/[(R+L)/2], where R and L represent homologous right and left regional cerebral metabolic rates for glucose (rCMRglc) or visual and verbal memory test scores. Metabolic asymmetry was correlated with short-term, but not long-term visual-verbal memory discrepancy in the healthy old (Pearson r=0.59, p<0.05), but not young subjects. Older subjects with higher left rCMRglc had higher verbal STM test scores and those with higher right rCMRglc had higher nonverbal-visual STM test scores. These results indicate that metabolic asymmetry in the parietal lobe is related to discrepancy between visual and verbal memory in old, but not young healthy adults.

179.8

DOES AGING COMPROMISE K^+ TRANSPORT IN HIPPOCAMPAL SLICES? E.L. Roberts, Jr.,* T.J. Sick,* and M. Rosenthal* (SPON: R. Kelley). Dept. of Neurology, University of Miami School of Medicine, Miami, FL 33101

Aging alters brain metabolic activity. For example, aged brain tissue did not augment oxygen consumption as much in response to increased extracellular K^+ activity (K^+_o). Also, following short anoxia, K^+_o homeostasis returned more slowly, and synaptic transmission recovered less completely, in hippocampal slices from aged rats. Hippocampal slices from rats 6-7 mon. and 26-27 mon. of age were studied to determine if aging produced deficits in ion transport in normoxic brain. Slices were placed into an interface chamber in which they were exposed to humidified 95% O_2, 5% CO_2, and were bathed in an artificial cerebrospinal fluid. K^+_o was recorded in stratum pyramidale of slices before, during, and after CA1 pyramidal cells were activated transsynaptically at high frequency (40 Hz) by Schaffer collateral stimulation. Stimulus trains were 2 sec. in duration, and were varied in intensity so that several values for maximum K^+_o during or immediately after a stimulus train were obtained. The rate of K^+_o disappearance after the stimulus train was analyzed by determining the times need for K^+_o to decay to half of its maximum value ($t_{0.5}$) and to its prestimulation baseline ($t_{1.0}$). We found that K^+_o decay times did not increase as brain age increased. Thus, energy for K^+ transport in both age groups may not depend on oxidative metabolism, or aerobic glycolysis in the aged brain may be increased to compensate for reduced oxidative metabolism. We conclude that decreased K^+ transport activity in aged brain tissue following short anoxic insults is not due to deficits in K^+ transport existing prior to anoxia. (Supported in part by a pilot study grant from NIA)

179.9

IMPAIRED DECISION-MAKING IN PERSONS AT RISK FOR HUNTINGTON'S DISEASE (AR). W.H. Riege, A.B. Lanto*, J.C. Mazziotta, M.E. Phelps, J.J. Fahl*. V A Med. Center Sepulveda, and Lab. of Nuclear Med., UCLA Sch. of Med., Los Angeles, CA 90024

To determine neuropsychological risk factors in AR persons, we used memory and problem-solving tasks together with evaluation of regional cerebral glucose use (CMRGlc) by positron emission tomography and F-18-fluoro-deoxyglucose. We compared the scores of 58 AR persons and 24 healthy controls on tasks related to decision-making (Abstraction, Progressive Matrices, Time for design reproductions, and Decision bias). AR persons scored low on these, although attention and learning were unimpaired. Even though decision indices were correlated ($p< 0.05$) with CMRGlc ratio of frontal-parietal, of caudate/hem ($r< 0.30$), and of putamen/hem ($r= 0.34$), only a few AR had low regional CMRGlc comparable to those of symptomatic HD persons. With a cutoff of 2 standard deviations below the mean CMRGlc, ten AR persons with basal-ganglia hypometabolism were identified who also scored low ($p<0.01$) in decision and abstraction tasks. Multiple contrasts (with errorwise correction) showed that these 10 AR used twice the time to solve problems, remembered fewer items, and resorted more to guessing than the remaining AR or control persons.

179.10

PET SCAN STUDY OF PATIENTS ON PHENCYCLIDINE. J.C. Wu M.S. Buchsbaum,* W.E. Bunney. Psychiatry Dept, UCI, Irvine, CA 92717.

Introduction: Phencyclidine intoxication is considered one of the best drug models of schizophrenic psychosis. Symptoms of phencyclidine (PCP) intoxication include thought disorders, hallucinations, and delusions. PCP has recently been found to be a noncompetitive antagonist of the NMDA glutamate system. Schizophrenia has been found to have some regional cerebral metabolic abnormalities such as decreased frontal, striatal, and thalamic metabolism on PET scans. If PCP psychosis is similar to schizophrenia, we hypothesize that there will be similar deficits in brain metabolism. Methods:Patients using PCP were recruited. Patients meeting DSM-III-R criteria for PCP abuse and dependence were studied. Patients performed the CPT attention task for 30 minutes after they received 5 mCi of 18F-deoxyglucose. Patients were then scanned in a NEUROECAT scanner (FWHM=7.8mm). Results:A preliminary analysis of 3 patients showed decreased frontal, striatal, and thalamic regional cerebral metabolism compared to a cohort of normal controls. The latest analysis of this study will be presented and discussed.

179.11

SPECT REGIONAL CEREBRAL BLOOD FLOW IN ASYMPTOMATIC HIV + DRUG ABUSERS. S. W. Woods, S. S. O'Malley*, L. H. Price, J. H. Krystal, P. B. Hoffer*, T. R. Kosten*. Dept. of Psychiatry, Yale U. Sch. of Med., New Haven, CT 06508.

An abnormal pattern of relative subcortical hypermetabolism has been reported early in the course of dementia due to human immunodeficiency virus (HIV) infection using positron emission tomography (Rottenberg et al. Ann Neurol 1987;22:700). In that sample, only 1/12 patients (PTS) had intravenous drug abuse (IVDA) as their risk factor for HIV. The present pilot study aimed to extend the previous report in HIV seropositive (HIV+) IVDA PTS not yet manifesting clinical dementia using the more generally available single photon emission computed tomography (SPECT) regional cerebral blood flow (rCBF) technique. METHOD: After giving informed consent, 5 methadone maintained (MM) HIV+ (2M, 3F, mean $\pm$ SD age 33 $\pm$ 10 yrs) and 5 MM HIV- (2M, 3F, 35 $\pm$ 4 yrs) IVDA PTS without clinical dementia underwent: 1) injection of the rCBF tracer Tc-99m HM-PAO (hexamethylpropyleneamine oxime, 20 mCi) followed by SPECT scanning using a neurodedicated multicrystal camera and 2) a comprehensive neuropsychological (NP) test battery. RESULTS: Preliminary analyses by two nuclear medicine physicians blind to HIV status revealed a relatively increased tracer uptake bilaterally in basal ganglia in HIV+ as compared to HIV- PTS (Mann-Whitney U-test, $p<.05$). HIV+ and - PTS showed some impairment on 39 and 25% of NP tasks, respectively. Further image analysis and SPECT/NP correlations will be presented. DISCUSSION: More research is indicated examining the potential role for SPECT rCBF imaging along with NP testing in the monitoring and possible early detection of neuropsychiatric impairment due to HIV infection in the IVDA population.

179.12

SPECT REGIONAL CEREBRAL BLOOD FLOW IN OCD. W.K. Goodman, L.H. Price, C.J. McDougle*, M.A. Riddle*, P.B. Hoffer*, S.W. Woods. Dept. of Psychiatry, Yale U. Sch. of Med., New Haven, CT 06508.

Positron Emission Tomography (PET) studies with ^{18}FDG have consistently shown increased metabolic activity in the orbital frontal cortex (either unilaterally or bilaterally) in Obsessive Compulsive Disorder (OCD) patients (Pts) compared to control subjects (Baxter et al., 1987, 1988; Nordahl et al., 1989; Swedo et al., 1989). Caudate hypermetabolism has been a less consistent finding. A PET study of Pts with Tourette's Syndrome (TS), which may be related to some forms of OCD, showed hypermetabolism of basal ganglia regions (Chase et al., 1984). The present study was designed to extend the PET findings in Pts with OCD (with/without chronic tic history) using the more generally available single photon emission computed tomography (SPECT) regional cerebral blood flow (rCBF) technique. METHODS: After giving informed consent, 11 drug-free, adult Pts with a principal diagnosis of OCD (DSM-III-R) (2F, 9M, mean $\pm$ SD age 33 $\pm$ 15 yrs) underwent injection of the rCBF tracer Tc-99m HM-PAO (hexamethylpropyleneamine oxime, 20 mCi) while lying still with eyes patched in a quiet room. SPECT scanning followed, using a neurodedicated multicrystal camera. After scanning, subjects were assessed regarding their obsessional state at the time of injection. Age- and sex-matched healthy subjects were studied under identical conditions. RESULTS: Data will be presented on the comparison of rCBF in OCD Pts and healthy subjects. The relationship between other clinical variables (e.g., tic history (N=5) & OCD severity) and rCBF will also be analysed. DISCUSSION: SPECT imaging is more widely available and less expensive than PET and seems well-suited to the evaluation of the function of brain regions (i.e., orbital frontal cortex and basal ganglia) reported abnormal in PET studies of OCD.

179.13

METABOLIC EVIDENCE OF BRAIN PLASTICITY IN CHILDREN FOLLOWING LARGE CEREBRAL RESECTIONS. H.T. Chugani*, M.E. Phelps and J.C. Mazziotta (SPON: E. Rubinstein). UCLA School of Medicine, Los Angeles, CA 90024, USA.

Children, unlike adults, can sustain large cerebral resections and yet show remarkably little functional deficit, presumably due to the reorganizational capability of the developing brain. We have used PET with ^{18}FDG in the resting state (eyes open, ears unplugged) to detect metabolic evidence of plasticity in 7 patients who underwent cerebral hemispherectomy as children for the control of intractable epilepsy. In 5 children studied within one year following surgery, PET revealed a typical pattern of hypometabolism in the cerebellar cortex contralateral to the side of hemispherectomy (crossed cerebellar diaschisis). However, in 2 patients studied 6 and 8 years after surgery, crossed cerebellar hypermetabolism was seen instead. Measurements of local cerebral metabolic rates for glucose (lCMRGlc) showed that the magnitudes of increase were 120% and 160% of values in the opposite (normal) cerebellum. In all 5 patients studied within one year of surgery, PET showed no evidence of metabolic activity in the surgically spared striatum and thalamus on the side of hemispherectomy, even though these structures appear normal on CT and MRI. Repeat PET in one of these children 2.5 years following surgery showed that glucose metabolic activity had returned in the largely deafferented caudate. These lCMRGlc changes may reflect altered energy demand in brain regions undergoing anatomic reorganization (e.g. collateral sprouting), such as seen in animal models of hemispherectomy. If such an anatomic-metabolic correlation can be substantiated in animal models, it would provide an intruiging perspective of brain plasticity with PET technology.

179.14

ALTERATIONS OF BRAIN GLUCOSE METABOLISM IN IODINE DEFICIENCY RAT. D.M.Zhang,Z.F.Yang*,D.R.Tian*,W.F Tang*,L.Y.Ma* and Z.P.Chen*(SPON:A.N.Epstein).Department of Anatomy Tianjin Medical College, Tianjin, People's Republic of China.

Iodine deficiency leads to irreversible brain damage during critical period of development. An animal model of iodine deficiency has been produced in rat with a low iodine diet (gestation through 70 days). To identify the distribution of the effect and to localize particulary vunerable regions, the (^{14}C) deoxyglucose method for the measurement of local cerebral glucose utilization was applied to iodine deficient rats at 70 days age. Each rat was injected (IV) with 2-DG 45 min prior to sacrifice. Of 14 brain regions (in 11 animals), all iodine deficiency rats showed reduced glucose utilization. The metabolic rate in the cortical regions was reduced from 29%. The reticular formation and the cerebellum were relatively less affected (4-6%). The auditory system showed the greatest decrease, with a 29% reduction in inferior colliculus and auditory cortex. These results demonstrate that iodine deficiency during development results in a generalized reduction in glucose utilization with the cortical structures and auditory system being particularly vulnerable.

179.15

DIAZEPAM REDUCES REGIONAL CEREBRAL METABOLIC RATE IN NORMAL SUBJECTS. J. T. Metz, H. de Wit*, L. I. Debbold*, S. J. Gatley*, and M. D. Cooper*. University of Chicago, Chicago, IL 60637.

As part of a series of studies with drugs of abuse, we assessed the effects of diazepam (valium) on regional cerebral metabolic rate of glucose (CMRglc), reaction time, and mood.

Eight normal male volunteers (aged 21-29) were studied. Subjects were instructed that they might receive a placebo, a tranquilizer, a stimulant, or alcohol. In each session, on a double-blind basis, they received a capsule containing 0.07 mg/kg diazepam (5 mg for a 70 kg subject), 0.14 mg/kg, or placebo and a citrus flavored beverage. 30 minutes after receiving the drug, they were injected with 6-8 mCi FDG. Subjects performed a visual monitoring task during FDG equilibration to assure that states of alertness were comparable in all subjects and conditions. Subjects also completed a Profile of Mood States before and after each scan. CMRglc was determined by the F-18-2FDG autoradiographic method with a PETT VI scanner. Fourteen ROIs were examined.

Subjects attained average blood levels of diazepam of 60.6 and 119.3 ng/mL at the two doses. Average CMRglc over the whole brain decreased significantly after both doses of diazepam (8.25 mg/100g/min [placebo], 7.36 [5 mg], 7.42 [10 mg]). All regions showed comparable effects. Subjects were less accurate in detecting target light flashes after both doses (96% [placebo] vs 86% [both doses]), but the median time to respond was unaffected (436, 433, and 436 msec at placebo, low dose, and high dose). Mood states were affected only by the higher dose of diazepam: subjects reported decreases in Anxiety and Depression.

This study shows that diazepam decreases overall CMRglc even at doses that do not affect mood or reaction time. These findings can be contrasted to previous findings with ethanol, where effects on CMRglc were correlated with mood.

179.16

CHRONIC HYPERAMMONEMIA REDUCES BRAIN GLUCOSE USE J.Jessy*, A.M.Mans, M.R.DeJoseph* & R.A.Hawkins, Dept. of Physiology and Biophysics, University of Health Sciences / The Chicago Medical School, North Chicago, IL 60064.

Ammonia is suspected to be at least partially responsible for the derangement of cerebral metabolism in hepatic encephalopathy. In portacaval shunted rats, a model of liver disease, characteristic changes include: elevated plasma and brain ammonia, increased brain glutamine and neutral amino acids, and increased transport of neutral amino acids across the blood-brain barrier. Most important is a marked decrease in cerebral function, as reflected by brain glucose and oxygen consumption. It is known that ammonia can alter blood-brain barrier transport and brain amino acid content, but whether hyperammonemia alone is responsible for diminished cerebral function has not been established. To answer this question rats were made hyperammonemic by intraperitoneal injections of urease. Glucose use and tryptophan transport were measured simultaneously in 5 min using a single injection, dual-label technique. After 48 h of urease treatment plasma ammonia was elevated (con. 0.15, exptl. 1.03 μmol/ml) as was brain glutamine (con. 5.4, exptl. 17.8 μmol/g) comparable to values observed in shunted animals. Hyperammonemia enhanced tryptophan transport by 177% and increased brain tryptophan content by 77% although plasma total tryptophan was unchanged. These changes were correlated with plasma ammonia and brain glutamine levels. Cerebral energy metabolism, as reflected by glucose use, was depressed and this depression was inversely correlated with brain glutamine content. These results show that some important metabolic derangements found in hepatic encephalopathy can be reproduced by hyperammonemia alone in otherwise healthy animals. Supported by NS 16389 and NS16737.

179.17

BRAIN FUNCTION, TRYPTOPHAN TRANSPORT, AND METABOLITES AFTER 6 HOURS OF PORTACAVAL SHUNTING. A.M. Mans, M.R. DeJoseph*, J.F. Giguere* and R.A. Hawkins, Dept. of Physiology, The Chicago Medical School, North Chicago, IL

Portacaval shunting in rats leads to decreased brain function (as indicated by lower glucose consumption), alterations in blood-brain barrier transport, and abnormal concentrations of many metabolites. We found previously that several of the most characteristic abnormalities were present as soon as 1 or 2 days after shunting. Because these factors may be etiological in hepatic encephalopathy, it is important to establish how early the changes occur, and which are correlated with decreased function. We repeated our measurements 6 hours after portacaval shunting or sham-operation. Brain glucose use showed a slight decrease ($p=0.052$) suggesting that brain energy metabolism and function was already affected. Tryptophan transport and brain content were increased. Plasma ammonia and glutamine were raised. Many plasma amino acids were elevated; branched chain amino acids were not decreased, as they are at later times after shunting. Therefore, changes in brain amino acids and transport were not dependent on decreased neutral amino acid competition effects, as has been suggested. Supported by NIH grants NS16389, NS16837.

179.18

EARLY, REVERSIBLE DECREASES OF α-KETOGLUTARATE DEHYDROGENASE ACTIVITY IN THE BRAINS OF THIAMINE-DEFICIENT RATS. M. Heroux* and R.F. Butterworth, Lab. Neurochem., Andre-Viallet Clin. Res. Ctr, Hop. St-Luc (Univ. of Montreal), Montreal, Que. H2X 3J4.

Administration of the central thiamine antagonist pyrithiamine (PT) to the rat results in neurological symptoms including nystagmus and loss of righting reflex which are promptly reversed by administration of thiamine. Measurement of thiamine-dependent enzymes in brain tissue of symptomatic PT-treated rats reveals early selective decreases of α-ketoglutarate dehydrogenase (αKGDH) in cerebral cortex (by 25%, $p<0.01$), cerebellum (by 30%,$p<0.01$), thalamus (by 56%, $p<0.01$) and pons (by 45%, $p<0.01$). Decreased αKGDH is accompanied by decreased concentrations of aspartate, glutamate and GABA and concomitantly increased alanine suggesting decreased entry of glucose carbon into the TCA cycle. αKGDH decreases precede the appearance of neurological symptoms by 2-4 days in all regions of brain. Activities of pyruvate dehydrogenase (PDH) were unaltered in all brain regions. Following thiamine rehabilitation of symptomatic animals, αKGDH activities returned to normal. These results suggest that the reversible oculomotor and cerebellar symptoms of thiamine deficiency result from selective decreases of αKGDH rather than PDH in brain. [Funded by MRC]

179.19

MK801 EFFECT ON QUANTITATIVE REGIONAL CEREBRAL GLUCOSE METABOLISM S. Kuo*, J. Dale*, H. Kaneda*, N. Kaneda*, T.N. Chase, C.A. Tamminga, Maryland Psychiatric Research Center, University of Maryland, Baltimore, MD 21228; NINDS, NIH, Bethesda, MD.

MK801 is a potent and selective PCP agonist. Its action in mammalian brain could help to clarify the behavioral and biochemical actions of selective PCP receptor stimulation. Regional cerebral glucose metabolism (RCGM) was assessed using 14C-2-deoxyglucose and autoradiography as described by Sokoloff. MK801 was administered intravenously 10 minutes prior to the 2DG at doses of 0.1 and 1.0 mg/kg. The semi-restrained animals demonstrated mild sterotypies and body weaving at the higher dose, but no apparant motor hyperactivity as seen with PCP. Regional analysis of glucose metabolism suggested two major findings: 1) MK801 significantly increases RCGM in the limbic regions, eg. hippocamous, subiculum, mediodorsal thalamus and cingulate cortex; and 2) MK801 decreases RCGM in sensory-processing brain areas, eg. auditory cortex; sensorimotor cortex; inf. colliculus, and lateral lemniscus. This pattern of alterations contrasts with PCP-induced metabolism changes cheifly by a lack of MK801-induced RCGM changes in extrapyramidal areas. These data suggest that PCP receptor stimulation enhances glucose metabolism in limbic structures and that this change may correlate with motor and mental sequelae of MK801.

179.20

QUANTITATIVE MODIFICATIONS OF THE DOUBLE-LABEL 2-DEOXYGLUCOSE AUTORADIOGRAPHIC METHOD ELIMINATE THE CONTRIBUTION OF THE FIRST TRACER TO THE SECOND FUNCTIONAL CONDITION. H.L. Loats, H.H. Holcomb, G.E. Alexander and M.R. DeLong. Johns Hopkins Medical Institutes, Dept. of Neurology; U. MD Psych. Res. Ctr.; Baltimore, MD; and Loats Associates, Westminster, MD.

Using [14C]- and [3H]-2-deoxyglucose we have improved the double-label autoradiographic method for measuring serial state changes in local glucose utilization rates (Friedman et al., J. Exp. Neurol., 1987) by removing the first injected tracer's contribution to the second functional condition. Using rats, we have applied the same or different vibrissae stimulation during two serial injections of radiolabeled 2-deoxyglucose (2DG). Vibrissae stimulation is initiated prior to injection of 120 microCuries/kg [14C]-2DG. A routine plasma sampling protocol is followed for 30 minutes; the rat is stimulated for 25 minutes. At the end of this first functional phase, [3H] 2DG, 7.2 milliCuries/kg, is rapidly injected and plasma samples are again taken in a conventional schedule. The second functional state either repeats or changes from the first as dictated by the experiment. At the conclusion of 60 minutes, the brain is removed and processed in a standard manner. Tissue defatting is accomplished with hexane prior to imaging. Each 20 micron section is autoradiographed twice, once with a filter to eliminate [3H] radiation and once without the filter. Plasma activity curves characterizing each tracer's history are used to calculate the first tracer's contribution to the first condition, the first tracer's contribution to the second condition and the second tracer's contribution to the second condition. Because the first tracer's rate during the second condition is the same as the second tracer's rate during the second condition, an image representing that utilization is generated and subtracted from the first tracer's combined utilization in both conditions.

179.21

TEMPORAL PARAMETERS FOR OPTIMAL SEPARATION OF EXPERIMENTAL CONDITIONS IN THE DOUBLE-LABEL 2DG METHOD. H.R. Friedman, C.J. Bruce, and P.S. Goldman-Rakic, Sec. of Neuroanatomy, Yale Univ. Sch. of Med., New Haven, CT 06510.

Double-label modifications of the 2DG technique using ^{3}H and ^{14}C-2DG greatly increase its range of application by permitting brain metabolism to be mapped under 2 separate experimental conditions in the same animal. However, the essential assumption - that the uptake of each label reflects primarily the condition that follows has been questioned by assertions that the 1st isotope labels both conditions in comparable amounts (McEachron et al., Soc Neurosci Abstr, 1988). We addressed this issue quantitatively by estimating the percentage uptake of the 1st label in the 2nd condition directly in a double-label paradigm in which ^{14}C-2DG was given 1st and the 2 conditions were 35 and 10 min long, respectively. During each condition, different vibrissae were manually stroked in awake rats. The cortical columns obtained for each label were commensurate with the whisker stimulated: no indication of the 2nd condition's whisker column was visible in the X-ray image of ^{14}C activity. Formulae derived to assess percentage-uptake of the 1st label in the 2nd condition were applied to radioactivity measurements taken across the cortical barrel fields in digitized ^{14}C and ^{3}H images. The data show that approximately 10% of the ^{14}C uptake was reflected in the 2nd condition. Our previous double-label 2DG studies used 30 min intervals for both the 1st and 2nd conditions (Friedman et al. EBR, 1987), and when we applied these formulae we found that 25 - 30% of the ^{14}C uptake was reflected in the 2nd condition. Lengthening the entire duration of the experiment (from 45 to 60 min) especially by lengthening the 2nd condition therefore appears to worsen the 1st label contamination of the 2nd condition perhaps via rephosphorylation. Indeed, McEachron et al. reported substantial labeling of their 2nd stimulation condition in a single-label 2DG experiment of 90 min duration. However, given optimal temporal intervals, double-label 2DG remains a valid and useful technique for obtaining fine dissociations of brain activity across 2 behavioral conditions in the same animal. Supported by PHS grants MH38546, MH 00298 & EY04740.

179.22

EFFECT OF STIMULATION OF AREA POSTREMA ON BLOOD FLOW TO CHOROID PLEXUS, BRAIN, AND EXTRACRANIAL TISSUES. J.L. Williams and D.D. Heistad. Univ. of Iowa and VA Med. Ctr., Iowa City, IA 52242.

The area postrema (AP) is involved in cardiovascular and volume regulation. We examined effects of stimulation of AP on blood flow to choroid plexus (CP), which produces cerebrospinal fluid (CSF), and on distribution of blood flow to brain and other tissues. AP was stimulated electrically in 11 rabbits and 7 dogs, and blood flow was measured with microspheres. Arterial pressure was maintained constant during AP stimulation. AP stimulation (25 μa) decreased blood flow (ml/min/100 g) to CP of the lateral ventricles from 321±43 (mean±SE) to 174±35 in rabbits and from 554±73 to 291±58 in dogs (P<0.05). In contrast, stimulation of AP had no significant effect on blood flow to cerebral cortex, thalamus, caudate, cerebellum, or brainstem in either species. Stimulation of adjacent medullary regions had no effect on blood flow to CP. In dogs, AP stimulation decreased blood flow to the kidney 34±8%, but had no effect on renal blood flow in rabbits. AP stimulation decreased blood flow to duodenum 55±5% and 46±5% in dogs and rabbits, respectively. Blood flow to masseter muscle did not change in either species.

In summary, stimulation of AP produces large changes in blood flow to CP. We speculate that AP may play an important role in the regulation of CSF production and perhaps in volume regulation in the brain.

179.23

ELECTRICAL STIMULATION OF THE SPHENOPALATINE GANGLION INCREASES REGIONAL CEREBRAL BLOOD FLOW INDEPENDENT OF GLUCOSE UTILIZATION IN THE CAT P.J. Goadsby Department of Neurology, The Prince Henry and Prince of Wales Hospitals, Little Bay NSW 2036 AUSTRALIA.

Recently physiological studies have demonstrated a neurogenic vasodilator innervation of the cerebral vessels from the facial nerve (Goadsby, *Am J Physiol*, in press, 1989). Anatomical studies, including investigations employing tracer studies have suggested that the facial nerve vasodilator fibers distribute through the sphenopalatine and otic ganglia. This study determined if the sphenopalatine ganglion exerted an influence over the cerebral circulation by directly stimulating it and measuring cerebral blood flow. Regional cerebral blood flow was determined using the tracer [^{14}C]-iodoantipyrine and regional brain dissection, and regional cerebral glucose utilization determined using the 2-deoxyglucose method, in the α-chloralose anesthetized cat to evaluate the effect of electrical stimulation of the sphenopalatine (pterygopalatine) ganglion. Unilateral stimulation resulted in increases in blood flow in the ipsilateral cerebral cortex of up to 45% (parietal cortex) with, in addition, increased flow in the white matter of the corpus callosum (42%). Flow was not altered in either the brainstem or basal ganglia (caudate nucleus). In contrast to these changes in cerebral blood flow no changes in cerebral glucose utilization were seen in any of the brain areas studied and in particular there were no changes in the areas in which blood flow increased. These data provide clear evidence that the innervation of the cerebral vasculature from the main parasympathetic ganglion can alter cerebral blood flow independent of cerebral metabolism.

179.24

INCREASE IN SPINAL CORD BLOOD FLOW ELICITED FROM DORSAL MEDULLARY RETICULAR FORMATION IN RAT. P.M. Lacombe*, C. Iadecola, K. Chida*, M.D. Underwood and D.J. Reis, Div. of Neurobiology, Cornell Univ. Med. Coll., NY, N.Y. 10021. (SPON: D. Labar)

Stimulation in the dorsal medullary reticular formation (DMRF) in rat increases cerebral blood flow (CBF) and glucose utilization globally. The increases are, in part, mediated by epinephrine released from the adrenals (*Am. J. Physiol.* 252:H1183, 1987). We examined whether stimulation of the DMRF also elevates spinal cord blood flow (SCBF) and, if so, whether the increases are dependent upon adrenal catecholamines. Rats were anesthetized (chloralose), paralyzed (tubocurarine) and artificially ventilated. The DMRF was stimulated electrically (40-75μA, 50 Hz, 1 sec on/1 sec off) and the elevations in arterial pressure were controlled. Local CBF (lCBF) and local SCBF (lSCBF) were measured autoradiographically using the ^{14}C-iodoantipyrine technique. In controls (n=5) lSCBF ranged from 19±3 ml/100g/min in the white matter (WM) of the thoracic lateral funiculus to 83±4 in laminae VI and VII of the lumbar gray matter (GM). As before, DMRF stimulation (n=6) increased lCBF in all regions (p<0.01). Stimulation also increased lSCBF in all segments bilaterally and symmetrically (p>0.05; paired t-test), averaging 206±7% of control (p<0.05). The elevations were greater in WM (280±15% in cervical ventral funiculus) than in GM (200±9% in cervical dorsal horn). Acute bilateral adrenalectomy (n=5) did not change resting lCBF (p>0.05; n=5), but reduced lSCBF increases from DMRF stimulation by 40-50% (p<0.05; n=5) in all spinal segments, the residual elevations averaging 160±5% (p<0.05). We conclude that stimulation of the DMRF produces a global elevation in SCBF which is, in part, dependent on adrenal catecholamines. Thus the DMRF is capable of powerfully driving the metabolism and blood flow of the entire neuraxis.

BRAINSTEM SYSTEMS

180.1

AN ANTEROGRADE PHA-L STUDY OF THE EFFERENT PROJECTIONS OF THE DORSAL RAPHE NUCLEUS IN THE RAT. Robert P. Vertes. Mercer University, School of Medicine, Macon, GA 31207

The PHA-L technique was used to trace the ascending and descending projections from the dorsal raphe (DR) nucleus. DR fibers terminated heavily in the following structures: nucleus incertus, laterodorsal tegmental nucleus, mesencephalic central gray, pedunculopontine tegmental nucleus, midbrain dopamine-containing cell groups (retrorubral nucleus, VTA and the SN-pars compacta), the central and rostral linear nuclei, the lateral hypothalamic area, the supramammillary nucleus, parafascicular, central medial, mediodorsal and reuniens nuclei of the thalamus, lateral preoptic area, substantia innominata, lateral septal nucleus, the caudate-putamen and the frontal cortex. The following structures were moderately innervated: dorsal hypothalamus, paracentral and central lateral nuclei of thalamus, amygdala, nucleus accumbens, hippocampus and the entorhinal cortex.

The DR was found to project strongly to DA-containing cell groups of the midbrain as well as their major sites of innervation (e.g., mediodorsal thalamic nucleus, the medial basal forebrain, the lateral septum, caudate-putamen and the frontal cortex). This system of projections is consistent with the recent demonstration that DA-containing neurons of the DR are destroyed by MPTP (Unguez and Schneider, Neurosci. Lett. 94:218, 1988) as well as earlier work showing that DR neurons are destroyed in Parkinson's disease.

180.2

PROJECTIONS OF CENTRAL TRIGEMINAL NUCLEI TO THALAMUS, CEREBELLAR CORTEX, AND SUPERIOR COLLICULUS OF THE RAT. G.W. Patrick, T. Hodgini*, A. Travis* and J.A. Stanek*, Dept. of Anatomy, Kirksville College of Osteopathic Med., Kirksville, MO 63501

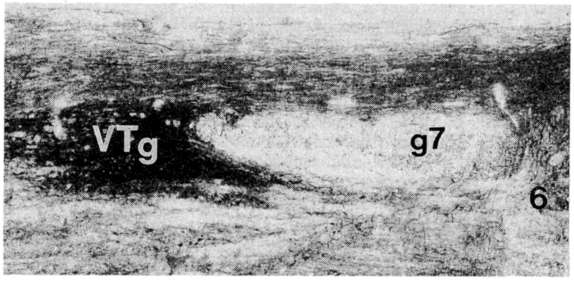
To further determine trigeminal projections, retrograde tracers were injected into anesthesized rats placed in a Kopf stereotaxic frame. Flurogold (FG) was injected into the ventrobasal thalamus (VBT), Fast Blue (FB) into superior colliculus (SC) and Diamidino Yellow (DY) into paramedian lobule (PML) of the cerebellar cortex. After a 24 hr. survival period, the animals were perfused with 10% neutral formalin. Sections were taken and analyzed by reflected light fluorescence with UV excitation. The main sensory nucleus (MSN), subnucleus interpolaris (Vi), and caudalis (Vc) contained single FG labelled cells projecting to contralateral VBT. Single labelled FB containing trigeminotectal neurons were seen in contralateral MSN, Vi and Vc. DY only containing trigeminocerebellar neurons were located in MSN and Vi. Double-labelled FG and DY containing cells were found in MSN and Vi, indicating individual neurons projecting to the VBT and PML. FG and FB double labelled neurons, trigemino-thalamic and -tectal, were present in MSN, Vi and Vc. Trigeminal neurons with collateral axons suggests a high degree of complexity of incoming signals and serve as a basis for understanding functional mechanisms.

180.3

THE HUMAN VENTRAL TEGMENTAL NUCLEUS. George Paxinos, Xu-Feng Huang* and Istvan Törk*. Schools of Psychology and Anatomy, University of New South Wales, Kensington, NSW 2033, Sydney, Australia.

In 1884 Gudden observed that in the rabbit the majority of the fibers in the mammillotegmental tract terminated in a distinct nucleus of the pontine tegmentum which he named after himself - das Guddensche Ganglion. This nucleus is now known as the ventral tegmental nucleus (VTg) and has been identified in all species studied except the human, where no trace of it has yet been detected, despite specific searches conducted by Gudden and others (see Petrovicky, Acta Anat. 80: 276, 1973). On the basis of acetylcholinesterase (AChE) reactivity, we now report that the VTg exists in humans as a densely AChE reactive nucleus rostral to the abducens nucleus (6) and the genu of the facial nerve (g7) as shown in the accompanying photograph of a sagittal section.

Supported by grants from the NH&MRC and Ramaciotti Foundation.



180.4

DISTRIBUTION OF NEUROPEPTIDE IMMUNOREACTIVITY WITHIN THE SUBNUCLEI OF THE NUCLEUS OF THE SOLITARY TRACT-DORSAL MOTOR NUCLEUS OF THE VAGUS IN THE PIGEON. **M.L. Berk and H.J. Karten. Dept. of Anat., Marshall Univ. Sch. of Med., Huntington, WV 25704 and Dept. of Neurosci., Sch. of Med., Univ. of Calif., San Diego, La Jolla, CA 92093.**

An immunocytochemical study was undertaken to localize substance P (SP), leu-enkephalin (LE), cholecystokinin (CCK), neurotensin (NT), and vasoactive intestinal polypeptide (VIP) within the nucleus of the solitary tract (NTS)-dorsal motor nucleus of vagus (DMN). Subnuclei of NTS: Many immunoreactive fibers are present in medial and lateral NTS subnuclei for all five peptides with some notable exceptions. Subnuclei medialis dorsalis pars anterior centralis and medialis dorsalis, pars intereminentialis contain few VIP, LE, and NT fibers, moderately dense SP fibers, and very dense CCK fibers. The lateral parasolitary subnucleus has few, if any, immunoreactive fibers for all peptides. Subnuclei of DMN: Numerous immunoreactive fibers are present in various DMN subnuclei. However, few VIP and CCK fibers are present within subnuclei anterior dorsalis parvocellularis, anterior dorsalis mediocellularis and post. intermedius mediocellularis. Subnucleus post. dorsalis magnacellularis contains a moderate number of SP fibers but few fibers of the other peptides. The relationships of the distribution of the peptides to their potential influence on organ function will be discussed.

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180.5

PROJECTIONS FROM THE MEDULLARY C1 AREA ONTO BRAINSTEM MONOAMINERGIC NEURONS. A.P. Nicholas* and M.B. Hancock. Department of Anatomy and Neurosciences, University of Texas Medical Branch, Galveston, Texas, 77550.

Phaseolus vulgaris-leucoagglutinin (PHA-L) was iontophoretically deposited in the rostral ventrolateral medulla of anesthetized Sprague-Dawley rats. Following survival times of 7-10 days,the rats were anesthetized and perfused with 4% paraformaldehyde. The brainstem was removed and sectioned (30 µm) with a Vibratome. PHA-L-immunoreactive (PHA-LI) elements were immunostained black with the PAP technique and nickel-intensified diaminobenzidine (Ni/DAB), while phenylethanolamine N-methyl transferase-immunoreactive (PNMTI), tyrosine hydroxylase-immunoreactive (THI) or serotonin-immunoreactive (5HTI) neurons were stained amber with the PAP technique and DAB alone. Black-stained PHA-LI cells were present at the deposition site among amber-stained PNMTI cells of the C1 cell group. PHA-LI varicosities were observed in contiguity with THI neurons of the A1, A2 and A5 cell groups, 5HTI neurons of the B1, B2 and B3 cell groups, and PNMTI neurons of the C1, C2 and C3 cell groups. Projections to adrenergic and noradrenergic cell groups from the C1 region were extensive, both ipsilaterally and contralaterally. This study demonstrates that the C1 area, a known vasopressor region, has widespead projections to local monoaminergic brainstem cell groups.

180.6

THE DISTRIBUTION OF BULBOSPINAL SEROTONINERGIC NEURONS THAT CO-CONTAIN ENKEPHALIN OR SUBSTANCE-P IN THE NORTH AMERICAN OPOSSUM. V.K. Reddy*, P.C. Cassini*, R.H. Ho and G.F. Martin. Dept. of Anatomy and Neuroscience Program, The Ohio State University, Columbus, OH 43210.

Our earlier studies have shown that many serotonin (5-HT), enkephalin (ENK) and substance-P (SP) immunoreactive neurons in the raphe nuclei and reticular formation project to the spinal cord in the opossum. In the rat and cat some of the 5-HT neurons in these nuclei contain ENK and SP. We attempted to localize spinally projecting neurons that co-contain 5-HT and ENK or 5-HT and SP in the opossum. In anaesthetized opossums, True Blue was injected unilaterally into the second or third cervical segment. Following an appropriate survival period, colchicine was injected into the brainstem 24 hrs prior to perfusion. Frozen (coronal) sections of the brainstem were cut and processed by the method of Wessendorf and Elde (J. Histochem. Cytochem., 33, 984, 1985) to demonstrate two antigens on the same section. 5-HT neurons that contain ENK or SP were present in several brainstem nuclei. The spinally projecting neurons that contain 5-HT and ENK were limited to the nuclei raphae magnus and obscurus and the nucleus reticularis gigantocellularis, pars ventralis. Spinally projecting neurons that contain 5-HT and SP were found in the same areas and in the nucleus raphae pallidus. Further studies are in progress to identify these neurosubstances in bulbospinal terminals in the opossum. (Supported by NIH 25095)

180.7

ENKEPHALIN-IMMUNOREACTIVITY AND MESSENGER RNA ARE EXPRESSED IN A DISCRETE PROJECTION FROM THE NUCLEUS OF THE SOLITARY TRACT TO THE NUCLEUS AMBIGUUS IN THE RAT. E.T. Cunningham Jr.†, D.M. Simmons, L.W. Swanson and P.E. Sawchenko, The Salk Institute, La Jolla, CA 92037, & †The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

We have described a circumscribed, partly somatostatinergic, projection from the nucleus of the solitary tract (NTS) to the rostral, or compact, part of the nucleus ambiguus (NA) in the rat. Complementary studies of the central sensory and motor representation of the esophagus support the notion that this pathway subserves reflex control of esophageal motility. Here, axonal transport, immunohistochemical and *in situ* hybridization techniques were used to determine if enkephalin (ENK), a peptide commonly coexpressed with somatostatin in medullary cell groups, is contained in the NTS-to-rostral NA projection. The results may be summarized as follows: (1). Cells immunoreactive (IR) for proenkephalin-derived peptides were found in the central, or esophageal-sensory, portion of the NTS in colchicine treated animals. (2). Preproenkephalin mRNA signal was found over the central part of the NTS, and appeared more abundant than that for preprosomatostatin. Combined retrograde transport-hybridization histochemical studies identified cells in the central NTS that project to the NA and contain either mRNA species. (3). Fibers labeled in the compact NA following deposits of the anterograde tracer PHA-L in the central NTS displayed a distribution similar to that of endogenous ENK-IR, with many also displaying ENK-IR. (4). Unilateral electrolytic lesions that included the central NTS virtually eliminated ENK-IR in the ipsilateral compact NA. Together, these studies suggest that ENK is prominently expressed in a putative interneuronal pathway from the central part of the NTS to the rostral NA that subserves reflex control of esophageal peristalsis.

180.8

MIDBRAIN RETICULAR NEURONES IN VITRO ARE SENSITIVE TO AMINES AND OPIATES. A. Khateb*, M. Serafin* and M. Mühlethaler. Dept. de Physiologie, CMU, 1211 Genève 4 , Switzerland.

Midbrain reticular neurones are known to play a major role in a network controlling sleep and arousal. Since they receive a variety of inputs implicated in the sleep/waking cycle, it is important to understand their sensitivity to different putative neurotransmitters. Using an isolated and perfused preparation as well as brainstem slices we have in particular investigated their sensitivity to amines and opiates. Compounds were bath-applied on cells that were characterized by the presence of a broad action potential, slow repetitive firing and an A current, and thus presumed to be cholinergic (Leonard and Llinas, Soc. Neurosci. Abst.: 14, 197,1988). Opiates inhibited these cells through a direct effect on receptors of the Mu type. The amines were also found to have mainly inhibitory effects with the exception of histamine which always had a direct excitatory effect. These data support the view of an interaction of opiates and amines with neurones of the ascending reticular formation. A balance in between excitatory and inhibitory amines impinging upon these cells could contribute to the control of behavioral states. (Swiss NSF grant no 3.288-0.85).

180.9

THALAMIC-PROJECTING NEURONS IN BRAINSTEM CHOLINERGIC NUCLEI INCREASE THEIR FIRING RATES ONE MINUTE IN ADVANCE OF EEG DESYNCHRONIZATION ASSOCIATED WITH REM SLEEP.
S. Datta, D. Paré, G. Oakson* and M. Steriade. Lab. of Neurophysiol., Laval Univ. School of Med., Québec, Canada.

Brainstem reticular neurons were recorded from PPT and LDT cholinergic nuclei during transition from slow wave sleep (S) to REM sleep in behaving cats. Neurons were localized within PPT/LDT limits on sections stained for NADPH-diaphorase and were antidromically invaded from reticular, LG, PUL-LP, intralaminar and medial thalamic nuclei. About 20% of tonically discharging brainstem-thalamic neurons increased their spontaneous discharges 1 min before EEG desynchronization with shift from S to REM sleep. Firing rates were 250-300% higher during REM sleep (median around 35 Hz) than during S. Because PGO waves occurred during the transitional S-to-REM (or pre-REM) epoch and some of these PPT/LDT neurons were also PGO-on (see Steriade et al., this meeting), we performed separate analyses and determined that the tonically increased firing rate preceded the first thalamic PGO wave by about 40 sec. This correlative evidence indicates that brainstem cholinergic neurons with identified thalamic projections are the best candidates for inducing disruption of synchronized spindling rhythmicity, one of the major aspects of EEG synchronization. Supported by MRC grant MT-3689.

180.10

DIFFERENT CLASSES OF PGO-ON NEURONS IN PEDUNCULOPONTINE AND LATERODORSAL TEGMENTAL BRAINSTEM CHOLINERGIC NUCLEI.
M. Steriade, D. Paré, S. Datta, G. Oakson* and R. Curro Dossi*. Lab. of Neurophysiol., Laval Univ. School of Med., Québec, Canada.

It is known that brainstem peribrachial neurons discharge short spike bursts 15-25 msec before the thalamic PGO wave and are otherwise silent. We now report that, in addition to a very low percentage (<5%) of such PGO-on bursting (130-170 Hz) cells, a great diversity of PGO-related neurons exists in brainstem cholinergic nuclei. We recorded PPT and LDT neurons with antidromically identified thalamic projections during REM-sleep of behaving cats. (a) A class of neurons discharged high-frequency (500-600 Hz) spike bursts 30-40 msec before the thalamic PGO wave, upon a background of tonically increased firing rates during REM sleep. This raises the possibility that such events are generated at a depolarized level, contrary to what is expected for a low-threshold rebound burst. (b) Other neurons discharged trains of tonic repetitive spikes preceding the thalamic PGO wave by 150-300 msec. (d) Still another population was REM-on, but PGO-off, suggesting that such neurons may belong to the category of GABAergic neurons recently described in the LDT nucleus and that their PGO-related silence may disinhibit neurons with tonic PGO-on discharges. Supported by MRC grant MT-3689.

180.11

AFFERENT CONNECTIONS OF THE PEDUNCULOPONTINE TEGMENTAL NUCLEUS IN THE RAT.
T.L. Steininger, D.B. Rye, and B.H. Wainer. Dept. of Pharm. and Phys. Science, Univ. of Chicago, Chicago IL 60637.

The cholinergic neurons of the pedunculopontine tegmental nucleus (PPTn) are a major source of ascending cholinergic fibers from the brainstem, and may modulate cortical activity through efferents to the thalamus and basal forebrain. Other evidence suggests that the PPTn is involved in the production of pontine-geniculate-occipital (PGO) waves that accompany the onset of rapid-eye-movement (REM) sleep. Knowlege of the afferents will provide additional insight to the function of this nucleus.

The retrograde tracer wheatgerm agglutinin-conjugated horseradish peroxidase (WGA-HRP) was pressure injected (5 to 15 nl) into the area of the PPTn. Retrogradely labeled neurons were consistently found in the posterior lateral hypothalamus, the paraventricular nucleus of the hypothalamus, zona incerta, sub-parafascicular nucleus of the thalamus, and the laterodorsal tegmental nucleus. Less heavily labeled areas included the midbrain central gray, pontine and medullary reticular formation, and the somatosensory relay nuclei in the medulla.

These anatomical data are consistent with previous studies that demonstrate that the PPTn is not involved in motor functions, and suggest that this cholinergic nucleus integrates visceral, sensory and behavioral state information. (NS 17661 and GM 07839)

180.12

PONTINE TEGMENTUM MECHANISMS IN THE GENERATION OF HIPPOCAMPAL THETA RHYTHM.
A. Nuñez*, I. de Andrés, C. Barrenechea* and E. García-Austt*. Dept. Morfología. F. Medicina. UAM and Dept. Investigación. H. Ramón y Cajal . Madrid. Spain.

Electrical stimulation of oral pontine tegmentum (PnO) elicits hippocampal theta rhythm (θ) (Vertes 1982). This report describes the findings of a study of the unitary activity of PnO neurons during θ and the effect of carbachol microinjections in PnO. Transmembrane potential propierties of medial septal (SEP) and diagonal band (DB) neurons were also examined through intracelullar "in vivo" records. Experiments were performed in urethane anesthetized and curarized rats. During θ evoked by sensory stimulation,the firing rate in 53% of PnO neurons increased and decreased in 18%. Rhythmical firing was not observed in PnO neurons, however, spikes were phase-locked with hippocampal θ in 28%. Microinjections of carbachol (1-4 %, 20-40nl) in contralateral PnO elicited θ (4-6 Hz) and firing rates similar to those described above. These effects were abolished by atropine injections in PnO. Electric stimulation of PnO (300 Hz, 100-200 μA) evoked θ, as well as, a depolarization of SEP neurons together with an increase of the rhythmical burst rate of DB neurons. We may conclude that the activity of PnO neurons contributes to hippocampal θ generation through a cholinergic system, modifying transmembrane potential of SEP and DB neurons. PnO neurons seems to act -through excitatory and inhibitory mechanisms- providing a tonic, non-rhythmic outflow to SEP and DB neurons. Finally, the presence of PnO neurons which spikes are phase-locked with θ suggests the existence of septal and/or hippocampal θ feedback .
Supported by PB-87-0130 CICYT Grant.

180.13

NEUROPHYSIOLOGICAL EVIDENCE FOR THE GATING OF HIPPOCAMPAL AUDITORY RESPONSES BY PONTINE RETICULAR FORMATION NEURONS.
P.C. Bickford-Wimer and R. Freedman, Departments of Pharmacology and Psychiatry, Univ. Colorado Health Sci. Ctr. and Veterans Admin. Med Ctr, Denver, CO. 80262

Rat hippocampal CA3 neurons show diminished response to the second of paired auditory stimuli, at interpair intervals up to several seconds. Both intrinsic inhibitory mechanisms and extrinsic inputs have been proposed to alter or "gate" the response of the hippocampus to sensory afferents. We found that pontine reticular formation neurons also exhibit a diminished response to paired auditory stimuli. Their initial response occurs at 20 msec, about 20 msec before the hippocampal response. Electrical stimulation of this brainstem area, followed by auditory stimulation, results in a diminished auditory response in the hippocampus, similar to the decrement seen when two auditory stimuli are paired. Primary auditory relay nuclei did not show diminished response to paired auditory stimuli, and their electrical stimulation did not decrease the response to subsequent auditory stimuli. The pontine reticular formatiom may thus participate in auditory sensory gating in the hippocampus.

180.14

BURST FIRING OF MEDIAL VESTIBULAR NUCLEI NEURONES IN VITRO.
M. Serafin*, P.P. Vidal, C. de Waele*, A. Khateb* and M. Mühlethaler, Dept. de Physiologie, CMU,1211 Genève 4 , Switzerland; *Laboratoire de Physiologie Neurosensorielle, CNRS, Paris, France.

The vestibulo-ocular reflex (VOR) generates a distinct pattern of eye movements in response to a differential input from the semicircular canals, consisting of slow and quick phases. During the quick phase of the vestibular nystagmus, type I medial vestibular neurones (MVN), located in the vestibular nucleus contralaterally to the side of head rotation, respond to a brisk disinhibition by a burst of spikes. Using guinea pig brainstem slices we found 3 main cell types: Type A displayed a single afterhyperpolarization and a presumed A current, type B had a double afterhyperpolarization and sodium and calcium plateau potentials. Type C neurones, which represented only a small minority, were the only ones whose intrinsic membrane properties could be at the origin of burst responses. These neurones, which in some respects had similar properties to type B neurones, usually discharged in a tonic manner at rest but responded with a burst when fired from a hyperpolarized level. When the sodium conductances were blocked by TTX (10-6 M) an underlying low threshold spike (LTS) was revealed. This LTS, very similar to the one described in thalamic neurones, was due to a calcium conductance as it was completely eliminated in presence of cadmium (2-3 mM). It is therefore tempting to speculate that, an LTS could play a role in the genesis of the bursting activity of MVN neurones during the quick phases of vestibular nystagmus in vivo. (Supported by a Swiss NSF grant no. 3.288-0.85).

180.15

VAGAL EFFERENT DISCHARGE IN RESPONSE TO A PERIPHERAL EMETIC STIMULUS IN THE FERRET. J.S. Davison*, R. Egizii, K.A. Sharkey* and G.E. Lucier. University of Calgary, Dept. of Medical Physiology, Calgary, Alberta T2N 4N1 Canada.

Intragastric administration of 1.5M NaCl is an effective emetic stimulus in the halothane anaesthetized ferret. At levels of halothane greater than 0.8 vol%, this stimulus does not elicit vomiting but the prodromal signs are still present. We have investigated vagal efferent discharge in response to intragastric 1.5M NaCl under these conditions. Single unit efferent activity was recorded in nerve strands dissected from the cervical vagus of ferrets anaesthetized with halothane (0.8-1.5 vol%), paralyzed with gallamine and artificially ventilated. All units were spontaneously active and responded to either gastric distention or chemical stimulation with 1.5M NaCl. The majority of units responded to gastric distention; 50% responded to chemical stimulation including those that were not mechanosensitive. The response to chemical stimulation was characterized by high frequency and prolonged discharge continuing after removal of the stimulus and was most evident at the lower anaesthetic levels. The ability of this emetic stimulus to activate vagal efferent discharge does not depend on activation of the full emetic episode. This modulation of vagal efferent activity may account for certain visceral prodromata elicited by emetic stimuli.

EPILEPSY: KINDLING I

181.1

ONTOGENY OF THE SENSITIVITY OF GABAERGIC INHIBITION IN VARIOUS EPILEPTIC CONDITIONS. H.B. Michelson, J. Kapur, and E.W. Lothman, Department of Neurology, University of Virginia, Charlottesville, VA 22908.

Using methods previously developed in our laboratory (J. Neurophys. 61:417, 1989) we examined the effects of a single seizure, of a model of recurrent triggered seizures and of a model of status epilepticus on hippocampal paired pulse (GABAergic) inhibition at various postnatal (PN) ages. Under urethane-anesthesia, population spikes were recorded in the stratum pyramidale of one CA1 region in response to stimulation of the contralateral CA3. The stimulus intensity and interpulse interval of paired stimuli were varied to systematically characterize the potency of paired pulse inhibition (PPI). The three seizure models were elicited with 20 Hz, 10 second trains, delivered at different rates. PPI was quantified 30 and 60 minutes after an isolated seizure, and hourly up to 4 hours after recurrent seizures and status epilepticus. The findings were that: 1) a single seizure caused a transient reduction of PPI, which lasted longer in younger animals; 2) after recurrent triggered seizures and with spontaneous seizures there were larger and longer lasting reductions of PPI than after a single seizure; 3) the changes associated with recurrent triggered seizures were smaller than those with status epilepticus; 4) profound losses of PPI were often, but not always, accompanied by spontaneous seizures. We conclude that GABA-mediated inhibition in the hippocampus at all postnatal ages is sensitive to post-seizure reductions in its potency and that this sensitivity is greater in young animals. This may be related to the propensity of the young brain for seizures. However, a diminution of GABAergic inhibition cannot be the sole mechanisms accounting for this propensity.

181.2

DEVELOPMENTAL PROFILE FOR THE ABILITY OF THE HIPPOCAMPUS TO SUPPORT ELECTROGRAPHIC KINDLING AND STATUS EPILEPTICUS. E.W. Lothman, H.B. Michelson, and J. Kapur, Dept. Neurology, University of Virginia, Charlottesville, VA 22908.

The pattern of seizures triggered by 10 second stimulus trains to the hippocampus differs markedly as a function of the time between trains. In urethane-anesthetized adult rats, when such trains are delivered every few minutes, successive afterdischarges lengthen in a fashion like that seen during kindling in awake animals. When the stimuli are spaced closely, producing a nearly "continuous hippocampal stimulation" (CHS), cyclical seizures appear during the course of CHS. Following CHS, spontaneous epileptic activity appears after a delay of 2 hours. The present experiments examined how these responses varied as a function of postnatal (PN) age. A microelectrode was positioned in the stratum pyramidale of one CA1 region. 10 sec, 20 Hz stimuli of 1 msec pulses at twice afterdischarge threshold were given to the contralateral CA3 region every 5 minutes (RRHS protocol) or continuously (CHS protocol). All ages showed an increase in afterdischarge durations during the RRHS protocol. The degree of lengthening was not different among the various ages examined (PN7, PN14, PN18, PN21, PN28 days of age, adults). During and after CHS, PN28 animals responded like adults. Some, but not all, of the PN18 animals showed adult-like cycling of seizures during CHS. However, none of these animals developed delayed, spontaneous seizures after CHS. At PN7, seizures were seen at the outset of CHS, but subsequent cycling of seizures did not occur and no delayed spontaneous seizures appeared after completion of CHS. These findings indicate that the processes which allow kindling of electrographic seizures in the hippocampus are present by PN7 whereas those which support status epilepticus mature later.

181.3

ELECTROPHYSIOLOGIC MARKER FOR THE KINDLED STATE: MAXIMAL DENTATE GYRUS ACTIVATION. J.L. Stringer and E.W.Lothman. Dept. of Neurology, Univ. Virginia Med. Ctr., Charlottesville, VA 22908.

Maximal dentate gyrus activation (MDA) consists of bursts of large amplitude (20-40 mV) population spikes that have been shown to be critical for the production of an afterdischarge in the hippocampus of rats. Electrophysiologic characteristics of MDA in fully kindled rats were compared to those in age-matched surgical control rats. Animals were anesthetized with urethane and trains of 20 Hz stimuli administered to the left CA3 region while recording in the right dentate gyrus. By systematically increasing the stimulus intensity, the thresholds for MDA and for afterdischarges were determined. In surgical control animals, the afterdischarge threshold was 6 times the threshold for MDA. In kindled animals these thresholds were equal; i.e. at the stimulus intensity threshold for elicitation of MDA an afterdischarge was produced. Once activated, the duration of MDA in kindled animals was significantly longer than in the surgical control animals ($p<0.01$, grouped t-test). These data suggest that kindling causes alterations in the processes which control the initiation and termination of MDA. The MDA parameters identified here provide a practical means to investigate basic mechanisms of kindling.

181.4

INTRAVENOUS PHENYTOIN BLOCKS AMYGDALOID KINDLED SEIZURES C. Shin, L. C. Rigsbee*, L. S. Butler* & J. O. McNamara, VA and Duke Univ. Med. Ctrs., Durham, N. C. 27705.

The efficacy of anticonvulsant drugs is one prerequisite for the validity of an animal model of epilepsy. Kindling is a model of human partial and 2^{o} generalized seizures (SZ). A strong correlation exists among drugs effective against SZ in humans and in kindling with one striking exception, i.e., phenytoin (PHT). PHT is an effective anticonvulsant in humans, but was found ineffective against kindled SZ by previous reports using intraperitoneal (IP) or oral administration. We reexamined its efficacy against amygdala-kindled SZ in rats using intravenous (IV) route to insure reliable access of PHT to the bloodstream.

IV PHT suppressed kindled SZ 60 min after injection in a dose-dependent manner: Motor SZ, by 50% at 12.5 mg/kg and 100% at 25 and 50 mg/kg; electrographic SZ, by 44% at 12.5 mg/kg and >90% at higher doses. No overt toxicity was observed at the time of testing. IV PHT produced a linear increase in serum concentration with increasing doses. At 50 mg/kg, serum levels were 48.7 $\pm$ 2.1 ug/ml after IV compared to 9.8 $\pm$ 8.4 after IP administration (mean $\pm$ SD).

Previous findings of inefficacy of IP PHT are most likely due to low and variable serum levels achieved. PHT powerfully suppresses kindled SZ, when adequate serum levels are reached. Thus the excellent correlation among drugs effective against SZs in humans and in the kindling model strengthens the validity of this model.

181.5

NMDA Receptor Plasticity in Kindling: Increased Number of Glycine Receptors and Glycine-Stimulated TCP Binding in Hippocampal Membranes. G.C. Yeh*, D.W. Bonhaus, J.V. Nadler and J.O. McNamara. Duke and V.A. Med. Ctr. Durham NC. 27705

Electrophysiologic studies indicate that kindling is associated with increased NMDA receptor-mediated synaptic transmission. Biochemical studies indicate that, while a change intrinsic to post-synaptic sites likely contributes to the electrophysiologic findings, there is no increase in the number of NMDA binding sites. Therefore, we tested whether an alteration in a second component of the NMDA receptor/channel complex, namely the glycine binding site, may account for the electrophysiologic findings. We measured glycine receptors (using [3H]glycine) and glycine activation of the NMDA channel (using [3H] TCP binding). Kindling was found to increase the density of glycine receptors by 41%, when assayed one month after the last evoked seizure ($p<.05$). At the same time the ability of glycine to activate the NMDA receptor-gated ion channel was increased by 29% ($p<.05$). No alterations in glycine binding or glycine-stimulated TCP binding were detected in animals sacrificed 24 hours after the last seizure. This is the first demonstration of plasticity of the glycine receptor. Up-regulation of the glycine recognition site on the NMDA receptor-channel complex may be one molecular mechanism that maintains the longlasting hyperexcitability in the hippocampus of kindled animals.

181.6

MORPHOLOGICAL EVIDENCE FOR SYNAPTIC REORGANIZATION INDUCED BY KINDLING IN THE STRATUM MOLECULARE OF THE CA1/SUBICULUM TRANSITIONAL REGION OF THE RAT HIPPOCAMPAL FORMATION Jose E. Cavazos and Thomas P. Sutula. Depts. of Neurology, Anatomy and Neuroscience Training Program. University of Wisconsin-Madison.

In the rat hippocampal formation studied with the Timm method there are two areas of punctate staining: 1) the terminal zone of the well-known mossy fiber pathway, and 2) the stratum moleculare of the ventral CA1/subiculum transitional region (Haug, Adv Anat Embr Cell Biol 48(1): 1-84, 1973). Synaptic reorganization is induced in the mossy fiber terminal zone by kindling, as demonstrated by development of mossy fiber terminals in the supragranular region of dentate gyrus (Science 239: 1147, 1988). To consider the possibility that kindling might induce synaptic reorganization in other brain areas, the stratum moleculare of CA1/subiculum was also examined after kindling. After 50 class V seizures evoked by olfactory bulb, amygdala or angular bundle stimulation, there was a dense band of Timm granules in the stratum moleculare of the CA1/subiculum, which was not present in implanted unstimulated controls or normal rats. Timm granules were also increased in CA1/subiculum at earlier stages of kindling. The results demonstrated that kindling induces morphological alterations in CA1/subiculum in addition to the mossy fiber pathway, and suggest that kindling may also induce synaptic reorganization in other pathways.

181.7

REGIONAL VARIATION IN THE DISTRIBUTION OF MOSSY FIBER SYNAPTIC REORGANIZATION INDUCED BY KINDLING: EVIDENCE FOR A RELATIONSHIP OF SYNAPTIC REORGANIZATION TO PATTERNS OF NEURAL ACTIVITY. Thomas P. Sutula and Jose E. Cavazos. Depts. of Neurology, Anatomy and Neuroscience Training Program. University of Wisconsin-Madison.

The factors that influence formation and maintenance of neural connections in developing and adult animals are uncertain. In adult animals kindling reorganizes connections of the mossy fiber pathway, as indicated by the development of mossy fiber terminals in the supragranular area of dentate gyrus. If synaptic reorganization in the dentate gyrus is a consequence of neural activity, the distribution of synaptic reorganization along the septotemporal axis should reflect patterns of activity in afferent pathways. To investigate this hypothesis, the distribution of mossy fiber synaptic reorganization after amygdala and perforant path kindling was studied with the Timm method at three different levels of the septotemporal axis. After perforant path kindling the increases of supragranular Timm granules were more prominent in septal than temporal dentate gyrus ($p<0.001$), in contrast to a more even distribution along the septotemporal axis after amygdala kindling. The results demonstrated regional variation in the distribution of synaptic reorganization that depends on kindling site, and suggest that mossy fiber synaptic reorganization is a consequence of patterns of neural activity during kindling.

181.8

STIMULATION-INDUCED STATUS EPILEPTICUS RELIABLY PROVOKES HIPPOCAMPAL MOSSY FIBER SPROUTING. M.M. Okazaki and J.V. Nadler. Depts. Pharmacology and Neurobiology, Duke Univ. Med. Ctr., Durham, NC 27710.

Degeneration of hippocampal CA4 neurons is a consistent feature of complex partial epilepsy in humans. Histochemical evidence suggests that, when a sufficient percentage of CA4 neurons is destroyed, mossy fibers sprout axon collaterals that grow into the molecular layer of the fascia dentata. These fibers appear to form a recurrent excitatory circuit that is not normally present. Detailed study of this aberrant circuit requires a reliable means of destroying enough CA4 neurons to provoke robust mossy fiber sprouting. The present study was undertaken to determine whether stimulation-induced status epilepticus (SISE) would serve this purpose.

Adult male rats were chronically implanted with a bipolar stimulating electrode in the angular bundle and a recording electrode in area CA3 of the ipsilateral dorsal hippocampus. At least a week later, the animals were electrically stimulated with pulse trains of 10 s duration (monophasic rectangular pulses, 0.2 ms, 20 Hz, current supramaximal for synaptic response) delivered every 40 s while they were unanesthetized and unrestrained. This procedure eventually resulted in continuous seizure activity that persisted after the stimulator was turned off. Two weeks later brain sections were prepared and stained for heavy metals by the Timm's sulfide silver method.

Heavy metal staining suggested the presence of robust recurrent mossy fiber sprouting in 8 of 9 rats that experienced at least 4.5 h of SISE. The sprouting was largely confined to the caudal third of the hippocampal formation, where the CA4 lesion was most extensive. In two animals stimulated for a comparable period with a current insufficient to induce SISE, there was little evidence of mossy fiber sprouting. Therefore SISE can serve as a useful tool for studying the role of axon sprouting in epilepsy. (Supported by NIH grant NS 17771.)

181.9

INTRACELLULAR CORRELATES OF THE KINDLING OF SYNCHRONIZED BURSTS IN RAT HIPPOCAMPAL SLICES. S.M. Bawin, M.D. Mahoney* and W.R. Adey. Departments of Physiology and Neurosurgery, Loma Linda University and Veterans Administration Medical Center, Loma Linda, CA 92357.

We previously demonstrated kindling of synchronized bursts by repeated sinewave stimulation (SW, 2-5 s, 60 Hz, 20-50 μA) in the CA2/3 area of rat hippocampal slices. The synchronized bursts involved the NMDA receptor system. Here we report the behavior of individual CA2/3 neurons during and following SW.

Intra- and extracellular responses were recorded concurrently. Test pulses (100 μs, 50-150 μA, 0.1 Hz) and SWs (every 5 min) were applied via bipolar electrodes in the stratum radiatum. Intracellular depolarizing steps (10-100 ms, 0.5-2 nA, 0.1 Hz) alternated with test pulses. Hyperpolarizing steps measured the membrane resistance.

Small depolarizations (5-10 mV) occurring during the first SWs were potentiated (20-25 mV) during repeated stimulation. Single action potentials (APs) then occurred at the beginning of SW, while bursts of APs, riding on small depolarizing waves, occurred later. Following these changes during SW, intra- and extracellular responses to test pulses started to exhibit delayed bursts or late EPSPs. There was no change in resting membrane potential, resistance, or responses to intracellular depolarizing steps. The early responses (fast EPSP or AP and population spike) were not potentiated.

Thus, SW selectively kindled late responses in CA2/3 pyramidal neurons. Activation of the NMDA receptors and associated calcium currents during SW may lead to long-lasting changes in the CA2/3 network of recurrent excitation.

181.10

CONTINUOUS HIPPOCAMPAL STIMULATION DECREASES CALCIUM CALMODULIN-DEPENDENT PROTEIN KINASE II ACTIVITY. J.B. Perlin, S.B. Churn*, E.W. Lothman[2], W.C. Taft and R.J. DeLorenzo. Dept. of Neurology and Dept. of Pharmacology and Toxicology, Medical College of Virginia-VCU, Richmond VA 23298 and [2]Dept. of Neurology, Univ. of Virginia School of Medicine, Charlottesville VA 22908.

Septal kindling leads to a permanent decrease in calcium-calmodulin-dependent protein kinase (CKII) activity (Brain Res. 377,47,1986). Recently, Lothman et al. (Epilepsy Res. 3,107,1989) demonstrated that 90 min continuous hippocampal stimulation (CHS) leads to progressively enhanced seizure activity which can become self sustaining (SSS). We investigated whether CKII is also decreased in this novel model of epilepsy.

Five male Sprague-Dawley rats (250-270g) and 5 paired surgical controls were stereotactically implanted with bipolar hippocampal electrodes. Test animals received a stimulus consisting of 10 sec trains of 50 Hz, 1 msec biphasic square wave pulses every 11 sec for 90 min. Animals were sacrificed following CHS but prior to SSS to eliminate effects of subsequent seizures on enzyme activity. CKII activity was measured in hippocampal homogenates by in vitro phosphorylation, SDS-PAGE, and autoradiography. CHS reduced hippocampal CKII activity by an average of 37 ± 14% ($p<0.0125$; Students Paired t-test). CKII activity was decreased in the stimulated animal from all 5 pairs (range 5 - 85% decrease). The decrement of activity among pairs was directly related to the intensity of the epileptiform discharges observed during monitoring intervals. In the same animals, no differences in cerebellar CKII activity were noted, but a trend toward decreasing activity in the cortex was observed. In summary, as with conventional kindling, CHS produces a decrease in CKII activity.

181.11

DYSREGULATION OF MEMBRANE SYSTEMS FOR PHOSPHORYLATION-DEPHOSPHORYLATION IN THE KINDLING MODEL OF EPILEPSY. J.T. Slevin, T.C.Vanaman*, and G.N. Barnes*. Veterans Administration and Univ of Kentucky, Lexington, KY 40536.

Phosphorylation-dephosphorylation events in hippocampal membranes may play a central role in learning, memory, and epilepsy.We have shown that rat hippocampal phosphoprotein phosphatase 2Ao (pp2Ao) apparently redistributes from cytosol to synaptic plasma membranes (SPM) by 2 min and that membrane protein kinase C (PKC) maximally decreases 4 min after electroshock (ECS); both return to control values by 10 min post-ECS. We report here that a similar but earlier decrease of SPM PKC occured after an ECS given to rats which had been kindled to Stage V by entorhinal stimulation 1 month earlier. Furthermore, there was an attenuation of the post-ECS redistribution of pp2Ao in the same kindled rats. Using an in vitro hippocampal slice preparation, similar effects on PKC and pp2Ao were seen in control and kindled hippocampi exposed to 5 min of Ca^{+2}-dependent, K^{+} depolarization. These data suggest that following epileptic discharge: 1) there may be a more rapid PKC activation/deactivation cycle in the hippocampus of kindled than control animals; and 2) neurotransmitter-induced activation of pp2Ao is altered in kindled animals. Since cAMP and Ca^{+2}/Cam dependent kinases are in vitro substrates for pp2Ao (Barnes etal, SocNeurosciAbstr, 1989), the above data suggest that cAMP and Ca^{+2}-regulated kinase activities may be permanently altered in SPM of kindled animals. Supported by VA Res.Ser. and NIH (5-R01-NS21868).

181.12

KINDLING INDUCES GREATER PROTEIN KINASE C ACTIVATION THAN LONG TERM POTENTIATION. L. J. Burdette, M. E. Gilbert & J. P. O'Callaghan. Graduate Hospital, NSI & U.S. Environmental Protection Agency, Philadelphia, PA & Research Triangle Park, NC

Based upon evidence that translocation of protein kinase C (PKC) from cytosol to membrane may be implicated in long term potentiation (LTP), synaptic potentiation and PKC activity were measured following kindling and LTP stimulation. Male Long Evans rats were implanted with electrodes in the perforant path and dentate granular cell layer. Initial input/output (I/O) curves were generated for a kindling, an LTP and a control group. The kindling group was stimulated daily until generalized seizures were elicited. The next day I/O curves were generated for all groups. LTP stimulation then was delivered to kindling and LTP groups and a third I/O curve was obtained 90 minutes later. Synaptic potentiation was determined by the difference in the population spike amplitude between initial, pre- and post-LTP I/O curves. Rats were sacrificed and hippocampal cytosol and membrane fractions were prepared. PKC activity was measured as the difference in activity with and without phosphatidylserine. Kindling potentiated baseline responses by 150%; further enhancement by LTP stimulation was minimal. At these same intensities, LTP rats showed 35-65% enhancement. The percentage of total PKC activity present in membrane was 68%, 50% and 32% in kindled, LTP, and control rats, respectively. These results suggest that differences in synaptic potentiation elicited by LTP and kindling stimulation reflect differences in PKC activation. In addition, the observation that membrane PKC activity was elevated in the contralateral hippocampus of kindled rats indicates that PKC translocation results from seizure propagation. (Supported by NSF BNS-8711242 to LB)

181.13

CHRONIC, NON-OSMOTIC PLASMA VASOPRESSIN AND VASOPRESSIN mRNA INCREASES FOLLOWING AMYGDALA KINDLING R.S. Greenwood, R.B. Meeker, and J.N. Hayward, Dept of Neurol. and Neurobiology Curriculum, UNC Sch. of Med., Chapel Hill, NC 27599

In the present study we sought to determine if amygdala kindling influences physiologic responses through synaptic interactions by measuring plasma vasopressin (VP) release and VP mRNA in magnocellular neuroendocrine cells.

Plasma VP levels, hematocrit and osmolality were measured in blood from amygdala kindled and non-kindled, adult male Sprague Dawley rats sampled through a chronic indwelling venous catheter. These blood samples were drawn before and after an amygdala stimulus both prior to beginning kindling and after kindling. In addition, five rats that had received kindling four months earlier and three control rats were processed for in situ hybridization. An [^{125}I]dCTP-labeled oligomer specific for VP mRNA (VP mRNA) was hybridized to 20 um cryostat sections followed by processing for high resolution autoradiography.

As previously reported (Greenwood et al, 1986), amygdala stimulation evoked an immediate but variable increase in plasma VP. Moreover, the resting plasma VP in kindled rats was significantly higher than plasma VP from rats before kindling (before kindling VP- 2.1+0.3 pg/ml vs kindled VP- 3.0+0.3 pg/ml $p<0.05$). No significant changes occurred in hematocrit or plasma osmolality. Quantification of the autoradiographs with a Bioquant Image Analysis System demonstrated a 300% increase in labeling in the supraoptic nucleus of rats that had received the kindling stimulus.

These results are consistent with a permanent alteration in genomic expression resulting from synaptic activation and enhanced mRNA translation. Supported in part by Javits Award NS-14311.

181.14

INCREASE OF PROSOMATOSTATIN mRNA FOLLOWING AMYGDALOID KINDLING VS. A SINGLE ELECTRICAL STIMULATION. H.Shinoda, J.P.Schwartz and N.S. Nadi[1]. Clinical Neuroscience and [1]Medical Neurology Branches, NINDS, NIH, Bethesda MD 20892

We have reported that prosomatostatin(SS) mRNA and SS were increased 3 days after the last stimulation in the cortex and striatum of amygdaloid-kindled rats (Mol.Brain Res., 1989). In order to investigate the potential role of neuropeptides in the kindled state, SS and proenkephalin (PE) mRNA were measured 1 day, 3 days, 1 week and 6 weeks after the last stimulation. In both kindled and single stimulated rats (200 uA, 1 msec, without seizure activity), SS mRNA increased in frontal cortex (44 and 34%), in temporal cortex (41 and 43%), and in hippocampus (34 and 37%) respectively 1 day after the stimulation and had returned to basal level by 1 wk.In striatum, SS mRNA increased slightly at 1 day, reached a peak of 70% increase by 3 days and returned to basal by 1 wk. PE mRNA did not change in these brain regions. These data show that SS mRNA is increased by a single electrical stimulation: the increase in SS thus cannot be directly relevant to the induction of kindling.

VISUAL SYSTEM: DEVELOPMENT AND PLASTICITY II

182.1

MORPHOLOGY OF RAT RETINAL GANGLION CELLS (RGCs) DURING FETAL AND POSTNATAL DEVELOPMENT. E.N. Yamasaki*, M.S. Rocha*, A.S. Ramoa. Lab. Mol. Pharmacol. II, Fed. Univ. Rio de Janeiro, Brazil 20540.

The retina of the adult rat contains α, δ and γ-like types of RGCs analogous to those found in the cat. We have examined how the adult form is achieved during development. We also asked which cells project to topographically incorrect portions of the superior colliculus (SC) and whether these errors affect development of RGC dendritic trees. Retinae were dissected from rats between embryonic day 20 (E20) and postnatal day 30 (P30) and maintained alive in a tissue-slice chamber. RGSs were identified by retrograde transport of rhodamine labeled microspheres injected into the SC and their fine morphology revealed by intracellular injections of Lucifer Yellow. At E20, RGCs were morphologically immature, with a few relatively simple, unbranched dentrites. A more elaborate pattern of dentritic branching was present in the neonatal retina and a few α, δ and γ-like cells could be distinguished by P5. Morphological features not present in the adult included a large number of dentritic spines and more highly branched dentrites. The axons often gave rise to collaterals or short side branches. These transient features reached a peak in number around P10 and declined during the following 2 weeks. Therefore, rat RGCs undergo extensive dendritic and axonal remodelling during development, as occurs in cat (Ramoa et al., J. Neurosci. 8:4238, 1988). Moreover, up to P5, RGCs terminating in topographically appropriate and inappropriate regions of the SC were morphologically indistinguishable, suggesting that initially a few cells of all RGC classes make projection errors. By P10, however, only a few γ-like cells remained from this population with inappropriate connections. Thus, mechanisms for elimination of targeting errors may differentially affect each RGC class. [Support: CNPq, FINEP and U.S. Army Res. & Dev. Comm. Contract DAMD17-88-C-8119 to Dr. E.X. Albuquerque (Dept. Pharmacol., Univ. Maryland Sch., Med. Baltimore, MD)]

182.2

SPIKING PROPERTIES OF DISSOCIATED GANGLION CELLS IN DEVELOPING CAT RETINA. I. Skaliora*, L.M. Chalupa, A.J. Gabor and R.P. Scobey. Depts. of Psychology, Neurology, and the Physiology and Neurobiology Graduate Groups, Univ. of California, Davis CA 95616.

We are investigating action potential patterns of retinal ganglion cells in postnatal and adult cats. Cells are dissociated using light papain treatment for 10 minutes and kept in culture at 10°C for up to 30 hours after removal from the eye. Intracellular recordings from isolated ganglion cells are obtained using the whole cell variation of the patch-clamp technique. Ganglion cells are identified by retrograde labeling with rhodamine beads previously injected into the superior colliculus and lateral geniculate nucleus.

After a giga-seal is obtained, voltage recordings are made (current clamp mode) followed by current recordings (voltage clamp mode). In response to depolarizing steps of current (1-80pA), two spiking patterns have been observed. Some cells show a sustained pattern of discharge lasting the duration of current application, while others show a transient pattern with rapid adaptation during the stimulating current. To date, both sustained and transient responses have been observed in different ganglion cells of the same retina as early as P5. This approach is being used to investigate the development of membrane conductances in the three main classes of ganglion cells of the cat retina.

(Supported by EY03991 from the NEI)

182.3

EXPRESSION OF A HIGH AFFINITY LAMININ RECEPTOR IN THE RAT NEURAL RETINA. G.Yang*, P.J.Douville*, and S.Carbonetto. (Spon:S.David) Center for Neurosciences Reseach, McGill University, Montreal General Hospital, 1650 Cedar, Montreal, Canada.

We and others have reported the presence of a high affinity (K_d=1nM) laminin receptor (LR) in neural cells. With polyclonal antisera to a synthetic peptide derived from the cDNA sequence of the LR (Yow et al, PNAS, 85:6394-6398,1988) we investigated immunocytochemically its developmental expression in rat neural retinas. LR immunoreactivity was not detected until postnatal day 6 (P6), when strong reactivity appeared in the ganglion cell layer (GCL), and much weaker immunoreactivity in the inner nuclear layer (INL) and some horizontal cells. By P14, LR immunoreactivity persisted in GCL but diminished in INL and horizontal cells. In the adult, immunoreactivity was exclusively localized in the GCL. The onset of LR expression coincided with differentiation of the inner plexiform layer (IPL) and correlated with immunostaining for neural adhesion molecule (NCAM) in the IPL. The timing and localization of LR immunoreactivity as well as its persistance in adult retina suggests that the antigen(s) identified by the antiserum may be involved in the establishment and maintenance of structural integrity of the GCL.

182.4

A POSITIONAL MARKER FOR THE DORSAL EMBRYONIC RETINA IS AN OPEN CONFIGURATION OF THE SO-CALLED 68KD-LAMININ RECEPTOR. U.C.Dräger, P.McCaffery*, S.A.Rabacchi, L.A.Kobierski*, H.-M.Chu*, R.L.Neve. Harvard Med. Sch., Boston, MA 02115.

In a search for determinants of positional information in the eye we made three monoclonal antibodies (MAb's) which label the dorsal part of the undifferentiated embryonic retina; two of the MAb's show a very strong dorso-ventral (D-V) difference, the third MAb labels the entire retina with only a slight dorsal preference. Through biochemical and molecular analysis we identified the antigen as a protein previously cloned as the 68kD-laminin receptor. A fourth MAb, which also strongly recognizes this laminin receptor in Western blots, shows immunohistochemically no preference at all for the dorsal retina. In order to understand the nature of the D-V difference seen by some but not all MAb's, we tested embryonic eyes bisected into dorsal and ventral halves: neither RNA blots nor Western blots reveal a D-V difference, indicating that the protein is present, but some of its epitopes are inaccessible in ventral retina. Tests for a biochemical correlate of the immunohistochemical D-V difference-- Western blots of D-V halves run on non-denaturing gels, of trypsin-digestion series and of formalin-fixed eyes-- have so far been negative, but we can demonstrate in Western blots much higher MAb-binding to fixed dorsal retina. Conversely, an immunohistochemical correlate of the biochemically even distribution of the antigen is seen in sections of formalin-fixed eyes treated with the denaturing reagents SDS or urea, which partially unmask the antigen in ventral retina; and in embryos fixed with ethylene glycol*bis*(succinimidylsuccinate), a homobifunctional cross-linker with very long spacer arm (16Å), which makes the antigen evenly accessible throughout the eye for all MAb's. These observations indicate that the D-V difference in the antigen is a non-covalently determined difference in conformation, probably a masking of epitopes in ventral retina by tighter coiling of the protein or by association with additional molecules. Supported by NIH grant EY01938.

182.5

NEUROPEPTIDE SYSTEMS IN THE DEVELOPING RAT RETINA. D.Zhang and H.H.Yeh. Dept. Neurobiology and Anatomy, Univ. Rochester School of Medicine, Rochester, NY 14642

Do different neuropeptide systems in the mammalian retina follow a similar pattern of development? We have previously described the development of corticotropin releasing factor (CRF)-like immunoreactive (-LI) amacrine cells in the rat retina. Here, we examined the ontogeny of other neuropeptide systems, including vasoactive intestinal polypeptide (VIP), substance P (SP) using a combination of immunohistochemistry and [3H]-thymidine autoradiography. Emphasis was placed on comparing their developmental patterns with that of the retinal CRF system. In the adult rat retina, these neuropeptide systems display distinctly different cellular morphology and patterns of fiber distribution.

During development, immunoreactivity to these neuropeptide systems were first detected at different times; CRF-LI appeared first, followed by SP- and VIP-LI. Eye opening coincided temporally with some changes in all these neuropeptide systems. Whereas CRF-LI cells already were adult-like by eye opening, VIP- and SP-LI cells have yet to complete their development. A center-to-periphery gradient was found in the CRF and SP systems but not in the VIP system. In addition, CRF and SP systems displayed a differential distribution; the immunoreativity was more prominent in the superior (S) and temporal (T) regions than in the inferior (I) and nasal (N) regions of the retina. Furthermore, quantitative determinations revealed that these peptide systems developed differently in terms of parameters such as changes in cell number and density. Preliminary results of our birthdating study suggest that, unlike CRF-LI cells which cease mitotic activity prenatally, VIP cells were still being generated postnatally.

Overall, our results indicate that different neuropeptide systems may correlate with different subpopulations of retinal cells and that they follow different programs in development.

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182.6

DIFFERENTIAL GABA- AND GAD- IMMUNOREACTIVITY IN THE CHICK RETINA DURING DEVELOPMENT. J.N. Hokoç; A.L.M.Ventura*; P.F. Gardino* and F.G. de Mello*. Instituto de Biofisica Carlos Chagas Filho, UFRJ, Rio de Janeiro. BRAZIL.

In early stages of retina development, GABA may be synthesized from putrescine. This synthetic pathway decreases in later stages, as glutamate decarboxylase (GAD) activity increases several fold (de Mello et al., '76). In this study, aldehyde-fixed chick retina sections from several stages of development were used to localize GABA and GAD-immunoreactivity (IR) in an attempt to discriminate GABA synthetic pathways. In mature retinas, GABA-IR cell bodies were localized in the distal and proximal inner nuclear (INL) and ganglion cell layers (GCL). Processes arborized in both outer (OPL) and inner plexiform layers (IPL). GAD-IR cells were seen in the innermost row of the INL and in the GCL, with processes arborizing in the IPL. During the development GABA was clearly detected in retina cell bodies as early as E8. However, GAD-IR neurons were not observed in retinas of embryos younger than E12. Thus, our data provide the first morphological evidence that GABA-IR in E8 may be accounted for an alternative GABA synthetic pathway.

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182.7

THE EFFECT OF GABA ON RETINAL MATURATION AND SYNAPTOGENESIS IN VIVO. E.Messersmith*,V.Blanchard* & D.Redburn. Dept. Neurobio. & Anat., UT Med.Sch.,Houston,TX 77225.

Previous work suggest that Type A horizontal cells may be pioneering elements for the outer synaptic layer of the rabbit retina, providing structural and/or chemical substrates necessary for appropriate synaptic connections between the three major cell types. During a critical period, lasting approximately one week after birth, Type A horizontal cells transiently express GABAergic properties. In order to examine the possible role of GABA during this critical period, GABA-A receptor antagonists, bicucculline and picrotoxin, were injected intraocularly at birth. Retinal development was significantly altered during the 5 days following treatment. Lamination of neuronal processes into inner and outer plexiform layers was disrupted. The normal complement of cells within the inner retina was reduced with a concomitant increase in photoreceptors cells and rosette formation. Bicucculine was more potent and gave more consistent effects than did picrotoxin. An injection of the GABA-B receptor antagonist, phaclophen, had little effect on retinal development. We suggest that GABA-A receptors and the transient expression of GABA by Type A horizontal cells may play an important role in normal synaptic development. Supported by NEI grant 1566-12.

182.8

ALTERATIONS IN THE TIMING OF RETINAL DEVELOPMENT DURING THE PROTRACTED DEVELOPMENT OF THE CALIFORNIA MOUSE. D.R. Sengelaub, M.A. Bimler*, J.A. Burke*, and A.R. Cover*. Program in Neural Science, Dept. of Psycholgy, Indiana University, Bloomington, IN 47405.

The Syrian hamster (Mesocricetus auratus) and the California mouse (Peromyscus californicus) are closely related cricetid rodents. While the hamster is three times larger in body size, their eye size and weight, total retinal cell number and distribution, retinal ganglion cell number, and overall brain size are quite similar. This similarity is interesting in that both the length and rate of development, two factors hypothesized to be important in producing species differences in retinal organization, are strikingly different between hamsters and California mice. Gestation (16 days in hamster vs. 33 in mice), the closed eye period (CEP= 30 days in hamster vs. 47 in mice), age of sexual maturity, and attainment of adult body size are significantly longer or delayed in the mouse. We have studied the protracted development of the California mouse to determine how formative events in retinogenesis have been orchestrated in the production of its hamster-like eye.

Generation of the ganglion cell layer of hamsters and mice has a similar timing and duration, with cells beginning to withdraw from the cell cycle at approximately 37% of the CEP. However, peak cell number and the onset of cell death in the retinal ganglion cell layer occur at approximately 67% of the CEP in the hamster, but are delayed in the mouse, occurring at about 80% of the CEP. Growth of the eye is remarkably similar in duration and rate between the two species, but again occurs throughout a later portion of development in the California mouse. It appears that the protracted development of the California mouse is accompanied by a delay in post-generational events in retinal development, resulting in a retinal topography and eye conformation similar to that of the hamster.

182.9

TERMINAL CELL BIRTH IN THE CAT RETINA D. H. Rapaport and A. J. Vietri* Department of Anatomy, University of Sydney, Sydney NSW 2006, AUSTRALIA.

Two zones of cell division have been recognized in the retina of the cat. To determine the identity of cells generated by them kittens received an injection of ^{3}H-thymidine, and the retinas were sectioned thinly and processed for autoradiography.

At P5 cell birth is occurring at the edge of the retina at high density. On average, 125 labelled cells are found within the peripheral 1mm of linear extent. The density of cell birth decreases rapidly to only 2 per linear 100µm at P21. The cells generated during this period are rods (RPR), bipolar (BP), and Müller (Mü) cells in approximate constant proportions of 3.7:4.7:1.9, respectively. The density of label in central retina is very low postnatally, ranging from .09-.52/linear mm within 5.7mm centered on the area centralis. At P5 most central labelled cells are BP (65%), 25% are PR, and 7% are Mü. By P11 there is a strong shift from BP cell birth (now 33%) to RPR's (55%) while Mü's remain approximately the same (10%). This trend continues to P21 where the proportions are now 75%-RPR, 20%-BP, and 5%-Mü.

Postnatally cell birth is mainly confined to peripheral retina and stops between P10-P15. There is no obvious sequence of birth. A sparse population of mitoses persists centrally, and with increasing age tends to generate mostly RPR's. Perhaps the persistence of RPR birth maintains their density during ocular growth in the cat, as apparently in goldfish (Johns, '82, J. Neurosci. 2: 179-198).

182.10

ACTIVITY AND THE MODULATION OF AXON LOSS IN THE CAT'S OPTIC NERVE. A.J. Scheetz, M.W. Dubin, University of Colorado at Boulder, and R.W. Williams, Yale University

In the cat, 100,000 retinal ganglion cells die during the first two postnatal months (Williams *et al.*, 1986). To determine the role of action potential activity in this ganglion cell death, we injected the action potential blocker tetrodotoxin (TTX), monocularly, from birth through day 60. Animals were perfused at the end of this continuous blockade, on day 60, and optic nerves prepared for electron microscopy. The size of the remaining ganglion cell population was estimated by counting axons in the optic nerves.

The axon population in the nerves of the TTX-treated eyes was 160,000 ± 1000 (n=3). This is precisely the same number as in normal cats, 6 weeks of age or older. Thus, neither activity nor the injection procedure itself affects the magnitude or timecourse of naturally-occurring cell death on the injected side. In marked contrast, the axon population on the uninjected side was significantly higher than normal, 182,000 ± 1000 (n=3). This reduced incidence of cell and axon loss is comparable to that seen in the remaining eye after early monocular enucleation, even though the normal number of axons from the treated eye remains in the TTX-injected case. There is a hypertrophy of layers of the lateral geniculate nucleus that receive input from the untreated eye (Dubin, personal observations). Active ganglion cells are probably at a competitive advantage and their axons are able to sprout into territories that are only loosely held by silent axons from the other eye. This increase in territory preserves active ganglion cells that would otherwise have died.

182.11

EVOLUTIONARY PLASTICITY IN THE FELINE RETINO-GENICULATE SYSTEM. Robert W. Williams, Yale University, Carmen Cavada and Fernando Reinoso-Suárez, Universidad Autónoma de Madrid.

The Spanish wildcat, *Felis silvestris tartessia*, is a relict of a once widespread species from which domestic cats descended (Kurtén, '65). Our aim is to evaluate evolutionary change in the visual system associated with a 40-50% reduction in body size that has taken place in this cat lineage over the past 25,000 years. To do this we compared neurons and fibers in the retina, optic nerve, and lateral geniculate nucleus (LGNs) of wildcats and domestic cats using quantitative EM methods, retrograde HRP labeling, and direct 3-D counting.

The decline in body size has been accompanied by a decrease in brain mass from ~34 g to 27 g. Correlated with this change there are 35% fewer ganglion cells in domestic cats than in wildcats—160,000 vs. 240,000. However, the size of the retina remains the same. Therefore, ganglion cells are more widely spaced in the domestic cat retina. There are also 35% fewer neurons in the domestic cat LGN—550,000 vs. ~880,000. Unlike the retina, this decline is matched by an equivalent decline in the size of the LGN—from ~39 to 26 mm^3. Therefore, the density of LGN neurons is similar in both species.

We conclude that the brains of domestic cats are smaller than those of wildcats because they contain fewer—not smaller—neurons. The stable 1-to-3.5 ratio between ganglion cells and LGN neurons in both species probably results from well conserved trophic interactions between neuron populations during development. Whether the rapid evolutionary change in brain structure has been achieved by variation in rates of neuron proliferation or cell death remains an open and intriguing question.

This work was supported by ICONA (Spain) and the NIH.

182.12

LOCALIZATION OF TAURINE-LIKE IMMUNOREACTIVITY (TLI) IN RAT OPTIC NERVE. Norma Lake, Luisa De Marte* and Carole Verdone-Smith*, Depts. of Physiology and Ophthalmology, McGill University, Montreal, Canada, H3G 1Y6.

The role of taurine in the vertebrate retina or optic nerve is unknown, however our recent EM morphometric studies have indicated that in addition to retinal cell degeneration taurine deficiency causes shrinkage of optic nerve axons, decreases in myelin thickness and frank loss of nerve fibres [Vis. Res. 128: 1071-76; Neurosci. Abs. 14: 358 (1988)]. We have developed an antibody to taurine, useful for immunocytochemistry by raising antisera in rabbits immunized with taurine as a hapten conjugated to a carrier protein. During early postnatal development TLI is very prominent in bundles of ganglion cell axons within the retina. In the adult optic nerve about 2mm behind the eye, the most prominent TLI occurs within glial cells. Preliminary studies show co-localization with glial fibrillary acidic protein (GFA) in some of these structures suggesting that some of the cells containing TLI may be astrocytes. One can speculate that the effects of taurine deficiency on the optic nerve may be secondary to glial cell dysfunction, in addition to the other etiologies we have proposed.

Supported by the MRC and RP Research Foundation of Canada.

182.13

THE MORHOLOGY OF ABERRANT MAMMALIAN RETINAL GANGLION CELLS. R.J.T. Wingate*, and I.D. Thompson* (SPON: Brain Research Association). University Laboratory of Physiology, Parks Road, Oxford OX1 3PT, Great Britain.

We have compared the detailed morphology of those hamster retinal ganglion cells which have an aberrant uncrossed projection from the nasal retina with those of the normal uncrossed and crossed populations. Populations are identified by the pattern of retinal decussation seen following a unilateral injection of rhodamine-latex microspheres into the optic pathways. Retrogradely labelled cell bodies are then located in an in vitro retina preparation and selectively impaled with glass micropipettes under a compound microscope and epifluorescent illumination. Detailed dendritic morphology is revealed by intracellular injection of Lucifer Yellow. Cells with an aberrant uncrossed projection to the mid-brain are found to have an abnormally sparse dendritic arbor with a significantly lower branching density. Such arbors are not seen in cells with appropriate decussation patterns. After monocular enucleation at birth, the increased nasal uncrossed population shows a more variable distribution of dendritic morphologies.

182.14

THE DISTANCE OF AXOTOMY FROM THE NEURONAL CELL BODY INFLUENCES RATE OF RETROGRADE DEGENERATION BUT NOT LONG-TERM SURVIVAL OF RETINAL GANGLION CELLS (RGCs). M.P. Villegas-Pérez*, M. Vidal-Sanz*, G.M. Bray, and A.J. Aguayo. Montreal General Hospital and McGill University, Montréal, Québec, H3G 1A4, Canada.

In the mammalian CNS, axotomy close to the cell body causes retrograde degeneration and death of many neurons. For example, more than 90% of RGCs die soon after optic nerve (ON) transection near the eye (Villegas-Pérez et al., J. Neurosci. 8:265, 1988). However, with axotomy further away from the cell body, RGC survival increases with the distance of ON transection (Villegas-Pérez et al., Soc. Neurosci. Abstr. 14:673, 1988). To study the effects of distance of axotomy on long-term RGC survival, we prelabeled RGC with diI and then transected the ON at 0.5, 3, 8, or 10 mm from the eye in adult Sprague-Dawley rats. The densities of surviving RGCs were assessed by counting diI-labeled neurons in 12 standard areas of each experimental retina in groups of animals examined at 1, 3, 6, 9, and 12 months after axotomy.

After 6 months, the number of surviving RGCs had fallen to approximately 5-10% of the normal RGC population in all of these retinas. Thus, the distance of ON transection from the eye appeared to influence the rate of RGC degeneration but not the long-term survival of these cells.

182.15

SYNAPSE FORMATION BY REGENERATING RETINAL GANGLION CELL AXONS DIRECTED INTO AN INAPPROPRIATE TARGET (THE CEREBELLAR CORTEX) IN ADULT HAMSTERS. T.J. ZWIMPFER*, A.J. AGUAYO, G.M. BRAY and D.G. LAWRENCE. Montreal General Hospital and McGill Univeristy, Montréal, Québec, Canada, H3G 1A4.

Regenerating retinal ganglion cell (RGC) axons of the adult hamster form synapses when guided to one of their appropriate targets, the superior colliculus (Carter D.A. et. al., Soc. Neurosci. Abstr. 14:654, 1988). To investigate the selectivity of regenerative synaptogenesis by adult mammalian CNS axons, regrowing RGC axons were guided into an inappropriate target, the cerebellar cortex, via a bridging peripheral nerve graft. Regenerating RGC axons penetrated the cerebellar cortex for distances up to 650 μm and arborized within the granule cell and Purkinje cell layers. Retino-cerebellar synapses were observed within the granule cell layer from 2 to 5 months after graft insertion. The morphology of the retino-cerebellar axon terminals and synapses was similar to that of both control and regenerated retino-collicular axons, although the mean area of retino-cerebellar axon terminals was larger.

These results indicate that in the absence of an appropriate target, regenerating CNS axons in adult mammals can form differentiated and persistent synapses with inappropriate target neurons.

SUBCORTICAL VISUAL PATHWAYS II

183.1

MODELING THE COMPUTATIONAL ORIGIN OF ROTATIONAL OPTIC FLOW DETECTORS FOR VISUAL PROPRIOCEPTION. G. C. Belmont* and J. I. Simpson, (SPON: H. Cohen). Dept. Physiol. & Biophys., NYU Med. Ctr., New York, NY 10016.

A computational model is being developed to account for the response properties of neurons of a visual proprioception system subserving compensatory eye movements and related behaviors. This system, which originates in the accessory optic system (AOS), detects global retinal image movement associated with self-motion, and its neurons have been characterized in the rabbit. They are speed and direction selective and the spatial organization of their direction selectivity is suited to detection of rotational optic flow about particular axes. This type of receptive field first appears in the AOS and is elaborated at the level of the visual tegmental relay zone, to which the AOS projects. At later synaptic levels rotation selective receptive fields underlie the visually-modulated complex and simple spike activity of cerebellar flocculus Purkinje cells, which can be divided into three classes on the basis of the orientation of the best-response axis of the climbing fibers. These axes are located close to the best-response axes of the semicircular canals and the rotation axes of the extraocular muscles. A previous model showed how the basic AOS response properties can be derived from those of on, direction-selective retinal ganglion cells. The current model seeks to describe how the AOS response properties are spatially combined to produce rotational optic flow detectors. Modulation of these detectors shows a near cosine relation with the angle between the best-response axis and the stimulus rotation axis and it is to this relation that the model is fit while incorporating data on the speed selectivity and retinal distribution of ganglion cells and the spatial distribution of direction selectivity within the receptive fields of neurons to which the AOS projects.

183.2

THE SYNAPTIC ORGANIZATION OF GABAERGIC AND NONGABAERGIC NEURONS IN THE PRETECTUM AND THE ACCESSORY OPTIC SYSTEM IN THE RABBIT: RETROGRADE TRACING COMBINED WITH GABA IMMUNOCYTOCHEMISTRY. J.J.L. Van der Want and J.J. Nunes Cardozo. Dept.of Morphology, The Netherlands Ophthalmic Res. Inst. P.O.Box 12141,1100AC Amsterdam, The Netherlands. (SPON: ENA)

Neurons in the pretectum and the accessory optic system (AOS) have been investigated ultrastructurally after retrograde labeling with WGA-HRP injected in the inferior olive and postembedding GABA-immunocytochemistry. The large number of GABA-positive neurons in the pretectum and the AOS suggests that some GABAergic neurons could be projection neurons. However, following injections of WGA-HRP in the inferior olive all retrogradely labeled neurons were GABA-negative. These projection neurons have large to medium sized cell bodies and are strongly contacted by GABA-positive terminals. In contrast to the GABA-negative cell bodies the GABA-positive cell bodies are surrounded by glial processes and receive few terminals. The GABA-positive terminals exhibit distinct morphological features: F-terminals, of axonal origin making axosomatic contacts and P-terminals, presumably of dendritic origin terminating mainly in the neuropil. GABA-negative terminals comprise F and P terminals and R terminals that are of retinal origin. The retrogradely labeled neurons showed striking similarities with respect to their ultrastructure and terminal organization, irrespective the localization in different nuclear areas.

183.3

PROJECTIONS OF THE LATERAL TERMINAL ACCESSORY OPTIC NUCLEUS (LTN) OF THE MARMOSET. R.H.I. Blanks, R.J. Clarke*, Y. Torigoe, S. Pham*, R.A. Giolli, Department of Anatomy and Neurobiol., Univ. Calif. Irvine, Irvine, CA 92717 and Departamento de Fisiologia e Farmacologia, Univ. Federale de Pernambuco, Cidade Universitaria, Recife, Brazile.

The projections of the largest of the monkey accessory optic (AOS) terminal nuclei, the LTN, have been studied using ^{3}H-leucine autoradiography (6 animals) and retrograde HRP techniques (10 animals). The largest projections are to the ipsilateral nucleus of the optic tract (NOT) and dorsal terminal nucleus (DTN). There is a small, contralateral projection to the olivary pretectal nucleus and LTN provided by axons crossing in the posterior commissure. A second, moderate projection is to the interstitial nucleus of the superior fasciculus (posterior fibers) (IN-SF$_p$) that ends abruptly in the marmoset midway between the LTN and ventral, medial terminal nucleus (MTN$_v$). A third, large projection is to a part of the ventral tegmental area termed the "visual tegmental relay zone" (VTRZ). This bundle also provides a large input to the mesencephalic and pontine reticular formations (PRF) and small fields to the dorsal MTN (MTN$_d$), MTNv and interstitial nucleus of Cajal. It crosses and provides some terminals to the contralateral VTRZ. A fourth major projection targets the supraoculomotor periaqueductal gray above the oculomotor nucleus; the IV and VI nuclei are unlabeled in all experiments. Lastly, there are two long-descending bundles: i) one courses within the medial longitudinal fasciculus to terminate within the dorsal cap of the inferior olive, ipsilaterally, ii) another travels ventral to the brachium conjunctivum and provides a moderate projection to the superior and medial vestibular nucleus, ipsilaterally. Retrograde HRP studies are incomplete, but all connections above have be confirmed except those to the vestibular nucleus and PRF. The present findings are in general agreement with the well-documented connections of the AOS nuclei of other mammals, and further support the general conclusion that the LTN provides a major relay of visual information to important oculomotor centers. (Supported in part by NSF grant BNS-8612919-02)

183.4

RESPONSES OF PRETECTAL NEURONS OF THE BEHAVING MONKEY U. J. ILG* (SPON: K. Behrend) Allg. Zoologie und Neurobiologie, University of Bochum, P.O. Box 10 21 48, D-4630 Bochum, F.R.G.

The pretectal region of vertebrates has been shown to contain neurons which do respond directionally selective to movements of large stimuli with ipsiversive preferred direction. These neurons can be localized in the Nucleus of the Optic Tract NOT (Hoffmann et al. 1988 Exp. Brain Res. 69: 635-644)

Single unit activity and eye movements were recorded from the NOT of awake and behaving monkey (M. fascicularis).

The recorded pretectal units are directionally selective with ipsiversive preferred directions for velocities up to 400 deg/sec of random dot pattern independent of luminance (0.1 to 14 cd/m*m tested). The latency of the response to the onset of movement in preferred direction varies betweem 80 and 100 ms. During OKN the activity of these neurons can be interpreted as a function of slip direction and velocity. During pursuit these neurons respond to target and background induced slip with contrary preferred directions.

183.5

RECIPROCAL CONNECTIONS BETWEEN ACCESSORY OPTIC SYSTEM AND PRETECTUM IN MONKEY (MACACA FASCICULARIS). M. Magnin*, C. Baleydier* and H. Cooper* (SPON: J. Bullier). Unité 94 I.N.S.E.R.M., 16 avenue Doyen Lépine, 69500 Bron, FRANCE.

Organization of the accessory optic system (AOS) and its connections with the pretectum are described in macaque monkey. Monocular intravitreal injections of tritiated amino acids confirm the existence of the three AOS nuclei as classically recognized in non primate species. Retinal AOS projecting cells, labeled by retrograde tracer injected in the lateral terminal nucleus (LTN) are distributed mainly within the foveal region of the contralateral eye.

Injections of tritiated amino acids in the pretectum demonstrate a major contralateral projection to the lateral and the medial terminal nuclei of the AOS (LTN and MTN) and a sparser projection to the ipsilateral LTN. From experiments of retrograde tracers (fluorescent dye or HRP) injected in the LTN, it appears that the contralateral projection originates from the pretectal olivary nucleus (OPN). Besides this crossed OPN-AOS connection which seems specific to primates, the classical projection from the nucleus of the optic tract (NOT) to the ipsilateral LTN was also found.

HRP injection in the pretectum reveals a bilateral, although mainly ipsilateral, reciprocal projection from the LTN to the pretectum

The direct connection between LTN and OPN suggests a possible role of the AOS in the control of the pupillary light reflex, supported by previous descriptions of numerous luminance responsive cells in the AOS.

183.6

Somatostatin Immunoreactivity within the Pretectal Complex of the Cat. J.T. Weber, L. Meyer*, A. Arimura and I-li Chen*. Tulane University Medical School, New Orleans, LA 70112.

Immunocytochemistry was used to identify the cellular components within the pretectal complex of the cat that presumably contain the neuropeptide, somatostatin (SOM). The anterior pretectal nucleus (APN), nucleus of the optic (NOT), and pretectal olivary nucleus (ON) contain the largest numbers of SOM-positive neurons; the posterior pretectal nucleus (PPN) contains relatively few SOM-positive neurons. Only one SOM-positive neuron was seen within the medial pretectal nucleus (MPN). SOM-positive fibers and terminals are found within all pretectal nuclei but are most numerous within ON and MPN. Comparisons of cell body areas reveal that in the NTO and ON the mean size of SOM-positive neurons are significantly larger than the mean size of Nissl stained neurons. For example, a sample of SOM-positive neurons within the NTO has a mean somal area of 352.6 u^2 (SD = 161.8) while a sample of Nissl stained neurons, in adjacent sections, has a mean somal area of 221.2 u^2 (SD = 103.7). No size difference between SOM-positive and Nissl stained neurons is apparent within the APN and PPN. These preliminary data suggest that a population of pretectal projection neurons contains SOM. This hypothesis is currently being tested with the combined use of immunocytochemistry and retrograde transport methods. Supported by NIH grant EY03731.

183.7

Ultrastructural Analysis of Retinal and Cortical Terminals within the Cat Olivary Pretectal Nucleus. B.Hutchins, and J.T.Weber. Baylor College of Dentistry, Dallas, Tx. 75246 and Tulane Medical School, New Orleans, La. 70112.

The cat olivary pretectal nucleus (ON) receives a rich supply of retinal projections within the head and tail regions (Hutchins and Weber, Br.Res., 331('85)150-154) as well as projections from the extrastriate cortex (Hutchins et al., Anat. Rec., 205('83)88). To test the hypothesis that retinal and cortical terminals may synapse on the same ON cell, the optic nerve was sectioned on the left side six days prior to sacrifice and HRP was placed throughout the extrastriate cortex on the right side in the same animal. Ultrastructural analysis of retinal projections within the ON head revealed primarily extraglomerular terminals which were in contact with medium to large diameter dendrites. Glomerular retinal terminals appeared to be in contact with small to medium diameter dendrites and all of the retinal terminals were of the RLP type. Cortical terminals were primarily of the RSD type although there were a few labelled RLD terminals. Retinal and labelled cortical terminals typically synapsed in close proximity along small diameter dendrites. In a few cases, degenerating retinal profiles were identified terminating on small HRP labelled cortical terminals. Thus, these data suggest retinal and cortical information can influence the same ON cell and there is direct modulation of cortical projections by retinal terminals. Supported by NIH grants EY06977 (BH) and EY03731 (JTW).

183.8

Transmitter and Peptide Content of the Isthmo-Optic Nucleus in the Pigeon (*Columba livia*) : A study of non-tectal afferents. W.Woodson*, T. Shimizu, and H.J. Karten, Dept.Neurosciences, Univ. California, San Diego.92093.

The only documented afferent to the isthmo-optic nucleus (ION) originates from the optic tectum (McGill et al ., *J. Anat. (Lond)*.100: 5,1966a,b). The cells of origin are found in layers 9 and 10 of the stratum griseum et fibrosum superficiale. Some of the neurons in this layer express γ-aminobutyric acid (Cuenod and Streit., *The Neurosciences: Fourth Study Program*. 989,1974). However, transmitter survey of the ION reveals that although we have not identified a neurotransmitter expressed by ION neurons, the neuropil contains not only large quantities of glutamic acid decarboxylase terminals but also moderate amounts of serotonin and tyrosine hydroxylase immunoreactivity. In addition, a survey of peptides indicates ION cells to be negative for all neuropeptides thus far tested, though the neuropil contains occasional processes immunoreactive for substance P, enkephalin, oxytocin, neuropeptide Y, and cholecystokinin octapeptide.

Injections of Phaseolus Vulgaris Leucoagglutinin (PHA-L) into the ION and/or surrounding tegmentum resulted in numerous terminals within the contralateral and ipsilateral ION. Also, following injections into either the raphe nucleus or hyperstriatum accessorium, fibers and terminal endings could be observed within the ION.

These results indicate that the centrifugal system upon the retina is not only modulated by the optic tectum but also by afferents from diverse areas in the central nervous system. (Supported by the Ford Foundation and NEI Grant EY06076-02).

183.9

ION-TYPE RETINOPETAL SYSTEM IN TELEOSTS AND BIRDS. H. Uchiyama and R.B. Barlow, Jr., Institute for Sensory Ressearch, Syracuse University., Syracuse, NY 13244

Centrifugal (efferent) pathways to the retina have been found in most vertebrates. Their morphological diversity (location of somata of retinopetal neurons in the brain, fiber connections, manner of intraretinal termination, etc.) indicates functional variety. However, some retinopetal systems share common features, such as a tectal input. A good example is the isthmo-optic nucleus (ION) which has been extensively investigated in birds. Therefore, we term these systems "ION-type" retinopetal systems. In addition to birds, these systems have been found in the cyclostomes, advanced teleosts and reptiles.

Here we present a study of the properties of the ION-type retinopetal systems of teleosts and birds, with particular emphasis on their central connections. In advanced teleosts (Acanthopterygians), retinopetal neurons are found in the diencephalon, while in birds retinopetal neurons are in the caudal-most mesencephalon (isthmic region). In both systems the retinopetal neurons receive a major input from the optic tectum. In a teleost (filefish) and a bird (Japanese quail), tectal neurons projecting to the retinopetal nucleus were labeled by HRP injections to the nuclei. Their dendritic patterns suggest that the tectal neurons integrate retinal and non-retinal inputs. Thus, the ION-type retinopetal system could be regarded as a tectal output to the retina. The function of the ION-type retinopetal system seems to be closely related to the visuomotor function of the tectum. (Supported by NIH EY-00667 and NSF 8709059)

183.10

BINOCULAR INTERACTIONS IN THE ACCESSORY OPTIC SYSTEM (AOS) OF THE PIGEON. D.R. Wylie and B.J. Frost. Dept. of Psychology, Queen's Univ., Kingston, Ont., Canada, K7L 3N6.

Several studies have demonstrated that the AOS is a self-motion analyser involved in processing visual information for compensatory head and eye movements. The nucleus of the basal optic root (nBOR), a component of the avian AOS, receives direct input from the displaced ganglion cells of the contralateral retina. The nBOR also receives input from the contralateral nBOR, thus providing an indirect route from the ipsilateral retina. In the pigeon, electrophysiological studies have shown that neurons in nBOR respond best to slow-moving wholefield visual stimuli moving in dorsal, ventral or temporal directions in the contralateral visual field. In this study we have demonstrated that some nBOR neurons respond to wholefield stimulation of the ipsilateral eye. In most cases the modulation by wholefield stimulation of the ipsilateral eye is much less than that of the contralateral eye, however, in some cases the response to stimulation of the ipsilateral eye is equal to or greater than that of the contralateral eye. These binocular neurons prefer either approximately the same direction in both eyes, or motion in opposite directions in the two eyes. Thus, it is suggested that some nBOR neurons specify a translation motion while others encode a rotation of the head. This research was supported by MRC grant #MA 7244 to B.J.F.

183.11

VISUAL RESPONESE PROPERTIES OF NUCLEUS ROTUNDUS CELLS IN THE PIGEON. S-Y Jiang*, Y-C Wang* and B.J. Frost. Departments of Psychology and Physiology, Queen's University, Kingston, Ontario, Canada, K7L 3N6.

The avian tectofugal pathway, which is similar to the mammalian colliculo-pulvinar-MT pathway, consists of the optic tectum (OT), nucleus rotundus (RT), and ectostriatum. Anatomical studies have shown that discrete zones within RT receive inputs from different tectal laminae, and in turn project to subregions of ectostriatum. Standard extracellular recordings were made with tungsten microelectrodes from Ketamine/Rompun-anaesthetized pigeons. Computer-generated stimuli were back-projected onto a tangent screen, and visual landmarks and receptive field boundaries were plotted. Based on recordings from 144 single units, several different classes were identified. (1) Luminance units: These units, which comprised 19% of the sample, had large receptive fields (RFs) and either systematically increased (11%), or decreased (8%) their firing to increases in luminance of the whole screen. (2) Looming units: These units (16%) also had large RFs, and responded to moving objects, but gave their largest response to expanding light or dark disks. They exhibited no X-Y directional preference and gave no "ON" or "OFF" responses. (3) Directional cells: These units gave standard directionally specific responses to Kinematograms. Some of these cells responded specifically to leading edge occlusion (Frost et al., 1989) with either excitation or inhibition. (4) Movement cells: These cells were similar to class (3) but exhibited no direction preference. Supported by NSERC of Canada.

183.12

VISUAL DEPRIVATION CHANGES DIRECTIONAL PREFERENCE OF ACCESSORY OPTIC SYSTEM (AOS) NEURONS IN CHICKENS. C.L. Natal*, J.C. Letelier*, X. Rojas* and J. Wallman. Biology Dept., City College, CUNY, New York, NY 10031.

The AOS appears to be a major input for non-horizontal optokinetic nystagmus (OKN). In month-old chickens, there is a strict division of the principal AOS nucleus into regions with upward and downward sensitvity. This segregation has been confirmed by 2-deoxyglucose autoradiography. Previous work found this anatomical parcellation much less evident in newly hatched birds, and that visual deprivation had effects on OKN and the pattern of 2-DG labelling, suggesting that the directional specificity of nBOR neurons is shaped by visual experience. To test this hypothesis, single unit recordings were made in normal and visually deprived chickens.

In normal chickens, we have recorded to date 21 "up" units and no units with nasalward sensitivity. In visually deprived chickens, only 7 units responded best to upward motion, but 23 were excited by nasalward motion. Even after 3 weeks of subsequent visual experience, fewer "up" units were found and the nasalward population was still conspicuous. These results argue that visual experience during a critical period either causes a shift of the directional sensitivity of AOS neurons in the upward direction, or that it prevents the shift from upward to horizontal sensitivity that occurs in the absence of vision. Supported by CNPq and NSF grants.

183.13

Retinal application of bicuculline eliminates direction sensitivity in the turtle's basal optic nucleus *in vitro*. A. F. Rosenberg and M. Ariel. Dept. of Behavioral Neuroscience, Univ. of Pittsburgh, Pittsburgh, PA 15260.

Direction sensitive (DS) retinal ganglion cells (RGC's) may converge onto cells in the mesencephalic basal optic nucleus (BON) to encode retinal slip, thereby providing a neural signal to drive optokinetic nystagmus (OKN). Direction sensitivity in the retina is mediated by GABA since it can be blocked by picrotoxin or Cl^- free ringers (Ariel & Adolph, 1985). In addition, GABAergic mechanisms play a role in the retinal processing required for OKN. Intravitreal injection of GABA antagonists have been reported to elicit a spontaneous nystagmus (Ariel et al., 1988). Therefore, DS-RGC's may be the primary input to the BON which in turn is involved in OKN.

We use a pharmacological approach to elucidate the connections occurring between the retina and the BON. The visual response properties of BON cells were characterized in an *in vitro* whole-brain, eyes-attached, telencephalon-removed preparation. All cells recorded were DS over a broad velocity range, had large receptive fields and responded best to moving large, textured visual stimuli presented to the contralateral eye. In general, these cells had little if any spontaneous activity and exhibited a poor response to light onset or offset. Application of 25-100 uM bicuculline, a $GABA_a$ receptor antagonist, via superfusion or bolus injection resulted in the elimination of directional sensitivity in BON cells. The cells became movement sensitive to any direction in addition to becoming much more responsive to onsets and offsets of light.

We conclude, therefore, that the directional information found in the BON is processed at the retina and is GABA mediated. Therefore, antidromic activation from the BON can become a useful marker for identifying DS-RGC's in the eyecup for anatomical or physiological studies.

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183.14

A VISUAL LAMINA IN THE MEDULLA OBLONGATA OF THE FROG. M.T.Wallace and E.R.Gruberg. Dept. of Biology, Temple University, Philadelphia, PA. 19122

Using single unit extracellular recording we have discovered a lamina of visually responsive units in the medulla oblongata of the frog, Rana pipiens. The lamina extends the entire rostrocaudal length of the medulla, and is confined to the medial medulla. The lamina is restricted to the neuropil of the rhombencephalic reticular formation. Most units within the lamina can be driven binocularly, and have very large receptive fields (greater than 90°). In addition, units in the rostral medulla tend to be unimodal (solely visual), while units in the caudal medulla are generally bimodal (visual and somatosensory). Unlike the visual centers in the midbrain and thalamus, this region does not appear to be organized along retinotopic lines. Unilateral ablation of the optic tectum and posterior thalamus abolishes visual responses in the medulla elicited by stimulation of the contralateral eye, suggesting that much of the visual activity in the medulla is mediated by the tectum and/or posterior thalamus. To determine the inputs to the visual medulla we have been using HRP histochemistry. The optic tectum, torus semicircularis, mesencephalic tegmentum, and the anterior and posterior thalamic nuclei project to the medulla oblongata.

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183.15

PREY CATCHING IS SPARED AFTER HEMISECTION OF THE OPTIC CHIASM IN THE FROG. R.F.Waldeck and E.R.Gruberg. Dept. of Biology, Temple University, Philadelphia,PA 19122.

We made hemisections of the optic chiasm and studied subsequent visual behavior and the distribution of retinotectal fibers. After cutting either the posterior or the anterior half of the optic chiasm frogs still respond to prey in the entire ground level visual field. However, they are unresponsive to visually presented threat stimuli in the entire visual field. The distribution of optic fibers in the tectum was determined using ^{3}H-proline autoradiography and horseradish peroxidase histochemistry. Animals with the posterior half of the chiasm severed have the dorsomedial and ventrolateral tectum labeled, while animals with the anterior half of the chiasm severed have complementary labelling. Although HRP labelling in posterior chiasm lesions is limited to the dorsomedial and ventrolateral area visually driven units can still be recorded throughout the contralateral tectum.

It was earlier shown that removal of the optic tectum leads to the loss of visually guided prey catching behavior in the contralateral monocular field. In our current work, it appears animals can respond to prey without direct retinal input to the optic tectum. Another retinorecipient area, such as the posterior thalamus, may play a mediating role in frog prey catching.

Supported by N.I.H. Grant EY04366

183.16

THE NATURE OF ELECTRICAL TRANSIENTS RECORDED IN FROG TECTAL NEUROPIL. A.C. Grant* and J.Y. Lettvin* (SPON: L.S. Frishkopf). Research Laboratory of Electronics, MIT, Cambridge, MA 02139.

In this study we have reexamined the properties of those transients evoked in the tectal neuropil of the frog, Rana pipiens, by excitation of RGC types 1 and 2. Both visual stimulation and electrical stimulation in the optic nerve and n. isthmi were used to generate responses in the tectum. First, unlike single unit RFs which are circular, tectal multiunit receptive fields (MURFs) are distinctly oval. The MURFs are oriented in space so as to define a visual "pole" which lies at or near the optic axis. Second, in addition to the common triphasic units, diphasic (first phase positive) and negative monophasic units are also present in the neuropil. Within a MURF the RFs of each unit type are distinct and always lie in the same order with respect to the visual "pole". Third, there is often a coincident firing of adjacent elements in the neuropil resulting in spikes with more than three phases. Fourth, excitation of two different tectal afferent fibers can produce the same transient. It is concluded that single units in the dorsal neuropil are not, as is generally assumed, generated presynaptically by an afferent axon's terminal arbors. Instead, they are generated by electrically active dendritic structures which have a marked anatomical anisotropy. Rapid-Golgi impregnations support this conclusion.

183.17

ANTEROGRADE AND RETROGRADE LABELING IN THE VISUAL SYSTEM WITH HERPES SIMPLEX VIRUS (HSV): VARIABLES INFLUENCING TRANSPORT. R. B. Norgren, H. C. Bubel*, A. Wander* and M. N. Lehman. Depts. Anat. & Cell Biol., Microbiol. and Ophthal., Univ. Cincinnati Med. Coll., Cincinnati, OH 456267

HSV has great potential in elucidating the structure and function of the visual system due to its transneuronal transport and complete filling of neurons with immunoreaction product. Factors influencing the transport of HSV in both the anterograde and retrograde direction were examined in the current study. Three strains of purified HSV were used: McIntyre and Shealey (type 1) and strain G (type 2). HSV was injected either into the vitreous body or into the superior colliculus (SC) of golden hamsters. Tissue was processed as previously reported (Norgren & Lehman, Brain Res. 479:374, 1989). After intraocular injection of the three strains HSV we found significant differences in the amount of labeling. Labeling in retinorecipient nuclei was most prominent with strain G, followed by the Shealey strain; the least amount was observed with McIntyre strain. No labeling of neurons was observed after intraocular injection of UV irradiated virus confirming that immunolabeling was detecting active replication of virus within retinorecipient neurons. After injection of McIntyre strain into the superior colliculus, labeled neurons afferent to the SC in brain and the retina were observed. In contrast, we were rarely able to demonstrate labeled neurons in either the brain or the retina of animals that received injections of Shealey strain into the SC. [Supported by NIH grant NS 24292 (MNL)]

183.18

PRIOR UNILATERAL OPTIC NERVE TRANSECTION ABOLISHES CAPSAICIN-INDUCED DEGENERATION IN DIENCEPHALIC AND PRETECTAL VISUAL STRUCTURES. S. Ritter and T. Dinh*. Department of VCAPP, Washington State University, Pullman, WA 99164-6520

Capsaicin is a neurotoxin capable of causing degeneration in discrete areas throughout the neuroaxis (Ritter and Dinh, JCN 271: 79). After neonatal capsaicin treatment, affected sites include the suprachiasmatic nucleus (SCh), the ventrolateral geniculate nucleus (VLG) and the medial and olivary pretectal nuclei (MPT and OPT). In adults, capsaicin-sensitivity is greatly attenuated or absent in the OPT and VLG, although still present in the SCh and MPT. In this experiment, we tested the hypothesis that capsaicin-induced terminal degeneration in the SCh, VLG, MPT and OPT results from destruction of retinal ganglion cell projections to these sites. The optic tract was transected unilaterally in anesthetized 15-16 day old Sprague-Dawley rat pups. At 20 days, 30 days, 3 or 6 mo of age, lesioned rats and controls were anesthetized and injected systemically with capsaicin or the vehicle solution. They were sacrificed 6 or 18 hr later and tissues processed using a cupric-silver stain for labelling degenerating neurons. In rats injected with capsaicin at 20 and 30 days of age, prior unilateral optic nerve transection eliminated capsaicin-induced degeneration in all four nuclei on the contralateral (denervated) side, but not on the ipsilateral side. Likewise, in lesioned adults, capsaicin-induced degeneration was present only in the ipsilateral SCh and MPT. These results suggest that capsaicin causes degeneration in the SCh, VLG, MPT and OPT by selective destruction of ganglion cell projections to these sites.

183.19

IMMUNOHISTOCHEMICAL STUDY OF ASTROGLIAL REACTION TO DEAFFERENTATION IN VISUAL NUCLEI OF RAT. R.Schmidt-Kastner*, K.Wietasch*, D.Meller*, U.Eysel (SPON: European Neuroscience Association). Dept. of Neurophysiology, Ruhr-Universität Bochum, D-4630 Bochum, F.R.Germany.

Astrocytes play an important role in deafferentation and plasticity (Gage et al., Exp.Neurol.102:2-12,1988). This study focusses upon astrocytic reaction to deafferentation in subcortical visual nuclei of rat where side comparisons are possible due to the almost complete crossing of retinal fibers. Deafferentation was produced in pentobarbital-anesthetized rats by unilateral optic nerve section (n=30) or by unilateral ischemic destruction of the retina using photochemically-induced vascular thrombosis (n=16). Brains from controls (n=20) and experimental animals surviving 1,2,4,6,7 or 21 days were studied with immunohistochemistry using antibodies against glial fibrillary acidic protein (GFAP) and vimentin (VIM). Antibodies against neurofilament peptides served to study damage of retinal axons and the Fink-Heimer technique to reveal terminal degeneration. In normal brains GFAP-staining in astrocytes was dense in the pretectal olivary nucleus, moderate in the dorsal lateral geniculate nucleus (dLGN) and low in the superior colliculus (SC). Degenerative changes of neurofilament staining and positive Fink-Heimer reaction first occurred at day 2. A subtle increase in GFAP-staining began to manifest in contralateral SC already 1 day after lesion. From day 2 on glial reactions were also seen in dLGN and pretectal area contralateral to lesion which persisted up to day 21. VIM-staining occurred in the affected optic tract and in glial processes of the deafferentated SC and dLGN. In conclusion, astrocytes in the subcortical visual system of rat react rapidly and with high spatial precision to deafferentation.

183.20

DISTRIBUTION OF GABA-A RECEPTORS IN SUBCORTICAL VISUAL NUCLEI OF MAMMALS. K. K. Glendenning, B. N. Baker*, S. L. Cochran* and R. B. Masterton. Dept. Psychology, Florida State Univ., Tallahassee, FL 32306.

Previously we have shown a systematic increase in the density of GABA-A receptors both in DGL and in LP through early mammals to prosimian primates. To explore the possibility this increase might be accompanied by related changes in other visual structures as well, we extended the study to include the major subdivisions of the superior colliculus, pretectum, VGL, DGL, and LP in 6 species.

The results suggest that the muscimol labeling totaled over the 4 major terminal nuclei of the optic nerve is more closely related with an animal's diurnal cycle than with any other behavioral, visual, structural, or other biochemical adaptation, while the heavy labeling in the superficial layer of superior colliculus alone is most closely related to the proportion of retinal cones.

The close relationship (r=.937, p<.006) between the density of GABA-A receptors in the dorsal lateral geniculate and in the lateral posterior nucleus is not reliably related to either behavioral or structural variation nor, among mammals alone, to phyletic grade. Therefore, it is possible that their parallel in GABA-receptor density may be due instead to their unique interdependence on cortex and a commonality of demands placed on thalamocortical input.
Supported by NIH-NINCDS # NS7726.

BIOLOGICAL RHYTHMS AND SLEEP: INVERTEBRATES

184.1

CIRCADIAN RHYTHM IN THE PERIOD PROTEIN OF DROSOPHILA. K.K. Siwicki*, D.M. Zerr* and J.C. Hall, Biology Department, Brandeis University, Waltham, MA 02254

The protein encoded by the period (per) locus of Drosophila, which influences the fly's behavioral circadian rhythms, is itself subject to daily oscillations in immunoreactive staining. Neurons, photoreceptors, and glial cells in the fly's head are strongly labelled by our anti-per antibody at "lights on", but are not detectably stained at "lights off" (in a 12 hour light: 12 hour dark cycle). The daily rhythm in the staining of the per protein continues in constant darkness for at least four days, with a period of about 24 hours in wild-type flies, and about 19 hours in mutant per-short flies. These results indicate that the period of the free-running circadian rhythm in the per protein is influenced by the per mutations.

The per protein is not detectable at any time of day in flies exposed to constant light, a treatment that renders flies arrhythmic in behavioral assays. In various light: dark regimes, we found a good correlation between per protein staining and behavioral circadian rhythms. Nonetheless, the distribution and daily cycling of the per protein appeared to be normal in several visual system mutants (norpA-P24, ninaE-oll7, and disco). Thus, the effects of light on the staining of the per protein may be mediated by extra-retinal photoreceptors.
(Supported by NIH grant GM-33205 to J.C.H. and M.Rosbash)

184.2

DROSOPHILA DUSKY (DY) MUTATIONS ALTER CIRCADIAN RHYTHMS. F. R. Jackson, L.M. Newby, and S.M. DiBartolomeis. Neurobiology Division, Worcester Fndn. Exptl. Biol., Shrewsbury, MA 01545.

The *Drosophila andante* (*and*) mutation lengthens freerunning periods for circadian and ultradian (courtship song) rhythms (Smith, R.J., Ph.D. Thesis, Cal. Tech., 1982; Kyriacou, C.P., pers. comm.). It maps to region 10E2-3 of the X chromosome, an interval that includes the *miniature-dusky* (*m-dy*) locus. Because the *and* allele is associated with a dusky-like phenotype (small and dark wings), we have examined the relationship between the two types of mutations.

Four new γ ray-induced *dusky* mutations (dy^{n1}, dy^{n2}, dy^{n3}, dy^{n4}) were isolated on the basis of wing size. Each of the four mutations fails to complement an existing *dy* mutation; i.e., they are alleles of the *dy* locus. Importantly, three of the new *dy* mutations affect circadian rhythms of eclosion and locomotor activity. Like *and* mutants, dy^{n1}, dy^{n3}, and dy^{n4} flies exhibit abnormally long circadian periods (eg., for locomotor activity, dy^{n1} = 25.7 ± 0.3 h, dy^{n3} = 25.2 ± 0.3 h, and dy^{n4} = 25.2 ± 0.1 h, whereas *and* = 25.4 ± 0.1 h and the wild type = 24.0 ± 0.2 h). The morphological and behavioral phenotypes associated with *andante* and *dy* mutations suggest they are alleles of a single locus.

To initiate molecular studies of these mutants, we have cloned approximately 100 kb of DNA encompassing the *m-dy* locus. Existing chromosomal rearrangements and RNA transcripts are currently being mapped within the cloned interval to delineate the physical limits of the locus.

184.3

ROLES FOR cAMP AND INTRACELLULAR CALCIUM IN THE GENERATION OF CIRCADIAN RHYTHMS IN BULLA. M.R. Ralph, S.B.S.Khalsa and G.D.Block. Dept. of Biology, University of Virginia, Charlottesville, VA 22901.

Cyclic nucleotides and calcium are thought to be components of biochemical pathways that mediate phase shifts of circadian rhythms in Aplysia (Eskin et al., 1982,1984) and Bulla (Khalsa & Block, 1988). We have investigated the involvement of these cellular components in the generation of rhythms in Bulla gouldiana. We have found that the cAMP analog, 8-Br-cAMP (2mM) produces phase shifts of the freerunning rhythm of compound action potential (CAP) frequency. Acute effects of the agent on CAP frequency and the phase response curve (PRC) are similar to hyperpolarization. Phase shifts induced by forskolin were similar in magnitude and direction.

However, the phase response curve for the phosphodiesterase inhibitor, caffeine, was different from cAMP in that large advances (+2 hr) were induced immediately prior to subj. dawn and large delays (-3 hr) directly following dawn. This feature of the caffeine PRC was closely mimicked by Thapsigargin, an agent that is reported to cause the release of Ca^{2+} from IP_3-sensitive stores. Since caffeine may also cause Ca^{2+} release from stores, these results suggest that two mechanisms may underlie the phase shifting effects of caffeine. One may be a cAMP-dependent process that may result in hyperpolarization of pacemaker cells. A second mechanism may involve the release and subsequent re-uptake of intracellular Ca^{2+}. The abrupt change from phase advances to delays at subjective dawn suggests that a spontaneous release of intracellular Ca^{2+} at dawn may be a normal component of the rhythm generation mechanism.

Supported by MH09483 to MRR, NS09621 to SSK and NS15264 to GDB.

184.4

CHLORIDE CONTRIBUTES TO PERIOD REGULATION IN THE BULLA OCULAR CIRCADIAN PACEMAKER. S.B.S.Khalsa, M.R.Ralph & G.D.Block. Biology Dept., University of Virginia, Charlottesville, VA 22901.

The eye of the mollusc Bulla contains a circadian pacemaker whose cells express a rhythm in membrane potential and in compound action potential frequency. In examining the contributing ionic conductances we have found that inhibiting chloride flux significantly shortens the period of the rhythm.

Replacement of extracellular Cl^- with sulfate, in a solution osmotically balanced with sucrose, shortens period in a Cl^- concentration dependent manner. Complete Cl^- replacement (100%) shortens period relative to control eyes by 2.6 hr. per cycle ($\Delta\tau$=-2.6, N=5); at 90% $\Delta\tau$=-1.5 hr. (N=3), at 80% $\Delta\tau$=-0.8 hr. (N=2), and at 75% no period shortening is apparent. This effect is not dependent upon the divalent sulfate ion, since similar period shortening was observed in solutions with the Cl^- replacing monovalent anions isethionate ($\Delta\tau$=-1.1 hr., N=4) and glutamate ($\Delta\tau$=-1.2 hr., N=4).

The Cl^- channel blocker 9-Anthracene Carboxylic Acid also shortened period in a concentration dependent manner; at 1 mM $\Delta\tau$=-1.8 hr. (N=3), at 500 μM $\Delta\tau$=-1.0 hr. (N=3) and at 100 μM $\Delta\tau$=-0.2 hr. (N=2). The Cl^- transport inhibitors Furosemide (500 μM, $\Delta\tau$=-0.5 hr., N=4) and Piretanide (100 μM, $\Delta\tau$=-0.3 hr., N=2) also shortened period. However, the more specific Cl^- channel blocker Diphenylamine Carboxylic Acid was ineffective.

Since the Cl^- transport inhibitors are known to affect Cl^- channels as well, it is not possible to discern the relative contribution of Cl^- conductance and Cl^- transport with our preliminary data, although a conductance mechanism seems most likely. However, a constant Cl^- conductance, and therefore a constant depolarization in its absence, is inconsistent with the previously observed period lengthening with depolarizing high K^+; Cl^- conductance may therefore be regulated in a circadian cycle.

Supported by NS09621 to SSK, MH09483 to MRR and NS15264 to GDB.

184.5

TEMPORALLY VARYING ANTIGENS IN THE EYE OF BULLA GOULDIANA. M.H. Roberts, Y. Chen*, and V. Bedian, Dept. of Biology, Clarkson University, Potsdam, NY 13676.

The eye of the marine snail Bulla gouldiana expresses a circadian rhythm generated by the rhythmic depolarizations of a population of electrically coupled neurons located at the retinal base, the basal retinal neurons (BRNs). BRN axons project down the optic nerve to the central ganglia where they modulate locomotor activity.

In order to identify: 1) components of the eye responsible for generating the rhythm, and 2) markers for the BRN axons within the brain, we raised monoclonal antibodies (MAbs) to the eye of Bulla. Following immunohistochemical screening, we performed immunoblot analysis of separated eye homogenates, in order to determine the molecular weights of the antigens recognized by our MAbs.

We have identified antigens specific to regions of the eye. Several antibodies recognize antigens specific to groups of neurons in the brain. Of particular interest is the antigen recognized by MAb B4D3.E3. This antibody, which stains the basal retinal region of the eye, as well as fibers along the periphery of the retina, displays a day/night variation in staining. The staining in eyes is high just after dusk and is low at dawn. This variation is also seen following "Western" transfer as a sharp band at approximately 10kD. We are currently investigating if the variation continues in constant conditions.

Supported by NS26272 to MHR.

184.6

A SECOND CIRCADIAN RHYTHM IN THE BULLA EYE. Michael Geusz* and Terry Page, Dept. of Biol., Vanderbilt University, Nashville, TN, 37235.

The eye of the mollusk Bulla gouldiana is a useful preparation for the study of the cellular basis of circadian rhythms. One group of cells in the eye, the basal retinal neurons, generates a circadian rhythm in compound action potentials (CAPs) in the optic nerve (Block et al., 1984, J. Comp. Physiol. A 155: 365-378). Recently, we discovered a circadian rhythm in small (10-40 uvolt) impulses in the optic nerve from a second population of cells in isolated eyes maintained in artificial seawater and darkness at 15°C. The frequency of these units, continuously recorded for 5-7 days, varied with a circadian rhythm that was approximately 12 hours out-of-phase with the rhythm in CAPs and damped out after 3-5 cycles. The activity reached a peak during the subjective night, when CAPs are absent, declined prior to the onset of CAP activity, and typically fell to a minimum within an hour of the peak CAP frequency. The phase of the rhythm was dependent on the prior light cycle. The amplitude of the rhythm was less stable and damped more rapidly than the CAP rhythm. Other results suggest that the small-impulses may be inhibited by light.

Supported by NIH Grant #NS15264 and BRSG #2S07.

184.7

AN APLYSIA EYE PROTEIN ASSOCIATED WITH CIRCADIAN PACEMAKER NEURONS AND RELATED TO THE PERIOD-PROTEIN OF DROSOPHILA. **S. Strack* and J.W. Jacklet, Neurobiology Research Center and Dept. of Biology, SUNY Albany, Albany, NY 12222**

We have reported staining of gastropod circadian pacemaker neurons with an antibody against the period (per) protein, which is known to control rhythms in Drosophila (Soc.Neurosc.Abst.1988,190.10). The putative per-like antigens were characterized by immunoblot analysis using the same antibody that was used for immunocytochemistry. We found a strongly immunoreactive 48 kD protein exclusively localized to Aplysia eyes, where immunocytochemistry shows specific staining in pacemaker cell bodies and their processes. Preliminary protein-fractionation experiments show this 48 kD protein is associated with membranes. The level of the per protein in Drosophila varies with the circadian rhythm (Siwicki et al., in preparation). By quantifying immunoblot-staining of the 48 kD Aplysia eye antigen from animals dissected at different times during the light-dark cycle, levels of the putative per-homolog were found to oscillate with the same phase as Drosophila per. Levels were minimal prior to lights-off (CT 11) and reached a three-fold higher peak before lights-on (CT 23). When Aplysia were kept in constant darkness, oscillations in protein amount continued for one to two cycles before plateauing at the peak level. These results suggest the per-like protein may function in the light entrainment pathway. The localization of the 48 kD per-related antigen to Aplysia pacemaker neurons and oscillations in abundance that mirror cycling of Drosophila per suggests to us that the clocks of these species share fundamental protein components. (Supported by NSF BNS 8819773)

184.8

CIRCADIAN RHYTHM PHASE SHIFTED BY REVERSIBLE TRANSCRIPTION INHIBITOR. S. Ramasubban* and A. Eskin. Dept. of Biochem. and Biophys. Sci., Univ. of Houston, TX 77204.

The role of protein synthesis in circadian mechanisms has been studied extensively using translation inhibitors, which phase shift and change the period of circadian rhythms. Transcription inhibitors, Actinomycin D and Aflatoxin B, have been shown to abolish rhythms (Karakashian and Hastings, 1962; Rothman and Strumwasser, 1977), but the interpretation of their effects is difficult due to the irreversible nature of these transcription inhibitors. We have begun an investigation of the role of transcription in the circadian rhythm of the isolated eye of Aplysia using a reversible transcription inhibitor, 5,6- dichloro-1-ß-d-ribobenzimidazole (DRB). DRB inhibits the synthesis of heterogenous nuclear RNA at the level of initiation (Tamm & Sehgal, 1978).

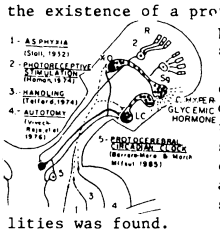
^{3}H-uridine incorporation into TCA precipitable material of the isolated Aplysia eye was inhibited about 85% by 0.1 mM DRB. Recovery after 3 h DRB treatments appeared complete following a 20 min wash; ^{3}H-uridine incorporation was measured for the 3 h following the wash. DRB appeared to have no effect on translation as measured by ^{3}H-leucine incorporation during a 2 h DRB treatment. DRB (2 h, 0.1mM) produced large phase shifts in the circadian rhythm at some phases (3.7 h delay, CT 6-8 , N=3) and no phase shifts at some other phases. Preliminarily, the DRB sensitive phases of the rhythm appear to be from CT 01 to CT 12. DRB had variable effects on the frequency of nerve impulses, producing at times a small inhibition. However, the effect of DRB on spiking did not correlate the effect of DRB on the rhythm.

Our results indicate that transcription is an important process in the circadian system and, moreover, that DRB will be a valuable tool for exploring the specific role of transcription in the circadian oscillator.

184.9

STRESSING AND CIRCADIAN GLYCEMIC RESPONSE IN CRAYFISH. B. Barrera-Mera and S. March Mifsut*. Depto. de Fisiología, Facultad de Medicina, U.N.A.M. Apdo. Postal No. 70-250, 04510 México, D.F. México.

The effect of autotomy, CO_2, and asphyxia used as hyperglycemic stimuli were tested in intact crayfish and in crayfish with damaged central nervous systems, in which the existence of a protocerebral control by a circadian pacemaker for visual activity and sugar regulation was also tested.



In contrast with the potent control of light-adapting response of retinal shielding pigments, in those preparations in which the surgical isolation of the protocerebrum was performed, a clear absense of hyperglycemic response to severe stimulation of sensory modalities was found.

As judged by the amplitude of the hyperglycemic response, these findings suggest that the amount of hyperglycemic hormone released from crayfish sinus gland would directly result from a heavy bombardment of afferent neural signals to the sinus gland coming both from peripheral and visceral sensory receptors, as it is illustrated in Fig. 1 in which exogenous and endogenous glycemic agencies are represented.

184.10

ROLE OF THE SINUS GLAND IN THE SYNCHRONIZATION OF THE ERG CIRCADIAN RHYTHM IN CRAYFISH. B. Fuentes-Pardo, J.A. Prieto-Sagredo*, E. Moreno-Sáenz† and J. Hernández-Falcón*. Depto. Fisiología, Fac. Medicina, UNAM. Apdo. Postal 70250, México, 04510 D.F.

Electroretinogram (ERG) of the crayfish depicts a circadian rhythm susceptible to be synchronized by photoperiods. To get insight on the role of the neuroendocrine system (NS) in this process, we recorded the ERG rhythm from both, intact (In) and to sinus gland-deprived (SGD) crayfish submitted to 12:12 photoperiods. Group 1 animals received the photoperiod in only one eye; group 2 an L:D photoperiod in one eye and D:L in the other. The results were: a) in the In animals, application of one photoperiod on one eye imposes its phase on the rhythm of non-stimulated eye; b) in the SGD animals the stimulated eye follows the imposed photoperiod, the other one shows an unclear circadian rhythm c) in the In animals the simultaneous application of the L:D to one eye and D:L to the other evokes phase shifts in both eyes but showing tendencies to interact; d) in SGD animals the L:D to one eye and D:L to the other causes a 180° phase shift between the activity of the eyes. We concluded that: a) in SGD animals, the overt rhythm is associated to a passive following of the light changes by photoreceptors; b) the phase relation between the ERG rhythms is maintained by the activity of the NS; c) the synchronization of the ERG rhythm by photoperiods reflects maximal phasing of the oscillators underlying the ERG rhythm and requires periodic activity of the sinus gland.

LEARNING AND MEMORY–PHARMACOLOGY NMDA

185.1

LONG-LASTING, APV-SENSITIVE, INCREASE IN FIELD EPSPs BY OXIRACETAM IN RAT HIPPOCAMPAL SLICES. R. Corradetti*, A.M. Pugliese*, L. Ballerini* and G. Pepeu (SPON: M. Raiteri). Dept. Preclin. and Clin. Pharmacol., Univ. Firenze, Italy.

The effect of the nootropic drug oxiracetam (Oxi) on field EPSPs was investigated in the CA1 region of rat hippocampal slices, obtained as previously described (Corradetti et al., J. Neurochem. 41, 1518, 1983), and superfused, in a submerged chamber, with oxygenated aCSF. Stimulation of the stratum radiatum, at 0.1 Hz, evoked field dendritic EPSPs, whose initial slope was measured. Bath applied Oxi (0.1-100 uM) increased the slope of EPSPs. This effect was antagonized by the NMDA antagonist D-(2)-aminophosphonovalerate (APV, 50 uM, n=7). The effect of Oxi on EPSP slope was maximal at 1 uM (+70± 11%, n=12). A 45±12 % increase persisted after 45 min wash, and subsequent high frequency stimulation (100 Hz, 0.4 s, x2) induced a moderate long-term potentiation (LTP). However, the total increase in slope (105%) was as in control LTP (99%), showing lack of additivity to Oxi effect. Consistently, after LTP, 1 uM Oxi slightly, yet reversibly, increased the EPSP. The enduring effect of Oxi, which also mimicked LTP for the need of NMDA-receptor activation, may be relevant for Oxi nootropic action. (Supported by CNR: 87.01425.04).

185.2

NMDA RECEPTORS AND MEMORY: PARALLELS BETWEEN THEIR ROLE IN LEARNING AND SYNAPTIC PLASTICITY. S.P.Butcher*, R.Hendry*, and R.G.M.Morris* (SPON: Brain Research Association) Dept. Pharmacol, Univ.Edinburgh, Edinburgh EH8 9JZ, Scotland.

The selective NMDA antagonist AP5 is known to block the induction of hippocampal LTP but have no effect upon its expression or maintenance. We have therefore studied whether AP5 impairs new spatial learning without affecting the recall of old information.

Rats were first trained in a water-maze to search for a fixed hidden platform (ending with 4 trials at 1 trial per day). They were then implanted with osmotic minipumps for i.c.v. drug administration (aCSF or 30mM D-AP5). For 18 rats, the platform was moved to the opposite location in the pool (eg from NE to SW) while for 12 it remained in the same place. During 8 retraining trials (1 trial/day), rats given D-AP5 were only impaired in the sub-group required to learn a new platform location. Furthermore, all rats were equally biased towards the former training quadrant on the 1st trial of retraining, measured as the distribution of time spent swimming around the pool. Hippocampal tissue levels of AP5 measured using HPLC were 0.23 ± 0.04 nmol/mg wet wt. (range 0.09 - 0.71), similar to values previously shown to cause spatial learning impairments.

These results cannot be explained in terms of a drug induced sensorimotor impairment and support the hypothesis that the underlying mechanisms of LTP have to be activated in spatial learning although not in recall.

185.3

EFFECTS OF PCP AND SIGMA RECEPTOR LIGANDS ON ACQUISITION OF A PASSIVE AVOIDANCE RESPONSE IN RATS. K. W. Jones*, L. M. Bauerle*, and V. J. DeNoble. (SPON: W. K. Schmidt) E. I. DuPont Medical Products Dept., P.O. Box 80400, Wilmington, DE 19880-0400.

Drugs with a strong binding affinity for the PCP receptor, such as PCP and MK-801, are known to disrupt learning and memory whereas the amnestic effects of compounds with a mixed PCP/sigma affinity are not as well delineated. Rats received s.c. doses of MK-801, PCP, ketamine, (+)-SKF 10,047, (+)-pentazocine, (+)-3-PPP, or DTG 30 min before passive avoidance (PA) acquisition training. PA acquisition consisted of placing male CD rats in the illuminated side of a two compartment apparatus. When they went into the dark side, they received two 3-sec 1.0 mA footshocks. Retention testing occured 24 hr later. The rat was placed in the illuminated side and the latency (≤ 300 sec) for entering the dark compartment was recorded. Drugs with a high PCP receptor affinity [MK-801 (0.1-0.3 mg/kg), PCP (0.54-1.7 mg/kg), and ketamine (5.4-17 mg/kg)], produced significant dose-dependent amnesia. The mixed PCP/sigma agonist (+)-SKF 10,047 (5.4-10 mg/kg) also produced amnesia. (+)-Pentazocine interefered with memory, but at a significantly higher dose (54 mg/kg). In contrast, drugs with a high sigma receptor affinity [(+)-3-PPP (1-17 mg/kg) and DTG (0.17-3 mg/kg)] had no effect on PA acqusition. These results suggest that the effects of mixed PCP/sigma agonists on PA acqusition are due to their effects on PCP receptor activity.

185.4

QUISQUALATE RECEPTOR BINDING IN HIPPOCAMPUS FOLLOWING CLASSICAL CONDITIONING OF THE RABBIT NICTITATING MEMBRANE. G. Tocco*, N. Uenishi*, J.K. Thompson*, C. Weiss, M Baudry@, R.F. Thompson (SPON: W. Boyar) USC, Dept. Psych., L.A., CA 90089-1061, @CLNM, UC Irvine,CA 92717.

Hippocampal pyramidal neurons exhibit a rapid within-trial increase in firing frequency during classical conditioning of the rabbit eyelid response. Another model for learning and memory in the hippocampus is Long Term Potentiation (LTP). Different types of glutamate receptors have been shown to be involved hippocampal LTP. While NMDA receptor activation triggers LTP, changes in quisqualate receptors might be responsible for the maintenance of LTP. We have therefore investigated the binding of different glutamate analogs to hippocampi of rabbits that were classically conditioned.

Both quantitative autoradiography in tissue sections and ligand binding to membrane preparations were used to evaluate changes in tritiated AMPA binding to quisqualate receptors following classical conditioning. Both hippocampi of conditioned and unpaired control rabbits were analyzed.

The results indicate that learning of the conditioned response is accompanied by an increase in tritiated AMPA binding in hippocampus suggesting that a modification in properties of quisqualate receptors is involved in the mechanisms of associative learning.

Supported by a McKnight award to R.F. Thompson.

185.5

EFFECTS OF A NOVEL NMDA ANTAGONIST ON LONG-TERM POTENTIATION AND MEMORY. D.L. WALKER* and P.E. GOLD. Neuroscience Program & Department of Psychology, Univ. Virginia, Charlottesville VA 22903.

N-methyl-D-aspartate (NMDA) receptor activation may be important for the establishment of long-term potentiation (LTP) and for learning and memory. A recently developed drug, NPC 12626, is a competitive NMDA receptor antagonist which crosses the blood-brain barrier. We examined the effects of this drug on perforant path - dentate gyrus LTP and on inhibitory avoidance and spontaneous alternation tests.

Rats injected with NPC 12626 (100 mg/kg) 150 min prior to high-frequency stimulation showed no signs of LTP for up to 60 min after tetanus whereas the population spike of control animals increased by 150%. In rats treated 90 min beforehand, LTP was blocked during the first 10 min but increased to control levels within 60 min. NPC 12626 did not itself affect the baseline response nor did it affect LTP when injected after tetanization.

Mice injected with NPC 12626 (35 mg/kg) 35 min prior to inhibitory avoidance training had significantly impaired retention performance (median latencies = 23 sec vs 202 sec for controls). Mice treated with NPC 12626 before spontaneous alternation testing exhibited significantly fewer alternations than did control animals (62±2.7% vs 74±3%).

These results support the view that NMDA receptor activation contributes to LTP and to learning and memory. (Supported by MH 31141 and ONR N0001489-J-1216; NPC 12626 was generously provided by NOVA Pharmaceuticals).

185.6

A COMPARISON OF THE MEMORY MODULATING EFFECTS OF MK-801 AND NALOXONE IN A NOVEL TEST OF PAVLOVIAN FEAR-CONDITIONING. G.A. Cicala*, L.B. Estall*, J.L. Azorlosa*, and S.J. Grant, Dept. Psychology and Program in Neuroscience, Univ. Delaware, Newark, DE. 19716

Compounds that act at NMDA and opiate receptors are known to modulate memory consolidation and retention. We have tested the effects of the non-competitive NMDA antagonist MK-801and the prototypical opiate antagonist naloxone on second-order Pavlovian fear conditioning in rats. This paradigm has the advantage of eliminating possible confounds of drug-induced pain enhancement and state-dependency since nociceptive stimuli are not present when the drug is administered during the second order conditioning session. This permits direct assessment of drug effects on associative processes.

Naloxone (1-4 mg/kg) produced significantly enhanced second-order conditiong. This was probably due to antagonism of endogenous opiates. MK-801 (0.25 mg/kg) blocked the second-order associations, but the endogenous pathways involved in this action are unknown. Test on the interaction between MK-801 and naloxone are in progress.

185.7

METHYLENEDIOXYAMPHETAMINE (MDA): EFFECTS ON CLASSICAL CONDITIONING. N.M. Bormann*, A.G. Romano & J.A. Harvey (SPON:V. Aloyo). Med. Coll. of PA/EPPI, Phil. PA 19129.

Acquisition and extinction of the rabbit's nictitating membrane response was used to examine the effects of MDA on learning. Doses of 1, 3 and 10 umol/kg were injected, s.c., 30 min before each of 4 training sessions consisting of 60 pairings of a tone CS and corneal air puff US. MDA produced a dose-dependent enhancement in acquisition which was significant at the lowest dose tested (1 umol/kg or 0.179 mg/kg). Four days later, subjects were tested for retention of CRs during 4 daily extinction sessions but no drug was injected. There were no differences between subjects previously given MDA or vehicle in the retention or subsequent extinction of CRs, suggesting the absence of state dependent learning under MDA. As a control for any nonassociative effects of MDA, separate groups were given vehicle or MDA (10 umol/kg), 30 min prior to each of 4 daily sessions consisting of unpaired presentations of CS and US. MDA had no effect on baseline responding, responding to the tone CS or on the amplitude of the UR elicited by the US. In previously trained animals, there was also no effect of MDA (10 umol/kg) on tone intensity thresholds for elicitation of CRs. In summary, MDA appears to have enhanced CR acquisition through an effect on associative learning without producing any detectable changes in the response evoking properties of the CS or US. Supported by NIDA Grant DA04944.

185.8

INTRAVENTRICULAR ADMINISTRATION OF THE NMDA ANTAGONIST APV DISRUPTS LEARNING OF AN ODOR AVERSION THAT IS POTENTIATED BY TASTE. G.B. Crooks, Jr., G.S. Robinson, Jr.*, T.J. Hatfield*, P. W. Graham* and M. Gallagher. Department of Psychology, University of North Carolina, Chapel Hill, NC 27599.

Previous research has shown that systemic administration of the non-competitive NMDA antagonist MK-801 impairs taste potentiated learning of an odor aversion (TPOA) at a dose that does not alter learning when either a taste or odor alone is paired with an effective unconditioned stimulus. In this study intraventricular administration of APV (2.5 µg. bilateral) was given prior to training under one of three conditions; during a 5 min drinking session rats were exposed to either a taste alone (0.1% saccharin), an odor alone (30 µl almond extract) or the compound taste/odor. Thirty min after the session malaise was induced (0.3M LiCl 190 mg/kg, i.g.). Aversions to the odor and taste were then tested separately. Administration of d-APV selectively impaired TPOA. Identical doses of l-APV were ineffective. Drug treated animals, however, acquired robust taste aversions. In a subsequent experiment NMDA-induced lesions of the amygdala basolateral nucleus (ABL) were likewise found to selectively impair TPOA. Further studies will examine whether intact NMDA function in the ABL is necessary for TPOA. Because ABL receives multimodal sensory inputs and exhibits LTP (Chapman, P.F., & Brown, T.H., Soc. Neurosci. Abstr., 14, 566, 1988), a NMDA mechanism at this site may be used to store information in this task. Supported by a NSF Predoctoral Fellowship to GC, a NIMH Research Scientist Award to MG (KO2-MH00406), and NIMH grant MH35554.

185.9

NMDA ANTAGONIST MK801 IMPAIRS ACQUISITION BUT NOT PERFORMANCE OF SPATIAL WORKING AND REFERENCE MEMORY. M.L. Shapiro and Z. Caramanos*. Department of Psychology, McGill University, Montreal, Quebec H3A 1B1.

NMDA receptor activation is necessary for inducing, but not for maintaining or expressing glutamate-dependent LTP in hippocampus. MK801 blocks NMDA receptors and LTP induction. If the same mechanisms enable both spatial memory and LTP, then blocking NMDA receptors with MK801 should impair the acquisition, but not the performance of memory tasks. To assess the effects of MK801 on memory, 2 groups of 8 SD rats were compared. An MK group was injected i.p. with .06 mg/kg MK801, and a SAL group with normal saline, 30 min. before behavior testing. Spatial working (WM) and reference (RM) memory were tested on an 8 arm radial maze. For each rat the same 4 arms of the maze were baited once per trial, while the other 4 arms were never baited. Re-entering baited arms during one trial defined WM errors, whereas entering unbaited arms defined RM errors.

After 45 days of testing, the SAL group performed near perfectly, while the MK group was severely impaired on both RM and WM tasks. Giving the well-trained, SAL group MK801 (.025, .06, .075, .10 mg/kg) affected neither WM nor RM. Higher doses (.12 & .15 mg/kg) produced slight ataxia and both WM and RM deficits. Thus, functioning NMDA receptors are necessary for acquiring both spatial WM and RM, but not for performing either WM or RM after training. The results suggest that spatial memory acquisition and LTP induction may share some common mechanisms.

185.10

EFFECTS OF NMDA ANTAGONISTS ON IMPAIRMENT OF THE PASSIVE AVOIDANCE RESPONSE IN MICE. M.J.Benvenga, A.V.Wing*, and R.A.DelVecchio.* Anaquest/BOC Health Care, Murray Hill, New Jersey, 07974.

Memory impairment is a phenomenon that has been linked to the density of N-methyl-D-aspartate (NMDA) binding sites in the hippocampus and other brain regions (Geddes, et.al., Brain Res, 1986). Consistent with this, 2-amino-5-phosphonovalerate (APV), a competetive NMDA antagonist, has recently been shown to impair memory retention in rats (Danysz, et.al., Neuropharm, 1988). The present study investigated the effects of 3(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP) and CGS 19755, isosteric antagonists of NMDA receptors, and PCP, an allosteric antagonist, on passive avoidance behavior in mice.

Male Swiss-Webster mice (25-30g) were injected either 30 minutes prior to or immediately following one-trial passive avoidance training (N=8/group). Groups were treated with CPP (0 [vehicle = distilled water], 1.0, 3.0, 5.0, 7.0, 10.0, 15.0 or 20.0 mg/kg), CGS 19755 (0, 1.0, 2.0, 4.0, 6.0, 10.0, 15.0 or 20.0 mg/kg) or PCP (0, 0.5, 0.75, 1.0, 2.0 or 5.0 mg/kg). All mice were tested 24 hours after initial exposure to the apparatus and the latency to respond on the second day was recorded. Median group latencies were compared by Mann Whitney U-tests.

The results indicated that both CPP and CGS 19755, competetive NMDA antagonists, produced a memory deficit similar to that produced by PCP, when administered prior to the training trial. Both CPP and CGS 19755 were approximately one-fifth as potent as PCP. When assessing the effect of posttrial administration, compared to PCP, much higher doses of CPP and CGS 19755 were necessary to disrupt the passive avoidance response. This suggests that isosteric antagonists affect memory acquisition differently than memory consolidation.

185.11

THE EFFECTS OF L-GLUTAMIC ACID DIETHYL ESTER ON DISCRIMINATION LEARNING IN RATS. R.Lalonde. Hôtel-Dieu Hospital, Neurology Service, Montreal, Quebec, Canada H2W 1T8.

Cortico-cortical and corticofugal pathways use excitatory amino acids as neurotransmitters. High levels of quisqualate receptors are found in the cortex, the striatum and the limbic system. For these reasons, it may be predicted that quisqualate receptor antagonists cause learning impairments. As a first test of this hypothesis, the effects of L-Glutamic Acid Diethyl Ester (LGDE) on a visuotactile simultaneous discrimination learning task were evaluated.

Male Sprague-Dawley rats (n=7) were injected with LGDE at 0,120,240 or 360 mg/kg while learning a discrimination in a T-maze between an arm covered with aluminum foil and an uncovered arm. It was found that rats at the two highest doses learned the task more slowly and committed more errors than at the two lowest doses.

These results indicate a role for quisqualate receptors in discrimination learning. It may be possible to dissociate between discrimination learning and other processes such as recognition memory (Mishkin, Malamut and Bachevalier, 1984, In:Neurobiology of learning and memory G Lynch et al. eds, pp. 65-77) by comparing LGDE with other antagonists.

185.12

NMDA ANTAGONISTS PRODUCE IMPAIRMENT OF SPONTANEOUS ALTERNATION BEHAVIOUR IN MICE. J. Parada-Turska‡, C. Ikonomidou‡ and W.A. Turski. Department of Pharmacology, Medical School, Jaczewskiego 8, PL-20090 Lublin, Poland.

The role of N-methyl-D-aspartate (NMDA) receptors in memory processes was examined using Y-maze and passive avoidance task. In the Y-maze, the total number of arm entries which represents locomotor activity, and alternation behaviour, thought to reflect working memory, were measured. All drugs were injected i.p. in adult male Swiss S mice 30 min prior to the test. Vehicle-treated animals performed 32.7 arm-entries and 59.4% alternations during an 8 min session. The competitive NMDA antagonist, CGS 19755 (cis-4-phosphonomethyl-2-piperidine-carboxylate), at doses of 1 and 2 mg/kg, influenced neither locomotion nor alternation behaviour. CGS 19755, at the dose of 4 mg/kg, lowered total number of arm-entries and alternations. Similarly, CPP (3-((+)-2-carboxypiperazin-4-yl)-propyl-1-phosphate), another competitive NMDA antagonist, reduced locomotor activity at doses of 2.5 and 5 mg/kg and attenuated alternation ability only at the highest dose of 5 mg/kg. On the contrary, the non-competitive NMDA antagonist MK 801 ((+)-5-methyl-10,11-dihydro-5H-dibenzo(a,d)cycloheptan-5,10-imine maleate), at doses of 0.1 and 0.2 mg/kg, dramatically enhanced total number of arm entries and reduced alternation behaviour. NMDA, at doses of 10, 25 and 50 mg/kg, showed no effect on locomotor activity and alternations in mice. NMDA, at the dose of 50 mg/kg, blocked both CGS-19755 and CPP-induced reduction of locomotor activity and alternation behaviour. NMDA had no effect on MK 801-induced enhancement of locomotor activity and reduction of alternation ability. These results suggest that blockade of excitatory transmission at NMDA receptors with antagonist-doses which do not affect locomotor activity does not impair working memory. In the passive avoidance task, mice were trained to avoid dark compartment entry (2 s, 0.6 mA, unscrambled DC current; 24 h before retention test). CPP, at doses of 1 and 2.5 mg/kg (30 min before training; i.p.), did not affect retention but impaired performance during the retention test at the dose of 5 mg/kg. MK-801, at doses of 0.02 and 0.05 mg/kg (30 min before training; i.p.), had no effect on retention and reduced retention at doses of 0.1 and 0.2 mg/kg. CGS-19755, 1 and 2 mg/kg, did not impair the performance during the retention test and reduced retention at the dose of 4 mg/kg. NMDA, 50 mg/kg, antagonized the effect of CPP, 5 mg/kg, CGS-19755, 4 mg/kg, and MK-801, 0.2 mg/kg. CPP, 5 mg/kg, and MK-801, 0.2 mg/kg, did not affect retention when administered immediately after training or 30 min before the test session. The results suggest that NMDA receptor antagonists decrease acquisition (learning) but do not impair retention (memory). Supported by the Polish Academy of Sciences.

LEARNING AND MEMORY–PHARMACOLOGY: MONOAMINES

186.1

ABILITY OF SEROTONERGIC RECEPTOR AGONISTS AND ANTAGONISTS TO ATTENUATE NBM LESION-INDUCED DEFICITS IN ONE-TRIAL INHIBITORY AVOIDANCE RETENTION. H.J. Altman[1], D.J. Jenden[2] and H.J. Normile[1], [1]Department of Psychiatry, Wayne State University School of Medicine, Detroit, MI 48207; [2]Department of Pharmacology, U.C.L.A. School of Medicine, Los Angeles, CA 90024.

A substantial body of evidence appears to suggest that the cholinergic and serotonergic nervous systems play an important and possibly interactive role in the mediation of the processes underlying learning and memory. The purpose of the present series of experiments was to determine whether the retention deficit normally exhibited by NBM-lesioned rats trained in a one-trial inhibitory avoidance task could be ameliorated by the administration of selective serotonergic receptor agonists and/or antagonists immediately following training. NBM lesions were produced by infusing ibotenic acid (bilaterally) into the NBM two weeks prior to behavioral assessment. Retention testing occurred 24 hours following training under extinction conditions. The results of this series of experiments appear to suggest that the retention deficit normally exhibited by NBM-lesioned rats is significantly attenuated by immediate post-train administration of the 5-HT1 agonist TFMPP, but not by the 5-HT2 antagonist ketanserin, in spite of the observation that both agents appear to significantly facilitate retention in non-lesioned animals. (Supported in part by NIA grant #AG07069 and ADRDA grant #RG87087).

186.2

THE EFFECTS OF SEROTONERGIC INTRAHIPPOCAMPAL NEONATAL GRAFTS ON LEARNING AND MEMORY IN RATS TRAINED IN THE STONE 14-UNIT T-MAZE. T.M. Ramirez[1]*, H.J. Altman[2], H.J. Normile[2], D.M.Kuhn[2], and E.C. Azmitia[1] (SPON: I.N. Montgomery). [1]Department of Biology, New York University, New York, N.Y. 10003, [2]Department of Psychiatry, Wayne State University School of Medicine, Detroit, MI 48207.

An increasing body of evidence appears to suggest that serotonin plays an important role in the mediation of the processes underlying learning and memory. An area of the brain which is known to be a major target area for serotonergic afferents emanating from the midbrain raphe nuclei is the hippocampus - an area of the brain that has long been suspected to be essential to the processing of information by the brain. The purpose of the present study was to determine the effects of intrahippocampal serotonergic neuronal grafts on learning and memory. The behavioral task used was the Stone 14-unit T-maze (a complex, positively-reinforced spatial discrimination task). Consistent with previous reports from a number of other laboratories on the effects of acute stimulation of the serotonergic nervous system on learning and memory, implantation of raphe-derived intrahippocampal neonatal grafts significantly impaired learning in rats trained in the Stone maze. The results of the present study confirm and extend previous reports which appear to suggest that augmentation or stimulation of serotonergic neurotransmission may interfere with new learning.

186.3

EFFECTS OF COMBINED SEROTONIN DEPLETION AND LESIONS OF THE NUCLEUS BASALIS MAGNOCELLULARIS ON ACQUISITION OF A COMPLEX SPATIAL DISCRIMINATION TASK. H.J. Normile[1], D.J. Jenden[2], D.M. Kuhn[1], W.A. Wolf[1]* and H.J. Altman[1]. [1]Department of Psychiatry, Wayne State University Sch. of Med., Detroit MI 48207; [2]Department of Pharmacology, UCLA Sch. of Med., Los Angeles, CA 90024.

Recent data suggest that the cholinergic and serotonergic neurotransmitter systems interact in the mediation of many physiological processes, including learning and memory. The purpose of the present study was to examine the effects of destruction of cholinergic neurons projecting from the nucleus basalis magnocellularis (NBM), alone or in combination with global serotonin (5-HT) depletion, on learning in rats trained in the Stone 14-unit T-maze - a complex, positively-reinforced spatial discrimination task. Destruction of cholinergic neurons within the NBM was accomplished by the infusion (bilateral) of ibotenic acid. Global 5-HT depletion was accomplished by the systemic administration of p-chloroamphetamine (PCA). The results show that PCA-induced 5-HT depletion enhanced acquisition. This effect was completely antagonized by NBM lesions, despite the fact NBM lesions alone did not produce a deficit in this task. The results further support the view that the cholinergic and serotonergic systems interact in learning and memory processes. (Supported by NIA grant #AG07069).

186.4

PLASMA GLUCOSE LEVELS PREDICT THE ATTENUATION OF EPINEPHRINE-INDUCED MEMORY ENHANCEMENT BY ADRENERGIC BLOCKADE. J.L. Hall & P.E. Gold. Dept. Psychology, University of Virginia, Charlottesville, VA 22903.

Posttraining epinephrine release or administration enhances memory. The effects of epinephrine on memory are blocked by pretreatment with alpha- or beta-adrenergic antagonists. Recent evidence indicates that epinephrine-induced elevations in blood glucose (BG) levels may contribute to the hormone's effects on memory. The present experiment determined whether adrenergic antagonists alter the BG response to epinephrine, thereby blocking epinephrine effects on memory.

BG levels were assessed in rats before and at several times after injections of phenoxybenzamine (an alpha-adrenergic antagonist; 2 mg/kg) or propranolol (a beta-adrenergic antagonist; 0.5 mg/kg) followed in some animals by epinephrine injections (0.1 mg/kg) 30 min later. At these doses, epinephrine enhances memory and the antagonists block the epinephrine-induced memory enhancement. Epinephrine resulted in maximal BG increases of 30 mg/kg within 30 min of injection. Neither antagonist itself significantly altered BG levels. However, phenoxybenzamine potentiated and propranolol attenuated the hyperglycemia resulting from epinephrine injection.

These findings suggest that aberrations in BG responses to epinephrine injection contribute to the disruption of memory enhancement after adrenergic blockade. Further, the opposite direction of effects of the two classes of antagonists (alpha- and beta-) on epinephrine-induced hyperglycemia suggests that the blockers' effects are mediated by different mechanisms. (Supported by MH 31141 and ONR N0001489-J-1216).

186.5

PD 122655: A NOVEL ALPHA-2 ANTAGONIST WITH COGNITION ACTIVATING PROPERTIES. R.E. Davis, L.L. Coughenour*, W.H. Moos, T.A. Pugsley, R.D. Schwarz, A.J. Thomas. Parke-Davis Pharmaceutical Research, Warner-Lambert Co., Ann Arbor, Mi. 48105.

Alpha-$_2$ antagonists are known to increase central noradrenergic function via presynaptic autoinhibition. Through this action these agents may be therapeutically useful in reversing the noradrenergic and cognitive decline accompanying aging and dementia. This led us to initiate an effort to discover, novel alpha-$_2$ antagonists with cognition activating properties. This effort yielded a series of chemically novel arylaminopyridnes with alpha-$_2$ antagonists properties typified by PD 122655, N-(3-chlorophenyl)-4- pyridinamine. PD 122655 binds with nM affinity at alpha-$_2$ sites and uM affinity at alpha-$_1$ sites with little or no affinity for other receptors (alpha-$_1$/alpha-$_2$ = 56). It possesses alpha-$_2$ antagonist properties in several peripheral systems and dose dependently enhances the synthesis of norepineprhine in discrete brain regions. PD 122655 also improves water-maze acquisition of hippocampally deficient mice, decreases scopolamine-induced and spontaneous swimming activity in rats and reverses clonidine-induced cognitive impairments in aged- rhesus monkeys. Based on this evidence PD 122655 appears to be an orally active alpha-$_2$ antagonist with cognition activating properties.

186.6

NEONATAL DSP4 REDUCES ENVIRONMENTAL INFLUENCES ON BRAIN NEUROCHEMISTRY AND OLFACTORY LEARNING IN DEVELOPING RATS. C.A. Cornwell-Jones, M.W. Decker, T. Gianulli*, E.L. Wright* and J.L. McGaugh. Dept. of Psychology, Syracuse Univ., Syracuse, NY, 13244, Center for the Neurobiology of Learning & Memory, and Dept. of Psychobilogy Univ. of Calif., Irvine, CA 92717.

Neonatal rats were injected with either DSP4 or 6-OHDOPA to determine whether these produce impairment in postweaning olfactory adaptation comparable to that produced by 6-OHDOPA (Cornwell -Jones et al., Behav. Neural Biol., 25:217, 1982). Controls housed in a new odor for 10 days after weaning with either 6-OHDOPA injected rats or other controls showed normal tolerance for the odor. In contrast, the odor was aversive to controls housed in the odor with DSP4-treated siblings. Drug-treated rats showed no effects of either olfactory or social experience. Housing controls with DSP4-treated rats depressed hippocampal NE and serotonin (5-HT) by 57% and 62%, and elevated olfactory cortex NE and 5-hydroxy-indole acetic acid (5-HIAA) by 32% and 174%. Controls' DSP4 treated cagemates showed parallel changes in regional 5-HT and 5-HIAA, but not NE levels. Experience-induced changes in NE may modulate environmental effects on brain and behavior.

186.7

INTERACTION BETWEEN DSP4 AND NALOXONE (NAL) IN AN ACTIVE AVOIDANCE TASK. R. Kunstmann[1], M.C. Bennett[2] and F.J. Hock[1]. (SPON: A. Frostholm) [1]HOECHST AG, P.O.B. 80 03 20, D-6230 Frankfurt/M. 80, FRG; [2]University of Colorado Health Sciences Center Dept. of Pharmacology, Denver, CO 80262

Male NMRI mice were given injections of the noradrenergic neurotoxin, DSP4 (25 mg/kg, iv) or a comparable volume of the vehicle 24-74 hr prior to behavioral testing. Animals were given 2 days of training on an active avoidance task. NAL was given (1, 3, or 10 mg/kg, ip) prior to training on day 1 and day 2, or prior to training on day 1 only. There was a dose-dependent impairment of acquisition by NAL in the vehicle-pretreated groups. NAL also modulated retention (day 2) performance of the active avoidance task. For the vehicle-pretreated groups, 1 mg/kg NAL facilitated and 10 mg/kg NAL impaired performance on day 2. DSP4 alone impaired acquisition but not retention of this task. NAL produced somewhat different effects in the DSP-4-pretreated mice than in the vehicle-pretreated mice. NAL (1 mg/kg) ameliorated the DSP4-induced impairment of acquisition; 10 mg/kg NAL did not significantly alter the acquisition; 10 mg/kg NAL did not significantly alter the acquisition of this group. For the DSP4-pretreated mice that received NAL before training on both days, the dose-response characteristics for retention scores were similar to those of vehicle-pretreated mice. However, for DSP4-pretreated mice that received NAL before training on day 1 only, the effective facilitating dose was shifted to the right.

186.8

DIFFERENTIAL EFFECTS OF PRETRAINING AND POSTTRAINING INTRA-AMYGDALA ADMINISTRATION OF APOMORPHINE ON LEARNING AND MEMORY. K.Vu*, J.B.Introini-Collison and J.L.McGaugh (SPON: B.J.Vasquez).Center for the Neurobiology of Learning and Memory and Department of Psychobiology, University of California, Irvine, CA 92717.

The present experiments examined the effects of intra-amygdally injected apomorphine on learning and memory processes as evaluated in inhibitory avoidance and the Morris water maze tasks. Apomorphine (0.1-3.0 µg) significantly impaired retention of the inhibitory avoidance when given immediately posttraining. Similarly, apomorphine (0.3 µg; 5 min pre-training) significantly impaired retention in the water maze. This latter effect was more manifest on the first trial of each day and was also observed when apomorphine was given immediately posttraining, suggesting a facilitatory effect of apomorphine on acquisition and an impairing effect on retention. This conclusion was corroborated when the most effective dose of apomorphine (0.3 µg) was given in the amygdala 5 minutes prior to training using multitrial training procedures.

These findings indicate that intra-amygdala injections of apomorphine impair retention in water maze as well as in inhibitory avoidance tasks. However, the findings that in both tasks apomorphine enhances acquisition, indicate that apomorphine has differential effects on learning and retention.

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186.9

CONDITIONING OF A DOPAMINERGIC DRUG-STATE. E.N Damianopoulos* and R.J. Carey (SPON: M.R. Lynch). Department of Psychiatry, SUNY and VA Medical Center, Syracuse, NY 13210

Two groups of rats received 2.0 mg/kg apomorphine (S.C.) either 10 min prior to placement in a drug conditioning box (paired group) or 30 min. after (unpaired group) in 10 consecutive one trial per day sessions. Two-weeks later, all animals were tested without drug or vehicle injection in the conditioning box. Paired but not unpaired animals exhibited hypermotility and inhibition of dopamine metabolites which characterized the behavioral and biochemical effects of the dopamine agonist apomorphine treatment. Conditioning effects were also observed for animals treated with haloperidol (1 mg/kg I.P.). These findings are consistent with the concept of a conditioned central drug state.

186.10

PAVLOVIAN CONDITIONING OF L-DOPA INDUCED ANTI-PARKINSONIAN MOVEMENTS. R.J. Carey. Department of Psychiatry, SUNY and VA Medical Center, Syracuse, NY 13210

In rats with unilateral 6-hydroxydopamine (6-OHDA) lesions of the substantia nigra, L-DOPA induced antiparkinsonian effects occur as contralateral rotational movements. In this study, drug-naive rats with unilateral 6-OHDA lesions were given 5 L-DOPA-carbidopa treatments (10 or 20 mg/kg L-DOPA with 1 or 2 mg/kg carbidopa). Some of the animals were exposed to a test environment for a 10 min. period during the L-DOPA treatment phase whereas other animals which received the identical L-DOPA treatment were exposed to the same environment but unpaired with the drug treatment. Two weeks after the final L-DOPA treatment, the animals were placed in the same environment without drug or vehicle treatment for a 10 min. test period. Animals which had received the environment-L-DOPA pairings exhibited contralateral rotation identical to that exhibited for L-DOPA whereas animals in the environment-L-DOPA unpaired treatment group exhibited the spontaneously occuring ipsilateral rotation.

186.11

EFFECT OF D-AMPHETAMINE ON SPINAL FIXATION IN RATS. M. J. Bartelt*, A. G. Hutchins*, and M.M. Patterson (SPON: D. Johnson). Dept. of Psychology and College of Osteopathic Medicine, Ohio Univ. Athens Ohio, 45701

It has been demonstrated that lasting hind limb flexion (spinal fixation) may be induced by external stimulation to the upper right hind limb in spinalized rats (Steinmetz 1981). The goal of the present study was to clarify earlier conflicting reports on the effect of d-amphetamine on spinal fixation. Palmer (1969) found d-amphetamine to decrease the time required for spinal fixation while F. Mouravieff-Lesuisse (1970), using the same dose of d-amphetamine (2mg/kg i.p.), reported no decrease in fixation time.

In the present study, 14 rats were anesthetized with Nembutal (50 mg/kg i.p.) and randomly assigned to either the d-amphetamine (2mg/kg i.p.) or saline/control group. The animals were administred the appropriate substance and then spinalized at T7. Immediatly following 20 minutes (normally a time too short to demonstrate reliable fixation) of upper hind limb stimulation (2-4mA, 100-pps, 7msec, repetitive dc pulses), fixation was measured by the amount of weight needed to remove the hind limb asymmetry.

At the 20 minute stimulation time we found a significantly greater amount of fixation in the d-amphetamine (mean wgt=8.4 gms) as compared to the saline/control group (mean wgt=1.9) at p<.01. These results, which support Palmer's (1969) work, suggest that catacholamines may be involved in the fixation of spinal reflexes.

186.12

MEMORY FOR APPETITIVE AND AVERSIVE EVENTS IMPROVED BY POST-TRAINING INJECTION OF DOPAMINE AUTORECEPTOR AGONISTS Mark G. Packard and Norman M. White, Department of Psychology, McGill University, 1205 Dr. Penfield Ave., Montreal, Quebec Canada H3A 1B1

Previous work has shown that post-training injection of the dopamine (DA) D2 receptor agonist, LY171555, improves memory, while a D1 agonist, SKF38393, does not. In the first experiment, a post-training SC injection of 0.06mg/Kg of apomorphine, a mixed DA receptor agonist, improved retention of a conditioned emotional response; lower and higher doses (0.01-1.2mg/Kg) had no effect. The low effective dose suggests that the observed memory improvement may result from activation of a DA autoreceptor. We tested this hypothesis using an appetitive task in a radial maze: a light cue signalled the location of food in 4 different arms on each trial; rats were required to visit each lit arm twice within a trial. All rats were given 1 trial per day for 10 days and were injected following trial 5. Apomorphine, LY171555 and BH-T920, a specific DA autoreceptor agonist, all improved acquisition of the task at 0.05mg/Kg relative to saline and delayed drug injection controls. These findings suggest that the memory improving action of DA agonists is effective for both aversive and appetitive tasks, and that the effect may be mediated by an event involving the autoreceptor. In view of the many reports that post-training amphetamine (which promotes DA release) improves retention, this event is unlikely to be simply the reduction in DA release caused by autoreceptor activation.

186.13

DOPAMINE AND MEMORY: EFFECT OF ACUTE SCOPOLAMINE TREATMENT ON DOPAMINE METABOLISM IN RAT HIPPOCAMPUS AND FRONTAL CORTEX. C. Missale, P. Liberini*, S. Sigala*, M. Pizzi*, M. Memo, P.F. Spano. Inst Pharmacol Exp Ther, Sch Med, Univ Brescia, Italy.

The aim of the present study was to investigate some neurochemical correlates of the transient memory disruption induced in rats by scopolamine. After the acquisition of the passive avoidance response, male Sprague Dawley rats were divided into groups of 10 and trated with various doses of scopolamine or saline. Two hours later animals were tested for retention of the acquired response. Rats were killed by decapitation and the contents of DA and its metabolites (DOPAC and HVA) were determined in the frontal cortex and dorsal hippocapmus by HPLC.

The results indicate that scopolamine induced a selective decrease in the content of DA metabolites in the hippocampus (- 46 % DOPAC and - 38 % for HVA) and frontal cortex (- 50 % for both DOPAC and HVA). This phenomenon paralleled, in term of time- and dose-dependence the drug-elicited amnesic effects, as measured by the passive avoidance test, thus supporting the idea of a role of DA in learning and memory.

186.14

p-CHLOROAMPHETAMINE BLOCKS THE MEMORY ENHANCING EFFECT OF PHYSOSTIGMINE IN RATS WITH N. BASALIS OF MEYNERT LESIONS. A.C. Santucci, V. Haroutunian, P.J. Knott, P.D.Kanof* and K.L. Davis*. Dept. of Psychiatry, Mount Sinai School of Medicine, New York, NY 10029.

The present study examined the effect of p-chloroamphetamine (PCA), a purported serotonergic releasing agent, on memory in rats with N. basalis of Meynert (nbM) lesions. Sham and nbM operated rats were trained on a passive avoidance task one month postoperatively. Thirty min prior to training subjects were injected with either PCA (2.5 mg/kg) or saline. Immediately after training subjects received injections of either physostigmine (0.06 mg/kg) or saline. All animals were tested 72 hr after training. Results indicated that 1) nbM lesions produced memory impairments, 2) posttraining physostigmine reversed these impairments, 3) pretraining PCA blocked the memory enhancing effect of physostigmine and 4) PCA alone impaired retention in both sham and nbM animals. In a parallel study 2.5 mg/kg of PCA decreased 5-HT and 5-HIAA levels in the frontal cortex of rats 60 and 120 min postinjection. These data indicate that the serotonergic and cholinergic systems interact on a functional level.

186.15

SYSTEMIC BUT NOT INTRA-AMYGDALA ADMINISTRATION OF FLUOXETINE FACILITATES MEMORY. S.To*, I.B.Introini-Collison and J.L.McGaugh (SPON: G.D.Novack). Center for the Neurobiology of Learning and Memory and Department of Psychobiology, University of California, Irvine, CA 92717.

In previous studies we have found that retention is influenced by treatments affecting several neuromodulatory systems within the amygdaloid complex. These experiments examined the role of the serotonergic system. Flood and Cherkin (Psychopharmacol. 93:36, 1987) reported that either pre- or posttraining systemic or icv injections of the serotonin uptake blocker fluoxetine enhanced retention in mice trained in a shock motivated T-maze task. The present experiments examined the effects of fluoxetine on retention of an inhibitory avoidance task and the Morris water maze in rats. Male Sprague Dawley rats were trained in a step-through inhibitory avoidance task and given fluoxetine (15 mg/kg; ip) posttraining. On the retention test 1 week later, the performance of fluoxetine-injected rats was significantly better than that of the saline controls. Similarly, fluoxetine (15 mg/kg; ip) administered 15 min pretraining significantly enhanced the rate of learning of the platform location in the Morris task. However, intra-amygdala fluoxetine (1.0, 3.0 or 10.0 µg) did not significantly modify retention in either task. These findings indicate that the serotonergic system in the amygdala is not involved in the modulatory influence of fluoxetine on memory.

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186.16

CAN SEROTONERGIC DEPLETION INFLUENCE BEHAVIOR FOLLOWING NBM LESIONS? A.L.Markowska, G.L.Wenk, and D.S.Olton. Department of Psychology, The Johns Hopkins University, Baltimore, MD 21218.

The present study investigated the effects of serotonergic depletion upon the performance of rats with lesions of the nucleus basalis magnocellularis in a nonspatial memory task. Lesions were made by injecting ibotenic acid into the NBM. Serotonin was depleted by systemic injection of p-chloroamphetamine (PCA). After four weeks of testing, the choice accuracy of PCA rats was not different from that of control rats (CON), while the choice accuracy of NBM rats and rats with combined treatment (NBM+PCA) was significantly lower and not different from each other. With prolonged testing (28 weeks), performance improved in CON rats and NBM rats, but not in PCA rats and NBM+PCA rats. These results show that damage to the serotonergic system did not produce an initial behavioral impairment, but did disrupt the recovery following NBM lesions. Pharmacological challenges to the cholinergic (scopolamine, physostigmine) and serotonergic (methysergide) systems describe further interactions between the two systems. Overall, these findings suggest an influence of both the NBM and the serotonergic system in processing nonspatial information. (Supported by NSF BNS 88-07010).

187.1

GENE EXPRESSION IN SPINAL CORD FOLLOWING BRIEF SENSORY STIMULATION. S. Williams*, W. Wisden* and S.P. Hunt* (SPON: D. Bousfield). M.R.C. Molecular Neurobiology Unit, Cambridge CB2 2QH, U.K.

Brief sensory stimulation can lead to rapid induction of the "immediate-early" onset gene, c-*fos* in spinal cord neurons (Hunt *et al.*, *Nature* 328:632, 1987). We have extended these observations to include other members of this gene 'family' and to map the appearance of gene product up to 24h after the initial stimulation. Rats were anaesthetised with Equithesin and the hind-paw stimulated by immersion in a water-bath for 20s at 52°C. mRNA for c-*fos*, NGF-IA, NGF-IB and c-*jun* A was detected at 5-15 min in the superficial dorsal horn and remained high for over 2h. By 4h, deeper-lying neurons were labelled but the superficial signal was greatly attenuated. Fos protein was seen to increase again from 4-16h bilaterally in a population of neurons in intermediate laminae. Local anaesthetic blockade of the ipsilateral sciatic nerve 1h after stimulation did not diminish this "second wave" of c-*fos* positive cells, implying independence from ongoing primary afferent discharge, and suggesting that the initial brief stimulation had triggered a long-term change in neuronal activity within the CNS.

187.2

FORMALIN INCREASES DORSAL HORN SUBSTANCE P CONTENT. K.E. McCarson and B.D. Goldstein. Department of Pharmacology and Toxicology, Medical College of Georgia, Augusta, GA 30912-2300.

Substance P (SP) found in primary afferent terminals has been proposed as a mediator of nociception in the dorsal horn of the spinal cord. The release of SP from these small diameter afferent neurons can be stimulated by a number of noxious stimuli. Formalin, a known nociceptive stimulus, has been shown to produce a biphasic effect with early (0-10 min) and late (20-60 min) increases in dorsal horn SP in immunohistochemical studies. This study was undertaken to determine if formalin affects the total dorsal horn content of SP. The right hindpaws of anesthetized rats received either an injection of 5% formalin or noxious mechanical stimulation (pinch) as a positive control. Saline injected and naive animals served as negative controls. After various time intervals the dorsal horns of the lumbar enlargement were assayed for SP using radioimmunoassay. Formalin produced a 262% increase in SP in the ipsilateral dorsal horn 10 minutes following hindpaw injection which decreased to baseline levels by 60 minutes. Hindpaw pinch increased SP in the ipsilateral dorsal horn by an average of 174% during the entire 60 minute period following the stimulus. Saline injection produced no significant effect. Contralateral dorsal horn SP levels were unchanged by any of the hindpaw treatments. The increases in SP produced by these nociceptive stimuli may be due either to increased release into the dorsal horn or increased cleavage of SP precursors in the primary afferent terminal. Supported by NS18664.

187.3

LACK OF CORRELATION BETWEEN THE LEVELS OF SUBSTANCE P (SP) IN THE DORSAL HORN (DH) AND PAIN-RELATED BEHAVIOR FOLLOWING FORMALIN. L.N. Holland and B.D. Goldstein. Department of Pharmacology and Toxicology, Medical College of Georgia, Augusta, GA 30912-2300.

The injection of dilute formalin into the hindpaw has been shown to increase SP in the rat DH as measured immunohistochemically. These SP levels have a time-course which is similar to the biphasic change observed in pain-related behaviors in the formalin test. Systemic morphine produces a decrease in this behavior, but causes a further increase in SP levels. Systemic morphine or lidocaine injected into the hindpaw without formalin will increase SP levels. We, therefore, measured the effect of lidocaine on pain-related behavior due to formalin, and determined SP changes which may be associated with lidocaine pretreatment.

The pain-related behavior due to formalin was measured by the Digiscan (Omnitech Electronics, OH) as duration of stereotypy for 60 minutes following a 5% formalin injection. The levels of the SP were quantified using immunohistochemistry at 60 minutes. An injection of 2% lidocaine with epinephrine into the hindpaw reduced the formalin-induced stereotypy time but resulted in a further increase in the levels of SP in the DH.

These data suggest that SP changes in the DH as a result of lidocaine pretreatment do not correlate with the reduction of pain-related behavior following formalin. Supported by NS-18664.

187.4

PREPRODYNORPHIN AND PREPROENKEPHALIN mRNAs IN THE SPINAL CORD DURING A MODEL OF NEUROPATHY, NERVE INJURIES AND PERIPHERAL INFLAMMATION. G. Draisci, K.C. Kajander, G.J. Bennett, M.J. Iadarola and R. Dubner. Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20892.

Previous studies have shown an increase in dynorphin gene expression during inflammation of the hind paw. In the present study, we analyze a model of neuropathic pain (caused by loose ligatures around the sciatic nerve which induces a partial deafferentation) and compare it to nerve crush, nerve transection and peripheral inflammation for alterations in spinal cord preprodynorphin (PPD) and preproenkephalin (PPE) mRNA levels.

Total lumbar spinal cord RNA was extracted at 2, 5, 10 and 20 days after sciatic nerve ligation and RNA blots were prepared. Blots were hybridized with ^{32}P-labeled probes for rat PPD (from J. Douglass and O. Civelli), rat PPE (from S. Sabol), and rat B-actin (from U. Nudel). Blot analysis was conducted at 2 days after sciatic nerve crush, transection and carrageenan inflammation of the hind paw.

A 2-fold increase in preprodynorphin mRNA occurred two days after ligation, reached a peak by 5 days (4-fold) and declined to 50% over control by 20 days. In addition to the ligation model, only inflammation produced an increase (4-fold) in dynorphin mRNA. No alterations were found at 2 days for transection or crush compared to sham operated rats. Only relatively small changes of preproenkephalin mRNA were found after inflammation (50% over control) and no changes were found with the other manipulations.

Our results show that in the sciatic nerve ligation model of neuropathic pain a rapid and sustained activation of dynorphin gene expression occurs. Thus, an increase in dynorphin mRNA occurs with both peripheral inflammation and peripheral neuropathy. This suggests that afferent input from small fibers and an intact connection with the periphery are important for dynorphin gene activation since these two features are common to both models.

187.5

PROLONGED ELEVATION OF MULTIPLE FOS IMMUNOREACTIVE PROTEINS IN RAT SPINAL CORD: WESTERN BLOT AND IMMUNOCYTOCHEMICAL ANALYSIS. M.J. Iadarola, C.L. Yeung*, G. Draisci and M.A. Ruda. Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20892.

We have recently found a rapid and transient increase in c-fos mRNA in lumbar spinal cord following carrageenan-induced inflammation of the hindpaw. In addition to the c-fos proto-oncogene, which codes for a nuclear transcription factor, other genes exist which code for Fos-immunoreactive proteins (Fos-related antigen or FRAs). In order to study the protein product of the c-fos gene and the FRAs in our model, we generated an antiserum to the synthetic peptide KVEQLSPEEEEKRRIRRERNKMAAA, a highly conserved region in Fos and the Fos-related antigens (FRAs). The antiserum was affinity purified against immobilized antigen and used for immunocytochemistry (ICC) and western blot analysis. Contrary to the short-lived elevation we expected from mRNA levels and previous ICC studies by others with nociceptive stimuli and an N-terminally directed antibody, a very prolonged increase (out to 4-6 days) was observed with ICC with our antibody. The initial pattern of cell labeling was complex during the first 8 hours, with several laminae in addition to I-II and V-VI showing immunoreactive cells. By 24 hrs and at later times the labeling was mainly found in laminae I-II and V-VI. The pattern of proteins seen on western blots was also complex. There was a rapid elevation (within 1 hr) in specific immunoreactive bands migrating at 55, 44, 41-38 kDa. The elevation in these bands was maintained through 24 hrs after which the 55 kDa band declined while the other bands (FRAs) remained elevated. These data indicate a specific and prolonged temporal sequence of increase in Fos proteins occurs in spinal cord induced by peripheral inflammation and suggest the participation of multiple Fos-reactive proteins in both triggering and sustaining expression of target genes in the spinal cord during inflammation.

187.6

SPINAL CORD DISTRIBUTION OF C-FOS PROTEIN IN VISCERAL VERSUS SOMATIC MODELS OF CHEMICAL INFLAMMATION. L.A. Birder*, J.R. Roppolo, M.J. Iadarola and W.C. de Groat (SPON: A. Moller). Univ. of Pittsburgh, Depts. Pharmacol. and Beh. Neurosci., Pittsburgh, PA 15261.

Activation of somatic nociceptive afferents (aff) stimulates expression of the c-fos oncogene in dorsal horn (DH) neurons of the spinal cord (SC). We have used immunocytochemical (IC) techniques to compare distribution of c-fos protein in the SC of the cat and rat following chemical irritation of the hindpaw or urinary bladder (UB) (intravesical 3-10% formalin for 2 hours). One decerebrate cat and neurally intact and spinal unanesthetized rats were studied. Animals were deeply anesthetized prior to perfusion and removal of the tissues. In both cat and rat UB irritation increased c-fos protein in lamina I neurons of the DH, in the dorsal commissure, lamina X and in the sacral parasympathetic nucleus (SPN) at the L_6-S_2 level of the SC. C-fos staining was markedly lower in the upper lumbar segments (L_1-L_3). Over distension of the UB produced only a slight increase in c-fos. Chemical irritation of the hindpaw increased c-fos in similar regions of the L_4-L_5 DH, excepting the intermediolateral area. These findings are consistent with the concept of viscerosomatic nociceptive aff convergence in the SC. They also reveal a difference between chemical and mechanical irritation of the UB and suggest that nociceptive input from the bladder is carried primarily by afferent pathways in the pelvic nerve to the lower lumbar and sacral segments of the SC.

187.7

THE CELLS OF ORIGIN OF THE SPINOHYPOTHALAMIC TRACT IN THE CAT. J.T. Katter, R. Burstein, & G.J. Giesler, Jr. Dept. Cell Biol. & Neuroanat., Grad. Prog. in Neurosci., Univ. Minn., Minneapolis, MN 55455.

Many spinal cord neurons in rats project directly to the hypothalamus. In this study, injections of retrograde tracers into the hypothalamus of cats were made in an attempt to define a similar projection. Injections of WGA-HRP, a mixture of WGA-HRP and B-HRP, or fast blue were centered in the hypothalamus in seven cats and did not spread into the thalamus, zona incerta, or midbrain. Retrogradely labeled neurons were observed bilaterally in all of the 17 examined spinal segments. Approximately 400-500 labeled neurons were observed in alternate sections in the most effective cases. Roughly 70% of these neurons were contralateral. Labeled neurons were found predominantly in the deep dorsal horn and in the area surrounding the central canal. A few were also noted in the superficial dorsal horn and ventral horn. Interestingly, the largest number of labeled neurons was observed in S1-2.

Overall, a smaller population of spinohypothalamic tract neurons was observed in cats than previously in rats. To test the sensitivity of our tracing techniques, WGA-HRP was injected into the thalamus, including the ventrobasal complex, in four cats. Thousands of spinal cord neurons were labeled in each cat. The results of this study suggest that a comparatively small number of spinal cord neurons project to the hypothalamus in cats. Supported by NS25932.

187.8

SENSITIZATION OF PRIMATE SPINOTHALAMIC TRACT NEURONS BY ELECTRICAL STIMULATION OF SENSORY AFFERENTS, P.M.Dougherty, C.Owens, D.Zhang and W.D.Willis, Dept. Anat. & Neurosci., Mar. Biomed. Inst., Univ. Texas Med. Br., Galveston, TX

Potentiation of evoked responses of somatosensory neurons by chemical, mechanical, and inflammatory stimuli present useful models of chronic pain. This study investigates the use of electrical stimulation of sensory root afferents in the production of sensitization of primate (M. fascicularis) spinothalamic tract neurons. Eleven physiologically identified cells located in spinal segments L2-L6 were studied in 10 anesthetized animals, using carbon-glass micropipettes (2-3MOhms). Upon mapping the receptive field size and response characteristics as well as the evoked field and single unit activity of graded dorsal root stimulation, a pair of conditioning shocks (100Hz, 1 s duration, 1 msec pulse width) at high intensity (200X threshold for field potential) was delivered by one dorsal rootlet (intact or cut). Receptive field and electrical-driven activities were again determined. The results suggest a preferential enhancement of low threshold (brush, press) vs. high threshold (pinch, squeeze) input in 8 cells. In addition, evoked field and driven spike activity demonstrated an increase in threshold and peak response in 10 cells. In summary, intense electrical stimulation of dorsal root afferents induces a sensitization of primate STT neurons potentially representing another model of chronic pain. (Supported by NIH grants NS11255 & NS09743 and the Bristol-Myers Co.)

187.9

ORGANIZATION OF VISCEROSOMATIC SPINAL AFFERENT INPUT TO C_3-T_4 SPINOTHALAMIC TRACT (STT) CELLS IN THE MONKEY. **S.F. Hobbs, D.C. Bolser, M.J. Chandler, & R.D. Foreman. Dept. of Physiol., Univ. Okla. HSC, Okla. City, OK 73190.**

To understand why visceral pain from the thoracic and upper abdominal organs is often felt as arising from chest muscle, we determined in STT cells the relationships between the following: 1) location of excitatory somatic fields, 2) input from hair, skin, and muscle, 3) visceral afferent input, and 4) spinal location of the ST cells. Responses of 89 STT cells from 19 chloralose-anesthetized monkeys were analyzed. In general, cells with somatic fields confined to the hand were maximally excited by skin pinch, but cells with somatic fields proximal to the hand were excited maximally by muscle input. Electrical stimulation of cardiopulmonary sympathetic or splanchnic afferents excited STT cells with chest or shoulder somatic fields more than cells with somatic fields confined to the forelimb. Also, cells excited by visceral input were excited more by muscle than skin or hair input. Cells with somatic fields confined to the forelimb were primarily found in segments C_7-C_8. Cells in segments C_3-C_6 and T_1-T_4 but not C_7-C_8 were excited by chest, shoulder, and/or forelimb input and by visceral input. These data indicate that visceral pain from thoracic and upper abdominal organs is often referred to the muscles of the chest because visceral input from these organs primarily excite STT cells whose major somatic input is from thoracic muscles. (OCAST & HL22732)

187.10

EVIDENCE THAT SUBSTANCE P MODULATES C-FIBER EVOKED DISCHARGES OF SPINAL CORD NOCICEPTIVE NEURONS IN THE RAT. D.E. Kellstein[1], D.D. Price[2], R.L. Hayes[3], and D.J. Mayer[1]. Depts. of [1]Physiology, [2]Anesthesiology, and [3]Neurosurgery, Medical College of Virginia, Richmond, VA 23298.

Previous studies suggest that the undecapeptide Substance P (SP) may function as a primary afferent neurotransmitter or neuromodulator of nociception. The present study investigated the effects of SP and an SP antagonist, [D-Pro^2,D-$Trp^{7,9}$]-SP (DPDT), on A- and C-fiber evoked firing of dorsal horn nociceptive neurons using extracellular electrophysiological recording.

Male Sprague-Dawley rats (450-550 g) were anesthetized with urethane (1.3 g/kg). A laminectomy was performed at spinal segments L4 and L5 and the dura was retracted. Single unit recordings were made at spinal segments L4 and L5 from wide dynamic range and nociceptive specific neurons of the dorsal horn (mean depth 1.07 $\pm$ 0.1 mm) during controlled repetitive electrical stimulation of the ipsalateral hind paw. Stimulation consisted of trains (6 pulses, 1 every 2 sec) of rectangular wave pulses supramaximal for C-fiber activation (3 mA, 2 msec duration). Following determination of pretreatment responses, SP or DPDT (4, 20, and 100 nmol in 10 μl) was applied onto the surface of the cord and evoked discharges were quantified at 1, 5, and 10 min after each dose. Whereas SP enhanced C-evoked firing with an apparent U-shaped dose response (100 > 4 > 20 nmol), DPDT inhibited C-evoked firing in a dose-related manner with an apparent ceiling effect at 20 nmol. Neither drug altered A-fiber evoked activity. In those neurons exhibiting C-evoked temporal summation, SP (4 and 100 nmol) flattened and maximized the temporal summation curve such that the response to first stimulation was nearly equivalent to the sixth. These findings suggest that SP may function as a neuromodulator of nociceptive transmission of C-fiber, but not A-fiber, mediated responses. Supported by PHS awards NS 24009-1A2 and DA 00576.

187.11

SPINAL CORD CODING OF PAIN: SPATIAL CODING REVEALED BY QUANTITATIVE 2-DEOXYGLUCOSE IMAGING. R.C. Coghill[1], D.D. Price[2], R.L. Hayes[3], and D.J. Mayer[1]. Dept. [1]Physiology, [2]Anesthesiology, and [3]Neurosurgery, Virginia Commonwealth University, Richmond VA 23298.

Direct evidence of spatial recruitment as a mechanism of coding nociception in the spinal cord requires simultaneous observation of whole populations of neurons which is not easily attained with electrophysiological techniques. We have analyzed spatial recruitment during nociceptive stimulation utilizing the quantitative ^{14}C-2-deoxyglucose (2-DG) technique of Sokoloff. Experiments were conducted in unanesthetized, T-2 spinalized rats paralysed with d-tubocurarine. Stimulation was delivered by cyclical immersion (5 sec in, 5 sec out) of the left hindpaw in a water bath. Groups of animals were stimulated with noxious (45°, 47°, 48°, 49°C) or non-noxious (35°C) water temperatures. At the beginning of the 45 minute period of stimulation 2-DG (50 μCi) was delivered through the jugular catheter, and sequential blood samples were drawn to monitor plasma glucose and plasma 2-DG levels during the 45 minute period of stimulation. Blood gasses, blood pressure, and core temperature were monitored to insure physiological normality. Spinal cords were removed, frozen, and sectioned at -20° C. Optic densities of autoradiographs of spinal cord sections were analyzed and converted to absolute rates of glucose utilization. Nociceptive stimulation produced somatotopically specific increases in glucose utilization; the ipsilateral dorsal horn was more active than the contralateral dorsal horn, and medial portions of the dorsal laminae (regions which represent the distal portions of the limb) were more active than lateral regions. Metabolic activity was at maximum levels at the fourth lumbar segment but, importantly, expanded rostrally and caudally in a graded manner as noxious temperature intensity increased. This spatial recruitment was more extensive for deeper (V-VII) as compared to superficial (I-IV) laminae. These findings indicate that substantial spatial recruitment occurs during painful stimulation and that this recruitment may serve as an important factor of nociceptive information encoding. Supported by PHS grant NS 24009-1A2.

187.12

EFFECT OF ERGOT ALKALOIDS ON CERVICAL SPINAL CORD NEURONS WITH CRANIOVASCULAR INPUT. P.Boers ,A.Lowy , G.A.Lambert ,H.Angus-Leppan and A.S.Zagami (SPON: J. Morley), Department of Medicine, University of New South Wales, Australia.

Previous work in our laboratory has demonstrated the presence of a region in the cervical spinal cord which responds to stimulation of craniovascular structures; this has been termed the dorsolateral area (dla). It has also been shown that intravenous injection of the antimigraine drug ergotamine can suppress or abolish both evoked potentials and single-unit responses, implying a central action of the ergot alkaloids. In order to test this hypothesis, single unit recordings were made in the dla of chloralose/urethane-anesthetised cats, and ergometrine was applied iontophoretically to these units. A length of the dura and falx containing the sagittal sinus (SS) was isolated and stimulated electrically with supramaximal shocks (40-120V, 250μsec, 0.2 s^{-1}). A tungsten-centred multibarreled glass electrode was used to record single-unit activity and to eject drugs. Units in the dla responded to electrical stimulation of the SS with A-delta and C-fiber latencies. About 1/3 of these responsive units had their response rate reversibly reduced to 75% or less of the control value by ergometrine currents of 50 nA. Another 1/3 showed a reduced response rate, with no recovery, and about 1/3 of units were unaltered in response rate. Most of the reversibly-inhibited units were also tested with iontophoretic application of D,L-homocysteic acid, and about 2/3 of these showed an acceleration in spontaneous firing rate. Iontophoretic ejection of equivalent currents from a NaCl-containing barrel were without effect on response rate. These results suggest that the previously-reported ability of ergot alkaloids to suppress craniovascular input may occur at a spinal cord level, a finding that has implications for migraine pathogenesis and therapy.

187.13

THE CERVICAL SPINAL CORD RELAYS CRANIOVASCULAR INPUT TO THE THALAMUS. H.Angus-Leppan*, P.Boers*, A.S.Zagami and G.A.Lambert* (SPON: S.Ghosh). Dept. of Medicine, Univ. of New South Wales, Australia.

Sensory information from the cranial vasculature is carried in the trigeminal nerve and is important in the transmission of headache pain. In general, the fibers of the this nerve terminate in the trigeminal nucleus, but it has been reported that elements in the cervical cord also receive such input. The thalamus, particularly the ventroposteromedial (vpm) nucleus, also contains neurons which receive craniovascular input, but it is unclear if this input is relayed in the cervical cord or the trigeminal nucleus. The experiments reported here have tested one of these possibilities. Cats anesthetized with chloralose/urethane were used. A length of the dura and flax containing the sagittal sinus (SS) was isolated and stimulated electrically with supramaximal shocks (40-120V, 250μs, 0.2sec^{-1}). Single unit recordings were made with glass-coated tungsten microelectodes in the vpm nucleus of the thalamus and the cervical cord. Cryoprobes cooled with circulating alcohol to -15° were applied to portions of the spinal cord to block neural conduction reversibly. Stimulation of the SS activated units in the dorsolateral area (dla) and the medioventral area (mva) of the cervical spinal cord and in the vpm nucleus of the thalamus, with A-delta and C-fiber latencies. Bilateral cooling of the surface of the cervical cord at the C2 level reversibly blocked the activation of a majority of vpm units. Block was slow to develop and slow to reverse. Selective cooling of the dla area reversibly blocked the response of neural elements in the mva. Units in both the mva and dla could be activated antidromically from the medial lemniscus. The results lend support to the idea that the cervical cord is a major relay centre for the perception of craniovascular pain and that both the dla and mva are intimately and sequentially linked in this relay process.

PAIN PATHWAYS: PRIMARY AFFERENTS

188.1

LOCAL APPLICATION OF CAPSAICIN OR NE-21610 DEPRESSES BOTH AFFERENT AND EFFERENT FUNCTIONS OF CUTANEOUS C-FIBRES IN THE RAT. B.Lynn* and W.Ye* (SPON: K.W.T.Caddy). Dept. of Physiol., University College, London WC1E 6BT.

Capsaicin (CAPS), the naturally occurring capsaicinoid in peppers, causes long-lasting local analgesia and loss of neurogenic inflammation when applied to nerves in the rat. We have examined the mechanism of these changes for CAPS and for the synthetic capsaicinoid NE-21610. Exposing the saphenous nerve of the rat to 0.1-1 mM CAPS or 2 mM NE-21610 caused a substantial immediate reduction in C-fibre, but not A-fibre, conduction. After exposure to 4 mM NE-21610 or 33 mM CAPS many C-fibres were abnormally small and were often not enclosed in their own Schwann cell mesaxon 1-2 weeks later. Electrophysiological examination of similarly treated nerves showed a long-lasting loss of around 75% of C-fibres of the polymodal nociceptor (PMN) type, even after 3 months. No other types, C or A fibre, were affected. Intradermal injection of capsaicinoids blocked afferent (heat and pressure) responses of C-PMNs (ED_{50}, NE-21610, 7-20 M; CAPS, <33 M) and antidromic vasodilatation (ADV) (ED_{50}, NE-21610, 10 M;CAPS, 2-7 M). ADV and substance P levels were substantially reduced for at least 2 days following 0.3 mM intradermal CAPS. It is concluded that capsaicinoids exert their analgesic and anti-inflammatory actions in adult rats by inactivating C-fibres of the PMN type.

188.2

PHARMACOLOGIC PROFILE OF NE-21610, A NEW ANALGESIC AGENT. E.F. Berman*, R.L. Bohne* and T. McPherson* (SPON: J.M. Meyer). Miami Valley Laboratories, The Procter & Gamble Co., Cincinnati, OH 45239-8707

NE-21610 (N-((4-(2-aminoethoxy)-3-methoxyphenyl)-methyl)-9z-octadecinamide) is an analog of capsaicin that has antinociceptive and anti-inflammatory activity after oral or parenteral administration. It does not appear to interact with opiate receptors or inhibit cyclooxygenase, but rather acts directly on peripheral sensory nerves. NE-21610 was evaluated in the mouse tail-flick, rat hot plate, mouse phenylquinone abdominal constriction, mouse formalin and rat Randall-Selitto paw pressure assays. The potency of NE-21610 in the phenylquinone and tail-flick assays after s.c. injection is similar to that of morphine. The potency of NE-21610 in the phenylquinone assay after oral dosing is better than that of codeine or aspirin. NE-21610 blocks both the early and late phase licking responses in the formalin assay, supporting our previous data indicating a direct action on C-fibers. Our results suggest that NE-21610 should be an efficacious drug for the treatment of moderate to severe pain and of inflammatory diseases.

188.3

EFFECTS OF NE-21610, A NOVEL CAPSAICIN ANALOGUE, ON THERMAL NOCICEPTIVE THRESHOLD IN THE RAT. R. B. Carter, Research and Development Dept., Miami Valley Laboratories, The Procter & Gamble Co., Cincinnati, OH 45239.

Capsaicin, the pungent principle of hot peppers, is the prototype for a new class of antinociceptive agents. The effects of one such analogue, NE-21610 [N-([4-(2-aminoethoxy)-3-methoxyphenyl]-methyl)-9Z-octadecenamide], were studied on the thermal nocirespons iveness of rats using latency to tail withdrawal from 45°-50.5°C water. Whereas NE-21610 (10-100 mg/kg, p.o.) produced dose-dependent increases in response latency at 47.5°C, it was inactive at 50°C. The thermal nociceptive threshold was determined by measuring latency to tail withdrawal as a function of water temperature. Latencies of control-treated rats were biphasic, decreasing rapidly with increasing temperature over a range of 45-47.5°C and more slowly from 47.5°-50°C. These data yielded a thermal nociceptive threshold in normal rats of 46.4°C. NE-21610 (100 mg/kg, p.o., 60 min post-dose) produced a rightward shift in the temperature-response function, indicating an increase in thermal threshold; latencies were likewise biphasic. The thermal nociceptive threshold in NE-21610-treated rats was calculated to be 48.3°C. By comparison, administration of the opioid agonist-antagonist pentazocine resulted in a threshold of 49.3°C. These data indicate that NE-21610 possesses antinociceptive efficacy approximately equivalent to that of an opioid mixed agonist-antagonist.

188.4

ANTINOCICEPTIVE ACTIVITY OF INTRATHECAL (I.T.) NEUROKININ (NK) AND N-METHYL-D-ASPARTATE (NMDA) ANTAGONISTS IN A MOUSE TONIC PAIN MODEL. C.W. Murray*, A. Cowan† and A.A. Larson. Dept. of Vet. Biol., Univ. of Minnesota, St. Paul, MN 55108 and †Dept. of Pharmacol., Temple Univ. Sch. of Med., Philadelphia, PA 19140.

While many lines of evidence support a role for the endogenous NK peptide substance P (SP, NK-1 receptor agonist) as a primary sensory transmitter of acute pain in mammalian spinal cord, its role in tonic pain is unclear. Excitatory amino acid (EAA) involvement in nociceptive processing is also uncertain. To test the hypothesis that NKs and EAAs contribute to signaling of *continuous (tonic) chemogenic* pain, we evaluated two NK antagonists ([D-Pro2,D-Trp7,9]-SP (DPDT-SP, 0.4–10 μg, non-specific) and [D-Pro4,D-Trp7,9,10,Phe11]-SP(4-11) (DPDTP-octa, 2–15 μg, somewhat NK-1 selective)), as well as dl-2-amino-5 phosphonovalerate (dl-AP5, an NMDA antagonist) and urethane (a kainic acid (KA) antagonist at the i.t. dose tested) for possible antinociceptive activity in the mouse formalin model. Neither NK nor EAA antagonists have previously been tested against tonic pain nor have they consistently reduced acute pain perception. DPDTP-octa is known to block SP-induced *behaviors* (reciprocal caudally directed biting and scratching (B&S)) but is *not* antinociceptive in *acute* models. In the current study, analgesia was defined as significantly reduced time spent licking a hind paw injected s.c. with 20 μl of 10% formalin compared to vehicle controls during a 25 min observation period under blind conditions (n=5–12/group; J. Pharmacol. Meth. 20:175). Our results indicate that both NK antagonists tested as well as 1 nmole of dl-AP5 were significantly antinociceptive. A 2.5 μmole dose of urethane (which inhibits B&S produced by 0.075 nmoles KA, i.t.) was inactive in this paradigm. A_{50} values for mean % analgesia, μg/mouse i.t. (95% CI) were: DPDT-SP, 1.7 (1.2-2.4); DPDTP-octa, 4.8 (2.9-7.5). Only minimal behavioral effects were produced by the NK antagonists. Neither agent alone produced NK-1 agonist activity (B&S) or motor impairment. dl-AP5 induced subtle postural and behavioral changes at 1 nmole. These data support the contention that NK, especially NK-1, and NMDA (but not kainate) receptors are involved in spinal processing of *tonic* chemical/inflammatory nociception in mice. The present results also suggest that inhibition of B&S is not predictive of antinociception in tonic pain models. [Grants DA04090, DA04190 and DA00124 to AAL and DA03945 to AC.]

188.5

CORTICOTROPIN RELEASING FACTOR (CRF) PRODUCES ANALGESIC AND ANTI-INFLAMMATORY EFFECTS BY MULTIPLE MECHANISMS OF ACTION. Kenneth Hargreaves*, Ann Costello* and Ronald Dubner. (Spon: E. Chudler). Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20892.

We have previously reported that CRF produces analgesia in post-operative patients and in rats (Brain Res. 422:154;1987). The present studies evaluated the mechanism(s) of this effect in the carrageenan (Carra) model of inflammation. Carra (2mg) was injected sc into hindpaws; test drugs were then injected sc into the neck and animals (8/group) were evaluated 3 hours later. Thermal hyperalgesia was determined by exposing hindpaws to a beam of radiant heat as previously described (Pain 32:77;1988). Edema was measured by a plethysmometer and hindpaw temperature was assessed by a thermocouple. Data were analyzed by ANOVA and Duncan's test. In a dose-response study, CRF was approximately 1,000 times more potent than indomethacin for blockade of hyperalgesia, edema and hyperthermia. As compared to hypophysectomized rats injected with vehicle, CRF (20 nmole/kg) in hypophysectomized rats blocked hyperalgesia (9.22± 1.8 sec vs 4.7± 0.8 sec: $p<0.01$), hyperthermia (28.5±.2°C vs 30.0±.3°C; $p<0.01$) and edema (1.87±.06 cc vs 2.50±.11 cc; $p<0.01$). In adrenalectomized rats, CRF retained its blockade of hyperalgesia (5.8±1.1 sec vs 3.2±0.5 sec; $p<0.05$) but its effects on edema and hyperthermia were absent. The anti-inflammatory, but not the analgesic, effects of CRF were also blocked in rats pre-treated with the glucocorticoid antagonist RU486 (20mg/kg). The analgesic effects of CRF, at 30 min after injection, but not at 180 min, were blocked by naltrexone (3 mg/kg; injected at 0 & 150 min). We conclude that CRF analgesia in the carra model of inflammation is opioid-dependent within the first 30 min, and is opioid-, pituitary-, and adrenal-independent at 180 min. In addition, the anti-inflammatory effects of CRF are due to the actions of adrenal glucocorticoids.

188.6

ANTAGONISM OF PGE_2-INDUCED HYPERTHERMIA IN RATS BY ANTINOCICEPTIVE PROSTAGLANDIN ANTAGONISTS. M.F. Rafferty[1], E.J. Drower[1], and D.L. Hammond[2]. [1]G.D. Searle & Co.,Skokie, IL 60077, and [2]Dept. of Anesthesia and Critical Care, U. of Chicago, Chicago, IL 60637.

We recently reported the antinociceptive activities in rat of some novel dibenzoxazepine derivatives, SC-19220 and SC-25469, which are selective antagonists of PGE in vitro (Drower et al., Eur. J. Pharm. (1987)133:249). In an attempt to determine whether PGE antagonism in vivo could be relevant to the behavioral actions of these compounds, we have assessed the ability of antinociceptive doses of both compounds to antagonize the hyperthermic effect of i.v. administered PGE_2 methyl ester. Since the hyperthermia is a function of the amount of PGE_2 which enters the CNS, information about a possible central site of action of these drugs could also be obtained. The general methodology was as described by Eguchi et al. (JPET(1988) 247:671). As previously reported, i.c.v. SC-19220 pretreatment (0.56-2.25 umoles) dose-dependently blocked the hyperthermic response. Likewise, i.c.v. SC-25469 was found to completely block the effect at a dose of 0.56 and 1.15 umoles. The apparent rank order of potency correlates with pA_2 values for antagonism of PGE_2-induced contractions in guinea pig ileum. Oral pretreatment with an analgesic dose of either compound (150 mg/kg, formalin test) blocked the hyperthermic effect. These results indicate that the antinociceptive effects of these compounds may in part be centrally mediated.

188.7

PROSTAGLANDINS INCREASE THE RELEASE OF SUBSTANCE P FROM CULTURED SENSORY NEURONS. M.R. Vasko, J. Crider and W. Campbell, Depts. Pharmacol. & Tox., Indiana U. Sch. Med., Indpls, IN 46223; U.T. Southwest Med Ctr, Dallas, TX 75235.

Evidence suggests that eicosanoids facilitate activation of sensory neurons and nociception. Since substance P (SP) is an important neuroactive substance in nociceptive sensory neurons, we studied whether eicosanoids could stimulate the release of SP from cultured sensory neurons.

Dorsal root ganglia from 11 day chick embryos were dissected, cells dissociated, then plated onto collagen coated culture dishes. Cells were grown in a modified Eagle's Medium supplemented with NGF for 5-7 days. Release studies were performed by incubating cells for repeated 5 min periods with Krebs-bicarbonate buffer containing either 3.5mM KCl, 50mM KCl or various concentrations of eicosanoids. After exposure to cells, the buffer was removed and assayed for SP content using RIA. Exposure of cells to 50mM KCl resulted in a Ca^{++}-dependent increase in SP release from a mean ± S.E.M. of 37.4 ± 5.9 pg/dish/5 min to 128.1 ± 15.9 pg/dish/5 min. Both PGE_2 and PGD_2 at 10^{-6}M caused an increase in SP release similar in magnitude to that observed for 50mM KCl. In contrast, 10^{-6}M LTB_4, a lipoxygenase metabolite of arachidonic acid, did not stimulate SP release. These results suggest that certain eicosanoids can increase SP release from sensory neurons; this may be a mechanism underlying the enhanced nociception by these substances. (Supported by NS 21697)

188.8

8(R),15(S)-diHETE SENSITIZES C-FIBRE NOCICEPTORS IN THE HAIRY SKIN OF THE RAT. D.M. White*, A.I. Basbaum, E.J. Goetzl* and J.D. Levine*. (SPON: A.M. Williamson) Depts. Anatomy, Medicine, Oral and Maxillofacial Surgery and Division of Neurobiology, UCSF, CA, 94143.

8(R),15(S)-diHETE, a product of the 15-lipoxygenase arachidonic acid pathway, is a potent hyperalgesic compound in behavioural studies. In this electrophysiological study we examined the effects of 8(R),15(S)-diHETE on the mechanical threshold and thermal responses of C-fibre mechanoheat nociceptors (C-MH) isolated from the saphenous nerve of pentobarbital anaesthetized rats. Mechanical thresholds were evaluated with calibrated von Frey hairs. Thermal responses were induced with a contact thermal stimulator. Intradermal injection of 8(R),15(S)-diHETE significantly decreased the mechanical threshold of C-MH's (n=25) from 7.4±0.4g to 4.6±0.6g. This decrease in threshold was completely antagonized by a stereoisomer, 8(S),15(S)-diHETE. The stereoisomer did not antagonize PGE_2-induced sensitization. To test whether 8(R),15(S)-diHETE sensitizes thermal responses we used a test stimulus of 37°C for 5min. There was a significant increase in the total number of spikes in the 5min period after 8(R),15(S)-diHETE, but not after the saline vehicle. 8(R),15(S)-diHETE may contribute to the hyperalgesia associated with inflammation by sensitizing C-fibre nociceptors via a specific binding site that is separate from the PGE_2 binding site.

188.9

CAPSAICIN ANALOGS DIFFERENTIALLY ACTIVATE AFFERENT C-FIBERS T. R. LaHann[1], G. Daniell[1] and P. Stark[2]*. Univ of Montana, Missoula, Mt[1], and the Univ of Minnesota, Minneapolis, Mn[2].

Topical or intracutaneous injection of capsaicin stimulates cutaneous c-fibers, eliciting pain and a neurogenic inflammation. IV injection of capsaicin into rats stimulates cardiopulmonary c-fibers and elicits the Bezold-Jarisch reflex (apnea, bradycardia and hypotension). IV injection of equimolar doses of the capsaicin derivative, vanillyloleamide (VO), failed to activate the Bezold-Jarisch reflex but topical and intracutanous administration of VO did elicit marked plasma extravasation, as quantitated by dye leakage from the vascular system into the skin. The comparison of the cardio-pulmonary and cutaneous irritant actions of capsaicin and its analog (VO) suggests that the c-fiber receptors mediating neurogenic inflammation differ from those receptors eliciting the Bezold-Jarisch reflex. Capsaicin analogs may thus be useful probes for defining the function and neurochemistry of afferent receptor subtypes. In addition, the selectivity of function demonstrated by VO suggests that modification of capsaicin's chemical structure may improve the overall therapeutic index.

188.10

CAPSAICIN-INDUCED ANTINOCICEPTION : CENTRAL AND PERIPHERAL SITES OF ACTION. A.Dray, J.Bettaney*, A.H. Dickenson* and N.Ashwood*. Sandoz Institute for Medical Research, London and Department of Pharmacology, University College, London, England.

An acute dose of capsaicin (ED50= 20 µmols / kg,s.c.) produces behavioral antinociception which peaks some 30min. after administration and lasts 1-3 hours.The plasma concentration at the peak effect is 0.8-2µM. We have examined whether spinal and peripheral sites contribute to this effect of capsaicin.

Polymodal spinal dorsal horn neurons were recorded in adult rats anesthetized with 1% halothane. The C-fibre input, evoked by stimulation of a hind toe, was selectively reduced a) following capsaicin injected into the peripheral receptive field b) by capsaicin injected at a distant site. This occured with a time course similar to that observed in antinociceptive tests c) by 100nM capsaicin administered intrathecally. A nociceptive response was also measured *in vitro*, from a ventral root of the neonatal rat spinal cord, following noxious heat stimulation of the attached tail. Prolonged capsaicin superfusion, to mimic drug conditions *in vivo*, consistently attenuated the nociceptive reflex when administered to the cord (0.2-1.0µM). When administered to the tail, it was only effective at higher concentrations (20-50µM) and was less consistent.

These data suggest that an antinociceptive dose of capsaicin selectively attenuates C-fibre input to the spinal cord and that a spinal action is important for this effect.

188.11

TWO MECHANICAL HYPERALGESIAS IN HUMAN NEUROPATHY. J.Ochoa,W.J.Roberts,M.A.Cline,R.Dotson & D.Yarnitsky*. Good Samaritan Med.Ctr., OHSU, Portland, OR 97210 USA.

Abnormal reduction of threshold for pain in response to non-noxious mechanical or thermal stimuli to human skin may be due to sensitization of unmyelinated nociceptor terminals (Cline,et al,Brain,89). But, in neuropathy, mechanical hyperalgesia has often been better explainable through secondary hyperexcitability of central neurons responsive to large caliber myelinated tactile input.

This study of 22 patients with chronic mono- or polyneuropathic mechanical hyperalgesia separates two subgroups with ostensibly different pathophysiology. Type D hyperalgesia disappears early during selective large caliber A-fiber block,in keeping with the commonly accepted basis. Nerve biopsy in 3 selected patients has revealed that type D may exist despite selective absence of small caliber fibers. Type S hyperalgesia persists after selective A-fiber block, while only C-fibers conduct. Nerve fiber pathology in one patient has revealed that type S may occur even in absence of myelinated fibers. D & S hyperalgesias may coexist in the same patient.

A clinical sign usually distinguishes the two hyperalgesias:type D is dynamic;type S is static. In type D, touch-induced pain is triggered only at on-off and may improve by sustained mechanical pressure. In type S, pain is maximally evoked by sustained pressure.

Unilateral interpretation of pathophysiological mechanisms behind mechanical hyperalgesias,a plural phenomenon, is bound to confuse the literature.

188.12

MK-801 BLOCKS THERMAL HYPERALGESIA IN A RAT MODEL OF NEUROPATHIC PAIN. G. Davar*, R. Maciewicz. Pain Physiology Laboratory, Mass General Hospital, Boston, MA 02114

The placement of loose constrictive ligatures around the sciatic nerve in the rat produces thermal hyperalgesia in the ipsilateral paw which begins at 2-3d and peaks at 7-10d. This hyperalgesic response has been proposed as an animal model of neuropathic pain (see Bennett GJ et al, Pain, 33(1988) 87-107), and is associated with changes in structure and function in spinal dorsal horn. These changes could result from neurotoxic damage to dorsal horn neurons produced by excitatory amino-acids (EAAs) released at the primary afferent synapse.

MK-801, a specific glutamate (NMDA) receptor antagonist has protective effects in rodent models of ischemic brain injury, and spinal cord damage. Using the loose ligature model of neuropathic pain, we have administered MK-801 immediately pre-operatively (1.0mg/kg i.p.) and then Q12h (0.5mg/kg) for 9d. Equal numbers of lesioned rats were given normal saline over the same time course. The latency to withdrawal from a thermal stimulus was examined in the ipsilateral and contralateral hindpaw of each animal. A difference score (DS) was calculated by subtracting the averaged latency values for the contralateral paw from the averaged latency values in the ipsilateral paw. Mean positive DSs were obtained in the MK-801 treated animals, while mean negative scores were obtained in the saline controls. A significant absolute difference in the mean DS was observed between the two groups. These data are evidence for a potential protective effect of MK-801 on the development of thermal hyperalgesia following sciatic nerve ligation in the rat. This effect of MK-801 in animals suggests a role for glutamate antagonists in the treatment of human conditions of neuropathic pain.

188.13

ANALGESIC EFFECTS OF ANTIBIOTICS ON AN ACUTE (HOT PLATE) AND A CHRONIC (DEAFFERENTATION) ANIMAL MODEL OF PAIN. C. Suaudeau*, R. de Beaurepaire and C. Cimetière* (Spon. E. MacKenzie). Laboratoire de Pharmacol., C.H.U., 14032, Caen, France.

Antibiotics are anti-infective agents not known to have analgesic properties. During previous experiments using the deafferentation animal model of chronic pain we incidentally observed that an antibiotic, chloramphenicol, was able to prevent the self-mutilation behavior observed in this model. We therefore extended the study to other antibiotics and also to an other model of experimental pain, the hot plate test.

In the first experiment (deafferentation) unilateral section of dorsal roots C5 to T1 were performed in rats and changes in the scores of automutilation were recorded after daily subcutaneous injections of four antibiotics (300mg/kg): chloramphenicol, thiophenicol, amoxicillin and doxycycline. Eight animals and eight controls (injected with distilled water) were used for each experimental group. The results show that chloramphenicol and amoxicillin have a significant preventive effect on the self-mutilation behavior, and that thiophenicol and doxycyclin have no significant effect (Student t-test for unmatched pairs). Therefore at least two antibiotics have an effect on this model of phantom limb pain.

In the second experiment 9 antibiotics randomly selected were tested on the constant hot plate test. Testing occured 45 minutes after subcutaneous injection. Eight treated and eight controls were tested in each group. Chloramphenicol and ampicillin have a significant analgesic effect at 100mg/kg (therapeutic dose routinely used in humans) and not at 30mg/kg. Amikacin, amoxicillin, cefaprin, kanamycin and oxacillin have a significant effect with higher doses (300 and/or 600mg/kg). Doxycycline and thiamphenicol have no significant effect at any dose. Therefore, in our experimental conditions, 7 out of 9 antibiotics produce analgesia in rats.

This is to our knowledge the first report of analgesic properties for antibiotics.

188.14

AGE-DEPENDENT CHANGES IN DENSITY AND ORGANIZATION OF CORNEAL FREE NERVE ENDINGS. *R. Beuerman and H. Thompson*. LSU Eye Center, New Orleans, LA 70112*

The thresholds for perception of noxious and non-noxious stimuli in elderly subjects are increased, possibly as a result of neural or perceptual changes that may be caused by peripheral or central alterations in sensory pathways. We used gold impregnation, TEM, and LM to examine quantitatively the corneal innervation in young (2.2-3.2 mos) and old (27.9-30.5 mos) Fischer rats from the NIA. In young rats, the density of the subepithelial plexus was relatively constant from limbus to center (4.1±0.2;±SEM), while in the aged rat, degenerative changes were seen. Aged rats had more axon leashes in the basal epithelial layer (38.6±2.87) than young rats (14.5 ±0-.89;p<0.01). Higher level axon terminal endings were more numerous in young rats (9.5±0.59 vs 5.1±0.30;p<0.01). The terminal endings in the aged rat had greater numbers of neurofilaments. The aged rat stroma showed numerous transformed spindle-shaped keratocytes with extensive rough endoplasmic reticulum. Degenerative changes of the limbal nerves were not prominent, but vacuolation of axonal profiles and occasional myelin figures were seen within the subepithelial plexus. Schwann cell basal lamina was thickened in the plexus and in the limbal nerves. Aging changes of the peripheral nerves may be responsible for the observed psychophysical changes, but additional work must be done to define the central connections. (EY04074)

PRESYNAPTIC MECHANISMS: CALCIUM

189.1

INTERACTION OF ADENOSINE AND CALCIUM ON NEUROMUSCULAR TRANSMISSION. D.F.Wilson* and P.Zhong* (SPON: R.G.Sherman) Zoology Department, Miami Univ., Oxford, OH 45056.

The interactions of adenosine and calcium on spontaneous transmitter release was examined in an attempt to identify the mechanism of action of adenosine on neuromuscular transmission. Intracellular recording techniques were used to monitor miniature end-plate potentials (MEPPs) in the isolated rat diaphragm-phrenic nerve preparation. The preparation was bathed in saline containing 10 mM K^+ to enhance calcium influx and transmitter release. In order to maximize the level of inhibition by adenosine a concentration of 2 mM adenosine was used. The calcium dependence of this inhibition was tested by comparing control vs adenosine preparations in the presence of 0.4, 0.6, 1.0, 2.0, 2.4 , or 4.4 mM calcium chloride. Frequency output increased with calcium increment in the presence or absence of adenosine. In the presence of adenosine the frequency of MEPPs was significantly lower than controls. A double reciprocal plot of calcium concentration vs MEPP frequency demonstrates that adenosine increases the slope of the double reciprocal curve without changing the maximum frequency. These results support the hypothesis that adenosine is a competitive inhibitor of calcium influx. (Supported by NIH grant NS-23195).

189.2

SPIKE BROADENING IN MAMMALIAN NEUROHYPOPHYSIAL NERVE TERMINALS. C.W. Bourque. Center for Research in Neuroscience. Montreal General Hospital and McGill University, Montreal, Quebec. Canada. H3G 1A4.

Increased firing rate and bursting activity strongly potentiate the release of peptides from the axon terminals of mammalian magnocellular neurosecretory cells (MNCs). To examine the mechanism underlying this effect, intraterminal recordings were obtained from the isolated rat neurohypophysis in vitro. Impaled axon terminals were identified by their constant-latency response to neural stalk (NS) stimulation, by collision tests, and by direct injection of Lucifer Yellow. Following NS stimuli or current pulses, the duration of action potentials recorded from each of 54 axon terminals was found to increase with firing frequency, with a maximum near 25 Hz. Removal of Ca^{2+} from the extracellular medium eliminated a shoulder on the repolarizing phase of the spike and abolished its frequency-dependent prolongation. A modulation of Ca^{2+} influx caused by changes in spike width may therefore underlie the effects of discharge rate and bursting activity on peptidergic secretion from MNCs. Supported by F.C.A.R., F.R.S.Q. and the M.R.C. of Canada.

189.3

HIGH FREQUENCY CONTINUOUS STIMULATION IS MORE EFFECTIVE THAN BURSTS FOR EVOKING LHRH RELEASE IN BULLFROG SYMPATHETIC GANGLIA. Y. Peng and J. P. Horn Department of Physiology, University of Pittsburgh, School of Medicine, Pittsburgh, PA 15261.

The preganglionic C input to paravertebral ganglia 9 & 10 co-releases acetylcholine and leuteinizing hormone releasing hormone (LHRH) in response to repetitive stimulation. LHRH produces a slow epsp in B and C postganglionic neurons. These experiements assess whether bursts of stimuli are more effective for evoking peptide release than are continuous stimuli, and whether release is intrinsically dependent upon the stimulus pattern.

LHRH release evoked by stimulation of the preganglionic C pathway was monitored indirectly by measuring the magnitudes of postsynaptic currents (epscs) recorded from ganglionic B and C cells with a single-electrode voltage clamp. Control experiments with teleost and chicken LHRH applied from a puffer pipette showed that the peak amplitude and integral of the postsynaptic current are both saturable sigmoidal functions of puffer pulse duration.

In normal Ca [1.8 mM], the magnitude of the slow epsc increases as the number and frequency of stimuli is increased. The minimal parameters for eliciting a detectable response were 50 stimuli at 0.5 Hz. Continuous stimulation (100 or 200 shocks) at 2Hz always elicits a response. The magnitude of the response saturates near 20 Hz. Bursts of high frequency (e.g. 5, 10, 20 Hz) stimuli elicited responses that were: A) bigger than those produced by continous low frequency stimulation (same mean frequency in each comparison) and B) smaller than those elicited by continuous stimulation at the high frequency used in bursting. Thus high frequency continuous stimulation is more effective than bursting. This implies that inactivation of presynaptic Ca channels does not limit LHRH release during sustained high frequency stimulation at 20 Hz. In 5.4 mM Ca, all frequency-response curves were shifted to the left and the optimal frequency was altered to 5 Hz. The ratio of responses to 20 and 2 Hz was lessened. These results suggest that peptide release does not depend intrinsically upon stimulus frequency but simply on the availability of calcium. Supported by a AHA fellowship(YP) and NIH grant NS21065 (JPH).

189.4

NIFEDIPINE, A L-TYPE CA++ CHANNEL BLOCKER, ENHANCES ACH RELEASE FROM POPULATIONS OF TERMINALS ALSO EXPRESSING SOMATOSTATIN-LIKE IMMUNOREACTIVITY. D.B. Gray, N. Manthay*, and D. Zelazny*. Dept. Physiology & Neurobiology, Univ. of CT, Storrs, CT 06269.

We demonstrated previously that K+-evoked ^{3}H-ACh release from ciliary ganglion nerve terminals on choroid smooth muscle of chick eye is inhibited by the DHP Ca++ channel agonist Bay K 8644 (Gray & Pilar, Soc. Neurosci. Abstr. 14: 646 (1988)). This inhibition may be mediated by release of endogenous somatostatin (SS) since incubation with a SS antagonist, Cyclo (7-aminoheptanoyl-phe-D-trp-lys)BZL (CyCam), reverses the effect of Bay K 8644. However, incubation of choroids with the DHp antagonist nifedipine did not result in either enhancement or inhibition of K+-evoked ACh release in over 3 min, implying that SS was not released in 55mM K+ incubation (without Bay K 8644).

We now report that with increased time resolution in the assay, nifedipine doubles evoked ACh release during the first minute in 55mM K+, followed by a slight depression of release in the next 3 min. Although the nature of this biphasic response is not yet fully understood, the fact that CyCam also enhances release (>25%) specifically in the first minute of high-K+ implies that endogenous SS may be released during this time. Furthermore, Bay K 8644-induced inhibition can be reversed by 10mM nifedipine, providing further evidence that SS release in high-K+ may be mediated by L-type Ca++ channels.

Supported by NIH grants #NS 10338 and #NS 07324.

189.5

DEPOLARIZATION ACTIVATED Ca^{++}-CURRENTS AND QUANTAL RELEASE FROM CHICK CILIARY GANGLION NEURONS. D.C. Brosius*, J.T. Hackett and J.B. Tuttle. Depts of Physiol. and Neurosci., Univ of Virginia School of Medicine, Charlottesvl., Va 22908

To record synaptic Ca^{++}-currents, muscle was grown on 1 mm spots of collagen and ciliary ganglion neurons added after myotube formation. Neurons were whole-cell patch clamped with the pipet containing (in mM) 120 CsCl, 20 TEA, 1 $CaCl_2$, 11 EGTA- CsOH, 1 $MgCl_2$, pH 7.4. Standard saline had 5 mM $CaCl_2$, 2.5 mM KCl, 10 mM Glucose and 2 µM TTX. Postsynaptic quanta were recorded using whole cell patch recording from the myotubes. In simultaneous recording from nerve and muscle pairs, four results were obtained: 1) Spontaneous quantal release of < 0.1 sec^{-1}; 2) cells depolarization to between -40 and -60 mV activated maintained quantal transmission; 3) minimum synaptic delay was 1.7 msec when evoked by calcium tail currents, but the delay was highly variable with low voltage command steps; 4) Ca-stimulated release in high K^+ was not blocked by 25 µM Nitrendipine. We conclude that these synaptic Ca^{++} currents may reflect the activity of channels different from the T, N, and L types. Supported by NSF BNS 87-08162 and the Am. Heart Assoc. (Va Affiliate).

189.6

EFFECT OF DIVALENT CATIONS ON CALCIUM-DEPENDENT POTENTIALS ORIGINATING IN LIZARD MOTOR NERVE TERMINALS. K. Morita and E.F. Barrett. Dept. of Physiology and Biophysics, University of Miami School of Medicine, Miami, FL 33101.

In the presence of 10 mM tetraethylammonium (TEA) and 2 mM Ca the action potential recorded intra-axonally near lizard motor terminals is followed by a series of Ca-dependent afterpotentials (depolarizations D1 and D2, followed by a prolonged hyperpolarization (h.a.p)). These afterpotentials could also be evoked when strontium was substituted for bath Ca, but higher [Sr] were needed to achieve afterpotentials comparable to those seen in Ca. Barium (1-2 mM), in the presence or absence of 2 mM Ca, increased the axonal input resistance, increased and prolonged the depolarizing afterpotentials, and reduced the slow h.a.p. In 2 mM Ca, the depolarizing afterpotentials and the slow h.a.p. were completely suppressed by 10 µM cadmium, 100 µM cobalt, 300 µM manganese or nickel, or 1 mM zinc. For most of these Ca antagonists suppression of D1 began at lower concentrations than suppression of D2 or the slow h.a.p., but 30-100 uM zinc suppressed D2 and the slow h.a.p. more effectively than D1. These results suggest that zinc's actions differ from those of other divalent Ca antagonists. These antagonists blocked the end-plate potential recorded in curare (no TEA) in the order cadmium > nickel > zinc. Support: NIH NS 12404.

189.7

TRANSMITTER RELEASE EVOKED BY CALCIUM RELEASED FROM THE PHOTOLABILE CHELATOR DM-NITROPHEN IN CRAYFISH PRESYNAPTIC BOUTONS. R.S. Zucker and R.M. Mulkey. Dept. of Physiology-Anatomy, University of California, Berkeley,CA. 94720.

Approximately 10 mM DM-nitrophen loaded with 30% calcium was injected into the excitatory motor axon of the crayfish opener muscle. Nitrophen diffused to terminals within about 300 microns of the injection site. Variable exposures from a xenon light source (1 - 20 sec), photolyzed 35 to 100% of the nitrophen and gave rise to transmitter release recorded postsynaptically in the muscle fiber. Transmitter release produced a depolarization of 2 - 15 mV depending on the light duration, intensity and amount of nitrophen contained in the terminals. Transmitter release lasted from 10 - 37 sec when terminals were exposed to enough light to completely photolyze nitrophen. The peak miniature excitatory postsynaptic potential frequency ranged from 1800 - 13,000/sec. Presynaptic action potentials given during release of calcium from nitrophen gave facilitated excitatory postsynaptic potentials of 200%. In a zero calcium medium, evoked release was blocked but transmitter release still occurred when internal calcium concentration was increased during nitrophen photolysis. A rise in internal presynaptic calcium due to release from a photolabile chelator results in an increase in miniature excitatory postsynaptic potential frequency and facilitation of transmitter release to a spike. Supported by NIH Grant NS 15114.

189.8

TRANSMITTER RELEASE EVOKED BY PRESYNAPTIC CALCIUM RELEASED FROM THE PHOTOLABILE CHELATOR DM-NITROPHEN AT THE SQUID GIANT SYNAPSE. K.R. Delaney and R.S. Zucker. Dept. of Physiology-Anatomy, Univ. of Calif., Berkeley, CA 94720.

About 10 mM of DM-nitrophen loaded 30% with Ca^{2+} was injected into presynaptic terminals of squid giant synapses. A brief intense light flash photolyzed about 20% of the nitrophen and gave rise to transmitter release monitored postsynaptically as an EPSP-like response, reaching an amplitude of up to 14 mV in 2-3 ms and decaying with a time constant of about 15-20 ms to a level about 20% of the peak at 18°C. The transient response is due to both transmitter depletion and reabsorption and re-equilibration of released Ca^{2+} with unphotolyzed nitrophen, as confirmed by fura-2 measurements of free Ca^{2+} in micro-cuvettes containing nitrophen and artificial axoplasm. The postsynaptic response was reversibly blocked by 3 mM kainic acid and was not seen when nitrophen was not loaded with Ca^{2+}. When Ca^{2+} influx was blocked in a zero-Ca^{2+} medium, presynaptic action potentials timed to occur during the release of Ca^{2+} had no effect on the postsynaptic response. Transmitter release began after a 'synaptic delay' that was highly temperature dependent, increasing from 0.5 ms at 22°C. to 4.0 ms at 8.5°C. We have thus dissociated the effects of presynaptic Ca^{2+} from presynaptic potential in eliciting transmitter release, and begun to characterize the process of neurosecretion elicited directly by rapid changes in presynaptic Ca^{2+} concentration. Supported by NIH Grant NS 15114.

189.9

CHARACTERIZATION OF CALCIUM CHANNELS IN RAT HIPPOCAMPAL MOSSY FIBER SYNAPTOSOMES. D. Terrian, D. Damron*, R. Gannon and R. Dorman*., USAF School of Aerospace Med., Brooks AFB, TX. 78235.

The inhibitory effects of calcium (Ca^{2+}) antagonists on K^+-evoked dynorphin (Dyn) A (1-8) release and Ca^{2+} entry in rat hippocampal mossy fiber synaptosomes were examined to characterize the pharmacological properties of these presynaptic Ca^{2+} channels and their relative contribution to opioid release. Based on the IC_{50} values calculated for these antagonists, the order of inhibitory potency on 35 mM K^+-evoked Dyn release was: flunarizine and cinnarizine ($< 10^{-5}$ M) > omega-conotoxin and gadolinium ($< 3 \times 10^{-5}$ M) >> nimodipine, nifedipine, verapamil and diltiazem ($> 10^{-4}$ M). Bay K 8644 (10^{-6} M) significantly enhanced (33.1%) K^+-evoked Dyn release. Amiloride and phenytoin had no inhibitory effect. The inhibitory potency of these agents on depolarization-induced Ca^{2+} entry, based on fura-2 measurements, was essentially the same as shown above for Dyn release. These results indicate that presynaptic N-type Ca^{2+} channels make a major contribution to the overall inward Ca^{2+} current that initiates Dyn release and may play a prominent role in excitatory hippocampal mossy fiber synaptic transmission. Supported by AFOSR-2312W3 (DT) and AFOSR- 89-0245 (RD).

189.10

CONCENTRATION-DEPENDENCE OF THE EFFECT OF BARIUM ON ENDOGENOUS GLUTAMATE RELEASE FROM RAT BRAIN SYNAPTOSOMES. R.A. Nichols and T.S. Sihra*. The Rockefeller University, New York, NY 10021.

Barium has been previously shown to substitute for calcium in the release of neurotransmitter. There has also been found an elevated resting conductance to Ba^{2+} in most preparations, when compared to Ca^{2+}. We have found that the effect of Ba^{2+} on resting synaptosomes is dependent upon its extracellular concentration. Endogenous glutamate release from rat brain synaptosomes was monitored by a continuous fluorometric assay. At low concentrations of divalent cation ($\leq$ 0.3 mM), Ba^{2+} behaved similarly to Ca^{2+} with respect to both resting and K^+-evoked glutamate release (30 mM K^+). At higher concentrations of Ba^{2+}, resting glutamate release was increased, such that at 1 mM Ba^{2+} the resting release rate was increased over an order of magnitude above the low resting release rate observed in the presence of 1 mM Ca^{2+}. At 3 mM Ba^{2+} the rate of glutamate release approached that obtained upon addition of 30 mM KCl and 1 mM Ca^{2+}. Following several mins of release stimulated by Ba^{2+} at concentrations $\geq$ 1 mM, addition of 30 mM KCl, with or without Ca^{2+} present, to depolarize the synaptosomes had only a small effect on the glutamate release, indicating that the Ba^{2+}-evoked release and the Ca^{2+}-dependent, K^+-evoked release originated from a common glutamate pool. The glutamate release evoked upon simultaneous addition of 30 mM KCl and Ba^{2+} was similar to that observed upon addition of 30 mM KCl and Ca^{2+}. Ba^{2+} had no significant effect on ^{86}Rb efflux, indicating that Ba^{2+} did not appreciably depolarize the synaptosomes. The effect of Ba^{2+} on glutamate release was sensitive to cobalt and cadmium (10 μM each), indicating that Ba^{2+} was largely entering through calcium channels.

189.11

OMEGA CONOTOXIN GVIA (ω-CgTx) REDUCES STIMULATED RELEASE OF [^{3}H] DOPAMINE FROM MOUSE STRIATAL SLICES. A. Munoz , J. Fiedler , H.B. Pollard and G. Feuerstein. (SPON.: R.M. STEINMAN) Dept. of Neurology, USUHS and Lab. Cell Biology & Genetics, NIH. Bethesda, MD 20892.

It has been suggested that ω-conotoxin GVIA, a novel peptide toxin, interacts with a neuronal voltage sensitive presynaptic calcium channel (VSCC) that mediates neurotransmiter release. We prepared mouse striatal slices to study the role of ω-CgTx sensitive VSCC in regulating dopamine release in the central nervous system.

Striatal slices of 0.25 mm thickness were loaded with [^{3}H]-dopamine and incubated with 5 μM ω-CgTx for 30 min at 37^{0}C. The release of [^{3}H]-dopamine was determined in the presence of 1 mM Ca^{++}, and the values calculated from the ratio between stimulation for 1 min with 35 mM K^+ (S_2) and spontaneous release in the presence of normal Krebs (S_1) (n = 6). Under these conditions, we observed that ω-CgTx reduced the K^+-evoked [^{3}H]-dopamine release from mouse striatal slices by 20% compared to controls. In the absence of Ca^{++}, no release of [^{3}H]-dopamine was observed in presence and absence of ω-CgTx. These data support the hypothesis that ω-CgTx-sensitive VSCC mediates dopamine release from neural tissue in the presence of Ca^{++}.

189.12

CALCIUM MOBILIZATION IN HIPPOCAMPAL MOSSY FIBER SYNAPTOSOMES: PHOSPHOLIPASE A2 MODULATION. D.S. Damron*, D.M. Terrian and R.V. Dorman* (SPON: P. Morell). Dept. Biological Sciences, Kent State University, Kent, OH 44242.

Hippocampal mossy fiber synaptosomes (0.5 mg protein) were loaded with Fura-2/AM, prior to stimulation with high K^+ buffer (45 mM KCl) in the presence and absence of phospholipase A_2 (PLA_2) modulators. The effects of PLA_2 inhibitors dibucaine and 4-bromophenacyl bromide (BPB) on the K^+-evoked rise of intraterminal free Ca^{2+} were examined. Dibucaine had a dose-dependent inhibitory effect on calcium mobilization, since 1, 10 and 100 uM dibucaine reduced the K^+-induced rise of $[Ca^{2+}]_i$ by 11, 26 and 65%, respectively. A similar effect was seen with BPB, since 50, 100 and 200 uM BPB inhibited the K^+-evoked accumulation of free Ca^{2+} by 19, 36 and 51%, respectively. The PLA_2 activator melittin increased $[Ca^{2+}]_i$, since 88, 380 and 880 nM melittin stimulated increases in $[Ca^{2+}]_i$ of 19, 47 and 81 nmoles, respectively. We suggest that the depolarization-induced accumulation of unesterified fatty acids, in particular arachidonic acid, may play a regulatory role in the mobilization of intraterminal free Ca^{2+} in this nerve terminal preparation. Supported by AFOSR-89-0245.

189.13

NEURONAL EXCITABILITY IN LONG-SLEEP (LS) AND SHORT-SLEEP (SS) MICE: *I.* SYNAPTOSOMAL K^+-STIMULATED CA^{2+} UPTAKE. C. C. Duncan, B. C. Jones*, and V. G. Erwin. University of Colorado School of Pharmacy, Boulder, CO., 80309.

The LS and SS lines of mice are genetically selected for their differential sensitivity to hypnotic effects of acute ethanol (EtOH). EtOH is known to inhibit rat brain synaptosomal uptake of calcium and may thus have an effect on stimulus-secretion coupling mechanisms. In this study, tissue from 60-90 day old LS and SS mice was employed to investigated the effect of EtOH (100, 400, 800, & 1600mg%) and barbiturates (diethylbarbital (DB) 75, 150, 300mM and secobarbital (SB) 35, 75, 150mM) on the voltage gated K^+-stimulated Ca^{2+} uptake by synaptosomes. Calcium uptake ($^{45}Ca^{2+}$) was measured at 1 or 3 seconds following synaptosomal exposure to either 4.7, 9.4, 18.8, or 37.6mM K^+ Krebs-Hepes buffer containing $^{45}Ca^{2+}$. Ca^{2+} influx was determined using liquid scintillation counting. In experiments with EtOH or barbiturates, the drug was included in the uptake-stimulating buffer. Synaptosomes from LS and SS mice showed differential sensitivity to K^+ concentration for stimulation of Ca^{2+} uptake with LS tissue being excited at lower concentrations of K^+ than SS. EtOH effectively decreased synaptosomal Ca^{2+} uptake in these lines with greater sensitivity of LS tissue compared to SS at EtOH concentrations of 400 and 800mg% (88 & 177mM). EtOH 100mg% was ineffective in decreasing synaptosomal Ca^{2+} uptake. The barbiturates also decreased Ca^{2+} uptake by this tissue with line-dependent differential sensitivity seen with DB (LS>SS), whereas SB decreased uptake equally in the two lines. These findings suggest that inhibition of voltage gated synaptosomal Ca^{2+} uptake may mediate in part, the hypnotic effects of EtOH and barbiturates. (USPHS AA03527,AA07330)

189.14

Ca^{2+} EVOKED PHOSPHORYLATION OF A 43KB PROTEIN IN STRIATAL SYNAPTOSOMES. J. F. Bowyer and N. Weiner. Dept. of Pharm., Univ. Colo. Hlth. Sci. Ctr., 4200 E. 9th Ave, Denver CO 80262.

The exposure of striatal synaptosomes, which have previously been incubated in Ca^{2+} free buffer, to 1.25mM Ca^{2+} can evoke the release of dopamine. Furthermore, this release mimics many aspects of the physiological release of dopamine. In these experiments the effects of 1.25mM Ca^{2+} exposure on the phosphorylation of synaptosomal proteins were investigated in an attempt to correlate phosphorylation changes with release. Synaptosomes were exposed to Ca^{2+}-free ^{32}P-phosphate (0.5mCi/ml) Krebs-Ringer bicarbonate buffer for 30min to label internal ATP stores. Subsequent exposure of the synaptosomes to 1.25-mM Ca^{2+} produced a 64% increase in the phosphorylation of a 43KB protein compared to Ca^{2+} free controls. The phosphorylation of the 43KB protein produced by Ca^{2+} was reduced to a 36% increase relative to control by tetrodotoxin. None of the other proteins between 90 and 40KB showed noticeable increases in phosphorylation from Ca^{2+} exposure at 10 min or less. Although Ca^{2+} exposure increased phosphorylation in a 43KB protein it decreased phosphorylation (to 80% control) in a 40KB band. It is possible that the 40KB protein is pyruvate dehydrogenase which can be dephosphorylated by a Ca^{2+} activated phosphatase. Maximal phosphorylation of the 43KB band appears within 5min. Whether or not the apparent phosphorylation of the 43KB protein plays a role in neurotransmitter release from synaptosomes is under investigation.

189.15

4-AMINOPYRIDINE INCREASES B-50 (GAP-43) PHOSPHORYLATION AND CALCIUM LEVELS IN RAT BRAIN SYNAPTOSOMES. F.M.J. Heemskerk*, L.H. Schrama, P.N.E. DeGraan*, W.E.J.M. Ghijsen*+, F.H. Lopes da Silva*+ and W.H. Gispen* (SPON: J. Eichberg). Div. Mol. Neurobiol., Rudolf Magnus Inst., Lab. Physiol. Chem., and Inst. Mol. Biol. & Med. Biotechnol., Univ. of Utrecht, Padualaan 8, 3584 CH Utrecht, +Dept. Zool., Univ. of Amsterdam, Kruislaan 320, 1098 SM, Amsterdam, NL.

The convulsant drug 4-aminopyridine (4-AP) enhances the phosphorylation of the growth-associated phosphoprotein B-50 (GAP-43) as revealed by immunoprecipitation after labeling of hippocampal slices with $^{32}P_i$ (DeGraan et al., J. Neurochem., 52:17, 1989). This effect could be blocked by the protein kinase C (PKC) inhibitor staurosporine. The time and concentration dependency of this phosphorylation effect are closely correlated with the changes in basal [^{3}H]-norepinephrine release from the slices evoked by 4-AP. In order to get more insight in the mechanisms underlying the 4-AP-induced effects, we studied B-50 phosphorylation and Ca^{2+} levels in synaptosomes and compared the effects to those evoked by depolarization with K^+. 4-AP stimulated B-50 phosphorylation within 15 sec in a Ca^{2+}-dependent manner; longer incubations revealed a sustained increase by 4-AP. The enhancement of the intrasynaptosomal Ca^{2+} level by 4-AP, as measured with fura-2, followed a similar time course as the B-50 phosphorylation. Moreover, the effect of 4-AP on Ca^{2+} levels could be inhibited by the sodium channel blocker tetrodotoxin, indicating the involvement of repeated spontaneous depolarizations. Our results indicate that the effects of 4-AP on B-50 phosphorylation are mediated by a Ca^{2+}-dependent activation of PKC through repeated spontaneous depolarizations. These findings further support the involvement of B-50 phosphorylation by PKC in the regulation of neurotransmitter release.

189.16

PROTEIN KINASE C-MEDIATED PHOSPHORYLATION OF THE GROWTH-ASSOCIATED PROTEIN B-50 (GAP-43) IS AN ESSENTIAL STEP IN NOREPINEPHRINE RELEASE. P.N.E. DeGraan*, L.V. Dekker*, D.H.G. Versteeg*, A.B. Oestreicher* and W.H. Gispen* (SPON: A.J. Schecter). Div. Mol. Neurobiol., Rudolf Magnus Inst. and Inst. Mol. Biol. & Med. Biotechnol., Univ. of Utrecht, Padualaan 8, 3584 CH Utrecht, NL.

The neuron-specific protein B-50 (GAP-43) is an endogenous substrate of protein kinase C (PKC) in the membrane of the presynaptic terminal and neuronal growth cones. Based on the close correlation between *in situ* B-50 phosphorylation and neurotransmitter release in hippocampal slices, we have recently suggested a role for PKC-mediated B-50 phosphorylation in neurotransmitter release (Dekker et al., *J. Neurochem.* 52:24, 1989). In the present study we investigated whether B-50 antibodies, which are known to interfere with PKC-mediated B-50 phosphorylation in synaptosomal plasma membranes, affect neurotransmitter release. Antibodies were introduced into rat cortical synaptosomes permeabilized with streptolysin-O, [^{3}H]-norepinephrine (NE) release was induced by increasing the free Ca^{2+} concentration from 10^{-7} to 10^{-5} M. B-50 phosphorylation was measured with [γ-^{32}P]-ATP as phosphate donor. Anti-B-50 IgGs inhibited Ca^{2+}-dependent NE release in a concentration-dependent way, whereas control IgGs and heat-inactivated anti-B-50 IgGs were ineffective. Under identical conditions anti-B-50 IgGs specifically inhibited PKC-mediated B-50 phosphorylation in permeabilized synaptosomes. We conclude that PKC-mediated B-50 phosphorylation plays a crucial role in the molecular mechanism of NE release in a step distal to the Ca^{2+} trigger. Since B-50 is present throughout the brain in regions enriched in synapses, B-50 phosphorylation may be more generally involved in transmitter release. We propose that B-50 plays a role in the mechanism of synaptic vesicle fusion with the plasma membrane during neurotransmitter release and in membrane fusion processes in the growth cone during neurite outgrowth.

PRESYNAPTIC MECHANISMS: FACILITATION AND DEPRESSION

190.1

FOCAL RECORDING OF FACILITATION IN FROG NEUROMUSCULAR JUNCTION. M.I. Glavinovic. Depts. of Anaesthesia Research and Physiology and Biomedical Engineering Unit, McGill University, Montreal, Canada H3G 1Y6.

It is well established that at low levels of release the facilitation is caused by greater quantal content (del Castillo & Katz, J. Physiol. 124, 574, 1954). While same is reasonable to assume at normal levels of release other factors cannot be excluded. Repeated nerve stimulation may lead to release over the areas previously activated, and thus to either greater or lower quantal responses depending on whether postsynaptic potentiation or desensitization predominate. This is an attempt to examine whether such changes do occur. End-plate potentials (EPP's) were recorded focally in cutaneous pectoris frog neuromuscular junctions at room temperature (20-22 C). Release was locally restored by current through the recording electrode which contained 1 M $NaCl$-$CaCl_2$. In all cases examined (11 end-plates) the facilitation was associated with greater quantal content. In majority of cases (7 end-plates) the quantal size of EPP's did not change appreciably, but in 4 cases it increased (5 to 17%). Facilitation was primarily caused by a greater quantal content, with a small contribution of greater quantal size.

190.2

PRESYNAPTIC FACILITATION WITH AND WITHOUT NERVE IMPULSES. A.I. Bain* and D.M.J. Quastel
Dept. of Pharmacology & Therapeutics, Univ. of British Columbia, Vancouver, Canada V6T 1W5

In mouse diaphragm, tetanic nerve stimulation induces a progressive increase in EPP quantal content (*m*), and minEPP frequency (*Fm*). Components of facilitation can be distinguished by time course and by the relation between *m* and *Fm*. An early component (0.1s tau) raises *Fm* and *m* by the same factor, as does a later component, complete at about 30 s. Subsequently, *Fm* rises more than *m*. Only the last phase corresponds to a residual ion (Ca?) model, which accounts for facilitation in the presence of Ba or Sr, at under 10 Hz. All components are insensitive to external Ca. With direct nerve terminal depolarization (tetrodotoxin present) EPPs elicited by brief (~0.7ms) maximally effective pulses, in low Ca, are similar in *m* to EPPs elicited by nerve stimulation. However, only the fast component of tetanic facilitation is normal; the others are virtually absent. Thus, the major components of normal tetanic potentiation are due neither to Ca entry nor to a 'direct' effect of depolarization.
(Supported by the Muscular Dystrophy Association of Canada and the MRC, Canada.)

190.3

EFFECT OF INCREASED PRESYNAPTIC CALCIUM BUFFERING CAPACITY ON SHORT-TERM FACILITATION S.N. Duffy*, J.L. Winslow* and M.P. Charlton, Dept. of Physiology, University of Toronto, Toronto, Ontario M5S-1A8.

Evoked neurotransmitter release from excitatory synapses innervating the dactyl opener muscle of the crayfish Procambarus clarkii increases during brief trains of closely spaced stimuli, a property known as short-term facilitation. Facilitated release may result from the summation of incoming Ca ions with residual free Ca ions from prior stimuli. To test this hypothesis, facilitation was evoked under conditions where synaptic Ca buffering capacity was elevated by incorporation of the cell permeant form of the Ca chelator BAPTA. Such treatment, which should buffer the $[Ca]_i$ transients, might be expected to affect the accumulation and disposal of residual free Ca and thus alter facilitation. Although BAPTA produced a significant (60-90%) decrease in transmitter output, the magnitude and duration of facilitation was not significantly affected. These results suggest that short-term facilitation at these terminals is mediated either through a Ca-independent mechanism or by Ca ions which remain bound to some intracellular site. This conclusion is substantiated by the results of a 3-D diffusion model of $Ca+B \rightleftharpoons Ca\text{-}B$ where B is a mobile BAPTA-type buffer.
Supported by Medical Research Council of Canada.

190.4

EFFECTS OF SODIUM ACCUMULATION IN PRESYNAPTIC BOUTONS OF CRAYFISH ON INTRACELLULAR CALCIUM CONCENTRATION MEASURED WITH FURA-2. R.M. Mulkey and R.S. Zucker. Dept. of Physiology-Anatomy, Univ. of California, Berkeley, CA. 94720.

Published evidence indicates that posttetanic-potentiation (PTP) in invertebrates may be due to internal sodium accumulation that occurs during the tetanic train. It has been proposed that sodium accumulation leads to raised presynaptic calcium through the effects of the Na/Ca exchanger. Recent experiments using Fura-2 have shown a rise in internal calcium during PTP. We used pharmacological methods (veratridine, a plant alkaloid which activates sodium channels and monensin, a sodium ionophore) to raise internal sodium in crayfish presynaptic terminals to determine if sodium accumulation could lead to an increase in internal calcium concentration. Free internal calcium was measured using the fluorescent dye Fura-2. Doses of veratridine (10, 20 & 100 μM) and monensin (1, 10 & 100 μM) in normal medium increased the free calcium from control values of 180 nM to a range of 1 to greater than 5 μM. Veratridine (20 & 100 μM) and monensin (100 μM) in a zero calcium medium with 2 mM EGTA produced a slight increase (100nM) in free internal calcium in the terminals. Upon return of normal medium, the free internal calcium in terminals rose to values in excess of 5 μM. This increase in internal free calcium upon return of normal medium was most likely due to the Na/Ca exchanger. This may be the source of the rise in free internal calcium during PTP. Supported by NIH Grant NS 15114.

190.5

BRADYKININ-EVOKED ACETYLCHOLINE RELEASE VIA INOSITOL TRISPHOSPHATE-DEPENDENT CALCIUM RISE. Haruhiro Higashida[1], Yasuhiro Myojo[1] and Akihiko Ogura[2]. [1]Dep. of Biophys. Kanazawa Univ. Sch. of Med. Kanazawa 920 and [2] Dep. of Neurosci. Mitsubishi Kasei Inst. of Life Sci. Machida 194, Japan.

The mechanism of the bradykinin(BK)-induced increase of acetylcholine was studied in NG108-15 mouse neuroblastoma x rat glioma hybrid cells and their synapses formed onto mouse muscle cells under culture condition. The BK-induced facilitation was observed even if the extracellular medium was changed to a lower Ca^{2+} concentration or Cd^{2+} (0.5-2 mM) added. Ba^{2+} (2-4mM) blocked the membrane hyperpolarization of hybrid cells in response to BK, but still the characteristic facilitation of MEPPs was observed in myotubes. Ionophoretic injection of inositol 1,4,5-trisphosphate (InsP3) into the cytoplasm of an NG108-15 cell briefly evoked MEPPs during the InsP3 -induced hyperpolarizing phase. However, the InsP3 -dependent facilitation was also observed whether or not InsP3 hyperpolized the cell. Real-time quantitative monitoring of the intracellular free Ca^{2+} concentration with fura-2 on a single NG108-15 cell showed that BK induced an elevation of the $[Ca^{2+}]i$ under any conditions mentioned above. These data suggest that the BK-induced initial facilitation results from the inositol 1,4,5-trisphosphate-dependent elevation of residual Ca^{2+}.

190.6

EFFECT OF CALCIUM ON DEPRESSION OF TRANSMITTER RELEASE AT THE FROG NEUROMUSCULAR JUNCTION. M.A. Sosa and J.E. Zengel. Depts. Neurosci./Neurosurg., Univ. of Fla. Coll. of Med. and VA Med. Ctr., Gainesville, FL 32610.

The amount of transmitter released by each nerve impulse varies as a function of previous synaptic activity. Under conditions of normal quantal release, repetitive stimulation leads to a decrease in the transmitter released per impulse. This depression of release has traditionally been attributed to a depletion of available transmitter. Depression could also result from an increase in intra-terminal Ca^{2+}, which could decrease release, perhaps by inducing a Ca-dependent inactivation of voltage-dependent Ca channels.

The purpose of this study was to describe depression in a quantitative manner and to examine the role of Ca^{2+} in the mechanism(s) underlying depression. End-plate potentials (EPPs) were recorded extracellularly from curarized frog (*Rana pipiens*) sartorius muscle in the presence of 1.8-7.2 mM Ca^{2+}. Typically, the nerve was conditioned with trains of 200 impulses at 20 imp./sec.

We observed what appear to be at least two phases of depression, an initial rapidly developing phase which reaches a maximum during the first sec of stimulation, followed by a second phase which develops more slowly during the remainder of the train. Increasing Ca^{2+} increased the magnitude and decreased the time course of both phases; this effect was more marked for the first phase. Addition of the Ca channel blocker Cd^{2+} increased the magnitude and decreased the time course of the first phase, while having the opposite effect on both parameters of the second phase.

These data suggest that there may be more than one mechanism underlying depression at the frog neuromuscular junction.

190.7

PROTEIN KINASE INHIBITORS AFFECT MOBILIZATION OF NEUTROTRANSMITTER IN MOTOR NERVE TERMINALS. W F Dryden and I G Marshall*, Dept. Pharmacology, Univ. Alberta, Edmonton, Alta, Canada, and Dept. Physiology and Pharmacology, Univ. Strathclyde, Glasgow, Scotland, U.K.

At least one component of neurotransmitter mobilization involves the transfer of quanta from secondary stores to the immediately available store within the nerve terminal. This may involve augmentation and/or potentiation, and require a phosphorylation step (Silinsky, Pharmacol Rev 37:81, 1985). To test this possibility, the depression of EPP amplitude during repetitive stimulation, and the recovery following a conditioning pulse were investigated in presynaptically blocked mouse phrenic nerve diaphragm preparations following incubation with a series of naphthalenesulfonamide protein kinase inhibitors (Inagaki et al. Mol Pharmacol 29:577, 1986).

Following 30-60 min incubation with 50 μM H-7 or 10 μM A-3 and in the presence of 0.3 μM alcuronium and 200 μM troxypyrrolium, rundown of EPP was enhanced at low frequencies, and at higher frequencies (30 - 50 Hz) failed intermittently or completely. In paired pulse experiments, recovery of EPP amplitude was delayed from 3 sec to > 5 sec. No such effects were seen following incubation with 10 μM W-7. At the concentrations used, H-7 and A-3 share specificity for only cyclic nucleotide dependent protein kinases indicating a phosphorylation step in the maintenance of transmitter release during repetitive stimulation.

HUMAN BEHAVIORAL NEUROBIOLOGY: EVENT RELATED POTENTIALS

191.1

MIDLATENCY AUDITORY EVOKED RESPONSES: COMPONENT ISOLATION AND TOPOGRAPHY. R.J.Erwin,M.Mawhinney-Hee* and R.C.Gur*. Dept. of Psychiatry,Univ. of Penn.,Philadelphia, PA 19104.

Evidence from both the human and cat model suggests that the P1 (50-65 msec latency) component of the midlatency auditory evoked response (MLR) is generated by the midbrain reticular activating system (Erwin and Buchwald, EEG suppl., 40:461, 1987). Other studies suggest that P1 is generated in temporal lobe (e.g., Reite et al., EEG, 70:490, 1988). To address this issue, the topographic distribution of the MLR component P1 was examined using a recovery cycle protocol.

MLRs were recorded from 20 normal adults in response to binaural clicks (.1ms, 50 db HL) presented at 3 rates: 1/sec, 5/sec and 10/sec. Recordings were from 19 scalp sites referenced to linked mandibles. MLR components were isolated using principal components analysis. Scalp distributions for each component and rate were examined by plotting factor scores on topological grids.

The distribution of P1 was characterized by a central maxima at the 1/sec rate and no distinct minima although amplitudes were lower over temporal regions. At faster rates of stimulation, the central positivity decreased and distinct frontal maxima and temporal minima were observed.

These findings indicate that separate MLR components, distinguished both by topography and parametric manipulations, exist at the P1 latency and could account for the conflicting reports regarding the P1 generator substrate.

191.2

NOVEL SOMATOSENSORY STIMULI GENERATE P3a EVENT-RELATED POTENTIALS S.Yamaguchi* and R.T.Knight (SPON:I.Kwee) Dept. of Neurology, Univ. of California, Davis, VAMC, Martinez, CA 94553

We recorded somatosensory event-related potentials (ERPs) to target and task-irrelevant novel stimuli. ERPs were recorded from twelve normal subjects in a somatosensory discrimination task. Subjects pressed a button to mechanical taps of the fifth finger (targets, P=.12), randomly interposed in sequences of taps to the second finger (standards, P=.76). Two infrequent novel stimuli were delivered; one was a mechanical tap to the third or fourth finger (tactile novels, P=.06), another was an electric shock at the wrist (shock novels, P=.06). Correctly detected targets generated a parietal maximal P300 (P3b, 19uV at Pz). Shock novels generated a central maximal P300 (P3a, 26uV at Cz). Tactile novels also generated a P3a with a scalp distribution comparable to the shock novels (12uV at Cz). P3a scalp topographies evoked by shock and tactile novels were different from the target P3b ($p<0.001$) although there was no significant latency difference between the P3b and P3a. Unlike the P3b, P3a amplitude to the shock novels habituated by 17% from the initial to the fifth shock. These results indicate that similar to the auditory and visual modality, task-irrelevant novel somatosensory stimuli generate a P3a ERP that may have independent intracranial generators from the P3b. Supported by NIH grant NS 21135.

191.3

EVENT RELATED POTENTIAL (ERP) ASYMMETRIES IN A DICHOTIC COMPLEX TONE TEST (CTT). C.E. Tenke, G.E. Bruder*, J.J. Sidtis, J.Towey*, H. Erhan*, M. Voglmaier*. Dept. of Psychophysiol., N.Y.S. Psych. Inst., N.Y., N.Y. 10032

The CTT is a nonverbal dichotic listening task that typically yields a behavioral left ear advantage in normal adults (Sidtis, 1981). Findings from neurological patients suggest a right hemisphere dominance for complex tone perception. The present study was conducted to provide a physiological measure of brain laterality by determining the form of ERP asymmetries produced by square wave complex tones during the CTT. ERPs of 10 right handed Ss with normal hearing were recorded from 12 scalp electrodes at midline and lateral locations, with a nose reference. EOGs were recorded for artifact rejection and eye movement correction. The following ERPs were evident: (1) a paired N100-P200 with frontocentral maximum; (2) a late positive complex with parietal maximum; and (3) a frontal negative and parietal positive slow wave. Preliminary analyses showed the following ERP asymmetries: (1) at frontal electrodes, there was significantly greater negativity over the right hemisphere than the left, which started at 80 msec poststimulus and peaked at 210 msec; (2) at central electrodes, there was a significant attenuation of P200 over the right hemisphere; (3) at parietal electrodes, there was an enhancement of the late positive complex over the right hemisphere in Ss with the largest left ear advantage. These results suggest lateralization at multiple stages of information processing. (Supported by NIMH grant MH36295).

191.4

LOCALIZATION AND TEMPORAL ACTIVITY FUNCTIONS OF BRAIN SOURCES OF THE HUMAN VISUAL ERP. G.V. Simpson, M. Scherg*, W. Ritter*, and H.G. Vaughan, Jr., Depts. of Neurology and Neuroscience, Albert Einstein College of Medicine, Bronx, New York 10461.

Characterization of the brain sources of human event related potentials (ERP) will increase our understanding of the brain processes in perception and cognition. This study examined the sources of human visual ERP using Scherg's method of Brain Electric Source Analysis (BESA), which yields a solution consisting of multiple dipole sources having stationary locations and orientations and time varying dipole strengths. The coordinates of the dipole sources in the spherical head model were then mapped onto the MRI of each subject's brain. This allowed visualization of the brain regions modelled by each of the equivalent-dipole sources. BESA also yields the amplitude function over time for each source (source waveform), thereby providing a real-time description of the activity pattern of each brain area represented by the dipole sources. The results showed multiple sources with sequential and overlapping activation. Localization of these sources in the MRI's indicated that the visual ERP was generated by the sequential and overlapping activation of multiple visual cortical areas consistent with the flow of cortical activation from striate to parietal and inferotemporal cortex, expected from primate vision research.

191.5

THE EFFECTS OF PRACTICE ON REACTION TIME AND P3 LATENCY IN A STIMULUS-RESPONSE INCOMPATIBILITY TASK. C. A. Christensen. Department of Psychology, Vassar College, Poughkeepsie, NY 12601.

Stimulus-response (S-R) incompatibility tasks have frequently been used to assess the functional significance of the P3 component of the ERP. In an earlier investigation (Pfefferbaum, et al., Electroencephal. clin Neurophysiol., 1986, 64: 424-437) we tested several S-R incompatibility tasks and observed that P3 latency was prolonged by incompatibility in some but not all tasks. We speculated that the effect is observed when speeded responding occurs in tasks that predispose to parallel stimulus and response processing, as when Ss are well practiced, for example. To evaluate this predicition 14 Ss were tested on a task in which others have failed to observe an S-R incompatibility effect on P3 latency. Ss saw an instruction stimulus (SAME or OPPOSITE) followed by an action stimulus (RIGHT or LEFT). Ss responded to the action stimulus by pressing the right or left of two response keys, depending on the instruction for that trial. Ss were tested for 10 blocks of 60 trials each. The EEG was recorded from Fz, Cz and Pz referenced to linked mastoids. Reaction times for both S-R compatible and incompatible trials diminished significantly across blocks showing a robust practice effect. P3 latencies at Pz were equivalent for compatible and incompatible trials for Block 1 but were significantly prolonged on incompatible trials by Block 10. The results indicate that P3 cannot reflect the duration of stimulus evaluation processes alone, as others suggest. In some instances, as when Ss are well practiced, it also reflects the duration of processes related to response initiation and execution.

191.6

FRONTAL LOBE CONTRIBUTIONS TO THE HUMAN AUDITORY P3a D. Scabini*, R.T. Knight and D.L. Woods, Neurology Dept., Univ. of California Davis, V.A.M.C., 150 Muir Rd., Martinez, Ca 94553

Novel auditory, visual and somatosensory stimuli generate a P300 (P3a) response which is maximal at fronto-central scalp sites. We investigated the contribution of human dorsolateral prefrontal cortex (PFCx) to P3a generation by recording event related potentials (ERPs) in an auditory discrimination task in controls (n=17) and in patients with unilateral PFCx lesions (n=13, left=8, right=5). Subjects were instructed to press a button to target tone pips (1.5Khz, 10% probability) randomly presented in a train of frequent tones (1Khz, 80% probability). P3a potentials were generated by delivery of task-irrelevant computer and environmental sounds (novels, 10% probability). In controls, the novel stimuli generated a P3a that was maximal at fronto-central scalp sites. The P3a was reduced in amplitude in the PFCx group at recording sites over lesioned hemisphere ($p<0.002$). The P3a reduction was maximal over lesioned right PFCx (Control, F3=9.45uv F4=9.45uv; right PFCx, F3=8.86uv F4=4.59uv; left PFCx, F3=6.86uv F4=8.00uv $p<0.001$). These data support PFCx involvement in generation of the scalp recorded auditory P3a. The results also indicate that PFCx may be asymmetrically organized for novelty detection. (Supported by NIH grant NS21135 and the VA Research Service)

191.7

Intracerebral distribution of human late potentials associated with target, novel and missing auditory stimuli. C.Alain*, F.Richer, A.Achim*& J.M.Saint-Hilaire*. Neurology dept., Notre-Dame Hospital, Montreal, Canada.

We have recorded late auditory potentials from lateral and medial regions in the frontal, temporal and parietal lobes of 14 patients with temporal lobe epilepsy implanted with depth electrodes. Tone sequences were presented in three tasks: 1) auditory target detection, 2) target detection with interspersed novel stimuli, and 3) detection of targets and stimulus omissions. Frontal and parietal intracerebral responses to target tones showed a triphasic complex with peak latencies of about N200, P270 and N360 ms. The latency of the positive peak was similar to that of scalp N2. Temporal responses had a positive potential at 300 ms which could be accompagnied by a negative peak at 200 ms or at 420 ms. The potentials evoked by novel stimuli had shorter latencies than those elicited by target tones. The respones in the frontal and parietal lobes were attenuated or absent during stimulus omissions. The results support the multiple generator hypothesis of late potentials and suggest that some potentials are stimulus driven and others not.

191.8

EVENT-RELATED POTENTIALS FOR COMPLEX FORMS: EFFECTS OF MENTAL ROTATION. D.S. O'Leary and D.L. Johnson*. Psychology Department, Univ. of Health Sciences/Chicago Medical Sch., North Chicago, IL 60064.

This study investigated differences in event-related potentials (ERPs) elicited by 16-sided random visual forms during passive viewing and mental rotation conditions. Subjects were 45 right-handed adult males. ERPs recorded from left and right hemisphere parietal leads (P3 and P4) showed significant task-related differences in a series of components beginning with a negativity at about 160 ms and persisting until a positivity at about 375 ms post-stimulus onset. Latencies were significantly shorter for P245 and N300 components during the rotation condition and significant increases in amplitude occurred for N160 and P375. The P245 component was of smaller amplitude in the rotation condition. A late negative-positive complex, seen only in the rotation condition, was of significantly shorter latency over the right hemisphere. Other hemispheric effects were seen (shorter latency and higher amplitude at P4 than at P3), but were of smaller magnitude than reported in a previous study.

191.9

IS THE MISMATCH NEGATIVITY WAVE OF THE HUMAN AUDITORY ERP INDEPENDENT OF ATTENTION?

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In the auditory modality, an infrequent, physically deviant stimulus in a sequence of repetitive "standard" stimuli elicits a negative event-related brain potential, termed the mismatch negativity (MMN). The MMN usually onsets around 100 msec after stimulus onset and peaks around 200 msec. Naatanen and associates have asserted that the MMN is unaffected by attention and reflects the operation of a strongly automatic mismatch detection process. This effect has been cited as evidence that the physical characteristics of stimuli are fully processed even when unattended.

Two dichotic listening experiments were designed to optimize the selective focussing of attention and the engagement of early stimulus selection. These conditions included 1) easily discriminable channels of tone pips distinguished by both frequency and ear of entry, 2) a rapid rate of stimulus presentation, and 3) a difficult target detection task for the attended channel that entailed responding to infrequent, slightly-fainter deviant tones. The first of these experiments, using interstimulus intervals (ISIs) of 120-320 msec and standard tone intensity of 55 dB SL, revealed a very early differentiation (ca. 20 msec) between the ERPs to the attended and unattended standard stimuli, indicating that attention was indeed highly focussed. The deviant tones elicited negative waves that onset just after 100 msec, peaked at around 200 msec, had frontal-central maxima on the scalp, and were larger contralaterally at central sites. However, in sharp contrast to previous reports by Naatanen's group, the negativity to unattended-channel deviants was drastically reduced (less than 1 uV) relative to the corresponding wave to attended-channel deviants (3-4 uV). The second experiment used shorter ISIs (65-205 msec) and louder tones (standard intensity 90 dB SL), with deviants again being occasional intensity decrements. Under these conditions, the MMN to unattended deviants was barely discernible, whereas the corresponding negative wave to the attended-channel deviants was clearly evident.

The present findings are at odds with the assertion that the MMN is independent of attentional influence, as well as with predictions that its amplitude should increase at short ISIs. The functional significance of this ERP component needs further examination.

191.10

GENERATORS OF THE NEUROMAGNETIC P3: J.D. Lewine, S.B.W. Roeder*, M.T. Oakley, D.L. Arthur, C.J. Aine, J.S. George and E.R. Flynn, P-6, Mailstop M715, Los Alamos National Laboratory, Los Alamos, N.M., 87545.

Neuromagnetic and neuroelectric measurements were obtained from nine subjects performing auditory and visual detection tasks. For these tasks, subjects had to count the number of times a target stimulus occurred within a sequence of target and non-target stimuli (auditory - 1000 vs 3000 hz tones : visual - horizontal vs vertical gratings). During half of the test blocks the target occurred with a probability of 80%, while during the remaining blocks it occurred with a probability of 20%. Electric potential data recorded at midline sites (referenced to linked ear lobes) revealed the expected, positive P3 complex when the target probability was 20%. In the auditory modality the peak-latency of the P3 was about 300 msec. In the visual modality the peak-latency was about 400 msec. Neuromagnetic recordings made with a 7-channel, 2nd-order squid gradiometer system revealed neuromagnetic correlates of the electrical P3 complex. Isofield maps constructed from data obtained at more than 16 dewar positions per subject suggest that the neural generators of the auditory and visual magnetic P3 are spatially distinct, and located within the temporal lobe (possibly within the hippocampus). Although the extent to which the generators of the magnetic P3 contribute to the electrical P3 remains to be determined, the data suggest that the magnetic P3 may prove to be a useful, non-invasive index of the functional integrity of the temporal lobe.

191.11

MONKEY P300-LIKE POTENTIALS IN A VISUAL PARADIGM: A ROLE FOR THE LOCUS COERULEUS-NORADRENERGIC SYSTEM? J. A. Pineda, D. Swick, and S.L. Foote, Depts. of Neuroscience and Psychiatry, UCSD, La Jolla, CA 92093 and Scripps Clinic and Research Foundation, La Jolla, CA 92037.

The similarities among human P300 potentials elicited in response to auditory, visual, or somatosensory stimulation suggest the existence of a common mechanism responsive to all sensory modalities. However, its neural substrates cannot be systematically investigated since the modality non-specific nature of P300 has not been extensively studied in animals, particularly non-human primates. A number of investigations have shown that monkeys exhibit auditory P300-like potentials similar to those recorded in human (Arthur and Starr, 1984; Paller et al., 1988; Pineda et al., 1987; 1988). To determine whether monkeys exhibit similar potentials in another sensory modality, visual evoked potentials (VEPs) were recorded from squirrel monkeys (Saimiri sciureus) using chronically implanted epidural electrodes. The results indicate that visually elicited monkey potentials resemble those reported for human P300s. Their sensitivity to stimulus probability and to trial-to-trial changes in stimulus sequence also resemble monkey auditory P300s. Additionally, since previous studies have suggested that the integrity of the locus coeruleus-noradrenergic system is critical for the production of auditory P300-like potentials (Pineda et al., 1989), the role of this system in generating or modulating VEPs was analyzed. VEPs were recorded before and after electrolytic lesions of the nucleus and before and after systemic administrations of the alpha-2 adrenergic agonist clonidine. P300-like potentials were substantially affected by the lesions, while drugs had less of an effect. The results support the hypothesis that monkey P300-like potentials reflect the activity of a modality non-specific mechanism and that the LC-NA may play such a role.

191.12

DISSOCIATION OF EARLY ATTENTION SENSITIVE ERP COMPONENTS DURING VISUAL-SPATIAL SELECTION.

G.R. Mangun(1), H.J. Heinze*(2) & S.A. Hillyard(1), University of California, San Diego, USA (1) & Medical School of Hannover, FRG (2)

Recent evidence suggests that the attentional modulations of the occipital P1 and N1 peaks of the visual event-related potential (ERP) during spatial selection may reflect functionally distinct processes; the P1 effect reflecting sensory pathway facilitation and the N1 effect indicating an orienting or "switching" of attention to stimuli at attended locations. To test the switching idea, we recorded ERPs from subjects attending a rapid sequence of bilaterally flashed letter pairs that were unpredictably followed by a unilateral pair appearing in either visual field. In one condition, subjects attended the letters pairs in one visual field (maintaining central fixation) and ignored all stimuli in the unattended field. The task was to determine whether the attended-side unilateral letters matched those in the attended field of the preceding bilateral array. In a second condition, the task was to determine whether the unilateral letters on either the attended or unattended sides matched those in the attended field of the preceding bilateral array. Thus, the occurrence of a unilateral stimulus in the unattended visual field required the subjects to switch their attention rapidly from the attended to the unattended field to detect target letter matches. If the N1 enhancement to attended-field stimuli reflects the switching of attention to an attended-location event, then one would expect an enlarged N1 component to unilateral stimuli on the "unattended" side that triggered a switch of attention.

ERPs to the bilateral stimuli displayed P1 components of enhanced amplitude when subjects attended the visual field contralateral to the recorded hemisphere. The ERPs to unilateral stimuli contained a broad positive deflection from 90-200 msec post-stimulus when in the attended versus unattended field. The early phase of this positivity coincided with the occipital P1 component and the later portion overlapped the N1 latency range. These attention effects were not significantly different as a function of whether or not subjects were required to ignore, or instead to switch to unilateral stimuli in the unattended visual field. These data support the idea that the P1 attention effect represents a facilitation of early visual inputs and question the generality of the proposal that the occipital N1 effect is an index of attentional switching.

191.13

EVENT-RELATED POTENTIALS RECORDED FROM SCALP AND HIPPOCAMPAL FORMATION IN HUMANS PERFORMING DETECTION TASKS. T. Scott, G. McCarthy, K.A. Paller, and C.C. Wood. VA Medical Center, West Haven, CT and Yale University, New Haven, CT 06516.

P300 is an event-related potential (ERP) thought to reflect neural activity associated with cognitive processing. In the *oddball* paradigm, P300 is elicited by target stimuli occurring in a random sequence with non-target stimuli. In this task, the amplitude of P300 is inversely proportional to the probability of the target. However, the relative contribution of event probability (Duncan-Johnson & Donchin, 1977) and temporal interval (Fitzgerald & Picton, 1981) in determining P300 amplitude is unclear, due to the confound between target probability and the temporal interval between successive targets. To examine the relative roles of probability and temporal interval on P300 amplitude and topography, we compared a condition in which target tones (p=.2) were presented in a random sequence with non-target tones to a condition in which identical targets were presented at the same intervals but with non-targets omitted. Based on recordings from 32 scalp locations, P300s in the two conditions were similar in amplitude and in scalp topography. These same two conditions were compared in recordings from electrodes implanted in the hippocampus and adjacent medial temporal lobe areas in a group of epileptic patients. Targets from both conditions elicited large negative ERPs with sharp spatial gradients in the region of the hippocampus. These results suggest that apparent probability effects observed for scalp P300 and related ERPs in the hippocampus may be due primarily to the temporal interval between task-relevant events. Furthermore, these results demonstrate additional task-dependent relationships between scalp-recorded P300 and ERPs elicited in the hippocampus.

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191.14

Auditory Evoked Potential Asymmetry and Patterns of Human Abilities, in Normal and Disabled Readers. D.W. Shucard, K.R. Cummins*, J.L. Shucard*, Dept of Neurology, SUNY at Buffalo, School of Medicine and Biomedical Sciences, 100 High Street (D-6), Buffalo NY 14203.

The purpose of this investigation was to examine the relationship between electrophysiological measures of hemispheric asymmetry and spatial and sequential abilities in normal and disabled readers. In a sample of 90 normal and 90 disabled readers, spatial and sequential ability scores were derived from the Wechsler Intelligence Scale for Children-Revised (WISC-R) subtests according to Bannatyne's reclassification system. Subjects were categorized according to the difference between their spatial and sequential ability scores. These scores were then compared with auditory evoked potential (AEP) amplitude asymmetry measures obtained to probe stimuli while the subjects were processing visual sequential information related to language.

The findings indicated that the normal and disabled readers who had the higher spatial-sequential difference scores (spatial-sequential >10.00) also had higher left than right hemisphere AEP Peak amplitudes. In the lower spatial-sequential difference group (spatial-sequential < 10.00), this pattern of AEP amplitude asymmetry was present only for the reading disabled subjects. The normal readers showed an opposite pattern of asymmetry with higher right than left hemisphere AEP amplitude.

These findings suggest a lack of flexibility in cognitive responsivity in the disabled readers as reflected in the consistent pattern of cerebral asymmetry obtained in disabled readers both for higher and lower spatial-sequential difference groups. Electrophysiological patterns of asymmetry obtained during cognitive tasks may be sensitive to the functional organization of the brain and may aid in our understanding of the relationship between human abilities and brain processes. Supported in part by NICHD Grant HD11681.

193

SYMPOSIUM. OPTICAL IMAGING OF CNS DEVELOPMENT, ORGANIZATION AND FUNCTION. *A. Grinvald, IBM, The Rockefeller Univ. & Weizmann Inst. (Chairperson); W.N.Ross, N.Y. Med. College; J.A. Connor, Roche Inst. of Mol. Biol.; L.B. Cohen, Yale Univ.; G.G. Blasdel, Harvard Univ.*

Optical imaging techniques have begun to provide new information about the development, organization and function of the nervous system, at levels ranging from single growth cones to the living mammalian brain. These imaging techniques employ a fast diode array (Ross, Cohen, Grinvald) or slower imaging devices offering a much higher spatial resolution (CCD: Connor, Ross, Grinvald; Video: Blasdel). The imaging is based on the use of specific molecular probes for calcium (Ross, Connor), and membrane potential (Cohen, Ross Grinvald), as well as changes in intrinsic optical properties of brain tissue resulting from electrical activity (Grinvald).

Ross will discuss high time-resolution measurements of activity-dependent calcium changes in neurons, focusing on the spatial distribution of the conductance changes that cause them and how they can indicate the spread of membrane potential changes in a cell (in cerebellar Purkinje cells in slices and in invertebrate neurons). **Connor** will focus on high spatial-resolution measurements of the distribution of calcium channels in developing neurons, the effects of excitatory amino acids on intracellular calcium in the dendrites and the soma of acutely isolated CA1 neurons, and the distribution of calcium transients during spontaneous and driven electrical activity in Purkinje cells and CA1 neurons in slices. **Cohen** will present measurements of action potential activity in a substantial fraction of the neurons in the *Aplysia* abdominal ganglion. Measurements during the gill withdrawal reflex indicated that a light touch to the siphon skin activated approximately 300 neurons, suggesting that the neuronal basis of this reflex may be very complex. **Grinvald** will discuss the functional architecture of Macaque V1 & V2 revealed by high resolution imaging based on reflection measurements without dyes. Dynamic patterns in cortical cell assemblies revealed with the aid of dyes will also be presented. The relative merits and limitations of the two methodologies will be discussed. **Blasdel** will describe the functional organization of monkey V1 revealed by high resolution video technology, allowing the visualization of many different activity patterns, in a single patch of cortex. Their analysis reveals an unexpected degree of lateral organization, one characterized by at least two fundamental subpatterns, in which response properties of neurons may correlate with separate perceptual functions.

194

SYMPOSIUM. RECENT ADVANCES IN THE BIOLOGY OF AFFECTIVE DISORDERS. C.B. Nemeroff, Duke Univ. Med. Ctr. (Chairperson); S.Z. Langer, LERS; J. Egeland*, Univ. Miami; J.M. Weiss, Duke Univ. Med. Ctr.; F. Sulser, Vanderbilt Univ.

In the past decade, considerable progress has been made in the biology of the most severe forms of affective (mood) illness, unipolar depression and manic-depressive (bipolar) disorders. In this symposium the neuroendocrinology, neurochemistry, and molecular genetics of affective disorders will be reviewed as well as the preclinical literature on behavioral and neuropharmacological approaches to the study of these disorders. Specifically Nemeroff will review the now well-documented neuroendocrine alterations that occur in drug-free depressed patients concentrating on the hypothalamic-pituitary-adrenal axis and growth hormone responses to provocative stimuli such as clonidine, an α_2-adrenergic agonist. Langer will describe neurochemical studies that demonstrate alterations in serotonergic and catecholaminergic neurotransmission in depression. Egeland will review studies using modern techniques of molecular genetics to identify the loci of genetic vulnerability to these devastating psychiatric diseases. Weiss will discuss the available animal models of depression and mania and critically assess their validity. Sulser will discuss preclinical neuropharmacological strategies to elucidate the mechanisms by which tricyclic antidepressants exert their therapeutic effects.

EXCITATORY AMINO ACIDS: EXCITOTOXICITY I

195.1

GANGLIOSIDES EXERT A GLUTAMATE RECEPTOR ABUSE DEPENDENT ANTAGONISM (RADA). M. Favaron*, H. Manev, A. Guidotti, and E. Costa. FGIN, 3900 Reservoir Rd., Washington, DC, 20007

In primary cultures of rat cortical or cerebellar neurones the persistent overstimulation ("abuse") of glutamate receptors leads to neuronal death. Natural gangliosides prevent glutamate-induced neuronal death without affecting the immediate physiological responses elicited by glutamate receptor stimulation. Hence, their action could be defined as Receptor Abuse Dependent Antagonism (RADA). Structural changes in the molecule of gangliosides (GM1) resulted in semisynthetic derivatives (PKS and LIGA), which are more potent RADA agents than the parent natural compound. Unlike GM1, the PKS and LIGA derivatives lack the amide-linked fatty acid tail of the ceramide structure. If the NH_2 group is left free (as in LysoGM1), the compound possess cytolytic activity and therefore no protective action. However, blockade of the free NH_2 group resulted in compounds (PKS 3, LIGA 4 and 20) with decreased cytotoxicity and a potent protective (RADA) effect against glutamate-induced neuronal death. The rank order of RADA potency is LIGA 4≥, LIGA 20>PKS 3 >GM1 (EC50 = LIGA 4 and 20, 5uM; PKS 3, 30uM; GM1, 55uM). The semisynthetic compounds act faster than natural ganglioside (10 min preincubation for PKS 3 and no preincubation required for LIGA 4 and 20, vs. 60-120 min for GM1). Moreover, the protective effects of LIGA 4 and 20 last longer than those of GM1. Since these compounds do not affect the electrophysiological responses and the stimulation of c-fos mRNA expression triggered by stimulation of glutamate receptors they may be considered a new class of RADA effectors with improved onset and duration of action.

195.2

ROLE OF DELAYED CA++ INFLUX AND PROTEIN KINASE C (PKC) TRANSLOCATION IN GLUTAMATE-INDUCED NEURONAL DEATH. H.Manev, M.Favaron*, G.Brooker, A.Guidotti and E.Costa. FIDIA-Georgetown Institute for Neurosciences; Dept. Biochemistry and Molecular Biology ; Georgetown University, Washington, DC, 20007.

We studied the mechanism of delayed glutamate-induced (50 uM, 15 min, no Mg++) neuronal death using primary cultures of rat cerebellar granule cells. In the interval between the end of glutamate receptor stimulation and the onset of neuronal death (post-glutamate period; PGP) neurotoxic concentrations of glutamate induced a sustained increase of: a) 45Ca++ uptake; b) intracellular Ca++ levels assayed by microscopic fluorimetric (fura 2) technique accompanied by "Attofluor" image analysis; c) PKC translocation measured by 3H-phorbol ester binding. These changes were insensitive to PGP treatment with blockers of glutamate receptor or voltage-dependent Ca++ channel, but were reverted by the removal for 60 min of extracellular Ca++. This Ca++ removal rescued from death about 70% neurons. Pretreatment with ganglioside GT1b failed to reduce the Ca++ influx during glutamate receptor stimulation, but prevented the successive increases in Ca++ levels, PKC translocation and neurotoxicity. Protection was also obtained by 24 h treatment with 100 nM phorbol 12-myristate 13-acetate which led to a large depletion of 80 kDa type II PKC isoenzyme. The activation of PKC following glutamate receptor stimulation may play a role in maintaining the elevation of intracellular Ca++ levels in PGP which activate enzymatic processes leading to neuronal death.

195.3

FREE RADICAL SCAVENGERS REDUCE EXCITATORY AMINO ACID RELEASE INDUCED BY SIMULATED ISCHEMIA. D.E. Pellegrini-Giampietro*, G. Cherici*, V. Carla'* and F. Moroni. Dept. of Pharmacology, Univ. of Florence, Italy.

The excitatory amino acids aspartate (Asp) and glutamate (Glu) are released in excessive amounts following hypoxic or ischemic injuries to the brain (Rothman, S.R. & Olney, J.W., *Ann. Neurol.*, 19: 105, 1986). Another possible pathogenetic event leading to ischemic neuronal damage appears to be the formation of toxic free radicals (Raichle, M.E., *Ann. Neurol.*, 13: 2, 1983). This prompted us to study whether there is a link between these two events.

The release of endogenous Asp and Glu (detected by HPLC) was studied from male Wistar rat hippocampal slices studied in an *in vitro* model of ischemia. The simulated pathology was achieved by incubating the slices, after electrophysiological viability control (Corradetti R. et al., *J. Neurochem.*, 41: 1518, 1983), for 10 min in a glucose-free Krebs-bicarbonate solution saturated with N_2 95% / CO_2 5%. This treatment induced an increased release of both Asp and Glu (14- and 20-fold respectively versus basal release). No release of the cytoplasmic enzyme LDH was detected. When the "ischemic" slices were exposed to compounds able to "scavange" free radicals such as mannitol (1 mM), catalase (50 µg/ml) or superoxide dismutase (50 µg/ml) the ischemic-induced release of Asp and Glu was significantly reduced. Compounds able to inhibit enzymatic free radical production such as indomethacin (50 µM) or corticosterone (50 µM) were also protective, although to a lesser degree. Furthermore, when "non-ischemic" slices were exposed to an enzymatic reaction leading to free radical formation (xanthine plus xanthine oxidase), endogenous Asp and Glu release was significantly increased. This release was again inhibited by mannitol, catalase and superoxide dismutase.

These results suggest that free radical formation during and after ischemic injuries to the brain lead to the output of excitatory amino acids. These two events may mutually contribute to the pathogenesis of the death of neurons, and the interruption of this vicious circle may be of therapeutic importance.

(Supported by the University of Florence and by the Region of Tuscany).

195.4

21-AMINOSTEROIDS REDUCE CORTICAL NEURONAL INJURY INDUCED BY IRON OR BY "ISCHEMIA" IN VITRO. H. Monyer, D.M. Hartley and D.W. Choi. Dept. of Neurology, Stanford Univ. Med. Sch., Stanford CA 94305.

Murine neocortical cell cultures exposed to 50 µM Fe^{2+} + 50 µM Fe^{3+} for 24 h developed substantial neuronal degeneration without overt glial damage. This iron neurotoxicity probably reflected a substantial contribution from iron-facilitated free radical formation, as it could be markedly reduced by 5 µM concentrations of the novel Upjohn 21-aminosteroids, U74500 and U74006, which have been shown to be potent lipid peroxidation inhibitors. As lipid peroxidation has been postulated to be important in the pathogenesis of ischemic neuronal injury, we examined the effect of these drugs against combined oxygen and glucose deprivation in the same cultures. Such in vitro "ischemia" for 45 to 60 min produced widespread neuronal degeneration in untreated cultures. Addition of optimal (5 µM) concentrations of U74500, U74006 and U75412 reliably attenuated resultant neuronal damage, although this attenuation (30 - 80% reduction of injury) was not generally as large as that produced by the NMDA antagonist, dextrorphan. In fact, lipid peroxidation may be an important downstream event following NMDA receptor activation, as the neurotoxicity of exogenously applied glutamate could also be partially attenuated by these 21-aminosteroids.

195.5

NIFEDIPINE ATTENUATES AMPA OR KAINATE NEUROTOXICITY. J.H. Weiss, J. Koh and D.W. Choi. Dept.of Neurology, Stanford Univ. Med. Sch., Stanford CA 94305.

Murine cortical cultures exposed for 5 min to glutamate or other NMDA agonists go on over the next hours to develop widespread neuronal degeneration. This late degeneration depends on the presence of extracellular calcium and may be at least partially mediated by calcium influx through NMDA channels. In contrast, 5 min exposure to the selective non-NMDA agonists, kainate and AMPA, produces little neuronal degeneration; substantial neuronal degeneration is seen only after exposure times of more than an hour. To test the hypothesis that calcium influx through voltage-dependent channels may contribute to the slow neurotoxicity of these compounds, we investigated the effect of the dihydropyridine calcium channel antagonist, nifedipine. Cultures exposed for 24 h to either 10 μM AMPA or 30 μM kainate developed widespread neuronal degeneration, apparent both by morphological examination and by the efflux of lactate dehydrogenase from damaged neurons. Addition of 10 - 100 μM nifedipine to the exposure solution produced concentration-dependent partial attenuation in this non-NMDA agonist-induced neurotoxicity. Calcium ion influx through voltage-dependent calcium channels may contribute to the neuronal injury induced by prolonged over-activation of non-NMDA glutamate receptors.

195.6

INCREASED EXCITATORY NEUROTRANSMISSION RESULTS IN THE DEATH OF CULTURED RAT HIPPOCAMPAL PYRAMIDAL NEURONS. April E. Abele*, Wendy K. Scholz, Kenneth P. Scholz, and Richard J. Miller (Sponsored by: S. Glusman). Department of Pharm. & Physiol. Sci., U. of Chicago, Chicago IL 60637

We investigated the consequences of increased glutamate mediated neurotransmission on the viability of cultured hippocampal pyramidal neurons(HPN). HPN were cultured from 17 day old fetuses according to the method of Banker and Cowan(1977) as modified by Scholz(1988). Cells were used after 10-20 days in culture. Immunohistochemical studies revealed that these cultures were composed primarily of neurons(3-5% astrocytes) that reacted with antibodies against glutamate. Approximately 5% of the neurons reacted with antibodies against GABA. Furthermore, electrophysiological studies revealed that the majority of synapses are excititatory in these cultures. We monitored $[Ca^{2+}]_i$ in single neurons using fura-2 based microfluorimetry. When Mg^{2+} was removed and >10nM glycine added, $[Ca^{2+}]_i$ showed large oscillations(see Miller et al. this volume). These fluctuations could be blocked by tetrodotoxin(TTX) or AP5 . We assessed the viability of the HPN following various treatments using a combined fluorescein/propidium staining procedure. When cells were incubated in Mg^{2+}-free medium for 15 minutes and examined 24 hours later, the percentage of dead cells in each culture increased from a baseline value of 10-15% to 30-40%. The increase in cell death induced by Mg^{2+}-free medium could be further enhanced by the addition of glycine to the medium during the Mg^{2+}-free period. Agents that reduced Mg^{2+}-free/glycine induced $[Ca^{2+}]_i$ oscillations such as TTX or 2-Cl-adenosine prevented the increase in cell death. Addition of exogenous NMDA or glutamate(100μM-1mM) in Mg^{2+}-free medium to the cultures resulted in the death of >75% of the cells present. Thus increased NMDA receptor mediated synaptic activity can produce "excitotoxicity" in cultured cells. This may be a model for cell death resulting from seizure activity.

195.7

GLUTAMATE NEUROTOXICITY IN VITRO: ANTAGONIST PHARMACOLOGY AND INTRACELLULAR CALCIUM CONCENTRATIONS. R.L. Michaels* and S.M. Rothman, Depts. of Biological Chemistry and Anatomy & Neurobiology, Washington University School of Medicine, St. Louis, MO 63110

The accumulation of extracellular glutamate (GLU) is likely responsible for neuronal death in a number of human diseases. However, the role of different GLU receptors in producing cell death has not been adequately determined. In addition, there is little information correlating free intracellular calcium levels (Ca_i) with eventual excitotoxic death. We used cultured neonatal rat hippocampal neurons to try to address these issues.

Approximately 75% of our neurons died after a 20 minute exposure to 500 μM GLU. The potent kainate/quisqualate antagonist CNQX (200 μM) blocked GLU currents by 85%, but did not significantly ameliorate GLU toxicity, while the noncompetitive N-methyl-D-aspartate (NMDA) antagonist MK-801 blocked GLU toxicity for at least two hours. Interestingly, kainate (1 mM) was still toxic for these neurons, but the kainate damage was also blocked by MK-801. Ca_i at the end of 20 minute exposures to GLU, kainate or 123 mM potassium (± various antagonists) correlated poorly with eventual cell death. As expected, GLU elevated steady-state Ca_i. Neurons treated with GLU + CNQX had normal Ca_i levels at 20 minutes, yet most still died 18 hours after return to control solution. MK-801 kept the Ca_i low and prevented eventual death. On the other hand, 123 mM potassium elevated Ca_i without causing significant delayed cell death.

These results are consistent with previous reports emphasizing the importance of NMDA receptors in GLU toxicity. However, they suggest that the link between Ca_i and eventual cell death is not so direct.
(Supported in part by NIH grants NS19988 and GM38838, AHA Grant 861413, and the Monsanto Corporation.)

195.8

A POSITIVE FEEDBACK LOOP GENERATING EXTRACELLULAR GLUTAMATE IN CORTICAL CULTURES EXPOSED TO GLUTAMINE. D. Crawford* and P.A. Rosenberg (SPON: S. Fischel). Dept. of Neurol., Children's Hosp., Harvard Med. Sch., Boston, MA 02115

The existence of a positive feedback loop contributing to glutamate neurotoxicity (GNT) in the forebrain has been postulated on the basis of the observation that a reduction in GNT is achieved by exposure of hippocampal (Rothman et al., Neuroscience, 22: 471, 1987) and cortical (Choi et al., J. Neurosci., 8: 185, 1988) cultures to glutamate antagonists following exposure to glutamate. By using astrocyte-poor (neuron-enriched) cultures of rat cerebral cortex (containing 50% neurons), it has been possible to demonstrate a positive feedback loop in which, in the presence of glutamine, cortical cultures generate extracellular glutamate by a process which can be completely blocked by the NMDA antagonist APV. Exposure of astrocyte-poor cultures to MEM plus 2 mM glutamine for 17-24 hours resulted in the accumulation of 255±158 μM glutamate (n=31) in the medium, and a proportionate reduction in glutamine. This phenomenon was neither seen in the absence of cultures nor in the presence of astrocyte-rich cultures (with 6% neurons). Coincident with this accumulation of glutamate is the death of most neurons in these cultures. Both the accumulation of glutamate and neuronal death can be blocked by APV. Supported by the Grass Foundation and NS00993.

195.9

L-CYSTEINE, AN ENDOGENOUS NMDA-MIMETIC EXCITOTOXIN THAT INDUCES GENERALIZED ISCHEMIA-LIKE NEONATAL OR FETAL BRAIN DAMAGE. J.W. Olney, J. Labruyere*, M.T. Price,Washington Univ., St.Louis, MO

Following systemic administration to infant or pregnant rodents, L-glutamate (Glu) and L-cysteine (Cys) induce a similar type of brain damage, but Glu damages only brain regions that lack blood brain barriers and Cys induces generalized damage throughout the infant or fetal brain (Olney et al., Br. Res., 45, 309, 1972). Recent evidence suggests that excitotoxic (including hypoxic/ischemic) brain damage in the immature brain is mediated primarily by the N-methyl-D-aspartate (NMDA) subclass of Glu receptor. Interestingly, injection of nmol amounts of NMDA into the immature rat brain causes disseminated Glu-like lesions affecting the frontoparietal neocortex, hippocampus, septum, caudate and thalamus, which is the same pattern of damage induced in immature rodent brain by hypoxia/ischemia or systemic Cys administration. In the isolated chick embryo retina, Cys and NMDA have identical cytopathological effects, and NMDA antagonists, including those acting at the NMDA, glycine, zinc, PCP or Mg^{++} sites, block Cys toxicity with potencies directly proportional to their potencies in blocking NMDA toxicity. Antagonists that act preferentially at non-NMDA receptors, such as CNQX, do not block Cys toxicity except when used in concentrations high enough to also block NMDA receptor activity. The powerful NMDA antagonist, MK-801, protects the infant rat brain against systemic Cys neurotoxicity. Based on evidence not detailed here, we consider Cys an unconventional excitotoxin that acts by a complex mechanism involving Zn^{++} and/or glycine components of the NMDA receptor channel complex. Since Cys is naturally present in brain, it can be added to the list of endogenous excitotoxins that might participate in neurodegenerative diseases. Moreover, since it penetrates both blood-brain and placental barriers, it is a valuable *in vivo* systemic tool for probing the vulnerability of developing CNS neurons to NMDA receptor-mediated excitotoxic processes. Supported by RSA MH 38894 (JWO) and HD 24237.

195.10

FOLATE AND QUISQUALATE CYTOTOXICITY IN FROG RETINA Z.H. Zhang,J.C. Blanks and S.R. Snodgrass. Neurol. Research Lab., Childrens Hospital Los Angeles and Doheny Eye Institute, Depts. Neurol. and Ophthal., Univ. So. Calif. Schl. of Med., Los Angeles, CA 90027

Various folates cause excitotoxic brain lesions. Only neural cells are folate-sensitive, and they vary greatly in sensitivity. All studies have found folic acid (FA) to be a more potent neurotoxin than 5-methyltetrahydrofolic acid (MTHF),the predominant extracellular folate. We incubated the frog retinal eyecup preparation with folates or excitatory amino acids, using morphological change to assess cytotoxicity. Thirty minute incubations with 1 mM FA produce cytotoxic changes in the inner nuclear layer, mainly in amacrine cells. Quisqualate (500 uM) destroyed multiple retinal elements, approaching the severity of kainate lesions. Our Quis lesions resembled those described by Sattayasi in the chick. FA and Quis cause translocation of Protein kinase C, shown by altered binding of ^{3}H phorbol dibutyrate. This simple model facilitates study of the role of protein kinases and excitatory amino acid receptors in cell death due to folates or Quis. The link between cytotoxicity and altered phorbol binding suggests that C kinase may mediate cytotoxicity.

195.11

REQUIREMENT FOR GLYCINE IN NMDA-INDUCED EXCITOTOXICITY IN RAT CORTICAL CELL CULTURE. D. McNamara* and R. Dingledine. (Spon: J.D. Mann). Dept. Pharmacology, Univ. North Carolina, Chapel Hill, NC 27599-7365.

The production of cation currents by NMDA in both mRNA-injected Xenopus oocytes and neurons appears to require glycine. Glycine also potentiates NMDA-induced excitotoxicity in rat cortical neurons (McNamara and Dingledine, Soc. Neurosci. Abs. 14: 236, 1988). NMDA is excitotoxic in the absence of added glycine, however, perhaps due to the presence of glycine released from cultured neurons. If this were the case, a selective glycine antagonist should abolish excitotoxicity incurred by exposure to NMDA. To test this hypothesis, E19 rat cortical neurons were exposed after 11 days in culture to 100μM NMDA and 0-1000 μM glycine for 5-60 min at 36 C. Following removal of the exposure solution, cells were incubated for 18-22 hours in growth medium containing 100μM D,L-APV to eliminate further action by residual NMDA and subsequent glutamate released from dying neurons. Degree of cell death was inferred by the amount of lactate dehydrogenase (LDH) activity released into the medium and expressed as a percentage of the total LDH available. In each of four experiments in which cells were exposed to 100μM NMDA with no added glycine for 10-30 minutes, 21% of the available LDH was released. When either 30μM 7-Cl-kynurenate or 100μM D-APV was included, however, no release of LDH above control was observed. 7-Cl-kyn also produced a shift to the right of the glycine dose-response curve in the excitotoxicity assay. In Xenopus oocytes 7-Cl-kyn has no effect on the potency of NMDA but is a competitive glycine antagonist with 40-fold selectivity over the quisqualate receptor (Kleckner and Dingledine, this meeting). These results indicate that glycine is required for NMDA-induced excitotoxicity, and suggest that glycine site blockers should be equally efficacious as NMDA antagonists in treating ischemic neuronal injury.

195.12

DOSE-DEPENDENT DECREASE OF VASOACTIVE INTESTINAL PEPTIDE (VIP) BUT NOT CHOLECYSTOKININ (CCK) FOLLOWING KAINIC ACID LESIONS OF THE STRIATUM.
M.F.Mazurek, K.J.Swartz, S.Garside*, M.F.Beal. McMaster Univ. Med. Ctr., Hamilton ONT. and Mass. Gen Hosp, Boston

We have recently shown that striatal concentrations of VIP are elevated 3- to 4-fold in Huntington's disease and 4- to 5-fold in rats with chronic quinolinate (QUIN)-induced lesions. Striatal levels of CCK, which like VIP is thought to arise for the most part from extrastriatal cell bodies, are normal in HD and increased only 2-fold in chronic QUIN-lesioned rats. We studied the dose-response profiles of VIP and CCK following excitotoxic striatal lesions induced by the selective glutamatergic agonists kainate (KA), QUIN, quisqualate (QUIS) and AMPA. KA produced a dose-dependent decrease in striatal VIP-like immunoreactivity, with levels reduced by 35% after 7.5 nmol KA lesion and by 43% following 10 nmol KA. CCK measured in the same samples was unchanged from control values. Neither VIP nor CCK was affected by increasing doses of QUIN, QUIS or AMPA. These results suggest that up to 40% of striatal VIP may be in a neuronal compartment that is sensitive to KA but not to the NMDA-receptor-agonist QUIN or the quisqualate agonists QUIS and AMPA. The VIP-containing cells appear to be up-regulated in Huntington's disease and in rats with chronic QUIN lesions Striatal CCK measured in parallel to VIP does not show the changes observed with VIP.

HUMAN BEHAVIORAL NEUROBIOLOGY: OTHER I

196.1

CATEGORY TYPICALITY EFFECTS IN ALZHEIMER'S DISEASE (AD). A. Cronin-Golomb, M.M. Keane, S. Corkin, J.H. Growdon. Dept. of Brain and Cognitive Sciences, MIT, Cambridge MA 02139 and Dept. of Neurology, Mass. General Hospital, Boston MA 02114.

Categorization deficits occur in AD, but no explanation has been suggested for deficient performance. We asked whether such deficits could be related to reduced category typicality effects in AD. In the normal effect, subjects are faster to judge high- (HTE) than low-typical exemplars (LTE) as members of a category. Subjects with AD of a wide range of dementia severity (N=8) and age-matched healthy control subjects (HCS: N=8) were compared on a category decision task. Words were derived from 12 categories, each of which included 6 HTE and 6 LTE. Subjects were asked,"Is the next word a kind of [category name]?" and responded 'yes' or 'no' to the word presented on the monitor. The measure was voice-activated reaction time (RT). Analysis of variance indicated that the two groups differed neither in overall speed nor in the effect of typicality; RTs were significantly faster for HTE than LTE ($p<.05$). Inspection of the data suggested that some AD patients have an enhanced typicality effect that may be accounted for by slowed RTs to LTE: AD patients were on the average 12% slower than HCS on HTE but 25% slower on LTE. Further, for the AD group, mean RT for LTE correlated strongly with dementia severity ($r=0.64$), whereas the correlation was relatively weak for HTE ($r=0.26$). The results suggest that category typicality effects are robust in AD. Categorization deficits in other tasks are probably not related to abnormally reduced typicality effects, but may occur when tasks involve LTE specifically.

196.2

DISRUPTION OF HAND AND JOINT KINEMATICS IN LIMB APRAXIA. H. Poizner, J.F. Soechting, M. Bracewell, L.J. Rothi, and K.M. Heilman. The Salk Institute, La Jolla, CA 92138, The University of Minnesota, The University of Florida, and V.A. Medical Center, Gainesville.

Apraxia is a disorder of the execution of learned, skilled movements in subjects with intact afferent and efferent systems, and its study can help illuminate the nature of cerebral organization in relation to movement. A major problem, however, in investigating the nature of this disorder has been the difficulty of obtaining objective measurement of movement in three-dimensional space. Three subjects with unilateral lesions in the left hemisphere and apraxia, two matched subjects with lesions in the right hemisphere, and five neurologically intact control subjects were asked to produce gestures requiring programming of complex trajectory timing and spatial relationships. Movements were digitized in three dimensional space and reconstructed graphically. Amplitudes and phase relations among relevant joint motions were computed, and joint and hand kinematics analyzed. Strikingly, the movements of subjects with right hemisphere lesions were virtually indistinguishable from those of control subjects. The apraxic subjects, however, showed marked distortions in both hand and joint kinematics even when imitating gestures or when given an actual tool to use. These data imply that the right hemisphere by itself may not contain all the information necessary to program the spatial or temporal aspects of skilled movements. Furthermore, since apraxic subjects showed errors in timing, spatial trajectory, and joint control even when imitating gestures and when manipulating tools, the basis of apraxia is likely not to rest on a disconnection between language and motor areas, but rather on a disturbance of spatiotemporal representations of skilled motor acts.

196.3

STRUCTURE OF OBJECTS IN CENTRAL VISION AFFECTS THE DISTRIBUTION OF VISUAL ATTENTION IN NEGLECT. M.J. Farah*, J.L. Brunn*, M.A. Wallace* and N. Madigan* (SPON: M. Oscar-Berman) Department of Psychology, Carnegie-Mellon University, Pittsburgh, PA 15213.

Is visual attention allocated to locations, objects, or both? The attentional deficit for one side of space in neglect suggests that location per se plays a role. By varying the structure of objects in central vision while holding the locations of stimulus targets constant we found evidence that attention is also allocated to objects per se. In one experiment, subjects with left neglect viewed pairs of meaningless forms that either straddled midline or were contained one within each of the two hemifields, and performed a visual search task for superimposed letters scattered throughout the visual field. There was less neglect of the left hemispace when background shapes straddled the hemifields. In a second experiment, more abstract 'objects' were used: subjects viewed either words or nonword letter strings, each letter of which was printed in a different color ink. Subjects named more colors on the left side of words than nonwords. These results imply that attention is allocated to entire objects, whether the objects are defined by low-level physical features or by familiarity of pattern, in addition to spatial location per se.

196.4

PULVINAR ACTIVITY IN HUMANS DURING A VISUAL ATTENTION TASK AS MEASURED BY PET IMAGES **David LaBerge and Monte S. Buchsbaum, Depts of Cognitive Science and Psychiatry, Univ of California, Irvine, CA 92717.**

We compared glucose uptake of the right and left pulvinars of 7 human subjects after they had performed a 32-minute visual identification that presented an attention-demanding display in one hemifield and a non-attention display in the other hemifield (and vice versa in a second session approximately a week later). The attention-demanding display, presented to one side of the central fixation dot, consisted of the letter O surrounded by other letters (G and Q) in a 3-by-3 item ensemble, and subjects were instructed to press a button if the center letter was the letter O, but not to respond if the center letter was a C or Ø. The non-attention display, presented to the other side of the dot, consisted of a single large letter O to be discriminated against the letter C or Ø, and subjects were to press a second button if the letter O appeared. The second session reversed the sides of the display types.

The pulvinar and mediodorsal (as a control) nuclei of each subject's thalami were traced on her MRI record, and then the glucose uptake within the corresponding areas of her PET images were compared across the two sessions. The results showed that, averaged across the two sessions, the pulvinar showed greater glucose uptake when it was contralateral to the attention-demanding display than when it was contralateral to the non-attention-demanding display. Parallel analyses of the mediodorsal nuclei, frontal white matter, and 20 occipital subareas revealed no significant task by hemisphere interactions.

The results support the hypothesis that the pulvinar operates interactively with cortical structures when an identification process demands attentional filtering. **(Supported by ONR and the MacArthur Foundation.)**

196.5

FOCUSING AND SHIFTING OF ATTENTION IN AUTISTIC SUBJECTS: ERP AND BEHAVIORAL FINDINGS. N. Akshoomoff*, E. Courchesne* and K. Ciesielski* (SPON: J. Johnson). Neuropsychology Research Lab., Children's Hospital, San Diego, CA 92123 and Dept. of Neurosciences, UCSD.

Abnormalities in the ability to focus and shift attention appear to exist in autistic individuals. ERPs were recorded from in a group of 10 nonretarded autistic subjects (mean age 23.5 years) and a group of 11 Performance IQ-matched normal control subjects (mean age 21.2 years). Subjects performed simple sustained visual and auditory attention tasks, as well as a divided attention task. They were then given a task which required them to rapidly shift their focus of attention back and forth between on-going sequences of auditory and visual stimuli. Cues that signal attention shift were randomly placed in these sequences. Within 1.5 sec following a cue to shift, controls made very few false alarms to stimuli in the "old" modality, and missed very few target stimuli in the "new" modality. Autistic subjects had many false alarms and misses within several seconds following a cue to shift. These time-related errors were not evident during the focused attention tasks. Control subjects showed ERP components associated with the on-going maintenance of focused attention (Nd, N270, Nc). A parietally maximal P700 response and a P3a response, which were specifically associated with the cue to shift attention, were also found. These ERP responses were absent in the autistic subjects. Thus in the autistic subjects there was no evidence of the normal neurophysiological responses which reflect shifts of attention between auditory and visual modalities and they demonstrated considerable difficulty disengaging from one focus of attention and moving to and engaging a new one.

196.6

THE ANTERIOR CINGULATE CORTEX MEDIATES RESPONSE SELECTION IN THE STROOP ATTENTIONAL CONFLICT PARADIGM. J.V. Pardo, P.J. Pardo*, K.W. Janer*, and M.E. Raichle. Washington University School of Medicine, St. Louis, MO 63110.

Regional cerebral blood flow was measured using positron emission tomography (PET) during the performance of the classic Stroop "color/word" task in eight healthy right-handed subjects. In the first condition subjects name the color of the words presented on a video monitor. All the words are the color names congruent to the color presented. In the second condition the color of the words presented are color names incongruent to the color presented. The difference in brain activity between these two conditions could reveal brain systems involved in the attentionally mediated resolution of the conflict between the habitual response of reading words vs. the task demands of naming color. The most robust responses occurred in the anterior cingulate cortex. Other responses noted were in the left premotor cortex, left postcentral cortex, left putamen, right superior temporal gyrus, and bilateral extrastriate cortices. These data provide support for the role of the anterior cingulate cortex in attentional processing through the selection and recruitment of processing centers appropriate for task execution. Furthermore, the extensive distributed network of activated regions suggests that the Stroop interference effect can not be explained simply in terms of stimulus encoding or response interference.

196.7

STEREOPSIS: LOCAL AND GLOBAL MEASURES. A. Ptito*, R.J. Zatorre, and W.L. Larson*. (SPON: B. Milner) Montreal Neurol. Inst. McGill Univ. and School of Optometry, U. de Montréal, Canada.

In man, an impairment of stereopsis may occur following damage outside the occipital lobes. If local and global stereopsis can be dissociated, this may imply that different mechanisms mediate these functions. We tested 35 patients with unilateral temporal-lobe lesions and 18 normal control subjects on 2 tasks. In the local stereopsis task subjects indicated which of two pins varying in disparity between 4 and 512 sec of arc was closer. Results showed no threshold impairment in any groups. In the global condition, random-dot stereograms varying in binocular correlation were presented in random order, and subjects indicated if the squares perceived in depth were in front of or behind the screen. At binocular correlations between 50% and 70%, left and right temporal lobectomy resulted in a deficit, which was more marked following right-sided excisions. This study suggests, therefore, that separate mechanisms come into play for local and global stereopsis, and is consistent with earlier work [Ptito & Zatorre, Neuropsychologia 26, 547, 1988] implicating temporal neocortex in global stereopsis.

196.8

RIGHT-NOSTRIL ADVANTAGE FOR ODOR DISCRIMINATION IN RIGHT HANDERS. R.J. Zatorre and M. Jones-Gotman*. Montreal Neurological Institute, McGill University. Montreal, Quebec, Canada H3A 2B4

To investigate hemispheric asymmetry in odor processing, olfactory discrimination was tested in 96 normal subjects using a same-different task with 8 pairs of odors. Half the subjects were right-handed and the other half left-handed. Each subject's detection thresholds for phenylethyl alcohol were also measured with a staircase method separately in each nostril, and the fused words dichotic listening test was given. Results indicated a marked asymmetry favoring the right nostril in odor discrimination among the right-handers ($p<.01$), but not among the left-handers. This cannot be accounted for by sensitivity differences, as there was no significant difference between the nostrils in detection thresholds. Direction of perceptual asymmetry on the dichotic test did not correlate with the olfactory asymmetry. These results replicate earlier data [Zatorre & Jones-Gotman Soc. Neurosci. Abs. 14, 219, 1988], and together with severe olfactory discrimination deficits observed following right orbitofrontal lesions, suggest a relative right-hemisphere superiority in certain aspects of olfactory processing.

196.9

NEUROTRANSMITTER DIFFERENTIATION IN VIVO BETWEEN CONTROL AND DEVELOPMENTAL LEARNING DISABILITY HUMANS
K.A.Bonnet,J.M.Luccioni,C.Walsh,A.J.Friedhoff, Millhauser Laboratories,New York University School of Med.,New York, New York 10016

Control and developmental learning disability young adults were studied on five successive days each with baseline records and with three hours of subsequent record after challenge with substances effecting selective potentiation in individual neurotransmitter systems. Baseline and postchallenge records consisted of resting EEG and auditory and visual evoked potentials. At baseline, the learning disabled (LD) individuals showed significantly greater right frontal reduction in frequency than controls, and reduced 300 ms component with increased 200 millisecond component in the P300 procedure, most definitive at the midline frontal region. Dopamine enhancement improved the 300 ms. component of the P300 in the controls, but the LD group showed decreased 300ms. component and increased 200 ms. component. Cholinergic potentiation produced significant increase in 200 ms. compoments and decreased 300 ms. componenets of P300, as well, in the LD group compared to controls. Serotonergic potentiaion potentiated the 300 ms. components in the P300 of the LD group only, and suppressed the 200 ms. components in both groups.

Supported by The Wacker Foundation, The Courtney Block Fund The Public Health Service of France

196.10

MRI AND VISUAL PSYCHOPHYSICAL STUDIES OF INHERITED DYSLEXIA. K. Gross-Glenn, R. Duara, A. Kushch*, S. Pascal*, W. Barker*, B. Jallad*, H.A. Lubs*. Univ. of Miami School of Medicine, Miami, FL 33101 and Mt. Sinai Medical Ctr., Miami Beach, FL 33141.

The role of visual functioning in the etiology of inherited dyslexia is poorly understood. Previous PET scan studies during oral reading (Duara, R., Gross-Glenn, K., Barker, W. et al., Neurol., 39 (suppl. 1):165, 1989) showed that significant differences between dyslexics and normal readers were localized to extra-striate visual cortex, specifically the lingual region. To further explore this problem we have combined MRI and visual psychophysical studies on right-handed adult dyslexics and normal readers. Morphometric area measurements were made on axial MRI scans (N=51) at the level of the Foramen of Monro. From these data laterality indices [200(R-L)/(R+L)] were calculated for homologous regions. No significant differences were observed in anterior areas, however mid-posterior regions revealed significantly more rightward asymmetry for dyslexics as compared with the leftward asymmetry observed in normal readers ($p=.005$). Normalized measurements of mid-sagittal corpus callosum area showed dyslexics to have a relatively larger splenium than normal readers ($p=.005$).

A subset of these individuals (12 dyslexics, 10 normal readers) participated in a visual psychophysical study. Spatial and temporal parameters were varied in a visual forward-masking paradigm that assessed contrast sensitivity thresholds for luminance sine wave gratings displayed on a video screen. Grating duration was varied and targets were presented either with or without a visual mask. These studies revealed very specific differences between dyslexics and normal readers that was limited to detection of masked high spatial-frequency gratings. The mask reduced perceptibility of low spatial frequency gratings for both groups equally. However, for high spatial frequency gatings, the time required to escape from the effects of the visual mask was significantly extended for dyslexics as compared with normal readers ($p < .05, .01, .04$ for targets durations of 17, 33, 100 msec, respectively). These studies point to very specific visual system differences in both functioning and anatomy for dyslexics as compared with normal adult readers.

196.11

RESOLUTION OF MULTIPLE SOURCES IN VISUAL EVOKED NEUROMAGNETIC RESPONSES. C.J. Aine, J.S. George, P.A. Medvick*, S. Supek* and E.R. Flynn. Life Sciences and Physics Divisions, MS M882, Los Alamos National Laboratory, Los Alamos, NM, 87545.

Although magnetoencephalography (MEG) offers increased spatial and/or temporal resolution relative to other noninvasive mapping techniques (e.g., ERPs and PET), the technique is limited by the assumptions of the models used to localize the source(s) of neuromagnetic field distributions. Visual neuromagnetic data suggest the existence of multiple sources when large areas of cortex are examined; therefore, we have developed procedures for resolving multiple, temporally synchronous and/or asynchronous sources.

Neuromagnetic field maps in response to small sinusoidal gratings placed in different locations of the visual field were obtained from four right-handed human subjects. In response to central field stimulation, left and right hemisphere sources with opposing orientations were resolved in three of the four subjects. When the stimulus was presented at 7° along the horizontal meridian in the right visual field, two sources were evident in the left hemisphere: one close to midline and another more lateral (approximately 5-6 cm from the midline). In other examples, two asynchronous sources were evident in the field distributions. In several subjects we have observed an apparent source at 100 msec poststimulus followed by a distinct second source at 120 msec. In conclusion, if multiple source models are utilized, MEG allows powerful inferences concerning spatial and temporal patterns of activation within the human visual system.

PRESYNAPTIC MECHANISMS II

197.1

PAIRING-SPECIFIC, ACTIVITY-DEPENDENT FACILITATION OF *APLYSIA* SENSORY-MOTOR NEURON SYNAPSES IN ISOLATED CULTURE. L.S. Eliot*, S. Schacher, E.R. Kandel, and R. D. Hawkins (SPONS: L.K. Simmons). Ctr. Neurobiol. & Behav., Columbia Univ., HHMI, & NYSPI, NY, N Y 10032.

When tetanic stimulation of a sensory neuron is combined with 5-HT, it produces longer-lasting facilitation of *Aplysia* sensory-motor synapses in culture than either tetanus or 5-HT alone (Montarolo and Schacher, *Soc. Neurosci. Abstr.* 14:909). We have now begun to analyze the temporal specificity of this effect. In cultures containing two sensory neurons (SNs) synapsing on a single LFS motor neuron, one SN received tetanus (20 Hz, 2s) paired with 5-HT, while the other received tetanus 1 min before 5-HT application. Paired training produced greater facilitation overall than unpaired training ($n = 14$, $F = 7.17$, $p < .02$). At 5', 10' and 15' the paired EPSP was more facilitated than the unpaired EPSP ($p < .02$ in each case; average = 114 ± 11% vs. 68 ± 7% of pre-tests). Similar results were obtained when a single SN was given two rounds of training, either paired followed by unpaired or vice-versa. Pairing produced an overall enhancement of facilitation at 5', 10' and 15' ($n = 20$, $F = 5.49$, $p < .05$). At each time point, the paired EPSP was more facilitated than the unpaired EPSP ($p < .05$, one-tailed in each case; average = 94 ±8% vs. 74 ±6%). These experiments demonstrate that pairing-specific facilitation can occur at single sensory-to-motor neuron synapses in culture, even in the absence of extra circuitry.

197.2

IP_3 RELEASES INTRACELLULAR CALCIUM IN *APLYSIA* SENSORY NEURONS. H. Blumenfeld, E.R. Kandel, and S.A. Siegelbaum. Ctr. Neurobiol. & Behav., Columbia Univ., HHMI, New York, New York 10032.

The sensory-motor neuron connection in *Aplysia* is modulated through two second messenger systems. 5-HT causes presynaptic facilitation through a cAMP cascade and FMRFa causes presynaptic inhibition through an arachidonic acid cascade. Is IP_3, which releases calcium from internal stores, also involved in the actions of these neuromodulators? To directly test the ability of IP_3 to release calcium in *Aplysia* sensory neurons, we injected IP_3 while measuring Ca_i with fura-2. Brief (10-50 ms) pressure pulses from electrodes containing 1.0 mM IP_3 caused a transient rise in Ca_i of 113 ± 11 nM (mean ± SEM; n = 21). Myo-inositol caused little or no response. Removal of external Ca eliminated Ca_i transients produced by action potentials but did not affect the response to IP_3. Since 5-HT and FMRFa do not increase resting Ca_i in sensory neurons, neither transmitter appears to stimulate significant IP_3 production.

We next asked whether 5-HT alters the Ca_i transient in response to IP_3, since 5-HT causes a 50% increase in the Ca_i transient produced by action potentials or voltage clamp depolarizations. The amplitude of the IP_3-induced Ca_i transients was not affected by 10 μM 5-HT (n = 13). However, in four out of seven experiments with IP_3 injections, 10 μM 5-HT caused a two-fold increase in the time constant of recovery of Ca_i.

197.3

PRESYNAPTIC INHIBITION OF TRANSMITTER RELEASE AT A GIANT SYNAPSE: I. FMRFa MODULATION OF ION CURRENTS. M. J. Zoran, H. J. Man-Son-Hing, L. R. Funte*, K. Lukowiak, and P. G. Haydon. Department of Zoology, Iowa State University, Ames, IA 50011.

The neuropeptide, Phe-Met-Arg-Phe-NH_2 (FMRFa; 1μm) modulates cholinergic connections between buccal neurons B5 and B19 of *Helisoma* in cell culture. Under conditions that inhibit neurite extension, sperical somata adopt synaptic functions. **Using giant synapses, we have demonstrated that FMRFa causes a presynaptic inhibition of acetylcholine release.** Neuron B5 (Pre) and neuron B19 (post) were voltage clamped using whole cell patch pipettes. Presynaptic B5 was depolarized from -60mV to command potentials ranging from -20mV to +10mV (duration 4s) to evoke desynchronized release of acetylcholine (ACh). Transmitter release was detected as tubocurarine-sensitive inward postsynaptic currents (p.s.c.s) in B19 (-80mV). Application of FMRFa (1μM) reversibly reduced the number of evoked p.s.c.s (n=9) while having negligible effects on postsynaptic sensitivity to ACh. The sub-cellular mechanisms involved in this FMRFa effect are multiple. First, FMRFa causes a reversible hyperpolarization of neuron B5 and increases the membrane (input) conductance. Second, FMRFa reversibly reduces a high voltage activated (HVA) calcium current within secretory membrane of neuron B5 (n=18)

Presynaptic inhibition of synaptic transmission also occurs at growth cones of neuron B5. Utilizing the calcium sensitive probe Fura-2, we have demonstrated that, like the giant synapse, FMRFa reversibly reduces AP-evoked intracellular Ca^{2+} transients in growth cones of B5.

197.4

PRESYNAPTIC INHIBITION OF TRANSMITTER RELEASE AT A GIANT SYNAPSE: II. FMRFa MODULATION OF SECRETORY APPARATUS. P. G. Haydon., M. J. Zoran, H. J. Man-Son-Hing, and K. Lukowiak. Department of Zoology, Iowa State University, Ames, IA 50011.

The neuropeptide Phe-Met-Arg-Phe-NH_2 (FMRFa; 1μm) modulates cholinergic connections between buccal neurons B5 and B19 of *Helisoma* in cell culture. **Using the synapse which forms between spherical somata we have demonstrated that FMRFa causes a decrease in the sensitivity of secretory apparatus to internal Ca^{2+}.**

The photolabile calcium cage Nitr-5 was dialysed into a voltage clamped presynaptic cell (B5) to control internal calcium independent of ion conductances. In response to UV light, calcium was released from Nitr-5 and ACh secretion was stimulated. Under conditions of elevated internal calcium, we applied FMRFa (1-10μM) from a puffer pipette to the synapse while measuring the rate of desynchronized ACh release, detected as inward postsynaptic currents (p.s.c.s) in the voltage-clamped postsynaptic cell (B19). **FMRFa caused a 65% reduction in the rate of p.s.c.s (n=9) without affecting the level of internal free calcium.**

Thus, FMRFa causes a presynaptic inhibition of transmitter release by modulating multiple subcellular processes. FMRFa reduces the influx of calcium in response to action potentials by regulating ion conductances of the secretory membrane (Zoran et al., Neurosci. Abst 1989; Man-Son-Hing and Haydon, Neurosci. Abst, 1989) and, as demonstrated here, reduces the sensitivity of the secretory apparatus to internal calcium. Supported by a grant from the NIH, NS26650.

197.5

CALCIUM INDICATOR LOADING IN MOUSE MOTOR NERVE TERMINALS. Z.-P. Fang* and N. Robbins (SPON: R. J. Lederman). Center for Neurosciences, School of Medicine, Case Western Reserve University, Cleveland, OH 44106.

Calcium-sensitive dyes have proven useful in measuring intracellular calcium concentrations. Recently the dye fura-2 was used to examine presynaptic calcium concentration at motor nerve terminals in invertebrates (K. R. Delaney, et al., Soc. Neurosci. Abstr. 14:1206, 1988). However, this methodology has not been applied to mammalian motor nerve terminals because of the difficulty of injecting the dye into small (<5um) myelinated motor axons.

We have developed a technique for loading fura-2 into the presynaptic nerve terminals at mouse neuromuscular junction. The soleus and sternocleidomastoid muscle were stained in vitro with fluorescent dyes to visualize the junction areas. The muscle was then transferred to a bath chamber on the stage of an upright microscope. A fine-tip glass micropipette (150-300MOhm with 100mM KCl) was back filled with 10mM fura-2 in 100mM KCl. A piezoelectric jolter was used to aid the penetration of the electrode tip through the myelin sheath and the nerve membrane at an internode close to the terminal. The dye was injected iontophoretically (-10uA) into the axon and the whole terminal arborization, including fine processes, was filled within a few minutes.

The technique of loading the calcium indicator into mouse nerve terminals will provide a means to study the presynaptic mechanisms in mammals. Work is in progress using fura-2 to examine calcium distribution and dynamics in motor nerve terminals of young and old mice. Supported by NIH Grant AG00795.

197.6

PREPARATION OF THE 'CHICK GIANT SYNAPSE' FOR ELECTROPHYSIOLOGICAL RECORDING: CA CURRENTS IN A VERTEBRATE PRESYNAPTIC NERVE TERMINAL. E.F. Stanley[1] and G. Goping[2], [1]LB, NINDS; [2]LCBG, NIDDK, NIH, Bethesda, MD 20892.

The small size of nerve terminals in most experimental preparations precludes the direct recording of ion currents. We have used the calyx synapse of the chick ciliary ganglion (CG) as a model for voltage clamp of the presynaptic nerve terminal.

The CG neuron is enveloped in a capsule of Schwann cells and the capsules are enmeshed in a collagen matrix within the ganglion. Electron microscopy was used to develop an enzymatic dissociation technique, based on collagenase and neutral protease, to isolate the CG neurons with the presynaptic nerve terminal intact. The nerve terminal is often very extensive, covering most of the CG neuron surface, but is very thin with a maximum thickness of less than 3 μm.

Standard patch clam techniques were used to record from the presynaptic nerve terminal. Ca currents were isolated by ion substitution and Na and K channel blocking agents. The main component of the calcium current had a high threshold, showed little inactivation during a 25 ms pulse, and appeared to wash out rapidly.

197.7

SPIKE BROADENING IS PARADOXICALLY ASSOCIATED WITH REDUCED JUNCTIONAL POTENTIAL AMPLITUDE AT A NEUROMUSCULAR SYNAPSE. A. N. Spencer, J. Przysiezniak and J. Acosta-Urquidi*. Bamfield Marine Station and Dept. of Zoology, Univ. of Alberta, Edmonton, Alberta, Canada, T6G 2E9.

Presynaptic modulation of action potential duration is known to regulate synaptic efficacy in both vertebrates and invertebrates. In the hydrozoan jellyfish Polyorchis penicillatus changes in the durations of motor neuron APs serve to synchronize swimming contractions. As APs propagate through the network of electrically coupled motor neurons their duration progressively decreases as they invade more hyperpolarized regions. The EJPs that are elicited by the propagating motor spike become increasingly larger and appear with reduced delay as the duration of the motor spike decreases. This decreased delay automatically compensates for the conduction time of the motor spike. Identified motor neurons can be isolated, grown in culture, and used for giga-ohm seal, whole-cell recording.

Current clamp recordings show that the duration of action potentials decreases with membrane hyperpolarization prior to stimulation. Voltage-clamp studies demonstrated that the loss of the plateau is due to the removal of inactivation of a transient potassium current (Ik-fast). This current peaked within 2 to 15ms, decayed with a time constant of 200ms, and was half-inactivated at a holding potential of -47mV. A rapidly activating (tau=2ms), and inactivating (tau=20ms) calcium current, which appears to be responsible for transmitter release, can be recorded at synaptic release sites. To determine the dynamics of voltage-gated calcium influx when APs of varying duration invade a pre-synaptic release site, we stimulated motor neurons using voltage commands shaped like APs. Short duration APs elicit large calcium currents with fast onsets, while long APs result in small currents with slow onsets. Although the total amount of calcium that enters during a long AP is greater than for a short AP, it is less effective at releasing transmitter since the calcium concentration at cytosolic binding sites may be mostly dependent on the rate of influx. This would be the case if Ca++ removal by diffusion, active pumping or sequestration is particularly rapid in these cells. Supported by NSERC grant A0 419.

197.8

SIGNAL TRANSDUCTION AND THE ADENOSINE RECEPTOR INHIBITORY TO ACETYLCHOLINE RELEASE IN FROG MOTOR NERVE ENDINGS. J.K. Hirsh* and E.M. Silinsky. Dept. of Pharmacology, Northwestern Univ. Med. Sch., Chicago, IL 60611.

The mechanism whereby adenosine receptors inhibit ACh release from motor nerve endings was studied. Electrophysiological measurements were made of acetylcholine (ACh) release in the frog nerve-cutaneous pectoris preparation. The role of protein phosphorylation in this process was examined using the protein kinase inhibitor H-7. H-7 reduced the epp and mepp amplitudes in parallel without altering levels of release. In addition, H-7 (30-100 μM) blocked the stimulatory effects of chlorophenylthio cAMP (250 μM) on ACh release. However, H-7 did not alter the effects of adenosine under conditions where we and others found that A, C, and G kinases are inhibited (Hidaka, H. et al, Biochemistry 23:5036, 1984; Caratsch, et al Naunyn-Schmeideberg's Arch. Pharmacol. 337:9, 1988). Additional studies examined the role of GTP-binding proteins in the action of adenosine. In preliminary experiments, GTP-γ-S- and Li-containing liposomes increased and decreased, respectively the level of inhibition produced by adenosine. The results suggest that adenosine receptors which inhibit ACh release from motor neurons may be coupled to GTP-binding protein(s) that are directly linked to a cellular effector.

197.9

BAPTISM OF FROG MOTOR NERVE TERMINALS DOESNOT IMPAIR THE INHIBITORY ACTIONS OF ADENOSINE ON ACETYLCHOLINE RELEASE. J.M. Hunt* and E.M. Silinsky (SPON R.W. Berry). Dept. of Pharmacology, Northwestern University, Chicago, Ill. 60611.

Motor nerve terminals in the frog cutaneous pectoris muscle were loaded with the Ca^{+2} chelator BAPTA (100-500μM BAPTA-AM, predissolved in 5μl DMSO.) The effectiveness of this treatment was assessed by examining whether the effects of agents which normally elevate cytosolic Ca levels are blocked (caffeine) or delayed (K^+ depolarization, Ca ionophores) by BAPTizing the nerve terminal.

The affinity of BAPTA for Ca is such that, in frog, cytosolic Ca levels are only modestly reduced. This is reflected as a decrease in miniature end plate frequency, mepp frequency, (see Kojima, H. and Tanabe, N. J. Physiol. 403:135, 1988) similar to that which is produced by maximal concentrations of adenosine. In BAPTized preparations we have found adenosine to exert its characteristic inhibition of acetylcholine release. Given the similar apparent ability of BAPTA and adenosine to reduce mepp frequency, the absence of an occlusive effect of BAPTA upon adenosine suggests adenosine is not inhibiting acetylcholine release by modulation of calcium levels in the nerve terminal.

197.10

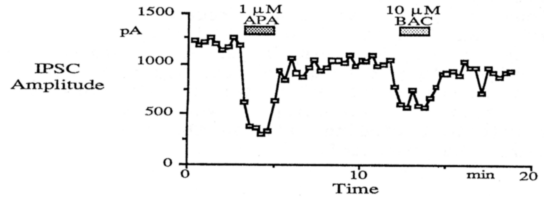
FOCAL APPLICATION OF SEROTONIN ENHANCES SPONTANEOUS AND EVOKED COMPONENTS OF INHIBITORY SYNAPTIC NOISE. J. Mintz* and H. Korn. Neurobiologie Cellulaire. INSERM U 261. Institut Pasteur. 75015 Paris. France.

Synaptic noise in central neurons comprises two components 1) single quanta most generally produced by spontaneous exocytotic events and 2) large multiquantal postsynaptic responses evoked by randomly occuring presynaptic impulses. Such is the case for inhibitory activity produced by glycinergic afferents at the Teleost Mauthner (M) cell (Korn et al., Proc. Natl. Acad. Sci. USA 84:5981, 1987). To clarify the presynaptic control of noise by 5-HT, experiments were achieved on goldfish (Carassius auratus) anaesthetized with tricaine and immobilized with gallamine. KCl-microelectrodes were used for M-cell current clamp and single electrode voltage clamp (chopping frequency 15 to 25 KHz) recording. 5-HT (5mM) was applied through a second micropipette, close to the M-cell membrane. 1) Resolution, in its quantal subunits (see ref.), of background activity recorded over long periods before and after 5-HT indicated that the amine markedly increased the intensity of synaptic noise by enhancing the number and amplitude of its largest (spike-dependent) components. 2) The frequency of single events remaining in the presence of TTX (20uM, applied on the 3rd ventricle) was also increased, although less dramatically, by about 15 to 30%. Thus, 5-HT controls the probability of transmitter release at terminal regions of inhibitory afferents, i.e. at presynaptic active zones.

197.11

3-AMINOPROPANEPHOSPHINIC ACID: A POTENT AGONIST AT PRESYNAPTIC $GABA_B$ RECEPTORS ON CULTURED GABAERGIC HIPPOCAMPAL NEURONS FROM THE RAT. N.L.Harrison, G.Hofer*, J.Ong*, J.L.Barker and D.J.B.Kerr* Laboratory of Neurophysiology, NINDS, NIH, Bethesda, MD and *Dept. of Pharmacology, The University of Sydney, N.S.W., Australia.

We have studied the actions of 3-aminopropanephosphinic acid (APA) and (-)baclofen (BAC) at inhibitory synapses between rat hippocampal neurons in culture. Simultaneous whole-cell recordings were made from pre- and postsynaptic neurons, using patch pipettes filled with an intracellular solution based on 145mM K gluconate. Individual bicuculline-sensitive IPSPs were elicited by a single electrically evoked presynaptic action potential. Both BAC and APA decreased the amplitude of evoked IPSPs and synaptic currents (IPSCs). Like BAC, APA did not reduce Cl^- current responses of postsynaptic neurons to GABA or increase somatic membrane conductance. APA was 10-20 times more potent than BAC (see Figure). Both BAC and APA reduced transmitter output from the presynaptic terminal, as assessed by calculating (mean IPSC amplitude)^2 / (variance). We conclude that BAC and APA reduce inhibition by acting at a presynaptic $GABA_B$ receptor on the terminals of GABAergic neurons.



SEROTONIN RECEPTORS III

198.1

HALLUCINOGENIC INDOLEALKYLAMINES ARE SELECTIVE FOR 5-HT_{2A} BINDING SITES. D.J. McKenna, D.B. Repke*, and S.J. Peroutka. Dept. of Neurology, Stanford University, Stanford, CA 94305 and *Syntex Research, Palo Alto, CA 94304.

The affinities of 21 indolealkylamine derivatives, some with hallucinogenic activity, were determined at 5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2B} binding sites using radioligand competition studies. Most derivatives have the highest potency at the 5-HT_{2A} receptor labeled by ^{125}I-R-(-)DOI in rat cortex and display 2 - 10 times lower affinity at the 5-HT_{2B} receptor labeled by ^{3}H-ketanserin in bovine cortex. 5-substituted derivatives display approximately equal potency at the 5-HT_{2A} site and at the 5-HT_{1A} site labeled by ^{3}H-8-OH-DPAT. The 4-hydroxylated derivatives are selective for the 5-HT_{2A} site vs. the other two sites, while the 6-substituted derivatives display weak affinity for all of the recognition sites examined. Derivatives lacking ring substituents and N-cycloalkyl-tryptamines display lower affinities for all of the recognition sites compared to derivatives substituted in the 4- or 5-position of the indole ring. The size of the N,N-dialkyl substituent is a secondary determinant of affinity, with groups larger than N,N-diisopropyl resulting in a marked reduction in affinity at both the 5-HT_{2A} and 5-HT_{1A} recognition sites. This study demonstrates that 4-hydroxy-indolealkylamines display selectivity for the 5-HT_{2A} recognition site labeled by ^{125}I-R-(-)DOI and provides further evidence indicating that this recently described 5-HT_2 receptor subtype may partially mediate the action of hallucinogenic agents.

198.2

INVOLVEMENT OF 5-HT AND SUBTYPES OF 5-HT RECEPTOR IN THE ACTIVATION OF ENTERIC NEURONS BY INTRALUMINAL CHOLERA TOXIN M. D. Gershon, A. L. Kirchgessner and P. R. Wade. Department of Anatomy and Cell Biology, Columbia Univ. P & S, New York, NY.

Previous studies by Lundgren and co-workers have suggested that 5-HT and enteric neurons participate in the mediation of the action of cholera toxin (CT) on the gut. We tested this hypothesis by investigating the effects of intraluminal application of CT (10 μg in 250 μl for 2.5-3 hrs) on the activity of neurons in the myenteric and submucosal plexuses of the guinea pig small intestine (3 cm) *in vitro*. Neuronal activity was assessed by measuring histochemically demonstrated cytochrome oxidase (CO) activity by computer assisted video microdensitometry. This parameter has been shown to vary directly with the rate at which action potentials are generated in enteric neurons. CT increased CO activity in neurons of both plexuses (myenteric > submucosal). This effect was blocked by lidocaine (4.3 mM), tetrodotoxin (1 μM), and desensitization to 5-HT (2 mM). It was also antagonized by inhibitors of specific subtypes of 5-HT receptor, including the 5-HT_3 antagonist, ICS 205-930 (0.1 μM), and the 5-HT_{1P} antagonists, N-acetyl- > N-hexanoyl-5-hydroxytryptophyl-5-hydroxytryptophan amide (10 μM). CT-stimulation of myenteric CO activity was also reduced by substituted benzamides (which interact with both 5-HT_3 and 5-HT_{1P} receptors), including BRL 24924 (1 μM), zacopride (0.1μM), and its stereoisomer, AHR 4965 (0.1 μM). These observations suggest that CT in the lumen of the bowel activates enteric neurons through a mechanism that involves the release of 5-HT. The released 5-HT appears to act on both 5-HT_3 and 5-HT_{1P} receptors. Blockade of either receptor subtype prevents the CT-stimulated increase in enteric neuronal activity. Supported by NIH grants NS 12969, NS 07062, and AH Robins Co.

198.3

PRE- AND POSTSYNAPTIC ACTIONS OF ZACOPRIDE ON ENTERIC NEURONS: RELATIONSHIP TO 5-HT RECEPTOR SUBTYPES. P.R. Wade* and M.D. Gershon. (Spon. T. Rothman) Dept. Anatomy & Cell Biology, Columbia Univ. P&S, New York, NY 10032

A substituted benzamide, BRL 24924, has been shown to antagonize responses of enteric neurons mediated by at least two 5-hydroxytryptamine (5-HT) receptor subtypes (5-HT_{1P} and 5-HT_3); BRL 24924 also mimics the ability of 5-HT to presynaptically inhibit nicotinic fast EPSPs. To explore the enteric neuronal actions of substituted benzamides further, we investigated the actions of zacopride, and its stereoisomers, AHR-4964 and -4965. Intracellular recording was used to study type II/AH neurons in the myenteric plexus of the guinea pig small intestine. Postsynaptic responses to microejected 5-HT include: (i) A "slow response" (mediated by 5-HT_{1P} receptors): a prolonged depolarization, antagonized by dipeptides of 5-hydroxytryptophan (5-HTP-DP), during which input resistance and neuronal excitability increase. (ii) A "fast response" (mediated by 5-HT_3 receptors): a brief depolarization, antagonized by ICS 205-930, during which input resistance decrease. (iii) A hyperpolarization (reported to be mediated by 5-HT_{1A} receptors). Presynaptic inhibition of fast EPSPs, which is mimicked by 8-hydroxy-dipropylaminotetralin but antagonized by 5-HTP-DP may be mediated both by 5-HT_{1A} and 5-HT_{1P} receptors. Zacopride and AHR-4965, but not AHR-4964, mimicked the slow response to 5-HT. This effect was prevented by desensitization to 5-HT (1μM) and thus is probably the result of an interaction with the 5-HT_{1P} receptor. AHR-4965 also mimicked the presynaptic inhibition of fast EPSPs by 5-HT. AHR-4964, -4965, and zacopride all reversibly blocked fast responses to 5-HT. It is suggested that substituted benzamides act on myenteric neurons at 5-HT_3 receptors and also at a strereospecific benzamide site that is linked to 5-HT_{1P} receptors. Supported by NIH grants NS 12969, NS 22637, NS 07062, the PMAF and A.H. Robins Company.

198.4

SEROTONIN MOBILISES INTRACELLULAR FREE CALCIUM IN RAT CEREBROVASCULAR SMOOTH MUSCLE CELLS IN CULTURE. Y. Wang, K.G. Baimbridge and D.A. Mathers. Department of Physiology, University of British Columbia, Vancouver, B.C., Canada, V6T 1W5.

The effect of serotonin on cytosolic calcium mobilization was studied in short-term cultured smooth muscle cells dissociated from rat cerebral arteries. Free cytosolic calcium levels, $[Ca^{2+}]i$ were measured using the calcium sensitive fluorescent dye fura2-acetoxy-methyl ester. The resting $[Ca^{2+}]i$ was 39±3.6 (mean ± S.E.M., 34 cells). Serotonin (10 nM - 100 μM) induced a transient increase in $[Ca^{2+}]i$ in 39/110 cells tested. The latency of the increase in $[Ca^{2+}]i$ following the application of 5-HT was 23±3 sec (n=58 responses in 39 cells). The effects of two potent 5-HT antagonists were examined in 10 cells to determine the receptor type which mediates these agonist induced changes in $[Ca^{2+}]i$. Simultaneous application of 5-HT (1 μM) and the 5-HT_2 receptor blocker ketanserin (10 mM) reversibly and powerfully attenuated the response of the cell to 5-HT. In contrast, the selective 5-HT_1 receptor antagonist cyproheptadine (10 mM) had no effect on the mobilization of cytosolic calcium by serotonin.

198.5

DISTRIBUTION OF SEROTONIN 5-HT_{1C} RECEPTOR mRNA IN ADULT RAT BRAIN. B.J. Hoffman and E. Mezey. Laboratory of Cell Biology, NIMH, Bethesda, MD 20892.

Based on in situ hybridization histochemistry (ISHH), we describe the anatomical distribution of the serotonin 5-HT_{1C} receptor mRNA. In addition to the very high levels in epithelial cells of the choroid plexus, 5-HT_{1C} receptor mRNA is found throughout the limbic system, in catecholaminergic cell areas and in regions of serotonergic neurons. Receptor transcripts are also present in the hypothalamus, numerous motor nuclei and the subthalamus. Our results correlate well with serotonin (5-HT) innervation and receptor binding. Receptor mRNA is present in many brain structures in addition to regions previously shown to have 5-HT_{1C} receptor binding. The distribution of this receptor mRNA suggests that the 5-HT_{1C} receptor may mediate a number of the central effects of 5-HT.

198.6

ISOLATION AND CHARACTERIZATION OF A HUMAN SEROTONIN 5-HT_2 RECEPTOR CLONE. H.-T. Kao*, M.A. Olsen* and P.R. Hartig, (SPON:T. Branchek) Neurogenetic Corporation, 215 College Road, Paramus, NJ 07652

Pritchett et al. (EMBO J. 13:4135 (88)) reported the isolation and characterization of a rat cDNA clone encoding the serotonin 5-HT_2 receptor. This clone was isolated by screening a rat cDNA library with oligonucleotides derived from the sequence of the rat 5-HT_{1C} receptor. We now report the isolation of a human 5-HT_2 receptor cDNA clone. A human brain stem cDNA library was screened with a cDNA clone for the coding region of the rat 5-HT_{1C} receptor. Several homologous clones were isolated, including one (clone 15) which exhibited over 90% amino acid identity with the rat 5-HT_2 receptor. Clone 15 shows greatest homology to the rat 5-HT_2 receptor in the transmembrane regions (98% identity vs. 83% identity to the rat 5-HT_{1C} receptor). Clone 15 shows significant divergence from the rat 5-HT_2 sequence in the COOH terminal tail region where 16 amino acid substitutions were observed. Pharmacological binding data on the transfected clone will be presented.

198.7

RAPID DESENSITIZATION AND RESENSITIZATION OF 5-HT_2 RECEPTORS. P.J. Pauwels*, P. Van Gompel* and J.E. Leysen Dept. of Biochemical Pharmacology, Janssen Research Foundation, B-2340 Beerse, Belgium.

Regulation of peripheral 5-HT_2 receptors was studied in calf aortic smooth muscle cultures. Confluent cultures were washed and incubated for at least 24 h in a (5-HT)-free, synthetic medium before (5-HT)-induced inositol phosphates formation was measured. The latter had the characteristics of a 5-HT_2 receptor according to the profile of agonist concentration response curves and the inhibition of the response by 5-HT_2 antagonists at nanomolar concentrations. Cultures pretreated with 10 µM 5-HT, washed and stimulated with 10 µM 5-HT for 1 h in the presence of 10 mM LiCl, showed a diminished response. Already after 15 min pretreatment, the response was significantly reduced by 20 %, after 1 h pretreatment the response was reduced by 80 %, and the response was completely abolished after 6 h treatment. Pretreatment with 10 µM of the selective 5-HT_2 receptor agonist 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane produced a similar time-course and degree of desensitization. In cultures pretreated for 2h and 24 h respectively with 10 µM 5-HT, and then kept for various time periods in a (5-HT)-free medium, the (5-HT)-induced inositol phosphates formation gradually returned to normal levels with half-times of 5 h and 12 h, respectively. The rapid desensitization followed by a relatively rapid resensitization points to decoupling/re-coupling or internalization/recycling phenomena.

198.8

5-HT1A AUTORECEPTORS: DEMONSTRATION OF A LARGE RECEPTOR RESERVE FOR 8-OH-DPAT AND PARTIAL AGONIST PROPERTIES OF NONBENZODIAZEPINE (NBD) ANXIOLYTICS. E. Meller, M. Goldstein and K. Bohmaker* (SPON: J.C. Miller). Dept. of Psychiatry, NYU Medical Center, New York, NY 10016.

Inhibition of 5-HT synthesis in rat brain by selective 5-HT1A agonists appears to be mediated by somatodendritic autoreceptors in the raphe nuclei. NBD anxiolytics display full intrinsic activity at 5-HT1A autoreceptors but are partial agonists at postsynaptic sites. Similar observations for dopamine (DA) agonists in the nigrostriatal DA system have been explained by a differential receptor reserve at pre- vs. postsynaptic sites. We now report that somatodendritic 5-HT1A autoreceptors display a large receptor reserve for the selective agonist 8-OH-DPAT.

Irreversible 5-HT1A receptor inactivation with N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ; 6 mg/kg) shifted the dose-response curves for 8-OH-DPAT-induced inhibition of 5-HTP accumulation 8- and 6-fold to the right in cortex (CTX) and hippocampus (HIPP), respectively; those for the NBD buspirone (BUS) were shifted only 2- and 1.6-fold, respectively. A steep nonlinear relationship between receptor occupancy and response for 8-OH-DPAT was obtained (50% of maximal response at 3.3 and 6.5% receptor occupancy for CTX and HIPP, respectively). Shallower relationships were obtained for BUS (50% of maximal response at 14.6 and 21.2% occupancy, respectively); the efficacy of BUS was about 0.25 that of 8-OH-DPAT. The relative efficacies of other NBDs will be reported. Supported by NS 23618.

198.9

SPECIES DIFFERENCES IN THE ABILITY OF 5-HT_{1A} AGONISTS TO INHIBIT FORSKOLIN-STIMULATED ADENYLATE CYCLASE. L.J. Cornfield, S.S. Nikam*, E.W. Taylor* & D.L. Nelson. Col. of Pharmacy, Univ. of Arizona, Tucson, Arizona 85721.

Several purported 5HT_{1A} agonists were tested in the forskolin-stimulated adenylate cyclase (FSC) assay in Sprague-Dawley rat hippocampal membranes. These compounds, which included spiroxatrine and certain aryltryptamines, produced shallow, multicomponent curves, suggesting the involvement of interactions other than 5HT_{1A}. Use of pindolol, a 5-HT_{1A} selective antagonist, resulted in only a slight shift to the right of these inhibition curves, also suggesting that only a small proportion of the observed inhibition could be attributed to a 5-HT_{1A} interaction. Since the usefulness of calf hippocampus in the FSC assay was recently proposed (Schoeffter & Hoyer, Br. J. Pharmacol., 95:975, 1988), we have investigated the inhibition of FSC in bovine, as well as New Zealand White rabbit, hippocampal membranes. Results for spiroxatrine showed steeper inhibition curves and greater shifts to the right with pindolol than in the rat, suggesting a greater involvement of 5-HT_{1A} receptors. Overall, the bovine hippocampus appeared to have the greatest amount of 5-HT_{1A} receptor interaction associated with the ability of spiroxatrine to inhibit FSC. Consequently, the effect of species choice is an important consideration when attempting to use FSC as a screen for novel 5-HT_{1A} compounds. (Sup. by NS16605 and NS01009)

198.10

IDENTIFICATION OF 5-HT_{1A} RECEPTORS IN HUMAN GANGLIONEUROBLASTOMA BUT NOT RELATED TUMORS. J. Balletti* and M. R. Pranzatelli. (SPON: T. Pedley). Departments of Neurology and Pediatrics, Columbia University, NY 10032.

In exploring the hypothesis that neurotransmitter receptors may be a useful biologic marker of human neural tumors, we measured 5-HT_{1A} sites in human neuroblastoma (n=12), ganglioneuroblastoma (n=4), and ganglioneuroma (n=3). 5-HT_{1A} sites were measured in saturation studies by radioligand binding assays using 6 concentrations of [^{3}H]DPAT and 10 µM unlabelled 5-HT to define nonspecific binding. 5-HT_{1A} sites were found on ganglioneuroblastomas but not on the other tumors. B_{max} was 87 ± 29 fmol/mg protein, K_d was 2.33 ± 0.58 nM, and n_H was 0.94 ± 0.03. Competition experiments *in vitro* with 8-OH-DPAT, DOI, RU 24969, methysergide, ketanserin, and methiothepin identified parallel trends but significant differences in IC_{50}s between 5-HT_{1A} sites in ganglioneuroblastoma and rat frontal cortex (n=3). These data suggest that differences in receptor populations on human neural tumors may be a useful clinical diagnostic marker. (M.R.P. supported by NCI-Cooperative Human Tissue Network, NIH grant NS01158 (CIDA), the Myoclonus Research Fund, the United Cerebral Palsy Education and Research Foundation (R381-88), and the William Randolph Hearst Foundation).

198.11

NEONATAL 5,7-DIHYDROXYTRYPTAMINE LESIONS IN THE RAT: RESPONSE TO SELECTIVE 5-HT_{1A} AND 5-HT_2 AGONISTS. M. R. Pranzatelli, V. Hlibczuk, and A. M. Dollison. Columbia University, Departments of Neurology and Pediatrics, New York, NY 10032.

To study the involvement of 5-HT receptor subtypes in brainstem hyperinnervation following neonatal 5,7-DHT lesions made by intraperitoneal (ip) but not intracisternal (ic) injection, we measured behavioral responses to selective 5-HT_{1A} (8-OH-DPAT) and 5-HT_2 (DOI) agonists compared to 5-HTP after either route of 5,7-DHT injection. Effects of DOI, such as shaking behavior and forepaw myoclonus, were enhanced by 5,7-DHT lesions made ic whereas 8-OH-DPAT-evoked behaviors, such as forepaw myoclonus and head weaving, were enhanced with either route of 5,7-DHT injection, somewhat more ip. In contrast, 5-HTP-evoked shaking behavior was increased only after ip 5,7-DHT-injections, which decreased other 5-HTP-evoked behaviors. Behavioral supersensitivity to 5-HTP, which was orders of magnitude greater than that elicited by direct receptor agonists, more clearly differentiated between rats with 5,7-DHT lesions and their controls, and between routes of 5,7-DHT injection, than responses to 5-HT agonists. 5,7-DHT-induced dysregulation of 5-HT receptors, including both presynaptic and post-synaptic changes, better explains these data than post-synaptic changes alone. (Supported by NIH grant NS01158 (CIDA); the Myoclonus Research Fund; the United Cerebral Palsy Education & Research Foundation (R381-88); the William Randolph Hearst Foundation.)

198.12

INVOLVEMENT OF VENTRAL MEDULLARY 5-HT_2 RECEPTORS IN CARDIORESPIRATORY CONTROL IN THE CAT. K.A. King* and J.R.Holtman, Jr (SPON: W. R. Revelette). Department of Pharmacology, College of Medicine, University of Kentucky, Lexington, Kentucky 40536.

Central serotonergic mechanisms have been implicated in the control of cardiorespiratory activity. However, the specific effects of activation of the various serotonin receptor subtypes are not entirely clear. The purpose of the present study was to characterize the cardiorespiratory effects of the 5-HT_2 receptor agonist, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane, (DOI), at the intermediate area of the ventral medulla. Application of DOI (0.3-100 µg) to this site produced dose-related hypertension (+29.4±3.7 mm Hg at 100 µg, N=5) with no change in heart rate. In addition, DOI (100 µg) produced decreases in the amplitude of the phrenic and recurrent laryngeal nerve signals to 82.5±10.8 and 77.0±13.2% of control, respectively (N=4). A decrease in respiratory rate from 11±2 to 5±1 breaths/min accompanied the inhibition of the nerve amplitude. Pretreatment of the intermediate area with the 5-HT_2 receptor antagonist, ketanserin (20 µg), antagonized both the hypertensive (N=7) and respiratory depressant (N=3) effects of DOI. These results demonstrate the involvement of 5-HT_2 receptors at the intermediate area in changes in cardiorespiratory function produced from the ventral medulla. (Supported by HL-36050 to JRH).

198.13

FURTHER CHARACTERIZATION OF THE UNIQUE 5-HT_{1R} BINDING SITE IN RABBIT BRAIN. D.L. Nelson and W.-C. Xiong*. Col. of Pharmacy, Univ. of Arizona, Tucson, AZ 85721.

Recently, we have reported a unique high-affinity binding site for [^{3}H]5-HT ([^{3}H]5-hydroxytryptamine) in the rabbit caudate nucleus (CN), designated 5-HT_{1R}, that is distinct from previously defined 5-HT_1 subtypes (Xiong & Nelson, FASEB J. 3: A119, 1989). While similar to the 5-HT_{1D} site, the 5-HT_{1R} was shown to differ both pharmacologically and in its sensitivity to GTP and Ca^{++}. The present work extends these findings, confirming the differences between the 5-HT_{1R} and 5-HT_{1D} sites, defined respectively in the rabbit and bovine CN. [^{3}H]5-HT binding was measured as described for the 5-HT_{1D} receptor (Heuring & Peroutka, J. Neurosci. 7: 894, 1987), using pharmacologic masks of 5-HT_{1A} and 5-HT_{1C} receptors. At the 5-HT_{1R} site the divalent cations Mn^{++}, Mg^{++}, and Ca^{++} all facilitated the ability of GTP to inhibit [^{3}H]5-HT binding, while at the 5-HT_{1D} site all three cations reduced the ability of GTP to inhibit binding. Treatment of the CN membranes with N-ethylmaleimide (NEM) showed differences between the tissues as well. A maximum of about 40% of 5-HT_{1R} binding could be inhibited by NEM while 100% of 5-HT_{1D} binding was inhibited. The pharmacologic profile of the 5-HT_{1R} was consistent with that expected for a 5-HT receptor, but further work remains to determine if it may represent the rabbit's version of the 5-HT_{1D} receptor. (Supported by NS16605 and NS01009.)

GABA AND BENZODIAZEPINES I

199.1

NEUROPEPTIDE Y IN CEREBRAL CORTEX AND CAUDATE PUTAMEN NUCLEI OF RAT BRAIN: ULTRASTRUCTURAL BASIS FOR INTERACTIONS WITH GABA. C. Aoki & V.M. Pickel, Neurobiol. Div, Cornell U. Med. Coll., NY, NY 10021.

Neuropeptide Y (NPY)-containing neurons are believed to be mostly GABAergic in cerebral cortex but not in the caudate-putamen nuclei (CP). Thus NPY and GABA may play different roles in the intrinsic circuitry of these two regions. We tested this possibility by comparing the ultrastructure of NPY-containing neurons between (1) cortex (somatosensory and anterior cingulate areas) vs. dorsolateral CP; and (2) GABAergic versus non-GABAergic NPY-neurons within each area. Single sections were dually labeled for GABA and NPY by combining immunoautoradiography with the immunoperoxidase method. The ultrastructural features of NPY-perikarya in both regions were morphologically similar regardless of whether the cells also contained GABA. In both regions, nearly all NPY- and NPY-GABA labeled terminals formed symmetric junctions, the majority of which were with unlabeled dendrites. Unlike most singly-labeled GABAergic neurons, the NPY-receptive GABAergic neurons were sparsely innervated by other unlabeled terminals at proximal dendrites and soma. Thus, NPY may play a more prominent role in inhibitory modulation of certain GABAergic neurons than would be predicted by the observed frequency of contacts in the two regions. One notable regional difference was the greater prevalence of axo-axonic associations involving NPY-terminals in cortex. This difference may indicate greater presynaptic modulation of the release of NPY or co-existing transmitter in cortex. Alternatively, NPY, particularly in cortex, may modulate the release or postsynaptic action of transmitters within neighboring terminals. Supported by NS07782 (NIH) to CA; MH40342(NIMH) & DA04600 (NIDA) to VMP.

199.2

TWO FORMS OF GLUTAMATE DECARBOXYLASE (GAD), WITH DIFFERENT N-TERMINAL SEQUENCES, HAVE DISTINCT INTRANEURONAL DISTRIBUTIONS. D.L. Kaufman[1], C.R. Houser[2,3], and A.J. Tobin[1,3,4] Departments of Biology[1] and Anatomy[2], Brain Research Institute[3], and Molecular Biology Institute[4], University of California, Los Angeles, California 90024

The human and rat genomes contain only one GAD gene, but human and rat brains contain two forms of GAD, with M_rs of approximately 68,000 and 61,000 (GAD_{68} and GAD_{61}). We have produced an antiserum against GAD_{68} by immunizing rabbits with bacterially produced feline GAD. Epitopes specific to GAD_{68} reside in the amino terminal segment, consistent with the derivation of GAD_{61} from GAD_{68} by proteolytic removal of an amino terminal peptide, a region enriched in PEST sequences. Immunoprecipitation and immunohistochemistry suggests that proteolytic processing may regulate both the activity and the intraneuronal distribution of GAD. Supported by NS 21908, NS 22256, and NS 20356, and VA Medical Research Funds.

199.3

IMMUNOCYTOCHEMICAL STUDIES USING A NEW ANTISERUM AGAINST BACTERIALLY PRODUCED FELINE GLUTAMATE DECARBOXYLASE.
C.R. Houser, J.E. Miyashiro*, D.L. Kaufman and A.J. Tobin. Depts. of Anatomy and Biology, Brain Research Institute and Molecular Biology Institute, UCLA, and VA Medical Center, Los Angeles, CA 90024.

A rabbit antisera to GAD that was produced in bacteria programmed with feline GAD cDNA and recognizes a polypeptide of M_r ~68k has been immunocytochemically characterized. Using standard methods in rat tissue perfused with 4% paraformaldehyde, the regional distribution of GAD-immunoreactivity was similar to that observed with other GAD antisera. However, in many brain regions, the new antisera (K2) produced strong staining of neuronal cell bodies that have been difficult to label with previous antisera unless colchicine or special fixatives were used. Staining patterns were compared to those of a monoclonal antibody to GAD (MAb-6, generously provided by D. Gottlieb) that reacts primarily with a polypeptide of M_r ~60k. Some cell body and axon terminal staining was observed with each reagent, but cell body staining was consistently greater with K2 whereas terminal staining was pronounced with MAb-6. These findings support the hypothesis that a larger GAD polypeptide is present in the cell body and is converted to a smaller form that is concentrated in axon terminals. Supported by NS21908, NS22256, NS20356 and VA Medical Research Funds.

199.4

ISOLATION AND FUNCTIONAL ANALYSIS OF THE PROMOTER FOR HUMAN GENE ENCODING GLUTAMATE DECARBOXYLASE (GAD).
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Primer extension, nuclease protection, and nucleotide sequencing experiments suggest that the 5' flanking sequence of the GAD gene resembles that of constitutively expressed genes: (1) it contains four identical "GC boxes"; (2) it lacks any CAAT and TATA consensus sequences; (3) the mRNA is heterogeneous, with multiple start sites for transcription, all about 200 bases upstream from the AUG initiation codon. In transient transfection experiments, three flanking genomic fragments all drive the expression of a luciferase reporter in three rat cell lines, C6, B104, and rat 1 fibroblasts. Of these lines, only C6 produces GAD and GAD mRNA. The longest of the three fragments extended 1750 bases upstream from the transcription start site. The sequences responsible for the specificity of GAD gene expression must therefore lie outside this region. Supported by NS 22256, NS 20356, and the Ursula Mandel Scholarship.

199.5

CHRONIC Ro15-1788 AND FG 7142 ADMINISTRATION AUGMENT GABAa RECEPTOR FUNCTION IN CULTURED NEURONS.
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After acute administration, Ro15-1788(Ro) is an antagonist at the benzodiazepine binding site on the GABAa receptor, and FG 7142(FG) is an inverse agonist. We assessed the effects of acute and chronic treatment with these agents on GABA-stimulated chloride uptake in primary chick cortical neurons in culture. Acute (1 hr) Ro(1 μM) treatment had no effect on uptake; however, after 2, 4, and 10 days(d) of treatment uptake was increased compared to vehicle (V) (Max: V 60.4; Ro: 2d 112.2; 4d 99.6; 10d 102 nmol/mg prot). Acute(1 hr) treatment with FG(1 μM) decreased chloride uptake (Max: V 54.6; FG 31.1 nmol/mg prot). However, chronic FG (1 μM) treatment at 2, 4, and 10 d markedly increased uptake (Max: FG 2d 182.6; 4d 164.8; 10d 172.2 nmol/mg prot). Neither compound altered non-GABA related chloride uptake, neuronal survival, or growth of non-neuronal cells. These results indicate that chronic inverse agonist treatment with FG 7142 augments GABAa receptor function and chronic treatment with Ro15-1788 may unmask inverse agonist effects.

199.6

ACUTE ETHANOL ADMINISTRATION DECREASES THE LEVEL OF GABA RECEPTOR α-SUBUNIT mRNAs IN RAT CEREBRAL CORTEX.
A.L. Morrow, P. Montpied*, E. I. Ginns*, B. M. Martin*, S. M. Paul* Clinical Neuroscience Branch, NIMH, Bethesda, MD 20892.

Pharmacologically relevant concentrations of ethanol have been shown to have direct and indirect effects on GABA receptor-mediated Cl^- flux in isolated synaptic vesicles and cultured neurons. To determine whether ethanol has an effect on the expression of genes encoding the GABA-A receptor, we have investigated the effects of acute and chronic (cf Montpied et al., this vol.) ethanol administration on the levels of GABA-A receptor (α-subunit) mRNA in the cerebral cortex. Rats were administered ethanol (2.0 g/kg, i.p.) or saline and sacrificed at various time points. Total RNA was purified from rat cerebral cortex, separated by gel electrophoresis and hybridized under high stringency with a 800bp human GABA-A receptor α-subunit ^{32}P-labelled cRNA probe, which labels multiple mRNA species in rat brain (J. Neurochem. **51**,652,1988).

Acute ethanol administration resulted in a rapid decrease in the two most abundant GABA receptor α-subunit mRNA's in cerebral cortex (4.4 and 4.8 kb mRNA's: Δ ~ 25-40%, $p<.05$, n=12-24). These effects were first observed 20 min following ethanol administration, were maximal 40 min post administration and were still present, although reduced, after 80 min. There was no effect of ethanol administration on the level of ribosomal RNA, poly(A)+ RNA, or the mRNAs coding for glutamic acid decarboxylase or β-actin. These data suggest that acute ethanol administration results in a rapid alteration in GABA-A receptor gene expression and/or mRNA processing.

199.7

CHRONIC ETHANOL EXPOSURE IN VIVO DECREASES THE LEVEL OF GABA-A RECEPTOR mRNAs IN RAT CEREBRAL CORTEX.
P. Montpied*, A. L. Morrow, P. Damshroder-Williams*, J. Karanian*, E. I. Ginns*, B. M. Martin*, S. M. Paul* (SPON: S. Koslow) Clinical Neuroscience Branch, NIMH, Bethesda, MD 20892.

Several lines of evidence suggest that ethanol's depressant effects are mediated in part by augmentation of GABAergic inhibitory neurotransmission. We have recently demonstrated that chronic exposure of rats to ethanol significantly decreases GABA-A receptor-mediated $^{36}Cl^-$ uptake in cerebral cortical synaptoneurosomes (J.P.E.T. **246**,158,1988). We have postulated that this adaptative response may be associated with an alteration of GABA-A receptor gene expression. To answer this question, a human GABA-A receptor α-subunit cDNA was used to synthesize a ^{32}P-labelled cRNA probe (J. Neurochem. **51**,652,1988). Rats were administrated ethanol by inhalation for 14 days, producing blood ETOH concentrations of 180 +/- 17.6 mg/%. Total RNA was extracted from the cerebral cortices of individual animals and analysed by Northern blots.

Chronic ethanol exposure resulted in a significant decrease in the levels of both GABA-A receptor (α-subunit) mRNA species in rat cerebral cortex, (4.8 Kb mRNA: 40%, $p< .05$; 4.4 Kb mRNA: 30%, $p< .05$, n=14). Glutamic acid decarboxylase mRNA, ribosomal RNA and poly(A)+ RNA levels were not significantly reduced following chronic ethanol exposure. Levels of β-actin mRNA were modestly reduced following chronic ethanol exposure (14% $p< .05$, n=14).

These data suggest that chronic ethanol exposure alters the level of GABA-A receptor gene expression and/or mRNA processing and may contribute to the development of ethanol tolerance and withdrawal.

199.8

NATURALLY OCCURING BENZODIAZEPINES IN CSF OF PATIENTS WITH HEPATIC ENCEPHALOPATHY
M. Olasmaa, A. Guidotti, J.D. Rothstein[1], S.M. Paul[2] and E.Costa FGIN-Georgetown Univ., Washington, D.C., [1]Dept. of Neurology, Johns Hopkins, Univ., Baltimore, M.D., [2]Clinical Neuroscience Branch, NIMH, Bethesda, M.D.

The reports that flumazenil, a benzodiazepine (BZ) antagonist, improves the neurological status of patients with hepatic encephalopathy (HE) led us to investigate the possible role of endogenous BZs in HE. Here we report that the content of BZ-like immunoreactivity in cerebrospinal fluid (CSF) of patients with HE was nearly four fold that of healthy subjects. An increase of the same order of magnitute was also observed in serum of patients with HE. In contrast, the corresponding values for patients with liver disease without encephalopathy and for patients with non-hepatic encephalopathy were comparable to those of healthy controls. Reverse phase HPLC analysis of control CSF revealed at least five separate peaks of BZ-like activity assessed by binding assay and three antibodies directed towards three specific BZs. The two peaks with the longest retention times corresponded to N-desmethyldiazepam and diazepam, whereas the three other peaks emerged in positions different from any known BZs that we tested. The material in the major peaks was more potent in displacing ^3H-flumazenil bound to membranes prepared from rat cerebellum than from spinal cord. This suggests that some of the BZ-like material present in CSF differs from diazepam. We are now investigating whether it is from dietary origin.

199.9

INCREASE OF DIAZEPAM BINDING INHIBITOR (DBI) IMMUNOREACTIVITY IN RAT BRAIN AREAS AFTER ACUTE NOISE STRESS. C.Ferrarese, T.Mennini[1], I.Appollonio*, M.Frigo*, M.Gobbi[1], N.Pecora*, M.Perego* and L.Frattola. Clinica Neurologica Universita'di Milano, Ospedale San Gerardo, Monza and (1)Istituto "Mario Negri", Milano, Italy.

DBI is a 10-kDa neuropeptide which allosterically modulates GABAergic transmission by binding to benzodiazepine recognition sites. Its brain levels are increased in benzodiazepine tolerant rats and it displays a proconflict activity when injected intraventricularly in rats (PNAS,80:3531,1983; PNAS,84:1444,1987).

We investigated the effects of acute noise-induced stress on brain levels of DBI and of its processing products. Rats were chronically habituated to handling and to the microwave box. On the day of the experiment stressed rats were sacrified at various times after noise-induced stress. After microwave fixation different brain areas were collected and stored at -80 c until the day of the assay. DBI was measured by a specific radioimmunoassay (RIA), its processing products were evaluated with a RIA for the synthetic 18 AA fragment of DBI, called octadecaneuropeptide (ODN). The different immunoreactivities were characterized by reverse-phase HPLC.

DBI levels were increased in parallel to its putative products of processing in cerebral cortex and hippocampus 15 minutes after acute stress and persisted elevated up to 90 minutes. No change of HPLC elution time was observed. The increase was significantly higher in left cortex and hippocampus compared to the right side. These data indicate that acute noise stress increases DBI turnover in rat brain; the increase is lateralized in left cortical and limbic areas.

199.10

β-ALANINE INDUCES CHANNELS OF HIGH CONDUCTANCE IN CULTURED SPINAL CORD NEURONS. D.A. Mathers, A. Grewal* and Y. Wang. Dept. of Physiology, Faculty of Medicine, University of British Columbia, Vancouver, B.C., Canada V6T 1W5.

β-Alanine (BALA) is a naturally occurring amino-acid, intermediate in structure between the putative inhibitory neurotransmitters γ-aminobutyric acid (GABA) and glycine. BALA exerts a marked inhibitory effect on the firing of certain cells in the spinal cord and brain of mammals. The molecular mechanisms which underlie this action were studied in cultured spinal cord neurons maintained in dissociated cell culture. Patch-clamp recordings were obtained from outside-out membrane patches using a List EPC-7 amplifier. Patches were voltage-clamped to a potential of -80 mV at 21-22°C. Patch electrodes contained 11 mM EGTA and $Tris^+$ replaced K^+. Bath application of BALA (10-40 μM) induced inwardly directed single channel currents in nearly all patches tested. These events inverted to outward currents at 0 mV, the chloride equilibrium potential. Amplitude histograms revealed that BALA-induced channels can adopt at least five conductance levels (8.3, 16.9, 26.5, 43.8 and 82.1 pS, means from 5 patches). The lower four conductance states are very similar to values previously reported for GABA and glycine in this preparation. The 81 pS level is, however, apparently unique to channels activated by BALA.

199.11

BENZODIAZEPINE RECEPTORS MODULATE 5-HT RELEASE: AN IN VIVO ELECTROPHYSIOLOGICAL STUDY IN THE RAT HIPPOCAMPUS. A. Lista*, P. Blier and C. de Montigny. (SPON: A. Gjedde). Dept. of Psychiatry, McGill Univ., Montréal, Canada.

Benzodiazepines facilitate the electrically-evoked release of [^{3}H]5-HT from slices of rat frontal cortex or hippocampus via the type 1 benzodiazepine receptor (BZ-R) (Lista et al., J. Neurochem. 51:1414, 1988). The present study was undertaken to investigate in vivo the effect of BZ-R activation on the efficacy of the 5-HT synapse in the dorsal hippocampus. Male Sprague-Dawley rats were anesthetized with chloral hydrate. Extracellular recordings were obtained from CA_3 hippocampus pyramidal neurons with five-barrelled glass micropipettes. 5-HT and GABA were applied by microiontophoresis and all other drugs were administered intravenously. The ascending 5-HT pathway was electrically stimulated and the effect of the stimulations was determined from peristimulus time histograms. The BZ-R agonists diazepam (0.05-2 mg/kg), lorazepam (0.05-2 mg/kg) and CL 218 872 (1-10 mg/kg) enhanced in a dose-dependent manner the effectiveness of the stimulation of the 5-HT pathway, whereas the BZP-R inverse agonist FG 7142 (0.05-1 mg/kg) dose-dependently decreased it. These effects of both diazepam and FG-7142 were blocked by the BZ-R antagonist flumazenil. Picrotoxine and bicuculline reduced the effectiveness of the 5-HT pathway stimulation; the subsequent administration of diazepam restored the efficacy of the stimulation. Neither the effect of 5-HT nor that of GABA, applied by microiontophoresis onto the same dorsal hippocampus pyramidal neurons, were modified by diazepam, FG-7142 or flumazenil. Hence, taken together, these data indicate that, in vivo, BZ-R ligands modulate 5-HT synaptic efficacy via a presynaptic mechanism by activating type 1 BZ-R coupled to the $GABA_A$-Cl channel complex.

199.12

COMPARATIVE EFFECT OF DIFFERENT BENZODIAZEPINE RECEPTOR LIGANDS UPON SINGLE CELLS' ACTIVITY. G.P. MEREU AND G. BIGGIO, Dep. Exp. Biology, University of Cagliari, Italy.

The action of structurally unrelated ligands for the benzodiazepine (BDZ) receptors upon the spontaneous activity of Substantia Nigra-Pars Reticulata (SNR) neurons was evaluated by using "in vivo" single cell recording and systemic (i.v.) administration. The tested drugs included both "positive" and "negative" modulators of the BDZ receptors, as well as preferential ligands for one of the receptor subtypes (1, 2 and P). SNR cells were inhibited by various BDZ ligands classified as "positive" modulators (or agonists). Rank order of their potency was: Flunitrazepam >> Zolpidem > Triazolam > ZK93423 > Alprazolam > Diazepam > Alpidem > Quazepam ~ Flurazepam. On the contrary, BDZ ligands considered "negative" modulators (or inverse-agonists) activated cell firing. Rank order of their potency was: β-CCM > DMCM > β-CCE > RO15-4513 > RO5-6993 >> FG7142. The two antagonists which were tested, Flumazenil (RO15-1788) and ZK93426, were almost inert per se, yet they antagonized the response of both positive and negative modulators, except RO5-6993, which was blocked by PK11195. The results indicate that in spite of their chemical origin, BDZ-ligands behave in the SNR model coherent with their action on Cl^- channels. However, their relative potency and intrinsic efficacy is likely related to their affinity and selectivity for BDZ receptor subtypes.

TRANSPLANTATION II

200.1

CURRENT CLINICAL STATUS OF THE FIRST MEXICAN AUTO- AND FETAL HOMOTRANSPLANTED PARKINSONIANS. I. Madrazo (1), R.E. Franco-Bourland*(2), F. Ostrosky-Solís*(3), M.C. Aguilera*(1), V. Díaz*(1) and C. Cuevas*(1). 1-Ctro. Med. La Raza, IMSS; 2-Inst. Nal. Nutrición SZ; 3-UNAM, México, D.F., Mex.

Three years after the initiation of adrenal medullary autotransplantations (AT), and 19 months after ventral mesencephalon and adrenal gland fetal homotransplantations (HT) to the caudate nucleus for the treatment of Parkinson's disease in our department, the clinical evaluations of the first 5 AT patients, and of the first 2 parkinsonians with fetal HT have revealed that they have maintained the level of recovery they attained approximately 4 months to 1 year after surgery. All patients were evaluated using internationally approved rating scales, and well defined neuropsychological and neurophysiological tests. Currently, of the 5 AT parkinsonians, the clinical scores of 3 of them continue to be rated as "good", those of another have remained steady at a "moderate" level, and the scores of the 5th patient still show his "poor" response to surgery. The scores obtained by the fetal ventral mesencephalon HT patient were "very good", and those obtained by the fetal adrenal gland HT patient were "good". We therefore conclude, that the patients' responses to any of the alternative transplantation procedures reached in the first 4-12 months postsurgery are likely to remain unaltered for at least 24-30 months in the case of the AT individuals, and for at least 15-16 months in the case of the parkinsonians with fetal HT.

200.2

BEHAVIORAL RECOVERY FROM MPTP-INDUCED PARKINSONISM IN MONKEYS AFTER INTRACEREBRAL TISSUE IMPLANTS IS NOT LINKED TO CSF CONCENTRATION OF DOPAMINE METABOLITES. K.S.Bankiewicz, R.J.Plunkett*, I.Mefford, I.J.Kopin, E.H.Oldfield*. Surgical Neurology, NINDS, NIH, Bethesda

Implantation of fetal dopaminergic (FDA) tissue into preformed cavities in the caudate nucleus leads to behavioral recovery. Recovery may be related to 2 sources of dopamine(DA): the grafted cells or sprouted fibers from host ventral tegmental DA neurons. After implantation of adrenal tissue(AD) behavioral recovery occurs but does not significantly differ from monkeys that are implanted with adrenal cortex, fat or cavitated only. However, their behavioral recovery is significantly less then that in FDA implanted animals. 7m after implantation, surviving AD was detected only in one monkey which had no behavioral recovery. All other AD implanted or controls showed behavioral recovery which was correlated with the presence of sprouted host DA fibers. We measured CSF concentrations of HVA, MHPG and 5-HIAA in all animals before and after MPTP administration and at intervals after tissue implantation or cavitation only. There was a dramatic drop of CSF HVA after MPTP (1100±220 pmol/ml baseline, 243±79 pmol/ml after MPTP). In 1 of 4 FDA implanted monkeys increased HVA sustained for 6m (800 pmol/ml before MPTP, 200 pmol/ml after, 450 pmol/ml 2m after implant, 740 pmol/ml 6m after implant). However in this monkey behavioral recovery was modest and the graft at autopsy was not TH-positive. In 3 AD implanted monkeys there was a transient increase of HVA after implantation and it then returned to post-MPTP levels. These results indicate that 1) implants do not have to be DA to induce functional recovery in MPTP-lesioned primates, 2) functional recovery does not correlate with HVA levels in CSF, 3) implant-induced host DA sprouting plays a role in behavioral recovery and 4) CSF catecholamine metabolites are not adequate indicaters of graft survival or functional recovery.

200.3

ADRENAL MEDULLARY IMPLANTS IN THE CAUDOPUTAMEN OF THE MUTANT MOUSE WEAVER. S. Roffler-Tarlov, R. Koeleveld, M. Kaniucki, A.M. Graybiel, and M. Bohn. Prog. in Neurosci. & Dept. of Neurosurg., Tufts Univ. Sch. Med., Boston, MA 02111; Dept. Brain & Cog. Sci., MIT, Cambridge, MA 02139; Dept. Neurobiol. & Anat., Univ. Rochester, Rochester, NY 14642

The disorder in mice caused by the autosomal recessive gene known as weaver results in a pattern of dopamine loss in the nigrostriatal system that is strikingly similar to that seen in idiopathic Parkinson's disease and in parkinsonism induced in man and in animals by the neurotoxin MPTP.

We report here the results of experiments in which adrenal implants were placed stereotaxically into the striatum of normal control mice and mice carrying the weaver mutation. The implants, enriched for adrenal medullary tissue, were taken from young normal donors. Pieces of adrenal medullary tissue were placed unilaterally into the control caudoputamen (CP), the dopamine-depleted CP of adult weaver mice and the developing CP of 7 day-old weavers, in which the nigrostriatal system had not yet degenerated. Grafted tissue survived in controls and in both adult and immature weavers, and included small numbers of tyrosine hydroxylase (TH)-positive cells that did not grow processes. The implants did not induce convincing reinnervation originating in the adult weaver host, in contrast to the results reported for similar implants in MPTP-treated adult mice (Bohn et al., Science 237:913, 1987). However, the implants in the 7 day-old weavers appeared to rescue partially TH-containing innervation. Viewed with TH-immunohistochemistry 4 weeks after implantation, the catecholamine innervation typical of the weaver's CP was markedly altered with a much greater density of TH-positive innervation in the dorsolateral CP bilaterally than in unimplanted weavers. The new pattern of innervation displayed a compartmental organization characterized by fields of heavy innervation surrounding pale-staining zones reminiscent of striosomes.

200.4

COMPARISON OF CAUDATE LESIONING AND ADRENAL MEDULLA GRAFTING IN 6-OHDA RATS. G.M. Friehs*, L.S. He*, R.G. Parker*, C.L. Ojakangas, D.A. Turner, S.J. Haines*, T.J. Ebner. (SPON: D. Onstott). Dept. Neurosurgery, Univ. of MN, Mpls., MN 55455.

A central issue concerning the efficacy of adrenal grafting for parkinsonian motor abnormalities is the mechanism underlying any improvement. In this study we compared the effects of adrenal medullary transplantation and radiofrequency lesions of the caudate nucleus in 6-OH dopamine (6-OHDA) rats. Using a randomized 2 by 2 design, 4 groups of 4 6-OHDA rats were studied for their rotational behavior to apomorphine: a) controls, b) radiofrequency (RF) lesion, c) heterologous adrenal medullary transplants and d) radiofrequency lesion followed by transplant. The RF lesion group showed a more than 90% reduction relative to control ($p<0.001$). The transplanted group showed a reduction in rotations of 28% ($p<0.05$). The caudate lesion plus graft group showed a persistent reduction (85%, $p<.001$), which did not differ from the RF lesion group. Immunohistochemical and histological findings documented the extent of the 6-OHDA lesion, the presence of the graft and lesion extent. The results show that disruption of the caudate nucleus alone is highly effected in reducing rotational behavior in this model. Supported by Minnesota Medical Foundation and AANS Research Foundation.

200.5

NORMALIZATION OF SUPERSENSITIVE D-1 AND D-2 DOPAMINE RECEPTORS IN THE 6-OHDA DENERVATED RAT AFTER TRANSPLANTATION OF FETAL MESENCEPHALON. J.K. Wamsley,* F.H. Gage,# V.L. Dawson,* L.J. Fisher,# and T.M. Dawson.** *Depts. Psych., Pharm., West. Inst. Neuropsych., Univ, UT, Sch. Med., SLC, UT; #Dept. Neurosci., Univ. CA, San Diego, La Jolla, CA; **Dept. Neurol., Hosp. Univ. Penn., Phila, PA

Transplantation of fetal substantia nigra (SN) into the 6-OHDA lesioned rat has been shown to result in the reversal of many of the behavioral dysfunctions associated with this rat model of Parkinson's Disease. Following transplatation and behavioral recovery one would expect normalization of supersensitive D-1 and D-2 dopamine (DA) receptors. Inorder to test this hypothesis we examined D-1 receptors labeled with [^{3}H]SCH 23390, D-2 receptors labeled with [^{3}H]sulpiride and the DA transport complex (DA-TC) labeled with [^{3}H]BTCP in the striatum of the DA denervated rat, with or without, homologous fetal SN transplants. In lesioned animals, which exhibited marked ipsilateral turning after subcutaneous amphetamine administration (<95% depletion of DA), there was a significant loss (-60%) of the DA-TC and a concomitant increase in D-1 receptors (+26.2%) and D-2 receptors (+57.6%) in the caudate-putamen. 10 to 12 months after transplantation a group of animals ceased rotating in the ipsilateral direction after amphetamine adminstration and in these animals D-1 and D-2 receptor densities were comparable to control values. These results provide evidence that functional recovery of SN transplants may be, in part, a receptor mediated phenomena.

200.6

EXPRESSION OF CELL ADHESION MOLECULES FROM THE L2/HNK-1 FAMILY IN RAT ADRENAL MEDULLA IN SITU, IN VITRO, AND AFTER INTRAVENTRICULAR TRANSPLANTATION. M. Poltorak*, K. Shimoda*, M. Schachner+, C.R. Rodgers* and W.J. Freed (SPON: N. Breslin). NIMH at St. Elizabeths, Washington, DC 20032 and +University of Heidelberg, FRG.

The cell adhesion molecules (CAMs) play an important role in regeneration and development of the nervous system and may be involved in the functional effects of adrenal medulla grafts. We have studied the expression of the CAMs L1/Ng-CAM, N-CAM, J1 molecules, MAG, and their common L2/HNK-1 epitope, in normal rat adrenal gland sections, in adrenal medulla cell cultures with and without NGF stimulation, and after intraventricular transplantation. In situ L1/Ng-CAM and N-CAM were observed on chromaffin cell clusters and surrounding extracellular matrix (EM). J1 immunoreactivity was associated only with EM. In vitro NGF stimulation enhanced both L1/Ng-CAM and N-CAM immunolabeling on chromaffin cells and their neurites, and L1/Ng-CAM on Schwann cells. The outgrowth of neurites was greater in chromaffin cell clusters containing S-100 positive Schwann cells as compared to dispersed single chromaffin cells. Chromaffin cells were grown on a layer of N-CAM and fibronectin positive fibroblasts, and often were associated with laminin immunoreactive material. These findings are currently being compared with the expression of CAMs in adrenal medulla grafts. Our results suggest that the increase in CAM expression on chromaffin and Schwann cells produced by NGF may be implicated in the survival of grafted tissue and its effects on host brain.

200.7

ENCAPSULATED PC12 CELLS SURVIVE CROSS-SPECIES NEURAL TRANSPLANTATION. P.A. Tresco *, S.R. Winn, P. Aebischer, C. B. Jaeger, L.A. Greene (SPON: J. McIlwain). AOL, Brown University, Providence, RI; CPR Purdue University, W. Lafayette, IN & Dept. Path., Columbia Univ., New York, NY, USA.

We are investigating polymer encapsulation as a means to immunoprotect neurosecretory tissue from rejection following cross-species transplantation into the CNS. Encapsulation with a permselective polymeric membrane (Mw cut-off of 50,000 Da) allows exchange of small molecules such as nutrients, growth factors and neurotransmitters to diffuse but prevents immunoglobulins and host cells from crossing the membrane. Acrylic copolymer U-shaped capsules were stereotaxically implanted into the striatum of six adult male guinea pigs and then loaded with PC12 rat pheochromocytoma cells. Five weeks later, PC12 cells were observed in 4 of the 6 capsules. The capsules contained well preserved tyrosine hydroxylase immunopositive cells and numerous mitotic figures. Histological analysis indicated that one of the failed implants most likely was a result of damage to the capsule wall. In this case, abundant lymphocytes were observed within and around the polymeric capsule. In contrast, only a mild astrocytic reaction was observed around intact polymer capsules containing PC12 cells. Unencapsulated PC12 cells did not survive transplantation into the guinea pig striatum. These findings indicate that polymer encapsulation provides immunoprotection of a neurosecretory cell line following cross-species transplantation without the need for immunosuppessive therapy. We conclude that the transplantation of encapsulated dopamine secreting cell lines may provide an alternative for the treatment of Parkinson's disease.

Supported by PHS grant NS 27694

200.8

POLYMER ENCAPSULATION OF DOPAMINE SECRETING TISSUE: AN IN VITRO EVALUATION. P. Aebischer, P.A. Tresco *, S.R. Winn, C.B. Jaeger, L.A. Greene. AOL, Brown University, Providence, RI, CPR Purdue University, W. Lafayette, IN and Dept. Pathology, Columbia University, New York, NY, USA.

Transplantation of polymer immunoisolated dopaminergic tissue may constitute an alternative for the treatment of Parkinson's disease. The encapsulation of secretory tissue within a permselective polymer capsule not only restricts the outgrowth of mitotically active cells but may prevent host rejection allowing cross-species transplantation. The effect of encapsulation on cell viability and dopamine secretion was assessed by loading a variety of cell types into acrylic copolymer capsules. These included dissociated mouse E14 mesencephalon, adrenal medulla, PC12 rat pheochromocytoma cells and NX31-T28 mouse hybrid cells. Basal and potassium-stimulated (56mM) release of catecholamines from the capsules was quantified under static incubation conditions by HPLC-EC. After one month of encapsulation, all cell types continued to release dopamine in the unstimulated state. High potassium treatment increased dopamine release from all cell types except NX31-T28. Encapsulated NX31-T28 cells did, however, increase their release of l-dopa under nicotine stimulation. The tumor cell lines increased their output of dopamine over time, whereas embryonic mesencephalon and chromaffin cells did not. Basal release of PC12 capsules (5 mm long, 0.8 mm ID) kept in culture for 2 months averaged 336 ng of dopamine per day. The increased output of dopamine from the encapsulated tumor cell lines is believed to be due to cell proliferation within the polymer capsule. We conclude that a variety of dopamine-secreting cell types survive encapsulation and continue to release dopamine over several weeks.

200.9

EVALUATION OF MELANOCYTES AS A POTENTIAL CELL SOURCE FOR DOPA REPLACEMENT. P. J. Kontur, A. Y. Deutch, A. Jotkowitz*, M. Lee*, A. B. Lerner*, R. Halaban* and R. H. Roth. Departments of Pharmacology and Dermatology, Yale University School of Medicine, New Haven, CT 06510.

Melanocytes contain the enzyme tyrosinase which catalyzes the hydroxylation of L-tyrosine to L-DOPA and the oxidation of L-DOPA to DOPA-quinone. Melanocytes may serve as an alternative to adrenal or neuronal cells for transplantation in animal models of Parkinson's disease because they have the capacity to synthesize L-DOPA. The ability of neonatal human melanocytes to secrete DOPA into culture medium and to survive after intrastriatal transplantation were evaluated. Significant levels of DOPA were detected in the medium using HPLC 30 min after incubation in media containing 10^{-5} - 4 x 10^{-4}M tyrosine. The levels continued to increase over 24 hrs. The ability of the melanocytes to survive after transplantation to the striata of non-immunosuppressed rats was evaluated in animals with or without unilateral 6-OHDA-induced lesions of nigrostriatal dopamine (DA) neurons. Melanocytes were seen in discrete cell clusters in the striata and did not appear to proliferate when examined 4-5 months post-transplantation. Melanocytes survived in both DA-denervated and intact striatal regions. These data suggest that melanocytes may serve as an alternative transplantable cell source of DOPA for the treatment of Parkinson's disease. Supported in part by USPHS Grant MH 14092 and the United Parkinson Foundation.

200.10

DEFICIENT LEARNING DURING HEPATIC ENCEPHALOPATHY IN THE RAT: EFFECTS OF FETAL STRIATAL GRAFTS. R. Tapia, A.L. Piña*, M. Díaz-Muñoz*, J.C. López-García* and F. Bermúdez-Rattoni. Inst. de Fisiología Celular, Univ. Nacional Autónoma de México, 04510-México D.F.

A useful model of hepatic encephalopathy in rats is provided by chronic treatment with carbon tetrachloride, which produces liver damage closely resembling human cirrhosis. We have studied the effect of such treatment on passive avoidance conditioning (PAC) and locomotor activity (LA). The cirrhotic animals showed a marked deficiency in the acquisition of the PAC and a 70% increase in LA, as compared to controls. Six of 8 cirrhotic rats homotopically transplanted with fetal striatum showed a notable improvement in the PAC 8 weeks after surgery, whereas the increased LA was not modified. Control cirrhotic rats with sham transplant did not recover the response. Striatal glutamate decarboxylase activity was decreased in the cirrhotic rats (J. Neurosci. Res. 20:376, 1988) and the grafting did not reverse this decrease. Striatal choline acetyltransferase activity was found unchanged in both the control and the grafted cirrhotic rats. Histochemical examination of AChE revealed an incomplete integration between graft and host tissue, although patches of low AChE-positive reaction were observed in the graft. This is the first experimental demonstration of a behavioral alteration due to liver failure and of its reversal by fetal transplants. Supported in part by the CoNaCyT (grant PCEXCNA-050290).

200.11

ACETYLCHOLINE RELEASE FROM INTRAHIPPOCAMPAL SEPTAL GRAFTS IS UNDER CONTROL BY THE HOST BRAIN: AN INTRACEREBRAL MICRODIALYSIS STUDY. O.G. Nilsson*, P. Kalen*, E. Rosengren*+ and A. Björklund. Departments of Medical Cell Research and Pharmacology+, University of Lund, Lund, Sweden.

Extracellular levels of acetylcholine (ACh) were measured in the hippocampal formation using *in vivo* microdialysis coupled to high performance liquid chromatography with electrochemical detection. Normal rats, rats with chronic (12 months) fimbria-fornix (FF) lesions, and rats with FF lesions plus intra-hippocampal septal suspension grafts (12 months post-lesion and grafting) were used. The effects on ACh release of handling (stroking), electrical stimulation of the lateral habenula (15 Hz, 0.3 mA), and addition of potassium chloride (KCl, 100 mM), or tetrodotoxin (TTX, 1 uM) to the perfusate were studied in awake animals. In order to reach stable and reliably measurable baseline levels of ACh, neostigmine (5 uM) was added to the perfusate during all manipulations. Baseline levels of ACh in normal rats before manipulations were on average 1.8 pmoles/30 ul of perfusate. Handling produced a 110% increase and lateral habenula stimulation increased ACh levels 4-fold. KCl produced a 5-fold increase in ACh levels and addition of TTX to the perfusate produced an approx. 75% reduction. While the FF lesioned rats had very low levels of ACh (about 30% of normal) and showed no reaction to the different manipulations, the grafted rats exhibited normal or supranormal baseline levels of ACh (about 2-5 pmoles/30 ul). Similar to the normal rats, they responded to handling (55% increase), habenula stimulation (3-fold increase), KCl (3-fold increase) and TTX (80% decrease). It is concluded that the intrahippocampal septal grafts restore baseline extracellular ACh levels and that the graft-derived ACh release is dependent on axonal impulse flow. The response to handling and habenula stimulation, moreover, indicate that the activity of the ectopically placed septal grafts can be regulated from the host brain and modified during ongoing behavior.

200.12

NERVE GROWTH FACTOR INCREASES SIZE OF FETAL BASAL FOREBRAIN GRAFTS IN CORTEX OF NUCLEUS BASALIS LESIONED RAT BRAINS. *P.R. MOUTON*, T. EBENDAL*, L. OLSON* (SPON:S. SWIHART). Dept. Neurobiology & Histology, Karolinska Institute, S-10401 Stockholm, Sweden.*

Nerve Growth Factor (NGF) may act on central cholinergic neurons as a trophic factor. This hypothesis was tested on immature rat basal forebrain, an area rich in cholinergic neurons and analogous to a primary region which degenerates in the brains of Alzheimer's disease patients.

Ibotenic acid lesions of the right nucleus basalis provided cholinergic denervation of the ipsilateral frontal cortex. Two weeks to two months later, some animals received multiple 2 ul implants of basal forebrain cell suspension into the right cortex. On the same day, continuous infusion of NGF or cytochrome-C was started via osmotic minipump and continued for one month. Animals were killed and their brains processed for histochemical analyses. Planimeter measurements revealed a reduction in transplant size of 34% in NGF-untreated rats, while NGF-treated rats showed an increase of 65% (mean values, relative to initial volume), suggesting a trophic role for NGF on developing basal forebrain tissue. Further studies are needed to confirm the cholinergic-specific nature of these findings.

200.13

MAGNETIC RESONANCE IMAGING OF RAT BRAIN IN VIVO: USE OF GADOLINIUM-DTPA IN NEUROPATHOLOGIES AND FETAL STRIATAL TISSUE TRANSPLANTS. A.B. Norman, S.R. Thomas*, R.G. Pratt*, R.C. Samaratunga*, M. Kolmonpunporn and P.R. Sanberg. University of Cincinnati College of Medicine, Div. of Neuroscience and Dept. of Radiology, Cincinnati, OH 45267.

The contrast agent gadolinium (Gd)-DTPA is used in humans to enhance various neuropathologies that are not normally distinguishable in magnetic resonance (MR) images. We investigated the ability of intravenously administered Gd-DTPA to enhance various neuropathological models in rat brain in vivo.

Male Sprague-Dawley rats were used in all studies. Unilateral models of various pathologies were employed: a) mechanical trauma induced by insertion of a needle into the brain; b) hyperosmotic disruption of blood-brain barrier (BBB) following intravenous and intracarotid infusion of mannitol; c) C6 glioma cell tumor; d) rat fetal striatal tissue transplants.

Enhancement of brain regions by Gd-DTPA requires disruption of the BBB or anomolous vascular formations. Gd-DTPA produced no observable enhancement of structures within the normal side of any brain. Hyperintense areas corresponding to mechanical trauma were evident at 24 hours, but not 2 hours or 5 days post-surgery. Mannitol infusions produced marked enhancement of brain regions in the frontal and hind brain. The tumors formed by the C6 glioma cells were greatly enhanced following Gd-DTPA administration. In two rats with 4 week transplants, no significant enhancement by Gd-DTPA was observed. Gd-DTPA is a useful tool for investigating in vivo the vascular changes produced by various neuropathologies in rat brain.

201.1

PHASE RESPONSES TO TEMPERATURE PULSES IN LABORATORY RATS. A.J.Francis* and G.J.Coleman* (SPON:B. Oldfield) Dept of Psychol., Latrobe Univ., Bundoora Vic 3083 Australia.

Compared to light, ambient temperature (Ta) appears to be a relatively weak zeitgeber for the mammalian circadian system. Species which have been convincingly demonstrated to entrain to cycles of high and low Ta (TaHL) are Macaca nemistrina (Tokura, H. and Aschoff, J., Am. J. Physiol., 245:800, 1983) and Rattus norvegicus (Francis, A. and Coleman, G., Physiol. and Beh., 43:471, 1988). The discrete effects of short pulses (2 hours duration) of high Ta (35 °C) upon the free running locomotor rhythms of male and female laboratory rats, maintained in dim LL and 21 °C, were examined to determine the phase-shifting properties of Ta as a zeitgeber. A phase response curve was obtained. Phase responses were largely delaying with a negative correlation between period length and magnitude of phase shift.

201.2

POTASSIUM CHANNEL BLOCKADE LENGTHENS THE PERIOD OF THE HAMSTER CIRCADIAN WHEEL-RUNNING RHYTHM. Harry Klemfuss and Daniel F. Kripke,* Veterans Administration Medical Center and University of California, San Diego.

We recently reported that dietary intake of potassium can influence the phase and period of circadian wheel-running in the hamster (Brain Research, in press). This finding led us to examine whether drugs affecting ion transport alter circadian rhythms in this species.

Adult male Syrian Golden hamsters, housed in running wheel cages in constant darkness, were given drinking water containing 20 mg/L of the potassium channel blocker 4-amino-pyridine (4AP).

The period of wheel-running was significantly lengthened by 4AP compared to water control. During days 4 to 11 of 4AP treatment, tau averaged 24.23 $\pm$ 0.17 (SD) hr by periodogram analysis vs. mean control tau of 24.08 $\pm$ 0.14 hr ($p<0.05$; n=14 and 9).

Linear regression on activity onsets produced similar estimates also indicating that 4AP significantly lengthened tau.

These results are consistent with the proposal that intracellular potassium may be involved in the control of circadian rhythms in the hamster, as has been suggested for plants and invertebrates. Preliminary results using verapamil (400 mg/L) suggest a similar lengthening of tau following calcium channel blockade.

Supported by the Veterans Administration and NIMH MH00117.

201.3

RESTRAINT CAN INDUCE PHASE DEPENDENT DELAYS IN THE HAMSTER CIRCADIAN CLOCK. O. Van Reeth*, J.M. Tecco* and F.W. Turek. Lab. of Interdisciplinary Research Institute in Human and Nuclear Biology, Université Libre de Bruxelles, Campus Erasme, Brussels, Belgium, and Department of Neurobiology and Physiology, Northwestern University, Evanston, IL 60208, USA.

Using the rhythm of locomotor activity as an assay of the clock, we have recently demonstrated that the phase-advancing and phase-delaying effects of dark pulses or triazolam on the hamster circadian clock are mediated through an acute stimulation of locomotor activity. These findings raise important questions about the role exerted by activity on the circadian system of mammals. In order to address this question directly, male golden hamsters free running in constant light ($\approx$ 600 lux) were submitted on 1-3 occasions (separated by at least 14 days) to a 3-h restraint procedure. The beginning of restraint was timed to occur at the following circadian times (CT): 0, 3, 6, 9, 12, 15, 18 and 21, with the onset of locomotor activity defined as CT12. While restraint during the periods of inactivity (i.e. between CTs 0 and 12) or reduced activity (i.e. between CTs 18 and 0) had little or no phase shifting effect on the circadian rhythm of locomotor activity, restraint during the period of normally intense activity induced phase-delays in the activity rhythm (CT 12-15: -52 $\pm$ 9 min, CT 15-18: -38 $\pm$ 10 min). The results of this experiment indicate that activity may have a feed-back effect on the circadian system, and thus may be part of the regulatory system for normal circadian time-keeping.

201.4

AGING ALTERS THE PHASE SHIFTING EFFECTS OF LIGHT PULSES ON THE HAMSTER CIRCADIAN CLOCK. P.C. Zee*, R.S. Rosenberg and F.W. Turek, Dept. of Neurobiology and Physiology, Northwestern University, Evanston, IL 60208.

We have recently reported age-related changes in the rate of reentrainment of the circadian rhythm of locomotor activity following shifts of the light-dark cycle in golden hamsters. One explanation for this differential rate of reentrainment is that aging alters the response of the circadian pacemaker to light. To test this hypothesis, the effects of aging on the phase shifting effect of single one hour saturating light pulses on the circadian rhythm of locomotor activity in golden hamsters was determined at circadian times (CT) 14, 16 and 19. All age groups (2, 5, 8, 13, 16 or 21 months at the start of the study), showed phase delay shifts of similar magnitude to light pulses administered at CT 14. However, in old (16-21 months) animals, phase advances in response to light pulses given at CT 16 and CT 19 were significantly greater in magnitude than in young animals. Unusually large (>300 minutes) phase shifts were seen in response to light pulses administered at CT 16 in 73% of animals in the two oldest groups, whereas, none of the animals younger than 6 months showed shifts of similar magnitude. These results indicate that the advance region of the phase response curve to light increases in amplitude with age in hamsters.

201.5

EFFECTS OF LIGHT ON THE CIRCADIAN CLOCK IN INBRED STRAINS OF GOLDEN HAMSTERS. M.M. Hotz and F.W. Turek. Dept. of Neuro. & Physiol., Northwestern Univ., Evanston, IL 60208.

In order to investigate genetic influences on circadian rhythmicity, four inbred strains of golden hamsters are being studied, measuring the circadian rhythm of locomotor activity. Significant strain differences have been observed in both the phase angle of entrainment to a light-dark cycle and the free-running period, indicating genetic influences on both parameters. The strain rank order is the same for both measures, suggesting common genetic control of these two parameters. To determine whether the strain differences in entrainment to a light-dark cycle may result from differences in how the clock is reset by light, we have studied the phase-shifting response to one hour light pulses in animals kept in constant darkness. Our preliminary results (50 light pulses per strain) show no significant differences between strains. This suggests that the between-strain variance in the phase angle of entrainment results from differences in free-running period and not from any genetic variance in the phase-shifting response to light. The lack of strain differences in the phase response curve to light is in sharp contrast to our previous findings of pronounced differences in the phase response curves to the benzodiazepine, triazolam, in these strains. This contrast indicates that light and triazolam act on the clock via distinct mechanisms.

201.6

THE HAMSTER CIRCADIAN PACEMAKER IS MOST SENSITIVE TO LIGHT PULSES OF 300 SECONDS IN DURATION. Dwight E. Nelson and Joseph S. Takahashi, Department of Neurobiology and Physiology, Northwestern University, Evanston, IL 60208

The physiological properties of the neural pathway that carries environmental light information to an internal circadian pacemaker in the mammal are poorly understood. To characterize the fundamental properties of this pathway for the golden hamster we have used the phase-shifting response of the activity rhythm to light as a functional assay. To quantify the ability of this system to integrate light signals over time, we have measured the stimulus-response relationships for light pulses of 3ms to 1h in duration. Golden hamsters were entrained to a 14L:10D cycle and then placed in darkness. After 7 days a single photic stimulus (503nm) with a duration of 3ms, 3s, 30s, 300s or 3600s was administered to each hamster at circadian time 19 and the steady-state phase shift was measured. Stimulus-response curves for the various durations showed different degrees of light sensitivity. The absolute threshold to irradiance was 1.4×10^{11} photons $cm^{-2}s^{-1}$. The greatest efficiency of light integration occurred in 30s to 1h pulses. The maximum sensitivity to total photons was to 300s pulses. This temporal summation is longer than that seen in any other visual system. These results suggest that the hamster pacemaker may also be differentially sensitive to identical "doses" of other inputs (such as pharmacological agents) that persist for different durations. (Supported by NSF PYI DCB-8451642 and Searle Scholars Award 85-H-107.)

201.7

PHASE OF CIRCADIAN RHYTHMS RESTORED BY SUPRACHIASMATIC NUCLEUS(SCN) TRANSPLANTS. F. C. Davis. Dept. of Biology, Northeastern Univ., Boston, MA 02115.

The SCN appear to contain a circadian pacemaker that regulates overt behavioral rhythms. A critical test of this hypothesis is the transplantation of donor-specific pacemaker properties such as period or phase. The transplantation of phase requires that entrainable oscillations are generated by the donor tissue at the time of transplantation. To determine this, the phases of restored rhythms on the day of transplantation were estimated by backward extrapolation of freerunning restored rhythms. Adult hamsters(Mesocricetus auratus) were kept in dim LL and lesioned 3-7 weeks before receiving fetal tissue. Pregnant hamsters were kept on a light/dark cycle and transplantations were performed at one time of day. The distribution of phases was non-random, indicating that the underlying oscillations were entrained at or before the time of transplantation. Eight of 11 phases fell within a 6 hr range around 0130 and 3 fell within a 2 hr range around 1100. Whether phase is related to maternal rhythmicity before transplantation or to the timing of the transplantation procedure is unknown. It is unlikely that the restored phases were determined by phases of the hosts before transplantation since the hosts received SCN lesions and were kept in dim LL for a variable number of weeks before receiving fetal tissue. Supported by NIH grant HD18686, and Northeastern Univ., Dept. of Biology.

201.8

LIGHT REGULATES FOS EXPRESSION IN THE SUPRACHIASMATIC NUCLEI. W.J. Schwartz and N. Aronin. Depts. of Neurology and Medicine, Univ. Massachusetts Medical School, Worcester, MA 01655

Fos, the product of the c-fos proto-oncogene, is a DNA-binding protein that activates gene transcription. Increased Fos expression has been hypothesized to couple transient extracellular stimuli to long-term changes in cellular function. Using an affinity-purified antibody to $Fos_{132-154}$ for immunohistochemistry (provided by S.M. Sagar and F.R. Sharp), we determined whether light affects Fos levels in the SCN of male Sprague-Dawley rats entrained to a 12h:12h light-dark cycle.

Fos immunoreactivity was clearly detected in the ventrolateral SCN during the light period (4h after lights-on). Both the number of cell nuclei stained and the intensity of their labeling were markedly diminished at this circadian phase if lights were not turned on and rats remained in the dark. Comparably low Fos levels were also found during the normal dark period (4h after lights-off). If instead lights remained on for these 4h, Fos expression was high and similar to that seen during the normal light period. Interestingly, cells in the intergeniculate leaflet were clearly immunoreactive for Fos when the lights were on, but no labeled cells were observed in the dorsal or ventral lateral geniculate and superior colliculus.

Thus, expression of the transcriptional activator, Fos, is physiologically regulated in the SCN by environmental light. This regulation appears selective for the visual system for light entrainment of circadian rhythms, since no change in Fos expression was detected in the visual pathways responsible for image formation and reflex oculomotor function. We speculate that transcriptional events may be part of the cellular basis for photic entrainment, and Fos may be a link in this signal transduction mechanism.

201.9

INDUCTION OF C-FOS EXPRESSION IN THE SUPRACHIASMATIC NUCLEUS (SCN) OF HAMSTERS BY LIGHT EXPOSURE. B. Rusak and H.A. Robertson, Depts. of Psychology and Pharmacology, Dalhousie Univ., Halifax, Nova Scotia, Canada B3H 4J1.

The SCN functions as a major circadian pacemaker and as a site of integration of photic entrainment information in mammals. Wewere interested in whether SCN cells would show spontaneous rhythms or light-evoked changes in the expression of proto-oncogenes, in particular c-fos. The protein products of these genes have been proposed to function as "tertiary" nuclear messengers, acting to alter the expression of other genes.

We prepared brains of Syrian hamsters sacrificed at various times in light-dark (LD) cycles or in constant darkness for immunocytochemical identification of Fos protein. There was little or no Fos immunoreactivity (ir) in the SCN of hamsters sacrificed during mid-L and mid-D phases, nor in animals sacrificed at any time in constant darkness. Light exposure (~30 lux) for 0.5-1.0 h caused a dramatic increase in c-fos expression in SCN cells. The pattern or Fos-ir closely resembled the distribution of retinal ganglion cell terminals in the SCN region. Pre-treatment with the NMDA receptor antagonist MK-801 blocked increases in Fos-ir. Other retinorecipient areas did not show similar increases in Fos-ir in response to light. These results suggest that rapidly induced changes in gene expression may play a role in mediating photic effects on circadian clocks. They provide a novel approach for the analysis of entrainment and the cellular clock mechanism.

201.10

EFFECT OF SUPRACHIASMATIC NUCLEI LESIONS ON BENZODIAZEPINE-INDUCED SLEEP IN THE RAT. Dale M. Edgar, Wesley F. Seidel and William C. Dement. Sleep Research Center, Stanford University School of Medicine, Stanford, CA. 94305.

The hypnotic efficacy of benzodiazepines (BZ) vary as a function of time of day. In humans, efficacy also depends on the amount of prior sleep (regardless of time of day). The neural substrate for these variations in BZ efficacy is not known, although it is assumed to involve the central neural circadian pacemaker-- the suprachiasmatic nucleus (SCN). This study examines the role of the SCN in the regulation of sleep induction by triazolam in rats.

Eight SCN-lesioned rats (SCNx) and ten intact rats were surgically prepared for physiological sleep recording. All animals were housed individually in cages equipped to monitor sleep-wake stages and drinking patterns continuously using "SCORE," a computerized sleep-wake and circadian rhythm bioassay system. Intact rats were entrained to a light-dark cycle (LD 12:12). SCNx rats were maintained in constant dark. Intact rats were administered triazolam (0.2 mg/kg I.P.) or vehicle (0.25% methylcellulose) at circadian time (CT) CT-5, CT-14 and CT-20. SCNx rats were similarly treated except that the absence of circadian rhythms precluded reference to CT.

Levels of sleep induced by TZ at this dosage varied as a function of circadian time in the intact rats. Triazolam was most effective at CT-20 increasing total sleep time an average of 20 min, and was least effective at CT-5. In contrast, SCNx animals showed no increase in sleep following treatment with TZ at this dose.

Attenuated sleep responses to BZs in SCNx animals implicates the biological clock in circadian dependent BZ receptor upregulation. Additionally, the induction of normal sleep by BZs may require sleep pressure which may be reduced in SCNx animals. Studies are underway to address these possibilities.

Research supported by NIA AG06490 and The Upjohn Company.

201.11

TEMPERATURE-DEPENDENCE OF SLOW WAVE SLEEP-LIKE EEG STATES IN THE CHRONIC CERVEAU ISOLE CAT. D. McGinty and R. Szymusiak. Neurophysiology Research, Sepulveda VAMC, Sepulveda, CA 91343.

Human slow wave sleep (SWS) and associated body cooling are increased after body heating while awake, and SWS may be elicited by preoptic/anterior hypothalamic (POAH) warming in cats. We tested the hypothesis that a normal physiological stimulus for SWS is brain warming, associated with waking, acting on specialized hypnogenic warm-sensitive POAH neurons. In the intact animal hypothalamic temperature (T_{hy}) cannot be manipulated without exposure to extreme ambient temperatures (T_a). However, in the chronic cerveau isole (CI) preparation thermoeffectors are disconnected from the hypothalamus, and T_{hy} varies with T_a. CIs were prepared by complete caudal midbrain transections using a subtentorial approach and were sustained for at least 4 weeks. After several days, animals exhibited alternating periods SWS-like EEG synchrony and waking-like EEG desynchrony. T_{hy} was then held at 37.5 or 39.5 °C during 8 hour recording sessions. Percent time in EEG sleep-like states was more than doubled (40% v. 17%) in the warm compared to the cooler condition. This study shows that forebrain warming is sufficient to tonically increase SWS. This result supports the hypothesis that SWS is driven by brain warming, normally associated with waking, and that this stimulus acts on forebrain structures.

201.12

EXPOSURE TO HEAT REVERSES PREOPTIC/ANTERIOR HYPOTHALAMIC LESION-INDUCED INSOMNIA IN CATS. R. Szymusiak, J. Danowski* and D. McGinty. Dept. Psych., Univ. Calif., Los Angeles and V.A. Med. Ctr., Sepulveda, CA 91343.

We have hypothesized that warm sensitive neurons of the preoptic/anterior hypothalamus (POAH) are a component of the basal forebrain hypnogenic mechanism. To test this hypothesis, POAH cell loss was induced in 6 adult cats by microinjections of neurotoxin. Postlesion amounts of slow-wave (SWS) and REM sleep were significantly reduced during 14 hr recordings at an ambient temperature (Ta) of 23°C. Reductions in SWS persisted through the seventh postlesion week. Loss of POAH warm-sensing neurons was indicated by a significant elevation in hypothalamic temperature (Thy) thresholds for panting; from a prelesion average of 39.5± 0.1°C to 41.3±0.3°C at 2 weeks postlesion.

At 2 and 4 weeks postlesion, cats were exposed for 6 hrs to Ta's of 13, 23 and 33°C. At the lower Ta's, amounts of SWS were significantly below prelesion levels. However, amounts of SWS at 33°C were significantly elevated compared to values at lower Ta's. At 4 weeks, SWS time at 33°C was equal to maximal prelesion values. Enhanced sleep at 33°C was accompanied by elevated Thy's, to an average of 40.1°C.

After POAH damage, abnormally high Thy's were required to elicit both panting and normal amounts of SWS, suggesting that loss of warm sensitive neurons produced both deficits. This supports the hypothesis that such neurons are an important facilitory input to SWS-regulating mechanisms.

202.1

INTER-ASTROCYTIC GAP JUNCTIONS INCREASE IN RAT RETINA FOLLOWING PHOTORECEPTOR DEGENERATION. M.S. Burns and N.K. Tyler*. Ophthalmology Research Laboratories, University of California, Davis., Davis, CA 95616.

Astrocytes are present in normal rat retina only in the inner retina, with processes frequently apposing retinal vessels. These astrocytes are connected by gap junctions, which have a frequency of 2.8 +/- 1.1 um per 1000 um of glial cell membrane contact length. Gap junctions are not found between Muller cells nor between Muller cells and astrocytes.

Urethane administration to newborn rats produces a photoreceptor cell specific degeneration which is complete in the central retina by 24 weeks. There is no apparent cell loss in the inner retina but the glial cells are reactive. The Muller cells retract from the inner limiting membrane and sometimes form desmosome like connections as though their normal polarity were reversed. Astrocytic hypertrophy and extensive production of intermediate filaments occurs in the innermost retina. By 56 weeks the interastrocytic gap junctions occupy close to 2% of the glial cell membrane contact length in inner retina, a 10 fold increase compared to normal. The size of individual junctions is increased two-fold.

We interpret this up-regulation of gap junctions as an attempt to increase spatial buffering of the extracellular compartment of the retina.

202.2

DOCOSAHEXAENOIC ACID (DHA) UPTAKE AND DISTRIBUTION WITHIN THE FROG RETINA. **W.C. Gordon* and N.G. Bazan, (SPON: R. Roskoski) LSU Eye Center and Neuroscience Center, New Orleans, LA 70112.**

Retinal uptake of ^{3}H-DHA shows different patterns among cell types. Rod outer segments (ROS) demonstrated two labeling patterns: a diffuse uptake throughout the layer, and a specific label that began at the base and spread apically as new membrane was synthesized. The label front co-migrated with the ^{3}H-leucine band. HPLC analysis of extracted retinas showed ^{3}H present only in DHA. Retinal protein showed no ^{3}H, implying that the migrating label results from a non-covalent association between the ^{3}H-DHA-rich molecules and rhodopsin. This pattern persists up to 50 days, then disperses. During this time labeling occurs in oil droplets of the pigmented epithelium and cone photoreceptors (PR), as well as at the PR terminals. Dense labeling occurs in the nerve fiber layer (>5 days), and in vitro retinas incubated 3 hrs with ^{3}H-DHA have dense cell profiles that resemble Muller cells (MC). This pattern is seen only after short incubation times, and also includes oil droplet-like regions near MC somas. We suggest that DHA in eye cups is incorporated by MC into DHA-rich molecules, delivered as needed to other cells, and then added to neural membranes and ROS. (Supported by NIH EY04428).

202.3

DEVELOPMENTAL EXPRESSION OF HUMAN CONE MATRIX SHEATH-SPECIFIC MOLECULES. **J.C. Blanks, K. Anderson*, C. Spee*, and G.S. Hageman*. Doheny Eye Institute, Los Angeles, CA; and Bethesda Eye Institute, St. Louis, MO.**

Cone photoreceptor outer segments (OS) in mature human retina are surrounded by cone matrix sheaths (CMS), domains of interphotoreceptor matrix that contain chondroitin and 6-sulfate glycosaminoglycan and an unidentified PNA-binding glycoconjugate. Little is known about expression of CMS-specific molecules during human retinal development, but cone-specific domains might serve as mediators of differentiation, development and cellular interaction of cone photoreceptors. We studied the expression of CMS-specific molecules as related to morphologic differentiation of cone photoreceptors. Eyes from preterm and one term infant were used. Cone photoreceptors were first noted at 13 wks. A single cilium was seen at the apical end of the cell, where junctions occur between photoreceptors and RPE. At 18 wks, these junctions were absent in the central retina and an obvious subretinal space was visible. Rudimentary OS were numerous. By 24 wks, cone photoreceptors were polarized and had inner segments, axons and synaptic pedicles. Domes of interphotoreceptor matrix were differentially associated with cone inner and outer segments. Most cone OS had stacks of disc membranes, evidence that their differentiation occurs prior to that of rods. Chondroitin 6-sulfate was first expressed at 17 to 18 wks and was associated solely with cone OS. PNA-binding glycoconjugates were present in the subretinal space at 12 weeks but concentrated accumulations were not seen until 17 to 18 weeks. These studies show that two major CMS constituents are expressed when OS first differentiate, about 10 weeks prior to differentiation of disc membranes. These findings suggest that CMS constituents may be necessary for OS differentiation and survival.

202.4

POSTNATAL DEVELOPMENT OF CHOLINERGIC AMACRINE CELLS IN THE KITTEN RETINA. J. F. DANN* (SPON: W. R. Levick). Vision, Touch & Hearing Res. Ctr., Dept. Physiol. Pharmacol., Univ. of Qld., St. Lucia 4067, Qld., Australia.

Rat monoclonal antisera directed against the ACh-synthesing enzyme, choline acetyltransferase (ChAT) at a dilution of 1:5 was applied to wholemounted kitten retinae which were Rat-PAP (dilution 1:100) reacted to determine the populations of immunoreactive (cholinergic) amacrine cells. From birth (postnatal day (P) 0) cholinergic amacrines were located either in the inner nuclear layer (INL) or were displaced to the ganglion cell layer (GCL). By P21 the number of cholinergic cells and their distribution in central and peripheral retina approached adult values. Between PO and P21 their number increased 5 fold in the GCL and 3.5 fold in the INL. In the periphery of the GCl the density increased dramatically between PO and P5 and decreased between P5 and P21. However, in contrast to the GCL, there was a steady increase in the number of cells expressing ChAT in the periphery of the INL.. The differences in the establishment of their density gradients and in soma diameter enlargement between PO and P21 indicate differential development of the two populations of cholinergic amacrines in the kitten retina.

202.5

DENDRITIC GROWTH OF DISPLACED CHOLINERGIC AMACRINE CELLS IN THE POSTNATAL RABBIT RETINA. R.O.L. Wong* and S.P. Collin. National Vision Res. Inst., Carlton, Vic. 3053 and Vision, Touch and Hearing Res. Ctr., Uni. of Queensland, Qld. 4067, Australia.

The relatively simple, symmetric and consistent dendritic tree morphology of cholinergic (Cb) amacrine cells enables a systematic analysis of their structure during postnatal development. The dendritic arbors of displaced Cb amacrine cells in adult and developing rabbit retinae were revealed by intracellular injection with lucifer yellow. Like immature retinal ganglion cells, developing Cb amacrine cells possess numerous short appendages (1-5μm) on their dendrites. The majority of these protrusions are lost by three weeks after birth, in parallel with the maturation of amacrine cell function (Dacheux, R.F. and Miller, R.F., *J. Comp. Neurol.* 198:327, 1981). If the protrusions are excluded, the number of dendritic branches remains constant after birth, unlike many ganglion cells which lose a large number of branches during postnatal growth. Comparisons of the area enclosing equivalent inner and outer regions of the developing and adult tree demonstrates that the increase in tree size is due to the elongation of both proximal and distal dendrites.

202.6

SOMATOSTATIN-LIKE IMMUNOREACTIVITY (SRIF-I) IN GANGLION AND OTHER CELLS OF THE DEVELOPING CAT RETINA. C.A. White and L.M. Chalupa. Dept. of Psychology, and Neurobiology and Physiology Graduate Groups, University of California, Davis, Davis CA 95616

In the adult cat retina, SRIF-I occurs in two types of cells: a wide-field amacrine cell and a large, granular-staining cell, both found predominantly in the ganglion cell layer (GCL) of the inferior retina (White et al., 1988a,b). The ontogeny of SRIF-I was studied in developing cat retinas using immunocytochemical techniques with a mouse monoclonal antibody (from the MRC Regulatory Peptide Group) to SRIF 14. Specific staining was eliminated by preabsorption of the primary antibody with synthetic SRIF 14. In vertical sections, SRIF-I was detected in cells of the GCL as early as embryonic day (E) 35 (gestation=65 days). In wholemounts at E42, E51 and E60, two types of SRIF-I cells were observed in the GCL. One type resembled the SRIF-I amacrine cell of the adult in both morphology and distribution. The second type, not seen in the adult, had irregularly shaped SRIF-I clusters within a concentric soma. Early in development the cells with irregular clusters were distributed throughout the retina. By P23, they were no longer present, and the adult pattern of wide-field amacrine and large granular-staining cells was evident. Central injections of rhodamine beads revealed that during early development at least some of the SRIF-I cells are ganglion cells.

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202.7

SYNTHESIS OF THE GROWTH-ASSOCIATED PROTEIN, GAP-43, BY DEVELOPING RETINAL GANGLION CELLS IN THE CAT. D.T. Hess*, C.J. Shatz, D.J. Schreyer and J.H.P. Skene. (SPON: M. Siegel) Dept. of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305.

Increased neuronal synthesis of GAP-43 is correlated consistently with developmental and regenerative growth of axons by vertebrate central and peripheral neurons (Ann. Rev. Neurosci., *12*:127, 1989). We have identified GAP-43 among proteins synthesized and transported rapidly by developing retinal ganglion cells (RGC) of the cat, a species in which the stages of growth of the retinofugal pathway are both temporally discrete and well-characterized morphologically (Ann. Rev. Neurosci., *9*:171, 1986). Intraocular injection of ^{35}S-methionine was employed to label proteins synthesized and transported by RGCs in fetal, neonatal and mature cats. A polypeptide was identified provisionally as cat GAP-43 based on its electrophoretic mobility in 2-D gels of samples prepared from optic nerve and primary optic targets. This polypeptide was recognized by a monoclonal antibody to rat GAP-43 on Western blots of target tissue. Immunohistochemistry with this antibody revealed GAP-43 within the primary optic pathway of fetal cats (and within other developing structures including the intermediate zone of the cerebral cortex). GAP-43 is relatively abundant within primary optic projections at embryonic day 43 (E43), after the majority of RGC axons have grown into their targets but prior to binocular segregation. It is significantly less abundant by E56, after binocular segregation is largely complete, and by birth is barely detectable. These observations suggest that elevated synthesis and transport of GAP-43 by developing mammalian RGCs is a concomitant of both initial innervation of and active axonal remodeling within primary optic targets.

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202.8

PHYSIOLOGICALLY IDENTIFIED RETINOGENICULATE X AXONS IN FERRETS WITH NEONATAL ABLATION OF VISUAL CORTEX: SIZES OF TERMINAL ARBORS DEPEND ONLY PARTLY ON TARGET SIZE. J. Hahm*, A. Roe, S. Pallas, and M. Sur (SPON: R. Held). Dept. Brain & Cognitive Sciences, M.I.T., Cambridge, MA 02139.

Ablating visual cortical areas 17, 18 and parts of 19 in ferrets at birth (E42) leads to extensive loss of X cells in the LGN, of medium sized ganglion cells in the retina, and of X retinogeniculate axons (Sur et al., Soc. Neurosci. Abst. 13: 590, 1987; cf., in cats, Tong et al., Science 217:72, 1982). We now report that a smaller ablation of visual cortex, including area 17 and parts of 18, in neonatal ferrets does not cause a similar loss of LGN X cells or of retinogeniculate X axons. LGN X cells can apparently project to alternative cortical targets following very early lesions of area 17. However, the LGN is shrunk in overall size, and soma sizes in the LGN corresponding to ablated area 17 are smaller than normal.

How are individual retinal X axon arbors in the LGN affected by these changes in their target? Single X axons (n=5) injected intracellularly with HRP have fewer boutons than normal and terminal arbors that are reduced up to 30% in volume compared with normal X arbors. The size of the LGN, however, is reduced by up to 75% following the neonatal lesions. These results indicate that retinogeniculate X arbor size is only partly influenced by target size, and that there is relative conservation of arbor size. Thus, each arbor occupies a proportionately larger volume of the LGN compared to normal (cf. Weber et. al., J. Comp. Neurol., in press, 1989). Since the cortical ablations occur before retinal axons have recognizable terminal arbors or have segregated into eye-specific laminae in the LGN (Hahm & Sur, Soc. Neurosci. Abst. 14:460, 1988), retinogeniculate X axons must have a significant intrinsic component in determining the size of their arbors.

Supported by NIGMS training grant GM07484 (JH, AR), fellowship EY06121 (SLP), and EY07023 (MS).

202.9

RETINAL AXON ARBORS IN A NOVEL TARGET: MORPHOLOGY OF GANGLION CELL AXONS INDUCED TO ARBORIZE IN THE MEDIAL GENICULATE NUCLEUS OF FERRETS. S.L. Pallas, J.-O. Hahm and M. Sur. Dept. of Brain & Cognitive Sci., M.I.T., Cambridge, MA 02139.

Retinal ganglion cell axons project into the auditory thalamus (MGN) following partial removal of retinal targets and deafferentation of the MGN in neonatal ferrets. We have used bulk HRP filling of retinal axons in vitro to study the morphology of individual axon arbors in operated ferrets reared to adulthood.

There are at least two types of retinal axons in the MGN. The first type, which constitute the majority of retinal axons in the MGN, make only en passant boutons on their way through the nucleus and do not branch. These axons often enter the MGN in fascicles. The second type have well-formed terminal arbors in the MGN and they tend to occur in clusters. The extent of their arbors (average of width and height) varies from 112 μm to 508 μm (mean=244 μm, n=7). In both types of axon, boutons vary substantially in size. Axon diameters of both types vary from 0.3 μm to 1.5 μm (mean=0.7 μm, n=18).

Physiological recordings of visual activity in the MGN of neonatally lesioned ferrets, as well as retinal backfills, suggest that retinal W cells provide the major input to the MGN (Sur et al., Science 242:1437, '88; Roe et al., Soc. Neurosci. Abstr. 13:1023, '87). In normal cats and ferrets, one population of retinal W cells projects to the LGN-C lamina and another population projects to the superior colliculus (Leventhal et al., J. Comp. Neurol. 237:216, '85; Stanford, J. Neurophysiol. 57:218, '87; Roe et al., unpub.). We hypothesize that the two axon types we observe correspond to these two W cell populations: those passing through the MGN may innervate the remaining fragment of LGN, and those which arborize in the MGN may normally innervate the superior colliculus, which has been ablated in our animals.

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202.10

LIMITED TOPOGRAPHIC SPECIFICITY IN THE TARGETING OF MAMMALIAN RETINAL AXONS. D.K. Simon and D.D.M. O'Leary (SPON: J.Volpe) Dept Neurosurgery, Washington U Sch Med, St.Louis, MO 63110

A central issue of developmental neurobiology is how vertebrate axons establish ordered connections within their target fields. The retinotectal projection has been a model system for studies of the development of topographic order in vertebrate axonal projections. In frogs and fish developing retinal axons reportedly grow directly to and arborize at topographically appropriate sites in the optic tectum. These findings have led to the generalization that topographic specificity in the targeting of vertebrate retinal axons is evident from the time they arrive in the tectum [Dodd & Jessell (1988) Science 242:692]. In contrast, we find in rats that developing retinal axons make major topographic targeting errors along both the medial-lateral and rostral-caudal axes of the superior colliculus, the mammalian homologue of the tectum. Localized injections of the tracer, DiI, covering 1% or less of the retina were made into peripheral retinal regions of E20 to P12 rats. In perinatal rats, axons originating from such an injection in temporal retina grow over the entire contralateral colliculus suggesting that many initially fail to recognize their target region in rostral colliculus. Many nasal axons branch along their entire course through the colliculus. A dense zone of arbors develops, but covers a much greater amount of colliculus than at maturity. Topographic order is established by P12. At this age, it is evident that some mistargeted axons make corrections and are retained; most do not and are lost. Cross sections of the optic nerves from the perinatal and P12 cases reveal that the labeled axons are dispersed throughout the nerve, indicating that preordering of axons contributes little to the development of topography within the colliculus. Our finding that the topographic targeting of retinal axons in rats lacks the specificity reported in lower vertebrates indicates that mechanisms other than directed axon growth are required to establish topographic order in axonal connections in the mammalian brain.(Support: NEI grant EY07025)

202.11

CHRONIC APPLICATION OF NMDA OR APV AFFECTS THE NMDA SENSITIVITY OF THE EVOKED TECTAL RESPONSE IN *RANA PIPIENS*. E.A. Debski, H.T. Cline and M. Constantine-Paton. Dept. of Biology, Yale University, New Haven, CT 06511.

Chronic treatment of three-eyed tadpoles with the NMDA antagonist APV desegregates eye-specific segregation zones in the optic tectum; chronic treatment with NMDA sharpens the boundaries of these zones (Cline et al., PNAS, 84: 432, 1987).

We have assessed the sensitivity of chronically treated animals to exogenously applied NMDA. In normal animals, perfusion of 6 μM NMDA blocks approximately half of the tectal response evoked by electrical stimulation of the optic nerve. Perfusion of 12 μM NMDA blocks most of the response (Debski and Constantine-Paton, Neurosci. Abstr., 14: 674, 1988). Animals chronically treated with NMDA show a decreased sensitivity to applied NMDA: application of 6 and 12 μM NMDA cause significantly less decrease in the tectal response than in normal animals. Conversely, at least part of the response in animals chronically treated with APV is more sensitive to exogenous NMDA. Preliminary results with animals chronically treated with L-APV indicate that their sensitivity is unchanged.

We conclude that chronic treatment with NMDA may either decrease the number and/or sensitivity of NMDA receptors in these animals. Chronic treatment with APV appears to have the opposite effect. Supported by NIH EY05829, EY05818 and EY06039.

202.12

Protein kinase activity is not required for the selective stabilization of coactive inputs in eye-specific segregation in the frog. H.T. Cline, M. Constantine-Paton. Yale Univ., Biology Dept. New Haven, CT. 06511

NMDA receptor activation is required for the development of eye-specific stripes and normal retinotopic maps in the optic tectum of frogs. It is thought that a calcium transient through the NMDA-gated channel might activate protein kinases and thereby initiate the stabilization of coactive retinal inputs. We exposed the tecta of three-eyed tadpoles to drugs thought to activate or block protein kinases. Exposure for 6 weeks to either phorbol esters, a protein kinase C activator, or sphingosine and H-7, both kinase blockers, did not alter the eye-specific stripe pattern. We are confident that the drugs were released from the Elvax and reached the tissue because ^{3}H-thymidine autoradiography reveals that tectal cell proliferation is increased following exposure to phorbol ester or sphingosine compared to stage-matched controls.

The role of the NMDA receptor in neural plasticity in the developing and mature brain invites comparison between potentiation in the hippocampus and neural map formation. In the hippocampus, the early post-tetanic component called slowly decaying potentiation (SDP) lasts for about 30-45 min and is independent of kinase activity. In the developing retinotectal projection, retinal terminals are mobile and synapses are transient. It is possible that the type of synapse stabilization seen in the developing retinotectal projection is comparable to SDP in the hippocampus, where coactive synapses are selectively stabilized, but for a limited period of time. Support by NIH EY06039&05818.

203.1

CLONING AND DNA SEQUENCING THE unc-29 AND unc-38 LEVAMISOLE RECEPTOR GENES OF THE NEMATODE CAENORHABDITIS ELEGANS. J.A. Lewis. Division of Life Sciences, University of Texas at San Antonio, San Antonio, TX 78285.

I have cloned the unc-29 and unc-38 receptor genes of the nematode C. elegans by Tcl transposon tagging. These genes are likely to encode structural peptides of the levamisole receptor, a nicotinic acetylcholine receptor present on nematode muscle. Spontaneous, putative transposon-induced mutants in these and other genes needed to make the receptor can be selected by means of the drug resistance that mutationally-induced receptor deficiency confers against the toxic muscle-contracting effects of levamisole, a potent nicotinic acetylcholine analog.

For unc-29, genomic probes derived from a Tcl insert revealed RFLP's in 6 unc-29 spontaneous mutants, with restoration of wild-size in revertants of several mutants. An RFLP was found in 1 of 5 unc-29 gamma-ray mutants in the same region. For unc-38, 4 of 5 different spontaneous Bergerac mutants were found to carry the same novel 4.7 kb HindIII Tcl-containing restriction fragment. Three of six gamma-ray unc-38 mutants showed a size difference in the HindIII fragment identified by Tcl tagging.

DNA sequencing reveals strong homology at the amino acid level of the putative unc-29 protein to ard, a putative Drosophila acetylcholine receptor structural gene, and somewhat less but still impressive homology to vertebrate nicotinic acetylcholine receptor subunits.

203.2

CLONING THE DOMAIN CONTAINING THE FOUR EXTRACELLULAR CYSTEINES OF SNAKE ACETYLCHOLINE RECEPTOR α-SUBUNIT. D. Neumann*, D. Barchan*, M. Horowitz*, E. Kochva* and S. Fuchs. (SPON:A.S. Gordon). Dept. of Chemical Immunology, The Weizmann Institute of Science, Rehovot, Israel, and Dept. of Zoology, Tel Aviv University, Tel Aviv, Israel.

Muscle acetylcholine receptor (AChR) of elapid snakes binds cholinergic ligands but unlike other muscle AChRs does not bind α-bungarotoxin (α-BTX). Previous studies indicate that the ligand binding site of the AChR includes cysteine residues at positions 192,193 of the α-subunit. In attempt to elucidate the structural basis for the unique binding properties of snake AChR, the domain of the α-subunit from Natrix and cobra AChR (amino acid residues 119-222), which contains the four extracellular cysteines (128,142,192,193) was amplified by reverse transcription of mRNA and the polymerase chain reaction, and then sequenced. Sequence comparison revealed that the cloned region of the snake α-subunit is highly homologous (75-80%) to other muscle AChRs and contains the two tandem cysteines at positions 192,193 like all other AChR α-subunits. In the presumed ligand binding site, in the vicinity of cysteines 192 and 193, four major substitutions occur in the snake sequence at positions 184 (Trp to Phe), 185 (Lys to Trp), 187 (Trp to Ser) and 194 (Pro to Leu). In addition, Asn 189 is a putative N-glycosylation site, present only in the snake. These changes or part of them may explain the lack of α-BTX binding to snake AChR.

203.3

cDNA CLONES CODING FOR SUBUNITS OF THE NEURONAL α-BUNGAROTOXIN-BINDING PROTEIN. R. Schoepfer, P. Whiting, W.G. Conroy,* J. Lindstrom. The Salk Institute, La Jolla, CA 92037

α-Bungarotoxin (α-Bgt) binds to the acetylcholine binding site of muscle-type nicotinic acetylcholine receptors (AChRs) and also binds to some vertebrate neuronal proteins with nicotinic binding properties. The function of these neuronal α-Bgt binding proteins (α-Bgt-BPs) is unclear; there is a large body of evidence indicating that the α-Bgt-BPs which have been most carefully studied are not ACh-gated cation channels. The subunit composition of neuronal α-Bgt-BPs is uncertain. It is certain that neuronal α-Bgt-BPs are functionally, structurally, immunologically, histologically, and pharmacologically distinct from functional vertebrate neuronal AChRs which do not bind α-Bgt.

Using an oligonucleotide probe based on the N-terminal amino acid sequence of a polypeptide of the α-Bgt-BP from chicken brain reported by Conti-Tronconi et al. (1985, *PNAS USA 82*, 5208) we have isolated a partial cDNA clone (Ch29) from a chicken brain cDNA library. The deduced amino acid sequence of Ch29 is identical at 19 of 21 amino acids of the reported sequence. By low stringency hybridization using Ch29, a second full-length cDNA clone (Ch31) was isolated which has 80% amino acid sequence identity to Ch29, suggesting that it codes for another subunit of the α-Bgt-BP. A unique sequence of Ch31 was expressed in bacteria. Antisera to this bacterially expressed protein efficiently precipitated authentic chicken brain α-Bgt-BPs. Thus, we have now determined part of the primary structure of one neuronal α-Bgt-BP subunit and the complete sequence of another.

203.4

SUBUNIT STOICHIOMETRY OF NEURONAL NICOTINIC ACETYLCHOLINE RECEPTORS. P. Whiting, J. Cooper,* W.G. Conroy,* and J. Lindstrom. The Salk Institute, La Jolla, CA 92037

Muscle-type nicotinic acetylcholine receptors (AChRs) have been shown by a variety of chemical and physical techniques to be pentamers consisting of two ACh-binding subunits (α) and three structural subunits (β, γ, δ) arranged like barrel staves around a central ion channel. Recently, using both monoclonal antibodies and cDNAs as probes, it has been shown that neuronal nicotinic AChRs are members of the same gene family as muscle AChRs. In contrast, however, the major subtype of neuronal AChRs appears to consist of only two types of subunits, ACh binding (α4) and structural (also termed β2 or non-α).

Using a chemical modification technique we have investigated the subunit stoichiometry of the neuronal AChR. Purified AChRs from *Torpedo* electric organ (control) and the brains of chickens, rats, and cows were denatured in 1% SDS and 4M urea, radioiodinated (to label tyrosine residues), and the subunits resolved by SDS-PAGE. After autoradiography, the subunits were excised, radioactivity quantitated, and the stoichiometry determined after correction for the number of tyrosines in each subunit. *Torpedo* AChR had the expected stoichiometry (α:β:γ:δ, 2.1:0.99:0.84:1). AChRs from brains of chicken, rats, and cows had stoichiometries (ACh-binding:structural subunit) of 1.1:1, 1.0:1, and 1.0:1, respectively. The calculated molecular weight of the structural subunits is ~54kD, the molecular weight of the ACh-binding subunits is ~68kD, and the molecular weight of the native neuronal AChR is ~300kD. Thus, the data is consistent with a tetrameric arrangement of two structural and two ACh-binding subunits.

203.5

INHIBITION OF ^{125}I-THYMOPOIETIN BINDING TO BRAIN MEMBRANES BY α-BUNGAROTOXIN AND NICOTINIC RECEPTOR LIGANDS. M. Quik, R. Afar*, T. Audhya* AND G. Goldstein* (SPON: P. Braun). Dept. Pharmacol., McGill Univ., Montreal, Can. & Immunobiol. Res. Inst., Annandale, NJ.

The identity of the nicotinic α-bungarotoxin (α-BGT) site in neuronal tissue is currently unclear because the toxin does not block nicotinic responses. It was thus of interest to determine whether agents other than acetylcholine could interact with the neuronal α-BGT receptor to possibly act as a regulator of this site. Current studies in our laboratory showed that the thymic polypeptide thymopoietin potently inhibited α-BGT binding to brain membranes (IC_{50} = 3.1 nM). In the present work, experiments were done using ^{125}I-thymopoietin. Binding studies showed that radiolabelled thymopoietin bound to brain membranes in a receptor specific fashion. The specific binding was saturable, reversible and of high affinity (Kd = 0.3 nM). Binding of ^{125}I-thymopoietin could be displaced, not only by cold thymopoietin, but also by α-BGT (Kd = 0.4 ηM) and nicotinic receptor ligands. On the other hand, a wide variety of other receptor ligands (muscarinic, adrenergic, dopaminergic) did not affect ^{125}I-thymopoietin binding. Thus, ^{125}I-thymopoietin binds with high affinity to a receptor site in brain; furthermore, binding is affected by α-BGT and nicotinic ligands. This may suggest that thymopoietin is a regulator of the neuronal nicotinic α-BGT receptor.

203.6

CYCLIC AMP-DEPENDENT PHOSPHORYLATION OF NICOTINIC ACETYLCHOLINE RECEPTORS IN CHICK CILIARY GANGLION NEURONS. S. Vijayaraghavan*, H.A. Schmid*, and D.K. Berg. Dept. of Biology, Univ. of Calif., San Diego; La Jolla, CA 92093.

Chick ciliary ganglion neurons have nicotinic acetylcholine receptors (AChRs) that mediate primary synaptic transmission through the ganglion. The ACh response of the neurons in culture can be enhanced by a cAMP-dependent process in which AChRs appear to be converted from a "silent" state to a functionally available one. We report here that 2 major species of subunits can be identified in AChRs immunopurified from the neurons in culture, and that both can be phosphorylated *in situ* in a cAMP-dependent manner. Moreover, cAMP analogs increase the ACh response and subunit phosphorylation to a comparable relative extent and do so after a similar lag period.

AChRs were immunoaffinity purified from ciliary ganglion neurons in culture, radioiodinated, and shown by SDS-PAGE to contain major subunit species with M_rs of about 50 and 58 kD. Both are phosphoproteins: incubating the neurons with $^{32}P_i$ prior to isolating AChRs produced ^{32}P-labeled subunits. Exposing the neurons to membrane-permeant cAMP analogs for 6 hours increased the amount of phosphorylation about 3-fold for both types of subunits, reaching levels of approximately 0.5 mole of P_i incorporated per mole AChR for each. No change was observed in the total number of AChRs or in the specific activity of the intracellular pool of ATP. AMP and a cGMP analog were unable to mimic the effects of the cAMP analogs. The ACh response was increased about 2-fold by a 6 hour incubation with cAMP analogs, and neither the ACh response nor the subunit phosphorylation showed an increase during the first 30 minutes of the incubation. These findings encourage the speculation that a cAMP-dependent process either directly or indirectly phosphorylates AChRs and thereby shifts the ratio of postulated "silent" and functionally available receptors on the neurons. (Supported by NIH grants NS12601 and NS25916)

203.7

TARGET-DERIVED COMPONENTS REGULATE THE NUMBER, FUNCTION, AND CYCLIC AMP MODULATION OF ACETYLCHOLINE RECEPTORS ON CHICK CILIARY GANGLION NEURONS IN CULTURE.
S.W. Halvorsen, H.A. Schmid*, A.E. McEachern, and D.K. Berg. Dept. of Biology, Univ. of Calif., San Diego; La Jolla, CA 92093.

Chick ciliary ganglion neurons have nicotinic acetylcholine receptors (AChRs) for synaptic input from preganglionic terminals, and innervate muscle tissue in the eye. Previous studies have shown that postganglionic axotomy greatly reduces the number of AChRs on the neurons *in vivo*, suggesting that contact with synaptic targets in the eye may be necessary for maintenance of AChRs on the neurons. We now report that a component of about 50 kD from eye tissue increases the number of AChRs on the neurons in culture. The component, or one of similar size, causes an even greater increase in the ACh response, and confers on the neurons the ability to have their ACh response increased by cyclic AMP (cAMP) analogs.

The 50 kD component was discovered by using gel filtration to fractionate extracts prepared from embryonic eye tissue. Testing the fractions on ciliary ganglion neurons in culture at low cell density indicated that a component of about 50 kD caused a 2-fold increase in the number of AChRs over a 1 week period without changing cell growth. AChRs were measured with a radiolabeled anti-AChR monoclonal antibody. Intracellular recordings revealed a 7-fold increase in the ACh-induced membrane conductance. In addition, the ACh response was doubled when the neurons were treated with a cAMP analog for 6 hours; no cAMP-dependent change in ACh response was observed for neurons grown without the 50 kD component. The active material is heat-labile and trypsin-sensitive, as expected for protein. Other tissues, including liver, have components of similar size that enhance the ACh response, but the specific activity of the 50 kD fraction from eye extract is 10-fold that of the liver fraction. The number of distinct active components in the 50 kD fraction is unknown. The component(s) may provide a means by which tissues regulate neurotransmitter receptors on the neurons that innervate them. (Supported by NS12601 and NS21725)

203.8

EFFECT OF DENERVATION UPON ACETYLCHOLINE RECEPTOR NUMBER ON THE SURFACE OF CARDIAC GANGLION CELLS.
P.B. Sargent and G.K. Bryan, Division of Biomedical Sciences, University of California, Riverside, CA 92521.

The effect of denervation upon the number of nicotinic acetylcholine receptors (AChRs) on the surface of autonomic neurons was examined in the cardiac ganglion of the frog *Rana pipiens*. ^{125}I-Neuronal bungarotoxin (kappa-bungarotoxin, toxin F, bungarotoxin 3.1) was used as a specific ligand for AChRs. Bath-applied neuronal bungarotoxin (n-bgt) reversibly blocked synaptic transmission in the cardiac ganglion, as monitored by intracellular recording. Excitatory post-synaptic potentials were reduced to subthreshold levels with 5 and 20 nM n-bgt in a dose-dependent manner. Binding studies showed that ^{125}I-n-bgt recognizes two classes of sites in ganglion homogenates; a high affinity binding site with a K_d of 5 nM and a B_{max} of 0.05 fmol/μg protein and a low affinity binding site with a K_d of 60 nM and a B_{max} of 0.3 fmol/μg protein. Comparison of the binding and electrophysiological results strongly suggests that the high affinity binding site represents the synaptic AChR.

Light microscopic autoradiography using 20 nM ^{125}I-n-bgt revealed an accumulation of grains at synaptic sites in normally innervated ganglia. The total number of grains at the surface of neurons denervated for 2-3 weeks was significantly reduced as compared with normal. This suggests that, in contrast to muscle, denervation of neurons leads to a decrease in the number of surface AChRs. The denervation-induced increase in acetylcholine sensitivity that is observed in cardiac ganglion cells may result from a change in receptor distribution, in receptor properties, and/or in cell surface acetylcholinesterase. (Supported by NIH NS24207).

203.9

LIGAND BINDING AND FUNCTIONAL PROPERTIES OF NICOTINIC ACETYLCHOLINE RECEPTORS EXPRESSED BY THE SH-SY5Y AND IMR-32 HUMAN NEUROBLASTOMA CLONAL LINES.
Ronald J. Lukas. Div. Neurobiol., Barrow Neurological Inst., Phoenix AZ 85013.

Pharmacological profiles were obtained for high-affinity 3-H-labeled acetylcholine (T-ACh) and 125-I-labeled alpha-bungarotoxin (I-Bgt) binding sites and for functional nicotinic acetylcholine receptors (nAChR) mediating 86-Rb efflux, all of which are expressed by cells of the human neural crest-derived SH-SY5Y and IMR-32 neuroblastoma (NB) clonal lines. For both NB lines (as is the case for the PC12 rat pheochromocytoma line) relatively poor inhibition (IC_{50} > 10 uM) of T-ACh and I-Bgt binding is observed for decamethonium and succinyldicholine (SCh), which inhibit T-ACh and I-Bgt binding to muscle nAChR by more than 50% at concentrations less than 1 uM. Suberyldicholine and SCh, which are strong and weak agonists, respectively, for muscle nAChR, are a poor agonist and an antagonist for functional nAChR responses measured in NB (and PC12) clones. Moreover, mecamylamine is a more potent antagonist of NB (and PC12) cell functional nAChR than is d-tubocurarine, and neither alpha- nor kappa-bungarotoxin produce significant inhibition of nAChR function at concentrations of 1 uM, in contrast to results obtained with muscle nAChR. The results indicate that SH-SY5Y and IMR-32 NB cell lines express high-affinity T-ACh and I-Bgt binding sites and functional nAChR that are more closely related to their neuronal analogues than to muscle nAChR.

203.10

HIGH AFFINITY STATE OF THE CLONED M_1 MUSCARINIC RECEPTOR IS FUNCTIONALLY COUPLED TO THE HYDROLYSIS OF INOSITOL LIPIDS IN TRANSFECTED MURINE FIBROBLAST B82 CELLS.
L. Mei, J. Lai, H.I. Yamamura and W.R. Roeske. Department of Pharmacology, University of Arizona, Tucson, AZ 85724.

The relationship between the M_1 muscarinic receptor (mAChR) density and the receptor-mediated hydrolysis of inositol lipids was studied in a series of 7 clones of transfected B82 cells. Carbachol(CCh)/[^{3}H](-)MQNB competition curve for the LK3-1 cells (receptor density of 12 fmol/10^6 cells) had a Hill coefficient (n_H) close to the unity while the competition curves in the clones with higher receptor densities (30-360 fmol/10^6 cells) had n_H less than one. The percentage of the M_1 mAChR which had high affinity for CCh (K_H of 1.9-7.7 μM) decreased as the receptor density increased, suggesting that endogenous factor(s) in these cells may be important for the agonist affinity state of the receptor. A significant correlation was observed between the M_1 mAChR density with high affinity for CCh and the maximum [^{3}H]IP_1 accumulation in these cells. In contrast to the K_L values of 34-53 μM, the K_H values were not significantly different from the CCh EC_{50} values (3.5-26 μM), or its functional dissociation constants (1.9-7.4 μM) obtained by the Furchgott method. These results suggest that the high affinity state for CCh may be the functional state of the M_1 mAChR in the transfected B82 cells. In addition, spare M_1 mAChR was observed in the clones with higher receptor density but not in those with low receptor densities. Supported by USPHS grants.

203.11

LIGAND BINDING AND FUNCTIONAL PROPERTIES OF A MUSCARINIC ACETYLCHOLINE RECEPTOR EXPRESSED BY THE TE671 CLONAL LINE.
Merouane Bencherif and Ronald J. Lukas. Division of Neurobiology, Barrow Neurological Institute, Phoenix AZ 85013.

TE671 human medulloblastoma clonal cells exhibit muscarinic acetylcholine receptors as revealed by specific, high-affinity binding of tritium-labeled quinuclidinylbenzilate (^{3}H-QNB). Functional studies have indicated that phosphoinositide hydrolysis is increased by carbamylcholine (1mM), the muscarinic agonist acetyl-beta-methylcholine bromide (1mM) but not by nicotine. Phosphoinositide metabolism is completely inhibited by atropine (1uM) and pirenzepine (10^{-6}M) but not by alpha-bungarotoxin (1 uM). Carbamylcholine stimulates inositol-1-phosphate accumulation with an EC_{50} = 10 uM. Atropine exhibits an IC_{50} = 1nM whereas pirenzepine was much less effective (IC_{50} = 300 nM) for displacement of ^{3}H-QNB binding and for inhibition of carbachol-stimulated accumulation of inositol-1-phosphate. These results indicate that a muscarinic acetylcholine receptor subtype with intermediate/low affinity for pirenzepine and coupled to phosphoinositide hydrolysis is expressed by TE671 human medulloblastoma cells.

203.12

Expression and Pharmacological Characterization of a Human Muscarinic Acetylcholine Receptor in Saccharomyces cerevisiae.
P. Payette*, F. Gossard*, M. Whiteway* and M. Dennis* (SPON: J. Magnan) Biotechnology Research Institute, Montreal H4P 2R2.

Muscarinic acetylcholine receptors are encoded by (at least) five distinct genes (M1-M5). The five structural subtypes fall into three pharmacological classes (m1-m3) and couple differentially to two G-protein-mediated response pathways. In an effort to develop systems which will permit detailed studies of ligand specificity and coupling selectivity of individual subtypes, we have examined the yeast S. cerevisiae as a host for expression of a gene encoding the human mAchR M1 subtype. The HM1 coding sequence was cloned into the yeast expression vector pVT102U, placing the gene under control of the promoter for the yeast alcohol dehydrogenase gene. Yeast transformed with this construct, but not with vector alone, produced HM1 transcripts as assessed by Northern blotting. Membrane preparations exhibited saturable binding of the muscarinic antagonist [3H]-N-methyl scopolamine to a single class of sites (KD = 179 pM, Bmax = 1-20 fmol/mg protein). A series of muscarinic agonists and antagonists inhibited binding with Ki values and a rank order of potency comparable to those described for m1 in tissues. We conclude that yeast can synthesize a mammalian G-protein- coupled receptor with appropriate pharmacological properties. (Supported by NRC and MRC.)

204.1

CELL INTERACTIONS DETERMINE ASYMMETRIC, ALTERNATING DISTRIBUTION OF SCP NEURONS IN LEECH CNS. S.S.Blair, M.Q.Martindale* and M.Shankland. Dept. Anatomy, Harvard Med. Sch., Boston, MA 02115.

The nerve cord of the leech is formed by a bilaterally distributed set of segmentally iterated precursor cells, which give rise to identifiable neurons that are for the most part bilaterally paired and identical from segmental ganglion to ganglion. However, some neurons are laterally asymmetric and segment specific in their distribution. The Rostral and Caudal Alternating SCP neurons (RAS and CAS) are two such neurons, identified by their staining with antisera against molluscan small cardiac peptide (SCP) and FMRFamide. Though born as bilaterally symmetric pairs, the cells within each ganglion "compete" so that only one neuron retains immunoreactivity; ablation of postmitotic AS neurons on one side leads to the retention of AS staining on the unablated side over 90% of the time, as long as the ablation is performed within a certain critical period.

An interesting feature of RAS and CAS distribution is that the side on which each neuron is retained normally alternates between successive ganglia with a fidelity of 95%. This is apparently determined by interactions between cells in successive ganglia. We ablated AS neurons or their precursors in single or small groups of ganglia; when CAS sidedness in one ganglion was biased by the ablation of the contralateral homologue, it influenced sidedness in the next posterior but not the next anterior ganglion, while the opposite appeared true of RAS. Since there is a corresponding difference in the projection of RAS and CAS axons in the nerve cord (posterior-contralateral for RAS, anterior-contralateral for CAS), it raises the possibility that AS neurons interact directly via axons to determine final cell fate. In this scheme, the axon would detect the AS cell asymmetry in the adjacent ganglion, and feed that information back to its cell body, reinforcing the peptidergic AS phenotype. AS distribution would thus be controlled by a combination of interactions within and between ganglia.

204.2

CONTACTS BETWEEN EMBRYONIC RETZIUS NEURONS AND GENITAL MESENCHYMAL CELLS MODIFY DEVELOPMENT. K.A. French, S.M. Jordan*, and W.B. Kristan, Jr., Dept. of Biology, UCSD, LaJolla, CA, 92093.

The Retzius (Rz) neurons in the central nervous system of the leech are alike in all mid-body segments, except in the segments containing the reproductive organs (segments 5 and 6). Although the Rz neurons in those two segments [Rz(5,6)] initially resemble their homologues, they begin to develop differently during the 10th day of embryonic life. On day 10, Rz(5,6) processes approach their future target organs, the reproductive ducts (RDs), and it has been shown previously that when embryonic RDs are removed on day 10, Rz(5,6) develop more like other Rz neurons.

During the 10th day of embryonic life, the RDs also undergo major morphogenetic changes. Early on day 10, the embryonic RD tissue is a mass of undifferentiated cells lying on either side of the ventral midline in segments 5 and 6. During the 10th day, while the processes of the Rz(5,6) reach the embryonic RDs and branch repeatedly, the RD tissue separates into two populations of cells: a tube, one epithelial cell thick, that demarkates the future basic morphology of the RDs and a cloud of apparently mesenchymal cells surrounding the epithelial tube near the midline. In embryos incubated with 5'-bromodeoxyuridine (BrdU) and labeled with antibody to BrdU, RD mesenchymal cells remained mitotically active on day 10 and for several days afterwards. During the next several days, RD mesenchymal cells appear to differentiate into the muscles of the RD walls, the eventual target organ of Rz(5,6). In serial sections of 10 day embryos, Rz processes were seen to contact the mesenchymal cells, but neither to contact the epithelial tube nor to extend beyond the mesenchyme. Between days 10 and 13, as the mesenchymal cells increased in number and extended toward the midline posterior to the epithelial tube, the processes of Rz(5,6) grew into this new territory. We hypothesize that Rz(5,6) processes follow a pathway marked by RD mesenchymal cells and that in this system - as in several other systems - contact with mesenchymal cells modifies the developmental program of embryonic cells, in this case Rz(5,6). This work was supported by a March of Dimes grant and NIH Research Grant HS25916 to WBK and a University of California, San Diego, BSRG grant to KAF.

204.3

MORPHOGENESIS OF AN IDENTIFIED LEECH MOTOR NEURON MAY INVOLVE BOTH STEREOTYPED AND PLASTIC DEVELOPMENTAL PHASES. W.B. Kristan, Jr. & J. Jellies. Dept./Biology, UCSD, La Jolla, CA, 92093.

Our interests in the ontogeny of behavior and neuromuscular organization have led us to examine the morphogenesis of cell 3, a behaviorally important excitor of dorsal longitudinal muscle in Hirudo medicinalis. HRP was injected into 118 cells 3 that were 10 to 40 days old (embryonic through early postembryonic). The initial axonal projection is highly stereotyped, but neuropilar processes added secondarily exhibit variability that implies plasticity. In the profuse arbor of anterior-projecting neurites contralateral to the soma, the number of primary neurites increases throughout embryogenesis, stabilizing close to the peak of 5.4/cell ±1.2(SEM) at 18 days. Neuritic complexity, measured as branch-points per neurite, also increases- to a high of 9.2/neurite ±1.08(SEM) at day 25. Overall complexity subsequently decreases postembryonically as only 1 or 2 of the primary neurites retains an elaborate branching pattern. Additionally, many cells 3 possessed extraganglionic neuritic branches during embryogenesis (≈35%of cells at late stages), which were lost postembryonically (<9% of cells). Thus, the final identifiable morphology of this motor neuron may arise by stereotyped axonal pathfinding followed by a period of overproduction of neurites that are then selectively pruned by as yet unknown mechanisms.
This work was supported by NIH NS25916 (WBK).

202.4

INDEPENDENT TARGET INNERVATION BY TWO AXONS ON THE SAME RETZIUS CELL DURING DEVELOPMENT OF THE LEECH. J. Jellies & W.B. Kristan, Jr. Dept./Biology, UCSD, La Jolla, CA, 92093.

Retzius (Rz) neurons in the two reproductive segments (5&6) of Hirudo medicinalis innervate reproductive structures rather than body wall muscle and skin, the targets for their segmental homologs. Rz(5,6) differ from their segmental homologs in morphology and physiology and development of the Rz(5,6) phenotype depends upon apparent contact with the reproductive primordia early in development. Rz(6) in the female segment innervates male tissue via a nerve (SNA(6)) found only between those two segments. Removing a single peripheral cell (the ARC) early in development prevents SNA(6) formation. We asked how the male innervation influences development of Rz(6) by ablating ARC at embryonic day 9, followed by intracellular HRP-filling at day 18 to determine the branching of Rz(6) axons. In each of 6 cases the anterior axon of Rz(6) branched laterally in the body wall, as in non-reproductive segments, and did not project toward male or female tissue. The posterior axon made its usual contact with female tissue and retraction of the CNS axons was induced. From these results we conclude that in early development; 1) Rz(6) peripheral axons can act independently to innervate different targets simultaneously, 2) the reproductive phenotype predominates when both peripheral targets are innervated by the same cell, 3) male tissue attracts axons to it only over relatively short distances.
This work was supported by NIH NS25916 (WBK).

202.5

GIANT DROSOPHILA NEURONS DIFFERENTIATED FROM CYTOKINESIS-ARRESTED EMBRYONIC NEUROBLASTS. C.-F. Wu, K. Sakai* and Y. Hotta*. Dept. of Physics, Univ. of Tokyo, Tokyo, JAPAN 113 and Dept. of Biology, Univ. of Iowa, Iowa City, IA 52242 USA.

Cells in cleavage-arrested early embryos of ascidians and nematodes can develop in correct lineage pattern several tissue-specific enzymes, antigens and ion channels, demonstrating differential segregation of cytoplasmic determinants for developmental potentials of daughter cells. However, effects of cleavage arrest on more complex features differentiated in late embryos have not been extensively studied, especially in species other than those of typical mosaic development. For example, some classes of neuronal differentiation might be initiated solely by intrinsic factors already present in the neuroblast even though normal differentiation occurs in daughter cells after a sequence of neuroblast divisions. We found that isolated neuroblasts from Drosophila gastrulae were able to differentiate neuron-specific properties even when cell divisions were inhibited by cytochlasin B. The resultant giant multinucleated neurons display thickened neurites with a variety of branching patterns and express action potential activity and neuron-specific antigens. These results indicate that the factors for initiating neuronal differentiation programs are present in a neuroblast, and that the expression does not depend on processes following further cell divisions.

202.6

MEMBRANE EXCITABILITY AND SYNAPTIC ACTIVITY IN CELL DIVISION-ARRESTED NEUROBLASTS IN *DROSOPHILA* CNS CULTURE.
M.Saito* and C.-F.Wu (spon:M.Gorczyca), Dept. of Biology, Univ. of Iowa, Iowa City, IA 52242

Drosophila is well-suited for molecular genetic analysis of channels and other proteins involved in neuronal signalling mechanisms. A growing collection of behavioral mutants is available which can be used to identify genes encoding such components in excitable membranes and to correlate their structural mechanisms to their functional activity *in vivo*. However, studies of *Drosophila* neurons have often been hampered by their small size. We found that gastrula neuroblasts in dissociated cultures can develop into giant multinucleated cells with typical neuronal morphology when neuroblast divisions are inhibited by cytochalasin B (Wu,Sakai & Hotta, this volume). These cells are more accessible to electrophysiological studies and may provide a useful model system for characterizing mutant phenotypes. We examined the electrical properties of these giant neurons by the whole-cell recording technique. Action potentials could be elicited by current injection in 1-13 day cultures. The firing pattern varied among cells but the frequency of spikes in general increased in response to stronger membrane depolarization. In voltage-clamp mode, some cell bodies showed large inward current followed by outward current. The amplitude of inward and outward currents varied from cell to cell. These giant neurons were capable of forming functional synaptic connections. In high density cultures, spontaneous excitatory synaptic currents of up to 30pA (holding potential -60mV) could be detected in some cells. Further experiments are underway to determine the ionic species and pharmacological sensitivity of these currents.
Supported by NIH grants NS 18500 and NS 26528.

204.7

EXPRESSION OF THE SINGLE-MINDED GENE IN DROSOPHILA. John R. Nambu and Stephen T. Crews. Dept. of Biology, UCLA, Los Angeles, CA 90024.

The single-minded (sim) gene encodes a nuclear protein which plays an important role in the development of the embryonic central nervous system. Mutations in the sim locus yield a recessive embryonic lethal phenotype, characterized by a collapse of the ventral nerve cord due to a loss or misplacement of midline nerve cells. The gene appears to be contained within 14 kb of genomic DNA and is comprised of at least seven exons. To expand upon previous studies of sim expression, we have generated a sim/lac-Z fusion construct which contains sim 5' upstream sequences and most of the sim coding region fused in frame to beta-galactosidase. This construct was used to transform flies, and expression of beta-galactosidase, as directed by the sim sequences, was monitored via X-gal or anti-beta-galactosidase staining. In wild type embryos, expression is detected after gastrulation in midline nerve cell precursors as well as in cells of the stomodeal and posterior midgut invaginations. The midline nerve cell precursors continue to stain during germ band retraction and sim-expressing glial cells are later found at the dorsal surface of the nerve cord. Sim expression is greatly reduced after nerve cell differentiation. Similar to previous immuno-cytochemical studies on the native sim protein, the sim/lac-Z fusion protein is localized to cell nuclei. The fusion construct is also expressed in sim mutant embryos, however the positions of sim-expressing cells during germ band retraction and neurogenesis appear to be altered. These and related studies are useful for further defining the roles of sim expression in the normal differentiation and function of the midline nerve cells, as well as in facilitating the identification of cis and trans regulatory elements.

204.8

A NEW MONOCLONAL ANTIBODY, 3H6, SPECIFICALLY STAINING THE MOLECULAR LAYER OF THE CEREBELLUM, USED TO STUDY CELL INTERACTIONS IN MURINE NEUROLOGICAL MUTANTS. A. M. Smith* and R. J. Mullen. Dept. of Anatomy, Univ. of Utah Sch. of Med., Salt Lake City, UT 84132.

The mouse MAb 3H6 is an IgM which specifically stains the neuropil in the molecular layer of the cerebellum in both mice and rats. The cognate antigen is first detectable at postnatal day 14 and remains on in the adult. The objective of these experiments was to determine where the antigen is localized and how it is regulated. Sections of fixed, polyester wax or paraffin embedded brain were immunohistochemically stained using Vector ABC with HRP.

In adult weaver (wv) mutants, which lose most of their granule cells, the epitope for 3H6 is not detected. However, in the heterozygote and wild-type it is present. The mutants Purkinje cell degeneration (pcd) and nervous (nr), which lose Purkinje cells postnatally, do not express this epitope in regions where Purkinje cells have degenerated. In postnatal day 42 pcd, when most degeneration has occurred, there is strong staining only in the cerebellar nodulus, the last region to lose Purkinje cells, while in the adult the staining is absent. In nr, where some Purkinje cells survive even in adults, staining is present only in close proximity to surviving Purkinje neurons. These preliminary data support our working hypothesis that the cognate antigen is on the granule cell parallel fiber and that continuing interaction with Purkinje cells is necessary to induce and maintain its expression. Examination of additional mutants and biochemical characterization of this antigen has begun. (Supported by NIH Grant EY07017.)

204.9

AN ANTIBODY THAT RECOGNIZES NEURONAL CELL NUCLEI: CELL SPECIFICITY AND DEVELOPMENTAL REGULATION. R. J. Mullen, A. M. Smith*, and C. R. Buck. Dept. of Anatomy, Univ. of Utah Sch. of Med., Salt Lake City, UT 84132.

An objective of our research has been to produce monoclonal antibodies directed against cell nuclei in the nervous system. BALB/c mice were immunized with purified brain nuclei from other species of mice. One anti-nuclear antibody, A60, is of particular interest as it appears specific to the nervous system, including the peripheral nervous system, and does not stain glia. The antibody recognizes most adult neuronal cell nuclei with three exceptions: cerebellar Purkinje cells, mitral cells in the olfactory bulb, and photoreceptor cells. Immunohistochemically, the cognate antigen is detectable as early as embryonic day 9 in mice. The epitope is not detectable in ventricular zones but becomes evident after the cells become post-mitotic and/or have migrated. In adults, the staining is most intense in nuclei although it is also detectable in the cytoplasm. It appears there can be significant variations in the intensity of staining in nuclei between neighboring cells. The antibody cross-reacts with rat, chick, and human. On Western blots, the antibody stains at least two bands in the 40-44 Kd range. The observations that the epitope is early-appearing, nervous system specific, cell type specific, and that amounts vary between cells of the same type, lead to the working hypothesis that the cognate antigen is involved in neuronal differentiation and regulation. Molecular characterization of this antigen has begun. (Supported by NIH Grant EY07017).

204.10

THE DEVELOPMENTAL EXPRESSION OF OZ42 IN WILD-TYPE AND WEAVER CEREBELLA. R.J. Smeyne[1], J. Napieralski[1], L.B. Pickford[2], R.V. Rouse[2], and D. Goldowitz[1]. Dept. of Anatomy[1], Thomas Jefferson University, Philadelphia, PA. Dept. of Pathology[2], Stanford University School of Medicine, Stanford, CA.

In the external granular layer (EGL) of the developing cerebellum, granule cells proliferate, exit their cell cycle, extend processes, and prepare for migration into the internal granular layer (IGL). We have suggested that the weaver (wv) gene deleteriously affects one of these processes; leading to the death of granule cells in the EGL. A method to dissect these developmental stages is immunocytochemical localization of stage-specific antigens. One antibody, OZ42 is suggested to specifically recognize a protein on post-mitotic premigratory cerebellar granule cells during the early stages of axon outgrowth. Fresh frozen sections of cerebellum from P0-P10 +/+, wv/+ and wv/wv mice were cut at 6μm, mounted onto slides, and immunostained for the presence of OZ42. Immunoreactive granule cells are first detected at P1 in the posterior lobules of +/+ and wv/+ cerebella. The expression of OZ42 progresses in a posterior-to-anterior direction, so that by P3 a discrete 1-2 cell thick layer of cells in the deep EGL is stained over the entire EGL. Immunopositive staining is qualitatively lighter in the wv/+ cerebellum. In the wv/wv EGL, OZ42-positive cells are not seen at P0 or P1. At P3, immunopositive cells are found throughout the EGL, rather than in the discrete layer seen in the +/+ & wv/+. At P10, in the +/+ and wv/+ EGL a well defined layer of immunoreactivity is seen through the entire medial-to-lateral extent of the cerebellum. In the P10 wv/wv vermis, few OZ42-positive cells are seen. More normal appearing OZ42-positive cells are present in the hemispheres. The expression of the antigen recognized by OZ42 is abnormal in the wv/wv cerebellum, and suggests that axonal outgrowth is affected in the wv/wv cerebellum, as previously suggested by the in vitro studies of Willinger et al.

204.11

ISOLATION OF GENES INVOLVED IN MOUSE CORTICAL DEVELOPMENT. J.L.R. Rubenstein*, M. Porteus*, E. Brice*, and R. D. Ciarenello. Lab. of Developmental Neurochemistry, Stanford Univ. Sch. of Med., Stanford, CA 94305

We have been developing techniques to use subtractive hybridization to isolate and characterize genes which are preferentially expressed in the mouse embryonic telencephalon. Our goal is to isolate genes which regulate the development of the mammalian neocortex. The method involves construction of cDNA libraries in which the cDNA is directionally inserted into an M13 phagemid vector. We have been successful in making several such libraries with complexities of 4×10^7 individual clones. cDNA libraries from adult and embryonic libraries have their cDNA inserts aligned in opposite orientations. The libraries are then converted to a single-stranded form, using m13 helper virus rescue. The single-stranded adult library is biotinylated and then mixed with the single-stranded embryonic library. Sequences common to both libraries will hybridize, and will be removed using streptavadin (Duguid et al PNAS 1988, Vol. 85, 5738-5742). cDNAs which are preferentially expressed in the embryonic brain will be analyzed with respect to their sequence, their homology to genes known to be involved in cell-type determination and cell-cell recognition, their transcription and their tissue specific expression.

205.1

REGULATION OF β_1- and β_2-ADRENERGIC RECEPTOR mRNA IN C6 GLIOMA CELLS. C. Hough* and D.-M. Chuang (SPON: B. Schrier). Lab. Preclin. Pharmacol., NIMH, Neuroscience Center at St. Elizabeths, Washington, D.C. 20032.

Exposure of C6 glioma cells to β-adrenergic (β-AR) agonist, isoproterenol (1 μM), leads to a rapid down-regulation of β_1- and β_2-adrenergic receptor mRNA. Using Northern blotting, we studied the nature of the signal pathway underlying this response. The down-regulation induced by isoproterenol is blocked by the presence of 10 μM β-adrenergic antagonist, alprenolol. Alprenolol, however, exhibits an effect of its own on C6 expression of β_1- and β_2-AR mRNA either alone or in concert with isoproterenol. Agonist-induced down-regulation of β-AR mRNA can also be prevented by the action of protein synthesis inhibitor, cycloheximide (10 μg/ml). While forskolin (10 μM) and phorbol 12-myristate 13-acetate (1 μM), each alone, can partially induce β-AR mRNA down-regulation, 8-BrcAMP (300 μM) and dibutyryl-cAMP (300 μM) did not. The data suggest that β-AR mRNA levels are regulated by more than one mechanism.

205.2

DEVELOPMENTAL EXPRESSION OF ACH RECEPTOR γ AND ε SUBUNIT GENES IN MOUSE MUSCLE. J.C. Martinou* & J.P. Merlie. Dept. of Pharmacology, Washington University, St. Louis, MO.

AChRs of fetal and neonatal muscle exhibit primarily channels of a low conductance class whereas adult muscles contain high conductance AChR channels. This change in properties appears due to a change in subunit structure of the AChR; γ subunit mRNA is replaced by ε subunit mRNA in the adult ($\alpha_2\beta\gamma\delta \rightarrow \alpha_2\beta\varepsilon\gamma$). In order to study the developmental regulation of the expression of these subunits, we measured the levels of mRNA encoding the ε and γ subunits during embryonic (E) and post-natal (PN) development in mouse. We found that γ and ε mRNA levels change in antithetic fashion during the first two post-natal weeks. γ mRNA is expressed at a high level during embryonic days E_{17} - E_{19}, then decreases to an undetectable level after PN 15. Conversely, ε mRNA is detectable at E_{19}, increases transiently about 10 fold between PN2 to PN12 then decreases in older mice to reach in one adult a level comparable to PN3. Mice at PN 4-5 express equal quantities of γ and ε mRNA. We also studied the expression of γ and ε mRNA in vitro, in aneural cultures of mouse embryonic muscles and in the muscle cell lines C2 and BC3H1. We found that ε was not detectable in myoblasts but appeared a few days (between 2 to 5) after cell differentiation in amounts comparable to that found in neonatal mice. The fact that these cells express ε without receiving any neural influence suggests that, *in vivo*, the nerve may not be required for the expression of the ε gene. However, our results do not exclude a role for the motorneuron in modulating selectively the expression of the γ and ε subunit genes.

205.3

DISTRIBUTION OF THE D_2 DOPAMINE RECEPTOR mRNA IN MOUSE BRAIN: IN SITU HYBRIDIZATION STUDIES USING A NOVEL OLIGONUCLEOTIDE PROBE. F. Szele, J.F. Chen, G. Bai and B. Weiss, Dept. Pharmacol., Med. Col. Pa., Philadelphia, PA. 19129

The D_1 and D_2 subtypes of dopamine receptors are differentially distributed in brain, mediate different behaviors and can be regulated independently. In an initial attempt to study the molecular events associated with these receptor mediated behaviors, we measured the mRNA for the D_2 receptor in mouse brain. An oligonucleotide probe directed at a portion of the mRNA coding for the D_2 receptor was designed and radiolabelled with [^{35}S]dATP. Specificity of the probe was determined using in situ hybridization and Northern blot analyses: 1) Hybridization of the probe to mouse striatal sections was RNase sensitive; 2) Excess unlabeled D2 probe eliminated the hybridization signal, whereas excess unlabeled proenkephalin probe did not; 3) Varying post-hybridization temperatures revealed a sharp, monophasic curve with an experimental T_m value of 46°, which was similar to the theoretical Tm; 4) Northern analyses demonstrated a single species of mRNA of about 2.5 Kb; 5) Emulsion autoradiography of brain sections revealed a high signal associated with cells, with both neurons and glia labeled. Levels of the D_2 receptor mRNA in mouse brain were highest in the striatum, olfactory tubercle, and substantia nigra pars compacta; moderate levels were found in the nucleus accumbens and hypothalamus; relatively low activity was seen in the cerebral cortex, septum, hippocampus, thalamus, cerebellum and pars reticulata. This distribution correlates well with that of D_2 receptors as determined from radioligand studies. The high specificity of this probe should allow investigations into the molecular mechanisms responsible for selective dopaminergically mediated behaviors. (Supported by MH42148)

205.4

CLONIDINE REGULATES PNMT GENE EXPRESSION IN CHROMAFFIN CELL CULTURES. M.J. Evinger, P. Ernsberger, T. Joh & D.J. Reis. Divs. Neurobiology and Molecular Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021.

Neurotransmitters regulate expression of the catecholamine (CA) synthetic gene phenylethanolamine N-methyltransferase (PNMT) in adrenal chromaffin cells. The antihypertensive agent clonidine binds to imidazole receptors on these cells (Ernsberger et al., these proceedings), thereby inhibiting CA secretion through a nonadrenergic mechanism (Powis and Baker, *Molec. Pharmacol.* 29: 134, 1987). We sought to determine whether PNMT gene expression may be regulated by the actions of clonidine on imidazole receptors in primary cultures of bovine adrenal medullary chromaffin cells.

Quantitative Northern blot hybridization of PNMT cDNA to total adrenal RNA reveals that clonidine stimulates PNMT mRNA production in a dose- (10 nM-100 μM) and time-dependent manner. Maximal PNMT mRNA production (5-6-fold stimulation above controls) at 20 hr is paralleled by a ~1.5-fold increase in enzymatic activity. Clonidine analogs active at imidazole receptors likewise stimulate PNMT mRNA with a potency less than that of clonidine: clonidine > cimetidine > rilmenidine, naphazoline, IAA.

Although clonidine inhibits nicotine-induced CA release, 100 μM clonidine does not attenuate the ability of nicotine (10 μM) or carbachol (200 μM) to stimulate PNMT mRNA production. Therefore, these studies indicate that clonidine can regulate PNMT gene expression in the adrenal medulla via activation of specific imidazole receptors.

205.5

TYROSINE HYDROXYLASE mRNA REGULATION FOLLOWING NIGRAL NEUROTOXIN LESIONS. G.M. Pasinetti, M.A. Myers*, D.G. Morgan, C.E. Finch. Andrus Gerontology Center and Dept. of Biological Sciences, Univ. of Southern California, Los Angeles, CA, 90089-0191.

Nigral 6-hydroxydopamine (6-OHDA) lesions induce atrophic changes in the remaining s. nigra dopaminergic neurons (tyrosine hydroxylase (TH) immunopositive neurons) including >50% loss of TH mRNA concentration per neuron measured by in situ hybridization 270 days post lesion (Pasinetti et al. Mol. Brain Res. 5:203-209 (1989). We extend these findings examining TH mRNA prevalence in the remaining s. nigra dopaminergic neurons, striatal TH activity and striatal TH protein, DA, DOPAC content at 2, 21, 90, 270 days following nigral 6-OHDA lesions. Nigral lesions were obtained by unilateral stereotaxic injection of 6-OHDA (8 ug/3 ul volume) in the rostral portion of the s. nigra pars compacta. TH mRNA concentration was assayed by in situ hybridization combined to immunocytochemistry using primary TH antisera (Dr. J. Reinhard, Wellcome Res. Lab.). Striatal TH catalytic activity was estimated by DOPA accumulation and TH protein was measured by TH immunoassay (ELISA). Striatal catecholamine content was assayed by HPLC with electrochemical detection. Nine months after nigral 6-OHDA lesion the cross sectional area and the TH mRNA concentration of the remaining s. nigra TH immunopositive neurons was reduced by about 40% vs. contralateral control values, which were the same as unoperated control values. These results confirm previous findings. The cross sectional area of the remaining nigral TH immunopositive neurons at 2,21,90 days after 6-OHDA lesions was reduced by 10%, 12% and 27% respectively vs. contralateral control values. Experiments are in progress to determine the time dependent correlation of the TH mRNA concentration in the remaining nigral dopaminergic neurons in respect with striatal TH enzymatic activity, TH protein and catecholamine content. (Supported by National Parkinson Foundation and United Parkinson Foundation to CEF and GMP and by John D. and Katherine T. MacArthur Foundation Research Program on Successful Aging to CEF).

205.6

STIMULATION OF ADRENAL MEDULLARY CELLS INDUCES EXPRESSION OF C-FOS PROTO-ONCOGENE. M.K. Stachowiak, M. Sar*, S. An*, E.K. Stachowiak*, A.M. Poisner*, M.J. Iadarola, J.S. Hong. Barrow's Neurol. Inst., Phoenix, AZ 85013, Univ. North Carolina, Chapel Hill, NC 27599, LMIN, NIEHS/NIH, Research Triangle Park, NC 27709.

Adaptation of adrenal medullary (AM) cells to increased catecholamine and peptide secretion during stress involves coordinated activation of genes encoding AM hormones and their biosynthetic enzymes. Here we report that the activation of the rat AM cells In vivo during insulin shock, and bovine AM cells in vitro by nicotine (NIC) or angiotensin II (ANG) produces rapid and transcient elevation of c-fos mRNA levels. Induction of the c-fos mRNA by by ANG and NIC were accompanied by the appearance of c-fos protein, initially in the cytoplasm and later in the nucleus and was colocalized with tyrosine hydroxylase. Nulear expression of the c-fos protein was also induced by veratridine, forskolin, and the calcium ionophore A23187. The role of Ca^{++} and protein kinase C in the regulation of the c-fos gene by ANG was further indicated by the inhibition of the effects of ANG with nifedipine and sphingosine, protein kinase C inhibitor. Activation of the c-fos gene may play a role in the coordinated induction of genes involved in the long-term adaptation of AM cells to increased functional demands.

205.7

REGION SPECIFIC INDUCTION OF C-FOS mRNA BY RESERPINE IN THE RAT. T. Shirao, K. Onel, M.J. Evinger and D.J. Reis. Div. of Neurobiology, Cornell Univ. Med. Coll., NY, NY 10021.

In vitro depolarization of adrenal medulla (ADR) induces the trans-acting transcriptional regulator c-fos and also the mRNA for tyrosine hydroxylase (TH). *In vivo* transynaptic depolarization by reserpine (RES) induces the mRNA for tyrosine hydroxylase in adrenals (ADR) as well as noradrenergic neurons of the locus ceruleus (LC) but not in dopaminergic neurons of the substantia nigra (SN). Since the cis-acting site to which the c-fos protein binds is present in the promoter of the TH gene, we sought to establish whether reserpine will induce fos mRNA in adrenal and brain and, if so, whether the pattern of fos expression is similar to that of TH.

RES (1-8 mg/kg i.p.) elicited a dose-dependent induction of fos mRNA, detected by quantitative Northern blot hybridization to a 3.2 kb portion of the mouse c-fos gene, in the ADR of Sprague-Dawley rats. Induction is rapid, appearing within 15 min, peaking at 30 min and returning to baseline by 2 h. 8 mg/kg RES elicits maximal response in fos mRNA--a 2-fold increase relative to adrenals of vehicle-injected rats. RES also induces fos mRNA in LC with comparable dosage range and time-course. However, no change in c-fos mRNA was detectable in SN or prefrontal cortex. RES also increases ADR c-fos mRNA in spontaneously hypertensive rats (SHR). We conclude that reserpine can induce c-fos mRNA and that the pattern of distribution parallels that of the induction of TH. Conceivably, c-fos may be an intracellular mediator leading to induction of TH in ADR and noradrenergic neurons in brain.

205.8

INDUCTION OF C-FOS mRNA IN BOVINE ADRENAL CHROMAFFIN CELL CULTURES. K. Onel, M.J. Evinger and D.J. Reis. Div. of Neurobiology, Cornell Univ. Med. Coll., NY, NY 10021

The proto-oncogene c-fos protein is a trans-acting transcriptional regulator. It binds to the cis-acting AP-1 site, the base sequence of which has been detected in the promoter regions of neural genes, e.g., TH and enkephalin. Since both genes are expressed in the neuro-ectodermally derived adrenal chromaffin cells, we sought to determine: (1) whether fos mRNA can be induced in this system; (2) whether physiologically relevant neural stimuli can mediate this induction.

Depolarization of bovine chromaffin cell cultures by 50mM K^+ induces a rapid and transient increase in fos mRNA levels within 15 min, as determined by northern blot analysis with a 3.2 kb fragment of the mouse c-fos gene. Maximal levels (14x above control) are reached within 30 min and return to control by 2 hr. The transcriptional inhibitor α-amanitin (1μM) blocks this response. Pretreatment with cycloheximide permits superinduction of fos mRNA by high K^+.

In vivo, the major neural input to the adrenals is cholinergic. To determine whether cholinergic stimuli influence fos expression in adrenals, primary chromaffin cell cultures were challenged with the general cholinergic agonist carbachol (200μM) and the nicotinic cholinergic agonist nicotine (50μM). Increases in steady state fos mRNA levels were observed within 15 min. The nicotinic antagonist hexamethonium blocks this induction, implicating nicotinic receptors in this response. The chelator EGTA also prevents induction of fos mRNA by cholinergic agonists, implying a Ca^{++}-dependent mechanism.

These data indicate that c-fos mRNA is rapidly altered in bovine adrenal chromaffin cells by cholinergic agents. As other mRNAs are also induced in in this system by these stimuli, it is important to resolve whether fos may act to regulate expression of these mRNAs.

205.9

THE MAS ONCOGENE IS DEVELOPMENTALLY REGULATED IN THE RAT CENTRAL NERVOUS SYSTEM. K.A. Martin[1], S.G.N. Grant[2], and S. Hockfield[1]. Sect. of Neuroanatomy, Yale University School of Medicine, New Haven, CT 06510[1] and Cold Spring Harbor Lab., Cold Spring Harbor, NY 11724[2].

Many oncogenes, and their cellular homologs, have structural and functional similarity to elements of signal transduction pathways that transmit information from the cell surface to the nucleus. This class of genes plays an important role in regulating cell growth and differentiation. MAS is the only identified oncogene that encodes a protein which belongs to the seven-transmembrane-domain family of G-protein coupled receptors. This family includes the adrenergic, muscarinic, and substance K receptors. MAS expression has been shown by in situ hybridization (Grant et al Soc. Neuro.Sci. Abs. 1989) to be restricted to specific subsets of neurons in the adult rat CNS. We have performed in situ hybridizations using ^{35}S-UTP labelled mRNA probes to analyze the developmental regulation of MAS. In the embryonic day 10.5 (E10.5) rat MAS is expressed throughout the CNS, including the mitotic neural precursor cells of the cerebral vesicles . High levels of MAS continue to be expressed in the precursor cells in the ventricular zone of the developing telencephalon throughout the major period of cortical neurogenesis, E15-E21. During this time cells that express MAS are also found in the cortical plate, which is populated by differentiated neurons. At postnatal day 5, MAS expression in the telecephalon is restricted to subpopulations of neurons in the hippocampus, dentate gyrus, and cortex, as it is in the adult rat. MAS early expression in neuronal precursors and later restriction to a subset of cells generated by these precursors suggests it may play a role in the determination of specific neuronal phenotypes. Supported by BNS8812163 (S.H.)

205.10

WITHDRAWN

205.11

NERVE GROWTH FACTOR (NGF) INCREASES CALMODULIN (CaM) mRNA AND CaM ACTIVITY IN PC12 CELLS **G. Bai*, J.F. Chen* and B. Weiss, (Spon. J. D. Winkler). Dept. Pharmacol., Med. Col. Pa., Philadelphia, PA 19129**

Although CaM is found in high concentrations in brain and has been shown to regulate a number neuronal processes, it is still unclear what role CaM plays in neuronal differentiation. PC12 cells, which can be induced into sympathetic-like neurons by NGF, were used in these studies as a model of neuronal differentiation. Cells were cultured on collagen-coated flasks, and NGF was added every 2 days. CaM activity was quantitated by its ability to activate a CaM sensitive phosphodiesterase. CaM mRNA was monitored by Northern analysis using a 36 mer radiolabeled oligonucleotide probe complementary to the coding region of rat CaM gene I. A Northern analysis showed that this probe hybridized to two transcripts of 1.7 and 4.0 Kb. Studies using actinomycin D (10 uM) to inhibit DNA transcription showed that the CaM mRNA had a half-life of approximately 8 hr. The addition of NGF (50 ng/ml) to the PC12 cells for 2 days increased both CaM mRNA and CaM biological activity per cell. The high CaM activity was maintained for more than 2 weeks, during which time NGF-induced outgrowth of neurites was apparent. A concentration response curve showed that NGF had an ED_{50} of about 8 ng/ml for increasing CaM activity. When CaM activity was calculated on a protein basis, a smaller effect of NGF was seen, since NGF increases protein concentration per cell. Our studies demonstrating that NGF increases CaM mRNA and CaM activity suggest that CaM may be involved in NGF-induced neuronal differentiation. (Supported by GM34334).

205.12

CALMODULIN mRNA AS A MARKER FOR GENETIC INTEGRITY OF MUTANT CEREBELLAR PURKINJE CELLS. A. Messer, J.A. Plummer-Siegard*, and Bonnie Eisenberg*. Wadsworth Ctr. for Labs. and Res., NYS Dept. of Health & Sch. of Public Health Sci., SUNY, Albany, N.Y. 12201.

Calmodulin (CaM) mRNA can be used to assess the nature and timing of deficits in cerebellar mutants with intrinsically affected Purkinje cells. In wild-type mouse cerebellum, the CaM mRNA shows developmental increases during the first 14 days postnatally, and is found predominantly within Purkinje cells, with lower levels in granule cells and interneurons.

The surviving Purkinje cells of the staggerer (sg/sg) mutant, which are grossly stunted and lack tertiary dendritic spines, contain no detectable CaM mRNA, as assayed by Northern blot or an enhanced biotinylated in situ hybridzation. This is in contrast to both Lurcher (Lc) Purkinje cells and sg/sg granule cells, which express near-normal levels of this mRNA up until the time they disappear.

Thus, in Lc Purkinje cells, CaM mRNA transcription must be separate from the primary chain of mutant events. In sg/sg however, CaM mRNA is apparently part of the primary chain of abnormal function in Purkinje cells. Regulation of this gene must still be secondary to another genetic process, since other brain cells within and outside of the cerebellum have the mRNA, but lack of CaM may affect further dendritic development as well as other functions, and/or be part of a "cassette" of differentiated Purkinje cell genes. (Supported by NS17633.)

206.1

INFLUENCE OF GRAVITY ON RESPONSE TO CALORIC STIMULATION OF THE PIGEON VESTIBULAR SYSTEM. H.P. Wit, H.A.A. Spoelstra*, and J.M. Segenhout*. Dept. of Exp. ENT, Univ. Hosp., P.O. Box 30.001, 9700 RB GRONINGEN, The Netherlands.

Responses to caloric stimulation under minimal influence of gravity show that the actual stimulus to the cupula-crista system in this situation is of mechanical (fluid expansion?) origin (Wit, H.P. and Segenhout, J.M., Acta Otolaryngol (Stockh), 105:338, 1988). By measuring gross electrode responses in the pigeon to caloric stimulation, both with and without gravity influence, by a special microphonic modulation technique (Wit, H.P. et al. Advances Oto-Rhino-Laryngol (Basel), 42:59, 1988), the relative influence of the above mentioned effect could be measured. It is of the same order of magnitude as the response due to convection (Bárány's theory). A specially designed Peltier-element miniprobe was used for controlled caloric stimulation of the horizontal semicircular canal. From the results the force on the cupula-crista system as a function of probe temperature and time could be derived using the simple overdamped torsion pendulum model.

206.2

PECULIAR POSTURE IN THE ADULT FLATFISH FOLLOWING HEMILABYRINTHECTOMY AND SELECTIVE OTOLITH LESIONS. W. Graf and R. Baker. Marine Biol. Laboratory, Woods Hole, MA 02543

The relative contribution of the two labyrinths and of single otolithic endorgans to maintain posture in the adult winter flounder, Pseudopleuronectes americanus was studied. Lesion of the down-side labyrinth (n=7) resulted in spiralling towards the lesioned side in some animals as observed in upright fish. Extirpation of the up-side labyrinth (n=14), in contrast, made the animals perform several full pitch-down rotations before settling. If "translated" into upright fish movements, this response would be a rotation about the dorso-ventral axis away from the lesioned side. Thus, two fundamental differences occur compared to upright fish. First, a change in direction of the lesion symptoms from movements about the rostro-caudal axis to rotations about the dorso-ventral axis. Second, a change in laterality from body movements towards the lesioned side to rotations away from the lesion. Selective neurectomy of the utricular nerve produced identical body movements as those following hemilabyrinthectomy (up-side: n=3; down-side: n=1). Sacculus (up-side: n=4) and lagena lesions (up-side: n=2) caused inconsistent movement patterns or none at all. We suggest that the peculiar bilaterally asymmetric labyrinthine organization in the adult flatfish reflects the functional contribution of the vestibular system for maintaining the flatfish as a flatfish. (Supported by NIH grant NS20358).

206.3

COMPUTER-ASSISTED VISUALIZATION*, ANIMATION* AND MODELING* OF VESTIBULAR* MACULAS. M.D. Ross*, L. Cutler*, G. Meyer*, J. Dayhoff*. (SPON. R. Fox) NASA-Ames Research Center, Moffett Field, CA 94035.

The 3-dimensional (3-D) organization of the vestibular macular neural network is under investigation by computer-assisted methods in preparation for studies of macular adaptation to microgravity. Fifth sections of a series of 570 ultrathin sections of rat utricular macula were photographed in a Phillips 400 trasmission electron microscope. Two small parts of the neural network and 16 receptive fields were traced from the montages, digitized into a PC, reconstructed as shaded solids and animated using an IRIS high performance workstation, then recorded on film using a Dunn camera and Abekas equipment. Results show that type I and type II hair cells are incorporated into receptive fields that overlap. No two receptive fields are identical. Synapses are spatially distributed and numerically different for each unit. Some portions of the network are innervated by 3 kinds of nerves based on their myelination patterns, but others are supplied only by nerves with long unmyelinated preterminals. Configurations of hair cell stereocilia differ with network complexity. Findings indicate that spatiotemporal factors are important in macular functioning, and that maculas are organized for parallel distributed processing of information. Redundancy and constrained randomness may contribute to robustness. Results are being included in symbolic, animated models.

206.4

REGIONAL VARIATIONS IN THE SYNAPTIC ORGANIZATION OF THE CHINCHILLA CRISTAE. A. Lysakowski and J. M. Goldberg. Dept. Pharmacol.-Physiol. Sci., Univ. of Chicago, Chicago, IL 60637.

The present ultrastructural study was motivated by two considerations. First, previous efforts at reconstructing the afferent innervation of the cristae (Fernández et al., 1988) led to the conclusion that there should be large differences in the innervation of type II hair cells in the central and peripheral zones. Second, it has been suggested that the relative contributions of calyx and bouton endings to afferent discharge are related to the relative number of afferent synapses made on these two structures (Baird et al., 1988). Data on both of these points were lacking. Results of the present serial-section EM study indicate that there are regional variations in type II innervation. As predicted from the afferent reconstruction, there is a 5- to 6-fold difference in bouton numbers in the two zones (3-5 boutons centrally as opposed to 25-30 peripherally). Despite differences in the numbers of boutons, type II hair cells make similar numbers of synaptic contacts in the two regions. There are two reasons for this. First, each central bouton receives multiple (3-4) ribbon synapses, while each peripheral bouton receives a single ribbon synapse. Second, many central type II hair cells make multiple ribbon contacts with the outer surface of adjacent calyces, while this type of contact seems rare in the periphery. Other features did not vary from region to region. For example, type I hair cells, in which ribbon synapses have been described as "exceedingly rare", were found to contain 10-20 ribbon synapses regardless of region. There were also no regional variations in numbers of efferent boutons contacting type II hair cells or calyces. These data should all prove useful in constructing a model of the functional organization of the cristae.

Supported by NS-01330 and NS-24281.

206.5

CNQX (6-CYANO-7-NITRO QUINOXALINE-2,3-DIONE) BLOCKS EXCITATORY SYNAPTIC TRANSMISSION IN THE VESTIBULAR LABYRINTH AND VESTIBULAR NUCLEUS OF THE FROG. S.L. Cochran, Dept. Life Sciences, Indiana State Univ., Terre Haute, IN 47809

Synapses between hair cells and VIIIth nerve afferents and between these afferents and second order vestibular neurons (VN's) are glutamatergic. At both, transmitter appears to act through kainate/quisqualate (KA/QUIS) receptors. Some components of EPSP's in VN's can be blocked by N-Methyl-D-Aspartate (NMDA) antagonists. CNQX reportedly blocks selectively KA/QUIS receptors and its effects have been studied at these synapses. In the labyrinth, bath-applied CNQX (25 μM) reduces the amplitudes of EPSP's without affecting their frequency of occurrence, suggesting subsynaptic blockade. NMDA agonists have little or no effect, while quisqualic acid causes an increase in EPSP frequency and afferent depolarization. In VN's high (100 μM) concentrations of CNQX reversibly block EPSP's arising from the ipsilateral VIIIth nerve and those from the contralateral vestibular nucleus. Low concentrations of CNQX (10 μM) block an initial component of the ipsilaterally-evoked EPSP and to a varying extent, components of the contralaterally-evoked EPSP. The resultant ipsilateral EPSP has a slower rise time and smaller amplitude than the control. These findings to date confirm that CNQX is a specific and potent blocker of KA/QUIS synaptic receptors, but its selectivity is dependent upon the concentration employed. Supported by NSF grant BNS-8616738 and NASA grant NAG-498 to SLC.

206.6

RESPONSES OF VESTIBULAR NUCLEUS NEURONS DURING EYE MOVEMENTS IN AWAKE RABBITS. J.S. Stahl and J.I. Simpson. Dept. Physiol. & Biophysics, NYU Med. Ctr., New York, NY 10016.

Neurons of the vestibular nuclei were recorded in the awake rabbit. We focused on neurons excited by rotation about the vertical axis toward the side of recording (Type vI) and identified by electrical stimulation as receiving from the flocculus (FRN, n=19), projecting to the midbrain (MPN, n=13), or having both relationships (FRN/MPN, n=4). These neurons were concentrated in the vestibular complex at the level of the caudal pole of the VIth nucleus. The neurons were tested during vestibular and optokinetic behaviors. The modulation of all neurons correlated strongly with eye movements. Qualitative inspection of the data failed to reveal neurons modulating strongly during VOR suppression (head and optokinetic drum rotated together) as do the vestibular and vestibular plus eye movement neurons in the primate. Also unlike the primate, neurons pausing for fast phases in the "on" direction were not seen; the response for "on" fast phases varied from nothing to bursts similar to those of rabbit motoneurons. Eye position and eye velocity sensitivities (r and k, respectively) were determined for the responses to sinusoidal rotation in the light (0.2 Hz, $\pm 2.5^{0}$). The ratio r/k was compared for the 3 groups and their combinations. The ratio differed significantly ($p<.001$) based on whether or not the neurons received from the flocculus (FRN, FRN/MPN versus MPN groupings). The respective values were 1.35 ± 0.67 (mean $\pm$ SD) vs. 0.52 ± 0.28. The value of r/k for the neurons not receiving flocculus input (the MPNs) is close to that of rabbit VIth nucleus neurons recorded during identical stimuli ($r/k=0.49 \pm .09$, n=22). In sum, the dynamics of neurons receiving direct flocculus input differ from at least two types of eye movement neurons lacking that input. Supported by NS-13742.

206.7

CAT VESTIBULAR SINGLE UNIT ACTIVITY DURING IMPOSED, FREE, AND ATTEMPTED HEAD MOVEMENTS. James H. Fuller. Dept. of Oral Anatomy, University of Illinois, Chicago, IL 60612.

Units were recorded in the caudal (P8-P9.5) portions of the medial and inferior vestibular nuclei in cats which could be rotated horizontally with the head fixed to the platform (whole body rotation, WBR), or with the head fixed in space (HFS); imposed rotations resembled active head movements (passive head saccades). Additionally, the cats were trained to make active head movements (free head saccades), or with the head fixed, to make ballistic, accurate, attempted head movements (head torque saccades). Units were selected based on the response to a brief interruption of free head saccades, which consisted of asymmetric and irregular stimulus-locked discharges (category 3 units; Fuller, in, Control of Head Movement, pp. 120-129, Ed. Peterson and Richmond, Oxford Univ. Press, 1988). The three relatively similar events (passive, free, and head torque saccades) were compared to distinguish the relative contributions of primary sensory, secondary sensory, and premotor signals contributing to the overall discharge patterns.

206.8

TIMING OF VERTICAL EYE MOVEMENTS INDUCED BY HORIZONTAL ROTATION AFTER CROSS-AXIS VOR PLASTICITY. B.W. Peterson, T.T. Khater*, K.D. Powell, K.J. Quinn*, J.F. Baker. Northwestern University Medical School, Chicago, IL 60611

The short (19 msec) latency of adaptive changes in horizontal VOR after monkeys have worn magnifying lenses for several days is taken as evidence for plastic changes in relatively direct VOR pathways (Lisberger, Science 242:771). To obtain comparable latencies for plastic changes in VOR direction, we recorded eye movements evoked by alternating 2.5 sec periods of ±10 °/sec horizontal whole body rotation (HWBR) in total darkness with a CNC eye coil system in 2 cats before and after "cross-axis" training. This training consisted of 2-3 hours of HWBR coupled with vertical pitch rotation of a projected random dot pattern with identical phase and 1.5-3 X the amplitude. The HWBR was either a 0.25 Hz, 12°/sec sinusoid or a sum of 6 sinusoids from 0.2 - 1.7 Hz.

Vertical VOR responses to ±10 °/sec HWBR were absent before but clearly present with gain ~ 0.2 after training. Latencies between changes in HWBR and vertical eye velocity, measured from computer-averaged records, were 50-74 msec after 0.25 Hz training and 35-45 msec after 0.2-1.7 Hz training. This difference may reflect frequency tuning in the adaptive process. Regardless of the training stimulus, latencies exceeded those reported by Lisberger and were long enough for more complex (eg trans-cerebellar) pathways to be involved. We are disinclined to invoke species differences in such a phylogenetically old behavior. Rather, since VOR plasticity appears to have 2 time constants on the order of 0.5 and 8 hours, we speculate that the faster learning, produced by our 2-3 hour sessions, may involve more complex pathways than learning that occurs over several days. Supported by EY05289, EY06485, EY07342

206.9

STATIC ROLL AND THE VESTIBULO-OCULAR REFLEX (VOR) T.C. Hain and U.E. Buettner. Depts of Neurology and Otolaryngology, Johns Hopkins University, Baltimore MD, 21205.

In 5 normal subjects we measured the effect of static tilt in roll on the time constant of the VOR, by recording both the horizontal and vertical components of eye velocity for rotation about earth vertical with the head rolled away from upright. We used steps of chair rotation at 60 deg/sec in darkness to elicit the VOR and the scleral eye coil to record eye movements.

The mean time constant of the horizontal VOR in the upright position was 19.2 ± 3.2 sec. The horizontal time constant decreased to 15.7 ± 4.0 sec for 30 deg roll and to 12.7 ± 2.7 sec for 60 deg roll. The time constant of the vertical component was 11.0 ± 1.4 sec for 30 deg roll and 7.5 ± 1.6 sec for 60 deg roll. The gain of the space-horizontal VOR did not vary with roll but an inappropriate space-vertical component appeared in the roll positions. This built up over about 5 seconds and then gradually decayed.

These data suggest that orientation to gravity modulates storage of semicircular canal signals, and that horizontal and vertical components of the VOR can decay at different rates.

206.10

GRAVITY INFLUENCES HUMAN VESTIBULO-OCULAR REFLEX GAIN DURING FAST ACTIVE HEAD MOVEMENTS. D.P. O'Leary, M. Gee* and L.L. Davis*. Dept. Otolaryngology-Head & Neck Surgery, Univ. Southern Calif., Los Angeles, CA 90033.

Human horizontal vestibulo-ocular reflex (HVOR) dynamics were analyzed during 18 seconds of active head shaking, sweeping in frequency from 2 to 6 Hz. Subjects fixating on a near target were tested three times in each randomized position: supine, sitting, prone, and on the right side. Head velocity was recorded with a headstrap-mounted rotational velocity sensor. Corrections were applied for systematic headstrap slippage. Eye velocity, computed by digital differentiation of electro-oculographic records, was corrected for near-target vergence during head movements. HVOR gains and phases (2-6 Hz) from 23 subjects were compared at the four test positions. Supine-prone gain differences exceeded +0.1 for all subjects, with reduced phase lags. Additional control experiments imply that these effects resulted from different gravitational force directions on inner ear receptors. However, a physically-equivalent force, linear acceleration, could also be relevant to VOR control during common head movements. We propose a new hypothesis: periodic linear acceleration forces, acting forward and backward on the head during locomotion, modulate the HVOR dynamics for finely-tuned head, eye, and body coordination. (Supported by The Deafness Research Foundation)

206.11

SUBJECTIVE RESPONSES DURING INTERACTION OF SMOOTH PURSUIT WITH OPTOKINETIC AND VESTIBULAR STIMULI. V. Honrubia, M.D.,[1] R.W. Baloh, M.D.,*[2] and R. Khalili, B.S.*[1] [1]Div. of Head and Neck Surgery and [2]Dept. of Neurology, Goodhill Ear Center, UCLA School of Medicine, Los Angeles, CA 90024.

Subjects were asked to visually track a moving laser light 1 degree in diameter that was projected from the back of the subject's chair onto the inner surface of 1) a rotating optokinetic (OK) drum with the subject stationary in the light, or 2) a stationary black drum while the subject was rotated in the dark. The laser target (LT) had a sinusoidal trajectory of ± 18 degrees at 0.2 and 0.4 Hz. In clockwise and counterclockwise directions, OK drum velocities ranged from 10 to 100 deg/sec and subjects were rotated with constant angular accelerations from 0.4 to 8.0 deg/sec^2. By pressing levers under each hand, subjects indicated the moment when the LT appeared to reverse direction. The signals were used to compute the velocity of the LT at the moment of direction reversal.

The perceived motion of the LT was influenced by the optokinetic and vestibular stimuli in a predictable manner. The perceived duration of the two half-cycles of sinusoidal LT movement was asymmetrical; the half-cycle of LT movement against the direction of the OK drum or in the direction of chair acceleration was longer. That is, the LT appeared to travel for a longer period when it moved in the same direction as that of the subject's sensation of self-motion (circular vection). The computed LT velocity at the apparent time of reversal was a power function of the magnitude of the interactive stimuli, with maximum effects leading to 100% error – the perceived LT motion became unidirectional. It was concluded that the brain judges the velocity of the LT in relationship to an internal reference that depends on the operational state of the vestibular system. The internal reference level can be changed by interactive stimuli, leading to predictable errors in the judgment of LT motion.

(Work supported by NIH-NINCDS grant NS09823.)

206.12

EFFECTS OF GRAVITY ON ASYMMETRICAL VELOCITY STORAGE. T. Raphan, H. Cohen, M.J. Dai*, B. Cohen. Depts. of CIS, Brooklyn College of CUNY and Neurology, Mount Sinai School of Medicine of CUNY, New York, N.Y

Rotation of the visual surround that induces vertical optokinetic nystagmus (VOKN) activates the velocity storage mechanism in the vestibulo-ocular reflex (VOR) and induces vertical optokinetic after-nystagmus (VOKAN). The VOKAN time constant depends on the orientation of the head with regard to gravity. The time constant is smallest in the upright position and largest in the 90° roll position, accompanied by a corresponding increase in the gain of VOKAN. Under these conditions, VOKAN has a longer time constant for slow phases in the upward than in the downward direction. When the head is tilted such that the roll angle relative to the spatial vertical is greater than 90° the upward and downward time constants tend to equalize. These modifications in the time constants are obtained while maintaining velocity storage along the animal's pitch axis as an eigenvector i.e., the OKAN is in the same direction as the OKN. The results indicate that some asymmetries observed in the dynamics of the VOR are due to gravitational effects on the physiological structure of the neural network that realizes the velocity storage integrator. The changes in velocity storage in the absence of gravity may have profound effects on inducing space sickness.

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207.1

MAGNETIC RESONANCE IMAGING OF THE CAUDA EQUINA SYNDROME ASSOCIATED WITH ANKYLOSING SPONDYLITIS
N.K. Gulati*, (SPON: B. Gallagher) Department of Neurology; Medical College of Georgia; Augusta, GA 30912

This is the first report of Magnetic Resonance Imaging (MRI) in a case of Cauda Equina Syndrome (CES) associated with Ankylosing Spondylitis (AS). Cauda Equina Syndrome has been reported as a late complication of AS and involves lumbosacral sensorimotor deficits with asymmetric hyporeflexic paresis. The patient reported is a 73 year old man who had an 18 month history of urinary incontinence. He recalled having back pain once during his mid 20's. He was found to have mild paraparesis, poor anal sphincter tone, and decreased sensation in the sacral dermatomes. Lumbosacral MRI revealed a widened subarachnoid space, a fluid-filled extradural sac lesion at L3, squaring of the vertebral bodies anteriorly with scalloping posteriorly, and a fine linear density along the anterior border of the vertebral column, perhaps representing ligamentous thickening or calcification. Plain spine radiographs revealed the classic 'Bamboo spine' of AS. It was felt that the patient's CES was secondary to AS which initially presented itself decades earlier. In conclusion, patients with CES and the MRI findings discussed above deserve careful investigation for long standing, unsuspected Ankylosing Spondylitis.

207.2

HYPOTHALAMIC EFFECTS ON MEDULLARY RETICULAR ACTIVATION OF DEEP BACK MUSCLE EMG. D.W. Pfaff, S.L. Cottingham, A. Korotzer & S. Schwartz-Giblin. Lab. of Neurobiol. & Behav., Rockefeller University, New York, NY 10021.

Medullary reticular stimulation activates deep back muscles (Am.J.Physiol. 246:R389). Midbrain central grey stimulation facilitates brainstem control over these muscles' EMG (Exp.Neurol.97:704 & Brain Res.421:397). Since this motor control hierarchy parallels lordosis behavior circuitry, we tested the hypothesis that medial hypothalamic lesions that decrease lordosis can also reduce medullary reticular activation of deep back muscle EMG. Urethane anesthetized rats were tested systematically for amplitude of lateral longissimus (LL) and medial longissimus (ML) EMG responses to NGC stimulus trains before and after electrolytic lesions of VM hypothalamus (N=18) or control sites (N=30). Bilateral VM lesions were able to greatly reduce EMG responses in LL and ML, often with a time course similar to previous behavioral results (J.Physiol.288:203). Surprisingly, lesions at the anterior VM pole were particularly effective, and may reflect importance of intra-VM neurons (J. Comp. Neurol. 1989). Although, on the average, various control lesions were less effective, the VM effect was not unique. EMG loss was not well correlated with cortical EEG changes. It appears that VM hypothalamic neurons can affect medullary reticular control of back muscle EMG, but must share this role with other forebrain elements.

207.3

A BRAIN STEM SITE THAT SUPPRESSES SYNCHRONOUS DISCHARGES OF GAMMA MOTONEURONES IN THE CAT. **P. H. Ellaway*, N. J. Davey*, M. C. Catley* and J. R. Baker* (SPON:Douglas G. Stuart). Dept. of Physiology, Charing Cross and Westminster Med. Sch., London W6 8RF, England.**

The background and reflexly induced discharges of gamma motoneurones in the decerebrated cat show no tendency towards synchrony, but do so following section of the dorsolateral funiculus of the spinal cord (Davey & Ellaway, J. Physiol. 72:249, 1988). We postulate that a reticulospinal tract may inhibit segmental inputs which, through shared connections to gamma motoneurones, cause synchrony of discharge. To locate the origin in the brainstem of this descending pathway experiments were performed on unanesthetised decerebrated, decerebellate cats with hemisection of the dorsolateral funiculus of the spinal cord contralateral to motoneurone recordings. Cross correlation of control recordings from pairs of individual gamma motoneurones (12 to 40 m/s) to the gastrocnemius muscle in this preparation showed no synchronous firing. Electrolytic lesions of the ipsilateral brainstem in the region of the nucleus reticularis magnocellularis (RMC) caused synchrony which could be as marked as that seen following total spinal cord section. Synchrony was evident as a peak in the correlogram indicating increased probability of discharge of one motoneurone within ± 2-10ms of the discharge of the other gamma motoneurone. Lesions of the midline nucleus raphe magnus did not produce synchrony and lesions of the locus coeruleus (LC) were less effective than those of RMC. We conclude that the descending spinal pathway originates in the medullary reticular formation close to RMC but that the LC may also be involved.

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207.4

SPONTANEOUS AND EVOKED ELECTRICAL ACTIVITY IN THE ISOLATED CNS OF THE NEWBORN OPOSSUM (Monodelphis domestica) R.R. Stewart, J.G. Nicholls, S.D. Erulkar and N.R Saunders*, Dept. Pharmacology, Biocenter, CH 4056 Basel, Switzerland and Dept. Physiol. & Pharmacol., Univ. Southampton, U.K.

At birth, the opossums are equivalent to rats at fetal stage XIV. Limbs and eyes are rudimentary; the forebrain and cerebellum have not developed. The animal's behavior consists of breathing and suckling. Newborn opossums were anesthetized with ether or by cooling; the brain and spinal cord were rapidly removed and maintained in oxygenated Krebs's fluid. When the spinal cord was stimulated electrically at one end, compound action potentials were evoked. Electrical excitability persisted for up to 4 days after isolation of the CNS. Evoked activity was reversibly blocked by 0.1 μM Tetrodotoxin. Following dorsal root stimulation at cervical and thoracic levels, volleys of action potentials were recorded in the ventral roots. These reflex responses were reversibly eliminated within 20 min by 20mM Mg^{++}. In some animals spontaneous rhythmical bursts of activity were recorded from ventral roots. Thus, the entire isolated CNS of the newborn opossum is hardy, rapidly penetrated by drugs and rhythmically active. It offers advantages for studying anatomical and functional development of a mammalian central nervous system.

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207.5

SPONTANEOUS ACTIVITY IN THE SPINAL CORD AND INFERIOR OLIVARY NUCLEUS OF NEONATAL RAT RECORDED *IN VITRO*. Kerry D. Walton, Department of Physiology and Biophysics, New York University School of Medicine, 550 First Ave., New York, NY 10016.

Harmaline directly effects the oscillatory properties of inferior olivary (IO) cells *in vitro* (Llinas & Yarom, J. Physiol. 376:163, 1986) and is a well-known tremorgen. However, *in vivo* studies in rat report that harmaline-induced tremor was not seen before P9 (Phillips et al, 1978). Harmaline has been used as a probe to study the functional development of the olivo-cerebellar-spinal system using *in vitro* spinal cord-brain stem-cerebellum isolated from P0-P6 rats. Spontaneous bursts of subthreshold oscillatory activity (3.5-4 Hz at 25-27°C) have been recorded from ventral roots and from motoneurons (Walton & Llinas, *Soc Neurosci. Abst*, 12, 1986). Addition of Harmaline (0.1mg/ml) elicited 8-10 Hz damped oscillations with waveforms similar to emgs recorded from harmaline-treated adult rats.

The spontaneous activity in the IO was characterized by 8 Hz damped oscillations and complex rhythmic activity. Fourier analysis revealed activity at 8Hz and its harmonics, with prominent activity near 30 Hz. In animals P3 and older, after addition of harmaline to the brainstem compartment, the 30 Hz oscillations disappeared leaving highly synchronous 8-11Hz oscillations. Addition of picrotoxin (50μM) seemed to increase the correlation of activity in adjacent IO regions and enhanced the harmaline effect. The results indicate that the conductances underlying IO rhythmic firing (1) are present at birth, (2) are sensitive to harmaline and picrotoxin by P3 but, (3) do not yield the uniform 10Hz osillations seen in adult. Also, the similarity in subthreshold rhythmic activity in these networks points to a role for such oscillations in synchronizing activity in the motor system. Supported by NS22975 and the Irma T. Hirshl Trust.

207.6

DESCENDING PROJECTIONS FROM THE DORSAL MESENCEPHALON W. R. Mehler, G. Holstege and R. J. Cowie[2] Dept. Anatomy Univ. Calif. San Francisco CA 94143 and [2]Dept. Anatomy Howard University Washington D.C. 20059

It is well known that the superior colliculus projects contralaterally to the spinal cord via the so-called tectospinal tract, distributing many collaterals to the medial tegmentum of caudal pons and medulla. Recently an ipsilateral tectobulbar pathway has also been demonstrated. However, the relation between the ipsi- and contralateral pathways remains unclear. To gain better insight in the organization of these different pathways, the retrograde HRP and the anterograde autoradiographic tracing techniques were used. Four cats were injected with HRP in the brainstem or spinal cord and 15 cats were injected with ^{3}H-leucine injections in various parts of the superior colliculus and/or the periaqueductal gray (PAG).

The results reveal that from the contralateral tectobulbospinal tract thick labeled fibers were not only distributed to the dorsal two thirds of the bulbar medial tegmental field, but some also to the lateral tegmental field. Furthermore, from the tectospinal tract, several recrossing fibers were observed, that terminated ipsilaterally in the medial and lateral tegmental field. The ipsilaterally descending (thin) tecto-tegmental fiber-system descended through the lateral tegmentum of caudal mesencephalon and pons, but then shifted medially to terminate in the ventral part of the medial tegmentum of caudal pons and medulla. These fibers originated in the deep layers of the superior colliculus and in the PAG. The results demonstrate that the thick fibers of the contralateral tectobulbospinal pathway terminate in different parts of pons and medulla than the thin fibers of the ipsilateral pathway. In our view the two descending pathways belong to different descending systems. The tectospinal tract takes part in the visuomotor system, controlling eye- and head movements, while the ipsilateral pathway belongs to the limbic descending system, involved in emotional behavior.

207.7

RETICULOSPINAL NEURONS IN THE LAMPREY: BIOPHYSICAL PROPERTIES AND INPUTS FROM THE MESENCEPHALIC LOCOMOTOR REGION. A.D. McClellan, Dept. of Physiology. & Biophysics, Univ. of Iowa, Iowa City, IA 52242

In the lamprey, spinal cord lesion and brainstem chemical microstimulation studies (McClellan, J. Neurosci. Meth. 21,251, 1987) suggest that reticulospinal (RS) neurons in the middle and posterior reticular nuclei (MRN and PRN) contribute to the initiation of locomotion. The purpose of this study was to examine the membrane properties of RS neurons, to characterize some of the inputs to these nuclei, and to determine the importance of these neurons in initiating locomotion.

Biophysical properties. Some RS neurons displayed postinhibitory rebound, delayed excitation, or spike-frequency adaptation. Most RS neurons fired at a rate which was dependent on injected current level with little adaptation or post-burst hyperpolarization.

Inputs to MRN and PRN. Pressure ejection of a retrograde tracer (HRP or WGA-HRP) into the MRN or PRN labeled neurons in the mesencephalon, ipsilateral and contralateral rhombencephalon, and spinal cord. The labeled neurons in the mesencephalon may correspond to the mesencephalic locomotor region (MLR), an area that can elicit locomotor activity when electrically stimulated. Pulsed microstimulation in the MLR could elicit 1:1 synaptic responses in RS neurons which changed amplitude when the membrane was artificially polarized, consistent with chemical transmission. Some of these MLR-evoked responses were depolarizing and capable of producing action potentials in RS neurons. Work is now in progress to determine if these evoked responses are monosynaptic and to examine their pharmacology.

Lesions in the MRN and PRN. Bilateral lesions which destroyed a significant part of either the MRN or PRN did not abolish locomotion, suggesting that neither nuclei is required for locomotor behavior. Lesion experiments are being performed to determine if both of these nuclei are necessary for the initiation of locomotion.

Supported by SCRF grant NBR 501-5 and NIH grant NS23216.

207.8

IDENTIFICATION OF A LIMITED REGION OF THE MEDULLA THAT MAY CONTAIN NEURON POPULATIONS GENERATING RESPIRATORY RHYTHM IN MAMMALIAN BRAINSTEM *IN VITRO*. J.C. Smith, J.J. Greer & J.L. Feldman. Systems Neurobiology Laboratory, Department of Kinesiology, UCLA, Los Angeles, CA 90024-1568.

Regions of the mammalian brainstem containing neuron populations generating respiratory rhythm have not been identified. We have developed a novel method for locating these regions using *in vitro* neonatal rat brainstem-spinal cord preparations and precision sectioning of the neuraxis with a vibratome. Brainstem-spinal cord preparations, or isolated medulla preparations, are maintained in physiological solution in the vibratome bath and spinal or cranial respiratory motoneuron discharge is recorded during serial sectioning of the medulla (50 μm thick sections) starting from either the rostral or caudal ends of the medulla. Analysis of the perturbations of the motor pattern induced by removal of caudal or rostral regions indicates that a very limited medullary region (~200 μm thick slice) extending from the level of the rostral end of the ventral respiratory group to the caudal end of Bötzinger Complex is essential for rhythmogenesis. Sectioning through this region induces instabilities in the respiratory rhythm and removal of the region results in cessation of rhythmic motor discharge. Perturbations of rhythmogenesis do not occur after removal of more rostral or caudal medullary regions; this result does not support the recent proposal (Onimaru et al., Neurosci. Lett., 78: 151, 1987; *ibid*, Brain Res. 44:314, 1988) that more rostral medullary regions are primary sites for rhythm generation. Our previous transection experiments (Smith and Feldman, Soc. Neurosci. Abs 14: 1060, 1988) indicate that cell populations in the ventral medulla are sufficient for rhythmogenesis, and a map of the neuron activity in the critical region indicates the presence in the ventrolateral reticular formation of several distinct classes of respiratory neurons with inspiratory and expiratory phase discharge patterns (Greer et al., this volume). Our recent anatomical tract tracing studies in the adult rat show a concentration of propriobulbar interneurons in the critical region projecting to other major medullary respiratory cell populations and this anatomical feature is consistent with the hypothesis that this region contains respiratory rhythm generating cells. Supported by NIH Grants HL 40959 and HL 02204.

207.9

INVOLVEMENT OF EXCITATORY AMINO ACIDS (EAAs) IN NEUROTRANSMISSION OF RHYTHMIC EXCITATORY DRIVE TO MOTONEURONS DURING LOCOMOTION IN THE RAT SPINAL CORD *IN VITRO*. B.J.Schmidt, J.C.Smith, and J.L.Feldman. Dept of Medicine, Univ. of Manitoba, Winnipeg Canada R3E 0W3, and Systems Neurobiology Lab, Dept of Kinesiology, UCLA, Los Angeles CA 90024.

We previously reported that the *in vitro* neonatal rat brainstem-spinal cord preparation is capable of generating rhythmic limb activity and motoneuronal drive potentials similar to those observed during locomotion in mammals *in vivo* (Smith et al FASEB J 2:2283, 1988; Schmidt et al Soc. Neurosci. Abst 14:427.2 1988). In the present studies we have used the *in vitro* system to investigate the role of EAAs in the transmission of oscillatory synaptic drive to spinal motoneurons during fictive locomotion. Studies in lower vertebrate systems indicate that EEAs mediate rhythmic excitation of spinal motoneurons during locomotion and the presence of EEA receptors on motoneurons in the mammalian spinal cord has been established. However, it is not known whether EAAs are involved in locomotor drive transmission pathways in the mammalian spinal cord. Intracellular recordings were obtained from identified lumbar motoneurons during fictive locomotion induced by the bath application of acetylcholine. The effect of EAA antagonists acting at N-methyl-D-aspartic acid (NMDA) and non-NMDA EAA receptors was examined. Block of NMDA receptor activation with 2-amino-5-phosphonovaleric acid (10-100 μM) and non-NMDA receptors with 6-cyano-7-nitroquinoxaline-2,3-dione (2-20 μM) abolished spike discharge and reduced the amplitude of the rhythmic locomotor drive potential in a dose dependent manner. The similar potency of NMDA and non-NMDA receptor block in reducing the drive potential amplitude (to < %10 of the control amplitude) is consistent with multi-component excitation of motoneurons and/or premotor neurons, mediated by several EAA receptor subtypes. These results suggest that endogenous EEAs are importantly involved in the transmission of rhythmic excitatory drive to spinal motoneurons during locomotion in mammals.

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LEARNING AND MEMORY: PHYSIOLOGY II

208.1

Long Term Learning-Specific Changes in the Distribution of Protein Kinase C within the Rabbit Hippocampus. J.L. OLDS, D.L. MCPHIE*, M.L. ANDERSON AND D.L. ALKON LMCN,NINDS, NIH, Bethesda MD. 20892 (SPON: M.E. Olds)

Pavlovian Conditioning has been previously shown to induce differences in the amount and distribution of Protein Kinase C (PKC) 24 h after classical conditioning (Olds et al Science 1989). Conditioned and control rabbits were studied 3 d after the end of the behavioral paradigm. Group 3C received 90 trials/day over 3 d (paired tone and airpuff). Group 3UP received the same same number of stimulii as Group 3C but in an explicitly unpaired manner. Group N remained in their home cages. Autoradiographic images of [3H]-PDBU, a highly specific radioligand for PKC, were analyzed for changes in the distribution of the label within the CA1 cell field. Images were compared between groups by constructing a ratio of specific-binding in the stratum pyrimidale to that in the stratum oriens (SP/SO) along a predetermined image transept-line. Group 3C showed a statistically significant decrease in this ratio (SP/SO=0.64±.08, N=5 each group) compared with Group 3UP (SP/SO=0.97±.02) and Group N (SP/SP=0.91±.05; $p<0.01$ one way ANOVA). This change in the distribution of PKC 3 days after the end of acquisition represents a learning-specific movement of the label from the area of the CA1 somata (24 h after conditioning) to the dendrites (3 days after conditioning). The time domain for this neurochemical change in the hippocampus is significantly longer than those previously reported and supports the hypothesis that PKC is critically involved in memory storage.

208.2

Isolated Pharmacological Activation of Three Neurotransmitter Systems Produces Differential Translocation of PKC in the Rabbit Hippocampus. D. L. MCPHIE* J. L. OLDS J. V .SANCHEZ-ANDRES* and D. L. ALKON (SPON:J.L. Olds) LMCN, NIH, Bethesda, MD, 20892.

The neurotransmitters GABA, Glutamate and Acetylcholine have been shown to play important roles in the hippocampus. In this study the relative contribution of each of these systems to the translocation of protein kinase C (PKC) was assessed. Physiological slices (500μm) were incubated for 20 min in transmitter agonist and antagonist combinations with tetrodotoxin(TTX) at 1μM to eliminate impulses and most chemical synaptic interactions. Controls of Artificial Cerebral Spinal Fluid (ACSF) and ACSF+TTX were also run. The slices were then frozen and sectioned at 20μm. Translocation of PKC was assessed by determining the specific binding of 3HPhorbol-12,13-dibutyrate (PDBU), a highly specific ligand for membrane associated PKC. (Worley et al., J. Neurosci. 6(1):199, 1986).

Glutamate incubation (10μM) produced the largest increase in 3H-PDBU binding, 41.6±1.5 nCi/g, followed by carbachol and GABA with valuesof 34±.97 and 34.8±1.6 respectively. All three of these conditions were significantly above controls ($N=20$-30:$p<.001$)ANOVA. Incubation with appropriate agonist + antagonist + TTX showed reversibility. Agonists applied without TTX produced smaller increases perhaps due to synaptic interactions.

This study shows a convergent effect on 2nd messenger systems by the three agonists tested and the putative translocation response of single neurons to each of the agonists. Taken together with evidence for PKC's integral invilvoment in memory storage (Alkon and Rasmussen, Science 239:988, 1988), these results suggest that known GABA, Acetylcholine and Glutamate inputs to the hippocampus may play an important role in learning-specific PKC distributional changes previously reported by this laboratory.

208.3

LEARNING-SPECIFIC CHANGES IN A CALCIUM-DEPENDENT-POTASSIUM CURRENT FROM CA1 HIPPOCAMPAL CELLS OF RABBIT. J.V. Sanchez-Andres* and D.L. Alkon (SPON: J.W. Cosgrove). LMCN/NINDS, National Institutes of Health. Bethesda, MD 20892.

Previously it was found in this laboratory that Pavlovian conditioning of the rabbit nictitating membrane produces a learning-specific decrease in the afterhyperpolarization of CA1 pyramidal cells (Disterhoft et al. 1986). We now report that the ionic basis of this phenomenon is a conditioning-specific decrease in a Ca^{++}-Dependent-K^+ current. Cells were studied from rabbits that received: (1) three days of classical conditioning (P); (2) three days of explicitly unpaired stimulus presentation (U) or (3) were left in their home cages (N). Single-electrode voltage clamp recordings were made from a total of 74 cells (P: 22; U: 19; N: 33). Cells from conditioned rabbits (Group P), unlike cells from the U or N groups, showed a highly significant change in the frequency distribution of blocked Ca^{++}-Dependent-K^+ currents (Chi^2, $P<0.01$). Additionally, we showed that application of phorbol 12,13 dibutyrate (PDBU), an activator of protein kinase C, blocked a Ca^{++}-Dependent-K^+ current. This effect was reversed by the protein kinase C inhibitor H7. Since this enzyme has already been implicated in memory storage both in the marine snail *Hermissenda crassicornis* and in the rabbit hippocampus (Alkon et al. 1988; Olds et al. 1989), we hypothesize that this enzyme may inactivate the Ca^{++}-Dependent-K^+ current and may be involved in the learning-specific changes observed in this study.

208.4

LONG-TERM CHANGES OF TEMPORAL PROPERTIES OF SINGLE CELLS IN AN ASSOCIATIVE LEARNING MODEL. C. Chen and C. Koch. Computation & Neural Systems Program, 216-76, Caltech, Pasadena, CA 91125.

Studies of neuronal plastisity focus exclusively on changes in the amplitude of the synaptic potential (*e.g.* in *Aplysia* or mammalian LTP). Associative learning in *Hermissenda* is mediated by cellular interactions between two sensory pathways and is stored by modulation of two potassium channels (g_A and $g_{Ca\text{-}K}$) in B-photoreceptors. Computer simulations of their biophysical properties reveal changes in timing of the light-mediated depolarization as a result of modulating the activation rate of these two conductances. The reductions of g_A and $g_{Ca\text{-}K}$ observed are in agreement with voltage-clamp data. The latency of the peak photoresponse is reduced by 20~100 *ms* and the spike frequency is increased from 8 *Hz* to 10 *Hz*. This result has two significant implications. 1. Both changes can increase the interaction between two sensory inputs to activate the intracellular messenger system for channel modulation. 2. The frequency increase will directly turn on the newly learned motor pathway, leading to clinging behavior. Thus learning-induced changes in properties of the ionic channels can modify the temporal dynamics of the neuronal response. To apply this result to machine learning, we are currently simulating a large neural network with variable time delays to learn temporal sequence of patterns.

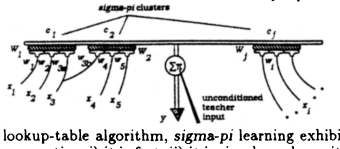
208.5

A BIOPHYSICAL MODEL FOR ASSOCIATIVE LEARNING IN CEREBRAL CORTEX. B. W. Mel* and C. Koch (SPON: A. Feng). Division of Biology, Caltech, Pasadena, CA 91125.

We propose a neural network model for associative learning in the cerebral cortex called *sigma-pi* learning, and discuss a biophysical implementation. The learning scheme is based on the *sigma-pi* neuron, which computes its activation level as a sum of contributions from independent multiplicative clusters of input synapses (see figure). Perceptron-based learning schemes (e.g. back-propagation) adjust hyperplanes in order to best fit a set of input-output examples. In contrast, *sigma-pi* units act as smoothly-interpolating lookup tables, explicitly storing input-output associations as distinct clusters of synapses.

$c_j = \Pi_i w_i x_i$

$y = \sigma(\Sigma_j W_j c_j)$.



Owing to its nature as a lookup-table algorithm, *sigma-pi* learning exhibits several biologically-desirable properties: i) it is fast, ii) it is simple: only excitatory weights are modified, and with only a simple Hebb-like rule, and iii) groups (e.g. columns) of *sigma-pi* units can directly pool their resources to increase total memory capacity. Several features of neural anatomy and physiology have influenced the form of *sigma-pi* learning, including i) the existence of dendritic channels (e.g. NMDA) capable of supporting AND-like computations among neighboring synapses, ii) dendritic spike activity in a variety of cell types, iii) the physiological observation of Hebb-like rules for synaptic plasticity, and iv) the availability of enormously large numbers of synapses (up to $10^9/mm^3$ in cortex). We show how the highly decoded sensory and motor representations of the cerebral cortex and the complex 3-D form of axons and dendrites are ideally suited to such a tabular learning scheme.

208.6

DIFFERENTIAL CONDITIONING MODIFIES AUDITORY PATTERNS OF 2-DEOXYGLUCOSE UPTAKE IN A SENSORY MAP. F. Gonzalez-Lima and J. Agudo*. Dept. Med. Anat., Texas A&M Univ., College Station, TX 77843, and Universidad de Valladolid, Spain.

We tested whether auditory responses to the same stimulus change due to reinforcement contingency. 2-deoxyglucose (2-DG) techniques applied during differential conditioning were used to discriminate sensory (tonotopic) from learned (reinforcing) effects of auditory stimuli. Rats were stimulated with two FM tones of low (4-5 kHz) and high (16-20 kHz) frequencies. Each tone was either reinforced (CS^+) or not reinforced (CS^-) with aversive reticular stimulation (US). Taking advantage of the inferior colliculus (IC) tonotopic organization, the IC was used as its own control to compare the effects of CS^+ vs. CS^-, i.e., differences between dorsal and ventral 2-DG bands in the same structure. The reinforced sound (CS^+) was found to evoke always significantly greater 2-DG uptake than the CS^-, independently of whether the CS^+ was the low or the high tone. The perimeter of the 2-DG band also became significantly greater when the sound was reinforced. Thus, enhanced 2-DG activity patterns in the IC were dependent on the learned signal value of sounds as opposed to their purely sensory stimulation. The findings visualized for the first time a direct "side-to-side" comparison of the representations of physical as well as learned behavioral parameters of a stimulus in a sensory map. Supported by NIMH grant R01 MH43353.

208.7

CONDITIONED STIMULUS DURATION: BEHAVIORAL ASSESSMENT OF A PREDICTION OF THE SUTTON-BARTO-DESMOND (SBD) MODEL. Diana E.J. Blazis & J.W. Moore. Neuroscience and Behavior Program and Department of Psychology, Univ. of Massachusetts, Amherst, MA 01003.

The SBD model is a neuron-like element that describes many features of rabbit nictitating membrane response (NMR) conditioning (Blazis & Moore, *Soc Neurosci Abstr* 14:842, 1987; Moore et al, *Behav Brain Res* 21:143, 1986), including the interstimulus interval (ISI) function for both forward-delay and trace conditioning. Changes in connection strength V depend on the magnitude of the eligibility trace $\bar{x}$. Appropriate ISI functions in the model are predicted from assuming that the rate of decay of $\bar{x}$ decreases as conditioned stimulus (CS) duration increases. For trace conditioning with a fixed trace interval (CS offset to unconditioned stimulus (US) offset) this assumption implies that V increases with CS duration, leading to the anomalous prediction that conditioning occurs more readily when a long rather than a short ISI is used. We tested this assumption with two intensities of an auditory CS. We used 4 groups of 8 albino rabbits. Each group received either an 83 or 63 db tone (1200 Hz) of duration 350 or 700 ms (denoted Short and Long, respectively). For all groups the trace interval was 300 ms, and the intertrial interval averaged 20 sec. The US was electrostimulation (DC, 1.5 ma, 50 ms) of the right eye. Subjects received 100 trials per day. Data presented here are the mean percent conditioned responding obtained over eight days of training. For groups receiving the 63 db tone, percent CRs obtained for group Short was greater than for group Long, but the difference was not significant (Long, mean = 31.87, SE = 10.10; Short, mean = 53.69, SE = 6.86, $t(14) = 1.79$, 2-tailed $p < .10$). For groups receiving the 83 db tone, percent CRs obtained for group Long was significantly greater than for group Short (Long, mean = 76.13, SE = 3.15; Short, mean = 54.26, SE = 6.25, $t(14) = 3.12$, 2-tailed $p < .01$). These results support the SBD model.

Supported by grants NSF BNS 88-10624 and AFOSR 86-0182.

208.8

ADAPTIVELY TIMED CONDITIONED RESPONSES AND THE CEREBELLUM: A NEURAL NETWORK APPROACH. J.W. Moore, J.E. Desmond, and N.E. Berthier. Dept. of Psychol., Univ. of Mass., Amherst, MA 01003.

Desmond and Moore (*Biol Cybern*, 58:353-358, 1988) described a network that learns to generate appropriately timed CRs in a variety of paradigms, including trace and long-delay conditioning, mixed CS-US intervals (Millenson, J., et al, *Learn & Mot*, 8:351-366, 1977), blocking, and conditioned inhibition. The model consists of two neuron-like computing units. One unit computes changes in connection stengths between CSs and the US and also generates the network output. The other unit computes the expected arrival time(s) of the US and reinforces computation of connection weights by the output unit. Both units receive CS input from a tapped delay-line which represents the temporal dimension of input to the network. A proposed implementation of the model in the cerebellum assumes Hebbian synaptic plasticity at synapses of parallel fibers (PFs) on the dendritic spines of Purkinje cells (PCs) in a manner consistent with physiological studies showing that concurrent stimulation of PCs by PF stimulation or application of glutamate (analogous to a CS) and climbing fiber (CF) stimulation (analogous to a US) causes long-term depression (LTD) of quisqualate (QA) receptors at the PF fiber/PC synaptic interface (Ito, M., *Ann Rev Neursci*, 12:85-102, 1989). We hypothesize that similar mechanisms for postsynaptic receptor depression exist at PF/Golgi cell synapses. As in the case of PF/PC synapses, these changes are assumed to require conjoint stimulation of Golgi-cell dendritic processes by PF activity induced by a CS with CF excitation induced by the US. Thus, our implementation assumes that CFs contribute to associative LTD of postsynaptic receptors at PF synapses in both PCs and Golgi cells.

This work was supported by grant AFOSR 86-0182 and NSF BNS 88-10624.

208.9

SINGLE UNIT ACTIVITY IN SPINAL TRIGEMINAL ORALIS AND ADJACENT RETICULAR FORMATION DURING CLASSICAL CONDITIONING OF THE RABBIT NICTITATING MEMBRANE RESPONSE.

T.N. Ricciardi,* W.G. Richards, and J.W. Moore. Neuroscience and Behavior Program and Dept. of Psychol., Univ. of Mass., Amherst, MA 01003.

Spinal trigeminal nucleus pars oralis (Spo V) subserves the nictitating membrane response (NMR). We recorded the activity of 66 single neurons in the vicinity of Spo V during classical conditioning of the NMR from 24 albino rabbits which had been pretrained on a two-tone disrimination (CSs: 1.6 vs .4 kHz counterbalanced, 78 db SPL, 350 ms duration; US: eye-shock, 1.0-1.5 mA, twin .2-ms dc pulses 4 ms apart; CS^+-US interval = 350 ms; intertrial interval = 20 sec). Neural and behavioral responses were recorded on tape and analyzed off-line. Twenty cells were in Spo V and 26 were in adjacent reticular formation (RF). Thirty-nine of these cells had activity patterns related to the occurence of CSs, the US, and/or CRs in various combinations: (a) Nine cells showed activity time-locked to CSs. Of these, 2 were located in Spo V, and 7 were in RF. (b) Twenty-seven cells responded with short latency to the US. In Spo V, 13 of these cells responded with increased activity, and 1 responded with decreased activity. In RF, 5 cells responded with increased activity, and 8 responded with decreased activity. (c) Fifteen cells showed increased activity time-locked to CRs, and 2 showed CR-related decreased activity. The timing of neural responses relative to CRs ranged from a lead of 110 ms to a lag of 32 ms. Five CR-related cells were in Spo V. They increased their activity in relation to CRs and also to the US. The remaining 12 CR-related cells, including the 2 with decreased activity, were in RF. Four of these responded to the US. This study has implications for brain implementation schemes of neural network computational models (e.g., Blazis, D. & Moore, J., *Soc Neurosci Abstr*, 14:143, 1987; Desmond, J. & Moore, J., *Biol Cybern*, 58:353-358, 1988).

This work was supported by grants AFOSR 86-0182 and NSF 88-10624.

208.10

TEMPORARY BLOCKADE OF THE RED NUCLEUS IN THE RABBIT AFFECTS PERFORMANCE OF CONDITIONED AND UNCONDITIONED NICTITATING MEMBRANE RESPONSES.

V.Bracha*, S.L.Stewart* and J.R.Bloedel. Division of Neurobiology, Barrow Neurological Inst., Phoenix, AZ 85013.

Previous studies showed that permanent lesions of the red nucleus (RN) specifically disrupt the classically conditioned but not unconditioned nictitating membrane response (NMR). In a series of experiments designed eventually to determine whether this effect is due to the selective involvement of the RN in the critical pathway for conditioned response (CR) or whether this nucleus exerts primarily an indirect action on this pathway, initial studies were performed to examine the effect of reversible lesions of the RN on execution of both CRs and URs.

Awake rabbits were trained using a standard delay paradigm: a 100 ms right corneal air-puff served as the unconditioned stimulus (US) and a 450 ms, 1kHz tone as the conditioned stimulus. Once the conditioned behavior was acquired the effect of RN block was tested by comparing the effects of injecting either 1µl of 4% lidocaine or 1µl of saline into the left RN in sessions in which the paired trials were alternated with US alone trials. Incidence, latency and amplitude of CRs and unconditioned responses (UR) before and after injection were compared.

Unilateral lidocaine injection substantially reduced both the incidence and amplitude of CRs in the contralateral eye. Although UR incidence was not affected, its time course and amplitude were markedly altered: UR amplitude decreased while time to response peak increased. These findings indicate that the effects of RN lesions may be the consequence of interfering with its role in regulating NMR performance rather than its selective involvement in the conditioned reflex pathway.(NIH Grant NS21958)

208.11

A PLASTIC SLOW-DECAYING RESPONSE COMPONENT IN ISOLATED TURTLE CEREBELLUM.

C.Y. Chan and A. Lee*. Dept. of Physiol., CUNY Med. Sch., New York, NY 10031.

Short-term plasticity in the cerebellar cortex may involve special physiological substrates. We report here a slow-decaying response component (SDC) recorded intracellularly in Purkinje cells (Pc) of the isolated turtle cerebellum. It appeared in the climbing fiber response (CfR) and mossy fiber response (MfR) evoked by peduncular stimulation, and CfR by strong pial stimulation. In normal Ringer, SDC was 0-4 mV and up to 300 ms in duration. It was graded, and habituated readily when activated at high frequency. Apparently, it was mediated by NMDA receptors, as it was blocked by 50-200 µM 2-amino-5-phosphonovalerate (APV), but enhanced in 0-Mg Ringer or by 5 µM glycine. Picrotoxin also enhanced it, suggesting involvement of Golgi or stellate cells. Though reflected in the Pc, the Mf-SDC probably occurred in the Mf-granule cell synapse, since 1) we found no sensitivity of Pc dendrite to iontophorezed NMDA; 2) depolarization of Pc dendrite did not enhance SDC; 3) APV blocked the disynaptic Mf-SDC but spared the parallel fiber response (PfR). Subtracting Mf-SDC from just-threshold CfR revealed no other SDC, suggesting that the Cf-SDC was merely a superimposed Mf-SDC. The Mf-SDC was inhibited by a prior (200-1000 ms) PfR or CfR. This interaction was enhanced in glycine or 0-Mg Ringer, but blocked by APV. Thus, the SDC appears to be the physiological substrate for Mf homosynaptic depression and depression of Mf pathway by Cf, possibly involving Golgi cells.

208.12

CHAOS IN THE OLFACTORY BULB OF THE CONSCIOUS RABBIT: REDUCED CORRELATION DIMENSION (D2) IN THE SURFACE POTENTIALS DURING RECOGNITION OF A FAMILIAR ODOR

JE Skinner, M Mitra*, CE Landisman*, and K Fulton*. (SPON: B. Saltzberg) Neurophysiology Section, Neurology Department, Baylor College of Medicine, Houston, Texas 77030.

Rabbits were implanted with an 8 x 8 electrode-array placed on the lateral surface of the olfactory bulb; the electrodes were separated by 0.5-mm intervals. After recovery from surgery, each animal was familiarized with the nose-cone and recording chamber during 2-hrs sessions conducted for 6 consecutive days. On the 7th day the same faint novel odor was presented briefly during each of 4 consecutive trials (3-min intertrial intervals). Compared to the surface potentials recorded during the 1st post odor-detection interval (i.e., determined by the first reduced inspiratory interval), those following the 4th post odor-detection interval showed a frequency-reduction and D2-decrease. All analyses were conducted by a completely automatic computer algorithm using the following parameters: epoch length, 0.5 to 1.5 sec; data points per electrode, 10^4; Tau-interval, 24 data points; embedding dimensions, 1 through 15. The values of D2 at rest prior to odor presentation ranged, between-subjects, from 6.60 to 6.58; the D2-values after odor-detection ranged from 6.50 to 6.08. We interprete our data to indicate that a lower dimensional chaotic attractor (D2-value) is used by the olfactory bulb during ODOR-RECOGNITION when the odor is familiar.

NEUROGLIA IN DISEASE

209.1

GLIOBLASTOMA INFILTRATION INTO CNS TISSUE IN VITRO: INVOLVEMENT OF TWO DISTINCT PROTEASES.

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Differentiated oligodendrocytes and CNS white matter have strong non-permissive substrate properties for regenerating neurites and migrating cells. This property is due to inhibitory proteins of 35 and 250 kD and is specifically neutralized by monoclonal antibody IN-1 (Caroni P. and M.E.Schwab; 1988. Neuron 1: 85-96). Using CNS tissue explants, cultured oligodendrocytes or CNS myelin as substrates, we have shown that the highly invasive rat C6 glioblastoma cells were not inhibited by these myelin-associated inhibitory components (Pagancetti P.A. et al.; 1988. J.Cell Biol. 107: 2281-2291). However, protease blockers specifically impaired C6 cell spreading on CNS myelin as well as C6 infiltration into CNS explants. At least two different type of proteolytic activities seem to be involved. One protease is a metalloprotease, since in the presence of o-phenanthroline or of synthetic oligopeptides C6 cells were unable to spread and migrate on CNS myelin or 35 or 250 kD inhibitors, a behavior similar to that of normal fibroblasts, astrocytes, or of B16 melanoma cells. C6 cell infiltration of CNS tissue explants was strongly reduced by the presence of these blockers. The other protease shows a thrombin-like specifity and plays a role in the early stages of C6 cell spreading on CNS myelin. Our results suggest that C6 cell infiltrative behavior into CNS white matter in vitro occurs by means of at least two distinct proteolytic activities, which probably act on the myelin-associated inhibitory substrates. (SPON: D. Kuffler)

209.2

MIGRATION OF TRANSPLANTED ASTROCYTES IN NEONATAL RATS.

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As part of a study to determine changes in neuroglial relationships during development, we were interested in determining the ability of transplanted astrocytes to migrate in neonatal rat cortex. Accordingly, a purified population of type I cortical astrocytes was obtained from postnatal day (PD) 1 rats (McCarthy and DeVellis, 1980) and characterized by immunocytochemistry using antibodies to glial fibrillary acidic protein and by electron microscopy. A confluent culture of astrocytes was fluorescently labeled with Fast Blue. A 1 µl suspension (10^6 cells/µl) of labeled cells was unilaterally injected into PD2 host animals using a Hamilton syringe fitted with a 26 gauge needle. The injection site was 1.0 mm anterior to bregma, 1.0 mm lateral to the midline, and 1.5 mm below the surface of the brain. Five days post-injection, labeled astrocytes were present beyond the limits of the injection site suggesting that there is a migration of transplanted astrocytes by this time. (Supported by EY05028 and EY07616).

209.3

MIGRATION OF GRAFTED TRANSFORMED ASTROCYTES.
W.J. Goldberg, E.R. Laws Jr. and J.J. Bernstein. Laboratory of CNS Injury and Regeneration, VA Medical Center and Departments of Physiology and Neurological Surgery, George Washington University School of Medicine, Washington, DC.

Grafted fetal astrocytes migrate throughout the host brain on basal lamina-lined surfaces and in parallel and intersecting white matter fascicles [Goldberg and Bernstein, J. Neurosci. Res. 20:38-45, 1988]. In the present experiments 10^6 C6 glioma cells were homografted as suspensions into freshly made implantation pockets in host rat cerebral cortex. Animals were prepared for transmission and scanning electron microscopy 1-7 days postimplantation [DPI]. A large mass grew out of the implantation pocket and by 3 DPI vacuolated C6 cells had migrated and/or invaded onto the glia limitans on the surface of the brain and into the corpus callosum, subependymal space, perivascular space and the cortex under the implantation pocket. By 7 DPI individual C6 cells had migrated into the corpus callosum and internal capsule and were observed in a perineuronal position in the hippocampus and other gray matter structures inferior to the corpus callosum [i.e. habenula]. Pockets were found surrounding each C6 cell and the processes of these cells had replaced host parenchyma. The preferred routes of migration were on basal lamina and parallel and intersecting nerve fiber bundles. Invasion occurred through gray and white matter. The movement of homografted C6 cells in the brain suggests that these cells actively migrate as individual cells as well and invade as a mass.

Supported by the Veterans Administration

209.4

DIFFERENTIAL SORTING OF ASTROCYTES EXPRESSING EPIDERMAL GROWTH FACTOR RECEPTOR FROM PRIMARY CULTURES OF BIOPSIED HUMAN GLIOMA.
S. Murphy and S. Thwin*. Dept. Pharmacology, College of Medicine, Univ. of Iowa, Iowa City, IA 52242.

Astrocytomas comprise the bulk of malignant brain neoplasms and carry a dismal prognosis. Gene amplification has been demonstrated in about 50% of these tumors, most consistently the gene coding for epidermal growth factor receptor (EGFr), suggesting involvement of EGFr-mediated growth regulation in the pathophysiological processes underlying malignancy. As the first step towards investigating the functional role of EGFr in neoplastic astrocytes, we have devised methods for deriving primary cultures from discarded human biopsies, and for selecting cells expressing EGFr and growing these on in culture.

Glioblastoma multiforme (0.1 g wet weight) were diced into cubes (0.4 mm) and seeded in small flasks. Within two days $GFAP^+$ processes, and then cells emerged from these explants, becoming confluent after 2-3 weeks. The majority of these cells were $GFAP^+$ and all EGFr-bearing cells were $GFAP^+$. The cultures were trypsinized, labelled with a monoclonal antibody against human EGFr (ICN ImmunoBiologicals) followed by FITC conjugated anti-mouse IgG, and then sorted by flow cytometry. Preliminary studies with A431 cells which express considerable amounts of EGFr revealed that trypsinization did not affect antibody labelling of EGFr. The glioma cells sorted on the basis of EGFr expression were harvested and replated. These cells were $GFAP^+$ and proliferated in culture.

This investigation was supported by Biomedical Research Support Grant RR 05572 from the NIH.

209.5

CHARACTERIZATION AND LOCALIZATION OF PERIPHERAL BENZODIAZEPINE RECEPTORS (PBR) IN HUMAN GLIOBLASTOMAS,
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Using radioligand binding techniques, six of six human glioblastoma (GBM) specimens had high concentrations of PBR ([^{3}H]-PK 11195 binding sites). The levels were significantly greater than in five normal human frontal cortex samples. The pharmacologic specificity of these sites differed significantly from PBR in human and rat kidney specimens.

Scatchard analysis revealed a small number of high affinity sites and a substantial number of sites of intermediate affinity. Under *in vitro* binding conditions the more numerous lower affinity site is the major contributor to specific binding measurements.

The ligand recognition site of the PBR in human GBM was photoaffinity labeled using [^{3}H]-PK 14105. SDS-PAGE revealed specific incorporation of label into a 17,300 molecular weight component. There was no specific incorporation into human frontal cortex, but a component of similar molecular weight was present in human kidney.

A seventh GBM was studied by *in situ* photoaffinity labelling and emulsion autoradiography of frozen/fixed sections. Light microscopy confirmed localization of PBR to malignant GBM cells.

We conclude that human GBMs consistently express PBR in greater density than in normal human brain. External imaging of these tumors with labeled analogues of PK 11195 thus appears feasible. Further characterization of the photoaffinity-labeled PBR should also provide useful information on the biology of glial tumors.

209.6

BENZODIAZEPINE (BZD) RECEPTOR IN CULTURED ASTROCYTES: PHOSPHORYLATION AND THE EFFECTS OF AMMONIA.
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Previous studies have shown that ammonium chloride treatment increases the affinity of the peripheral-type BZD receptor (Brain Res., in press; Soc. Neurosci. Abstr. 14:1082, 1988) and alters protein phosphorylation in astrocytes (Brain Res 437:161, 1987). Since phosphorylation has been shown to affect the status of a number of membrane receptors, we determined a) whether the astrocyte BZD receptor might be affected by membrane phosphorylation and b) whether an altered state of phosphorylation might be a mechanism whereby ammonium chloride induces changes in receptor affinity. Primary astrocyte cultures were obtained from neonatal rat cortices. Phosphorylation of astrocyte homogenates by ATP and $MgCl_2$ was carried out for 5 min at 30°C in the presence of calcium, calmodulin and 8-Br-cAMP to activate calcium- and cAMP-dependent protein kinases. Particulate fractions were obtained by centrifugation at 20,000 x g for 15 min and receptor properties were assessed by using 3H-Ro5-4864 as the ligand. Scatchard analysis showed a 38% decrease ($p<0.01$) in Kd in fractions that had been phosphorylated. No significant change in receptor number was observed. Preliminary data suggest that no further reduction in Kd occurred when preparations from ammonium chloride-treated cells (10 mM, 22 hrs) were subjected to phosphorylation. These results indicate that phosphorylation affects the status of the astrocyte peripheral-type BZD receptor; furthermore, phosphorylation of this receptor or of closely associated membrane components may be responsible for the increase in affinity of the BZD receptor after exposure to ammonium chloride. (Supported by NIH grant DK38153 and the Veterans Administration)

209.7

ASTROCYTE ATP CONCENTRATIONS ARE REDUCED BY FACTORS CIRCULATING IN THE BLOOD OF REYE'S SYNDROME PATIENTS.
J.E. Olson. Department of Emergency Medicine, Wright State University School of Medicine, Dayton, Ohio, 45401.

Reye's Syndrome (RS) is characterized by the rapid onset and progression of neurological dysfunction caused by cerebral edema associated with astrocyte swelling. Plasma levels of free fatty acids and ammonia are significantly elevated. Use of salicylates during the prodromal illness may increase the likelihood of developing RS. We have demonstrated that astrocyte cell volume regulation following osmotic swelling is dependent upon cellular energy (J. Cell. Physiol. 128:209-217, 1986). In this study, we investigated the influence of fatty acids, ammonia, and salicylate on astrocyte energy state.

Astrocytes from primary culture were incubated for 30 min in hypoosmotic phosphate-buffered saline containing no additive (control cells) or 0.03-10 mM octanoate, dodecanoate, ammonia or salicylate. Protein and ATP contents then were determined in each sample.

Ammonia had no effect at any concentration tested. Relative to control cells, salicylate reduced the ATP content by 16% at 1.0 mM and by 92% 10 mM. Dodecanoic and octanoic acids reduced the ATP content by approximately 50% at 1.0 mM and 10 mM, respectively.

The results indicate that free fatty acids impair astrocyte energy metabolism at concentrations observed in RS. Salicylate also may contribute to the metabolic deficit. In contrast, ammonia does not appear to alter astrocyte energy metabolism. These effects may be important in the pathogenesis of cerebral edema in Reye's Syndrome.

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209.8

INTERLEUKIN-1 (IL-1) IN ALZHEIMER'S: ALUMINUM AS A POTENTIAL INDUCER.
L.S. Kamaraju*, S.K. Sundar*, D.E. Schmechel*, M.A. Cierpial & J.M. Weiss (SPON: E. Ellinwood). Departments of Psychiatry and Medicine, Duke ADRC, Duke University Medical Center, Durham, NC 27710.

Some investigators believe that aluminum (AL) salts form the bulk of the inorganic material in the cores of senile plaques. Since Al is a potent inducer of IL-1 in macrophages, we investigated 1) IL-1 concentrations in brains of Alzheimer's disease (AD) patients, and 2) whether Al can induce production of IL-1 by astrocytes in vitro and in vivo.

Assay of glia-rich preparations from two AD brains showed, in fact, high levels of IL-1. In vivo treatment of C6 glioma cells with Al resulted in the secretion of copious amounts of IL-1 in contrast to undetectable levels in control cultures. Likewise, intraventricular infusion of Al into rats resulted in high levels of IL-1 in glial-rich cell fractions.

Although IL-1 has been recently detected in the brains of AD patients, its potential role has yet to be determined. In experimental animals, Al has been postulated to produce cognitive changes by decreasing glucose metabolism and IL-1 has been shown to inhibit glucose sensitive neurons; therefore, Al may exert effects on cognitive functioning via the induction of IL-1.

209.9

DELAYED SCHWANNIAN REMYELINATION OF CENTRAL AXONS AFTER INTRASPINAL ETHIDIUM BROMIDE + IRRADIATION
P.A. Felts[1]* & K.J. Smith[1,2], [1]Anatomy & Cell Biology, Eastern Virginia Medical Sch., Norfolk, 23501, & [2]Neurology, U.M.D.S., London, SE19RT.

Transcutaneous x-irradiation (40Gy) is known to prevent remyelination of central demyelinated lesions for 8 weeks (Blakemore & Patterson, '78), but long term examination has been prevented by radiation-induced skin and grey matter lesions. We have developed a technique which restricts the radiation to precise areas of the exposed spinal cord. Radiation occurs at 6.28 Gy/min, using a 10x20mm source of ^{90}Sr (0.1Ci) behind a shield of variable aperture. The ß particles are approximately 50% absorbed by 1mm of tissue, effectively restricting radiation to the superficial cord.

22 adult rats (Sprague Dawley) received dorsal column injections of ethidium bromide (0.6-1.2ul, 0.5mg/ml), with 40Gy of irradiation over a 3X5mm area. Glial and myelin dissolution were prominent by 7d, and by 21d the lesion consisted of naked, demyelinated axons. Remyelination was prevented for at least 1m (beginning in non-irradiated controls at 14d), but long term examination established that the vast majority of affected axons were eventually remyelinated by Schwann cells. Supported by NIH NS21670 to KJS.

209.10

PROLIFERATION OF SCHWANN CELLS OF UNMYELINATED FIBRES IN RAT SCIATIC NERVE DURING WALLERIAN DEGENERATION OF NEIGHBOURING NERVE FIBRES.
D.R. Archer* and J.W. Griffin. Department of Neurology, Johns Hopkins University Medical School, 600, N. Wolfe St., Baltimore, MD.

Following axotomy axons of the distal stump undergo Wallerian degeneration. Proliferation of Schwann cells whose axons are undergoing Wallerian degeneration is well known; in the rat Schwann cell division peaks 3 days after axotomy. The purpose of this study was to investigate the influence of Wallerian degeneration on neighbouring intact nerve fibres. We sectioned the L4 and L5 ventral roots in 4-week-old rats. At times up to 14 days after axotomy the rats were injected with ^{3}H-thymidine 2, 4 and 6 hours prior to sacrifice. Nerve segments from the sciatic nerve 2 cm distal to the L5 DRG were examined by electron microscopy and autoradiography. At both 3 and 4 days after labelling Schwann cells of intact, unmyelinated axons were seen in mitosis. Mitotic figures were rare in contralateral control nerves. These observations suggest that degenerating nerve fibres release a mitogenic factor capable of acting over a short distance to stimulate proliferation of nearby Schwann cells of unmyelinated fibres.

209.11

PATTERN OF GFAP IMMUNOSTAINING IN EARLY POSTNATAL RAT SPINAL CORD.
S. A. Gilmore and T. J. Sims. Dept. of Anatomy, Univ. of Arkansas for Medical Sciences, Little Rock, AR 72205-7199.

Immunoperoxidase stained, transverse sections of immature rat spinal cord were examined to determine the location and distribution of cells positive for glial fibrillary acidic protein (GFAP), an intermediate filament protein of astrocytes. The elucidation of these data is necessitated by the paucity of such information in normal rats to serve as a baseline for evaluation of astrocytic reactions in the injured spinal cord. The lumbosacral region (LSR) was studied in Charles River CD rats ranging in age from 3 to 21 days. At 3 days GFAP immunoreactivity was associated with punctate structures near the surface of white matter, suggestive of processes cut transversely. This was characteristic of the ventral and the ventrolateral funiculi. A few process bearing cells were noted in the dorsolateral white matter. The latter were noted throughout the LSR, whereas the punctiform distribution was limited to the more rostral sections. By 6 days GFAP immunoreactivity was present in the punctate pattern throughout the LSR. In addition, the dorsolateral white matter in caudal sections contained GFAP positive cells which appeared to have processes in a bipolar arrangement. More rostrally, at the gray/white interface in this region, many cells with multiple, thin, branching GFAP positive processes were noted. The intensity and the numbers of immunoreactive cells were markedly increased throughout the white matter by 9 days of age. With respect to the gray matter, a gradient still persisted at this age in that GFAP positive cells were present ventromedially in rostral sections but were not yet apparent in the caudal ones. Thereafter, GFAP immunoreactivity increased throughout the LSR with a tendency for a ventral to dorsal gradient. This pattern will be compared with that of vimentin immunoreactivity on contiguous or adjacent sections. Supported by NIH grant NS 04761.

209.12

DIFFERENTIAL REGULATION OF PROTEOGLYCAN BIOSYNTHESIS BY SOLUBLE MEDIATORS OF INFLAMMATION IN PRIMARY ASTROCYTE CULTURES.
P.C. Johnson-Green*, K.E. Dow and R.J. Riopelle. Queen's University, Depts. of Pediatrics and Medicine, Kingston, Canada K7L 3N6.

Proteoglycans on the cell surface and in the extracellular matrix have been implicated in cell adhesion and in the regulation of neurite outgrowth.

Primary astrocyte cultures from 3 day old rats were labelled with $^{35}SO_4$ and ^{3}H-glucosamine after pretreatment with Interleukin-1 (IL-1) (5 units/ml) or macrophage conditioned media (MCM) (5:1, DMEM/F12:MCM). Media and cell layers were extracted with guanidine-HCl and glycosaminoglycans (GAGs) were isolated by pronase digestion and ion exchange HPLC, and identified.

Untreated (control) cells released heparan sulphate (HS) (16% of total), chondroitin sulphate (CS) (46%), dermatan sulphate (DS) (13%) and hyaluronic acid (HA) (25%) into the media. The cell layer contained the same 4 GAGs, although in different proportions (53% HS, 33% CS, 5% DS and 9% HA). IL-1 treated cells released more GAGs into the media (140% HS, 167% CS, 167% DS and 168% HA of control), but the amount of GAGs in the cell layer was similar to the control. Conversely MCM treated cells released similar amounts of GAGs into the media as compared with control, whereas the cell layer contained 55% less HS.

The differential effects of MCM on cellular and released GAGs suggest that known proteolytic activity of MCM does not account for the observations, unless cell-associated proteoglycans are a preferred substrate for proteolysis. MCM may contain other factors that influence cellular production of GAGs and interfere with the IL-1 in the MCM. Our results also suggest that the effects of MCM on GAG content cannot be solely explained by IL-1.

These observations begin to address molecular mechanisms that influence axonal and oligodendroglial regenerative responses following insult to the CNS.

Supported by MS Canada and the Rick Hansen Fund.

209.13

ESTRADIOL INCREASES GLIAL PEROXIDASE ACTIVITY IN THE HYPOTHALAMIC ARCUATE NUCLEUS.
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In adult female rats, treatment with estradiol valerate (EV) produces anovulation and neuropathological changes in the hypothalamic arcuate nucleus (Brawer et al, Endocrinology 1978;103: 501). In the present study, a single intramuscular injection of 2 mg EV (n=4) resulted in a three fold increase in numbers of diaminobenzidine(peroxidase)-positive granules in the arcuate nuclei relative to vehicle treated controls (n=5,p<.01). The peroxidase activity was localized to astrocytes by double-label immunohistochemistry utilizing antiserum to glial fibrillary acidic protein. Diaminobenzidine staining occurred over a pH range of 4-10.5 and was resistant to the catalase inhibitor, aminotriazole, and to tissue pre-heating, indicating that a nonenzymatic pseudoperoxidation reaction has been induced in these cells by estrogen administration. Possible mediators of this reaction are metalloporphyrins known to be present in rodent hypothalamus. Enhanced peroxidase activity in arcuate astrocytes may play a role in estradiol-related hypothalamic damage by oxidizing estradiol to potentially toxic semiquinone radicals as occurs in peripheral estrogen target tissues.

210.1

MORPHOLOGY OF ASTROCYTES AND PRESUMED OLIGODENDROCYTES IN THE INTACT RAT OPTIC NERVE USING INTRACELLULAR INJECTION OF LUCIFER YELLOW (LY) AND HORSERADISH PEROXIDASE (HRP). Arthur M. Butt* and Bruce R. Ransom, Dept. Neurology, Yale Univ. Sch. Med., New Haven, CT 06510, *Biomedical Sciences Division, King's College London, Kensington, London, UK. (SPON: J. Ebersole)

Using intracellular injections of LY or HRP to visualize glial cells in the rat optic nerve(RON), two clearly distinct macroglial cell types were observed in over 500 cells examined from rats 1-30 days old. One cell type had the typical chacteristics of fibrous astrocytes. The other cells were presumed to be oligodendrocytes because their morpology corresponded well to the imagined picture of this cell type based on EM studies. Mature astrocytes had ~50-60 fine, radially directed processes 100-200μm in length which terminated in bulbous end-feet at the pial surface, at blood vessels, or within the nerve parenchyma (possibly paranodal end-feet). All astrocytes contributed to the subpial and perivascular glial limiting membranes and their morphology appeared to depend in part on their position within the nerve. Individual astrocytes often extended processes which spanned the entire transverse diameter of the RON(~500μm). Presumed oligodendrocytes, in RONs >2 weeks of age, had 20-30 processes ~150-200μm in length which ran exclusively along the long axis of the nerve parallel to the axons. The processes of these cells never branched or terminated at the pia or blood vessels, had no end-feet, and were connected to the cell body by fine, 10-15μm long, connecting processes. These longitudinal processes are most likely the external tongue processes of the myelin sheathes, and their length would correspond directly to internode distance (a parameter which has only been estimated to date). In many instances fine circular terminations could be detected on the longitudinal processes and these may represent paranodal loops. Each presumed oligodendrocyte would therefore myelinate 20-30 axons within 10-15μm of the cell body with an internodal distance of about 150-200μm. These studies on the 3-dimensional morphology of glial cells *in situ* should provide important new insights into the relationship between their form and their function. Supported by NIH grant NS 15589.

210.2

RADIAL GLIA GIVE RISE TO PERINODAL PROCESSES. T. J. Sims, S.A. Gilmore and S.G. Waxman, Dept. of Anatomy, University of Arkansas for Medical Sciences, Little Rock, AR 72205 and Dept. of Neurology, Yale Medical School, New Haven, CT 06510.

This study examined the associations between radial glia processes and the axolemma at nodes of Ranvier in the spinal cord of the mature axolotl salamander. Fixation of the spinal cord was accomplished by perfusion of the truncus arteriosus with a mixture containing 2% glutaraldehyde, 2% paraformaldehyde, 0.5 % acrolein in 0.9 M cacodylate buffer at pH 7.3. Spinal cords were cut into 2 mm segments and were embedded in Spurr plastic. Thick and thin sections were cut in sagittal or transverse planes and examined by light and electron microscopy. Radial glia represent a large proportion of the total glial population in the spinal cord of the mature axolotl. The radial glia in a location dorsal to the central canal project long processes to the dorsal surface of the spinal cord. These processes coalesce into large fascicles in the midline and form the dorsal median septum. As these processes pass through the white matter they give rise to branches that terminate by becoming apposed to the axolemma at nodes of Ranvier. Radial glia are present also in the midline ventral to the central canal. Processes from these cells extend a short distance to the glial limitans overlying the ventral medial septum. These processes give rise to short branches, as do their dorsal counterparts, and many of these branches terminate on nodal axolemma of myelinated axons in the anterior white commissure. Radial glia are thought to differentiate into astrocytes during development of the nervous system in mammals, and perinodal processes derived from astrocytes have been observed in a variety of species. The observations in this study support the view that radial glia, as well as astrocytes, give rise to perinodal processes. The origin of perinodal processes from radial glia in the salamander indicates that this axo-glial relationship is an important functional interaction preserved across a large portion of the phylogenetic scale. Supported by NIH grant NS-04761

210.3

THREE-DIMENSIONAL IMAGING OF SUBSTRUCTURAL FEATURES OF SELECTIVELY STAINED NODES OF RANVIER BY HIGH VOLTAGE EM AND COMPUTER-AIDED RECONSTRUCTION FROM SERIAL SECTIONS. M. H. Ellisman, T. J. Deerinck*, D. Hessler*, and S. J. Young, Lab. for Neurocytology, U.C.S.D., LaJolla, Ca. 92093

The node of Ranvier is the site of complex and rapid physiological events related to saltatory propagation of the action potential. In this region, myelinating cells and the axon form intimate associations via specializations of the cytoplasmic membrane systems, cytoskeleton, and plasma membrane. We have used selective staining and reconstructions from serial sections to study these specializations. Selectively stained thick sections were viewed at 1MeV with the aid of a High Voltage Electron Microscope (HVEM). ***Phosphotungstic Acid***, included in the primary fixative and followed by conventional osmium staining delineates the axolemma at the node and paranode, the ends of the paranodal loops, and the junctional feet of the glial-axonal-junction (GAJ). ***Lanthanum Nitrate***, in the secondary fixative, enhances the paranodal GAJ and provides negative staining of the junctional feet. ***Hatchett's Brown Reaction*** results in electron dense material associated with the nodal axolemma but not the paranodal region. This stain also delineates the paranodal loops. ***Tannic Acid*** in the primary fixative better defines the extracellular matrix associated with the nodal membrane and its relation to the Schwann cell microvilli and the nodal axolemma. ***Osmium Impregnation*** enhances contrast of differentiations of the axoplasmic reticulum which form subsurface cisternae adjacent to the GAJ's of the paranodal axolemma. 3-dimensional HVEM images reveal the distribution of each substructural feature in relation to other nodal components. We have also used computer-aided reconstructions from serial sections to follow axoplasmic intermediate filaments and found many to associate with the axolemma at the GAJ. These reconstructions have also allowed us to track the axoplasmic reticulum in this paranodal region. 3-D views of the node of Ranvier and selectively stained constituents provide a more complete structural framework for understanding the physiological processes occurring at this important site. Supported by PHS NS14718, RR04050, RR00592.

210.4

CULTURED MAMMALIAN ASTROCYTES SYNTHESIZE COLLAGEN TYPE IV: EVIDENCE THAT ASTROCYTES MAY PARTICIPATE IN BASEMENT MEMBRANE FORMATION AT CNS BOUNDARIES. J.R. WUJEK, H. HALEEM-SMITH*, E. FREESE AND Y. YAMADA*. Lab. of Molec. Biol., NINDS, NIH and Lab. of Dev. Biol. and Anomalies, NIDR, NIH., Bethesda, MD 20892.

Basement membranes are formed at the interface between astrocytes of the central nervous system (CNS) and non-CNS structures, such as meninges and blood vessels. Meningeal cells and endothelial cells synthesize collagen type IV, a major component of basement membranes. Immunocytochemical studies have not shown the appearance of collagen type IV on cultured astrocyes (Rutka et al., J. Neuropath. Exp. Neurol., 1988, 45: 285) We have used RNA and protein analysis to investigate the ability of astrocytes to synthesize collagen type IV. Primary cultures of cerebral cortex astrocytes were established from neonatal rats. Northern blot analysis of astrocyte RNA, using a cDNA probe to alpha-1 collagen type IV (Oberbaumer et al., Eur. J. Biochem., 1985, 147: 217), revealed the presence of collagen type IV mRNA (similar in size to collagen type IV mRNA in differentiated F9 teratocarcinoma cells). The amount of collagen mRNA increased during extended growth of astrocytes from 2 weeks up to 10 weeks in culture, . Western blot analysis of astrocyte cell lysates showed a protein band at 190 kDa, corresponding to collagen type IV. These results show that astrocytes, *in vitro*, synthesize collagen type IV. We suggest that astrocytes, *in vivo*, may participate in the formation of basement membranes at the interface between CNS and non-CNS tissues (eg. meninges and blood vessels).

210.5

MONOCLONAL ANTIBODIES WHICH BIND SELECTIVELY TO ASTROCYTIC EPITOPES. Dennis M. D. Landis, Lori A. Weinstein*, Christine I. Skordeles*, and H. Ross Payne*. Department of Neurology and Center for Neurosciences, School of Medicine, Case Western Reserve University, Cleveland, OH 44106

We have been interested in obtaining monoclonal antibodies which bind selectively to astrocytic epitopes, and which would be useful in studies of astrocyte function, development, and lineage. Plasmalemmal fractions from primary cultures of neonatal rat forebrain astrocytes have been used as immunogens in ten fusions, and the resulting hybridomas have been screened by ELISA for binding to the immunogen and to live cultured astrocytes, and by immunocytochemical staining. Forty three clones have been selected, and we are in the process of characterizing each. Several antibodies bind to surface determinants on virtually all GFAP+ cells in culture. MAb 8C10 stains fixed and unfixed cultures and tissue; the tissue pattern suggests that it binds to an epitope distributed over most of the astrocytic surface. Comparison of staining patterns in cerebellum, hippocampus, and spinal cord suggests that this mAb may be a useful probe for identifying regional heterogeneity in the extent of astrocytic processes and for measuring astrocyte surface areas. Other antibodies bind to subpopulations of astrocytes in culture and in tissue. MAb 4C6 binds to scattered, flat GFAP+ cells in culture, but stains myelin in fixed tissue. Its pattern on Western blots appears to represent a hitherto undescribed epitope, and the mAb might represent a tool for studying oligodendrocyte lineage. Several mAb's stain intracellular epitopes in a sub-populations of cerebellar astrocytes, or are selective for astrocytic nuclei. MAb 5E6, for example, selectively stains astrocytes in white matter.

These antibodies may prove to be useful reagents in the analysis of astrocyte structure, function, lineage and development.

210.6

ISOLATION AND CHARACTERIZATION OF DOG GLIAL HYALURONATE-BINDING PROTEIN. G. Perides and A. Bignami. Harvard Medical School and VA Medical Center, Boston, MA 02132

Using antibodies raised against human glial hyaluronate-binding protein (GHAP), we identified its homologous in the dog central nervous system. The procedure to isolate the dog protein however, is considerably different. Instead of only one affinity chromatographic step which was used for human GHAP (Perides et al. J Biol Chem 264:5981, 1989), two ion exchange chromatography columns had to be introduced followed by the HA-Sepharose. Briefly, the protein was extracted from dog spinal cord with HCl at pH 2.2. Then it was enriched by binding to CM-Sepharose followed by a DEAE-Sepharose. Subsequently, the protein was affinity purified by binding onto a hyaluronic acid Sepharose column and eluted with 4M guanidine HCl. This brain-specific extracellular matrix glycoprotein consists of two isoelectric variants (pI 4.3-4.4) as the homologous human protein.

Enzymatic deglocosylation, peptide mapping with trypsin, V8 protease and Lys-C specific endoproteinase as well as hyaluronic acid binding experiments will be presented and the results will be compared to those obtained with human GHAP. Supported by NIH grant NS 13034 and by the Veterans Administration.

210.7

GLIAL HYALURONATE-BINDING PROTEIN (GHAP) IN TISSUE CULTURE. D. Dahl and A. Bignami. Harvard Medical School and VA Medical Center, Boston, MA 02132.

GHAP is a structural glycoprotein of brain extracellular matrix produced by white matter astrocytes. The in vitro expression of GHAP was studied in primary cultures prepared from mechanically dissociated postnatal rat cerebella. Cells stained with GHAP antibodies were first observed in 4-5 day cultures. The small and phase-dark cells were either isolated or collected in clusters. All cells were vimentin-positive and only few also stained with anti-GFAP. In 6-7 day cultures, the morphology of GHAP-positive astrocytes varied considerably from small cells similar to the vimentin-positive precursor to large cells with many processes. Flat, epithelioid, (type-1) astrocytes were GHAP-negative. The relation of GHAP-positive astrocytes with type-2 astrocytes remains to be determined, but type-2 astrocytes of stellate appearance (Aloisi et al. J Neurosci Res 21:188, 1988) were GHAP-negative. It is suggested that astrocytes form two distinct populations: GHAP-positive astrocytes mainly in white matter and GHAP-negative astrocytes mainly in gray matter, each group originating from a different precursor. Supported by NIH grant NS 13034 and the Veterans Administration.

210.8

IDENTIFICATION OF AN INTERFERON-γ RESPONSIVE ELEMENT IN THE HLA-DRA GENE BY TRANSFECTING CULTURED TYPE 1 ASTROCYTES. H. Moses* and J. Ting* (SPON: R. Rohwer). Curr. in Neurobiology and Dept. of Microbiology and Immunology, Univ. of North Carolina, Chapel Hill, NC 27599.

Previous studies have shown that Ia expression on type 1 astrocytes is induced with interferon-gamma(IFN-γ) treatment. This is of interest because class II major histocompatability complex(MHC) antigen expression has been detected in the brain during autoimmune and neurodegenerative disease processes. The purpose of this report is to use a molecular biological approach to identify the IFN-γ responsive DNA region of DRα in primary glial cells. Using a nested series of 5' deletion mutants, we have found that the IFN-γ responsive region in type 1 astrocytes is contained within basepairs -135 to -109. The deletion mutants contain the upstream promoter region of the DRα gene linked to a reporter gene, chloramphenicol acetyl transferase.

The deletion mutants all contain a putative CAAT box, a TATA box, and the transcriptional start site. The IFN-γ induction of DRα is not seen using deletion mutants that are less than 109 base pairs upstream from the start site. Furthermore, any additional region beyond -135 does not provide any greater induction with IFN-γ. With the deletion mutant containing the-109 to -135 base pair region, we have observed about a five fold increase of promoter function with IFN-γ treatment.

Preliminary experiments with a substitution mutant of the IFN-γ responsive element resulted in a loss of induction with IFN-γ treatment. Further experiments are in progress to determine if the IFN-γ induction of DRα is transcriptionally regulated, and finally site-specific mutagenesis studies should identify precisely the IFN-γ inducible sequences. As far as we know, this is the first report that delineates the IFN-γ responsive region in primary cells, specifically astrocytes.

210.9

ULTRASTRUCTURAL IDENTIFICATION OF ANTI-GFAP IMMUNO-REACTIVE CELLS IN THE REGION OF THE DEVELOPING AUDITORY DECUSSATION. Sherry Vinsant*, Constance Linville*, Darrell Agee* and Judy K. Brunso-Bechtold (SPON: Wayne Silver). Program in Cell Biology and Neuroscience, Department of Anatomy, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27103.

For some time we have been interested in mechanisms involved in the development of the hindbrain auditory decussation, the trapezoid body. During the early stages of the development of this decussation, light microscopy reveals the existence of a midline glial boundary (MGB) extending from the 4th ventricle to the base of the brain, as well as processes perpendicular to the boundary, both of which are immunoreactive to antibodies to glial fibrillary acidic protein (GFAP). Electron microscopy reveals a population of cells in the midline region which extend large processes through the MGB, often accompanied by bundles of axons. These cells contain glycogen granules and receive numerous synaptic contacts. In an effort to determine the identity of these cells we have used EM-immunocytochemistry to localize anti-GFAP immunoreactivity by the ABC-peroxidase method. Our results indicate that at least some of the cells with large processes and synaptic contacts are anti-GFAP immunoreactive. In addition, the processes of the MGB are immunoreactive at the EM level. (Supported by NS23092).

210.10

ASTROCYTE RESPONSES TO EGF AND CYTOKINE INTERACTIONS

K.R. Huff and L.L.V. Ibric*. Childrens Hospital of Los Angeles, University of Southern California School of Medicine, Los Angeles, CA 90027

We have studied changes in proliferation, Epidermal Growth Factor (EGF) binding, and major histocompatability (MHC) antigen expression of purified cells from neonatal rat or mouse cerebral cortices to treatments with EGF and cytokines. Astrocytes proliferate locally in immune mediated disease states, and the signals may come from growth factors released in injured nervous tissue alone or interacting with cytokines brought by immune cells crossing the vascular space.

We have found that astrocyte proliferation is markedly inhibited by gamma interferon (INF), is modestly increased by tumor necrosis factor (TNF), and is unchanged by interleukin-2 (IL-2) unless combined with INF which causes reduction. Immunofluorescence studies were performed on fixed GFAP containing cells, and fluorescence activated cell sorting (FACS) analysis was done of lightly trypsinized suspended unfixed cells using mAbs to class I and II MHC antigens, which demonstrated response to cytokines and EGF in immune mediation parameters as well. INF and TNF induced both class I and II expression, and EGF markedly induced class I expression; however, combined with INF or TNF reduced EGF induction of MHC. In addition, INF increased EGF binding despite the inhibition of astrocyte proliferation and EGF-induced class I MHC expression.

EGF may work as a concert member of local signals producing functional astrocyte changes in disease states, or it could be a coordinator of signals involving both changes in proliferation and changes in immune mediation in the absence of mitogenic effects.

210.11

TIME COURSE OF MITOGEN INDUCED ALTERATIONS IN THE CELL CYCLE OF CULTURED RAT ASTROCYTES.

D.L. Davies and R.B. Owens*. Depts. of Anatomy and Pathol., Univ. of Arkansas for Med. Sci., Little Rock, AR 72205-7199.

As part of an endeavor to analyze the proliferative and morphologic responses of astrocytes to endogenous mitogens, the cell-cycle of astrocyte enriched cultures was examined using flow cytometric techniques. An extract of homogenized adult rat brain was used as a mitogen to elicit a proliferative response in secondary cultures derived from neonatal rat cerebra. For flow cytometric cell-cycle analysis, cell nuclei were stained with propidium iodide after 6, 12, 18, 24 and 30 hrs of exposure to the brain extract. In control cultures, the proportion of cells in the quiescent/presynthetic (G_0/G_1), synthetic (S), and postsynthetic + mitotic (G_2+M) phases did not vary significantly during the 30 hr study interval. In contrast, in brain extract treated cultures, a decrease in the proportion of cells in G_0/G_1 was noted and a two fold increase in the number of cells in S phase was measured at 18 hrs. Furthermore, an increase in the proportion of cells in the G_2+M phases was observed at 24 and 30 hrs after introduction of the brain extract. These findings suggest that the brain extract increased astrocytic proliferation by recruitment of G_0/G_1 cells into the S phase, a finding compatible with studies of cultured chick neuroblasts and glia (Burakat, I. *et al.*, Cell Tissue Kinet. 18:45, 1985).

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210.12

EXPRESSION AND DISTRIBUTION OF G-PROTEIN IN CEREBRAL CORTICAL CELLS. F.L. Jordan. Dept. of Oral Biology. College of Dentistry, The Ohio State University, Columbus, OH 43210.

There is much evidence that G-protein (Gp) functions as a transducer of external stimuli in mammalian cells; however, Gp function in brain has not been extensively studied. In an effort to develop a system in which to investigate this, the developmental expression and cellular distribution of Gp in rat cerebral cortex was determined. Gp was assessed in intact cerebral cortical tissue through gel electrophoresis and Western blot analysis, and the cellular localization was revealed through immunohistochemical staining in primary cultures of dissociated cells. Gp was detected in the intact tissue as early as E16 and increased through the neonatal and young adult stages. The protein could also be detected in cortical cultures, and localized via immunohistochemical staining as early as day 4 after plating of neonatal cells. In mature cultures Gp was detected in all cell types - neurons stained most intensely, some astrocytes (type 2) also exhibited significant staining, while most other cell types were detectably stained at lower levels. An overall pattern of developmental expression of higher levels in developing cells than in mature cells was observed. It can be concluded that Gp is expressed very early in cerebral cortical development, is expressed at higher levels in immature cells versus mature cells, and is present in essentially all mature cell types.

210.13

STUDIES OF THE DYNAMIC PROPERTIES OF MICROGLIAL CELLS BY TIME-LAPSE VIDEO MICROSCOPY. J. A. Glenn*, F.J. Jordan, P.L. Booth* and W.E. Thomas (SPON: M. Whitehead). Dept. Of Oral Biology, College of Dentistry, The Ohio State University, Columbus, OH 43210.

In continuing efforts to investigate the overall role of brain microglial cells, dynamic properties of these cells were studied. Utilizing a primary culture system of dissociated cerebral cortical cells from rat, viable cells equivalent to ramified microglia of intact brain tissue were identified and their activity monitored over time periods of 12-48 hr using time-lapse video recording. The most striking initial observation was the highly dynamic nature of these cells - microglia constantly rearranged their overall morphology by changes in the shape and size of the cell body and through the retraction and extension of processes. In addition, simultaneous with this activity the cells were mobile and exhibited apparently random migration usually over a spatial area several times the diameter of the cell body. In this dynamic state microglia were also observed to constantly ingest particles of cellular debris present in the cultures. Under certain conditions microglia displayed a cellular transformation in which they converted to a small sperical dark appearing cell form lacking processes. Similar cells in these cultures have previously been demonstrated to be predominately phagocytic. It is suggested that the ramified microglia possess the ability to convert into a highly phagocytic cell form.

210.14

CHARACTERIZATION OF CEREBRAL CORTICAL MACROPHAGES IN TISSUE CULTURE. W.E. THOMAS AND F.L. JORDAN. Dept. of Oral Biology, College of Dentistry, The Ohio State University, Columbus, OH 43210.

In an effort to establish an amenable preparation for the investigation of cerebral cortical macrophages, these cells have been characterized in tissue cultures of dissociated cells from rat. Previously determined unique morphological features were used to identify macrophages in living cultures where they were continuously monitored using time lapse video microscopy. Initially it was noted that the identified cell form usually exhibited mitosis. The resulting daughter cells displayed intense surface activity, including membrane bubbling and ruffling. The daughters then spread over the culture surface and assumed a very broad flat appearance, and remained in this state for extended time periods thereafter. Such flattened cells were observed to extend finger-like projections or processes up into the culture medium to entrap fragments of cellular debris which were then engulfed. This activity proceeded constantly. Individual flat cells apparently at random would pull up from the culture surface, round up to assume the originally identified cell form, and then undergo the pattern of mitosis described above. It was concluded that the characterized cortical macrophages are distinct from microglial cells, exhibit a specific pattern or cycle of cellular activity, and may be present in brain cell cultures in greater numbers than previously considered.

210.15

RAPID PROLIFERATION OF ASTROCYTIC PROCESSES IN CHICK COCHLEAR NUCLEUS FOLLOWING AFFERENT ACTIVITY BLOCKADE K. S. Canady* and E. W Rubel (SPON: N. Cant), Depts. of Physiology & Biophysics, Psychology and Otolaryngology, Univ. of Washington, Seattle, WA 98195

Cochlea removal in young chicks results in a rapid increase in astrocytic processes immunoreactive for glial fibrillary acidic protein (GFAP) in the cochlear nucleus, nucleus magnocellularis (NM). This increase is evident by 1 hour and reaches 80-100% by 6 hours after receptor removal (Rubel & MacDonald, *Soc. Neurosci. Abstr.*, 13:80; 1987). Analysis of Golgi impregnated glial cells has confirmed that the increase in GFAP reflects an increase in glial processes (MacDonald & Rubel, *Soc. Neurosci. Abstr.*, 15, 1989). The rapidity of this glial response suggests that growth of glial processes may be regulated by activity in NM neurons or 8th nerve axons. We tested this hypothesis by unilaterally blocking 8th nerve action potentials with perilymphatic injections of tetrodotoxin (TTX) in neonatal chicks. Immunocytochemistry was used to visualize GFAP within astrocytic processes in NM. The contralateral NM was used as a within-animal control. Far-field auditory evoked potentials were measured in a separate group of chicks before and after injecting TTX into the perilymph to confirm elimination of the auditory brain stem response.

Tissue sections taken from 6 chicks after 6 hours of unilateral 8th nerve activity blockade showed an increase in GFAP-positive processes in and around NM ipsilateral to afferent activity blockade. We conclude that astrocytes are sensitive to changes in neuronal activity, and that 6 hours of activity deprivation is sufficient to stimulate moderate growth of astrocytic processes. Supported by PHS grants NS24518 & GM07108.

210.16

NORMAL AND PROLIFERATING GLIAL PROCESSES OF N. MAGNOCELLULARIS IN NEONATAL CHICKS: A SILVER IMPREGNATION STUDY. G. H. MacDonald* and E. W Rubel, Hearing Development Laboratories, Univ. Washington Medical Center., Seattle, WA. 98195

Increased GFAP immunolabelling observed in Nucleus Magnocellularis (NM) after deafferentation by ipsilateral cochlea removal (CR) (Rubel and MacDonald, *Soc .Neurosci. Abs.*, 13:80, 1988) was further probed by silver impregnation methods. Chicks were perfused 4 hrs. after unilateral CR; brainstems were impregnated by del Hortega's silver nitrate method and embedded in celloidin. Computer stored 3-dimensional images were drawn of glial cells in and around NM. Distinct glial cell distribution patterns were found in control NM, with fibrous astrocytes and oligodendrocytes predominating. Other glial cells displayed microglial morphology, while distinctly protoplasmic astrocytes were rarely observed.

The impregnated glial cells in the deafferented NM doubled in number and displayed significant morphological changes. Presumptive fibrous astrocytes filled NM and the region between NM and N. Laminaris with primary processes of increased diameter and orientation parallel to NM axons. Although the number of primary glial processes was unchanged, the number of branch points nearly doubled and branches were much closer together on the processes, and process length increased approximately 80%. Some responding glial cells resembled protoplasmic astrocytes, or variants of other glial cell types. These findings support previous GFAP immunolabelling which indicated a rapid change in fibrous astrocyte processes after CR. We conclude that swelling and proliferation of processes are part of a glial response to decreases in synaptic activity. The results also suggest that glia types other than fibrous astrocytes may be involved in the response. Supported by NIH grant NS24518

210.17

PEPTIDE AMIDATING MONOOXYGENASE (PAM) IMMUNOREACTIVITY IN GLIA AS WELL AS NEURONS C.H. Rhodes and R.A. Angeletti, Univ. Pennsylvania and Albert Einstein Col. Med.

Anti-peptide antisera were raised against synthetic peptides corresponding to sequences near the N-terminus (NPAM), middle (MPAM), and C-terminus (CPAM) of PAM. Specificity of antiserum NPAM was demonstrated by biochemical and immunohistologic experiments. Protein purified from rat brain homogenate by elution from an NPAM-immunoaffinity column was enzymatically active and had an appropriate molecular weight on SDS-PAGE. Antiserum NPAM adsorbed by immobilized peptide produced no immunohistochemical staining. PAM is extensively processed, and pre-treatment of tissue with SDS or trypsin produced different patterns of staining, presumably because different forms of the enzyme are exposed by the treatments. In addition to staining neurons in most areas of the rat brain, NPAM produced staining of ependyma, intrafasicular glia (oligodendroglia), Bergmann glia, and subpial and subependymal fibers.

210.18

PHOSPHORYLATION OF VIMENTIN AND GFAP BY PROTEIN KINASE C AND CYCLIC AMP-DEPENDENT PROTEIN KINASE IN ASTROCYTES. B.C. Harrison and P.L. Mobley. *Univ. Texas Health Science Center, San Antonio, TX 78284*

Cultured rat astrocytes undergo changes in morphology in response to treatment with the protein kinase C (PK-C) activator phorbol 12-myristate 13-acetate (PMA). Exposure to PMA also produces changes in the phosphorylation of proteins including the cytoskeletal proteins glial fibrillary acidic protein (GFAP) and vimentin. The changes in morphology resemble that produced by treatment with 8-bromo cyclic AMP (an activator of cAMP-dependent protein kinase, PK-A), and this treatment also increases the phosphorylation of both GFAP and vimentin. Studies were then conducted to determine if PMA and 8-bromo cAMP treatments were phosphorylating similar sites on these proteins. After a 2 hour labeling period with ^{32}P-orthophosphate, cultures were exposed to 300 nM PMA, 2 mM 8-bromo cAMP or the vehicle (control) for 15 min and then subjected to two-dimension gel electrophoresis. GFAP and vimentin were cut from the gels and digested with trypsin. The peptide fragments were then subjected to 2-D tryptic mapping with electrophoresis in the first and chromatography in the second dimension. It was determined that PMA and 8-bromo cAMP treatments produced different tryptic maps for both GFAP and vimentin. Therefore, these studies suggest that PK-C and PK-A result in the phosphorylation of both GFAP and vimentin, but that the kinases differ as to their sites of phosphorylation of these proteins.

211.1

Head and Eye Movement Related Responses of Single Units in The Lateral Reticular Nucleus of Rhesus Monkey. J.O. Phillips* and A.F. Fuchs. (SPON: F.R. Robinson). Regional Primate Research Center and Depts. of Psychology and Physiology and Biophysics, University of Washington, Seattle, WA 98195.

The lateral reticular nucleus (LRN) is a major pre-cerebellar nucleus, which receives input from many regions of the CNS including the vestibular nuclei and several pre-oculomotor structures. To determine whether the LRN could transmit signals important for vestibular reflexes or voluntary eye movements, we recorded LRN unit activity in monkeys trained to fixate and track a point target while head fixed or during whole body sinusoidal rotation (.3 to 1.4Hz) about a vertical axis.

26 units have been subjected to a preliminary analysis. All showed a modulation of firing rate in response to whole body rotation. 3 units responded to smooth pursuit eye movements alone. 23 units responded to rotations during which eye movements were suppressed. Most of these showed an increase in average firing rate with peak head velocity, and were considered to be driven by vestibular stimuli. However, the signal relayed by the LRN is much less reliable and precise than that relayed by the vestibular nuclei under similar conditions.

This study was supported by National Institues of Health grants EY00745 and RR00166

211.2

THE EFFECT OF VESTIBULAR AND OPTOKINETIC STIMULATION ON FASTIGIAL NUCLEUS NEURONS IN THE ALERT MONKEY. U. Büttner, G. Markert-Schwab* and A.F. Fuchs. Dept of Neurology, Ludwig-Maximilian-University, Marchioninistr. 15, D 8000 München 70 (FRG)

The dorsal vermis of the cerebellum interacts via the fastigial nucleus with the vestibular nuclei. Both the dorsal vermis and the vestibular nuclei participate in visual-vestibular interaction. In this study the visual-vestibular interaction was investigated in the fastigial nucleus of alert monkeys (M. mulatta, fascicularis), chronically prepared for single unit and eye position (scleral search coil) recordings. Vestibular stimulation was achieved by rotating the monkey about a vertical axis in darkness at 0.2 Hz (± 40 deg/s). For optokinetic stimulation a surrounding cylinder was rotated in the horizontal plane at constant velocity (20 - 80 deg/s) for 60 s, or sinusoidally (0.2 Hz, ± 40 deg/s). The majority of neurons in the fastigial nucleus responding to vestibular stimulation were type II with an irregular firing pattern and no modulation with individual eye movements (Gardner and Fuchs, J Neurophysiol 38:627, 1975). Most of these irregular neurons also responded to constant velocity optokinetic stimulation. As in the vestibular nuclei, optokinetic and vestibular stiumuli had to be in opposite directions to elicit an activation (on-direction). Activity increased with stimulus velocity above 20 deg/s. No, or only little, modulation occurred during sinusoidal optokinetic stimulation. About 20 % of the neurons (both type I and II) had a regular firing pattern. All regular neurons responded to constant velocity (but not sinusoidal) optokinetic stimulation, with a higher sensitivity on average than the irregular neurons. The results show that 'vestibular' neurons in the fastigial nucleus respond to different modalities of stimulation. A comparison with dorsal vermis activity shows that the fastigial nucleus does not simply reflect the activity in the overlying cerebellar cortex.

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211.3

MIDLINE SECTION OF COMMISSURAL CONNECTIONS BETWEEN THE VESTIBULAR NUCLEI IN THE MEDULLA DOES NOT AFFECT THE GAIN OF THE FAST COMPONENT OF THE VOR. E. Katz, B.Cohen, H.Cohen, J.Buettner*, Depts. of Neurol. & Physiol. Mt. Sinai School of Medicine New York,10029 & Dept. of Physiol. University of Munich.

Midline section of the medulla from the caudal border of the abducens nucleus to the obex, extending from 1 to 4 mm below the surface causes a partial or complete loss of the slow component of the VOR in the cynomolgus monkey (Katz, E. et. al., Soc. Neurosci. *14*: 173, 1988). In a typical animal, the time constant of the response to a step of constant velocity rotation in darkness fell to approximately 5-7 sec, and all functions attributable to velocity storage were lost or severely attenuated. The amplitude and maximum velocity of saccadic eye movements were unaffected. No significant changes were observed during a post-operative follow-up of one year, demonstrating the importance of the commissural connections for maintenance of velocity storage.

It has been postulated that vestibular commissural connections are utilized to adapt the gain of the fast component of the VOR in response to visual-vestibular conflict or after labyrinthectomy. In this study we adapted the VOR in a monkey with a midline commissural section and a consequent loss of velocity storage. The animal wore X0.5 reducing and X2.2 magnifying lenses for 6-9 hrs on separate days following base line testing. VOR gain adaptation was achieved through head and eye movements while the partially restrained monkey fed itself. Normal monkeys are able to alter the VOR step gain by about 30% in this paradigm during the same periods (Cohen, H. et. al., Soc. Neurosci. *13*: 1226, 1987). Similar changes in gain were recorded from the commissurectomized monkey when tested with steps of angular velocity (15-180 deg/s). On average the VOR gain increased 35% after 9 hrs. with magnifying lenses, and decreased 30% after 6 hrs. with reducing lenses. The data show that commissural connections caudal to the abducens nuclei, responsible for maintenance of velocity storage, are not essential for the animal's ability to adapt the gain of the fast component of the VOR.

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211.4

SIMULATIONS OF A THREE-DIMENSIONAL VOR WITH STABLE GAZE. T. Vilis, D. Crawford*, and D. Tweed* (SPON J. Hore). Depts. of Physiology and Ophthalmology, University of Western Ontario, London, Canada, N6A 5C1.

The VOR data of the companion poster were compared to simulations of a model that incorporates the rotational kinematics of the eye (Tweed and Vilis, J. Neurophysiol. 58:832, 1987). Such a model is necessary to produce fixed slow phase axes, independent of eye position. The simulations show that the pattern observed in the slow phase eye position is a geometric consequence of rotation about a fixed axis. Actual and simulated eye position quaternions move along great circles in four-dimensional space with the vector components projecting to three-dimensional ellipses. These ellipses are tilted with respect to the axis of rotation, resulting in an eye position trajectory with a component perpendicular to the axis. The change in eye position depends on current position. For example rotations about a vertical axis produce a torsional change in eye position whose magnitude depends on vertical eye position. Such changes in eye position are required to produce the observed stable gaze direction in space.

Quick phases are targeted to a surface of desired eye positions that anticipates the action of slow phases by a mechanism in which primary position is shifted by the vestibular input.

211.5

QUICK PHASE PLANES ANTICIPATE VIOLATIONS OF LISTING'S LAW PRODUCED BY SLOW PHASES. D. Crawford*, T. Vilis,and W. Cadera*. Depts. of Physiology and Opthalmology, University of Western Ontario, London, Canada, N6A 5C1.

Three-dimensional eye positions were recorded in 4 monkeys during head rotations about various axes. Axes of eye rotation during slow phases of the VOR were independent of eye position and were either collinear with or deviated systematically from axes of head rotation. These deviations were accurately predicted by the 3 × 3 "gain" matrix computed from three orthogonal inputs at 0.5 Hz. The matrix was close to the negative identity but had a torsion to torsion component of -0.8 and small non-zero entries off the main diagonal. The gaze direction was usually stable in space. As expected, changes in eye position quaternions were mainly along the axes of eye rotation. However, when the initial eye position vector did not align with the eye rotation axis, the position vector rotated clockwise about the origin when viewed down the line of the eye velocity vector. Thus, surprisingly, even slow phase axes without torsional components produce violations of Listing's law. Quick phases not only corrected these violations, but directed the eye across the standard Listing's plane to the surface of position vectors that anticipated the violations, i.e. primary eye position rotated in the direction of head rotation. Quick phase axes are not just the reverse of slow phase axes. These results are modeled in a companion theoretical poster.

211.6

VESTIBULO-OCULAR REFLEX (VOR) DIRECTION ADAPTATION TO A PHASE SHIFTED OPTOKINETIC STIMULUS. K.D. Powell, S.A. Rude, K.J. Quinn*, B.W. Peterson, J.F. Baker. Dept. of Physiology, Norhwestern Univ., Chicago, IL 60611.

VOR gain and direction can adapt to reduce retinal slip. Can VOR *phase* also adapt to reduce retinal slip? To answer this question 5 alert cats were rotated horizontally for 2 hr. while viewing a phase shifted vertical optokinetic stimulus. In this paradigm the VOR must produce a vertical direction component which is phase shifted from the horizontal component to minimize retinal slip.

Vertical and horizontal VOR at .05 - 1.0Hz were measured with EOGs in the dark before and after adaptation. Horizontal whole body rotation at 0.25Hz was coupled to vertical rotation of a field of spots (a thousand points of light). The optokinetic sinusoid was 90° or 45° out of phase with the vestibular stimulus. The adaptive response was calculated as the difference between the pre and post adaptation vertical VOR during horizontal rotations. To determine if the horizontal VOR phase changed, the pre and post adaptation horizontal VOR were compared.

In all experiments, after 2 hr. adaptation, horizontal rotation produced a phase-shifted vertical VOR. In 90° phase shift experiments the vertical VOR to horizontal rotations adapted with an average gain of .09 and phase of 86° at .25Hz. An average vertical VOR gain and phase of .13 and -22° were measured at .25Hz after adaptation to a 45° phase lag. In no experiments did horizontal VOR phase change more than 6°. The phase shifted adaptive vertical VOR phases showed band-pass filter behavior, but gains are lower than in previous 0° phase direction adaptation, suggesting that phase shifted stimuli elicit a similar but less robust VOR adaptation. Supported by EY05289, EY06485, EY07342.

211.7

EFFECT OF LINEAR ACCELERATION ON THE VERTICAL VOR AND VISUAL SUPPRESSION. D.E.Angelaki, J.H.Anderson and B.Blakley. Depts. Otolaryngol. and Physiol., Univ. of Minn., Mpls., MN 55455.

It was shown in the cat (Blakley and Anderson, Soc. Neuro. Abst., 1986) that centripetal linear acceleration along the animal's vertical axis changes the VOR during vertical canal stimulation. The animals were positioned on or 45 cm. off the axis of rotation and ramp changes in angular velocity used. The eye velocity was approximated with a single exponential. Compared with on-axis, the response off-axis had a time constant and amplitude that were greater when the centripetal acceleration was directed ventrally and were less when the it was directed dorsally. Based on a model for the VOR with velocity storage (cf., Raphan et al., Exp. Brain Res., 35:229-248, 1979), these changes can be predicted if the feedback in the storage mechanism is modulated by the otolith input.

During the same rotations in the light and with the visual surround fixed to the animal, the dynamics of the VOR were different. A first order fit suggested that the time constants for both up and down eye movements were decreased by one to two seconds. For down eye movements, the time constant was about 2 sec or less and the centripetal acceleration changed the VOR to a greater extent in the dark compared to visual suppression. The model, with an optokinetic input and suppression, can account for this. (Supported by NS-12125 and NS-16567.)

211.8

CHANGES IN THE VESTIBULO-OCULAR REFLEX ASSOCIATED WITH SIMULATED STIMULUS CONDITIONS OF SPACEFLIGHT. L.Michaud*, D.E. Parker* and D.L. Harm* (SPON: D.J. Anderson). Neurophysiology Research Laboratory, NASA John son Space Center, Houston, TX 77058.

The Otolith Tilt-Translation Reinterpretation (OTTR) model of adaptation to weightlessness suggests that graviceptor tilt signals are reinterpreted by the brain as translation during prolonged exposure to microgravity. Exposure to a Tilt-Translation Device (TTD) that simulates the stimulus rearrangement present during spaceflight should facilitate the translation interpretation and suppress the tilt interpretation. Ten naive subjects participated in both a TTD and control (tilt in darkness) condition. Subjects were restrained on a one degree-of-freedom moving base. Linear vection was induced by a combination of Y-axis pitch tilt and X-axis linear translation of an optokinetic visual scene. Electrooculography recordings of compensatory vertical eye movements (VVOR) were obtained before, during, and immediately after each condition. Following TTD exposure subjects should exhibit diminished VVOR in response to pitch oscillation compared to VVOR before exposure. In 7 subjects, VVOR was significantly decreased after a 30 min. TTD profile, compared to no change in the control condition. Results indicate similarities between post-TTD VVOR and astronauts' postflight VVOR. Concepts for adapting astronauts to the stimulus rearrangements associated with microgravity prior to spaceflight is supported.

211.9

ADAPTIVE CHANGES IN VOR DIRECTION IN HUMANS. T. T. Khater*, B. W. Peterson, and J. F. Baker. Rehab. Institute of Chicago, and Northwestern Univ. Med. School, Chicago, IL 60611.

This study was designed to determine whether pairing vertical (vert.) image motion with horizontal (hor.) body rotation induces a plastic change in VOR direction in humans as it does in the cat, and to examine how behavioral state affects this change. Two normal subjects sat in a servo controlled rotating chair with the head held in a chair mounted restraint. Eye movements were recorded using d.c. EOG. After recording baseline hor. and vert. VOR data, the subject was adapted for two hours, by pairing ±12°, 0.25 Hz sinusoidal hor. chair rotation with ±18°, 0.25 Hz vert. optokinetic stimulation. Vert. and hor. VOR were recorded in total darkness every 15 minutes during adaptation and at the end of the two hour adaptation period.

Our subjects clearly acquired a vert. VOR component in response to 0.25 Hz hor. rotation in the dark. The gain of the acquired response was 0.14 in the first subject and 0.19 in the second, and the phases of both were ≈ 0°. The time constant of adaptation resembles the 30 minute time constant observed in the cat. Furthermore, as in the cat, deadaptation was clearly faster than adaptation, appearing to be complete in 15 min. in both subjects. There is also a correlation between VOR gain and state of consciousness. When one of the subjects tried to fixate on an imaginary object while in total darkness, her hor. and vert. VOR gains were 2.20x and 2.26x larger (respectively) than when she was not doing any mental exercises. Finally, there appears to be a component of the learned vert. response that could not be overcome by fixation, because when subjects were rotated while fixating a stationary LED, both reported that the LED appeared to move vertically in phase with the hor. chair rotation. Supported by EY05289, EY06485, and EY07342.

211.10

GRAVITOINERTIAL FORCE (G) LEVEL AFFECTS HORIZONTAL OPTOKINETIC AFTER-NYSTAGMUS P. Dizio and J.R. Lackner Ashton Graybiel Spatial Orientation Laboratory, Brandeis University, Waltham, MA 02254.

We have found that the decay time constant (T) of horizontal post-rotary nystagmus is lower in 0G and 1.8G than in 1G; T for vertical nystamus is attenuated only in 0G. Peak velocity of vestibular nystamus is unaffected by variations in G. To test whether an effect of G on velocity storage (Raphan & Cohen, 1977) is responsible for this we measured optokinetic after-nystagmus (OKAN) in 0, 1 and 1.8G in parabolic flight. Blindfolded subjects (n=4) were rotated about about a vertical axis at 60 °/s until vestibular nystagmus abated; then the eyes were alternately uncovered for 45 s to induce optokinetic nystagmus and covered (5 s after a transition to the desired G level) for 20 s to measure OKAN. There were at least three trials per subject, per G level, in each direction.

T for OKAN was 6.5, 9.9, and 8.2 s in 0, 1, and 1.8G. Only the 1G and 0G values are significantly different. G level did not affect the initial level of OKAN, the values being 19.8 , 16.7, and 18.7 °/s in 0, 1, and 1.8 G. There was no evidence of vertical eye movement during horizontal OKAN. We conclude 1)velocity storage activity in the plane of stimulation is attenuated in higher and lower than normal G levels, 2) the plane of velocity storage does not seem to change as a function G.

Supported by NASA grant NAG 9-295.

211.11

HEAD POSITION AND THE HUMAN VERTICAL VESTIBULO-OCULAR REFLEX (VOR). R.W.Baloh* and J.L.Demer.(SPON:R.C.Collins). Dept.of Neurology and Jules Stein Eye Institute, UCLA Sch.of Med.,Los Angeles,CA 90024-1769

Previous studies in cat found a decreased vertical VOR gain elicited by pitch with the animal on its side compared to pitch with the animal upright. These findings could have important implications in the pathogenesis of space motion sickness. We studied the vertical VOR and visual-vestibular interaction in 6 normal human subjects elicited by voluntary pitch in the upright and lateral positions (head-right and left). Subjects were trained to produce sinusoidal (0.4 - 2 Hz) and step pitch movements guided by a frequency modulated sound signal. Eye and head movements were recorded with a magnetic search coil.

There was no significant difference between average gain (eye vel./head vel.) of the VOR in the head upright and head lateral positions. Vertical VOR gain in any position could be more or less than 1. By contrast, gain during pitch in the light with an earth-fixed visual target was always near 1. Fixation suppression of the vertical VOR was highly correlated with the subjects' ability to perform vertical smooth pursuit. When asymmetric (in 3 subjects), upward pursuit was better than downward pursuit and the downward VOR was inhibited better than the upward VOR.

We conclude that combined otolith-canal stimulation is not sufficient for accurate vertical compensatory eye movements; vision is also required.

211.12

EFFECTS OF HEAD AND BODY POSITION ON TORSIONAL OPTOKINETIC AND VESTIBULAR EYE MOVEMENTS IN HUMANS. M.J. Morrow* and J.A. Sharpe. Division of Neurology, Playfair Neuroscience Unit, The Toronto Hospital, University of Toronto, Toronto, Ontario M5T 2S8

We studied torsional OKN and VOR in 5 normal subjects, aged 29 to 46, using magnetic search coil oculography. Patients were tested in three positions: sitting, head upright; sitting, head supine; and lying, head supine. OKN was elicited with a full-field drum rotating at constant velocity. VOR was measured to active roll plane head movements in all three positions, and to passive sinusoidal rotation in the two head-supine positions. VOR cancellation was measured during passive head movement.

OKN gains were low and OKAN was minimal. OKN and OKAN were unaffected by body or head position. Passive head-supine rotation produced higher VOR gains in lying than in sitting position. VOR gains were higher with an earth-fixed surround, and were slightly lower with a cancellation target. VOR gains during active head rotation were highest with the head upright.

Uniformly low OKN gain in each head position showed no effect of otolith or neck proprioceptive input. Higher passive VOR gains in the lying position may reflect an effect of neck proprioceptive input on the torsional VOR. Higher active VOR gains in the upright position indicate a dynamic otolith contribution. (Supported by NIH Grant EY-06040 and MRC of Canada Grant MT5404).

211.13

VESTIBULO-OCULAR REFLEX INITIATION IN INTERNUCLEAR OPHTHALMOPLEGIA. Janine L. Johnston* and James A. Sharpe (SPON: R.D.G. Blair) Playfair Neuroscience Unit and Departments of Medicine and Ophthalmology, The Toronto Hospital, University of Toronto, Toronto, Canada M5T 2S8

Horizontal vestibulo-ocular reflex (VOR) latency was recorded in 3 patients with unilateral internuclear ophthalmoplegia (INO) using a magnetic search coil technique and compared with 12 normal subjects. Patients were secured in a vestibular chair and accelerated at unpredictable timing and directions, achieving a mean head velocity of 30 deg/sec and acceleration of 320 deg/sec/sec. Trials were conducted in darkness without a target, and while fixating a stationary target. Mean VOR latency was 9 ms, and did not differ from VOR latencies for normal subjects (8 ± 5 ms). VOR gain in the first 40 ms after head motion was reduced (0.21; normal: 0.87 ± 0.13). After a latency of 71 ± 10 ms, VOR gain increased signficantly (0.67). In patients with INO, damage to direct VOR pathways resulted in low VOR gain, but did alter latency. Recovery of VOR gain after 70 ms may represent transmission through indirect, modifiable VOR pathways. This is significantly longer than the VOR latency of presumed indirect pathways in monkeys (Lisberger & Pavelko: J. Neurosci.1986) and may reflect abnormal conduction through the medial longitudinal fasciculus or neighbouring tegmental pathways. (Supported by the R.S.McLaughlin Foundation and MRC of Canada Grant MT5404)

211.14

Vestibulo-Ocular Reflex (VOR) and Adaptive Plasticity with Aging. G.D.Paige. Washington Univ, St Louis, MO 63110.

Vestibular deterioration with aging is known to occur anatomically. However, VOR changes with aging remain unclear. Further, nothing is known about potential deterioration in adaptive control which normally corrects VOR performance when visual-vestibular mismatches arise during head movements. The horizontal VOR was assessed (coil technique) in normal humans 18-89 years of age before and after 8 hr of adaptation to 2x lenses. Subjects were oscillated at 0.025-4.0 Hz, 50 o/s pk head velocity, and for 0.025 and 0.25 Hz, at 50-300 o/s pk velocity. For younger (Y, under 60) subjects, VOR gain was near 0.90 at 2.5-4.0 Hz, dropping to 0.55 as frequency decreased to 0.025 Hz. Phase showed a 3 deg lead at 4 Hz which disappeared as frequency dropped to 0.25 Hz, but returned to reach 14 deg at 0.025 Hz. The VOR in older (O, above 65) subjects was similar except for larger phase leads at 0.1-0.025 Hz. As head velocity rose from 50 to 300 o/s (0.025 and 0.25 Hz), gain in Y subjects was unchanged, but dropped in O subjects for 0.025 Hz. Phase lead rose with increasing head velocity at both frequencies, more so in Y subjects at 0.025 Hz but more so in O subjects at 0.25 Hz. After wearing 2x lenses, VOR gain rose by 45% at 0.025-0.5 Hz in the Y group, but only 30% in the O group. Gain change was less at higher frequencies; 22% for Y and only 2% for O subjects at 4 Hz. Gain change also declined with increasing head velocity. In conclusion, the VOR shows changes with aging as well as declining adaptive capabilities. (Supported by NIH grant AG06442.)

211.15

ADAPTIVE PLASTICITY OF THE SQUIRREL MONKEY VESTIBULO-OCULAR (VOR) REFLEX IN 3-D. S.L.Bello, G.Paige and S.M.Highstein, Dept. Otolaryngology., Washington Univ. Sch. Med., St. Louis, MO 63110.

Vertical, horizontal and torsional eye movements were recorded (3-D search coil tech.) in squirrel monkeys during earth horizontal head rotation in each of 3 head planes(yaw,pitch,and roll). The VOR was assessed over a broad frequency(0.025-4Hz) and amplitude(50-300deg/sec) range. Subjects were studied before and after 6Hrs. of multi freq. forced rotation while wearing 0.5x or 2x spectacles on alternate days both for head yaw and pitch. For head roll, a full field contrast disc was rotated in phase(0x) or 180 deg. out of phase(2x) relative to head. VOR gain and phase in all three planes were studied for all adaptation experiments. The pre-adapted Tor. and Vert. VORs showed greater low frequency phase leads than the Hor. VOR. The Tor. VOR showed roughly 1/2 the overall gain of the others. For each plane of head rotation, a robust coplanar change in VOR gain was observed, for both 0.5x(0x for roll) and 2x adaptation paradigms. However, the effect was less pronounced at high frequencies(>1Hz) and amplitudes (>100deg/sec).

211.16

CERVICO-OCULAR REFLEX IN HUMANS WITH NORMAL VESTIBULO-OCULAR REFLEX. S.E. Thurston[1,2], K.R. Becker*[3] and C.V. Ackley*[1]. Depts. of Neurology[1], Ophthalmology[2] and Otolaryngology-Head and Neck Surgery[3], Geisinger Medical Center, Danville, PA 17822.

Cervico-ocular reflex (COR) gain is low in normal humans but may increase to compensate for changes in the vestibulo-ocular reflex (VOR). Previous studies do not agree on the direction of normal COR eye movement re trunk movement. We measured gaze (eye in space), head, eye (gaze-head) and trunk position and velocity using magnetic search coils in 5 subjects as follows: 1) head and trunk rotated together (VOR), 2) trunk rotated with head stationary in space (COR) and 3) head rotated with trunk stationary (combined VOR+COR). Sinusoidal, horizontal rotations in the dark were at frequencies of 0.01-1.0 Hz with peak-to-peak amplitudes of 10-40 deg. VOR gain (eye velocity/head velocity) and phase were normal in all subjects. The COR was usually not measurable. COR gain (eye velocity/trunk velocity) never exceeded 0.07 and did not vary significantly with instructions to imagine a target stationary in space or moving with the trunk. Direction of COR eye movement was not consistently related to direction of trunk movement. Gains for combined VOR+ COR (eye velocity/head velocity) were higher at all frequencies than for the VOR alone and were greater than accounted for by adding the individual COR and VOR gains. These data confirm that the COR is negligible in humans with a normal VOR.

211.17

THE DIRECTION OF BALANCE INFLUENCES THE PERCEIVED UPRIGHT. Thomas A. Stoffregen, Gary E. Riccio*, and Eric J. Martin*. Dept. Psychology, University of Alabama, Tuscaloosa, AL 35487, and Dept. Kinesiology, University of Illinois, Urbana, IL 61801.

The perception of "upright" is thought to be based on detection of gravitoinertial force by the otolith organs. This kinetically defined direction can be contrasted with the kinematically defined direction of balance, the direction in which the torque acting on an inverted pendulum is zero. It can be argued that the direction of balance is more relevant to the control of orientation. In natural situations the direction of balance is determined by the direction of gravitoinertial force, but the two can be manipulated independently in the laboratory. We placed subjects in a whole body motion device that rotated around a fore-aft axis. The dynamics of the device were based on a simple inverted-pendulum model of human stance, such that the device was unstable: without control by the subject, its angular acceleration (rate of rotational falling) would be proportional to its angular position, or tilt, with respect to the direction of balance. We could vary the direction of balance with respect to the direction of gravitoinertial force. Subjects controlled the device so as to keep it "upright" across a pseudo-random disturbance. Subjects estimated their mean tilt from "upright" during each trial. These estimates were correlated with the direction of balance and with the direction of gravitoinertial force. Results showed that the perceived upright was significantly influenced by the direction of balance, and that the influence of balance was significantly greater than the influence of gravitoinertial force. These findings are at odds with classical approaches to the perception and control of spatial orientation, in which the perception of gravitoinertial force is deemed to play a fundamental role.

211.18

THE EFFECTS OF VARYING GRAVITATIONAL LOADS ON CUTANEOMUSCULAR TEST REFLEXES IN HUMAN LOWER LIMB MUSCULATURE (Q and H). *Szturm T., Jell R.M., *Kriellaars D. Dept. of Physiology, University of Manitoba.

It has been established from animal experiments that the coupling of otolith end organs and motoneurons subserving lower limb extensors/flexors involve a multi-link pathway which is shared by "Flexion Reflex Afferents". In previous human studies of the reflex effects of sural nerve stimulation during natural otolith stimulation using static tilt in pitch, a highly significant tilt-dependent modulation of the magnitude of the early component of the cutaneomuscular test reflex was demonstrated in lower limb flexors and extensors (Szturm et al Exp. Neurol. (1987) 97:529-541, (1988) 99:178-186). To seek further evidence in support of the otolith dependence of the cutaneomuscular reflex response we have examined its sensitivity to varying gravitational loads made possible by parabolic trajectory flight on the NASA operated KC-135 aircraft. Subjects, with head and body restrained, were placed in either; (1) a normal sitting position n=3, (2) rolled 90° R ear down, n=2. For each stimulus (pain threshold, delivered at 0.5 Hz) an epoch of 750 ms. of EMG was collected (150 ms. pre-stimulus and 600 ms. post-stimulus), rectified/low passed filtered (30 Hz). From the pool of EMG sweeps, those during elevated g. (1.8 g) and during microgravity period were averaged.

Mean EMG levels varied little between elevated g. and microgravity for all subjects expect one in position (1). For position (1) the magnitude of the early components of the test reflexes (calculated at peak and relative to mean EMG) varied slightly between G levels, but no significant or consistent changes were observed. This result is consistent with a utricle mediated tilt dependence of the reflex. In one subject, a late response developed in elevated g., consisting of an alternating sequence of excitation and inhibition (7-9 Hz). This late response, depending on the muscle, was not present, or was significantly reduced in microgravity. For position (2), although slight changes in the early components were observed between G levels, a larger sample size is needed to show significance. No differential effect of G level on the late component was observed. Sponsored by National Research Council of Canada

211.19

SOME VESTIBULOSPINAL NEURONS ALSO STAIN WITH ASPARTATE-LIKE IMMUNOREACTIVITY. G.A. Kevetter and A.R. Coffey*. Dept. Otolaryngology and Anatomy and Neuroscience. Univ. TX Med. Branch, Galveston, TX 77550.

In an effort to further characterize vestibulospinal pathways in the gerbil, immunocytochemistry was combined with retrograde identification of neurons. Small injections of 20% horseradish peroxidase (HRP) were made into the C5-C6 cord of anesthetized gerbils. Sections were reacted with nickel acetate-diaminobenzadine, giving a black reaction product. Sections were incubated in polyclonal antisera to aspartate (1:200 or 1:500, Chemicon) for 24 hours. They were then incubated in biotinylated anti-rabbit, followed by avidin-biotin-peroxidase complex, and finally reacted with diaminobenzidine to give a brown reaction product. Brown cells, stained with aspartate-like immunoreactivity (ASP-lir), were located in all four major vestibular nuclei. These included small and medium cells in the medial (MVN) and descending (DVN) vestibular nuclei and medium and large ASP-lir cells in the lateral (LVN) and ventromedial (VMVN) nuclei. In MVN, more cells were stained for ASP-lir caudally than rostrally. After the small injections of HRP into the cervical cord most cells were labeled in the caudal two-thirds of MVN and the adjacent DVN. Double-labeled cells (containing both the black particulate reaction product from retrogradely-transported HRP and also the diffuse brown reaction product from ASP-lir staining) were located in MVN, especially along the border with DVN. (Supported in part by BNS-NSF-84-18559).

211.20

CONTRIBUTION OF MEDIAL VESTIBULOSPINAL NEURONS (VSNs) TO SPATIAL TRANSFORMATION IN THE VESTIBULO-COLLIC REFLEX (VCR). S. I. Perlmutter, Y. Iwamoto, J. F. Baker, B. W. Peterson, Northwestern Univ. Med. School, Chicago, IL USA

We are investigating the neural substrates of spatial motor patterns of the VCR by recording VSN and neck muscle EMG activity. In 2 alert and 11 decerebrate cats (heads fixed), 2nd and higher order VSNs were identified by their responses to electrical stimulation of the labyrinth and descending MLF. The direction of rotation producing maximal activation (MAD) was determined from 0.5 Hz rotations in many vertical and horizontal planes. Connections of VSNs to neck motoneurons are being studied with spike-triggered averaging and cross-correlations.

Alert and decerebrate cat data were similar and combined (79 VSNs). Type II responses were more common in higher order than 2nd-order cells. Four VSNs exhibited complex behavior suggesting otolith input. Of 74 neurons with responses consistent with a linear sum of canal inputs, 25% had MADs aligned with the ipsilateral posterior (18), anterior (1) or horizontal (0) canal. Another 46% received convergent input from orthogonal canal(s) that shifted their MADs >10° from that of the primary ipsilateral input canal (9% vertical-vertical canal, 23% vertical-horizontal canal, 14% all 3 canals). 28% of VSNs responded as if their primary input were from contralateral canal(s). Low frequency responses of several cells suggested additional weak otolith input.

In alert cats, tonic eye position sensitivity was clear in 7/30 2nd-order VSNs. In 1 cat, 3/9 2nd- and 0/4 higher-order cells had axon collaterals identified by ascending MLF stimulation.

Significant spatial transformation of vestibular signals occurs on VSNs, even at the 2nd-order level. VSNs had more convergent input than VOR relay neurons reported last year. EY06485, EY07342

211.21

RESPONSE PROPERTIES OF VESTIBULAR NEURONS PROJECTING TO UPPER CERVICAL SPINAL CORD. S. Nonaka*, R.H. Schor, V.J. Wilson, Y. Yamagata*, B.J. Yates. Rockefeller Univ., New York, NY 10021 and Univ. Pittsburgh, Pittsburgh, PA 15213.

Different spatial tilts (e.g. roll vs pitch) evoke neck responses (vestibulocollic reflex) which have different temporal properties (Baker et al., 1985). Furthermore, the dynamics of the reflex suggest that it receives important input from irregular vestibular afferents (Bilotto et al., 1982). We examined the response properties of neurons in the lateral, medial, and descending vestibular nuclei of decerebrate cats which could be antidromically activated from mid-C1, but not from C5. The tilt direction evoking maximal modulation, and the response dynamics of these neck-projecting neurons, were examined using planar and rotating (wobble) sinusoidal tilts (0.02 to 2 Hz). The response properties of this neck population resemble those of vestibular neurons in an earlier study whose projection was not identified (Kasper et al., 1988); a larger fraction of the neck sub-population has advanced phase (> 90° at 1 Hz), suggesting a contribution from irregular afferents. Most neurons projecting to the neck exhibited the same temporal response to varying spatial stimuli. Some neurons exhibiting spatio-temporal convergence (Baker et al., 1984) were observed, but apparently too few to account for vestibulocollic reflex behavior; this behavior may be due to convergence of inputs with different spatial and temporal properties at other levels of the reflex pathway. (Supported by NIH grants NS02619, NS24930, NS08506).

211.22

OCULAR COUNTERROLLING IN PARABOLIC FLIGHT: PREDICTIVE TEST OF SPACE MOTION SICKNESS? S.G. Diamond and C.H. Markham, Dept Neurology, UCLA Sch Medicine, Los Angeles, CA 90024.

An earlier study examined 4 subjects who had symmetric ocular counterrolling (OCR) in ground-based 1G testing. During parabolas flown on a NASA KC-135 aircraft, 3 of the 4 had no eye torsion while upright in 0G or 1.8G. Tilted, they had no OCR at 0G, and more OCR at 1.8G than at 1G. None of these 3 became sick during flight.

The fourth subject had leftward eye torsion at 0G in upright and tilted positions. This bias was also seen at 1.8G, where he had less OCR than at 1G when tilted to the side inducing rightward OCR. He did become sick in flight.

These results suggested that asymmetry of the otolith system may be well compensated in the usual 1G environment on earth, but that exposure to unaccustomed gravitational states may unmask this compensation. The sudden asymmetric vestibular responses thus stimulated may be the cause of the unique motion sickness observed in space flight.

To test this hypothesis, 7 subjects with symmetric OCR in 1G underwent testing on the KC-135 for 20 parabolas to examine the correlation of asymmetric OCR in non-1G states with space motion sickness. Three subjects were former astronauts; some had been sick in space and others not. The OCR test attempted to ascertain blind which were which. Two subjects were prospective astronauts; the test attempted to predict their motion sickness in space. The remaining subjects were drawn from the NASA subject pool.

211.23

MOTION SICKNESS AND MOTOR STRATEGY. D.G.D. Watt, I. Nevo*, T. Yang* and A.V. Smith*. Aerospace Medical Research Unit, McGill University, Montreal, Canada H3G 1Y6.

Motion sickness occurs frequently in altered gravity environments such as orbital or parabolic flight. Under these conditions, coordination of eye, head and body movements is often unusual, with the eyes and head rotating with the torso when reorienting to a new target. Are these inappropriate motor strategies a cause of motion sickness?

10-17 subjects took part in each of 12 experiments over 24 weeks. Each session required a different pattern of eye, head and body coordination to be repeated for 30 minutes (e.g. sweep gaze back and forth between targets located 130 degrees to either side of straight ahead, at 0.7 Hz). A questionnaire and 5 vestibular tests were administered before and repeatedly after the rhythmical movement.

All experiments caused dizziness, postural instability and oscillopsia. Motion sickness could develop if the subject was distracted during the repetitive movement, but more often appeared when normal activity resumed. Vestibular responses were decreased, the greatest changes tending to occur in those subjects who became motion sick.

These results suggest that some (perhaps many) forms of motion sickness are associated with transiently altered vestibular function resulting from inappropriate motor strategies. The signs and symptoms may serve as a warning against these counter-productive strategies. Thus, "motion sickness" might be better labelled Dysadaptation Syndrome. (Supported by Medical Research Council of Canada)

211.24

ANALGESIC AND B-ENDORPHIN (BE) RESPONSES TO MOTION-SICKNESS IN MONOSODIUM GLUTAMATE-TREATED RATS. A.C. Scallet, S. Wilson*, R. L. Rountree*, W. Henry, Jr.*, A. Andrews*, and C.A. Walker[1], Natl. Ctr. for Toxicol. Res., Jefferson, AR 72079-9502 and [1]Univ. of Arkansas-Pine Bluff, Pine Bluff AR 71601.

Drugs, x-irradiation, and motion-sickness produce emetic responses and/or taste aversions. Area postrema (AP) lesions attenuate x-irradiation and drug-induced, but enhance motion-sickness-induced taste aversions in rats. To develop other indices of motion-sickness, we measured analgesia (55°C hotplate) before and after 30 minutes of cross-coupled acceleration as well as BE levels. We also evaluated animals with lesions of the AP (and other circumventricular organs, CVOs) produced by neonatal MSG treatment. Motion-sickness produced a brief (<30 min) increase in analgesic latency which was greater in MSG-treated than control rats (105% vs 51%, $p<0.01$). MSG-treated rats showed the expected decrease in hypothalamic BE (51%, $p<0.01$), but in correspondence to the analgesic effects, motion-sickness produced a further and larger relative drop of hypothalamic BE in MSG than control rats (52% vs 16%, $p<0.01$). These results identify analgesia as a useful endpoint for motion-sickness, suggest that BE may mediate certain motion-sickness responses, and confirm that CVO lesions enhance rather than block such responses. Supported by U.S.A. FDA and NASA Grant NAG 2-427.

212.1

SPATIAL PATTERNING OF HAIR CELLS IN THE VESTIBULAR AMPULLAE OF *Pseudemys scripta*. E.H. Peterson, L.L. DiCaprio* and A.M. Brichta. Department of Zoology, College of Arts & Sciences; Basic Science Unit, College of Osteopathic Medicine. Ohio University, Athens, OH 45701.

As part of a project aimed at understanding vestibular control of head movement in *P. scripta*, we are characterizing the spatial organization of ampullary hair cells using scanning electron microscopy of sonicated and unsonicated cristae, and differential interference contrast imaging of sectioned cristae. Complete reconstructions of hemi-cristae reveal that each bears 1800-2000 hair cells. Receptor somata and apical surfaces differ in size, shape, and density; their stereocilia vary in number, thickness, length, packing geometry, and position on the hair cell apical surface. These hair cell parameters covary to produce several spatial patterns across the surface of the crista, including (1) a central region in which receptors are large and sparsely packed, with very short stereocilia arranged in a distinctive geometric array, and (2) a peripheral ring where hair cells are small, with few, irregularly spaced stereocilia. Such hair cell spatial patterning parallels the morphological gradients exhibited by vestibular primaries in this species (Brichta and Peterson, 1989, Soc. Neurosci. Abs.), suggesting that mechanoelectric transformations occurring in the ampullary neuroepithelium may differ with spatial locus. Supported by NIH grant NS23498.

212.2

LOCATION SPECIFIC ARCHITECTURE OF PRIMARY AFFERENTS INNERVATING THE VESTIBULAR AMPULLAE OF *Pseudemys scripta*. A.M. Brichta and E.H. Peterson. Department of Zoology, College of Arts & Sciences; Basic Sciences Unit, College of Osteopathic Medicine. Ohio University, Athens, OH 45701.

To help understand vestibular control of head movements we are characterizing the functional architecture of vestibular primary afferents in a turtle, *P. scripta*. We have begun by assessing the morphology and spatial distribution of their peripheral processes using an in vitro horseradish peroxidase technique (Brichta & Peterson, 1987 Soc. Neurosci. Abs. 13:634). We traced each primary afferent to its cell soma in the vestibular ganglion, then reconstructed its peripheral terminal and characterized it in terms of morphological variables thought to have functional significance: axon diameter, soma area, number, size, and density of endings per terminal, and size and location of collecting territories.

There are three morphological classes of peripheral terminals: calyx, dimorphic, and bouton (Brichta & Peterson, 1988, Soc. Neurosci. Abs. 14:329). Calyceal terminals usually contact 3-5 hair cells and have small collecting territories (<660um²); terminals with single calyces are relatively rare. The terminals of dimorphic endings (calyces plus bouton sprays) also have small collecting areas (<1150 um²). Calyx and, probably, dimorphic endings are restricted to the central, 'flattened' portion of each hemicrista. Bouton terminals are found throughout the hemicrista, and their architecture varies with location. Bouton terminals with very large collecting areas (>2,500um²) are located toward the apex; some small (786um²) collecting areas are seen in the central region. Thus the center of each hemicrista bears all three terminal types, and central terminals tend to have the most restricted dendritic fields. At the far periphery there is an abrupt transition to bouton terminals with flattened collecting areas (<620 um²), characteristic 'spindly' architecture, and small varicosities.

This regional variation in primary architecture, taken together with parallel spatial patterns in *Pseudemys* vestibular hair cells (Peterson et. al., 1989, Soc. Neurosci. Abs.), suggests that there may be location specific differences in the transfer properties of the ampullary neuroepithelium. Supported by NIH grant 23498.

212.3

RESPONSE DYNAMICS OF BULLFROG HORIZONTAL CANAL AFFERENTS REFLECT THE LOCUS OF CRISTA INNERVATION. L. Hoffman* and V. Honrubia (SPON: M. McGinn), UCLA School of Medicine, Los Angeles, CA. 90024-1624.

The physiological characteristics of 142 afferents innervating the bullfrogs' horizontal semicircular canal cristae were studied. A subset of these neurons were labeled intraaxonally with horseradish peroxidase, and their axon diameters were measured and loci of innervation within the crista reconstructed. Morphophysiological data were evaluated in the context of the distribution within the crista of afferents of diverse axon diameters, determined in parallel experiments using extracellular labeling techniques.

The response dynamics of horizontal canal afferents were found to be continuously distributed across the range of values for the coefficient of variation of spontaneous firing (CV). Gains ranged from 0.2 to 6.7 $sp \cdot s^{-1}/^{\circ} \cdot s^{-1}$ and were positively correlated with CV for afferents with CV < 0.6. The gains of the more irregularly-firing afferents (CV≥0.6) exhibited broad variation (0.3 to 8.6 $sp \cdot s^{-1}/^{\circ} \cdot s^{-1}$), and were uncorrelated with CV. Response phases ranged between -1° and 76° (re: head velocity) and exhibited a similar association with CV as did gain. The afferents intraaxonally labeled exhibited a positive correlation between CV and axon diameter (r=0.61, n=15), indicating that the unlabeled afferents with the lowest CV values and smallest gains and phase leads corresponded to those with the smallest axon diameters. The smallest extracellularly-labeled afferents (<1 μm) were found to innervate the peripheral regions of the horizontal crista's bulbed portion. Intraaxonally-labeled neurons for which response dynamics were obtained had axon diameters that ranged between 1.2 and 8.3 μm, representative of the thickest 63% of the horizontal canal afferent population. In this sample of intraaxonally-labeled neurons, those with gains between 1.0 and 3.5 $sp \cdot s^{-1}/^{\circ} \cdot s^{-1}$ innervated the crista's bulbed end; of these, afferents with the smaller gains innervated the perimeter regions. These afferents exhibited the smallest phase leads (23° to 31°), and had axon diameters that ranged between 1.2 and 5.7 μm. Labeled afferents with gains between 4.4 and 7.1 $sp \cdot s^{-1}/^{\circ} \cdot s^{-1}$ innervated the crista's narrow end. The phase leads of these afferents were also larger, ranging between 45° and 65°, and ranged in axon diameter between 1.5 and 8.3 μm. This diversity in innervation loci of afferents with similar axon diameters was comparable to that observed in extracellularly-labeled specimens. (Support: NS 09823)

212.4

VERTICAL CANAL-RELATED VESTIBULAR NUCLEI NEURONS RESPOND TO TIME-VARYING LINEAR HORIZONTAL HEAD ACCELERATION. G. A. Bush[1] and A. A. Perachio[1,2]. Depts Otolaryngol.[1] and Physiol. & Biophysics[2], Univ. Texas Med. Branch, Galveston TX 77550.

Vertical canal-related neurons in decerebrated rats were identified by their responses to vertical angular head acceleration. They were then tested for their responses to sinusoidal linear head acceleration applied in the plane of the utricular maculae, across a frequency range of 0.2 to 1.4 Hz. The stimulus consisted of translational motion (peak acceleration of either ±0.08g or ±0.15g) along the sagittal head axis as well as vectors oriented ±90° to the midsagittal plane. Cells were also characterized according to their responses to ipsilateral electrical stimulation applied at the oval window. Most of the vertical canal-related neurons tested to date respond to linear acceleration. These cells generally show either large or modest response gain increases across frequencies while the response peaks (re: peak head acceleration) exhibit, respectively, an increasing phase lag or a modest phase lead as the stimulus frequency is increased. In cells with dynamic response characteristics, a response gain increase of 13 dB associated with a phase shift of as much as 120° occurred as the frequency was increased from 0.2 to 1.4 Hz. Both monosynaptically and polysynaptically driven neurons received convergent otolith/canal input. The responses of vertical canal-related neurons to translational linear acceleration resemble the dynamic tilt responses described previously (Schor et al. J. Neurophys., 53:1444, 1985) for lateral vestibular nuclei cells. (Supported by NASA grants NAG2-26, NTG 44-008-801; NIH grant NS24391)

212.5

GABA-LIKE IMMUNOREACTIVITY IN THE GUINEA PIG VESTIBULE:POST EMBEDDING LIGHT AND ELECTRON MICROSCOPY FINDINGS. [1]López,I.,*[2]Juiz,J.M.,*[3]Altschuler, R.A. and [1]Meza,G. Dept. Neurociencias, IFIC, UNAM[1] México, Dept. Histología, Alicante,[2]España and Kresge Institute, U. Michigan[3] Ann Arbor,USA.

Based on neurochemical, pharmacological and immunocytochemical evidence, a neurotransmitter role for GABA has been proposed at the synapse between vestibular hair cells and its afferent nerve endings. We have used postembedding immunocytochemical techniques to further explore the localization and distribution of GABA in the guinea pig vestibule.

Animals were perfused with a mixture of aldehydes. The vestibular organs were dissected, dehydrated and embedded in plastic. Semithin and ultrathin sections were incubated with GABA antiserum* and processed for postembedding immunoperoxidase and immunogold (IMG) staining, respectively.

By light microscopy (LM), immunoreactivity was found both in type I and type II hair cells and in some myelinated fibers in the subjacent connective stroma. With the E.M. as LM, GABA-like IMG reaction was located in hair cells (Type I and II), and it was also encountered in the afferent chalice contacting type I hair cell.

These results confirm the GABAergic nature of hair cells and suggest that a GABA-mediated interaction may exist between type I hair cell and its chaliceal afferent synapse.

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212.6

4-Aminopyridine in the Semicircular Canal

Anthony J Ricci,BA*Charles H Norris,PhD,Paul S Guth,PhD

4-Aminopyridine (4-AP) is believed to block voltage sensitive potassium channels at millimolar concentrations. It is a novel blocker in that it binds to closed channels and can be released via depolarization. As such it would be expected to prolong depolarization and thus increase the firing rate. However, in the isolated semicircular canal of the frog (Rana Pipiens) 4-AP has quite different effects. In millimolar concentrations, this drug rapidly inhibits over 80% of spontaneous firing, while only blocking 20% of evoked firing. Carbachol, an acetylcholine agonist known to increase spontaneous firing in the canal, is almost totally inhibited by 4-AP. In micromolar concentrations the actions differ. Spontaneous activity is enhanced slightly while evoked is still inhibited, though to a lesser degree. The action of Carbachol is still inhibited. In some instances the 4-AP action is not apparent until the bolus of Carbachol is administered. 4-AP may aid in differentiating between spontaneous and evoked activity. It also may elucidate the stimulatory role of acetylcholine. This work was supported by grants from Southern Hearing & Speech & NIH Grant NS-22051.

212.7

COEXISTENCE OF CHOLINE ACETYLTRANSFERASE AND CALCITONIN GENE-RELATED PEPTIDE IN VESTIBULAR EFFERENTS OF THE GERBIL.

Perachio, A.A.[1,2] and Kevetter, G.A.[1,3], Depts. of Otolaryngology[1], Physiology and Biophysics[2], and Anatomy and Neurosciences[3], Univ. TX Medical Branch, Galveston, TX 77550.

In previous work, we identified the location of two groups of neurons whose cell bodies were retrogradely labeled following injections of horseradish peroxidase or fluorescent microspheres in the utricular maculae of the labyrinth in the gerbil. The larger of these two groups was labeled bilaterally following a unilateral injection and was located dorsolaterally to the facial genu. A smaller group was observed ventral to the genu and medial to the root of the facial nerve. The dorsal group of neurons were found to be acetylcholinesterase positive. In the present study, we demonstrated the coexistence of choline acetyltransferase (polyclonal antisera, Chemicon) and calcitonin gene-related peptide (polyclonal antisera, a gift from Dr. Cary Cooper) within retrogradely labeled efferent cell bodies. The smaller number of more ventrally located efferent neurons were not found to be immunoreactive to either set of antisera suggesting a functional subdivision among the vestibular efferents in this species. (supported by NASA NAG 2-26 and NIH 24391)

212.8

CENTRAL PROJECTIONS OF THE INDIVIDUAL VESTIBULAR SENSORY END-ORGANS OF THE CHINCHILLA.

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The central projections of afferent fibers from individual vestibular receptors were studied using horseradish peroxidase labelling in 42 ears from 32 chinchillas. The specificity of the labelling procedure was documented in the peripheral nerves and ganglion cells. Differences in the site of projection of fibers from each end-organ within the vestibular root, tract and each vestibular nuclei were demonstrated.

The superior vestibular nucleus (SN) was more profusely innervated by the end-organs than were the other nuclei (between ~500 to ~700 fibers from each end-organ). The lateral vestibular nucleus (LN) received a similar number of fibers from all the end-organs (~500 each) except from the utriculus (~700). The medial vestibular nucleus (MN) appeared to receive the least number of fibers of all the nuclei (~300 from each end-organ). The descending vestibular nucleus (DN) received uniform innervation from all the end-organs (~500 each) except from the utriculus (~300).

Fibers to the SN and the DN arrived as two different types of bundles. Bundles to the DN consisted of 10-20 medium size fibers, while those to the SN had one large fiber surrounded by others of smaller diameters. The LN received fibers of different diameters that projected singly, not in bundles, and made axosomatic contacts with the large cells (Deiters'). Some of these fibers were branches of the vestibular tract, while others were large primary afferents that directly innervated this nucleus. Innervation of the MN consisted of thin fibers arriving with a curvilinear trajectory through the LN and DN and forming a dense netlike pattern. There were no identifiable fiber projections in the ventromedial SN nor in the dorsomedial LN and DN. The MN was innervated throughout at the level of the rostral vestibular root. There were no projections in the caudal MN.

There were more projections to the cerebellum and interstitial nucleus (IN) from the maculae than from the cristae. The major projections to the restiform body were from the sacculus. The IN was the most densely innervated of all the nuclei. The utricular fibers were rostral to those of the sacculus in the IN.

(Supported by NIH-NINCDS grant NSO9823 and the Goodhill Ear Center.)

212.9

CHANGES IN THE GLUTAMATE BINDING SITES IN THE RAT VESTIBULAR NUCLEI FOLLOWING HEMILABYRINTHECTOMY.

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Neuronal mechanisms underlying functional recovery following hemilabyrinthectomy are still poorly understood. Recently it has been recognized that vestibular nerve terminals are glutamatergic (Raymond et al., Progress in Brain Res. 76, 1988) and glutamate binding sites have been characterized in the rat vestibular nuclei (Touati et al., Exp. Brain Res., 1989). It could be hypothesized that this transmitter system is involved in the recovery of the activity of deafferented vestibular nuclei.

In the rat, we investigated the synaptic loss and the glutamatergic deficit in the vestibular nuclei one week after unilateral vestibular nerve section. The synaptic density was quantified by measuring the immunoreactive surface of the whole population of terminals using an antibody against synaptophysin (p38)[1], a synaptic vesicle-specific protein. Our results indicate a loss of 35 % of the synaptic surface in the medial vestibular nucleus. At one-week after hemilabyrinthectomy, measurements of the high affinity glutamate uptake in the deafferented vestibular nuclei show a decrease of 31 %[2].

We investigated if this synaptic and glutamatergic deficit can be correlated with changes of the postsynaptic glutamatergic binding sites analysed by quantitative autoradiography.

Preliminary results do not indicate hypersensitivity of the glutamate receptors but are consistent with the involvement of glutamate in the regulation of the neuronal properties following vestibular compensation.

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212.10

THE EFFECTS OF N-METHYL-D-ASPARTATE ANTAGONISTS ON GUINEA PIG VESTIBULAR NUCLEUS NEURONS IN VITRO FOLLOWING CHRONIC IPSILATERAL LABYRINTHECTOMY.

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In order to determine whether N-methyl-D-aspartate (NMDA) receptors contribute to neuronal activity in the medial vestibular nucleus (MVN) following compensation for ipsilateral labyrinthectomy, we recorded extracellularly from MVN neurons in vitro, in brainstem slices from compensated guinea pigs, while perfusing with NMDA antagonists. Coronal slices of the rostral medulla including the MVN were incubated in a standard artificial cerebrospinal fluid. Slices were perfused with the selective NMDA receptor antagonists ((+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]-cyclohepten-5,10,-imine maleate) (MK801) or (3-((±)-2- carboxypiperazin-4-yl)-propyl-1-phosphonic acid) (CPP) (both 10^{-8}M). Of 10 neurons tested with MK801, 6 showed a decrease in firing rate, 1 showed an increase and 3 showed no change. Of 10 neurons tested with CPP, 4 showed a decrease in firing rate, 1 showed an increase and 5 showed no change. These results show that NMDA antagonists reduce the activity of some MVN neurons in vitro, following ipsilateral labyrinthectomy, suggest-that some component of the resting activity exhibited by these neurons may be mediated by NMDA receptors.

212.11

THE EFFECTS OF NEUROPEPTIDE HORMONES ON GUINEA PIG VESTIBULAR NUCLEUS NEURONS IN VITRO FOLLOWING CHRONIC UNILATERAL LABYRINTHECTOMY.

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In order to determine whether neuropeptide hormones may influence vestibular compensation for unilateral labyrinthectomy by acting on vestibular nucleus neurons, we examined the effect of adrenocorticotropic hormone, fragment 4-10 (ACTH4-10) and alpha-melanocyte stimulating hormone (alpha-MSH) (both 10^{-8}M) on medial vestibular nucleus (MVN) neurons in vitro, in brainstem slices from guinea pigs which had compensated for unilateral labyrinthectomy. Coronal slices of the medulla containing the MVN were incubated in a standard artificial cerebrospinal fluid. MVN neurons were recorded extracellularly before and after perfusion with ACTH4-10 and alpha- MSH. Neurons responsive to ACTH4-10 and alpha-MSH were found in the MVN ipsilateral and contralateral to the labyrinthectomy. Both increases and decreases in firing rate in response to these hormones were observed in different MVN neurons. These results suggest that neuropeptide hormones may influence compensation by acting directly or indirectly on MVN neurons.

212.12

EFFECTS OF VESTIBULAR NERVE TRANSECTION ON THE TANGENTIAL NUCLEUS OF THE HATCHLING CHICK.

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A previous quantitative electron microscope (EM) study described a normal developmental process of synaptic reorganization in the hatchling chick tangential nucleus (Peusner, 1984). Briefly, vestibular spoon endings retract from the principal cell bodies, while small terminals of uncertain origin proliferate. Total somatic surface covered by terminals is conserved. To identify the factors influencing synaptic reorganization, we have transected the entire vestibular nerve in hatchlings. Vestibular compensation was rapid: subjects were able to stand by 2½ days post-op, and by 7 days the righting reflex, head tilt, locomotion and balance were near normal. Subjects were prepared for EM at 1 day, 7 days and 8 weeks post-op. At 1 day, degenerative changes in vestibular fibers and axosomatic terminals were seen. However, some spoon endings with normal synaptic contacts were present. By 7 days degenerating terminals were not observed, and many terminals were present on principal cell bodies. At 8 weeks principal cells contacted by axosomatic terminals were seen. A quantitative EM study is underway. Supported by NIH grant RO1 NS18108.

212.13

CEREBELLAR MUTATIONS IN MICE AFFECT THE SIZE OF GABAergic TERMINALS IN THE NUCLEUS DEITERS. J. Bäurle*, U. Grüsser-Cornehls* and B. G. Grover*. (SPON: ENA). Dept of Physiology, Freie Universität Berlin, Arnimallee 22, D-1000 Berlin 33 (FRG)

The main source of GABAergic input to Nucleus Deiters are cerebellar Purkinje cells. In this study GABAergic terminals in Nucleus Deiters were investigated in two extreme types of cerebellar mutation in mice: the Weaver mice completely lacking granule cells and some Purkinje cells, the PCD mice displaying a total loss of Purkinje cells.

In material immunoreacted for GABA, camera lucida drawings of labelled terminal structures were made using plan-apo-oil-phase-contrast-optics (Zeiss). Quantitative evaluations of the cross-sectional area of terminals were performed with a computerized image analyzer (Kontron Mini-Mop, Zeiss).

Distinct differences in terminal sizes were revealed between the mutants and their controls. These differences were most pronounced between the two mutant types, whereas terminal sizes of the C57BL/6J-wildtype did not deviate from those of the B6CBA-wildtype. Terminal sizes in 6-month-old PCD mice vary from 0.2 - 2 μm^2, in controls from 0.2 - 3 μm^2 and in Weavers from 0.2 - 6 μm^2. The increase and subsequent decrease in terminal sizes with age found in Weavers and their controls was not as marked in PCD mice.

The increased number of large terminal structures in Weaver mutants is probably one of the plastic changes in cascade caused by the degeneration of granule cells, while the loss of such in PCD mice supports the assumption that the source of large terminals are cerebellar Purkinje cells.

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212.14

THE INTERCONNECTIONS OF THE PARIETO-INSULAR VESTIBULAR CORTEX (PIVC) IN THE SQUIRREL MONKEY. W. O. Guldin,* S. Akbarian* and O.-J. Grüsser. (SPON: ENA). Dept. of Physiol., Freie Universität Berlin, Arnimallee 22, 1000 Berlin 33, Germany.

The interconnections of the PIVC and the vestibular 3a area have been investigated with retrograde tracer techniques in Saimiri sciureus. By recording extracellular single units responsive to vestibular stimulation, the loci of injections were determined. Thereafter small amounts of various flourescence tracers as well as horseradish peroxidase were injected iontophoretically or by pressure via a capillary.

For the thalamic afferents we could not point out a specific nucleus of origin. Our data suggest that vestibular input to the thalamus is widespread over several nuclei: the ventral posterior inferior nucleus, the medial part of the ventral posterior nucleus, as well as parts of the oral and medial pulvinar nucleus.

For the cortico-cortical interconnections most outstanding was the finding of the extremely dense afferent and efferent connections of PIVC to the vestibular area 3a. Area 3a is also connected to those parts of area 7 known to contain a small amount of units responding to semicircular canal stimulation. We could also detect input to the PIVC from the frontal eye fields, the areas 8 and 6, as well as from the cingular cortex. Noteworthy is the fact that we never found any input from the visual cortical areas V1 - V4 and only sparse input from the temporal lobe.

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MUSCLE I

213.1

IMMUNOHISTOCHEMICAL EVALUATION OF THE CONNECTIVE TISSUE SKELETON OF CHICKEN GOLGI TENDON ORGANS. A. Maier and R. Mayne. Department of Cell Biology and Anatomy, University of Alabama at Birmingham, Birmingham, AL 35294.

Tendon organs from leg and forearm muscles of White Leghorn chickens were examined with a library of monoclonal antibodies to determine the composition of their connective tissue skeleton. Their capsules were positive for constituents of basal lamina (collagen type IV, laminin, heparan sulfate proteoglycan), and for tenascin and collagen type VI. All intracapsular connective tissue bundles reacted with antibodies against collagen type I and chondroitin-4-sulfate. In addition, some also reacted with collagen types III and VI. At the tendinous end of the receptors the capsule and the collagenous bundles blended gradually with tendons. At the muscular end, terminating, in series muscle fibers that attached at receptors split to produce finger-like extensions that were separated by deep longitudinal fissures. Into these fissures extended tongues of connective tissue containing tenascin, collagen type I and fibronectin. Distally the connective tissues extended toward receptors, becoming continuous with the tenascin in the capsule and just internal to the capsule, fibronectin in the capsule, and collagen type I in the collagenous bundles. This arrangement of connective tissues suggests that muscle fibers that attach in series at the muscular ends of tendon organs exert a force during muscular contraction not only on the collagenous bundles, but also on the receptor capsule.

Supported by a grant from the Muscular Dystrophy Association.

213.2

REMODELING OF NEUROMUSCULAR JUNCTIONS IN MUSCLE OF THE C57Bl/KsJ-dbm DIABETIC MOUSE K.M. Klueber and L. Stahr* Dept. Anatomical Sciences & Neurobiology, University of Louisville, School of Medicine, Louisville, KY 40292

Diabetes adversely affects neuromuscular junctions (NMJ's). Ultrastructural evidence reveals degeneration of both the axons and myofibers resulting in denervation (Feczko and Klueber, Am.J.Anat 182:224-240, 1988). In partial denervation, axons sprout to form new synaptic contacts. The objective of this study was to determine if axonal sprouting with NMJ remodeling occurs in diabetic muscle. Extensor digitorum longus muscles (EDL) from 80 and 126 day old diabetic and control mice (n=5/group/age) were stained using a combined silver and cholinesterase stain (Slack and Pockett, Pflugers Arch 391:306-8, 1981). Examination of the whole mount diabetic EDL's revealed 2.2% of the axon terminals in 80 day and 3.0% of the axon terminals from 126 day (N= 322 and 228 NMJ's, respectively) had terminal sprouts. The sprouts either made synaptic contact with a myofiber near the original terminal or extended for a distance from the terminal without making contact. In diabetic muscles, very small NMJ's were observed which were absent in the controls. These small NMJ's may be synaptic contacts made by axonal sprouts which formed during degeneration of the parent axonal terminal. Thus, remodelling of NMJ's in diabetic muscle in response to degeneration of the axonal terminals and myofibers occurs during the progression of the disease. Funded by:USHS 1R29DK41553-01

213.3

ACETYLCHOLINESTERASE IN MUSCLES OF DIABETIC AND GALACTOSEMIC RATS. K.A. Skau. Div. Pharmacol. & Med.Chem., College of Pharmacy, University of Cincinnati, Cincinnati, OH 45267.

Experimental diabetes in animals produces abnormalities of motor nerves that might be expected to result in functional and biochemical changes in skeletal muscle. Galactosemia in rats produces similar neuronal abnormalities but does not have the complication of reduced insulin-stimulated glucose uptake. This study compared the acetylcholinesterase (AChE) molecular forms in skeletal muscle of diabetic (streptozotocin 65 mg/kg iv) and galactosemic (40% galactose diet) rats. At various times of diabetes and galactosemia the rats, with age-matched controls, were sacrificed and hemidiaphragm (HD), extensor digitorum longus (EDL) and soleus (SOL) muscles were homogenized. Solubilized AChE molecular forms were separated on sucrose density gradients and assayed with a radiometric assay. The predominantly red SOL exhibited an increase in all forms of AChE in both diabetes and galactosemia apparently independent of muscle weight differences. HD and EDL showed more variable results that might be related to changes in muscle mass. These results may represent differential regulation of AChE in different types of muscles.

(Supported by USPHS grant DK 38856.)

213.4

EXPRESSION OF DEVELOPMENTAL AND MATURE MYOSIN ISOFORMS DURING DIAPHRAGM MATURATION. LaFramboise, W.A.*, Daood, M.J.*, Guthrie, R.D.*, Ontell, M. Univ. of Pittsburgh, Sch. of Med., Pittsburgh, PA 15261.

Rat diaphragm myosin heavy chain (MHC) composition was studied at embryonic (E) days 18 and 21, postnatal days (D) 4,21,30,60, and in the adult (>115 days) utilizing electrophoretic (6% SDS-PAGE) and immunocytochemical methods (n=4). Embryonic, neonatal and slow (Type I) MHC were present on E-18 through D-4 with either technique. A small percentage (14±5%) of E-21 myofibers reacted with a polyclonal antibody which in the adult rat diaphragm reacts with MHC-IIx and IIb fibers (Bulter-Browne and Whalen, Paris). At this stage, no fibers reacted with monoclonal antibodies specific for MHC-IIa or IIb (Schiaffino, Padova). An additional MHC band was present in D-4 gels. D-4 fibers reacted with the slow, neonatal and the IIx/IIb antibodies while the reaction with MHC-emb was reduced. Fibers also reacted with the antibody against MHC-IIa by D-4 (27±7%). By D-21, the embryonic band was not present, the neonatal band was diminished, and a second adult fast band appeared in the gels. A MHC-IIb band appeared by D-60. At this stage 34±8% of the fibers reacted with a monoclonal antibody specific for MHC-IIb. The adult pattern of MHC expression was established between 60 and 115 days postnatal. Mature diaphragms contained fibers of Types I, IIa, IIb or IIx MHCs (Type I:35±9%, IIa:31±3%, IIb:4±2%, IIx:30±6%). While it has previously been established that the adult rat diaphragm contains a high percentage of IIx fibers (Schiaffino et al., in press; Bar and Pette, 1988), this study establishes that the MHC-IIx appears prior to MHC-IIb and that MHC-IIb is transiently abundant in the diaphragm.

213.5

MODULATION OF SLOW MYOSIN EXPRESSION IN A MOUSE FAST-TWITCH MUSCLE. K. Condon and W.J. Thompson. Dept. of Zoology, University of Texas, Austin, TX 78712.

Previous investigations have noted the complete or nearly complete loss of slow muscle fibers in the extensor digitorum longus muscle of C57BL/6J mice during postnatal life. The basis of this loss and role of the nervous system in it are examined using monoclonal antibodies to myosin heavy chain isoforms. At postnatal day 3 (d3) there are approximately 175 slow fibers (12% of the total number of myofibers) distributed in a mosaic pattern through the deep region of the muscle. By d18 all myofibers express adult myosin isoforms and slow fibers still account for approximately 11% of the myofibers. However, in adults (10+ weeks) slow fibers account for only 0-3% of the myofibers. Moreover, many of the remaining slow fibers in these muscles co-express a IIA myosin isform suggesting the loss of slow fibers results from their transformation to a fast fiber type. To determine the role of innervation in this selective loss, sciatic neurectomy was performed in neonates at d2. Denervation results in a steady increase in the percentage of fibers expressing slow myosin (45% at d18, 83% at 5 weeks, and 98% at 33 weeks). Moreover, the increase progressively spreads from the deep to the superficial region of the muscles. Neonatal denervation also results in progressive atrophy of the myofibers such that in adults the cross-sectional area of the muscle is 10% of innervated muscles. Denervation of adult muscles by repeated evulsion of the common peroneal nerve produces markedly different results. No changes in myosin isoform expression are observed prior to 4 weeks. By 14 weeks the distribution and frequency of slow fibers (11-17%) resembles that seen at d3, suggesting re-expression of slow myosin in the original slow fibers. Atrophy of adult muscle is markedly less than in neonates with a reduction in cross-sectional area to 45% of innervated muscles. These results suggest that the loss of slow myosin during postnatal life is probably under neural control and that the expression of myosin heavy chain isoforms under aneural conditions is affected by the developmental history of the individual muscle fibers.

213.6

UNIFORM ENERGY CAPACITY OF FIBERS WITHIN MOTOR UNITS OF SNAKE MUSCLE. R. S. Wilkinson, B. W. C. Rosser, and P. M. Nemeth. Depts. of Cell Biology & Physiology, Neurology, and Anatomy & Neurobiology, Washington Univ. Sch. Med., St. Louis, MO 63110.

The thin transversus abdominis muscle of the garter snake contains ~100 fibers comprising 7-9 motor units of 3 types. We have measured homogeneity of enzyme content among the fibers of one motor unit in order to determine whether, and with what precision, a motor neuron controls gene expression in this muscle.

Terminals of one motor axon were labeled with the activity-dependent probe sulforhodamine 101. Fibers underlying the terminals were excised, as were control fibers identified as the same type (slower twitch) by physiological criteria. Fibers were assayed for key energy-related enzymes: β-hydroxyacyl-CoA dehydrogenase (βOAC); lactate dehydrogenase (LDH); and adenylokinase (AK). Homogeneity of enzyme activities (mol/kg dry weight/hr) was characterized by the coefficient of variation (CV = 100 x S.D./mean) among fibers. For each enzyme, the CV was smaller among fibers of a motor unit than among the control fibers of the same type (βOAC, 6.1% vs 17.7%; LDH, 7.4% vs 14.8%; AK, 11.8% vs 17.5%. Indeed, enzyme variation among fibers of a motor unit was no greater than that within one fiber at various points along its length (averages: βOAC, 8.4%; LDH, 9.8%; AK, 15.1%). These results demonstrate the precise biochemical alliance among muscle cells within one motor unit. Supported by NIH grants NS24752, DK38375 and the MDA.

213.7

METABOLIC HOMOGENEITY AMONG CAT TIBIALIS POSTERIOR MUSCLE UNIT FIBERS. C.E. Blanco*, M. Fournier and G.C. Sieck (SPON: M.A. Arbib). Dept. of Biomedical Engineering, USC, Los Angeles, CA 90089.

Metabolic variability among muscle unit (MU) fibers has been reported to be either higher than (Martin *et al., Am. J. Physiol.* 225:C43, 1988) or identical to (Nemeth *et al., J. Neurosci.* 6:892) that found along the length of individual fibers. These conflicting results may reflect differences in techniques used to quantify enzyme activity (quantitative histochemistry vs. microbiochemistry). Quantitative histochemistry was used to measure succinate dehydrogenase (SDH) activity in cat tibialis posterior (TP) muscle unit (MU) fibers identified by glycogen depletion. SDH variability along the length of fibers (~ 30 fibers/animal) was measured in 20 serial sections (10 μm thick) across a distance of 1000 μm. The mean coefficient of variation (CV) for SDH activity along the length of type II fibers was 12.4±4.0%. This compared to a mean CV of 15.6±5.7% for five MU (2 FF, 1 FInt, and 2 FR units; ~ 44 fibers/unit). Within a single section the CV for all type II fibers (~ 280/animal) was 57.1±10.4%. Thus, metabolic variability within a muscle unit was similar to that observed along the length of muscle fibers. We conclude that in the cat TP, the metabolic homogeneity among MU fibers reflects the predominance of neurogenic influences. (Supported by NIH grants HL34817 and HL37680.)

213.8

METABOLIC VARIABILITY ALONG DIAPHRAGM MUSCLE FIBER LENGTH. T.S. Cheung*, M. Fournier, C.E. Blanco*, and G.C. Sieck. (Spon: J.D. Schlag). Dept. Biomedical Engineering, USC, Los Angeles, CA 90089.

The coefficient of variation (CV) of succinate dehydrogenase (SDH) activity among muscle unit fibers in the cat diaphragm (DIA) is 20.1±6.7% for slow-twitch (type I) units and 26.6±6.9% for fast-twitch (type II) units. The purpose of this study was to determine whether this metabolic variability differed from that found along the length of individual DIA fibers. Muscle cross-sections (10 μm thick) were cut in the following sequence: 1 section stained for myofibrillar ATPase (used to classify fiber as type I or II); 4 sections reacted for SDH with substrate; and 2 sections reacted for SDH without substrate (tissue blanks). Five such sets were cut at 130 μm intervals across a total length of 870 μm. SDH variability was assessed for ~45 fibers in each of 5 DIA. The CV along fiber length was 13.4±2.1% for type I and 17.5±3.2% for type II. Within a single cross-section, the CV of fiber SDH activity was 21.5±5.4% for type I and 41.8±14.1% for type II. We conclude that SDH activity is not uniformly distributed along the length of DIA fibers and that this metabolic variability accounts for most of the differences in SDH activity among muscle unit fibers. (Supported by NIH grants HL34817 and HL37680).

213.9

VARIATION OF ENZYME ACTIVITIES IN RAT MUSCLE FIBERS WITH RESPECT TO THEIR LOCATION AND SIZE. B.W.C. Rosser, B.J. Norris* and P.M. Nemeth. Depts. of Neurology, and of Anatomy and Neurobiology, Washington Univ. Sch. Med., St. Louis, MO 63110.

We examined metabolic capacities of muscle fibers with different diameters and intramuscular locations, and from functionally distinct muscles in adult rats. Fibers were typed histochemically and dissected from adjacent lyophilized cross-sections for quantitative fluorometric assays of malate dehydrogenase (MDH, oxidative), lactate dehydrogenase (LDH, glycolytic) and adenylokinase (AK, high energy phosphate). Fibers from either type 1 or 2a populations were similar in diameter and in MDH, LDH and AK activities, from the deepest regions of the soleus muscle to their most superficial location in the overlying lateral gastrocnemius (LG). However, 2b fibers from the superficial regions of the LG were on average 1.65 times larger in diameter than the 2b fibers found deeper in the muscle. The larger 2b fibers had on average a fifth the MDH activity, but 1.25 and 1.41 fold greater LDH and AK activity than the smaller 2b fibers.

Type 2a fibers have a high capacity for both oxidative and glycolytic metabolism and, therefore, were studied from several functionally distinct muscles (psoas, LG, extensor digitorum longus, biceps brachii, superficial masseter, diaphragm and extraocular) of a single rat. Mean fiber diameters varied 2.46 fold, and enzyme activities varied 1.83, 2.57 and 1.69 fold for MDH, LDH and AK, respectively. While there was a strong inverse correlation (r=-0.94) between mean fiber diameters and MDH activities, fiber size was not related to either mean LDH or AK activities among the disparate muscles.

The results reveal distinct relationships between enzyme activities and fiber size, but they are limited to certain fiber types and enzymes. Thus, we conclude that other parameters related to muscle function are also involved in determining cellular metabolic capacity. Supported by NIH grant DK38375.

213.10

IMPAIRED Na^+ PUMP CAPACITY AND UPREGULATION OF PUMP NUMBER IN SKELETAL MUSCLES FROM SPONTANEOUSLY HYPERTENSIVE RATS (SHR). JG Pickar*, RC Carlsen, SD Gray*, Dept. Human Physiol., Univ. of Calif., Davis, CA 95616

Slow twitch oxidative soleus skeletal muscles from spontaneously hypertensive rats (SHR) with well established hypertension exhibit a deficit in Na^+-K^+ pump function. We have used electrophysiological and radioligand binding techniques to characterize this deficit in 24-28 week old animals. Muscle resting membrane potentials, determined in situ, were significantly more depolarized in SHR than in normotensive (WKY) rats (83.0 ± 0.5mV vs 88.2 ± 0.4mV). SHR membrane potentials were also significantly less hyperpolarized following a 4min period of repetitive stimulation (86.5 ± 1.0mV vs 94.3 ± 0.5mV). Addition of epinephrine (10^{-6}M) to soleus muscles in vitro generated a 8.6 ± 2.2mV hyperpolarization in WKY, but only a 1.2 ± 0.5mV hyperpolarization in SHR. Addition of insulin (4U/L) produced a 5.5mV hyperpolarization within 10min in WKY, but had no effect in SHR. SHR muscles were less depolarized by ouabain (10^{-3}M) than were WKY muscles, but neither the time course of depolarization nor the extent were significantly different. Despite these indications of an apparent reduction in Na^+ pump capacity in SHR skeletal muscles, ligand-binding studies using ^{3}H-ouabain indicated that SHR soleus contained 72% more high affinity binding sites than were found in age-matched WKY muscles. Binding affinities were similar in the two strains. The presence of an increased number of binding sites coupled with an apparent reduction in pump capacity suggests that hypertension may be associated with a defect in pump structure. (Supported by NIH HL37080).

213.11

CONFIGURATION OF THIN, INTERMEDIATE AND THICK FILAMENTS AT THE EQUATOR OF CHICKEN INTRAFUSAL FIBERS. R. Zak*, A. Maier, and R. Mayne* (SPON: B.J. Davis). Department of Medicine, University of Chicago, Chicago IL 60637, and Department of Cell Biology and Anatomy, University of Alabama at Birmingham, Birmingham, AL 35294.

The configuration and distribution of thin, intermediate and thick filaments at the equator of chicken intrafusal fibers were examined with immuno- fluorescence light microscopy, using monoclonal antibodies against actin, desmin and myosin heavy chains. Actin was localized within equatorial nuclei and in the spaces external to them; however, the fluorescence was much more intense within the nuclei than in the extranuclear spaces. Desmin and myosin heavy chains were only observed in the extranuclear spaces. Although all three filaments were present at the pole and at the equator, the fluorescent pattern produced after incubation with the various antibodies varied considerably between these two regions. Fluorescence at the pole was in the form of striations, while at the equator it was confluent. Incubation with an antibody against tropomyosin produced striations at the pole and confluent staining at the extranuclear portion of the equator, but failed to give any reaction within nuclei. This suggests that the extranuclear and intranuclear staining after incubation with anti-actin is due to different isoforms. The peculiar organization of filaments at the equator would be expected to result in mechanical properties that differ substantially from those of the pole.

Supported by a grant from the Muscular Dystrophy Association.

GEOMETRIC PATTERN OF TAPERING IN FAST MUSCLE FIBERS OF CAT TIBIALIS ANTERIOR. E. Eldred, M. Ounjian,* R.R. Roy, and R.V. Edgerton. Departments of Anatomy and Cell Biology, and Kinesiology, UCLA, Los Angeles, CA 90024.

Many fibers in the cat tibialis anterior muscle as seen through glycogen depletion of a motor unit and tracing through histological sections are seen to taper over a centimeter before ending (Ounjian et al., Soc. Neurosci. Absts 13:1213, 1988). Whether the diameter or the cross-sectional area varies linearly as length could give insight as to formation of the taper and relay of contractile force to the endomysium. For 12 endings in a fast motor unit comparison was made of correlation coefficients of linear regression curves for the relationships of length along the taper to: 1) fiber cross-sectional area as measured by an image-processing system, 2) the diameter, assuming the area to be a circle, and 3) measurements of the perimeter.

Coefficients were significantly higher for area/length suggesting that: 1) the taper cannot arise from a progressive loss of myofibrillae restricted to a peripheral zone of constant depth: 2) total force relayed to endomysium per unit of length is constant; and 3) the concentration of force per unit of circumference increases curvilinearly with approach toward the fiber tip. This suggests in turn a corresponding gradation in concentration of the endomysial envelope.

213.13

QUANTITATIVE ANALYSES OF MUSCLE FIBERS IN THE PRIMATE TONGUE. R. DePaul* (Spon: J. Abbs), Department of Speech, University of Wisconsin-Whitewater & Speech and Motor Control Laboratories, University of Wisconsin-Madison, 1018 Roseman Bldg., Whitewater, WI 53190

While primate intrinsic tongue muscles are of unquestionable importance for mastication, respiration, deglutation, and in humans, speech, there have been few advances beyond 19th century gross morphology. Distribution of muscle fiber types, their relative sizes, and variations in size and fiber type for different muscle sites appears critical for understanding tongue motor control.

Whole tongues were obtained from three young adult male macaca Fascicularis monkeys, blocked, sectioned, quick frozen in liquid nitrogen-cooled isopentane, and sectioned serially to provide cross-sectional views of superior longitudinal (SL), inferior longitudinal (IL), transverse (TV), and vertical (V) intrinsic muscles. Sections were processed by ATPase and reverse ATPase methods, and muscle fibers (n = 7758) in a select set of sections from each of the four intrinsic muscles were measured using an locally-developed computer program.

Overall, muscle fibers in these intrinsic tongue muscle fibers were smaller (Mean diameter 20.8 to 37.4 u) than in limb muscle, with Type II fibers only moderately larger than Type I. Type II fibers were strongly predominant, ranging from 70.3 to 84.8 %, which is a somewhat greater proportion of Type II fibers than most limb muscles. Importantly, from a functional view, all muscles manifested not only an striking increase in percentage of Type I to Type II fibers from anterior to posterior sites, but a parallel increase in fiber size. From anterior to posterior, fiber size increased from 8.9% for the transverse muscle to 64% for superior longitudinal. These data argue that the anterior and posterior portions of these muscles are distinct in their innervation and actions, with functional implications for differential movements of the tip and back of the tongue. Research supported by NIH Grants NS-13274, HD-03352, and NS-16373, and a grant from the University of Wisconsin-Whitewater.

213.14

PRELIMINARY MORPHOLOGICAL STUDIES EXAMINING COMPARTMENTS IN TWO-JOINT HUMAN MUSCLES. M.J. DeCamp,* M.T. Chopp,* S.L. Wolf, R.L. Segal, A.W. English (SPON: C.E. Coogler). Div. Phys. Ther., Dept. Rehab. Med. & Dept. Anat. & Cell Biol., Emory Univ. Sch. Med., Atlanta, GA 30322.

To acquire anatomical evidence of compartmentalization within human muscles, innervation patterns of lateral gastrocnemius (LG), extensor carpi radialis longus (ECRL), and flexor carpi radialis (FCR) muscles were examined in conjunction with muscle fiber architecture and attention paid to the distribution of muscle nerves. Consistent patterns of innervation were found within each of the three muscles. In LG, a single nerve enters the muscle proximally and contains 3 longitudinal and 2 recurrent branches, proximally. ECRL is innervated by 2 different branches of the radial nerve, each running roughly perpendicular to the muscle fascicles. Each branch is further divided into superficial and deep compartments. FCR was also clearly partitioned by its muscle nerve. Differentiation between motor and sensory axons was not possible. Thus, these muscles may not function as homogeneous units, but as aggregations of compartments.

213.15

MUSCLE FIBER LENGTHS, PINNATION ANGLES AND DEFORMATION OF APONEUROTIC SHEETS IN THE CAT MEDIAL GASTROCNEMIUS MUSCLE DURING NORMAL MOVEMENT. A.A. Caputi*, J.A. Hoffer and I.E. Pose*. Depts. of Clinical Neurosciences and Medical Physiology, University of Calgary, Faculty of Medicine, Calgary, Alberta T2N 4N1, Canada.

The ultrasound transit-time technique has revealed major differences between length changes in the medial gastrocnemius muscle (MG) and its muscle fibers during the cat step cycle (Griffiths & Hoffer, *SN Abstr 13:* 1214; Hoffer et al., *Progr Brain Res.*, in press). These discrepancies cannot be explained by a simple model where tendon compliance is in-series with the muscle fibers. We have used the ultrasound technique to investigate how three factors, *muscle fiber length, pinnation angle* and *deformation of aponeurotic sheets*, act in concert to determine the length of the muscle belly during the different phases of the step cycle (F->E3).

Twelve cylindrical piezoelectric crystals were implanted near the ends of muscle fibers at four levels of the MG muscle in the mid-saggital plane, to monitor fiber length, fiber pinnation angle and aponeurotic sheet deformation during level, up- and downhill locomotion, at speeds from 0.2 to 1.5 m/s. Fiber pinnation angles as function of time were computed by triangulation. Muscle length, tendon force and local EMG near crystals were also recorded. The following observations emerged:

1. Features of *muscle fiber* movement varied systematically depending on *location* within the muscle. The shorter *proximal* fibers worked over a wider range (100-150% of their length in early F) than the longer *distal* fibers (90-110%).
2. *Pinnation angles* changed over a wider range for proximal fibers (20-60°) than for distal fibers (10-30°). In general, pinnation angles were *steepest* when the fibers were at their *shortest;* however, this relationship was not obligatory.
3. The deep and superficial *aponeurotic sheets* had two lengthen/shorten episodes: in the *unloaded* phases (late F, E1) and during *stance* (E2-E3). The timecourse and extent of stretch in each episode varied with distance along each aponeurotic sheet.

Quantitative analysis of the relationship among these variables is being carried out taking into account architecture, task, speed, level of activation and external load.

Funded by a Muscular Dystrophy Association of Canada grant to JAH. AAC was an AHFMR fellow. IEP was an Alberta Paraplegic Foundation fellow.

213.16

NEUROMUSCULAR STRATEGIES UNDERLYING FAST MOVEMENT IN A TURTLE, *Pseudemys scripta*. R.J. Callister and E.H. Peterson. Department of Zoology and College of Osteopathic Medicine. Ohio University, Athens, OH 45701.

The head retractor muscle RCCQ in *P. scripta* comprises three fiber types (Callister et al., '89, J. Morph. 199:266-286). Fg fibers account for over 50% of total fibers and up to 80% of the muscle cross section. Some fibers of all types terminate in tapered endings within the muscle belly and are short (20-85% of total muscle length), but most Fg fibers and some FOG and SO types run the full muscle length (approx. 6cm). We have suggested that they may be specialized for producing rapid head displacements (Callister and Peterson, '88, Soc. Neurosci. Abs. 14: 1233). Examination of sudan black stained wholemounts and single fibers treated with NBT reveals that 3/4 RCCQ bellies are innervated by multiple segmental nerves whose intramuscular territories do not overlap. On single fibers, motor axons form focal MEPs that consist of 1-5 longitudinally arranged varicose strips. Single fibers bear 2-13 MEPs at 1-13mm intervals along their entire length; inter-terminal spacing is remarkably constant (approx. 5mm). Thus single fibers receive multiterminal and multisegmental innervation. Rapid head retraction would appear to require near simultaneous depolarization of MEPS along the fiber length, but we do not know how this synchrony is effected within the spinal cord. Supported by NIH grant NS23498.

214.1

IMMOBILIZATION DECREASES THE FATIGABILITY AND INCREASES THE FORCE PRODUCED BY CAT MOTOR UNITS. G.A. Robinson, R.M. Enoka and D. G. Stuart, Departments of Physiology and Exercise & Sport Sciences, University of Arizona, Tucson, AZ 85724.

Mechanical properties from individual tibialis posterior motor units were studied after a 3-week period of hindlimb immobilization in 11 adult cats and compared to those of units from 5 non-immobilized cats. All cats were preconditioned for 3 weeks in a communal environment. Immobilization of the maximally shortened test muscle using an external metal brace reduced its wet weight by an average of 17% compared to the contralateral control. Motor units were characterized into one of four types described previously for normal tibialis posterior muscle (FF, F(int), FR or S; McDonagh et al., J. Neurophysiol., 44: 696, 1980). Measurements of peak twitch force, time to peak twitch force, rate of twitch force development, peak tetanic force and fatigability were altered significantly in at least one motor unit type after immobilization, namely: 1) time to peak twitch force was lengthened in FF and F(int) units; 2) the rate of twitch force development increased in FF units; 3) the magnitude of peak twitch force increased in FF units, with a trend for increases among F(int) and FR units; 4) the magnitude of peak tetanic force increased in FF units, but decreased in S units, and 5) fatigability in FF, FR and S units was reduced. The changes in force production may reflect changes in muscle connective tissue whereas the decreased fatigability is probably related to the type of fatiguing stimulation used in the study. Supported by USPHS grants NS 20544, NS 25077, NS 20762, RR 05675, NS 07309 and HL 07249.

214.2

THE EFFECTS OF LIMB IMMOBILIZATION ON MUSCLE STRENGTH AND ENDURANCE. N.C. Rich. PHS Dept., Miami Univ., Oxford, OH 45056

The purpose of the investigation was to examine muscle strength, endurance, girth and fatfold changes of the upper arm following 14 days of immobilization. Five males and 5 female subjects were placed in two groups: (1) control and (2) isometric exercise. Measurements included: (1) forearm flexion and extension strength, (2) forearm flexion and extension endurance, (3) girths of arm, &(4) fatfolds. Each subject wore a fiberglass cast on the non-dominant arm from the deltoid to hand for 14 days. The exercise group performed 3 sets of 10-6 second contractions for both the flexors and extensors. Non-dominant strength decreased from 23.0 to 17.0 kg (26%) for flexion and from 15.0 to 12.9 kg (14%) for extension in the control group. The exercise group values decreased 16.2% and 13.5%, respectively. Flexion and extension endurance values decreased 22.4% and 17.9% in the control group and 13.7% and 16.1% in the exersize group. There were no differences in the girth or fatfold measurements. Thus, the data indicate that the performance of isometric exercise for both agonists and antagonists may prevent deleterious effects of immobilization.

214.3

DESIGN OF A MAGNETIC MUSCLE STIMULATIOR (TETANY) FOR CLINICAL USE. R.G. BICKFORD, D.BRITTAIN, P. FORTESCUE*, L. ALEXANDER*, EEG LABS, UCSD AND VA HOSPITAL, LA JOLLA, CA 92161.

Painless tetanic muscle stimulation has significant application in clinical rehabilitation. Magnetic stimulators, although painless, presently lack adequate repetition rate, frequency, power, and ability to operate without overheating. These problems can be solved using (1) high capacitor charging voltage (2) regenerative circuitry with transfer to a subsequent cycle of unused charge (4) appropriate trigger circuit for fast switching.

A stimulator of this kind yielding 400 joule, 100 sec pulses repeatable up to 30 per sec has been tested on the arm and leg muscles normal (6 subjects). Functional tetanus capable of raising the leg against gravity was produced using a 9 cm dia, 10 turn coil placed over the appropriate muscle. It runs on a duty cycle 100% without overheating. The stimulus is painless and thus provides an important clinical advantage over the comparable electric muscle stimulator (FES method).

Supported by Orthopedic Div. Funds (Dr. Akeson) and BIK Systems.

214.4

MODIFICATION OF HUMAN MUSCLE PROPERTIES BY ELECTRICAL STIMULATION AFTER SPINAL CORD INJURY. R.B. Stein, J. Jefferson* and T. Gordon. Div. of Neurosci. and Dept. of Physiotherapy, Univ. of Alberta, Edmonton, Canada T6G 2S2.

The optimal method for training paralysed muscles for use in functional tasks remains unclear. Protocols involving from half an hour 2-3 times a week to 8 hours daily have been recommended. In this study tibialis anterior muscles of complete, spinal cord injured subjects were stimulated at 20 Hz and 50% duty cycle in successive 6 week periods for 15 min., 45 min., 2 hrs. and 6-8 hrs. daily. Fifteen min. of stimulation had little if any effect on the parameters measured: twitch and tetanic tensions, contraction and half-relaxation times, fatigue index (% of force produced after 2 or 3.5 minutes of stimulation, compared to control values) Forty-five min. of stimulation produced a substantial slowing of the muscle with increase in fatigue resistance. Longer periods of stimulation produced relatively small further changes. None of the patterns tested increased the force output of the muscles, but the increase in fatigue-resistance should be useful for tasks such as walking, produced by electrical stimulation. Supported by MRC and MDA of Canada.

214.5

ULTRASTRUCTURAL ANALYSIS OF ELECTRICALLY STIMULATED CANINE SKELETAL MUSCLE AFTER RESUMPTION OF NATIVE NEURAL IMPULSE ACTIVITY. B.C. Marts*, K.M. Klueber, J.W. Brown*, and J. Hoffpauir* (SPON:R. Gurd) Dept. Anat. Sci.& Neurobiology, U of Louisville, Sch of Med, Louisville, KY 40292 and Med Sciences Prog, Indiana U Sch of Med, Bloomington IN 47405

Do the ultrastructural and histochemical changes noted in skeletal muscle after electrical stimulation persist following cessation of stimulation? The objective of this study was to examine the cytoarchitecture of canine skeletal muscle after stimulation and following a period of normal neural impulse activity. The rectus abdominis muscles of 3 female dogs were stimulated electrically (2 Hz, 5.9V,.2 msec pulse duration; 97 days) after which neural impulse activity was resumed (155 days). Myosin ATPase activity demonstrated 44%, 100% and 98% Type I myofibers prior to, after stimulation (97 days) and normal neural activity (155 days), respectively. Following stimulation myofibers were characterized by disorganization of sarcomeres, target and ring fibers, focal degeneration, split fibers, abnormal mitochondria and myonuclear profiles which persisted after return of normal neural impulse activity. Among these fibers, evidence of regeneration (centrally placed nuclei, polyribosomes, activated satellite cells) was noted. The cytoarchitectural changes in muscle induced during stimulation persist 5 months after its cessation contrary to data reported by others. Funded by: Dept of Surgery, Indiana U. Sch. of Medicine.

214.6

EFFECT OF pH ON EMG MEDIAN FREQUENCY AND CONDUCTION VELOCITY IN THE HAMSTER DIAPHRAGM. L.Brody*, M.Pollock*†, S.H. Roy, C.J.DeLuca, and B.Celli*†, *NeuroMuscular Research Ctr, Pulmonary Ctr and Boston VA Medical Ctr†, Boston Univ., Boston, MA 02215.*

The physiological determinants of EMG fatigue parameters are uncertain. H^+ ion accumulation at the sarcolemma is believed to play a key role in determining the electro-physiological correlates of fatigue. To establish a causal relationship between muscle pH and EMG, studies must go beyond merely recording concurrent changes in these measures.

This paper describes an in-vitro method to externally manipulate muscle pH while measuring the resultant effect on surface-detected median frequency (MF) and conduction velocity (CV) parameters. Hamster muscle diaphragm strips (n=4) were isolated with the phrenic n. intact and placed in an oxygenated Krebs bath (26 °C). The muscle was clamped to a non-compliant load cell to measure isometric contractile tension. Tetanic contraction was developed via 40 Hz supermaximal stimulation of the phrenic n. Differential signals were recorded from 3 EMG detection surfaces for computation of CV (via the phase shift in the EMG signals) and MF. Repeated trials were conducted at bath pH's of 7.4, 7.0 and 6.6. Bath pH was altered by aerating pre-determined concentrations of O_2 and CO_2 into the bath.

Results indicated that the MF and CV decreased by an average of 15% and 13% respectively when the pH was changed from 7.0 to 6.6. Each increased by an average of 19% when the pH was changed from 6.6 to 7.0. No appreciable change in MF or CV occured when the bath pH was changed from 7.4 to 7.0 (or vice versa). This suggests that EMG changes are independent of extracellular pH because the muscle is effectively buffered at pH above 7.0. These results support the premise that MF and CV parameters are causally related to intracellular pH.

214.7

THE ELECTROMYOGRAMS MEDIAN FREQUENCY PREDICTS THE MOTOR UNITS RECRUITMENT STRATEGY. M. Solomonow, C. Baten*, J. Smith*, R. Baratta* & R. D'Ambrosia*. Bioengineering Laboratory, LSU Medical Center, New Orleans, LA 70112.

Since the median frequency (MF) of the EMG power density spectrum is linearly related to the average conduction velocity in the muscle (Stulen & DeLuca, IEEE-BME, 28:515-523, 1981), then the MF should increase only during the orderly recruitment phase of increasing force contraction but not during the final rate increase phase. Motor units of the isometric cat M. Gastrocnemius muscle were orderly recruited with concurrent rate increase at various control strategies with electrical nerve stimulation (Solomonow et al. IEEE-BME, 34:692-703, 1987). The MF of each M-wave picked with bipolar intramuscular electrodes was calculated and plotted vs. time. The MF increased only during the orderly recruitment phase of motor units but not during the final rate increase. Direct increase of the firing rate of all the motor units resulted in constant MF. It was concluded that the MF rise can predict the recruitment strategy of various muscles performing various functions.

214.8

CHANGES IN DIAPHRAGM CONTRACTILE PROPERTIES AFTER COMPENSATORY LOADING. W.Z. Zhan and G.C. Sieck. Dept. of Biomedical Engineering, USC, Los Angeles, CA 90089.

The influence of chronic compensatory loading (CL) on the contractile and fatigue properties of the diaphragm (DIA) was studied in adult hamsters. After paralysis of the right hemidiaphragm, EMG activity of the left side increased by 60% and inspiratory duration increased by 25%. The DIA forces generated during eupnea were 34% of maximum before paralysis and 56% after. After 2 weeks of CL, the isometric contractile and fatigue properties of the left hemidiaphragm were determined using an in vitro preparation and direct muscle stimulation. Tension measurements were normalized for muscle weight. Contraction time decreased by 13.4% and 1/2 relaxation time by 21.8% in the CL animals. Normalized maximum tetanic tensions were increased by 19.7% in the CL animals. The ratio of peak twitch tension to maximum tetanic tension decreased by 16%. No significant change in fatigue resistance was observed. These results suggest that after prolonged CL, there is an increased contribution of type II muscle fibers to the total force generating capacity of the DIA. (Supported by NIH grants HL34817 and HL37680).

214.9

EFFECT OF CHRONIC COMPENSATORY LOADING ON DIAPHRAGM MUSCLE FIBERS. G.C. Sieck and W.Z. Zhan. Dept. of Biomedical Engineering, USC, Los Angeles, CA 90089.

The influence of chronic compensatory loading (CL) on the metabolic and morphometric properties of diaphragm (DIA) muscle fibers was examined in adult hamsters. After paralysis of the right hemidiaphragm, EMG activity of the left side increased by 60% and inspiratory duration increased by 25%. Ventilatory forces generated by the DIA increased from 34% of maximum force generating capacity in controls to 56% in the CL animals. After 2 weeks of CL, the DIA was excised and rapidly frozen. Alternate serial sections were stained for ATPase (pH 9.0) and succinate dehydrogenase (SDH). Fibers were classified as type I or II based on the ATPase stain. Fiber SDH activity was quantified microphotometrically. After CL, the mean SDH activity of type I fibers increased by 30% and type II by 12%. The proportions of type I and II fibers were unchanged. However, the number of type II with higher SDH activity increased. The cross-sectional area of these higher oxidative type II fibers also increased. These results suggest that CL selectively affects the oxidative capacity of those DIA fibers that must increase their activation to sustain adequate ventilatory forces. (Supported by NIH grants HL34817 and HL37680).

214.10

MOTOR UNIT RECRUITMENT AND RATE CODING INFLUENCES ON MUSCLE TENSION AND EMG. A.J. Fuglevand*, D.A. Winter*, A.E. Patla, and D. Stashuk* (SPON:E. Cafarelli). Depts. of Kinesiology and Systems Design Engineer., University of Waterloo, Waterloo, Ont., CANADA N2L 3G1.

A motor unit (mu) pool model was employed to examine the relation between surface EMG and isometric tension. The model was comprised of three elements; a motoneuron, an isometric tension, and an EMG model. The size-principle based model predicted recruitment and firing patterns in 100 mus. EMG and tension were simulated for 11 levels of excitation from 5 to 100% maximum excitation (ME). ME was the excitation required to bring the last mu to saturation firing rate (SFR). Two recruitment ranges were tested, recruitment limit < 50% ME and recruitment limit > 70% ME. Five conditions of SFR were also tested. Linear EMG-tension relationships ($R^2 > .99$) were exhibited when recruitment operated over a large excitatory range. If the recruitment limit was less than 50% ME, the EMG-tension relation was nonlinear and nonphysiological. The selected range of SFRs had little effect on the shape of the EMG-tension relation. However, if no SFR limit was imposed, EMG increased as a fractional exponent of tension. If all mus were assigned similar SFR, and recruitment operated over a large range, a parabolic EMG-tension relation was demonstrated.

214.11

LONG TERM EFFECTS OF LOW FREQUENCY STIMULATION ON TENSION-FREQUENCY RELATIONS IN FAST-TWITCH MOTOR UNITS. R.K. Powers and M.D. Binder, Dept. of Physiol. & Biophys., Univ. of Washington, Seattle, WA 98195.

To investigate the long term effects of low frequency stimulation on tension-frequency (T-F) relations, single fast-twitch motor units from the flexor digitorum longus muscle were activated by stimulating ventral root filaments in barbiturate-anesthetized cats. T-F relations were determined by delivering trains of 25 pulses at frequencies of 10 to 80 Hz, first in ascending then in descending order. A series of 4 - 11 ten second 20 Hz trains was then delivered at 0.07 Hz, and T-F relations were determined at various times after this conditioning stimulation. T-F runs were generally followed by a 600 msec train at 200 Hz to specify the unit's maximum tension at that time. A standard fatigue test was applied at the end of the protocol in order to identify the unit as FR, FI or FF. The conditioning stimulation acted to shift the entire T-F relation along the frequency axis, and this shift could be quantified as a change in the activation frequency needed to produce 50% of the maximum tension (f50). Immediately after conditioning, f50 was reduced (-2.2±6.9 pps), but increased above control by 30 minutes (11.8±8.8 pps) and remained increased for several hours. The magnitude of these shifts was related to unit type - the initial decrease in f50 was seen primarily in FR units (-6.7±6.1 vs. 1.0±6.4 pps in FI + FF units), while the later increase was significantly greater in FI and FF units (17.1±6.6 vs. 4.4±5.5 pps in FR units). Preliminary analysis of unit EMG potentials indicates that these changes in tension production were often accompanied by long lasting decreases in muscle fiber conduction velocity.

Supported by NIH grant s NS25206 and NS26840.

214.12

EMG POWER SPECTRUM: A NON-INVASIVE MUSCULAR BIOPSY? M. Bilodeau*, A.B. Arsenault, D. Gravel*, D. Bourbonnais, F. Kemp*. School of Rehabilitation, University of Montreal and Research Centre, Montreal Rehabilitation Institute, Montreal, QC, Canada, H3S 2J4.

It has been proposed that the mean power frequency (MPF) of the EMG power spectrum increases gradually with force of contraction, this being more pronounced if the muscle investigated has a high proportion of fast twitch fibers. To validate this proposition, the Triceps Brachii (TB, 65% fast twitch fibers) was compared to the Anconeus (AN, 65% slow twitch fibers). Subjects (n=13) produced 10 isometric ramp elbow extensions from 0 to 100% of maximal voluntary contraction (MVC). EMG signals were recorded with miniature surface electrodes during these 5 sec. contractions. The MPF was obtained on 0.25 sec. windows taken at levels of 10, 20, 40, 60, 80 and 100% MVC. ANOVAs on the AN data showed that MPF increased ($p < .01$) up to 60% MVC. In contrast, the MPF for TB decreased ($p > .05$) down to 40% MVC. It thus seems that changes of the MPF with different force levels cannot be used directly to estimate the fiber type composition of two different muscles.

(Supported by the Quebec March of Dimes and FRSQ)

214.13

DISCHARGE VARIABILITY AND PHYSIOLOGICAL PROPERTIES OF HUMAN MASSETER MOTOR UNITS. M.A. Nordstrom* & T.S. Miles* (SPON: R. Tulsi). Department of Physiology, University of Adelaide, Adelaide 5000, Australia, and Department of Physiology, University of Arizona, Tucson AZ 85724.

Correlations were sought between motor unit discharge variability in the human masseter and physiological properties of the units which were determined using the spike-triggered averaging (STA) technique. Motor unit activity was recorded by an intramuscular electrode. Subjects bit on bars which measured the isometric biting force, and controlled the mean firing rate of a selected unit at 10 Hz for 15 minutes with the aid of feedback. The discharge variability was assessed from the normalized Standard Deviation (SD) for a mean interspike interval (ISI) of 100 ms. Variability measures were obtained for 37 units from 2-minute epochs at the beginning and end of the contraction. In 81% of units, ISI variability increased after 15 minutes of continuous activity. No significant correlations were found between initial ISI variability and recruitment threshold, twitch tension or TTP. However, there was a significant correlation between motor unit fatiguability and its initial discharge variability. This represents a previously unrecognized link between motoneurones and their muscle units. Supported by the NH & MRC of Australia. M. Nordstrom is a CJ Martin Fellow of the NH & MRC.

214.14

MEMBRANE POTENTIAL NOISE IN TONICALLY-CONTRACTING MOTOR NEURONS IN HUMANS. T.S. Miles*, K.S. Türker*, M.A. Nordstrom* and J.J. Seguin, Department of Physiology, The University of Adelaide, Adelaide S.A. 5000, Australia.

With the help of feedback, humans can run a single motor unit at a prescribed mean rate with only minor variations in the durations of the interspike intervals (ISIs). The relationship between the mean ISI and its Standard Deviation (SD) is linear over much of the unit's frequency. If the membrane potential of a tonically-firing motor neuron rises linearly towards the firing threshold during the ISI, with synaptic noise superimposed on this trajectory, the SD will be proportional to the ISI if the amplitude of the synaptic noise remained constant. The characteristics of the membrane noise could then be inferred from the variation in the ISIs. We determined the minimal variation of ISIs at various firing rates in masseter and limb motor neurons in order to estimate the minimal noise on their membranes. To ensure that the motor neuron was running at a constant rate, a computer program selected for analysis only the interval which followed 2 consecutive ISIs of specified duration. Our noise estimates are comparable to measurements made from intracellular recordings from CNS neurons in awake animals.

This project was supported by the Australian National Health and Medical Research Council.

214.15

TWITCHLIKE EMG-TORQUE MODEL IN HUMAN ANKLE FLEXORS. T. Sinkjær, E. Toft*, S. Andreassen*. Dept. of Medical Informatics and Image Analysis, Aalborg University, Badehusvej 23, DK-9000 Aalborg, Denmark.

The twitch of resting motor units changes with use and is a poor index for determining the contractile speed of active muscles (Dubose et al., Muscle & Nerve 10:744, 1987). In this study a twitchlike EMG-torque model of an active muscle was developed and compared to electrically elicited muscle twitches. The model was characterized by contraction time (CT), half relaxation time (HRT), maximal amplitude (AMP), and DC-gain. Eight subjects 22-30 years old were examined. The subject sat in a chair with the left foot strapped to a platform (Sinkjær et al, J. Neurophysiol., 60:1110,1988), and followed a tracking command around different percentages of Maximal Voluntary Contraction (MVC) in the ankle dorsiflexors. At the different %MVC a nonparametric model was calculated, describing the torque around the ankle joint from the rectified and filtered anterior tibialis EMG. CT and HRT: The best linear fit showed a CT of 89 ms at 0% MVC declining to 73 ms at 50% MVC. In the electrically elicited twitch CT was on average 71 ms. HRT was 115 ms at 0% MVC, declining to 95 ms at 50% MVC. HRT was on average 60 ms in the electrically elicited twitch. AMP and DC: An increase was observed from 0 to 20% MVC. Above 20% MVC both parameters declined at increasing contraction. A second order fit predicted the DC-gain to be suppressed to 0 Nm/μV at 90% MVC. The model proposes faster motor units to be recruited at increasing contraction levels, and the contractile properties in a muscle limit the maximal force developed (Toft et al., Exp. Brain Res., 74:213,1989).

POSTSYNAPTIC MECHANISMS II

215.1

CROSS-TALK BETWEEN PHOSPHOINOSITIDE- AND CYCLIC AMP -COUPLED RECEPTORS IN THE RAT HIPPOCAMPUS. M.Parenti, G.P.Ceresoli*, S.Consolo*, E.Palazzi* and A.Groppetti*. Dept.Pharmacology, Univ.Milan and "Mario Negri" Inst. Milan, Italy.

The stimulation of α-1 adrenergic receptors in rat hippocampal slices with phenylephrine (PHE) led to an enhanced phosphoinositide (PI) breakdown and an increased cAMP accumulation. This dual response could either be due to distinct subtypes of α-1 sites coupled to different second messenger-generating systems or, alternatively, a unique class of receptors linked to a common effector could generate a signal able to affect other cell receptor-coupled transduction mechanisms. Indeed cross-talk between hippocampal receptors was confirmed by the finding that diolein, a diacylglycerol (DAG) analogue that stimulates protein kinase C (PKC), enhanced cAMP formation, suggesting that PI-linked receptors, through DAG generation and activation of PKC, could positively influence adenylate cyclase (AC) activity. This is true for α-1 receptors since PHE-induced increase of cAMP was effectively antagonized by different PKC inhibitors, e.g. H-7 and staurosporine.

These observations support the view that hippocampal α-1 receptors coupled to PI breakdown "talk" to AC linked receptors. These, in turn, can communicate with the first since the elevation of cAMP by forskolin significantly decreased PHE stimulation of PI hydrolysis.

215.2

THE EFFECTS OF ETHANOL ON MEMBRANE PROPERTIES OF NEURONS OF THE VENTRAL TEGMENTAL AREA *IN VITRO*. M.S. Brodie and S.A. Shefner. Abbott Laboratories, Abbott Park, IL and University of Illinois College of Medicine, Chicago, IL.

Within the ventral tegmental area (VTA) are dopamine-containing neurons which have been studied electrophysiologically *in vitro* and *in vivo*. These neurons have been implicated in the central mechanisms which mediate the rewarding properties of drugs of abuse, including ethanol. Ethanol excites dopaminergic neurons of the VTA. We have made intracellular recordings from neurons of the VTA in order to characterize the excitatory effects of ethanol. Rat brain slices of the mesencephalon were prepared as described previously, and were maintained submerged in a superfused recording chamber. Neurons were selected which had electrophysiological characteristics similar to those previously established for dopamine-containing mesencephalic neurons recorded intracellularly *in vitro*. Concentrations of ethanol tested ranged from 80 to 160 mM. In our studies using extracellular recordings, 90% of the putative dopamine-containing VTA neurons were excited by ethanol; in our intracellular studies a larger percentage of neurons were either not affected by or were inhibited by ethanol. Those neurons which were excited by ethanol exhibited a slight depolarization (1-4 mV), with a decrease in slope resistance. Time-dependent anomalous rectification, a prominent characteristic of these neurons, was enhanced in neurons excited by ethanol. Furthermore, the action potential amplitude and the afterhyperpolarization amplitude were reduced during ethanol administration. These changes in the membrane properties of VTA neurons may be responsible for ethanol-induced excitation. Grant Support: PHS AA05846.

215.3

$GABA_A$ RECEPTORS MEDIATE MEDIAN PREOPTIC - EVOKED INHIBITION OF SUPRAOPTIC NEUROSECRETORY NEURONS IN RAT. R. Nissen and L.P. Renaud, Centre for Research in Neuroscience, Montreal General Hospital and McGill University. Montreal, Canada, H3G 1A4.

The hypothalamic supraoptic nucleus (SON) receives a projection from the median preoptic nucleus (MnPO). Extracellular recordings from the majority of SON neurons display a reduction in excitability following electrical and chemical stimulation of MnPO. In order to characterize the transmitter involved in this response, we utilized a transpharyngeal approach in urethane anesthetized male Long Evans rats to obtain further data from SON cells and the effects of locally applied inhibitory transmitter antagonists. Bicuculline methiodide (100μM) reversibly abolished the stimulus evoked inhibition of 17/18 vasopressin (VP) and 2/2 oxytocin neuron tested. Neither timolol (25μM), a β adrenergic antagonist, nor strychnine (100μM), a glycine receptor antagonist, had an effect on 5/5 VP neurons where the inhibition was abolished by bicuculline. These observations demonstrate that the MnPO projection to SON vasopressin and oxytocin neurons is predominantly inhibitory and is mediated by $GABA_A$ receptors. (Supported by MRC and FRSQ).

215.4

PHARMACOLOGY OF INHIBITORY RESPONSES RECORDED IN BASOLATERAL AND LATERAL AMYGDALOID NEURONS *IN VITRO*. H. C. Moises and M. S. Washburn (SPON: J. McReynolds). Dept. of Physiology, Univ. of Michigan , Ann Arbor, MI 48109.

Stimulation of forebrain afferents to the amygdala evokes a complex sequence of excitatory and inhibitory synaptic responses in basolateral and lateral amygdaloid neurons (Washburn and Moises, Neurosci. Abstr., this volume). In hippocampus and cortex a similar profile of inhibitory responses to synaptic stimulation occurs which involves the activation of $GABA_A$ and $GABA_B$ receptors. The purpose of this study was to determine the pharmacology of inhibitory events in the amygdala and to examine the ionic mechanisms involved.

Responses to stimulation of the external capsule or nucleus basalis were recorded intracellularly from neurons in the basolateral or lateral nuclei in horizontal and coronal slice preparations of the rat amygdala. Stimulation of either site evoked a short latency epsp followed by an early ipsp with maximal amplitude approximately 35 ms after the stimulus and a reversal potential close to -70 mV. This was typically followed by a late ipsp which peaked around 170 ms after the stimulus and reversed at approximately -90 mV. Bath application of the $GABA_A$ antagonist bicuculline methiodide (10 -40 μM) or the chloride channel blocker picrotoxin (20 μM) consistently blocked only the early ipsp. In contrast, the $GABA_B$ antagonist phaclofen (700 nM) selectively blocked the late ipsp. Pressure ejection of GABA (1-10 mM) produced inhibitory responses similar to those obtained with synaptic stimulation. This included a bicuculline- and picrotoxin-insensitive hyperpolarization which was associated with an increase in membrane conductance and which reversed around -90mV. A second hyperpolarization was observed that was also accompanied by an increase in membrane conductance, but had a reversal potential of -70 mV and was blocked by $GABA_A$ antagonists. In addition, GABA produced depolarizing responses that were not evoked by synaptic stimulation and which might have resulted from the activation of dendritic GABA receptors. Pressure ejection of the $GABA_B$ agonist baclofen (3 mM) consistently produced long-lasting hyperpolarizations which were insensitive to bicuculline, reversed near -90 mV and were associated with an increase in conductance.

These results suggest that amygdaloid neurons display inhibitory reponses to synaptically released GABA that are mediated by $GABA_A$ and $GABA_B$ receptors. These responses to GABA appear to involve an increase in chloride and potassium conductance, respectively. (Suported by NIDA grant DA-03365)

215.5

EXTRACELLULAR CYCLIC AMP (cAMP) DECREASES $GABA_A$ RECEPTOR-MEDIATED CHLORIDE CURRENT IN CULTURED RAT HIPPOCAMPAL NEURONS. N.A. Lambert‡ and N.L. Harrison. Laboratory of Neurophysiology, NINDS, NIH, Bethesda, MD 20892 and ‡Dept. of Neurobiology, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272.

We have studied the effects of cAMP and the membrane-permeant cAMP analogs 8-bromo-cAMP (8-Br-cAMP) and 8-(4-chlorophenylthio)-cAMP (CPT-cAMP) on the $GABA_A$ receptor-mediated chloride current (I_{GABA}) in E19 cultured rat hippocampal neurons. Whole-cell recordings were made using a pipette solution based on 145mM K gluconate and 5mM ATP. Outward currents were recorded in response to GABA from neurons held at -40mV in voltage-clamp. GABA was applied by pressure (1-2 p.s.i.; 20-100ms) from a micropipette containing 50μM GABA.

External perfusion with 8-Br-cAMP or CPT-cAMP caused a reversible, dose-dependent decrease in I_{GABA}; 8-bromo-cGMP was inactive. Peak I_{GABA} in the presence of 2mM 8-Br-cAMP was 52 ± 7% (mean ± S.E.M., n=5) of control; 1mM CPT-cAMP reduced I_{GABA} to 45 ± 5% (n=6) of control. However, 50 μM L858051, a water-soluble analog of the adenylate cyclase activator forskolin, did not decrease I_{GABA}, even in the presence of the phosphodiesterase inhibitor IBMX (100μM). CPT-cAMP also decreased I_{GABA} when the protein kinase A (PKA) inhibitor H-8 was present in the extracellular medium (50μM) or in the recording pipette (10μM).

External cAMP was also found to decrease I_{GABA}. The potency of cAMP was similar to the potency of the membrane-permeant analogs. Recording from cells with pipette solution containing 1mM cAMP did not cause a steady decline in I_{GABA}, while external perfusion with cAMP decreased I_{GABA} as before.

We conclude that the effects of membrane-permeant analogs of cAMP on I_{GABA} do not depend upon their ability to penetrate cells or activate PKA, and that effects of these analogs on receptors and ion channels should be interpreted with caution.

215.6

INCREASE IN THE EXTENT OF DYE COUPLING BETWEEN CA1 HIPPOCAMPAL PYRAMIDAL NEURONS BY RAISING EXTRACELLULAR BICARBONATE ION CONCENTRATION. J.Church, H. McLennan* and K.G. Baimbridge. Dept. Physiology, Univ. of British Columbia, Vancouver, B.C., Canada V6T 1W5.

Dye coupling between hippocampal neurons after intracellular injection of dyes such as Lucifer Yellow (LY) has been observed repeatedly. Reducing pH_i by raising P_{CO2} lowers its incidence suggesting that dye coupling is not artifactual. Cerebrospinal fluid pH is, however, determined by the active regulation of both CO_2 and HCO_3^-. We have therefore examined the possibility that changes in $[HCO_3^-]_o$, and thus pH_o, may modulate the extent of dye coupling between CA1 pyramidal neurons in the rat hippocampal slice.

In control conditions (26mM HCO_3^-, pH 7.4 after equilibration with 5% CO_2), 12 of 27 neurons (44%) injected with LY were dye coupled, usually to only one other neuron. In contrast, during perfusion with medium containing 72mM HCO_3^- (pH 7.9) for between 6 and 115 min, 30 of 34 neurons (88%) were dye coupled, often to two or three but occasionally to as many as eight others. This increase was reversed on reperfusion with control medium. The results suggest the possibility that raising $[HCO_3^-]_o$ increases pH_i, which in turn modulates the degree of dye coupling.

215.7

EXCITATORY AMINO ACID-STIMULATED PROTEIN PHOSPHORYLATION IN RAT HIPPOCAMPAL PYRAMIDAL NEURONS IN CULTURE. Wendy K. Scholz and H. Clive Palfrey* Dept. of Pharmacol. and Physiol. Sci. Univ. of Chicago, Chicago, IL. 60637.

Protein phosphorylation has been implicated in the transduction of glutamate stimulation leading to such phenomena as LTP and excitotoxicity. To investigate the biochemical mechanisms underlying glutamate receptor signaling, a neuronal culture system of predominantly hippocampal pyramidal neurons was used. These were prepared from 17d embryonic rat hippocampi by a modification of the procedure of Banker and Cowan (1977). Protein phosphorylation in pyramidal neurons cultured for 10-18 days was examined after labeling endogenous ATP by incubating cultures with $^{32}P_i$ and treating with glutamate or other agonists. Treatment with glutamate (100 uM), NMDA (100 uM plus 1 uM glycine in Mg2+-free media) or kainate (100 uM) resulted in phosphate incorporation into specific proteins that was 10-30% over basal (unstimulated) levels. In contrast to high K+ stimulation of pyramidal neurons, which caused a general increase in $^{32}P_i$ incorporation, glutamate stimulated incorporation into specific proteins of M_r 48, 64, 87, 135, and 200 kD, as assessed by polyacrylamide gel electrophoresis (PAGE). The 48 and 87 kD proteins were tentatively identified as GAP43/B50/neuromodulin and the brain 87 kD phosphoprotein of Wu et al. (1982). This incorporation was completely blocked by CNQX (a kainate/quisqualate antagonist) plus APV (an NMDA antagonist) added 5 min. before glutamate. Closer examination, using 2-dimensional PAGE, of the time course of phosphorylation following glutamate and NMDA treatment showed greatest incorporation into the most highly phosphorylated state of the 48 and 87 kD proteins at less than 5 sec. Incorporation into these proteins decreased at 1 and 2 min, showing greatest incorporation into the less highly phosphorylated forms. These two phosphoproteins are known to be protein kinase C (PKC) substrates. The involvement of PKC in glutamate stimulation is also supported by measurements of second messenger levels, specifically diacylglycerol (DAG) and $[Ca2+]_i$. DAG showed a 1.5 to 3-fold and $[Ca2+]_i$ an 8-fold transient increase following glutamate treatment.

215.8

CONVERGENCE OF THE EXCITATORY ACTIONS OF NEUROTRANSMITTERS IN RAT PREFRONTAL CORTEX. R.C. Araneda* and R. Andrade.(SPON: V. A. Chiappinelli). Dept. of Pharmacology, St. Louis Univ. School of Med., St. Louis, MO 63104

Many neurotransmitter receptors which inhibit adenylate cyclase couple to a common population of potassium channels in central neurons. While such convergence has implications for the mechanisms by which neurons integrate incoming receptor mediated signals, little is known about the extent to which receptors coupled to other transduction mechanisms converge upon common membrane mechanisms. To begin addressing this question we have used intracellular recording in in vitro slices from the rat prefrontal cortex to analyze the actions of serotonin (5-HT), noradrenaline and acetylcholine, three neurotransmitters coupled to the inositol phospholipids in this region.

Bath administration of 5-HT, norepinephrine and the cholinergic agonist carbachol elicited a qualitatively similar pattern of membrane effects. These included a slow depolarizations, an increase in the reactivity of the cells to depolarizing stimuli and the appearance of a slow depolarizing afterpotential following a burst of spikes. Pharmacological analysis revealed that these effects were mediated by $5\text{-}HT_2$, α-adrenergic and muscarinic cholinergic receptors, all receptor subtypes previously shown to increase the turnover of inositol phospholipids. In contrast to these results, activation of $5\text{-}HT_{1A}$ and $GABA_B$ receptors, two receptor subtypes not associated with the PI cycle, failed to elicit these membrane responses and instead resulted in a slow hyperpolarization.

These results indicate that neurotransmitter receptors which share the ability to increase the turnover of inositol phospholipids also converge upon a discrete set of membrane mechanisms to exert their actions. While these studies cannot resolve whether such convergence occurs at the level of the inositol phospholipid cascade, the similarity of electrophysiological actions of the neurotransmitter receptors examined suggest that the convergent regulation of cellular membrane properties might be a widespread phenomenon in central neurons. Supported by the PMA foundation and USPHS grant MH43985.

215.9

ELECTROPHYSIOLOGICAL EVIDENCE OF PROTEIN KINASE C (PKC) INVOLVEMENT IN EXCITATORY AMINO ACID RESPONSES. A. Baskys, N. K. Bernstein*, A. J. Mendonca*, N. W. Milgram and P. L. Carlen. Playfair Neurosci. Unit, Toronto Western Hosp., Addiction Res. Foundation, Div. of Life Sci. and Dept. of Physiology, Univ. Toronto, Toronto, Ont. M5T 2S8, Canada.

Excitatory amino acid (EAA) receptors are known to interact with the phosphoinositol second messenger system (Sladeczek F. et al., *Nature*, 317:717, 1985). To examine the role of this system in EAA-mediated responses, we perfused N-methyl-D-aspartate (NMDA) and quisqualate (Quis) at submicromolar concentrations (100-750 nM) on dentate granule neurons in hippocampal slices taken from 4-6 mos. old Fischer 344 rats. Both NMDA and Quis reduced the Ca^{2+}-dependent K^+ current (I_{ahp}) with respective IC_{50}s of 500 and 100 nM. NMDA but not Quis responses were blocked by the NMDA receptor blocker APV (50 uM). TTX did not block either response. Tetanic suprathreshold stimulation of the perforant pathway (400 Hz, eight 20 ms trains, 50-100 uA) reduced the I_{ahp}. This effect could be blocked by APV. The PKC inhibitor, isoquinolinesulphonyl - 2 - methyl - piperazine dihydrochloride (H-7, Sigma, 10 uM), abolished the NMDA- or Quis-induced suppression of the I_{ahp} suggesting that this reduction is mediated by a mechanism involving PKC. We further tested the effects of H-7 on the spatial learning behaviour of Long-Evans rats in the Morris water maze, showing that aquisition was not affected in the H-7 - treated rats. However, retention was better in animals which did not receive H-7. Supported by OMHF, MRC and NSERC.

215.10

LOCAL CIRCUIT EPSPS MEDIATED BY EXCITATORY AMINO ACIDS IN RAT NEOCORTEX. Alex M. Thomson *. Dept. Physiology Royal Free Hospital School of Medicine, Rowland Hill St., London NW3 2PF, UK (SPON: Brain Research Association)

Previous studies employing paired recordings and spike triggered averaging, in neocortical slices, indicated that excitatory postsynaptic potentials (epsps) evoked in layer II/III neurones by action potentials in layer III/IV cells were mediated by both NMDA (N-methyl-D-aspartate) and non-NMDA receptors, since only the later component was blocked by NMDA antagonists, while the entire epsp was reduced by broad spectrum excitatory amino acid (EAA) antagonists. CNQX (6-cyano-7-nitroquinoxaline-2,3-dione), which blocks non-NMDA EAA receptors and the NMDA-facilitating glycine receptor, reduced the entire epsp in the absence of added glycine, but more selectively blocked the early epsp component when glycine was applied. Responses to glutamate which are mediated predominantly by non-NMDA receptors under control conditions, were enhanced by added glycine. The enhanced response was blocked by NMDA antagonists and the naturally occurring glycine antagonist kynurenate. These results indicate that a single EAA transmitter can act at two receptor types on the post-synaptic cell and that the degree to which each receptor is activated depends on the endogenous levels of modulators such as glycine and kynurenate.

215.11

QUISQUALATE AND CARBACHOL: STIMULATION OF HIPPOCAMPAL CELL FIRING AND PHOSPHOINOSITIDE HYDROLYSIS. N.J. Pontzer and F.T. Crews. Dept. of Pharmacology, Univ. of Florida, Box J-267, Gainesville, FL 32610.

The relationship between agonist stimulated phosphoinositide (PI) hydrolysis and neuronal excitation in the CNS is not yet known. We have thus characterized quisqualate and carbachol stimulated PI hydrolysis in hippocampal slices from the rat. Lithium, which disrupts the phosphatidylinositol cycle, was used to further characterize the contribution of phosphoinositide metabolism to electrophysiological responses. Drug treatments were applied in artificial cerebral spinal fluid (ACSF) at 32°C, and firing rates from area CA1 were obtained 20-30 min after exposure when stable responses were usually obtained. For biochemical experiments, similar slices were labelled with [^{3}H]-inositol, and after washing out unincorporated label, stimulated in ACSF with agonist for 60 min at 32° C in the presence of 8 mM lithium. Both quisqualate and carbachol stimulated neuronal firing in a biphasic manner. Quisqualate had an EC_{50} of 3-4 μm for the stimulation of firing and above 10 μm responses rapidly desensitized. Carbachol stimulated firing with an EC_{50} of 1.4 μM and above 5 μM responses desensitized. Quisqualate stimulated PI hydrolysis with an EC_{50} of ≈2 μm and a maximum release of ≈10 % of total incorporated [^{3}H]-inositol. Carbachol stimulated PI hydrolysis was best fit (non-linear fit to 1 and 2 site models) by a two-site concentration-response curve with EC_{50}'s of 1.4 and 119 μm and a maximal response of 20.5%. These data demonstrated a possible relationship between PI hydrolysis and neuronal firing. Lithium (0.3-8 mM) reversed carbachol induced desensitization while having no effect on carbachol stimulated firing rate. In contrast, 2-8 mM lithium reduced quisqualate stimulated firing in a concentration-dependent manner. Our results are consistent with a model in which the PI second messenger system mediates quisqualate stimulated neuronal firing and carbachol mediated desensitization. Carbachol stimulated cell firing is caused by a non-PI mechanism, but results in PI hydrolysis, perhaps by calcium influx.

215.12

ANTIDEPRESSANTS MAY DIRECTLY AFFECT GTP BINDING SITES IN VITRO. H.Yamamoto*, M.Mikuni, A.Kagaya*, Y.Kuroda* and K.Takahashi* (SPON:T.Higuchi) Div. of Mental Disorder Res., National Institute of Neuroscience, NCNP, Kodaira, Tokyo 187, Japan.

Antidepressants(AD) in the CNS exhibit a variety of properties including amine uptake inhibition and the ability to induce β-down regulation. These observations may be insufficient to explain the therapeutic action of AD lacking the above properties. In an attempt to reveal the common profile of the AD, we studied the effects of AD on GTP binding sites of rat cortex clude membranes in vitro. In the presence of AD(final conc.300uM), in vitro GTP binding was shown to be transformed to a high Kd,high B_{max} state, but not in the presence of neuroleptic or anxiolytic agents. Ribosylation catalyzed by toxins affected selectively to the affinity of basal GTP binding with no effect on basal GTP binding capacity. A diminished increase in Kd value induced by AD was obtained following ribosylation of Gs, Gi or Go. Similarly the activity of GTPase with AD were inhibited in a dose-dependent manner. Inhibition of basal GTPase activity by AD probably reflected high Kd state of GTP binding sites sensitive to toxins. Our results indicate that AD may directly affect GTP binding sites, which can be G proteins. In view of the key role in the mood disorder and its therapeutic drugs, such direct interaction may represent an important mechanism.

215.13

SECOND MESSENGER MEDIATION OF SOMATOSTATIN (SS) ELECTROPHYSIOLOGICAL EFFECTS IN CA1 PYRAMIDAL NEURONS IN VITRO. P. Schweitzer*, S. Moore*, S. Madamba* and G.R. Siggins. Research Institute of Scripps Clinic, La Jolla, CA.

We previously found that SS augments the M-current (I_M), a voltage-dependent K^+ current blocked by muscarinic agonists, in hippocampal pyramidal neurons (HPNs) (Moore et al, Science 239:278, 1988). Several reports suggest that SS and muscarinic effects are mediated by second messengers such as G-proteins (see Mihara et al. J.Physiol. 390:335, 1987) or inositol triphosphate (Dutar and Nicoll, J. Neurosci, 8:4214, 1988). To test second messenger involvement in SS effects on I_M, we used single electrode voltage-clamp and computer averaging to measure membrane currents in rat hippocampal slices. In HPNs held at rest, SS superfusion (1 μM) often elicited a small outward current that was blocked by pertussis toxin (PTX) pretreatment in 4 of 6 cells. However, PTX did not affect the SS-induced increase in I_M at -40 mV holding potentials. The SS-induced I_M increase was blocked by the phospholipase A2 (PLA2) inhibitor quinacrine (5-8 μM; 10 of 10 cells) and by the cycloxygenase inhibitor indomethacin (10 μM; 4 of 4 cells), usually without recovery. However, neither PGE_2 nor $PGF_{2\alpha}$ altered I_M. These results suggest that the SS outward current at rest, but not SS I_M augmentation, is mediated by a PTX-sensitive G-protein. The SS-evoked I_M effect may involve some component of the PLA2 system, probably not PGE_2 or $PGF_{2\alpha}$. Supported by MH-44346, AA-06420, and AA-07456.

215.14

SOMATOSTATIN HYPERPOLARIZES NEURONS IN RAT DORSOLATERAL SEPTAL NUCLEUS BY A PERTUSSIS TOXIN-RESISTANT MECHANISM. M. J. Twery, L. A. Wong, and J. P. Gallagher. Dept. of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, TX 77550.

Somatostatin (SS) has been reported to have neuroendocrine and neurotransmitter-like actions which involve a guanine nucleotide regulatory (G-) protein sensitive to inactivation by pertussis toxin (PTX). The present study investigated whether a similar G-protein mediated the hyperpolarizing effects of SS on DLSN neurons using a submerged brain slice preparation and intracellular recording techniques. Rats (n=9) were administered PTX intracerebroventricularly (2.5 μg/5 μl) 2-5 days prior to testing. In tissue obtained from PTX treated rats, SS superfused (0.01-1 μM) or ejected from micropipettes (50-100 μM) produced membrane hyperpolarization (2-17 mV) and decreased the membrane resistance (15-40%) of DLSN neurons (n=15). Hyperpolarizing effects of SS persisted in the presence of tetrodotoxin. Current-voltage relations of the SS-induced, PTX-resistant, hyperpolarization indicated a reversal potential (-96.8 ± 4.2 mV, N=3) close to the potassium equilibrium potential. Membrane hyperpolarizations produced by SS in DLSN neurons from PTX treated tissue were similar to those recorded in tissue obtained from untreated rats. Potassium-mediated, hyperpolarizing responses to the selective $GABA_B$ agonist, baclofen, however, were selectively blocked by the PTX treatment used in the present study. Thus, direct hyperpolarizing effects of SS on postsynaptic DLSN neurons do not appear to involve a G-protein sensitive to inactivation by PTX. A PTX sensitive receptor transduction mechanism(s) however does mediate the actions of baclofen in these cells. (Research supported by USPHS MH-39162 to J.P.G.).

215.15

NICOTINIC RECEPTOR-MEDIATED INHIBITION OF RAT DORSOLATERAL SEPTAL NUCLEUS (DLSN) NEURONS IS CALCIUM-DEPENDENT. Linda A. Wong and Joel P. Gallagher. Dept. of Pharmacology and Toxicology, University of Texas Medical Branch at Galveston, Texas 77550.

Our previous studies using in vitro intracellular recording techniques revealed that nicotinic agonists such as nicotine, dimethylphenylpiperazinium (DMPP) and acetylcholine (in the presence of atropine) hyperpolarized postsynaptic DLSN neurons through an increase in a potassium conductance. To further characterize the K^+ conductance underlying this nicotinic-induced inhibition, brain slices were treated with media containing one of various classes of K^+ channel blockers, viz., Cs^+, 4-AP, Ba^{2+}, TEA, and apamin. Our preliminary data indicate that the agonist-induced response can be blocked by Ba^{2+} (2mM), TEA (20mM) and apamin (10μM). Since all three agents are known to block Ca-dependent K^+ channels in other neuronal preparations, it is likely that nicotinic agonists activate a Ca^{2+}-dependent K^+ conductance in DLSN neurons. Since this response persisted in media containing no added calcium and 6mM Mg^{2+}, it is possible that an intracellular calcium source is implicated. The ability to block the nicotinic receptor-mediated hyperpolarization via intracellular injection of EGTA or superfusion of divalent calcium channel antagonists, Ni^{2+}(400μM) or Cd^{2+}(200μM), lend further evidence implicating the role of calcium in the agonist-induced response. In summary, our present findings suggest that nicotinic agonists mobilize intracellular calcium which in turn activates a potassium channel. (Research supported by the National Tobacco Council, Inc.)

215.16

THE MUSCARINIC DEPOLARIZATION PRESENT IN RAT CORTEX IS POTENTIATED BY CELL STIMULATION. R.Andrade and R.C. Araneda. Dept of Pharmacology, St. Louis Univ. School of Med., St. Louis, MO 63104.

Functional studies in vivo have shown that cholinergic stimulation results in a marked facilitation of excitatory stimuli in mammalian cortex. In an attempt to better understand the cellular mechanisms which underlie this facilitation we have examined the cellular effects of cholinergic agonists in rat prefrontal cortex using intracellular recording techniques in in vitro rat brain slices.

Bath administration of carbachol (.3μM-30μM) was found to elicit a modest dose dependent depolarization which generally failed to directly excite cells in this region. Surprisingly however, when administration of this agonist was paired to low frequency stimulation, either brief depolarizing current pulses or evoked synaptic potentials, the ability of carbachol to depolarize these cells was found to be greatly potentiated. We have used current and single electrode voltage clamp to determine the membrane mechanisms underlying this potentiation. In these cells, in addition to its well establish effects on calcium and voltage activated potassium currents, carbachol induced the appearance of a slow calcium-dependent inward aftercurrent following a burst of spikes. This aftercurrent exhibited a slow decay lasting several seconds which allowed for its cumulative activation by low frequency stimulation. This resulted in an activity-dependent depolarization which could add to that directly elicited by carbachol. Low frequency stimulation failed to potentiate depolarizations elicited by current injection or bath applied glutamate suggesting that this potentiation is not simply an effect secondary to the carbachol induced depolarization. Since both atropine (1 μM) and pirenzepine (100 nM) both reduced or blocked the inward aftercurrent, this effect appears to be mediated by muscarinic cholinergic receptors of the M1 subtype.

These results suggest a novel mechanism by which the association of brief excitatory stimuli to cholinergic stimulation can lead to increased cell excitability. Supported by the PMA Foundation and USPHS grant MH43985.

215.17

M-CURRENTS IN PATCH-CLAMPED FROG PARASYMPATHETIC GANGLION NEURONES: EFFECTS OF MUSCARINE AND ADRENALINE. A.A. Selyanko*, J.A. Zidichouski and P.A. Smith (SPON: D.P.J. Boisvert). Bogomoletz Institute of Physiology, Kiev, U.S.S.R. and Dept. of Pharmacol., University of Alberta, Edmonton, Canada.

Whole-cell patch-clamp recording was used to study neurones which were enzymatically dissociated from Rana pipiens intra-atrial parasympathetic ganglia. Although muscarine produces inward current in sympathetic ganglion B-cells by depressing a slow non-inactivating K current (I(M)), this current is usually considered to be absent from parasympathetic ganglion neurones. Contrary to this, we found that under the conditions of our experiments, a Ba sensitive I(M) could be detected in frog cardiac ganglion neurones. This current had similar kinetics, magnitude and voltage-dependence to the I(M) recorded in sympathetic C-neurones and was clearly unaffected by agonists when ATP or cyclic AMP was included in the patch-pipette. Small inward currents were produced by 10μM muscarine or 10-100μM adrenaline in the absence of intracellular nucleotides. With intracellular cyclic AMP, both agonists produced (outward) K currents. Unlike the currents produced in sympathetic C-neurones, these currents failed to exhibit inward rectification. These results demonstrate the possible importance of phosphorylation and/or dephosphorylation in the production of agonist responses. Supported by MRC & AHFMR (Canada).

215.18

M-CURRENT POTENTIATION AND EFFECTS OF ADRENOCEPTOR AGONISTS ON FROG SYMPATHETIC NEURONES. P.A. Smith, H. Chen*, J.A. Zidichouski and A.A. Selyanko*. Dept. of Pharmacology, University of Alberta, Edmonton, Canada & Bogomoletz Institute of Physiology, Kiev, U.S.S.R.

When B-cells from Rana pipiens paravertebral sympathetic ganglia are studied with the whole-cell patch-clamp technique, muscarine always produces inward current as a result of M-current (I(M)) suppression (Selyanko et al., Soc. Neurosci. Abs. 14, 280, 1988). In about 40% of the B-cells, adrenaline produced inward current by suppressing I(M) whereas in about 9% of the cells an outward current occurred as a result of I(M) potentiation. Unpublished data using extracellular recording and precedents from other literature led us to suspect that I(M) inhibition may result from alpha-1-adrenoceptor stimulation whereas the potentiation might be mediated via a beta-adrenoceptor. Isoprenaline (10-25μM) produced a small outward current in 9/26 B-cells which appeard to involve I(M) potentiation yet produced inward current in 7 other cells. Phenylephrine (10-25μM) produced inward current in 6/11 B-cells and outward current in 1 cell. Although the predominant effect of adrenoceptor agonists seems to be I(M) inhibition, the results show that I(M) can occasionally be potentiated in neurones as it can in smooth muscle cells (Sims et al., Science, 239:190, 1988). However, there is only weak correlation between adrenoceptor subtype and type of response observed. Supported by MRC and AHFMR.

215.19

EFFECTS OF NPY ON B- AND C-CELLS IN FROG SYMPATHETIC GANGLIA. J.A. Zidichouski, H. Chen* and P.A. Smith. Dept. of Pharmacology, Univ. of Alberta, Edmonton, Alberta, Canada.

The whole-cell patch-clamp technique was used to study neurons isolated from Rana pipiens sympathetic ganglia. These ganglia contain two cell types which are identified on the basis of their response to muscarine; B-cells display an inward current (due to I(M) suppression) and C-cells exhibit an inwardly rectifying K^+ current (Selyanko et al., Soc. Neurosci. Abs. 14, 946, 1988). NPY immunoreactivity has been reported in C-cells but not in B-cells (Horn et al, J. Neurosc. 1987, 7, 1717). NPY has been shown to reduce Ca^{++} currents in C cells but not in B-cells (Schofield & Ikeda. Eur. J Phamacol. 1988, 151, 131). We futher examined the effects of NPY on both cell types to test whether it has other actions which are cell selective. In C-cells, 300nM NPY induced an outward current which inwardly rectified, reversed at E(K) and appeared similar to that induced by muscarine and adrenaline. However, in B-cells we observed two distinct effects on I(M). In 5 of 8 B cells a decrease in I(M) was observed. In the remaining 3 cells a small increase in I(M) was observed. Whilst the effect of NPY on C-cells may relate to the presence of autoreceptors, the significance of its effects on B-cells remains to be evaluated. I(M) is known to be modulated by a variety of peptides so that the effects of NPY may reflect a lack of absolute receptor specificity. Supported by MRC and AHFMR. We thank W.F. Colmers for the gift of NPY.

215.20

INTRACELLULAR DEMONSTRATION OF A NOVEL SLOW EPSP IN THE SYMPATHETIC B NEURONS OF THE FROG. Parviz Yavari* (SPON: T. E. Frumkes). Dept. Physiol. Cell Biophys., CPS of Columbia U., New York, NY 10032.

Last year at this meeting I presented evidence for the occurrence of a non-cholinergic slow EPSP in the bullfrog 9th and 10th paravertebral sympathetic ganglia recorded by the sucrose-gap technique. Here I describe intracellular experiments on the B neurons of the frog sympathetic ganglia demonstrating a cellular homolog for this EPSP. Recordings were made in vitro from 113 B cells in ganglia 9/10 of the frog (38 bullfrogs, 7 Rana pipiens). Tetanic stimulation of the sympathetic chain just below ganglion 6 (0.2-2V, 0.5 ms, 5-100 Hz for 1-2 sec) elicited a slow EPSP in 74 of 113 cells. In 55 cells the cholinergic nature of the slow EPSP was tested by determining the effect of high doses of atropine (10-100 μM) and d-tubocurarine (100 μM). In only 34 of these cells was a total blockade of the slow EPSP achieved. In the remaining 21 cells, the slow EPSP remained partially resistant to these antagonists indicting the non-cholinergic nature of these responses (amplitude 4.6 ± 0.7 mV; duration 3 ± 0.3 min, M ± SEM, n=21). The majority of these EPSPs were also resistant to pressure application of an LHRH antagonist (Jan & Jan, J. Physiol. 327:219, 1982). The novel slow EPSP could be further subdivided into (i) a very long latency response (latencies of 10-30 sec; M± SEM = 18 ± 2 sec, n= 8) with a slow rise to peak, and (ii) a shorter latency response (3 -6 sec) with a faster rise to peak. The novel EPSP(s) described here may reflect activation of preganglionic sympathetic B fibers containing CGRP (Horn & Stofer, Neurosci. Abs, 13:297, 1987) or sensory fibers containing SP/CGRP (Stofer & Horn, Neurosci. Abs. 14:355, 1988), both systems having been shown to make synaptic contact with B cells in frog ganglia.

216.1

EPSPs EVOKED BY VENTRAL ROOT AFFERENTS IN RAT MOTONEURONS: INVOLVEMENT OF NMDA AND NON-NMDA RECEPTORS. E. Shen*, M. Y. Wang* and N. J. Dun (SPON: D.D.Christ). Dept. of Pharmacol. Loyola Univ. Med. Ctr., Maywood, Il 60153

Intracellular recordings were made from antidromically identified motoneurons (MNs) in transverse spinal cord slices (500 μm) of neonatal (< 20 days) rats. Stimulation of ventral roots evoked an excitatory postsynaptic potential (EPSP) in over 30% of MNs tested. As the EPSPs were not abolished by dihydro-ß-erythrodine (1 μM) or d-tubocurarine (10 μM), the responses were not due to the activation of MNs by recurrent axon collaterals. Further, the EPSPs persisted in MNs when the dorsal half of the slice was transected from the ventral half. The EPSPs consisted of two components; an early EPSP of short latency (1.4 ± 0.4 ms) and/or a late EPSP whose latency (3 to 10 ms) showed some variability. The early and late EPSPs were reversibly antagonized by kynurenic acid. Further studies showed that the early EPSPs were abolished by the non-NMDA antagonist DNQX (0.5 -1 μM) and not affected by Mg-free solution. The late EPSPs were depressed by NMDA antagonists ketamine and APV (1-10 μM) and increased in Mg-free media. The results indicate that ventral root afferents make di-or poly-synaptic connections with the MNs which are confined within the ventral horn area and generate an EPSP mediated by an excitatory amino acid acting on NMDA and non-NMDA receptors. (Supported by NS24226)

216.2

PARTICIPATION OF NMDA AND NON-NMDA RECEPTORS IN THE SLOW EXCITATORY SYNAPTIC TRANSMISSION IN THE RAT SPINAL DORSAL HORN IN VITRO. M. Randic and G. Gerber* (SPON: N.A. O'CONNELL). Dept. of Vet. Physiol. and Pharmacol., Iowa State University, Ames, IA 50011, USA.

Using current-clamp and voltage-clamp techniques in spinal slices we have studied further the contribution of NMDA and non-NMDA receptors to the early and late components of the slow excitatory synaptic responses elicited in the rat (18-24 days) dorsal horn neurons by repetitive stimulation of dorsal roots (Kangrga et al., Neurosci. Abstr., 14:96, 1988). We distinguished early and late components of the slow excitatory synaptic currents (EPSCs) on the basis of their voltage-dependence and sensitivity to Mg^{2+}-ions and NMDA and non-NMDA receptors antagonists. In the presence of Mg^{2+}, the early component of the slow EPSC increased with membrane hyperpolarization with a linear I-V relationship, whereas the late component decreased. In a zero-Mg^{2+} medium, the early component was potentiated, but the late component was reduced or unaltered. SP-induced depolarization was also depressed in a zero-Mg^{2+} solution. Superfusion of slices with CNQX reduced (<25%) the early component of the slow synaptic response. This component was, however, more reduced (60%) by D-APV. In a zero-Mg^{2+} solution, or at membrane potentials positive to -55mV in 1mM Mg^{2+}, D-APV reduced or even abolished the early component. We propose that slow excitatory synaptic response has two components, an early transient component that requires activation of NMDA and non-NMDA receptors, and a late longer-lasting peptidergic component. Supported by NIH, NSF, and USDA.

216.3

FAST EXCITATORY SYNAPTIC TRANSMISSION IN THE RAT SPINAL DORSAL HORN IN VITRO IS MEDIATED BY NON-NMDA AND NMDA RECEPTORS. G. Gerber* and M. Randic, Dept. of Vet. Phys. and Pharmacol., Iowa State University, Ames, IA 50011, USA.

In the spinal cord 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) selectively blocks responses to acidic amino acids acting at receptors of the non-NMDA type, whereas D-2-amino-5-phosphonovalerate (APV) is a selective NMDA-receptor antagonist. Using current-clamp and single electrode voltage-clamp techniques we found that in more than half of dorsal horn neurons superfusion of rat (18-23 days) spinal slices with CNQX ($5x10^{-6}$M in 1 mM Mg^{2+}-medium) completely and reversibly blocks fast excitatory synaptic responses evoked in dorsal horn neurons by stimulation of dorsal roots (2-6V for 0.02-0.2 ms). In the remaining cells, the synaptic responses were markedly reduced. Inward currents induced by α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and quisqualate were reduced to a greater extent than those induced by kainate or NMDA. In 1 mM-Mg^{2+} medium, APV ($5x10^{-5}$-10^{-4}M) reduced the synaptic responses in most of examined cells but had little effect on their early rising phase. The effect of APV was more prominent in a zero-Mg^{2+} solution and at membrane potentials positive to rest. Under voltage-clamp, the excitatory postsynaptic currents also showed fast (CNQX-sensitive) and slow (APV-sensitive, Mg-sensitive) components, both of which had similar thresholds but differed in their latency and voltage-sensitivity. These results support the concept that both non-NMDA and NMDA receptor channels are simultaneously activated by transmitter released from stimulated primary afferents. Supported by NIH, NSF, and USDA.

216.4

INTRACELLULAR RECORDINGS FROM GUINEA PIG AUDITORY CORTICAL NEURONS MAINTAINED IN VITRO: ELECTROPHYSIOLOGICAL AND PHARMACOLOGICAL OBSERVATIONS. C.L. Cox, R. Metherate, N.M. Weinberger and J.H. Ashe Dept. of Psychology, Univ. of Calif., Riverside, CA 92521, Center for the Neurobiology of Learning and Memory, and Dept. of Psychobiology, Univ. of Calif., Irvine, CA 92717.

Intracellular recordings were obtained from 106 auditory cortical neurons (Vm= -65.5 ± 1.1 mV) in *in vitro* slices from guinea pig. Electrical stimulation to the underlying white matter or the pia elicited depolarizing or hyperpolarizing responses in 36 of 65 cells so tested. These responses varied as expected of synaptic potentials when the membrane potential was altered by intracellular injection of depolarizing or hyperpolarizing current. Most of these cells responded with an EPSP that could result in cell discharge when stimulation was of adequate strength. Several cells responded with an EPSP that was followed by an IPSP, and a few with only an IPSP.

Initial observations suggest that the quisqualate/kainate receptor antagonist, 6,7-dinitroquinoxaline-2,3-dione (DNQX) is capable of reducing and eventually blocking the EPSP. In 42 of 74 cells, the cholinergic agonists acetylcholine or methacholine produced a slow membrane depolarization that was usually accompanied by an increase in membrane resistance.

These findings set the stage for an intracellular analysis of the modulatory effects of ACh on auditory receptive fields that we have previously shown to occur in the auditory cortex *in vivo*.

216.5

PHENCYCLIDINE PRODUCES LONG-LASTING ELECTROPHYSIOLOGICAL CHANGES IN RAT STRIATAL NEURONS RECORDED IN VITRO. C.S. Bailey, S.G. Howard and M.S. Levine. MRRC, UCLA, CA 90024.

Effects of phencyclidine (PCP) on striatal neurons were examined using intracellular recordings in brain slices from 22 adult Sprague-Dawley rats. Baseline values were obtained for threshold currents that evoke excitatory postsynaptic potentials (EPSP) and action potentials (AP) when slices were bathed in Ringer's. Excitability in response to depolarizing current injection (EX) was also measured. Slices were then bathed with PCP (1, 10, 25, 50, 100 or 300 μM) and measurements were taken 15 min later. They were subsequently washed with Ringer's for up to 80 min. Measurements were taken at 15 min intervals during the wash. All concentrations of PCP produced increases in EPSP and AP threshold. These increases occurred more frequently with lower concentrations (1-25 μM). Recovery of both EPSP and AP threshold to baseline values occurred in <10% of the responding cells. EX increased in 76% of cells tested at 1-25 μM but in only 52% at 50-300 μM. Recovery of EX occurred in <20% of the cells tested. These findings indicate that PCP produced long-lasting decreases in synaptic transmission while increasing neuronal excitability. These effects may involve a mechanism common to the generation of both action potentials and excitatory postsynaptic potentials such as blockade of cation conductance. In addition, higher concentrations of PCP also produced more complex interactions, perhaps reflecting toxicity. Supported by USPHS Grants DA 3017 and HD 5958.

216.6

INTERACTION OF METAPHIT, AN ANALOG OF PHENCYCLIDINE, WITH THE RELEASE OF ^{3}H-DOPAMINE FROM RAT STRIATAL SLICES. I. Zimanyi*, A. Lajtha and M.E.A. Reith (SPON:I. Wajda). Center for Neurochemistry, N.S. Kline Institute, Ward's Island, New York, NY 10035

The effect of metaphit (MET), an isothiocyanate derivative of phencyclidine (PCP) was investigated on the release of ^{3}H-dopamine evoked by electrical (1 Hz, 60 shocks) stimulation (overflow), amphetamine (AMPH 0.1-10 uM), dopamine (DA 0.1-10 uM) or veratridine (VER 5 uM) from rat striatal slices. MET itself (10 and 25 uM) increased the basal release (outflow) of ^{3}H and decreased the overflow of ^{3}H at 25 uM concentration (S_2/S_1 was 1.0 and 0.4 in the absence and presence of MET, respectively). The AMPH and unlabeled (DA) induced outflow was inhibited by MET. The nomifensine (NOMI) induced outflow was further elevated additively by MET. The electrical stimulation evoked overflow of ^{3}H in the presence of AMPH and unlabeled DA was enhanced by MET, in the presence of NOMI MET additively enhanced the stimulation evoked release. The VER induced release was greatly decreased by MET. The above data shows the complexity of the effect of MET on the release of ^{3}H-DA: 1. it acts similarly to the DA uptake inhibitors on the outflow of ^{3}H-DA (additive effect with NOMI), 2. it inhibits the carrier mediated exchange of DA induced by AMPH or DA, 3. it inhibits the voltage-dependent sodium channels (VER induced release). This work was supported by an NIH grant DA 03025 and by a grant from BRSG.

216.7

IMMUNOHISTOCHEMICAL DISTRIBUTION OF *N*-ACETYL-ASPARTYLGLUTAMATE AND L-AP4-SENSITIVE SLOW SYNAPTIC TRANSMISSION IN TURTLE CEREBELLUM.
N.T. Slater, J.R. Moffett, L.J. Larson-Prior, and J.H. Neale. Dept. of Physiology, Northwestern University Medical School, Chicago, IL 60611 and Dept. of Biology, Georgetown University, Washington DC 20057 U.S.A.

A novel, slow excitatory postsynaptic potential (sEPSP) has recently been demonstrated to follow repetitive activation of parallel fibers (PFs) in the intact turtle cerebellum *in vitro* [1]. This PF-sEPSP is selectively blocked by L-2-amino-4-phosphonobutyrate (L-AP4, 20-100 μM), but not by L-serine-*O*-phosphate (200 μM), suggesting that the receptor mediating the PF-sEPSP differs from a "classical" AP4 receptor. Because L-AP4 also blocks EPSPs in mammalian piriform cortex which have been proposed to be mediated by the dipeptide *N*-acetylaspartylglutamate (NAAG), the potential role of NAAG in mediating the PF-sEPSP in turtle cerebellum was explored. We have used affinity-purified NAAG anti-sera to determine the histochemical localization of NAAG in turtle cerebellum. Purkinje cell somata and proximal dendrites, as well as somata of the deep cerebellar nuclei, showed dense NAAG-like immunoreactivity (NAAG-LI). The granular layer also exhibited heavy NAAG-LI. No distinct fibers were immunoreactive, either in the cerebellar peduncle, the granular layer, or the molecular layer. Although the molecular layer displayed NAAG-LI above background levels, label could not be localized to either fibers or terminals of the PFs. No label was seen in interneurons of either the granular or molecular layers. These data therefore do not support the conclusion that NAAG mediates the PF-sEPSP. The observation that PF-sEPSP is also blocked by the excitatory amino acid antagonists kynurenate (1-2 mM) and CNQX (10 μM)[1], suggests the following hypothesis: the PF-sEPSP is mediated via activation of postsynaptic non-NMDA receptors, and is selectively inhibited by presynaptic L-AP4 receptor activation. Supported by DK37024 & NS25682.
[1] Slater, N.T. & Larson-Prior, L.J. (1989) *Biophys. J.* 55(2): 178a.

216.8

Excitatory Amino Acid (eaa) Antagonists Block Kindling Induced Synaptic Excitability in the Basolateral Amygdala (bla).
A.C. Anderson, E. Asprodini* and P. Shinnick-Gallagher. **Dept. of Pharmacology, Univ. of Texas Med. Branch, Galveston Tx 77550**

Synaptic transmission in amygdaloid kindled rats was examined using dl-2-amino-5-phosphonovaleric acid (dl-apv, 50uM), 7cl-kynurenic acid (7clkyn, 10uM), and 6-cyano-7-nitroquinoxaline-2,3-dione (cnqx, 5uM). Control intracellular recordings revealed an excitatory post synaptic potential (epsp) with a superimposed spike, a fast inhibitory post synaptic potential (f-ipsp) and a late hyperpolarizing potential in 50% of the cells. In some control neurons a burst could be evoked by suprathreshold stimuli. Kindled cells showed epileptiform bursting following threshold stimuli in 83% or an epsp with single spikes in 17% of the cells but no ipsp's were recorded. Dl-apv or 7clkyn blocked spikes and burst activity both evoked and spontaneous in 100% of control and 63% and 57% of kindled cells respectively. Resulting in a small residual epsp (75%) or completely blocked transmission (25%). These drugs had no effect on 12% and 25% of kindled cells respectively. The f-ipsp present in two control cells was reversibly decreased by dl-apv. Cnqx blocked burst activity and epsp in 100% of all neurons. The data suggest that bursting or spiking activity in bla neurons is mediated by the n-methyl-d-aspartate receptor, but that activation of the quisqualate receptor is also of primary importance. (Supported by NS24643)

216.9

CATIONS ALTER THE ACTION OF CIS-2,3-PDA AS AN ANTAGONIST OF SPONTANEOUS ACTIVITY OF AFFERENT FIBERS IN THE LATERAL LINE OF XENOPUS LAEVIS.
S.C. Bledsoe, Jr. and S.J. Allen*. Kresge Hearing Research Institute, Univ. of Michigan Medical School, Ann Arbor, MI 48109.

This study evaluated the effects of different cations on the action of cis-2,3-piperidine dicarboxylic acid (cis-PDA), a glutamate receptor antagonist, on spontaneous activity at the hair cell/afferent nerve synapse in the lateral line of Xenopus laevis.

The in vitro methods for isolating and recording afferent nerve responses from a single lateral line organ and applying drugs dissolved in frog Ringer solution to the serosal surface of the skin were as previously described (Bledsoe and Bobbin, Neurosci. Lett. 32:315, 1982). In each experiment cis-PDA from 0.0625 to 5.0 mM was tested on spontaneous activity in standard Ringer solution and then again in Ringer solution which contained an additional 0.5 mM calcium or other cation.

Increasing concentrations of cis-PDA up to 2.0 mM progressively decreased the rate of spontaneous activity in a dose-dependent manner. Concentrations in excess of 2.0 mM, however, reversed this trend, perhaps due to agonist-like actions of the cis-PDA. An additional 0.5 mM calcium added to the Ringer solution enhanced the suppressive effects of cis-PDA, as did 0.5 mM manganese. Barium and strontium, which should substitute for calcium as charge carriers, actually partially blocked the effects of cis-PDA. Increasing the potassium concentration from 2.0 mM to 2.5 mM had a smaller, but similar effect. Cobalt (0.5 mM) completely blocked the decrease in spontaneous activity normally seen with cis-PDA. Magnesium (0.5mM) had no effect on the action of cis-PDA on spontaneous activity.

Although other interpretations are possible, these results are best explained by actions of the cations on pre- and post-synaptic elements with a predominant effect of charge screening by calcium on the post-synaptic nerve.

216.10

ACTIONS OF BACLOFEN AND KAINATE ON MONOSYNAPTIC GABA IPSPS IN RAT HIPPOCAMPAL SLICES
C.H.Davies* and G.L.Collingridge*. (SPON: Brain Research Association) Department of Pharmacology, University of Bristol, Bristol BS8 1TD, U.K.

In the presence of 40 μM D-2-amino-5-phosphonovalerate (APV) and 20 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) which block synaptic excitation, stimulation in stratum radiatum evokes a pure monosynaptic biphasic IPSP in nearby CA1 neurones. These IPSPs exhibit paired-pulse fatigue that may be due to neurotransmitter acting on presynaptic receptors. We have looked for some possible candidates.

(-)Baclofen (0.25-5 μM) depressed this IPSP (and IPSC) by 50-100 % and hyperpolarised cells, whereas (+)baclofen was inactive. Phaclofen (1 mM) reversed these effects of (-)baclofen and also depressed the late phase of the IPSP.

Kainate (1-10 μM) depressed both the early and late phases of the IPSP in parallel and tended to depolarise cells. In contrast α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA; 10 μM) depolarised cells but did not depress the IPSP.

The results can be explained by these drugs acting directly on presynaptic GABA terminals to inhibit GABA release, in addition to having postsynaptic actions.

216.11

BACLOFEN BINDING TO $GABA_b$ RECEPTORS CAN BE STIMULATED OR DISPLACED BY CALCIUM CHANNEL LIGANDS.
M.I. Al-Dahan* and R.H. Thalmann, Baylor College of Medicine, Houston, TX 77030.

Synaptic plasma membrane from adult male rat cerebrum was prepared and [^{3}H]-baclofen binding was assayed by filtration as described earlier (Al-Dahan and Thalmann, J. Neurochem. 1989, 52, p.313) with the major exception that saponin (0.5%) rather than Triton was used to permeabilize the membranes. The dihydropyridine (DHP) calcium channel antagonist nifedipine displaced up to 75% of the [^{3}H]-baclofen binding (IC50=16±5nM) while the DHP calcium channel agonist Bay K8644 stimulated binding up to 30% (EC50=115±48nM). The DHP agonist +202-791 also stimulated binding up to 30% (EC50=7.6±2.5nM) while its enantiomer had no consistent effect. These compounds did not compete for the $GABA_b$ site, since a Scatchard analysis revealed that nifedipine (10 nM) primarily affected $GABA_b$ receptor density rather than affinity (control Bmax = 935±48fmol/mg protein; Kd=54±8nM; while in the presence of 10nM nifedipine, Bmax was reduced to 638±49mg/k and Kd=49±7nM). The calcium channel antagonist D600 also displaced up to 30% baclofen binding (IC50=27±17nM), while the antagonist diltiazem stimulated binding up to 35% (EC50=75nM). Although these results could reflect the forward coupling of $GABA_b$ receptors with calcium channels in brain, they do not rule out other, possibly more direct interactions between $GABA_b$ receptors and calcium channel ligand binding sites. Supported by NINCDS grant NS21713.

216.12

EFFECTS OF BACLOFEN AND BICUCULLINE ON DENTATE GRANULE CELL EXCITABILITY SUGGEST A PRESYNAPTIC LOCUS FOR GABAb RECEPTORS.
S.C. Steffensen*, Y.M. Kim*, and S.J. Henriksen. (SPON: N. Ling) Department of Neuropharmacology, Research Institute of Scripps Clinic, La Jolla, CA 92037.

In vitro studies have suggested both pre- and post-synaptic actions for GABA receptors in the hippocampus (N. Harrison et al, Neurosci. Letts, 85(1988) 105-109). The purpose of our studies was to further characterize GABAergic mechanisms influencing dentate granule cell excitability in the anesthetized rat. Field potentials were elicited by perforant pathway stimulation and population spike (PS) amplitudes of 5-8mV (half-maximal stimulation) were obtained at recording depths where simultaneous granule cell single-unit recordings were encountered. Control curves of test vs. conditioning PS amplitudes using a paired-pulse (PP) paradigm revealed a triphasic function with absolute inhibition occurring between 10-40ms, potentiation (e.g. 220% of control at 80ms) between 40-160ms and partial inhibition between 160-560ms (e.g. 60% of control at 560ms). Microiontophoretically applied ±baclofen (0.1-1.0mM in saline, 50-200nA) had minimal effects on primary excitability (10% increase in test PS amplitude) but produced a dose-dependent antagonism of PP inhibition at both short and long interstimulus-intervals (ISIs). In fact, higher concentrations and/or ejection currents of baclofen *potentiated* PSs at short (10-40 ms) ISIs (eg 200% of control). These effects were competitively antagonized by concurrent microiontophoretic application of the putative GABAb antagonist, phaclofen (50-75mM in saline, 50-100nA). In contrast, microiontophoretic application of the the GABAa competitive antagonist bicuculline (0.7mM in saline, 50-100nA) produced an incomplete antagonism of PP inhibition at short ISIs and complete antagonism at long ISIs. Unlike baclofen, bicuculline produced a marked increase in primary excitability (up to 200% of control) and single-unit firing rate. Baclofen either slightly decreased or had no effect on firing rate. These results together with in-vitro studies suggest that baclofen, a GABAb agonist, produces this 'paradoxical' decrease in PP inhibition by actions on GABAb receptors located presynaptic to dentate granule cells. (Supported by AA07456 and KO2/DA00131 to S.J.H.)

216.13

Opioid peptides and GABA inhibit A-delta and C fiber-evoked epsps recorded from substantia gelatinosa neurons in an adult rat spinal cord slice.
M. Yoshimura and T. M. Jessell, Center for Neurobiology and Howard Hughes Medical Institute, Columbia University, New York, NY 10032

The actions of opioid peptides and GABA on A-delta and C fiber afferent evoked monosynaptic epsps have been analyzed by intracellular recording from substantia gelatinosa (s.g.) neurons in a transverse slice preparation of adult rat spinal cord.

Superfusion of slices with the mu opiate receptor agonist DAGO (100nM - 1 uM) hyperpolarized about 60 % of s.g. neurons that received A-delta and/or C fiber input and depressed afferent-evoked epsps by 40 - 100 %. The effect of DAGO was reversed by application of naloxone (100 nM). DAGO also suppressed A-delta and C fiber evoked epsps in s.g. neurons in which hyperpolarizing response to DAGO were not detected. The delta opiate receptor agonist DPDPE (100 nM - 1 uM) did not alter the membrane potential of s.g. neurons and did not suppress A-delta and C fiber epsps. The kappa receptor agonist dynorphine (1 uM) produced a small (~ 30 %) inhibition of afferent evoked epsps but did not affect the membrane potential of s.g. neurons.

GABA (1 - 2 mM) hyperpolarized 60 % of s.g. neurons and suppressed A-delta and C fiber evoked epsps in both GABA-sensitive and -insensitive neurons. The suppression of epsps in GABA-insensitive s.g. neurons was not reversed by the GABA-A receptor antagonist bicuculline (10 uM). The GABA-B receptor agonist baclofen (10 - 20 uM) hyperpolarized many s.g. neurons and suppressed afferent-evoked epsps. However, at lower concentrations (500 nM - 1 uM) baclofen suppressed epsps without affecting the membrane potential of s.g. neurons.

These results suggest that opioid peptides and GABA modify fine fiber-evoked input to some s.g. neurons, in part, by a direct post-synaptic action. However, our results are also consistent with a pre-synaptic inhibition of excitatory transmitter release from A-delta and C fiber afferents.

216.14

WITHDRAWN

216.15

GADOLINIUM REDUCES NERVE-EVOKED, BUT NOT SPONTANEOUS RELEASE OF ACETYLCHOLINE (ACh) AT THE MOUSE NEUROMUSCULAR JUNCTION.
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The lanthanide gadolinium (Gd^{3+}) has been proposed to block a component of neuronal calcium currents preferentially. This component has been proposed by some to be the "N" component of I_{Ca}. A number of heavy metal polyvalent cations which are known to block neuronal Ca channels alter neurotransmitter release both at rest, and following nerve stimulation. Inasmuch as the type of Ca channel responsible for transmitter release from somatic motor axon terminals is unclear, it was of interest to determine whether Gd^{3+} would affect Ca-dependent release of ACh from motor axon terminals in response to nerve stimulation, or spontaneously. Mouse hemidiaphragms were cut to prevent contractions, and end-plate potentials (EPPs) and miniature end-plate potentials (MEPPs) were recorded. Gd^{3+} concentrations of 10-100 μM reduced the amplitude of nerve-evoked EPPs. At higher concentrations (50, 100 μm), Gd^{3+} ultimately blocked the EPP completely. At no concentration tested, did Gd^{3+} affect the frequency of occurrence of MEPPs. Thus, Gd^{3+} affects nerve-evoked, but not spontaneous release of ACh from mouse motor nerve terminals. In this regard, Gd^{3+} differs in its effects from several other heavy metal Ca channel blockers such as Pb^{2+}, La^{3+} and Hg^{2+}. (Supported by NIH grant ES03299.)

216.16

EFFECT OF ω-CONOTOXIN GVIA ON SYNAPTIC TRANSMISSION IN THE CA1 FIELD OF THE RAT HIPPOCAMPUS.
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ω-Conotoxin GVIA (ω-CgTx),a peptide isolated from the venom of a marin mollusc, has been studied on CA1 pyramidal hippocampal neurons using intracellular recordings in the in vitro slice preparation. We observed that ω-CgTx applied in the superfusion medium (0.1-1μM) quickly (≃ 1min) and irreversibly blocked the EPSP and IPSPs elicited by stimulation of the Schaffer collaterals/commissural fibers (n=6). It also blocked the slow cholinergic EPSP induced by stimulation of the cholinergic afferents (n=3). These synaptic events being due to the postsynaptic action of neurotransmitters released from terminals, we studied the postsynaptic effects of GABAergic and cholinergic agents in the presence of ω-CgTx. These effects were unaffected by ω-CgTx. In addition, ω-CgTx had no effect on the membrane polarization or on the afterhyperpolarization which follows a train of spikes (n=9). These results suggest that ω-CgTx acts at a presynaptic level, blocking the synaptic transmission in the hippocampal slice. This effect probably results from the action of ω-CgTx on the N-type calcium channels which seem to be presynapticaly located in this structure.
Supported by a grant from Bayer Pharma France.

216.17

FREQUENCY-DEPENDENT INHIBITION BY ω-CONOTOXIN GVIA (ω-CT) OF NEUROGENIC ENHANCEMENT OF THE ELECTRICALLY PACED GUINEA PIG LEFT ATRIA (GPLA).
R.A. Keith, D. LaMonte and A.I. Salama. Dept. of Pharmacology, ICI Pharmaceuticals Group, ICI Americas Inc., Wilmington, DE 19897.

A 5 sec field stimulation (FS, 1 mSec, 25 V, 0.5 - 40 Hz) of the electrically paced (punctate electrode, 2 Hz, 4 mSec, 4V) GPLA caused a delayed enhancement of the contractile response. Tetrodotoxin (3×10^{-7} M) or propranolol (3×10^{-6} M) inhibited FS enhancement by greater than 90% suggesting adrenergic neuronal mediation. ω-CT, an inhibitor of neuronal N- and L-type voltage-sensitive calcium channels (VSCC) has been shown to inhibit neurotransmitter release. ω-CT caused a concentration-dependent and irreversible inhibition of FS enhancement but had no effect on the paced response. The inhibition of FS enhancement by ω-CT was more pronounced at lower frequencies, e.g., 10^{-8} M ω-CT inhibited 4 Hz by 97 $\pm$ 2% and 40 Hz by 16 $\pm$ 2% (n=8). The frequency-dependent inhibition by ω-CT suggests that ω-CT sensitive (low frequencies) and ω-CT insensitive (high frequencies) VSCC mediate neurotransmitter release. Neomycin, an aminoglycoside antibiotic that displaces [^{125}I]ω-CT, reversibly inhibited both the paced response and the FS enhancement. Pre-exposure of GPLA to 10^{-2} M neomycin prevented the irreversible inhibition of FS enhancement caused by 10^{-8}M ω-CT, suggesting a common neuronal site of interaction for ω-CT and neomycin.

216.18

DEPOLARIZATION-DEPENDENT ACTIONS OF DIHYDROPYRIDINE CALCIUM CHANNEL LIGANDS IN THE IN VITRO RAT HIPPOCAMPUS.
M.H. O'Regan*, J.D. Kocsis, N.P. Poolos, and S.G. Waxman. Dept. of Neurology, Yale Univ. Sch. of Med. and VA Med. Ctr., West Haven, CT. 06516. Extracellular field potential analysis was utilized to examine the central nervous system (CNS) effects of dihydropyridines (DHP). In the absence of light, application of the DHP nimodipine or nifedipine (10-40μM), putative Ca^{++} channel antagonists, increased the excitability of CA_1 pyramidal cells as evidenced by an increase in the magnitude of the population EPSP and the appearance of multiple population spikes. Both actions were dependent upon membrane depolarization, achieved via elevated K^+(5 mM), low frequency (20 Hz stimulus trains, or high intensity (30-40 V) stimulation of the Schaffer collaterals. The effects of nifedipine were abolished upon exposure to high intensity light, while the effects of nimodipine were not. Application of the agonist DHP, Bay K 8644 (0.1-2 μM) in the dark, had similar excitant actions. Upon washout plus exposure to light, these effects were first abolished and then reversed, resulting in a persistent decrease in the EPSP although the postsynaptic membrane remained sensitive to glutamate. These results demonstrate that DHPs can directly alter synaptic transmission in the mammalian CNS, with implications for the neuroprotective activities of these agents.

216.19

FACILITATION OF HIPPOCAMPAL 5-HYDROXYTRYPTAMINE RELEASE BY L-TYPE VOLTAGE SENSITIVE CALCIUM CHANNEL AGONISTS: DEPENDENCY ON MAO INHIBITION.

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Bay K 8644, a dihydropyridine (DHP) agonist of L-type voltage-sensitive calcium channels (L-VSCC) has been reported to enhance 5-hydroxyptamine (5-HT) release from rat cortex slices (Middlemiss and Spedding, *Nature* 314: 94; 1985). This effect was observed in slices incubated and superfused with medium containing the monoamine oxidase (MAO) inhibitor pargyline. There is, however, other evidence suggesting that Bay K 8644 has no effect on neurotransmitter release (e.g., Dooley et al., *Eur. J. Pharmacol.* 148: 261, 1988). Since inhibition of MAO alters the balance between the cytoplasmic and vesicular transmitter pools, we investigated the effects of the DHP agonists Bay K 8644 and (+)-202-791 and of the DHP antagonist (-)-202-791 on 5-HT release evoked from rabbit hippocampal slices by K^+ (25 mM) in the presence and absence of pargyline (10 uM). The slices were pretreated, incubated with (^{3}H)-5-HT and superfused in medium with or without pargyline. They were stimulated twice and DHP ligands (1 uM) were added 15 min before the second stimulation. Bay K 8644 and (+)-202-791 significantly enhanced the evoked overflow of tritium only when pargyline was present. When MAO was not blocked, however, both DHP agonists were without effect on the evoked transmitter release. The L-VSCC antagonist, (-)202-791, either in the presence or in the absence of pargyline, did not affect the release of 5-HT.

These results indicate that the 5-HT-releasing actions of DHP agonists are critically dependent on MAO inhibition which provides a cytoplasmic 5-HT pool.

EXCITATORY AMINO ACIDS: RECEPTORS IV

217.1

USE OF (+)[^{3}H]-7-N$_3$-MK-801 FOR PHOTOAFFINITY LABELLING THE NMDA RECEPTOR. M.S. Sonders*, P. Barmettler†, J.F.W. Keana†* and E. Weber (SPON: M. Seger) Vollum Institute for Advanced Biomedical Research, Oregon Health Sciences University, Portland, OR 97201, and †Department of Chemistry, University of Oregon, Eugene, OR 97403

Relatively little is known about the structure of the NMDA receptor. In order to identify the constituent proteins of this ligand-gated ion channel we have synthesized a radioactive photoaffinity derivative of MK-801, a highly selective non-competitive NMDA antagonist which binds at the PCP site. This ligand, (+)[^{3}H]-7-N$_3$-MK-801, has a specific activity of 30 Ci/mmol, displays reversible binding characteristics and a pharmacological profile virtually identical to those of MK-801 itself (Keana et al., Soc. Neurosci. Abstr. 14:199.25, 1988). In guinea pig brain membranes (+)[^{3}H]-7-N$_3$-MK-801 binds to a single class of apparent sites with a K_D of 2.8 nM and a B_{max} of 1.2 pmol/mg protein. It also displays a binding maximum at pH 8 and a rank order of inhibitor potencies characteristic of the high affinity PCP binding site (MK-801> (-)MK-801>TCP>dexoxadrol>PCP>(-)cyclazocine >(+) cyclazocine≥(+)SKF 10,047> (-)SKF 10,047>D-ketamine>L-ketamine>levoxadrol).

Photoaffinity labelling was performed on crude guinea pig brain membranes in 5 mM Tris HCl buffer, pH 8, containing 0.1 mM PMSF, 1 mM EDTA and 50 µg/ml bacitracin. Membranes (0.2-0.4 mg/ml) were incubated in the dark for >4 hours with (+)[^{3}H]-7-N$_3$-MK-801 (<10 nM) in the absence or presence of 10 µM PCP and then rapidly filtered and washed on GF/C filter discs. The filters were then irradiated with long wave UV light, rewashed and the radioactivity was solubilized from them with 0.5% SDS. Samples were concentrated by either lyophilization or precipitation with ice-cold acetone and electrophoresed on SDS-polyacrylamide gels under reducing conditions. Fluorograms of stained gels indicate a single radioactive protein band which migrates with an apparent molecular mass of 115 kd. This band was absent from lanes of samples that had been incubated with PCP or that had not been irradiated.

This work has been supported by grants MH40303, MH42068 and by Cambridge NeuroScience Research, Inc., Cambridge, MA.

217.2

HYBRID ARREST OF GABA AND NMDA RECEPTOR EXPRESSION IN XENOPUS OOCYTES INJECTED WITH RAT BRAIN mRNA. **S.J. Myers*, C.C. Joneckis*, R.A. Nicholas*, and R. Dingledine, (Spon. D.B. Hoch). Dept. of Pharmacology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7365.**

The ability of several oligodeoxynucleotides to block the translation of specific rat brain mRNAs was investigated in *Xenopus laevis* oocytes. Antisense oligonucleotides (30 mers) were mixed with whole rat brain poly A+ mRNA and injected into stage V oocytes (35 ng RNA and 6-26 ng oligo per cell). After 3 days oocytes were voltage clamped (-60 mV) and inward current responses to 100µM bath applications of γ-aminobutyric acid (GABA), kainate (KA), N-methyl-D-aspartic acid (NMDA), and carbachol were measured. Oocytes injected with mRNA alone evoked 16 ± 3, 54 ± 7, 46 ± 5, and 25 ± 5 (Mean ± SEM) nA responses to GABA, KA, NMDA, and carbachol, respectively. Oligo 840 selectively (0.06-2.7 pmols/cell) inhibited GABA and NMDA evoked currents over those of KA and carbachol. Four other oligos tested under similar conditions failed to produce similar results. In separate experiments RNAse H and DNAse were added 20 min apart to an oligo 840:mRNA mixture *in vitro* (37 C) prior to injection. Under these conditions GABA and NMDA currents were specifically inhibited by 96% and 67%, respectively, whereas KA and carbachol responses were unaffected. Northern analysis of whole rat brain mRNA probed with oligo 840 detected seven distinct bands with M_r sizes from 1.6 to 10.9 kb. Three of these bands have apparent M_r's similar to that of GABA$_A$ α-subunit mRNAs. These results together suggest that oligo 840 recognizes GABA and perhaps NMDA receptor mRNAs in a sequence specific manner.

217.3

PARTIAL PURIFICATION AND CHARACTERIZATION OF A [3H]CPP RECOGNIZING PROTEIN. M.D.Cunningham, M. Gurfinkiel*, P.S.Johnson*, K.Bushell*, and E.K.Michaelis. Depts of Biochemistry, Pharmacology, and the Center for Biomedical Research, University of Kansas, Lawrence, Kansas, 66045.

We have used a combination of CHAPS and polyoxyethylene-10-tridecyl ether, in the presence of glycerol and high concentrations of protease inhibitors, to solubilize [3H]CPP recognition sites from rat brain synaptic membranes. Ligand saturation analyses of intact membranes show a single binding site with a Kd and Bmax of 0.5uM and 3 pmol/mg which changes to 0.6uM and 6 pmol/mg upon solubilization.

[3H]CPP binding activity was purified by ibotenate affinity chromatography, and a fraction eluted with MgSO4 contains saturable binding with Kd and Bmax of 0.8uM and 11 nmol/mg. [3H]CPP displacement profiles by various acidic amino acids and amino acid analogs indicate the presence of recognition sites for NMDA antagonists but not agonists; binding also appears to be modulated by Mg+2 ions and glycine. This fraction displays no evidence of GAD enzyme activity nor [3H]GABA binding activity.

SDS-PAGE of the MgSO4-eluted fraction reveals strong enrichment of a 58kDa protein. Western blot analysis shows antibodies we have previously raised against rat brain glutamate binding protein (JBC 263:417) do not recognize the 58kDa protein. This protein may play an importanat role in NMDA receptor-ion channel regulation. [Supported by grants DAAL 03-88-K0017 from ARO and AA04732 from NIAAA].

217.4

SPIDER TOXIN (JSTX)-BINDING PROTEIN IN RAT HIPPOCAMPUS. K. Shimazaki*, T. Nakajima*, and N. Kawai* (SPON: Y. Sahara). Dept. Neurobiol., Tokyo Metropol. Inst. Neurosci., Tokyo 183, and Dept. Anal. Chem., Fact. Pharmaceut. Sci., Tokyo Univ., Tokyo 113, Japan.

In order to purify the glutamate receptor of the brain we have used synthesized spider toxin (JSTX-3), which specifically blocks glutamatergic synapses. After a crude synaptic membrane fraction was prepared from rat hippocampus, a JSTX-3 binding protein was solubilized by Triton X-100. Specific binding of [^{3}H]-biotinyl JSTX-3 to the solubilized fraction was saturable with a K_d of 5 µM and a B_{max} of 12 pmol/mg protein. Binding was inhibited by unmodified JSTX-3, but not reduced by Asp-Cad-Put, an inactive analog of JSTX-3. The JSTX-3 binding protein molecules migrated with M_r = 17,000 and M_r = 66,000 in FPLC analysis using a Superose 6 column. SDS-polyacrylamide gel electrophoresis of the affinity-purified JSTX-3 binding protein showed a single band with silver stain, migrating with M_r = 70,000-80,000. This is the first demonstration of JSTX-3 binding protein isolated from the mammalian brain and our findings should facilitate the molecular characterization of the glutamate receptor.

217.5

SOLUBILIZATION AND PARTIAL PURIFICATION OF AMPA BINDING SITES FROM RAT AND BOVINE BRAIN.
C.Hunter,K.Wheaton* and R.J.Wenthold. Laboratory of Molecular Otology, NIDCD, NIH, Bethesda, MD 20892.

Binding sites for AMPA, proposed as a selective agonist for the quisqualate excitatory amino acid receptor, were solubilized from rat or bovine brain using 1% Triton X-100 in 0.5M potassium phosphate buffer containing 20% glycerol. The solubilized binding site had an M_r on gel filtration of 425,000 and was stable for several days at 4°C. Binding and pharmacological analyses indicated that the solubilized binding sites were similar to the membrane bound sites in rat and bovine preparations. [3H]AMPA binding to soluble and membrane preparations was increased in the presence of potassium thiocyanate. Scatchard analyses showed that both preparations contained high and low affinity binding sites. Competition studies with both preparations displayed a similar rank order of potency. A 54 fold purification of the soluble preparation from the rat was achieved by ion exchange chromatography and gel filtration. The characterization and purification of these soluble binding sites is potentially useful for the molecular characterization of this putative amino acid receptor subtype.

217.6

ISOLATION AND CHARACTERIZATION OF A cDNA ENCODING A PUTATIVE KAINIC ACID RECEPTOR SUBUNIT IN FROG BRAIN.
K.Wada[1*],Y.Nakatani[2*],K.Kusano[3],C.Dechesne[1],D.Hampson[1], S.Shimasaki[4*],K.Wheaton[1*],C.Banner[2*] and R.Wenthold[1]. [1]Lab. of Molecular Otology, NIDCD, [2]Lab. of Molecular Biology, NINDS, [3]Lab. of Neurochemistry, NINDS, NIH, Bethesda, MD20892 and [4]Neuroendocrinology Lab., The Salk Institute, San Diego, CA92138.

A putative kainic acid receptor in frog brain(Rana pipiens berlandieri) has been purified by domoic acid affinity chromatography and shown to contain a subunit of 48KDa protein (Hampson and Wenthold, J. Biol. Chem. 263, 2500,1988). Micro amino acid sequence was determined from the major V8 protease fragments of the 48KDa protein, and used to make oligonucleotide probes. Several clones were isolated from frog brain cDNA library using the probes. The deduced amino acid sequence from the overlapping isolated clones revealed that the cDNA encodes the 48KDa protein composed of 466 amino acids. Northern hybridization showed that two major transcripts of ~6 and ~4 kb were expressed specifically in brain. A characteristic hydrophobic feature found in other ligand gated ion channel subunits was conserved in the 48KDa protein. Some weak amino acid sequence similarities were found against brain nicotinic receptor subunits in particular segments. In situ hybridization showed that localization of transcripts for the 48KDa protein was compatible with the data of immunohistochemistry and receptor autoradiography studies for kainic acid receptor in frog brain. cDNA expression studies in oocytes are currently under way to determine the function of the cDNA.

217.7

PURIFICATION AND RECONSTITUTION OF THE KAINATE RECEPTOR FROM GOLDFISH BRAIN.
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Goldfish brain membrane fragments provide a good source of kainate(KA) receptors (100 pmol/mg pr) as determined by [3H]KA saturation binding. Previous results demonstrate that 50% of binding sites are recovered when solubilized in *n*-octyl-β-D-glucopyranoside (OG); the pharmacological profiles of membrane bound and solubilized receptors are similar (Henley and Oswald BBA 937:103, 1988).

KA receptors from whole goldfish brain were solubilized in a Tris buffer containing 2% OG and subsequently purified in a two step chromatographic procedure: ion exchange (DEAE) followed by domoate affinity chromatography (Hampson and Wenthold, *JBC* 263:2500, 1988). [3H]KA binding studies of crude solubilized brain revealed a single class of binding sites with a K_d of 360 nM and a B_{max} of 50 pmol/mg pr. In contrast, KA receptors purified in 20 mM Tris containing 10% glycerol and 0.1% Triton-X had a K_d of 200 nM and these preparations contained 6500 pmol/mg pr thus yielding a 130 fold purification. Displacement of [3H]KA by competitive ligands was similar in both; the K_i values for crude solubilized and purified receptors respectively were kainate 53 nM and 52 nM; kynurenic acid 4.8 mM and 6 mM; L-glutamate 35 mM and 54 mM. Silver stained SDS gels run under reducing conditions revealed a major band which migrated with a molecular weight of 49 kDa. However, there was also a 43 kDa band which was usually much less intense.

Purified KA receptors reconstituted into artificial lipid vesicles by an OG dialysis procedure bound [3H]KA with a K_d of 208 nM, similar to the purified soluble receptor. However, the specific activity (19800 pmol/mg pr) was apparently 3x greater in the reconstituted preparation. Preliminary studies indicate that when the purification and reconstitution are done in 20 mM Hepes with 100 mM NaCl, 50 mM KCl, and 5 mM $MgCl_2$ there is greater recovery of total numbers of binding sites.

217.8

DEVELOPMENT OF METHODOLOGY FOR RAPID PURIFICATION AND PHYSICO-CHEMICAL CHARACTERIZATION OF GLUTAMATE-BINDING PROTEINS FROM SYNAPTIC MEMBRANES.
K. Kumar*, K. Eggeman*, Y. Cong*, and E. Michaelis. Depts. Pharmacol. & Biochem., and Cent. for Biomed. Res, Univ. of Kansas, Lawrence, KS 66045

We have previously reported on the purification of a 71 kDa glutamate-binding protein (GBP) from rat brain synaptic membranes [Chen et al., J. Biol. Chem. 263, 417-426, 1988]. This glycoprotein has ligand binding characteristics suggestive of a role as a glutamate receptor in synaptic membranes. The protein has neither enzymatic activity nor the characteristics of membrane carriers for glutamate. In order to complete the biochemical and physico-chemical characterization of this protein, including amino acid analyses and amino acid sequencing, we have developed a new method for rapid purification of the protein to near homogeneity and removal of associated detergents. The new methodology involves affinity column chromatography through an L-glutamate-derivatized Trisacryl column and selective elution by 5 mM L-glutamate or 5 mM NaN_3. This fraction is highly enriched in glutamate binding activity, ~1,000-fold enrichment, and consists primarily of two protein species, ~70 and ~60 kDa proteins. This preparation has been used to estimate the sedimentation coefficient by sucrose density centrifugation in the presence of 5-20% sucrose in H_2O and D_2O and the Stokes radius by chromatography on Sephacryl S400 HR. Detergent removal and selective elution of the ~70 kDa protein is accomplished through HPLC on a cyanopropyl-derivatized column. A homogeneous preparation of this protein that is useful for chemical studies is obtained by this procedure. [Supported by grants DAAL-03-88-K0017 from ARO and AA 04732 from NIAAA].

217.9

AFFINITY PURIFICATION OF A KAINIC ACID BINDING PROTEIN FROM *XENOPUS* CNS.
A. Ambrosini*, J.M. Henley* and E.A. Barnard*(SPON. B.R.A., UK).MRC, Mol. Neurobiol. Unit, MRC Centre, Hills Rd, CB2 2QH Cambridge UK.

Kainic acid (KA) is a potent agonist at a subtype of excitatory amino acid receptors in the vertebrate CNS. Recently, Hampson and Wenthold presented the purification, by affinity chromatography, of a 48 kDa protein identified as the putative KA receptor(1). We describe here the affinity purification of the [3H]KA binding site from a particularly rich source, the *Xenopus* CNS. In this tissue, [3H]KA binds with high affinity to a large population of binding sites (K_d=54 nM; B_{max}=28 pmol/mg protein). These membrane-bound sites have been solubilised in 1% n-octyl-β-D-glucopyranoside (OGP). In detergent extracts [3H]KA binds with a K_d of 46 nM and a B_{max} of 22 pmol/mg protein and both membrane-bound and solubilised binding sites have similar pharmacological characteristics (2). We employed the domoic acid-affinity chromatography (1) to purify these OGP-solubilized [3H]KA binding sites. All of them were retained by the affinity resin and [3H]KA binding activity could be recovered after specific elution with 60 μM unlabelled KA and dialysis. Several bands were consistently identified on silver stained SDS gels of the specific eluate. Radiation inactivation experiments performed on *Xenopus* CNS tissue (in collaboration with dr. M. Nielsen, Denmark) suggest the [3H]KA membrane binding site is an oligomeric protein of 240 kDa.

The pharmacological and biochemical properties of these purified proteins and their identity as part of the putative KA receptor will be described.

1. Hampson D.R. and Wenthold R.J. (1988) J. Biol. Chem.,263, 2500-2505.
2. Henley J.M. and Barnard E.A. (1989) J. Neurochem., 52, 31-57.

217.10

CHARACTERIZATION AND PARTIAL PURIFICATION OF A CHLORIDE- AND CALCIUM-DEPENDENT GLUTAMATE-BINDING PROTEIN FROM RAT BRAIN.
N. Brose*[1], S. Halpain[2], C. Suchanek*[1] and R. Jahn[1] (SPON: G. Neuweiler). [1]Dept. Neurochemistry, MPI for Psychiatry, D-8033 Martinsried, FRG and [2]Rockefeller University, New York, NY 10021.

A glutamate-binding protein was solubilized from rat brain synaptic plasma membranes using sodium cholate. Its properties were characterized after incorporation into proteoliposomes. Glutamate binding was dependent on calcium and chloride ions. The effects of the two ions were synergistic and maximal at concentrations of 10^{-5} M calcium and 10 mM chloride ions. Glutamate binding was not affected by inhibitors specific for NMDA and kainate receptor subtypes, but was inhibited by quisqualate and DL-APB. Furthermore, binding was abolished by DIDS and dithiothreitol. These properties resemble those of the chloride- and calcium-dependent binding site. Starting from the detergent extract, the glutamate-binding protein was purified 123-fold using fractionated ammonium sulfate precipitation, chromatography on hydroxyapatite and on DEAE-Sephacel as sequential purification steps. SDS-PAGE of the purified protein fraction showed two major bands migrating with M_r values of 51,000 and 105,000. Glutamate binding to the partially purified protein is not due to a sequestration process or product binding to N-acetylated α-linked acidic dipeptidase. A rabbit antiserum was generated by immunization with the partially purified binding protein. The antibodies react with a band of 51,000 Da. Small amounts of this antiserum lead to a significant inhibition of glutamate binding. Glutamate-binding activity was completely removed from solubilized synaptic plasma membranes by immunoprecipitation, indicating that the 51,000 Da protein is an essential component of the binding site.

218.1

ACTIVATION OF PERIPHERAL NOCICEPTORS BY GLUTAMATE.
D.L. Tanelian and M.B. MacIver. Dept. of Anesthesia, Stanford Univ. Sch. of Medicine, Stanford, CA 94305.

Glutamate has been shown to activate CNS, spinal cord and dorsal root ganglion neurons, but to date has not been shown to stimulate mammalian sensory afferents. Using the in vivo rabbit cornea model of nociception (Tanelian, D.L. and Beuerman R.W., Exp. Neurol.,84:165, 1984), a population of peripheral afferents has been identified which are activated by glutamate and selectively blocked by glutamate antagonists in a reversible fashion.

Corneal C-fiber afferents responded to topical application of glutamate (100 uM) in a tonic fashion which lasted for the duration of stimulus application and in some cases, slightly beyond. The addition of glycine (100nM) potentiated the effect of glutamate, but produced no effect on its own. N-methyl-D-aspartate (NMDA:100 uM) was as effective an agonist as glutamate (100uM). The antagonists 2-amino-5-phosphono-valerate (APV:100uM) and ketamine (100uM) completely abolished the responses to glutamate and NMDA in a reversible fashion. This evidence establishes the existence of glutamate receptors on the peripheral terminals of mammalian sensory afferent fibers. Preliminary data suggests that they may be an NMDA receptor subtype. However, further studies are needed to completely characterize the receptor subtype (s). Supported by the Parker B. Francis Investigatorship in Anesthesiology and the American Cancer Society.

218.2

NMDA RECEPTOR ACTIVATION REGULATES THE PHOSPHORYLATION STATE OF DARPP-32. Shelley Halpain, Jean-Antoine Girault, Stevin Zorn, and Paul Greengard. Laboratory of Molecular and Cellular Neuroscience. The Rockefeller University, New York, NY 10021.

Medium-sized spiny neurons of the striatum receive glutamatergic input from the neocortex. The excitatory response of these cells to glutamate may be inhibited in vivo by dopaminergic inputs from the substantia nigra. DARPP-32, a protein enriched in medium-sized spiny neurons, is phosphorylated by cAMP-dependent protein kinase on a threonyl residue. Previous studies indicated that dopamine and other activators of cAMP-dependent protein kinase could stimulate phosphorylation of DARPP-32 in striatal slices. We sought to examine the effect of glutamate agonists on DARPP-32 phosphorylation. Rat striatal slices were preincubated with ^{32}P-ortho-phosphate to label the cellular ATP pools. Addition of 50 μM forskolin for 5 min resulted in a 200% increase in threonine phosphorylation of DARPP-32. This forskolin stimulation was prevented by co-treatment with 100 μM N-methyl-D-aspartate (NMDA). The specific NMDA receptor antagonist D-aminophosphonopentanoic acid (D-AP5) blocked the effect of NMDA. These results indicate that, in the striatum, activation of NMDA receptors and activation of dopamine receptors have opposite effects on the state of phosphorylation of DARPP-32. When DARPP-32 is phosphorylated on a threonyl residue it acts as a potent inhibitor of protein phosphatase-1. Thus, the stimulation of DARPP-32 threonine phosphorylation by forskolin or dopamine in situ presumably results in the inhibition of protein phosphatase-1. Activation of NMDA receptors would remove this inhibition. We suggest that such a mechanism may be involved in the opposing effects exhibited by dopamine and glutamate on the excitability of medium-sized spiny neurons.

218.3

PHARMACOLOGICAL PROPERTIES OF THE NMDA RECEPTOR SYSTEM COUPLED TO THE EVOKED RELEASE OF [^{3}H]GABA FROM STRIATAL NEURONS IN PRIMARY CULTURE. D.E. Kemp and S. Weiss (SPON: W.J. Becker). Neuroscience Research Group, University of Calgary, Calgary, Alberta.

The actions of a series of endogenous excitatory amino acid (EAA) agonists and synthetic antagonists at the NMDA receptor system coupled to the evoked release of [^{3}H]GABA from striatal neurons in primary culture was examined. EAA agonists displayed the following rank order of potency in evoking [^{3}H]GABA release: glutamate > homocysteate > aspartate, NMDA > cysteine sulfinate. Glutamate, homocysteate and cysteate were equieffective, while at saturating concentrations, aspartate and NMDA reached 75 and 65%, respectively, of the maximum efficacy of the former three agonists. The release of [^{3}H]GABA evoked by 100μM NMDA was attenuated in a dose-dependent manner by the following antagonists (IC_{50}, μM): MK-801 (0.067), PCP (0.151), CGS-19755 (3.81), APV (18.8), kynurenate (100) and γ-DGG (100). The antagonist properties of MK-801 and PCP were not competitive with NMDA, while NMDA dose-response curves performed in the absence and presence of increasing concentrations of CGS-19755 resulted in parallel rightward shifts (pA_2 = 5.95). CGS-19755 produced similar rightward shifts of the homocysteate dose-response curve (pA_2 = 5.89). At glutamate concentrations < 100μM, CGS-19755 and APV were potent antagonists of glutamate-evoked release, however at glutamate concentrations > 100μM these agents were ineffective blockers. The blockade of NMDA-evoked release of [^{3}H]GABA by kynurenate was not competitive in nature. Glycine (ineffective alone) induced a dose-dependent (EC_{50}, 1.3μM) reversal of 100μM kynurenate blockade of the NMDA response; glycine did not reverse the effects of any of the other classes of antagonists. Although with increasing kynurenate a rightward shift of the glycine dose-response curve was observed, at 300μM kynurenate the reversal was incomplete. This study suggests that three distinct sites for antagonists exist on the NMDA receptor system mediating EAA-evoked release of [^{3}H]GABA from striatal neurons.

Supported by the Medical Research Council of Canada.

218.4

PHENCYCLIDINE ATTENUATION OF NMDA-INDUCED DOPAMINE RELEASE IN THE RAT STRIATUM. P. Marek and S. Howard, MRRC, Department of Pharmacology, UCLA, CA 90024

The effects of phencyclidine (PCP) and N-methyl-D-aspartic acid (NMDA) on striatal concentrations of dopamine and DOPAC were studied using in vitro microdialysis. Samples were collected from urethane anesthetized rats and analyzed using an HPLC-EC assay. NMDA and PCP were administered locally with microdialysis probe for a 30 sec period and their effects were measured in 10 minute samples collected for one hour period following drug administration. 1 mM NMDA produced a 2000% increase in extracellular dopamine concentration and a 20% decrease in extracellular DOPAC concentration.

Administration of 25 μM PCP prior to NMDA reduced the effect of NMDA by 50%. Administration of 1 mM APV for 30 sec prior to NMDA produced a complete block of the NMDA effect. PCP induced dose dependent increase in extracellular dopamine concentration without changing DOPAC level. This grant was supported by NIH grant DA 03156.

218.5

NMDA RECEPTOR AND METABOLIC PATHWAY INVOLVED IN GLUTAMATE-INDUCED SRIF RELEASE FROM CORTICAL NEURONS. L. TAPIA-ARANCIBIA and H. ASTIER. URA 1197 CNRS, University Montpellier II, France.

We have recently shown that glutamate (Glu) stimulates somatostatin (SRIF) release from rat cortical neurons in primary cultures presumably by activation of a NMDA receptor type (J. Neurochem. in press). The present experiments were designed 1/ to further characterize the related receptor. 2/ to examine the metabolic pathways involved in the Glu-stimulated SRIF release. SRIF was measured in the media by RIA after a 15 min incubation of glia-free cortical neurons with the agonists or antagonists in Mg^{2+}-free Locke medium. Under these conditions Mg^{2+} decreases the stimulatory effects of Glu. NMDA and Glu-induced SRIF release were blocked in a dose-related manner by DL-2-amino-5-phosphonovaleric acid (APV) and thienyl-phencyclidine (TCP), and potentiated by glycine or serine (10 μM) ; these potentiations were reversed by kynurenic acid (200 mM). Zn2+ (50 mM) was also able to significantly decrease the stimulatory effect of NMDA.

The metabolic pathways involved were studied by using : mepacrine an inhibitor of phospholipase A_2 (PLA_2) ; H7 a specific inhibitor of Prot Kinase C ; indomethacin (Ind) a blocker of the cyclooxygenase pathway and nordihydroguairetic acid (NDAG) , a lipoxygenase inhibitor. Mepacrine (0.5 mM) completely inhibited the stimulatory effect of Glu (10 μM), while H7 (100 μM) had no effect on SRIF release. Ind and NDAG (100 μM) significantly reduced the inhibitory effect of Glu and NMDA (10 μM). Furthermore, melittin, a PLA_2 stimulatory peptide, also increased SRIF release in a dose-dependent manner, this effect being blocked by mepacrine. Preliminary results show that Glu is also able to stimulate [^{3}H] arachidonic acid release from the cortical neurons.

These data suggest that 1/ a NMDA receptor-channel complex is involved in the stimulatory effect of Glu on SRIF release 2/ the activation of PLA_2 seems to participate in this response.

This work was supported by a grant from the INSERM (88-4002).

218.6

TTX AND STX INHIBIT RESPONSES OF SPINAL CORD NEURONS IN CELL CULTURE TO NMDA. M.J. McLean and A.W. Wamil* (SPON: W.D. Dettbarn). Dept. of Neurology, Vanderbilt Univ. Med. Ctr., Nashville, TN

We studied the effects of the marine toxins tetrodotoxin (TTX) and saxitoxin (STX) on the responses of mouse spinal cord neurons in monolayer cell culture to N-methyl-D-aspartate (NMDA) and glutamate (GLU). Neurons were superfused with phosphate buffer containing 7-10 mM Mg^{++} to suppress spontaneous activity. Pressure application of NMDA or GLU from a patch clamp pipette near a neuron impaled with a microelectrode for conventional transmembrane recording of potential produced a concentration dependent depolarization and firing of action potentials. Action potentials fired in response to application of NMDA or GLU or elicited by depolarizing current pulses applied through the recording microelectrode were abolished reversibly by TTX or STX. Responses to NMDA were diminished by TTX (IC_{50} 5 X 10^{-10}M) and by STX (IC_{50} 10^{-9}M). Half-maximal inhibition of GLU (10^{-5}M) responses by TTX occurred at about 10^{-8}M). Responses to NMDA were abolished by AP-7 and MK-801. About 60% of the GLU response was abolished by AP-7. These findings indicate that TTX and STX can reversibly block sodium influx through ion channels activated by NMDA and the excitatory amino acid neurotransmitter GLU. (Supported by a Mallinckrodt Scholarship and NIH Grant NS 01194 to MJM and a Cissy Patterson Fellowship to AWW.)

218.7

THE RELATIVE POTENCY OF LANTHANUM AS A BLOCKER OF VOLTAGE-GATED CALCIUM CURRENTS AND OF CURRENTS EVOKED BY EXCITATORY AMINO ACIDS. D.B. Reichling and A.B. MacDermott, Dept. Physiology and Center for Neurobiology and Behavior, Columbia Univ., NYC, NY 10032

Glutamate can promote voltage-depedent Ca^{2+} entry into neurons by directly activating Ca^{2+}-permeable NMDA receptors and indirectly activating voltage-gated Ca^{2+} channels (VGCC). La^{3+} blocks current through VGCC. To determine if La^{3+} selectively blocks VGCCs during EAA activation of non-voltage clamped neurons, we tested the effectiveness of different concentrations of La^{3+} on VGCC currents and currents evoked by excitatory amino acids (EAA). First, the potency of La^{3+} as a blocker of VGCCs was assessed. Cultured neurons from the dorsal horn of rat spinal cord were continuously perfused with bath (5 mM Ba^{2+} added) and inward currents were evoked by depolarizing voltage steps from −60 mV. Maximal blocking of VGCC was achieved with concentrations of La^{3+} between 1 and 10 uM. The second set of experiments tested if these concentrations of La^{3+} had any effect on EAA-gated currents. To minimize the activation of VGCC, the neurons were voltage clamped. 1 uM La^{3+} reversibly depressed the response to 50-100 uM NMDA by 51%; 10 uM La^{3+} caused 73% depression. In contrast, the responses to 50 uM kainate and 10 uM quisqualate were not depressed by either concentration of La^{3+}. In some cells, 10 uM La^{3+} increased the response to kainate and quisqualate. Thus, doses of La^{3+} which effectively blocked VGCCs did not reduce responses to kainate and quisqualate and only partially reduced responses to NMDA. Therefore, La^{3+} may be a useful tool to distinguish between direct effects of EAAs and indirect effects due to activation of VGCCs.

218.8

THE EFFECTS OF N-METHYL-D-ASPARTATE (NMDA) ON CALCIUM FLUX AND NEUROTRANSMITTER RELEASE FROM RAT BRAIN NEURONS. J.J. Woodward, R.A. Gonzales, J.E. Dildy*, S.M. Rezazadeh*, J.L. Morris*, and S.W. Leslie. Division of Pharmacology and Institute for Neuroscience, University of Texas at Austin, Austin, TX 78712.

The effects of the glutamate receptor agonist, N-methyl-D-aspartate (NMDA) on calcium flux and neurotransmitter release were investigated using several different preparations of brain neurons from Sprague-Dawley rats. Stimulation of freshly dissociated whole brain neurons from neonatal (0-1 days old) rats with NMDA (25-200 uM) rapidly increased intracellular free calcium concentrations ([Cai]) as measured by fura-2 spectroscopy. These increases were blocked or reversed by magnesium (1 mM) or the selective NMDA receptor antagonist 2-amino-5-phosphonovalerate (AP5, 100 uM). Similarly, brain slices prepared from the corpus striatum or the frontal cortex of adult Sprague-Dawley rats released significant amounts of endogenous dopamine (striatum) or preloaded tritiated norepinephrine (cortex) over a two minute stimulation with 50-1000 uM NMDA. Release of endogenous dopamine or tritiated norepinephrine was totally blocked by magnesium (1 mM). In contrast to these findings, calcium entry into whole brain neurons freshly dissociated from adult Sprague-Dawley rats was not stimulated by NMDA (25-1000 uM) as measured by fura-2 spectroscopy and $^{45}Ca^{2+}$ flux experiments. NMDA also did not stimulate the release of endogenous dopamine from these cells. However, stimulation of freshly dissociated adult brain neurons for thirty seconds with potassium (15-45 mM) significantly increased $^{45}Ca^{2+}$ uptake as well as [Cai] as measured by fura-2. In addition, these cells released significant amounts of endogenous dopamine upon potassium depolarization. These results suggest that the NMDA receptor-operated calcium channel may be sensitive to disruption by experimental manipulations during tissue isolation. (Supported by grants NIAAA AA08089 (J.J.W.); AA07297 (R.A.G.); AA05809 and RSDA AA00044 (S.W.L.); and NIAAA training grant AA07471 (J.E.D. and J.L.M.).

218.9

EXCITATORY AMINO ACID-INDUCED SYNAPTIC ACTIVITY BETWEEN CULTURED RAT HIPPOCAMPAL PYRAMIDAL NEURONS ASSESSED USING FURA-2 MICROFLUORIMETRY. Richard J. Miller, Wendy K. Scholz and Kenneth P. Scholz (Spon L. Noronha-Blob). Dept. of Pharm. and Physiol., Univ. of Chicago, Chicago, IL 60637.

Fura-2 microfluorimetry was used to record spontaneous activity in cultures of rat hippocampal pyramidal neurons prepared by the methods of Banker and coworkers (1977) as modified by Scholz et al., (1988). These cells possess NMDA and quisqualate-kainate subclasses of excitatory amino acid receptors as well as receptors for many other neurotransmitters that are endogenous to the hippocampus. Intracellular recording demonstrated that these cells form primarily excitatory synapses when cultured from 17 day embryonic rats. Cell bodies had a basal $[Ca2+]_i$ of 75-150 nM. When the culture dish was perfused with Mg2+-free medium supplemented with 10-1000 nM glycine, neurons exhibited large spontaneous fluctuations in $[Ca2+]_i$ of 50-300 nM. These fluctuations could be blocked by 10 uM APV and 1 uM TTX, indicating that they may be due to enhanced spontaneous synaptic activity that is a consequence of removal of the Mg2+ blockade from the NMDA receptor. In addition, removal of Mg2+ may affect transmitter release or the threshold for generation of action potentials. Activation of receptors that produce presynaptic inhibition in the hippocampal slice preparation also reduce these spontaneous fluctuations of $[Ca2+]_i$. These include receptors for adenosine and neuropeptide Y as well as GABAb receptors. It is possible that the $[Ca2+]_i$ fluctuations observed under these conditions may serve as a useful model of epileptiform activity at hippocampal synapses.

218.10

NMDA RECEPTOR-MEDIATED INCREASES IN CYTOSOLIC CALCIUM IN CEREBELLAR GRANULE CELLS: MODULATION BY GLYCINE AND MAGNESIUM. E.F. Nemeth and T.N. Parks, Natural Products Sciences, Inc., Salt Lake City, UT 84108.

A method for measuring cytosolic free Ca^{2+} ($[Ca^{2+}]_i$) in large, homogeneous populations of normal CNS neurons was developed. Dissociated cerebellar granule cells from 8 day-old rats were prepared by enzymatic treatment and trituration, plated onto polylysine-coated plastic squares, and cultured in the presence of cytosine arabinoside for 7-9 days. Cells were loaded loaded with fura-2 by incubation with fura-2/AM, washed, and the plastic squares transferred to a cuvette containing Mg^{2+}-free buffer for fluorescence recording. The addition of glutamate, aspartate, NMDA or KCl caused rapid, dose-dependent increases in $[Ca^{2+}]_i$ whereas the addition of a variety of other CNS transmitters were without effect on $[Ca^{2+}]_i$. Maximal increases in $[Ca^{2+}]_i$ were obtained with 25 uM NMDA. The response to 40 mM K^+, but not that to 25 uM NMDA, was inhibited by 1 uM nifedipine. The addition of glycine (0.1-1 uM) caused no change in $[Ca^{2+}]_i$ but greatly potentiated the responses to NMDA and to aspartate. In contrast, increases in $[Ca^{2+}]_i$ elicited by NMDA were blocked by prior addition of Mg^{2+} (1 mM). Increases in $[Ca^{2+}]_i$ elicited by NMDA were also inhibited by MK-801 (IC_{50} = 35 nM) or AP-5 (IC_{50} = 20 uM). The effects of glycine, Mg^{2+}, and certain drugs on NMDA-induced increases in $[Ca^{2+}]_i$ in cerebellar granule cells thus parallels their effects on NMDA-induced changes in electrical activity as determined by electrophysiological methods. Measurements of $[Ca^{2+}]_i$ by this means thus provide a valid and simple assay for investigating NMDA receptors and for rapidly assessing the effects of various drugs on functional responses mediated by such receptors.

218.11

ZINC MAY ENTER CORTICAL NEURONS THROUGH NMDA CHANNELS. J. Koh and D.W.Choi. Dept. of Neurology, Stanford Univ. Sch. of Med., Stanford, CA 94305.

Zinc (Zn^{++}) is released at forebrain excitatory synapses with neuronal activity, where it may regulate the activation of glutamate receptors. We have been interested in the additional possibility that such Zn^{++} might enter postsynaptic neurons through open NMDA channels, where it could modulate intracellular processes, or in excess, cause neuronal injury. We examined Zn^{++} entry with a fluorescent chelator specific for Zn^{++} (toluene sulfonamide quinoline, TSQ) (Frederickson et al., J. Neurosci. Meth. 20: 91-103, 1987). Murine cortical cell cultures were exposed for 2 min to 100 μM Zn^{++} in a HEPES buffered salt solution lacking Ca^{++}. Neuronal perikarya and processes showed intense fluorescence after TSQ application. However, when the same Zn^{++} exposure was done in the presence of 30 μM MK-801 or 1 mM D-APV, the staining was markedly reduced. A voltage-dependent Ca^{++} channel blocker, nifedipine, did not attenuate the TSQ signal even at 100 μM. Furthermore, the less intense signal produced by exposure to 50 μM Zn^{++} for 1.5 min could be markedly increased by co-application of 1 mM NMDA. These data suggests that Zn^{++} may enter neurons at least in part through open NMDA channels. If Zn^{++} can be translocated from presynaptic to postsynaptic neurons, it could play a physiological role as a "trans-synaptic messenger."

218.12

MODULATION OF NMDA RESPONSE BY HALOPERIDOL AND *m*-NITRO-PCP. E.S. Rocha[1*], A. Ramoa[1], E.X. Albuquerque[1,2]. (SPON: E.P. Christian) [1]Lab. Mol. Pharmacol. II, UFRJ, Brazil and [2]Dept. Pharmacol., Univ. Md. Sch. Med., Baltimore, MD 21201.

Electrophysiological studies have shown that haloperidol potentiates the MK-801 antagonism of NMDA responses (Monnet *et al., Soc. Neurosci. Abst.*, 1988). We examined the effect of haloperidol on the response properties of single NMDA receptors. Application of NMDA (10-30 μM) to outside-out patches of membrane, excised from cultured fetal rat hippocampal neurons, elicited ion currents which were generally affected by haloperidol. Haloperidol (300 nM-1 μM) potentiated the NMDA response in about half of the patches studied by increasing the frequency of NMDA-induced openings. Potentiation by haloperidol was weaker than that observed with other NMDA receptor potentiating agents such as glycine (1-10 μM) and *m*-NITRO-PCP (5-10 μM) (Ramoa and Albuquerque, *FEBS Lett. 235*:156, 1988). Haloperidol and *m*-NITRO-PCP did not affect channel lifetime. At higher concentrations, however, both haloperidol (5-30 μM) and *m*-NITRO-PCP (5-10 μM), but not glycine (100 μM), markedly reduced the frequency of NMDA openings. This effect was reversed at positive potentials. Channel lifetime (intra-burst openings) was differently affected by both agents: it was reduced by *m*-NITRO-PCP but remained relatively unaltered with haloperidol. Thus, both agents modulated NMDA receptor function in a concentration-dependent manner. At low concentration, they may allosterically enhance the NMDA response. (Support: CNPq & FINEP, Brazil; DAMD17-88-C-8119)

218.13

MODULATION OF NMDA CURRENTS BY INTRACELLULAR PHOSPHORYLATION. M.C. BARTLETT*, M.W. SALTER, & J.F. MACDONALD. Playfair Neurosci. Unit, The Toronto Hosp. & Dept. of Physiol., Univ. of Toronto, Toronto, Ont. M5T 2S8.

NMDA currents "wash out" to about 50% of their initial amplitude during whole-cell patch clamp recordings, an effect which is prevented or reversed by dialysing the neuron with an ATP regenerating solution (MacDonald et al, *J. Physiol.* 1989, in press). Cultured hippocampal neurons were patch-clamped using the whole-cell configuration. NMDA currents were evoked by pressure applications of 250 μM L-aspartate. The intracellular solution consisted of (in mM): 120 CsCl, 35 CsOH, 11 EGTA, 1 $CaCl_2$ (or 80 μM EGTA, 25 μM $CaCl_2$), 2 TEA, 2 $MgCl_2$, 10 HEPES. In addition, the ATP regenerating solutions contained: 4 K-ATP, 20 phosphocreatine, 50 U/ml creatine phosphokinase. Adenosine-5'-0-(3-thiotriphosphate ($ATP\gamma S$) thiophosphorylates proteins which are then resistant to hydrolysis by phosphatases. Replacement of ATP with $ATP\gamma S$ also prevented the wash out of NMDA currents but the non-hydrolyzable analogue β,γ-methylene ATP was unable to retard wash out. To test for the possible involvement of a specific protein kinase we examined the effects of the phorbol ester 12-0-tetradecanoyl phorbol-13 acetate (TPA), an activator of protein kinase C. TPA (10 nM or 3 μM) was applied by bath perfusion. When neurons were dialysed with the ATP regenerating solution TPA caused a reversible depression of NMDA currents. This depression was blocked when TPA was co-perfused with the protein kinase C inhibitor, H7 (20 μM). In contrast, NMDA currents recorded following wash out were not blocked by TPA. Thus, while phosphorylation is required to maintain NMDA currents activation of protein kinase C can, paradoxically, depress NMDA currents. Supported by the Savoy Foundation and the MRC of Canada.

218.14

PHORBOL ESTER ENHANCEMENT OF NMDA-INDUCED CURRENTS IN XENOPUS OOCYTES. John P. Leonard, and Stephen R. Kelso Department of Biological Sciences. University of Illinois at Chicago, Chicago, IL, 60680.

The N-methyl-D-aspartate (NMDA) subclass of receptors for glutamate, has recently drawn attention as a result of its involvement in a range of processes including associative forms of synaptic modification. We wished to determine whether the properties of this receptor could be modulated by C-kinase, which is implicated in LTP in hippocampus.

We have taken advantage of the Xenopus oocyte system for expressing functional channel proteins. Xenopus oocytes were injected with rat brain mRNA and currents were recorded using two-electrode voltage-clamp techniques. All cells (n>30) exhibited NMDA responses with several properties previously shown to be characteristic of the NMDA class of receptors. The response was reversibly inhibited by 0.1 to 1 mM Mg^{2+}, by 2 mM Cd^{2+}, and the specific NMDA antagonist, 2-amino-5-phosphonovaleric acid (APV, 10 μM). In addition, in 20 cells, application of the protein kinase C activator, 4-β-phorbol- 12,13-dibutyrate (PDBu) increased the NMDA-evoked current recorded at -80 mV to > 200% of control. Controls, often in same cell, used α-phorbol, a stereoisomer that does not activate C-kinase. This effect did not require external Ca^{2+}, as substitution for Ca^{2+} with Ba^{2+} yielded similar results.
(Supported by NIH Grants NS26432 (JPL) and NS24591 (SRK))

218.15

"DESENSITIZATION" OF NMDA RESPONSES MAY INVOLVE CHLORIDE IN XENOPUS OOCYTES. Stephen R. Kelso and John P. Leonard. Department of Biological Sciences. University of Illinois at Chicago, Chicago, IL, 60680.

The N-methyl-D-aspartate (NMDA) subclass of glutamate receptors has recently been expressed and studied in oocytes injected with rat brain mRNA. The response to NMDA application typically shows a rapid early inward current that decays in several seconds to a relatively stable level. This decay is reportedly due to desensitization.

Xenopus oocytes were injected with rat brain mRNA and currents recorded using two-electrode voltage-clamp techniques. In some experiments, the transient current could be evoked more than once during a single application of NMDA, suggesting that the receptor did not actually desensitize. A transient inward current would occur immediately after removal of NMDA channel block by either 1 mM Mg^{2+} or 100 μM APV. DNDS, a stilbene derivative that blocks chloride channels, nearly eliminated the transient component. Replacement of extracellular Ca^{2+} with Ba^{2+}, which is a poor activator of $I_{Cl(Ca)}$, also abolished the transient component. In contrast to the case in normal saline, DNDS did not affect the NMDA response in Ba^{2+} saline. We suggest that a significant portion of the NMDA current typically seen in these oocytes is carried by an inward chloride current (as E_{Cl} = -25 mV) triggered by Ca influx through the NMDA receptor/ channel.
(Supported by NIH Grants NS26432 (JPL) and NS24591 (SRK))

218.16

INTRACELLULAR CALCIUM INCREASES THE RATE OF RECOVERY FROM N-METHYL-D-ASPARTATE (NMDA) DESENSITIZATION. Gary D. Clark, David B. Clifford and Charles F. Zorumski; Washington University School of Medicine, St. Louis, MO 63119

Desensitization of NMDA evoked currents in postnatal rat hippocampal neurons is dependent on the presence of extracellular calcium. Using standard patch clamp techniques we have found that in the presence of 1μM glycine responses to NMDA (1mM) attenuate 17 $\pm$ 11% in 19μM calcium and 78 $\pm$ 15% in 3mM calcium. The site of action of calcium in this process is not certain. In the presence of intracellular calcium buffers, NMDA currents desensitize more prominently than when calcium chelators are omitted. Additionally, in the absence of calcium chelators many NMDA currents desensitize initially but exhibit a secondary increase in current during a prolonged NMDA application. The secondary increase is not seen in the presence of 1,2-bis(o-aminophenoxy)ethane -N,N,N',N'-tetraacetic acid (BAPTA) and is not the result of a calcium activated K^+ or Cl^- current. We have also found that in the presence of calcium chelators recovery from NMDA desensitization occurs as a monoexponential process with a time constant of about 60s. In the absence of calcium chelators the time constant of recovery is 12s. These results suggest that increases in intracellular calcium do not promote and actually diminish NMDA desensitization by increasing the rate of recovery.

218.17

Activation kinetics of NMDA, kainate and quisqualate receptors in mouse hippocampus. M.L. Mayer and L. Vyklicky Jr*. Unit of Neurophysiology and Biophysics, LDN, NICHD, NIH, Bethesda, MD 20892.

Excitatory synaptic currents in the CNS often have two components with distinct temporal and pharmacological properties. The epsc due to activation of quisqualate receptors shows fast activation, and decays with a time constant of 1-4 ms; the NMDA receptor mediated component of the epsc has a slower rise time, and a decay time constant of 50 - 150 ms.

We used a fast perfusion system to apply excitatory amino acids to embryonic hippocampal neurons in dissociated cell culture (e.g. PNAS 86, 1411-1415, 1989), and will describe experiments in which the equilibrium dose response relationship and the kinetics of activation and deactivation of NMDA, kainate and quisqualate receptors was studied following rapid application and removal of transmitter candidates.

The activation of NMDA receptors is relatively slow (minimum time constant 7-10 ms at 1 mM NMDA) compared to responses to kainate and quisqualate (minimum time constant ≈ 1.5 ms at 3 mM kainate), suggesting that the slow rise time of the epsc may reflect the gating process at NMDA receptors. However, since NMDA receptor responses deactivate with a time constant of 4-8 ms, the long duration of the epsc most likely reflects rebinding of transmitter in the synaptic cleft.

219.1

EFFECTS OF TTX AND AMILORIDE ON THE SODIUM CHANNEL GATING. M.Ichikawa* and G.Matsumoto* (SPON:K.Kawano). Electrotechnical Lab., Tsukuba, Ibaraki, 305, Japan.

Both TTX and amiloride are known as the sodium channel blocker. It has been understood that these reagents work as a plug into a channel pore and have no appreciable effect on gating current. We measured the sodium gating current of squid giant axons in the presence and absence of one of these reagents or both of them. External 300nM TTX blocked sodium ionic current completely and reduced the gating charge by about 20%. Internal 2mM amiloride exhibited the similar effect on sodium channels. Detailed comparative studies on the effects of TTX and amiloride show, however, that TTX was more influential on the gating current than amiloride was; the total charge more reduced and the falling phase more quickened. When both TTX and amiloride were applied at the same time, the gating current was completely blocked. This result suggests that these reagents have a cooperative effect on the sodium channel gating mechanism.

219.2

KINETIC EVIDENCE FOR TWO Na CHANNELS IN FROG SKELETAL MUSCLE. R. Hahin Biological Sciences Dept. Northern Illinois University, DeKalb, IL 60115

Sodium currents were studied using the vaseline gap voltage clamp method. Frog muscle Na currents activate and inactivate following an initial delay. Inactivation occurs via a double exponential decay for all voltages. Deactivation of Na currents exhibits two components and retains the components after eliminating inactivation with Chloramine-T treatment. These observations are interpreted as the activation, inactivation and deactivation of two subtypes of Na channels. Plots of the slow deactivation component magnitude vs the duration of the eliciting pulse provide a way to determine the kinetics of the putative slow Na channel in muscle. Ammonium substitution for Na in the Ringer produces a voltage dependent activation and inactivation of current which exhibits only one decay phase, suggesting that adjustments of the ionic environments of the channel can mask the presence of one of the channel subtypes so that the kinetically fast Na channel which is the majority carrier of the current can be seen in isolation.

219.3

SODIUM CURRENT EXPRESSED IN Y-79 HUMAN RETINOBLASTOMA CELLS AFTER IN VITRO DIFFERENTIATION. M. Gomez*, G. Waloga* and E. Nasi* (SPON: B. Schnapp). Dept. of Physiol. Boston Univ. Sch. of Med.: Boston, MA 02118.

Y-79, a cell line derived from human retinoblastoma, can be induced to differentiate by altering the culturing conditions, making it possible to investigate the ontogenesis of membrane conductances during the process of differentiation. We had previously reported that undifferentiated Y-79 cells under whole-cell voltage clamp exhibit a small inward current which is carried by both Ca and Na ions (probably sharing a common conduction pathway) and is only partially blocked by tetrodotoxin (TTX). We have recently induced neuron-like differentiation in Y-79 cells by culturing them on a poly-D-lysin and laminin substrate. Differentaited Y-79 cells express a large inward current which is abolished by removal of external Na and is blocked completely and reversibly by TTX. Its fast kinetics resemble the Na current that underlies the action potential in neurons. This could reflect a modification of the original population of channels or the expression of a novel sodium-selective channel.

(Supported by NIH grants EYO-477, EYO-7559 and a fellowship from C.E.S., Colombia S.A.)

219.4

PUMILIOTOXIN-B ACTIVATION OF SODIUM CURRENT IN GUINEA PIG HIPPOCAMPAL NEURONS. R.E.Sheridan, S.S.Deshpande*, F.J.Lebeda, J.P.Apland, D.J.Braitman* and M.Adler. Neurotoxicology Branch, U. S. Army Med. Res. Inst. of Chem. Def., APG, MD 21010.

The actions of Pumiliotoxin-B (PTX-B), extracted from the skin of the frog *Dendrobates pumilio*, were examined in hippocampal slices and in dissociated hippocampal neurons isolated from the adult guinea pig. Application of 0.5-1 μM PTX-B to hippocampal slices caused repetitive field discharges in response to orthodromic stimulation of the CA1 region. In the CA3 subfield, toxin application caused spontaneous epileptiform events. Prolonged toxin incubations reduced excitability in both regions. While washout of toxin transiently restored hyperexcitability, further washing (>30 min.) eliminated all toxin effects. In whole-cell patch clamp of isolated CA1 pyramidal cells, 1-2 μM PTX-B shifted the midpoint of Na^+ current activation 14 mV more negative than normal and increased the apparent voltage sensitivity threefold. PTX-B did not change the rate of Na^+ current inactivation and had no effect on the delayed K^+ current. These experiments suggest that PTX-B binds to the open state of the sodium channel and shifts the activation voltage of toxin-modified channels to more negative potentials.

219.5

EFFECT OF ALIPHATIC ALCOHOLS ON THE BINDING OF [^{3}H]BATRACHOTOXIN BENZOATE TO VOLTAGE-SENSITIVE SODIUM CHANNELS IN SYNAPTONEUROSOMES. C.R. Creveling and M.E. Bell*. Lab. of Bioorganic Chemistry, NIH, Bethesda, MD 20892.

Treatment of synaptoneurosomes from guinea pig cerebral cortex with straight chain alcohols (n-alkanols) from C 1 to C10 inhibited the specific binding of [^{3}H]batrachotoxin-A benzoate ([^{3}H]BTX-B) to voltage-sensitive sodium channels in a concentration-dependent manner. The potency of n-alkanols increased progressively from methanol (Ki=0.88M) to nonanol (Ki=4.5x10^{-5}M). No further increase in potency was observed with decanol. n-Alkanols from C11 to C23 were inactive. The correlation between Ki values and published anesthetic potencies of n-alkanols from C2-C10 approached unity. The inhibition was competitive. n-Alkanols increased the equilibrium dissociation constant without affecting the apparent maximum number of binding sites. The dissociation rate of the [^{3}H]BTX-B/sodium channel complex at equilibrium was accelerated by n-alkanols in a concentration-dependent manner. The apparent competitive inhibition of [^{3}H]BTX-B binding and the accelerated dissociation rate induced by n-alkanols are similar to that observed with other local anesthetics. These results are consistent with an indirect allosteric mechanism whereby the modification by an anesthetic agent of a site(s) closely associated with but distinct from the BTX binding site results in a decrease in the affinity of the BTX-site for BTX. It is suggested that this change in the BTX binding site results from a shift of the ion channel from a conducting to a non-conducting state following the modification of a specific protein(s) by the anesthetic agent. Similar effects of n-alkanols have been obtained with ligands for the central GABA-gated chloride channel suggesting a wider distribution of the protein(s) responsible for the allosteric effect.

219.6

PERMETHRIN ENHANCES DIVALENT CATION INFLUX THROUGH VOLTAGE SENSITIVE SODIUM CHANNELS IN THE POND SNAIL *L. stagnalis*. C.A. Ferguson* and G. Audesirk. Biology Dept. University of Colorado at Denver, 1200 Larimer St., Denver, CO 80204.

Permethrin, a synthetic pyrethroid, has been shown to exert its toxic effect by delaying the inactivation of voltage sensitive sodium channels, thus prolonging open time. Permethrin has also been shown to decrease neurite initiation and elongation in cultured neurons, both thought to be calcium dependent mechanisms.

Voltage clamp studies were done to determine the effect of permethrin on divalent cation influx in the RPD-1 neuron of *L. stagnalis* using barium as the charge carrier. 250uM permethrin causes a forty percent increase in inward barium current over controls, measured at the end of a 200 msec clamp step. TTX (1uM) alone does not affect barium current in control (non-permethrin exposed) neurons. However, upon addition of 1uM TTX to the permethrin, inward barium currents are reduced to control values. This would suggest that the observed increase in inward barium current seen with permethrin is a result of divalent cations flowing through voltage sensitive sodium channels whose open time has been prolonged by permethrin.

219.7

STEADY-STATE ACTIVATION PROPERTIES OF BATRACHOTOXIN-MODIFIED SODIUM CHANNELS IN LIPID BILAYERS. L.D. Chabala, B.W. Urban*, L.B. Weiss, W.N. Green and O.S. Andersen. Depts. of Physiology & Biophysics and Anesthesiology*, Cornell University Medical College, N.Y., N.Y. 10021.

Batrachotoxin-modified sodium channels from canine synaptosomes were incorporated into neutral lipid bilayers. The steady-state activation curve was a sigmoidal function of membrane potential with a midpoint potential (V_a) that varied as a function of symmetrical [NaCl] (-109 mV in 0.1 M NaCl to -75 mV in 1.0 M NaCl). The apparent gating charge (z_a=3.4-3.9 elementary charges) showed no systematic variation. The positive shifts in V_a along the voltage axis as [NaCl] increased were interpreted in terms of screening of negative charges (Gouy-Chapman theory) near the gating machinery. The external surface has the larger apparent charge density, which was quantified by experiments in asymmetrical NaCl. At constant [NaCl], V_a could vary by as much as 20 mV, and spontaneous shifts in gating were often observed that resulted in a shift of the activation curve along the voltage axis with little change in z_a. At low salt (0.1 M), symmetrical additions of 0.005 M Ba^{+2} caused a depolarizing shift in V_a, ~two-fold larger shifts were seen when Ba^{+2} was added only to the extracellular side. Ba^{+2} is screening negative charges, but, from the magnitude of the shift, it appears that Ba^{+2} may also bind to the gating machinery, since Ba^{+2} addition generally reduces z_a.

219.8

FURTHER CHARACTERIZATION OF SINGLE SODIUM CHANNELS FROM DIFFERENT HUMAN BRAIN TISSUES. C. Frenkel*, DS Duch*, and BW Urban. Anesth & Physiol Depts, Cornell U Med Coll, New York, NY 10021; Inst Anästh, Univ Bonn, 5300 Bonn, FRG.

Continuing previous work, sodium channels from diseased and non-diseased human brain were incorporated into planar lipid bilayers in the presence of 0.25 and 1 uM batrachotoxin (BTX). The following single channel properties were examined, serving as an indicator of whether the corresponding distinct functional domains of the channel protein remained similar or unchanged in the two tissues: single channel conductance and subconductance states (ion pathway), steady-state activation (voltage sensor), fractional open times outside the gating region (BTX-binding site), tetrodotoxin block (TTX-binding site) and pharmacologic interactions (hydrophobic protein domains). Effects of surface charge were tested by changing electrolyte concentrations. The single channel properties did not depend on BTX concentration (close to K_D) used. No statistically significant differences were noted comparing preparations from the different tissues. These results indicate either that sodium channels from a variety of brain tissues do not differ in fundamental characteristics or that the present technique is highly selective for a particular type of sodium channel. Single channel properties did not change over a time period of 20 months, demonstrating a viable and extremely stable sodium channel system suitable for structure-function and pharmacologic studies.

219.9

THE VOLTAGE-SENSITIVE SODIUM CHANNEL IN HUMAN BRAIN. C.M. Lu, R.E. Powers*, AND G.B. Brown. Neuropsychiat. Res. Prog., Dept. Psychiat. & Biochem., Univ. of AL. at B'ham, B'ham., AL. 35294.

The voltage-sensitive Na-channel has been extensively studied in animal models, but very little is known about human Na-channels. Therefore, we have undertaken to provide a pharmacological profile of human brain Na-channels by assessing the binding characteristics of several Na-channel specific neurotoxins.

In preparation for similar studies involving human brain tissue, we examined the post-mortem stability of Na-channel in rat. Rat brain tissue was left intact for 0,4,8, and 12 hrs post-mortem before preparing synaptoneurosomes as described[1]. The channel stability was determined by measuring total specific binding of ^{3}H-batrachotoxin A 20-α-benzoate (^{3}H-BTX-B) at different post-mortem times as mentioned. The channel protein was found to be quite stable even if the brain tissue was not dissected until 12 hrs post-mortem. For the effect of long-term storage time, tissue was stored in a -70°C freezer for up to 12 mo. before making the synaptoneurosomes. Data collection is still in progress at this point. Saxitoxin (STX) binding behavior has been examined in human brain tissue. Synaptoneurosomes were prepared from frontal pole of a normal human brain[2]. This brain was dissected 7 hrs post-mortem and had been stored at -70°C. Binding reactions were initiated by addition of 150 µl of 2 nM ^{3}H-STX to a mixture of 150 µl synaptoneurosome preparation plus varying concentrations of unlabeled STX. The K_d was found to be 3.9 nM with a B_{max} of 2.34 pmol/mg protein. Preliminary results with ^{3}H-BTX-B binding suggest a slightly lower binding affinity for human Na channels than for rat. These results demonstrate the feasibility of preparing synaptoneurosomes from post-mortem human brain and open the way to the development of a pharmacological profile for human brain Na-channel. 1. Brown, G.B. (1986) J. Neursci. 6(7): 2064. 2. All experimental procedures have been reviewed and approved by the Human Use Committee of UAB.

219.10

THE ROLE OF ALTERED SODIUM CURRENTS IN ACTION POTENTIAL ABNORMALITIES OF CULTURED DORSAL ROOT GANGLION NEURONS FROM TRISOMY 21 HUMAN FETUSES. P. Caviedes*, B. Ault* and S. I. Rapoport (SPON: A. NORONHA). Laboratory of Neurosciences, National Institutes on Aging, NIH, Bethesda, MD 20892, U.S.A.

Trisomy 21 (Down Syndrome) results in abnormalities in electrical membrane properties of cultured human fetal dorsal root ganglion (DRG) neurons. Action potentials show faster depolarization and repolarization rates, with decreased spike duration, compared to diploid neurons. To analyze the faster depolarization rate of trisomic neurons, we studied the sodium currents of cultured human fetal DRG neurons from trisomy 21 and control subjects, using the whole cell patch clamp technique. A fast, tetrodotoxin (TTX)-sensitive Na^+ current; and a slow, TTX-resistant component were identified. The inactivation curves of both current types in trisomic neurons showed a 10 mV shift towards more depolarized potentials compared to control neurons. Thus, whereas essentially all of the fast Na^+ channels were inactivated at normal resting potentials in control cells, approximately 10% of these channels were available for activation in trisomy 21 cells. Further, the fast current showed accelerated activation kinetics in trisomic neurons. The slow Na^+ current of trisomic neurons showed slower deactivation kinetics than control cells. No differences in maximal conductances were observed between trisomic and control neurons. The data indicates that acceleration of the depolarizing phase of the action potential in trisomy 21 neurons at resting potentials is primarily due to activation of residual fast Na^+ channels that have accelerated activation kinetics.

219.11

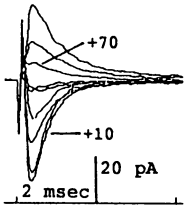
TTX-SENSITIVE AND -RESISTANT SODIUM CHANNELS IN A HUMAN NEUROBLASTOMA CELL LINE THAT DIFFERENTIATES IN RESPONSE TO RETINOIC ACID. R.E. Weiss* & N. Sidell*‡ (SPON: L. Kruger). Depts. of Pediatric Cardiology and *Pathology, UCLA School of Medicine, Los Angeles, CA 90024.

Na currents were recorded from human neuroblastoma cells (LA-N-5) before and after treatment with $4x10^{-6}$ M retinoic acid (RA) and 10^{-3} M dibutyrl cAMP with the whole-cell voltage clamp technique. Cells were bathed in a Tyrodes solution at 18° C. K currents were blocked with internal Cs. The holding potential was -80 mV. Peak Na currents were recorded at test potentials near 0 mV. The mid-point of steady state inactivation was between -40 and -50 mV. Dose-response experiments demonstrated that 10-20% of the Na current was TTX resistant, requiring >10 µM TTX for complete block. All Na currents were blocked by Co at a half-blocking concentration of 0.1-0.2 mM. After 10 days post RA treatment, the kinetic properties of the Na current appeared unchanged. Preliminary results suggest that the ratio of TTX-resistant to TTX-sensitive Na channels declines.
Supported by grants from MDA and AHA to R.E.W. and NIH to N.S.

219.12

PATCH-CLAMP CHARACTERIZATION OF NATIVE Na CHANNELS FROM ELECTROCYTES OF THE ELECTRIC EEL *Electrophorus electricus*. S. Shenkel* (SPON: W.S. Agnew). Dept. of Cellular & Molecular Physiol., Yale Univ. School of Medicine, New Haven, CT 06510.

Macroscopic and single-channel currents were recorded from inside-out patches of innervated electrocyte membrane at 22-24°C. The figure shows currents in response to steps from -40 to +100 mV. Peak currents of 40 to 90 pA were obtained between 0 and +10 mV. Currents were characterized by an activation midpoint potential near -5 mV and an apparent gating charge of ~ 3e. The mean current was half inactivated at -87 mV. The single-exponential inactivation time-constants became shorter between -50 mV (~1 msec) and 0 mV (~0.3 msec) and gradually increased with stronger depolarizations to ~1 msec at +130 mV. Under biionic conditions the selectivity ratio P_{Na}/P_K was concentration dependent. The single-channel slope conductance was ~16 pS (mM: 200 Na_{pip}/200 K_{bath}) at -40 mV.



219.13

DEAE, A PROCAINE METABOLITE, RAISES FIRING THRESHOLD OF PYRAMIDAL CELLS. L.R. Cole*, and J.F. Butterworth IV. Dept. of Anesthesia, Wake Forest Univ. Medical Center, Winston-Salem, NC 27103

Procaine, a local anesthetic which is rapidly metabolized in serum to diethylaminoethanol (DEAE) and p-aminobenzoic acid, has been shown to produce long-lasting analgesia after intravenous infusion.[1,2] These findings could be explained if procaine metabolites had pharmacological activity. The present study was undertaken to determine whether DEAE alters excitability of brain cells similarly to procaine, its parent compound.

Microelectrode current clamping methods were used to measure threshold and spike amplitude before and after drug application to rat hippocampal slices.

Procaine greatly increased threshold (300-400%) at concentrations (0.5 & 1.0mM) which minimally reduced spike amplitude, and did not inhibit impulse conduction. Likewise, 5mM DEAE increased threshold (300-400%) but permitted impulse conduction.

Our findings demonstrate that DEAE, at a concentration obtainable after prolonged procaine administration, alters excitability of brain cells. Thus, persisting DEAE levels may underlie the prolonged effects of procaine infusions.

1. J Pharmacol Exp Ther 94:359-366, 1948
2. J Am Geriatr Soc 25:1-19, 1977

219.14

EFFECTS OF CYANIDE ON THE EXCITABILITY OF CULTURED NEUROBLASTOMA CELLS. S.S.Deshpande*,M.Adler,R.E.Sheridan and D.J.BRAITMAN*, Neurotoxicology Branch, U.S. Army Med. Res. Inst. of Chem. Def., Aberdeen Proving Ground, MD 21010

Mechanisms underlying alteration of excitability by sodium cyanide (CN) were investigated in hybrid NG108-15 neuroblastoma cells differentiated with 1mM dibutyryl cAMP. These cells exhibit action potentials as a result of a sequential increase in conductance to Na^+ (Na-spike) and Ca^{2+} (Ca-spike). After 15 minutes perfusion, CN (1-5mM) produced a selective inhibition of the Ca-spike elicited by anodal break stimulation. After 30-45 minutes CN exposure,Ca-spike threshold increased, the spike amplitude was depressed 20-30% and the time course was prolonged. This depression was further enhanced during repetitive stimulation as shown by a decline in amplitude of successive responses which led to a complete block. These effects were reversible; complete recovery was observed after 45-60 minutes of perfusion with CN-free medium. Higher CN concentration (10mM) produced depression of Na^+ as well as Ca^{2+} components of the action potential. Although the role of cellular anoxia in inducing these effects is not clear, the preferential action of CN in suppressing the Ca-spike suggests an alteration of Ca^{2+} conductance as shown earlier, in mouse dorsal root ganglion cells (Duchen, M.R. and Somjen, G.G., J. Physiol, 401, 61-P, 1988).

219.15

ANTITOXIN COMPONENTS OF SCORPION VENOM (LEIURUS QUINQUESTRIATUS) G.B. Brown, R. J. Bradley and J.E. Gaupp*. Neuropsych. Res. Prog. and Dept. of Psych., Univ. of AL at B'ham, Birmingham, AL 35294

Scorpion venoms from a variety of species are typically multicomponent mixtures of polypeptides, the individual components of which often share significant sequence homology with other constituents of both the same and other venoms. Thus, families of sodium channel scorpion polypeptide neurotoxins (α, β, γ toxins) have been classified on the basis of sequence homologies, immunological cross-reactivity, and common sodium channel effects (e.g. Kopeyan et al., FEBS Lett. 181: 211-217, 1985). Due to the presence of numerous homologs with varying activities, we hypothesized that there may be present in the venom components (albeit at low concentrations) that bind the Na channel receptor with high affinity, yet lack efficacy. Such components would represent natural experiments with relevance to structure-activity relationships of scorpion neurotoxins and might provide useful antitoxins. We now report the presence of such components in the venom of L. quinquestriatus with antitoxin activity apparently directed against scorpion α-toxins.

Whole venom (Sigma Chemical Co.) was dissolved in 0.1 M NH_4OAc, pH 6.9 (40 mg/ml) and fractionated on a Rainin Dynamax C18 column (1x25 cm) using a 2-step gradient between 15 and 80% isopropanol in ammonium acetate. Fractions eluting at 15% and at 80% isopropanol were identified in a screening assay as having an inhibitory effect on α-scorpion toxin-enhanced BTX-B binding to rat brain sodium channels. The latter fraction was the more potent and has been further studied. Pretreatment of isolated rat phrenic nerve with this fraction at a concentration corresponding to 0.2 mg whole venom/ml had no effect on the compound action potential (CAP), but did inhibit the effect of L. quinquestriatus toxin V (an α-toxin) on the CAP. Further purification of this component by gradient HPLC on a C4 reversed phase column has yielded an active fraction that is ≥ 80% pure by native and SDS PAGE. We are currently working to obtain sequence information for this interesting component.

219.16

CONDUCTION MEASURED IN SINGLE DEMYELINATED AND REMYELINATING AXONS USING OPTICAL TECHNIQUES. P. Shrager, C. Rubinstein* and E. Brunschweiger*. Dept. of Physiology, Univ. of Rochester Medical Center, Rochester, NY 14642.

In amphibian and mammalian peripheral axons sodium channels are present in demyelinated internodes, though at much lower densities than at nodes of Ranvier (Brain Res. 483: 149 (1989)). In the present study we have followed conduction in single fibers optically. *Xenopus* sciatic nerves demyelinated by lysolecithin were stained with the dye RH155. Light absorption measurements were made at 705±25 nm. In control nerves signals were confined to the nodal region (velocities, 7-24 m/s). At one week post-injection some axons were covered by loose, disrupted myelin while others had been completely demyelinated by macrophages. Optical records showed that action potentials could propagate >1 mm in these cells (at ~1 m/s), though successful conduction entirely through the lesion (~3 mm) was very rare. At 10-12 days post-injection proliferating Schwann cells begin to envelop the demyelinated fibers and at 14-15 days the first few lamellae of new myelin can be seen. Conduction through these zones remains at about 1 m/s, but increasing numbers of signals traverse the entire demyelinated region. Calculations allow an assessment of the relative importance of internodal sodium channels and of new, thin myelin in this process. Supported by the NIH (NS17965) and the National Multiple Sclerosis Society (RG-1774).

POTASSIUM CHANNELS III

220.1

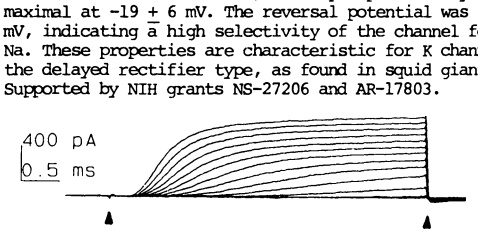
The MBK1 mouse K^+ channel protein is encoded by a single uninterrupted exon. K. G. Chandy*, #C. Williams*, R. H. Spencer*, +B. Tempel, #G. A. Gutman* (SPON: M. Cahalan). Dept. of Med. & #Dept. of Microbiology & Molecular Genetics, Univ. of CA Irvine, CA 92717; +Geriatric Res , Education and Clin. Ctr, VA Med. Ctr, Seattle.

Several classes of K^+ channels have been identified in diverse cell types. The molecular mechanisms responsible for generating these physiologically diverse K^+ channels are best understood in *Drosophila*. Four different K^+ channel proteins arise from the *Shaker* locus through alternative splicing of a single primary transcript. Expression of cDNAs encoding these proteins in *Xenopus* oocytes gives rise to voltage-dependent K^+ currents with different physiological properties. In contrast, we report here that the voltage-dependent K^+ channel gene from mouse brain, MBK1, is encoded by a single, uninterrupted exon. Furthermore, two genes closely related to MBK1 are encoded at distinct genomic loci. Restriction map data suggest that one of these genes encodes the mouse homologue of the second rat brain K^+ channel (RBK2) cDNA, implying that it may also have an intronless coding region. If other mammalian K^+ channels have intronless coding regions, their genomic sequences may be readily expressible in *Xenopus* oocytes, thereby facilitating the experimental association of K^+ channel genes with the biophysical behavior of the proteins they encode. These data suggest that discrete genes may be responsible for K^+ channel diversity in mammalian cells.

220.2

MOUSE BRAIN GENE MBK1 CODES FOR A VOLTAGE-SENSITIVE K CHANNEL OF THE DELAYED RECTIFIER TYPE. K. M. Houamed*, B. L. Tempel and W. Almers, Depts. of Physiology & Biophysics and Pharmacology, Univ. of Washington, Seattle, WA 98195

Because MBK1 is highly homologous to the *Shaker* gene of *Drosophila*, MBK1 was suggested to code for a voltage-sensitive K channel (Tempel et al., Nature 332, 847). Indeed, mRNA transcribed from MBK1 in vitro caused the appearance of voltage-sensitive K channels (30 µA at 10 mV) in *Xenopus* oocytes. Cell-attached patch recordings were made from 4 cells with 5 µm tip pipettes filled with Ringer. In the Fig., 16 voltage pulses (arrows) to between -50 and 90 mV were applied from a holding potential of -100 mV. Channels opened with a delay. Activation was rapid, being half-maximal within 1.8 ± 0.1 ms at 50 mV. No inactivation is evident (< 3% in 1 s at 50 mV). The open probability was half maximal at -19 ± 6 mV. The reversal potential was 89 ± 8 mV, indicating a high selectivity of the channel for K over Na. These properties are characteristic for K channels of the delayed rectifier type, as found in squid giant axons. Supported by NIH grants NS-27206 and AR-17803.



220.3

A NON A-TYPE POTASSIUM CHANNEL WITH DELAYED RECTIFIER PROPERTIES: ISOLATION THROUGH EXPRESSION CLONING IN *XENOPUS* OOCYTES. G.C. Frech, A.M.J. VanDongen, G. Schuster, A.M. Brown and R.H. Joho. (SPON: D. Kunze). Department of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, TX 77030.

Voltage-activated potassium (K^+) channels are an exceptionally diverse and widely distributed class of ion channels. We have isolated from the rat brain and expressed in *Xenopus* oocytes a new type of K^+ channel genc (non A-type) with properties of a delayed rectifier. Size-fractionated rat brain poly(A)$^+$ RNA was microinjected into *Xenopus* oocytes to identify fractions expressing potassium channels. Oocytes injected with RNA enriched for sizes between 3.3-4.2 kb expressed a depolarization-induced outward potassium current (I_K). This RNA was used to construct, in a transcription-competent vector, a directional cDNA library enriched for full-length inserts (Frech, G. & Joho, R. *Gene Anal. Techn.* 6:33, 1989). DNA from pools of cDNA clones were used as templates for *in vitro* synthesis of mRNA. After microinjection of oocytes with transcripts, we detected I_K currents in several pools of 10,000 recombinants. Using the oocyte system as a screening assay and by selecting and subdividing "positive" pools containing 1000, 100, and 12 clones, a single functional clone drk1 (with a 3.4 kb insert) was isolated. The electrophysiological properties of this K^+ channel were reminiscent of the delayed rectifier. The expressed channels showed sigmoidal voltage-dependent activation, inactivated only partially with a time constant of several seconds, and were selective for K^+ over Na^+. The DNA sequence of drk1 was determined and showed a open reading frame encoding a protein of 853 amino acids. Comparison of drk1 to K^+ channel sequences from *Drosophila*, rat and mouse shows that drk1 is most closely related to Shab from *Drosophila* (70 % identity in the core region), whereas A-type channels from *Drosophila* and rat brain do not show a close relationship. The fact that A-type channel genes of rat and *Drosophila* are more related to one another than to our new K^+ channel indicates that A-type and non-A-type K^+ channels are evolutionarily older than the common ancestor of flies and mammals.

220.4

CHARACTERIZATION OF A CLONED DELAYED RECTIFIER POTASSIUM CHANNEL FROM RAT BRAIN EXPRESSED IN *Xenopus laevis* OOCYTES. A.M.J. VanDongen*, G.C. Frech*, R.H. Joho*, and A.M. Brown,
Dept of Molecular Physiology and Biophysics,
Baylor College of Medicine, Houston, Texas, TX77030.

Voltage dependent K^+ channels are diverse and widespread. We have recently isolated a cDNA clone (*drk1*) encoding a K^+ channel from rat brain by expression cloning, using *Xenopus* oocytes (Frech *et al.*, this meeting). This channel was expressed in oocytes by injection of *in vitro* transcripts and functionally characterized using 2-electrode voltage clamp (2EVC) and patch clamp techniques. Depolarizing voltage steps from holding potentials between -80 and -30 mV produced large (> 10 μA) outward currents, which did not inactivate within 500 msec. In 2EVC experiments, threshold for activation was -20 mV, time to half maximal activation ranged from 100 to 10 msec, and activation kinetics were sigmoidal. Using 10 second voltage steps, partial inactivation did occur with a time constant of several seconds. Reversal potentials were estimated from instantaneous current-voltage relationships. Substituting external Na^+ for N-Methyl-D-Glucamine$^+$ had a negligible effect, while varying external K^+ concentration shifted the reversal potential with a slope of 48 mV per decade. K^+ currents were more sensitive to 4-AP (IC_{50}=0.5 mM) then TEA (IC_{50}=10 mM). The density of expressed channels was so large that in cell-attached patch experiments using pipettes of 1 Mohm, macroscopic K^+ currents (up to 500 pA) were recorded. Single channel current were recorded using small pipettes and oocytes injected with less RNA. The unitary current amplitude at +40 mV was 0.5 pA, the mean open time was 5 msec and the probability of opening versus time resembled macroscopic currents. Functionally, *drk1* expresses a channel that activates more slowly than any of the cloned K^+ channels expressed in oocytes thus far and more closely resembles a delayed rectifier than an A channel. Since *drk1* is also structurally distinct from reported K^+ channel cDNAs, we have identified a novel K^+ gene.

220.5

FUNCTIONAL CROSS-HYBRIDIZING CLONES OF A RAT BRAIN DELAYED OUTWARD RECTIFYING POTASSIUM CHANNEL. J.A. Drewe, G. Frech, T. VanDongen, A.M. Brown and R.H. Joho. (SPON: P. Rutecki). Dept. of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, TX 77030.

The cloning and expression of a rat brain delayed outward rectifying potassium channel by Frech *et al* is being presented at this meeting. Families of related gene products have been found for both the rat sodium channel and for the *Drosophila* transient potassium channel. Low-stringency cross-hybridization techniques were used to isolate related clones from other rat brain cDNA libraries. The potassium channel clone was nick translated and used to screen 500,000 cloning events. The hybridization conditions were 50% formamide at 30°C, washing with 290 mM NaCl, 18 mM PO_4 and 2 mM EDTA (2X SSPE) at 35°C. Thirteen positive clones were isolated, three of which with indications of low homology. RNA has been transcribed *in vitro* from ten of these clones and injected into *Xenopus* oocytes for expression. Variations have been found in the electrophysiological kinetics of the expressed currents. These are in the process of being correlated with differences in the primary sequences. These results are likely to provide information about the molecular bases for structure-function for this rat brain delayed outward potassium channel.

220.6

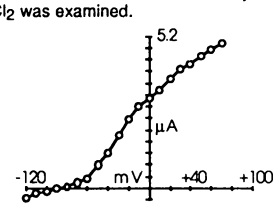
PROPERTIES OF A CLONED RAT BRAIN POTASSIUM CHANNEL EXPRESSED IN XENOPUS OOCYTES. M.J.Christie, J.P.Adelman,* J.Douglass* and R.A.North Vollum Institute, Oregon Health Sci. Univ. Portland, OR 97201.

A clone (RBK-1) which was isolated from a rat hippocampus cDNA library encodes a voltage activated potassium channel which is sensitive to blockade by 4-aminopyridine and tetraethylammonium, (Christie et al, Science 244:221, 1989). Therefore, the current expressed in Xenopus oocytes has features in common with both delayed rectifier and A-currents in hippocampal neurons. Further properties of the current were examined in the present study. Recordings of membrane current were made during depolarizing pulses using oocytes injected with RNA transcribed in vitro from RBK-1. The concentration of blockers producing a 50% reduction of the current was 5.5 ± 0.4 nM (n=3) for charybdotoxin and 6.1 ± 0.8 (n=6) for α-dendrotoxin. β-bungarotoxin (300 nM) did not block the current, or affect the inhibition produced by α-dendrotoxin (10 nM). The expressed current differs in toxin sensitivity from both delayed rectifier and A-currents in hippocampal neurons. The predicted amino acid sequence of RBK-1 contains a consensus sequence for phosphorylation by A-kinase. Application of forskolin (3 μM) or injection of cAMP (4 mM) into oocytes did not affect the outward current. When the phosphorylation site of RBK-1 was changed from serine (ser^{445}) to alanine by site directed mutagenesis, the induced currents were indistinguishable from the wild type.

220.7

EXPRESSION OF A POTASSIUM CHANNEL FROM RAT BRAIN cDNA IN *XENOPUS* OOCYTES. D.L. Lewis, D.E. Clapham* and E.G. Peralta*. Dept. Pharm., Mayo Clinic, Rochester, MN 55905 and Dept. Molecular Biology, Genentech, Inc., San Francisco, CA 94143.

An oligonucleotide probe encoding amino acids 72-98 of a putative mouse potassium channel protein was used to screen a rat forebrain cDNA library under high stringency conditions. A single clone was identified which encoded a 495 amino acid protein displaying >90% identity with the mouse protein sequence. In vitro transcripts encoding this putative rat forebrain potassium channel were synthesized following insertion of the cDNA into a plasmid vector containing an SP6 promoter sequence. *Xenopus* oocytes were injected with 25ng mRNA. Whole cell currents were recorded using a two microelectrode voltage clamp on day 3. A non-inactivating, voltage-dependent outward potassium current was recorded (Fig). This current activated at potentials ≥-60mV. Control oocytes never showed this outward current. The sensitivity of this current to TEA, 4-AP and $BaCl_2$ was examined.



220.8

CLONING AND EXPRESSION OF K^+ CHANNELS FROM RAT BRAIN R.Swanson, K. Folander, J. Antanavage, C. Bennett, J. Williams, *L. Kaczmarek, R.B. Stein, and J. Smith Merck Sharp and Dohme Research Labs, West Point,PA and *Dept. of Pharmacology, Yale Univ. Sch. Med., New Haven, CT

K41, a cDNA encoding the putative carboxy terminal half of a rat brain K^+ channel (Kaczmarek, 1988), was extended in the 5' direction by constructing a specifically primed cDNA library. Two classes of upstream sequences were isolated. One, represented by clone A, contains sequences identical to the 5' end of K41 while the other, represented by clone B, is homologous but nonidentical. Clone A, when ligated to K41, induces a delayed rectifier type I_K in Xenopus oocytes. It activates in a voltage-dependent manner with a midpoint of activation at +20mV. Time to peak current (at 40mV) is 38ms at room temp, and the currents show only partial inactivation during depolarizing pulses as long as 10s. Clone B, when ligated to K41, induces the expression of a more rapidly activating I_K with a time to peak (at 40mV) of 12ms at room temp. It activates at voltages more negative than does clone A, with a midpoint of activation near -25mV, and also shows little inactivation during prolonged depolarizing steps. Both currents are blocked completely by 1 mM 4AP and are relatively insensitive to block by TEA (IC_{50}=100 mM).

220.9

ISOLATION OF A cDNA CLONE CODING FOR A SECOND POTASSIUM CHANNEL EXPRESSED IN RAT BRAIN. D. McKinnon. Department of Pharmacology, Washington University Medical School, St. Louis, MO 63110.

A second potassium channel expressed in rat brain is identified by cloning and characterization of cDNA clones. Two cDNA clones, isolated from rat brain libraries, encode a 499 residue protein that is 80% identical with a previously described rat brain potassium channel and 68% identical with a *Drosophila* potassium channel. This new channel is called BK2 to distinguish it from the previously described potassium channel (BK1). The BK2 gene, unlike the *Drosophila* potassium channel gene complex, appears to produce a single, large RNA transcript. Southern analysis of rat genomic DNA indicates that the BK1 and BK2 transcripts are the products of independent genes. Analysis of the distribution of BK2 transcripts in rat brain by in situ hybridization histochemistry suggest that the BK2 gene is ubiquitously expressed by CNS neurons. Identification of this putative second mammalian potassium channel cDNA indicates the existence of a potassium channel gene family, confirming electrophysiological data on the diversity of potassium channels expressed in rat brain.

220.10

CLONING AND EXPRESSION OF A DELAYED RECTIFIER TYPE K^+ CHANNEL FROM NEONATAL RAT HEART AND DES-PRIMED RAT UTERUS K. Folander, J. Smith, J. Antanavage, C. Bennett, R.B.Stein and R. Swanson (SPON:R. Gould) Merck Sharp and Dohme Research Labs, West Point, Pennsylvania

Northern blot analysis of rat poly A^+ mRNAs, probed with oligonucleotides derived from the sequence of a K^+ channel from rat kidney (Takumi et al, 1988), identified bands of the same size (0.7kb) in the poly A^+ fractions of RNA from kidney, neonatal heart, and DES-primed uterus. No signal was observed in mRNA from brain, adult heart, or non-primed uterus. The polymerase chain reaction was used to clone the cDNAs encoding the channel from neonatal heart and DES-primed uterus. The nucleotide sequences of both clones are identical to that from kidney. Injection of RNA transcripts of the clones into Xenopus oocytes resulted in the expression of outward K^+ currents, measured using standard two microelectrode voltage clamp technique. The currents slowly activate over a time course of seconds in response to depolarizing potentials of $\geq$-20mV. Currents were K^+ selective as determined by tail current analysis in different K^+ concentrations. TEA (20mM) partially blocked the slow outward currents and the antiarrhythmic drug, clofilium, blocked the current with an IC_{50}=75uM. Antisense oligonucleotides derived from the clones specifically inhibit the expression of the slow outward current observed in oocytes injected with mRNA from the parent tissues (eg., kidney or uterus).

220.11

SIZE FRACTIONATED RAT BRAIN mRNA INDUCES VARIOUS VOLTAGE DEPENDENT POTASSIUM CHANNELS IN XENOPUS OOCYTES. J.H. Hoger*[1], B. Rudy[2], N. Davidson[1],& H. Lester[1]. Divs. of Biology & Chemistry, Caltech, Pasadena, CA 91125. and Dept. of Physiology & Biophysics, N.Y.U. Med. Ctr., N.Y., N.Y. 10016.

The great diversity of voltage dependent K channels encoded in rat brain mRNA was studied by two microelectrode voltage clamp experiments of Xenopus oocytes injected with unfractionated and sucrose gradient size fractionated poly(A) RNA. Our main conclusions are: (1) injection of the principal I_A current encoding fractions (5-7Kb) gives tetraethylammonium (TEA) sensitive channels unlike most of the I_A in unfractionated RNA. (2) The predominent delayed rectifier (I_k) currents are encoded in a (7-10 Kb) fraction. TEA (40mM) blocks only 50% of this I_k. This I_k does not inactivate during a 3sec depolarization at +30mV. (3) The I_k encoded in the (3 -7Kb) fractions contain components of different TEA sensitivity as compared to the unfractionated RNA. A component in these fractions undergoes inactivation at +30mV with a time constant of 2 sec.

Overall, these studies suggest a high degree of diversity in rat brain K channels. Supported by NIH GM-10991, GM-29836, GM-26976, Klingenstein Foundation, and an American Cancer Society Fellowship to JHH.

220.12

Cloning and characterization of human potassium channel genes. M. K. Mathew* §, M. Ramaswami* §, M. Gautam*§, C. A. Kamb* ‡, B. Rudy † & M. A. Tanouye §. § Division of Biology, California Institute of Technology, Pasadena, CA; ‡ Department of Biochemistry and Biophysics, University of California, San Francisco, CA; † Department of Physiology, New York University, New York, NY.

The polymerase chain reaction (PCR) can be used to amplify a region of DNA between two defined sequences. Using sequences conserved at the amino acid level between the *Shaker* potassium channel gene from *Drosophila* and a mouse homolog, six different sequences were amplified from human genomic DNA [Kamb *et al.* Proc. Natl. Acad. Sci., in press]. These amplified fragments were then used to probe several cDNA libraries prepared from human brain. cDNAs corresponding to five of the six amplified fragments were isolated and sequenced. All of the sequences are very similar to *Shaker* at the amino acid level and suggest similar structural organization for the encoded proteins. The nucleotide sequences, however, are quite dissimilar even among the various human clones indicating that they are derived from distinct genes. Amino acid sequence similarities are maximal in the putative voltage sensing element (S4) and in flanking hydrophobic segments that may define transmembrane helices. The connecting loops, on the other hand, are less conserved.

Correlation of the functional properties of these channels, as assayed after expression in Xenopus oocytes, with their sequences should allow the formulation of hypotheses regarding the structural basis of potassium channel function and diversity.

220.13

Molecular Cloning of a Rat Heart Potassium Channel cDNA. J. C-L. Tseng-Crank* and M. A. Tanouye. (SPON: W. D. Crank) Division of Biology, California Institute of Technology, Pasadena, CA 91125.

Cardiovascular-specific K channels are potential targets of anti-arrhythmia and anti-hypertensive drugs. We initiated investigation of rat heart K channels by using PCR method to amplify K channel-specific sequences from cDNA libraries. Amino acid sequences conserved among *Drosophila*, mouse, and rat K channels were selected to make degenerate oligonucleotide primers. A 220 bp fragment corresponding to transmembrane domains H4 to H6 was cloned and named RHKp. The amino acid sequence of RHKp is highly conserved compared to the corresponding region in a rat brain clone RCK1 (Baumann et al., EMBO J. 7:2457, 1988), with differences mainly in the loop connecting H4 and H5. One significant change from Tyr to Lys is found in H5. The DNA sequences are 77% homologous, with differences mostly in the third base position. Southern analysis suggests that in addition to the gene corresponding to RHKp there is another gene with substantial sequence homology. Using RHKp to screen heart library yielded 2 cDNAs of insert sizes 3.2 Kb and 1.8 Kb. *Xenopus* oocyte expression experiment may provide evidence correlating this gene to the known heart K channels.

220.14

THE SHAKER GENE SUPERFAMILY OF PUTATIVE POTASSIUM CHANNELS IN THE MAMMALIAN BRAIN. M. Pak*, A. Ratcliffe*, A. Wei, A. Butler*, D. Gottlieb and L. Salkoff. Dept. of Anat. & Neurobiol., Wash. U. Sch. Med., St. Louis, MO 63110

Previously we demonstrated in Drosophila the existence of a family of Shaker-like genes, Shab, Shaw, and Shal. We now report that similar genes exist in mammals and that each Drosophila gene may correspond to a separate subfamily of genes in mammals. In Drosophila the Shaker gene primary transcript is extensively alternatively spliced to produce a subfamily of highly similar potassium channel proteins (Papazian et al., Science, 1987). In mammals, however, there is no evidence that extensive alternative splicing exists. Instead, a Shaker subfamily of separate but highly similar genes may produce the minor variation of protein products (McKinnon, JBC, 1989). In mammals, similar subfamilies may exist representing perhaps all of the Drosophila genes. These subfamilies apparently produce proteins which vary only slightly in features relative to the differences seen between families. The diversification of the Shaker gene superfamily probably occurred prior to the separation of vertebrates and invertebrates.

220.15

CLONING OF *APLYSIA* POTASSIUM CHANNEL WITH HOMOLOGY TO *DROSOPHILA SHAKER*. P. Pfaffinger*, B. Zhao*, M. Knapp*, J. Brunet*, D. Dugan*, and E.R. Kandel. HHMI, Columbia Univ., N.Y., N.Y. 10032.

Modulation of K^+ channels contributes to various forms of synaptic plasticity in *Aplysia*. To begin to examine K^+ channel modulation on the molecular level, we have cloned a K^+ channel from *Aplysia* that has high homology to the *Drosophila Shaker*, mouse and rat K channels. Homology screening was performed using PCR on total nervous system RNA. To screen, we constructed oligonucleotide pools for all possible codings of two short stretches of conserved amino acid sequence (one sense and one antisense). Using these two pools as primers, PCR amplified a single DNA band of 180 bp, the predicted size based on other K channel clones. This fragment was sequenced, and showed >85% identity to other K channel clones. We have made specific oligonucleotides to this *Aplysia* K channel and are using PCR to clone the complete coding region out from this central sequence. Amplification in the 3' direction, using oligo dT plus restriction sites as the 3' primer, has identified five different 3' ends, one of which has a possible A kinase phosphorylation site. These 3' ends are apparently generated by alternative splicing since Southern Analysis suggests this is a single copy gene. In the 5' direction, we have cloned past the transmembrane spanning regions. The domain containing the transmembrane spanning regions has >70% identity to other channels. We are now completing the cloning of the 5' end and attempting expression in oocytes.

220.16

A NEW METHOD FOR RNA PHENOTYPING ALLOWS MAPPING OF POTASSIUM CHANNEL mRNA ISOFORMS IN IDENTIFIED *APLYSIA* NEURONS. B. Zhao*, E.R. Kandel, and P. Pfaffinger* (SPON: J. Goldman). HHMI, Columbia, N.Y., N.Y. 10032.

K^+ channels differ in their distribution, function, and modes of regulation in different identified cells. We are exploring how expression of different K channel mRNA's, cloned from *Aplysia* nervous system, contribute to differential channel function and modulation in identified cells. Toward this end we have developed a new technique to determine the level for these mRNA's in individual neurons. We prepare total RNA from identified neurons. Following oligo dT primed reverse transcription, the cDNA is amplified using primers for specific K^+ channel mRNA isoforms. By diluting the cDNA until the specific K channel can no longer be amplified, we can measure the levels of expression for that specific mRNA in individual cells using a Poissonian analysis. This method is more sensitive for the measurement of rare transcripts that code for channel proteins than *in situ* hybridization and more precise than conventional RNA phenotyping. Preliminary experiments on one K^+ channel isoform show it to be expressed in R2, sensory clusters and bag cells. Normalized to calmodulin this isoform is present in these cells at 100 times greater levels than in mRNA prepared from total nervous system. Extending this work to other K^+ channels mRNA's and other cells should provide insights into the relationship between the expression of different isoforms and the electrical properties of specific neurons.

220.17

AGE- AND TISSUE-RELATED EXPRESSION OF POTASSIUM ION CHANNEL mRNA LEVELS IN THE RAT. CJ Luneau*, JB Williams*, and RB Stein*, (SPON: D Pettibone), Merck, Sharp & Dohme Res. Labs, West Point, PA

To assist concurrent efforts towards molecular cloning of mammalian K+ ion channels, total polyadenylated RNA pools were prepared from heart and brain of various aged Sprague-Dawley rats, and from various tissues of adult rat, and other species. Partial cDNA's of 4 different putative rat brain K+ channels (E.Levitan and L.Kaczmarek, unpublished, and R.Swanson, et al, unpublished) and a cloned heart delayed rectifier type K+ channel (K.Folander, et al, unpublished) were used as probes on RNA blots. Specifically hybridizing bands unique to each clone were identified and range from 0.7 to 9.5 kb. Developmental regulation of specific mRNA is observed for 2 of the 5 channels. Two of the channels appear to be specific to brain; the other 3 are also observed in other tissues. Careful electrophysiological measurements from expression of whole mRNA pools and the cloned individual channels will be necessary to dissect the role of each in defining the electrical excitability of the rat heart and nerve cell at various developmental stages.

220.18

EXPRESSION OF TRUNCATED I_A CHANNEL SUBUNITS IN MUTANT OR TRANSGENIC DROSOPHILA. A. Mallart, G. Gisselman‡, S. Sewing‡, B.W. Madsen‡, C. Bourret‡, D. Angaut-Petit‡, F. Müller-Holtkamp‡, A. Ferrús‡ and O. Pongs‡. Ruhr Universität Bochum, F.R.G.; C.N.R.S., Gif/Yvette, France and Instituto Cajal, Madrid, Spain.

Shaker (*Sh*) mutants of *Drosophila melanogaster* lack or have abnormal transient K current (I_A) in muscle or nervous tissues. Some *Sh* mutants, like Sh^{102}, behave as antimorphs in gene dosage tests. Since the *Sh* locus encodes a transcription unit which expresses a family of I_A channel subunits, it was postulated that mutated *Sh* gene products give rise to abnormal I_A channel subunits. We found by cloning and sequencing the appropriate part of the Sh^{102} DNA that the mutation caused a base exchange giving a termination codon thereby generating truncated I_A subunits lacking the 6th presumed transmembrane helix and the entire carboxy terminus. To explain the dominant antimorphic behavior of Sh^+/Sh^{102} heterozygotes, we hypothesized that the truncated I_A channel subunits produced in Sh^{102} form multimeric assemblies with their normal counterparts to give inactive channels. This was tested by inserting similarly truncated cDNA in wild type flies using a heat shock expression vector which lead to the transformation of wild type into *Sh* flies as shown by a 30% reduction in larval muscle I_A, increased transmitter release in neuromuscular junctions and leg shaking behavior in adult flies.

220.19

DECREASED POTASSIUM CHANNEL CONDUCTANCE, ENCODED BY THE *SHAKER* LOCUS IN *DROSOPHILA*, INCREASES VOLATILE ANESTHETIC REQUIREMENTS M.Maze, J.Tinklenberg, I.S.Segal, R.W.Aldrich (SPON: K.L.Chow) Depts. of Anesthesia and Neurobiology, Stanford University, Stanford CA 94305 & PAVAMC CA 94304

Evidence is accumulating for the pivotal role of potassium (K^+) conductance for anesthetic action(FASEB J 3:A1202,'89). The K^+ channel encoded by the *Shaker* (*Sh*) locus on the X chromosome of *Drosophila melanogaster* (*D.m.*) may be similar to the one altered by volatile anesthetics. Alleles for the *Sh* locus which express different degrees of dysfunctional K^+ conductance ($Sh^{null} > Sh^{KS133} > Sh^5$) and the wild-type (WT) were tested for their sensitivity to the volatile anesthetic, forane. *D.m.* were equilibrated to a measured concentration of forane and a heat stimulus was applied to individual flies for a maximum of 6 sec. The flies were designated as nonresponders if no purposeful movement was noted. The concentration of forane was adjusted and after equilibration the testing procedure was repeated. After testing at a minimum of 4 concentrations, the IC_{50}'s (forane concentration at which 50% of the flies were nonresponsive) were derived for each cohort (n=150). Compared to the WT (0.50%), Sh^{null} (1.57%) had a 3-fold, Sh^{KS133} (1.30%) a 2.6-fold and Sh^5 (0.68%) a 1.3-fold increment in the IC_{50}. This rank order of insensitivity to forane correlates with the degree to which K^+ conductance is decreased and provides further evidence for the important role played by K^+ conductance in volatile anesthetic action.

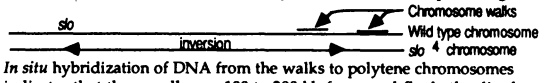
Supported by NIGMS #30232 and the Veterans Administration.

220.20

MOLECULAR ANALYSIS OF THE *SLO* LOCUS: A GENE AFFECTING A CA^{2+}-ACTIVATED K^+ CURRENT. N. S. Atkinson*, G. A. Robertson and B. Ganetzky, Lab. of Genetics, Univ. of Wisc. Madison, WI 53706

The transient Ca^{2+}-activated K^+ current, I_{Cf}, is required for the normal repolarization of the action potential in the dorsal longitudinal flight muscles (DLM) of *D. melanogaster*. The slo^1 mutation eliminates I_{Cf} causing action potentials to be much broader than normal. A 37°C heat pulse immobilizes slo^1 homozygotes for extended periods. The *slo* gene may encode a protein that 1) is involved in regulation or assembly of the channel that conducts I_{Cf}, 2) conveys the Ca^{2+} signal to the channel or 3) is the channel itself. We are utilizing a mutational approach to clone *slo* because such an approach does not rely on assumptions concerning the nature of the gene product.

We have used behavioral screens to isolate a number of new mutant *slo* alleles and have demonstrated that the behavioral and physiological phenotypes result from a single mutational event. As a prerequisite to cloning, the gene was localized cytologically to the right arm of chromosome 3 with the use of five chromosome rearrangements. The slo^4 mutant allele is the result of an inversion, one end of which is in the *slo* locus and the other end of which is in the *E(spl)* region. Two chromosome walks from this region were furnished to us by E. Knust and J. Campos-Ortega.



In situ hybridization of DNA from the walks to polytene chromosomes indicates that these walks are 100 to 200 kb from, and flank, the distal end of the inversion. Extension of the existing chromosome walks to the distal endpoint of the inversion will provide us with an entry point into the *slo* gene. This approach will enable us to elucidate at a molecular level the nature of the *slo* product and its role in the production of I_{Cf}.

221.1

KAPPA OPIATE RECEPTORS: MAJOR NEUROENDOCRINE ROLE AND ATYPICAL RESPONSE TO CHRONIC STIMULATION IN ONTOGENY. C. Kuhn, W. Adamson* and R. Windh.* Dep't of Pharmacology, Duke University Medical Center, Durham, NC 27710.

The present study contrasts the normal ontogeny and development of tolerance at mu and kappa opiate receptors, and evaluates the contribution of these characteristics to the neuroendocrine adaptations which occur during perinatal opiate treatment. The ontogeny of growth hormone (GH) and corticosterone (CS) responses to the mu/delta agonist morphine (MOR) and the kappa agonist U50,488 were determined. Also, GH and CS responses to U were measured 48 hrs after 5 days of chronic U(.5 mg/kg/day up to 2.5 mg/kg/day) on days 5-9 or 20-25. For both hormones, U responses appeared earlier than MOR responses, but CS responses preceded GH (day 2 vs day 5). GH tolerance was not observed at any age, while CS tolerance was observedin weanling pups but not earlier. These results show that kappa responses reliably precede mu, as postulated earlier . The resistance to kappa tolerance contrasts with the marked and specific mu CS tolerance we have observed in neonatal or adult rats. These results show that adaptations of neural systems to chronic perinatal opiate treatment are not easily predicted by the timing of treatment relative to the first appearance of the response, but instead appears determined by the receptor subtype mediating the response and the particular response under investigation.

221.2

A COMPARISON BETWEEN THE ANTINOCICEPTIVE EFFECTS OF (S,S) AND (R,R) U-50, 488 FOLLOWING SYSTEMIC AND INTRAVENTRICULAR ADMINISTRATION. L.B. Estall*, P. de Costa*, K. Rice* and A. Pert (SPON: A. Gran da). BPB, NIMH and LC, NIDDK, Bethesda MD 20892.

Although kappa receptor-specific opiate agonists produce antinociceptive effects in a variety of tests, there is still confusion regarding the precise neural focus of their actions or pharmacological specificity. In these studies, we have evaluated and compared the antinociceptive properties of the active and inactive enantiomers of U-50, 488 (a selective kappa agonist) following either systemic or intraventricular injections. (S,S) U-50, 488 (the active enantiomer) increased reaction latencies in the hot-plate, paw-pressure and tail-flick tests. (R,R) U-50, 488 (the inactive enantiomer) did not alter the reaction times in any test. Surprisingly, however, the effects of the active enantiomer were relatively resistant to antagonism by either naloxone or MR-2266. Intraventricular injections of (S,S) U50, 488 (100 nmoles) also increased reaction latencies in the hot-plate and paw-pressure tests. The inactive enantiomer, however, was equally effective. Nor-BNI did not antagonize the effects of (S,S) U50, 488 in any test. These findings suggest that kappa agonist antinociception does not appear to involve supraspinal mechanisms, but is probably mediated either peripherally or at the spinal level.

221.3

SITES OF DOPAMINE RELEASE INHIBITION BY KAPPA AGONISTS: LACK OF LOCAL EFFECT IN THE NUCLEUS ACCUMBENS. B.A.Donzanti, M.A.Burian, J.S.Althaus and P.F.VonVoigtlander (SPON: E.C.Krimmer). NEOUCOM, Rootstown, Ohio 44272 and The Upjohn Company, Kalamazoo, Michigan 49001.

The selective kappa opioid agonist, U-50488H has been shown to reduce the release of dopamine in the striatum and nucleus accumbens (Dichiara et al., 1985). In the present experiments, microdialysis probes were inserted into the nucleus accumbens of freely moving rats. Perfusates were analyzed for dopamine, DOPAC, HVA and U-50488 by HPLC-EC. U-50488H (2.5-10mg/kg, s.c.) resulted in a dose related reduction of dopamine, DOPAC and HVA release. Overtly, the animals displayed an initial hyperactivity followed by hypomotility and excessive urination. In contrast, when U-50488H (up to 1mM) was perfused through the probe only a transient increase in dopamine release was noted. This coincided with a period of hypermotility. The effects of U-50488H on dopamine synthesis, levels and metabolism were assessed in aromatic amino acid decarboxylase (NSD 1015) treated rats. U-50488H did not cause the marked alterations of these parameters typical of the action of dopamine autoreceptor agonists. Together these results indicate that U-50488H acts at sites distant from the nucleus accumbens to inhibit dopamine release and that modulation of dopamine receptor mediated feedback is not involved.

221.4

REGULATION BY KAPPA OPIOID RECEPTORS OF TUBEROHYPOPHYSIAL DOPAMINERGIC (THDA) NEURONS AND THE SECRETION OF α-MELANOCYTE STIMULATING HORMONE (αMSH). J. Manzanares, K.J. Lookingland and K.E. Moore. Department of Pharmacology and Toxicology, Michigan State University, East Lansing, MI 48824.

The secretion of αMSH is tonically inhibited by THDA neurons projecting to the intermediate lobe of the pituitary. The purpose of the present study was to examine the role of kappa opioid receptors in regulating THDA neurons and the secretion of αMSH in male rats. The activity of THDA neurons was estimated by measuring in the dissected intermediate lobe of the pituitary: 1) the concentration of 3,4-dihydroxyphenylacetic acid (DOPAC) and 2) the accumulation of 3,4-dihydroxyphenlalanine (DOPA) after the administration of a decarboxylase inhibitor (NSD 1015). Systemic administration of U50,448, a kappa opioid agonist, caused a dose- and time-related increase in plasma levels of αMSH and a decrease in DOPAC concentrations and DOPA accumulation in the intermediate lobe. These actions of U50,488 were blocked by an intracerebroventricular injection of nor-binaltorphimine, a selective kappa opioid receptor antagonist, but not by naltrexone. These results suggest that the kappa agonist U50,488 increases αMSH secretion by decreasing the inhibitory tone of THDA neurons projecting to the intermediate lobe of the pituitary. (Supported by NIH grant NS15911).

221.5

SIGMA RECEPTORS REGULATE CONTRACTIONS IN GUINEA PIG ILEUM LONGITUDINAL MUSCLE/MYENTERIC PLEXUS. B.G. Campbell, M. Scherz*, J.F.W. Keana* and E. Weber. Vollum Institute, Oregon Health Sciences Univ., Portland, OR 97201 and Univ. of Oregon, Eugene, OR 97403.

Sigma receptors are specific, highly localized binding sites in limbic and sensorimotor structures of the brain as well as certain endocrine organs and peripheral structures. These sites interact with many psychotropic agents including the psychotomimetic benzomorphan opiates, the psychotomimetic drug phencyclidine (PCP) and its analogs, as well as numerous typical and atypical antipsychotic drugs such as haloperidol, chlorpromazine and the novel drugs BMY 14802, rimcazole and remoxiperide. Systematic investigations into the function of sigma receptors have been hampered by the lack of a wide spectrum of selective sigma receptor ligands and the lack of an in vitro bioassay system in which to study sigma receptor function.

We report here the characterization of 11 congeners of the selective sigma receptor ligand DTG (N,N'-di[o-tolyl]guanidine --see B. Tester, this meeting, for details of synthesis and structure-activity relationships) and the development of an in vitro bioassay demonstrating sigma receptor-mediated responses. DTG and 9 congeners with potent sigma receptor affinity dose-dependently and by a neuronal mechanism inhibit muscle contractions of the isolated guinea pig ileal longitudinal muscle/myenteric plexus preparation (LMMP) evoked by electrical stimulation or by exogenous serotonin acting via neuronal 5-HT3 receptors. Two additional DTG congeners with very low sigma recetpor affinity do not inhibit LMMP contractions evoked by either stimulus. The bioactivity of these and numerous other compounds strongly correlated with their binding affinity for CNS sigma receptors, suggesting the presence of functional sigma receptors in the LMMP. Supported by grants from NIMH (MH40303 and MH42068) and Cambridge NeuroScience Research.

221.6

1,3-DI(2-TOLYL)GUANIDINE AND PHENCYCLIDINE CAUSE SIGMA RECEPTOR MEDIATED INHIBITIONS OF SPONTANEOUS PURKINJE CELL FIRING RATE IN RAT CEREBELLUM. M. B. KIM*, P. C. BICKFORD-WIMER, R. FREEDMAN, AND B.J. HOFFER. (SPON: G.W. KINDT) V.A. Medical Center 1055 Clermont St. Denver, CO 80220; Department of Pharmacology UCHSC 4200 E. Ninth Ave. Denver, CO 80262

The relationship of sigma "opiate" and PCP receptors in the central nervous system has been the subject of much recent debate. In this study the physiological effects of the sigma agonist 1,3-di(2-tolyl)guanidine) (DTG) were investigated in the cerebellum using electrophysiological techniques. Local application of DTG via pressure micro-ejection from glass multibarreled micropipettes was compared with that of phencyclidine (PCP). Similar to PCP, local application of DTG caused a dose dependant and reversible inhibition of Purkinje cell firing rates. Dose response curves after local application of the two drugs demonstrated that they had equipotent ED50s of about 20 PSI-SEC. Previous investigations with PCP have demonstrated that it acts as an indirect noradrenergic (NE) agonist in the cerebellum. The possible interaction of DTG at NE synapses was investigated in animals pre-treated with 6-hydroxydopamine to destroy NE terminals. Paired animal experiments were performed to compare the potency of PCP and DTG between control and lesioned cerebella using the same multibarreled mircropipette. Cumulative population dose response curves to both DTG and PCP were significantly shifted to the right in 6OHDA treated rats ($p<0.02$ and 0.01 respectively, two tailed Kolmogorov-Smirnov test for two populations) suggesting that endogenous NE is important for the observed Purkinje cell responses to both PCP and DTG. Taken together, this data further supports the commonality of certain types of PCP and Sigma mediated effects in the cerebellum. Supported by USPHS grant .DA 02429 and the VAMRC

221.7

(+)-PENTAZOCINE ATTENUATES (-)-ETHYLKETOCYCLAZOCINE-INDUCED EEG AND EEG SPECTRAL POWER. N.C. Paquette, K.L. Marquis, N. Khazan and G.A. Young. Dept. of Pharmacol. & Toxicol., Univ. of Maryland Sch. of Pharm., Baltimore, MD 21201.

Benzomorphans have been shown to have both opioid and nonopioid components whose effects can be further delineated using enantiomers. We hypothesized that the nonopioid effects interact or suppress the opioid component in the racemates. Thus, an enantiomer with known nonopioid effects should suppress the opioid effects of a selective opioid enantiomer. I.v. administration of (-)-ethylketocyclazocine (EKC), a selective kappa opioid agonist, at 0.5 mg/kg produced high-voltage EEG bursts with peak frequencies in the 4-6 Hz band that were associated with exophthalmos and behavioral stupor. When (+)-pentazocine, (4.0 mg/kg), the nonopioid sigma enantiomer, was given, the EKC-induced high-voltage EEG bursts were attenuated and absolute spectral power in the 4-6 Hz band was decreased with a correlated decrease in behavioral stupor. These findings complement a previous study which demonstrated that haloperidol, a presumed sigma antagonist, unmasked opioid effects of U-50,488H, a kappa and sigma ligand, on EEG power spectra in rats (Young et al., FASEB J. 3:A421, 1989).

(Supported by NIDA Grant DA-01050.)

221.8

DESENSITIZATION AND SENSITIZATION TO THE BEHAVIORAL EFFECTS OF SUBSTANCE P AND EXCITATORY AMINO ACID (EAA) AGONISTS IN THE MOUSE SPINAL CORD: INVOLVEMENT OF OPIOID RECEPTORS. X. Sun* and A.A. Larson (SPON: D.H. Smullin). Dept. of Vet. Biol., Univ. of Minn., St. Paul, MN 55108.

We have previously demonstrated that repeated intrathecal (i.t.) injections of substance P (SP) in mice result in desensitization to the caudally directed biting and scratching behavior (CBS) elicited by SP. To determine whether desensitization develops selectively to this effect of SP, we tested various EAA agonists, which also produce CBS behavior when injected intrathecally, for their ability to evoke sustained CBS behavior after multiple challanges. Like SP, repeated i.t. injections of N-methyl-d-aspartate (NMDA) typically result in some desensitization but only after an initial potentiation. In contrast, repeated i.t. injections of kainic acid (KA) or quisqualic acid (Quis) result in marked sensitization. These results suggest that repeated i.t. injections of SP and EAA agonists result in behavioral profiles that are distinct in time course and intensity. SP-elicited desensitization is blocked by the opioid antagonists naloxone and beta-funaltrexamine (beta-FNA). To determine whether endogenous opioids are also involved in the sensitization or desensitization to EAA agonists, we evaluated the effect of opioid antagonists on these processes. Five nmol of 1,3-di-O-tolyguanidine (DTG), a sigma-selective antagonist, enhanced the development of SP desensitization but prevented NMDA desensitization. The delta antagonist ICI 174864, at a dose of 0.25 nmol, prevented NMDA desensitization but significantly inhibited sensitization to the effect of Quis. These data indicate that sigma opioid receptors may be involved in NMDA and SP desensitization whereas delta opioid activity antagonizes the development of Quis sensitization and NMDA desensitization. Supported by USPHS grants DA04090, DA04190 and DA00124.

221.9

GUINEA-PIG VAS DEFERENS CONTAINS *SIGMA* BINDING SITES BUT NOT PHENCYCLIDINE BINDING SITES. X.-Z. Wu* and T.-P. Su., Neuropharmacology Laboratory, Addiction Res. Ctr., NIDA , Baltimore, MD 21224.

The guinea-pig vas deferens was proposed to contain both *sigma* receptors and phencyclidine (PCP) receptors as *sigma* and PCP drugs potentiated electrically-induced twitches of the isolated tissue preparation (Vaupel, D.B. and Su, T.-P., Eur. J. Pharmacol. 139:125, 1987). This study examined the presence of these two receptors in the guinea-pig vas deferens using radioligand binding assay. [^{3}H]*d*-SKF-10047 bound saturably to a single class of binding sites in the homogenates of the guinea-pig vas deferens with a K_d of 311 nM and a Bmax of 1090 fmol/mg protein. IC50's of several representative *sigma* drugs in displacing [^{3}H]*d*-SKF-10047 from this binding site correlated well with the IC50's obtained from *sigma* binding in the brain (r = 0.96). However, attempts to label the PCP receptor in this tissue using [^{3}H]TCP (N-[1-(2-thienyl)cyclohexyl]piperidine) yielded negative results. The IC50's obtained from the *sigma* binding assay in the vas deferens did not correlate with the potentiating potencies observed in the previous study. Therefore, although the previously observed effects induced by *sigma* and PCP drugs in the vas deferens may be mediated in part through *sigma* receptors, the possibility exists that the responses are mediated through an as yet unknown mechanism.

221.10

LACK OF EVIDENCE FOR SIGMA RECEPTORS IN THE MOUSE VAS DEFERENS (MVD) PREPARATION. Susan J. Ward[1], Lee Hildebrand[1,*], Lorraine C. Fleissner[1,2] and Diane L. DeHaven-Hudkins[2]. Neurobiology, Sterling Research Group, [1]Rensselaer, NY 12144 and [2]Great Valley, PA 19355

It has been suggested previously (Weber et. al., Eur. J. Pharmacol. 138: 447, 1987, Neurosci.) that putative sigma agonists potentiate electrically stimulated twitch contractions (ESTC) of the MVD preparation, but only in concentrations 100 - 10,000 times greater than their Ki's in sigma binding assays. Thus, the purpose of the present study was to evaluate lower, potentially more relevant, concentrations of ligands for possible sigma receptor effects in the MVD.

Putative sigma agonists and antagonists all inhibited 0.1 Hz ESTC in nM concentrations. Inhibitory activity plateaued between 20 and 60% for all compounds except DTG, which had a shallow dose response curve. Subsequent to the plateau, higher concentrations (30 uM) of rimcazole and BMY-14802 fully inhibited ESTC.

	Sigma Binding	MVD			
Compound	Ki (uM)	IC_{50} ± s.e. (nM)	Emax	Slope	n
Haloperidol	8.5	172 ± 82	68 ± 11	1.5 ± 0.6	4
DTG	86.1	1944 ± 1300	100 ± 0	0.7 ± 0.1	4
BMY 14802	173	50.6 ± 11.3	39 ± 8	4.1 ± 1.5	4
Rimcazole	249	51.8 ± 31.2	38 ± 9	2.6 ± 0.9	4
(+)3-PPP	319	179 ± 59	44 ± 5	2.9 ± 0.4	8
(+)Pentazocine	871	16.9 ± 1.3	22 ± 3	2.4 ± 0.3	4

There was no correlation between inhibitory potency or maximal effect in the MVD and binding potency at sigma sites in guinea pig brain (r = -0.36 and -0.69 respectively). The inhibitory effects of (+)3-PPP on ESTC were not antagonized by the putative sigma antagonists DTG (10 uM), haloperidol (1 uM), rimcazole (3 uM) or BMY 14802 (3 uM), nor by yohimbine, (+)SCH-23390, (-)sulpiride, or naloxone. It is concluded that the inhibitory effects of sigma ligands in the MVD are not mediated by sigma receptors. Although the mechanism of sigma ligand effects in the MVD has not been established, the data caution against a presumption that effects of low concentrations of sigma ligands are sigma-receptor mediated.

We thank Drs. D.P. Taylor (Bristol-Myers) and R.M. Ferris (Burroughs-Wellcome) for the supplies of BMY-14802 and rimcazole.

221.11

AGE-RELATED DIFFERENCES IN SIGMA BINDING PARAMETERS AND BEHAVIORAL RESPONSES TO A SELECTIVE SIGMA LIGAND. M.K. Hemstreet*, R.R. Matsumoto, W.D. Bowen and J.M. Walker. (SPON: T.O. Bruhn). Brown University, Department of Psychology and Section of Biochemistry, Providence, RI 02912.

Sigma receptors were assayed using [^{3}H]-di-o-tolylguanidine in whole brain minus cerebellum of young adult rats (2-3 months old) and middle aged rats (5-6 months old). These binding studies showed that the older rats had poorer sigma binding parameters (Bmax=368 ± 34; Kd=53 ± 7) than the younger animals (Bmax= 557 ± 43; Kd=26 ± 2). The older animals also exhibited a decreased behavioral response to the selective sigma ligand, di-o-tolylguanidine (DTG). Unilateral microinjection of DTG into the substantia nigra pars reticulata of rats produced fewer contralateral turns in middle aged animals, compared to younger rats (t=2.83; P<0.02). Likewise, the postural changes produced by unilateral microinjection of DTG into the red nucleus were much weaker in the older animals (t=2.75; P<0.02). These data suggest that changes in the number and affinity of sigma binding sites that occur during development have subsequent effects on movement.

221.12

SIGMA DRUGS CAN BLOCK CARBACHOL-STIMULATED PHOSPHOINOSITIDE TURNOVER IN NCB-20 CELLS. J. Adams* and E. Weber. Vollum Institute for Advanced Biomedical Research, Oregon Health Sciences University, Portland, OR 97201.

The neurohybrid NCB-20 cells should provide an appropriate model for investigating the functional role of the haloperidol-sensitive sigma site (Largent, et al., EJP 124, 183, 1986; Adams et al., manuscript in preparation). It has recently been shown that sigma drugs block agonist-stimulated phosphoinositide (PI) turnover in rat brain synaptosomes (Bowen, et al., EJP, 149, 399, 1988). We here conducted studies to further characterize the nature of this effect in NCB-20 cells. Cells were pre-labeled with [3H]myo-inositol and the accumulation of [3H]inositol-1-phosphate ([3H]-IP1) was assayed in the presence of LiCl (Berridge, et al., Nature, 312, 315, 1984). Di-ortho-tolyl-guanidine (DTG), at 100 uM, completely attenuated the response elicited by 1mM carbachol (the effects of carbachol were mediated strictly through muscarinic receptors in these cells). There was no effect of 100 uM DTG on basal levels of [3H]-IP1. The inhibition by DTG was dose-dependent with an ED50 value of approximately 20 uM. The reduction of carbachol-stimulated [3H]IP1 accumulation by DTG was reversible, did not require a pre-incubation period, and was immediate. Carbachol dose-response curves performed in the presence of 50 uM DTG or haloperidol show a decrease in maximum response indicating that the inhibition by these two sigma ligands is noncompetitive in nature. DTG, however, did not antagonize PI hydrolysis stimulated by bradykinin in NCB-20 cells. The DTG congener, dimethyl-guanidine, which is inactive in sigma binding assays, was without effect at 1 mM, while the potent DTG congener, diadamantylguanidine, was as effective as DTG at inhibiting the carbachol response. These data suggest that sigma ligands can negatively modulate some component(s) of the PI signalling pathway. Supported by NIMH grants MH40303 and MH42608 and a grant from Cambridge NeuroScience Inc.

221.13

DETERMINATION OF EFFICACIES AT THE μ-OPIATE RECEPTOR IN A NEUROBLASTOMA CELL LINE. L. Toll, Life Sciences Division, SRI International, Menlo Park, CA 94025.

The μ-opioid receptor is the site mediating the analgesic activity of morphine and the majority of fused ring and peptide opiates. Some fused-ring opiates, so-called "mixed agonist/antagonists" such as nalorphine, are thought to be κ-agonists and μ-antagonists. It is unclear, with these compounds, which site mediates analgesic activits and, in fact, what their activity or efficacy is at the μ receptor. The neuroblastoma cell line SH-SY5Y has both μ and δ receptors and a μ receptor mediated inhibition of adenylate cyclase activity has been demonstrated. We have confirmed this, finding a maximum of 50 to 70% inhibition of forskolin-stimulated cAMP accumulation by morphine and other μ-agonists. The mixed agonist/antagonist nalorphine was less effective, causing only 25% inhibition at 100 μM. The morphine-induced inhibition was inhibited by naloxone but not by the selective δ-antagonist ICI 174,864. To determine efficacies of various opioid compounds at the μ receptor, we have determined potency in the inhibition of cAMP accumulation in intact cells, and under the same conditions, determined binding affinities at the μ receptor using the μ-selective peptide antagonist [^{3}H]CTOP. Relative efficacies, based on the relationship between potencies and binding affinities for selected compounds, will be discussed.

221.14

A NALTRINDOLE ISOTHIOCYANATE (NTI-NCS), A SELECTIVE NONEQUILIBRIUM DELTA OPIOID RECEPTOR ANTAGONIST. M. Sultana*, A.E. Takemori*+, P.S. Portoghese. Department of Medicinal Chemistry, College of Pharmacy, and Department of Pharmacology+, Medical School, University of Minnesota, Minneapolis, MN 55455.

Naltrindole (NTI) is a recently developed nonpeptide opioid antagonist that is highly selective for δ opioid receptors (Eur. J. Pharmacol. 146:185, 1988). In an effort to convert NTI into a selective, nonequilibrium δ opioid receptor antagonist we have synthesized its 5'-isothiocyanate (NCS) substituted analogue (NTI-NCS). Incubation of NTI-NCS (100 nM) with the mouse vas deferens preparation (MVD) for 30 min followed by thorough washing afforded an 18-fold shift (IC_{50} ratio) of the concentration-response curve for the δ-selective agonist, [D-Ala^2, D-Leu^5]-enkephalin (DADLE). On the other hand, the IC_{50} ratios of morphine (μ-selective) and ethylketazocine (κ-selective) did not differ significantly from unity in the guinea pig ileum preparation (GPI) after exposure to NTI-NCS. When administered i.c.v. (10 nmol) in mice 24 hours prior to antinociceptive testing (abdominal stretch) with the δ agonist, [D-Ser^2,Leu^5,Thr^6]enkephalin (DSLET), NTI-NCS shifted the dose-response curve by a factor of 50 (ED_{50} ratio). In contrast, NTI-NCS failed to shift the response of morphine and produced only a small shift (ED_{50} ratio = 3) with the U50488H. These experiments demonstrate the nonequilibrium, selective blockage of δ opioid receptors by NTI-NCS.

221.15

THE AFFINITY LIGAND "UPHIT" ACYLATES THE κ OPIOID RECEPTOR *IN VIVO*: RECEPTOR BINDING AND PHARMACOLOGICAL EVIDENCE. L. Band,*† B.R. de Costa,*§ V. Bykov,*† A. Pert,*†† S. Iyengar,§§ P.L. Wood,§§ K.C. Rice,§ R.B. Rothman† (SPON: C.J. Smith). † Unit on Receptor Studies, LCS, and †† BPB, NIMH, Bethesda, MD 20892; § Laboratory of Medicinal Chemistry, NIDDK, Bethesda, MD 20892; §§ G. D. Searle & Co., St. Louis, MO 63198.

An isothiocyanate analog, (1S,2S*trans*-2-Isothiocyanato-4,5-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)cyclohexyl]-benzeneacetamide), of U50,488, designated "UPHIT," has been developed for site-directed acylation of κ opioid receptors. The (S,S) isomer displaced [^{3}H]U69,593 binding *in vitro* with IC_{50} values of 25.92 ± 1.74 nM, whereas IC_{50} values obtained for the (R,R) isomer and racemate were 827.42 ± 4.80 and 115.10 ± 1.22 nM, respectively. Twenty-four hours after i.c.v. injection of the racemate (0-100 μg/10 μl dimethyl sulfoxide), guinea pig brain membranes were prepared and pretreated with the site-directed acylators BIT and FIT, depleting membranes of μ and δ binding sites, respectively. Binding assays revealed that the 100 μg dose eliminated 98% of [^{3}H]U69,593 and 40% of [^{3}H]bremazocine sites without reducing [^{3}H]FOXY or [^{3}H]DADL sites. Injection of (-)-U50,488 and the (R,R) isomer of UPHIT produced no change in binding parameters. Pretreatment with 50 and 75 μg of the (S,S) isomer (i.c.v.) attenuated an increase in ACTH release induced by sytemic injection of 8 mg/kg of U50488H in rats. These results indicate that UPHIT binds selectively and irreversibly to κ sites and acts as an irreversible κ receptor antagonist.

221.16

CHRONIC HALOPERIDOL TREATMENT DIFFERENTIALLY REGULATES DOPAMINE AND SIGMA RECEPTORS. R. R. Matsumoto, W. D. Bowen and J. M. Walker. Department of Psychology and Section of Biochemistry, Brown University, Providence, RI 02912.

Haloperidol is a neuroleptic with equal affinity for dopamine and sigma receptors. A recent study in our laboratory showed that microinjection of haloperidol in the red nucleus of rats produces dystonic reactions through sigma receptors. We now report that chronic haloperidol treatment differentially regulates dopamine and sigma receptors. In this study, rats received daily s.c. injections of haloperidol (5 mg/kg) or an equal volume of saline for up to 20 days. The striata from these animals were assayed for dopamine receptors using [^{3}H]-sulpiride as the radioligand while the rest of the brain (minus cerebellum) was assayed for sigma receptors with [^{3}H]-di-o-tolylguanidine. This drug regimen down-regulated sigma receptors at 10 and 20 days and produced the expected up-regulation of striatal dopamine receptors in the same animals. The simultaneous up-regulation of dopamine receptors and down-regulation of sigma receptors by haloperidol suggests that the compound acts as a dopamine receptor antagonist and a sigma receptor agonist. This finding suggests that haloperidol and other similarly acting sigma ligands act as agonists at sigma receptors.

221.17

INTERACTION OF UMESPIRONE (KC 9172), A NOVEL ANTIPSYCHOTIC/ANXIOLYTIC AGENT, WITH SIGMA RECEPTORS. H. KRAHLING*, M. RUHLAND*, Y. ITZHAK* (SPON: A. FISHER), Kali-Chemie Pharmaceutical Division, D - 3000 Hannover 1, W. Germany and Dept. of Biochemistry & Mol. Biology, REPSCEND Labs, University of Miami School of Medicine, Miami, FL, 33101 USA.

Umespirone (KC 9172) is a potent atypical antipsychotic with anxiolytic properties derived from: 3,7-diazabicyclo[3.3.1]nonan-2,4,6,8-tetraons. In the present study we examined the interaction of umespirone with the sigma and PCP receptors postulated to be involved in psychotomimetic activity. Sigma receptors in rat brain membranes were labelled with (+)[3H]-3-PPP and (+)[3H]-SKF 10047, and PCP receptors with [3H]PCP-3-OH and [3H]TCP. Umespirone competes for the binding of the sigma ligands in a biphasic manner: IC50's 0.75 and 330 nM (vs.(+)[3H]-3-PPP). Inclusion of umespirone in the saturation binding assays of (+)[3H]-3-PPP indicated a noncompetitive type of interaction: i.e., control binding: Kd = 27 nM; Bmax = 53 pmol/g tissue. In the presence of umespirone (50 nM) Kd = 28 nM; Bmax = 33 pmol/g tissue. Umespirone (10 μM) does not inhibit the binding of the PCP ligands. The high selectivity of umespirone for the "sigma/haloperidol" receptor site, compared to other antipsychotic/anxiolytics, may attribute not only to its therapeutic effects but also to the reduced side effects liability of this compound.

221.18

PRENATAL INFLUENCE OF MORPHINE AND NALTREXONE ON THE EXPRESSION OF OPIATE AND MUSCARINIC CHOLINERGIC RECEPTORS R.Simantov, S.Amir and J.Dymshitz* Dept. of Neurobiology and Genetics, Weizmann Inst. of Sci., Rehovot, Israel

Prenatal exposure to opiate agonists and antagonists may retard or accelerate, respectively, the early development of the brain. We sought to investigate whether application of morphine or naltrexone alters the expression of opiate and muscarinic cholinergic receptors in several regions of fetal rat brain, and in striatal neuronal cultures. When injected at early stages of embryonic development (E5-E14), both opioids had no effect on opiate binding levels in any brain region. However, naltrexone applied during the period of rapid opiate receptor expression (E14-E18) increased the number of opiate binding sites. Similar effect was observed in neuron-enriched striatal cultures. Morphine injected on days E14-E18 or applied in culture had an opposite but more moderate effect. The kinetics of morphine-induced receptor down-regulation indicated that this process requires several days. However, exposure of the cultures to 10 μM of morphine for 30 mins or 2 hrs increased the opiate binding levels by 20-40%, whereas naltrexone had no such effect. In addition, both opioids did not change the expression of muscarinic cholinergic receptors, monitored with ^{3}H-QNB. The study indicates that during the early period of brain development opiate receptors may be affected by morphine and naltrexone directly through the receptors and not via a general hormonal system of the pregnant mother.

221.19

STRESS-INDUCED TOLERANCE TO DELTA RECEPTOR AGONIST DPDPE AND SELECTIVITY OF THE IRREVERSIBLE DELTA LIGAND, DALCE. Daniel J. Calcagnetti*, Wayne D. Bowen and Stephen G. Holtzman. Dept. of Pharmacology, Emory University, Atlanta, GA 30322 and Brown University, Section of Biochemistry, Providence, RI 02912.

Experiments were conducted to 1) provide evidence of the selectivity of [D-Ala2, Leu5, Cys6] enkephalin (DALCE) as an antagonist of delta receptor ligands and 2) use DALCE as a tool to explore the role of delta receptors in restraint stress(RS). Dose- and time-response curves were generated for the respective delta and mu selective opioid agonists DPDPE (3-30μg) and DAGO (0.03-0.3μg) to increase paw-lick latency (PLL) in the hot-plate test in rats after ventricular (ICV) injection. ICV injected DALCE (0.4-10 μg, 24 hr) did not alter baseline pain sensitivity. DALCE dose-dependently blocked the increase in PLL produced by DPDPE (30μg) but not that induced by an equivalent analgesic dose of DAGO (0.3 μg). Lastly, we determined whether 1 hr of RS would a) alter delta receptor sensitivity as indexed by DPDPE-induced analgesia and b) attenuate the ability of DALCE to functionally antagonize DPDPE-induced analgesia. Rats were tested for analgesia induced by DPDPE (30-120μg) 24 hr after one of four treatments: vehicle + no RS; vehicle + RS; DALCE (10μg) + no RS; DALCE + RS. Exposure to RS alone produced tolerance to DPDPE-induced analgesia. DALCE antagonized DPDPE similarly regardless of RS condition. The effects of both RS and DALCE were surmounted by the highest dose of DPDPE. We conclude that DALCE is a selective delta antagonist and that RS can induce tolerance to the analgesic effect of DPDPE. [Supported by NIDA grants DA00541(SGH) and DA03776(WDB) and RSA DA00008 (SGH)].

221.20

SENSITIVITY TO OPIOIDS IN INBRED MICE: NALTREXONE PRECIPITATED WITHDRAWAL JUMPING. J.C. Chow*, I. B. Finn*, J. M. Carney* and T. W. Seale (SPON: R. Gilmore). Dept. of Pharmacology, Univ. of Kentucky Coll. of Med., Lexington, KY 40536, and Dept. of Pediatrics, Univ. of Oklahoma Hlth Sci. Ctr., Oklahoma City, OK 73104

C57Bl/6ByJ, C57Bl/6J, C57Bl/10SnJ, DBA/2J, BALB/cByJ, A/J, AKR, SWR, CBA/J and C3H/HeJ mice were pre-treated (i.p.) with morphine (32mg/Kg), normorphine (32mg/Kg), etonitazine (0.01mg/Kg), levorphanol (3.2mg/Kg) and fentanyl (0.5mg/Kg) and injected 30 min later with naltrexone (NTX). Nine of the ten strains responded (+) to morphine (M). Levorphanol (L) and fentanyl(F) responsiveness was similar to morphine in the nine strains, with the exception of C57Bl/10SnJ. DBA was consistently hyporesponsive (-) to the five opioid agonists studied. Etonitazine(E) and normorphine(N) failed to produce NTX-precipitated withdrawal jumping in three and five of the nine strains respectively.

	6ByJ	6J	10SnJ	DBA	BALB	A/J	AKR	SWR	CBA	C3H
M	+	+	+	-	+	+	+	+	+	+
N	+	-	+	-	+	+	-	-	-	-
E	+	+	+	-	+	+	-	+	-	-
L	+	+	-	-	+	+	+	+	+	+
F	+	+	+	-	+	+	+	+	+	+

The data indicate that the genetic determinants of precipitated jumping are complex and consistent with the existence of multiple mu-receptor subtypes. (Supported in part by DA04028 and NIDA contract 271-87-8133).

PAIN MODULATION: BIOGENIC AMINES I

222.1

EVOKED RELEASE CHANGES IN SEROTONIN AND NOREPINEPHRINE: IN VIVO DIALYSIS OF RAT DORSAL HORN. R. M. Bowker, Dept. Anat., Mich. State Univ., E. Lansing, MI 48824 and R. H. Abhold, Dept. VCAPP, Wash. State Univ., Pullman, WA 99164.

Employing an in vivo dialysis technique basal levels of serotonin (5HT) and norepinephrine (NE) were collected by in vivo dialysis of the rat dorsal horn during chemical and electrical stimulation. In anesthetized rats a dialysis probe was placed in the lumbar spinal cord and 20-minute samples were collected from the dorsal horn and measured during direct chemical stimulation and electrical stimulation of nucleus raphe magnus (NRM) (100 μA, 100 μsec pulses, 10 Hz for 10 min). Chemical infusions of 100 mM KCl and 100 mM glutamate resulted in an increased release of NE (321.4 ± 41.2% and 488.9 ± 79.2%, respectively). On the other hand, 5HT levels decreased (49.6 ± 4% and 76.1%, respectively). Electrical stimulation of the NRM produced an increased release of both NE (66.5 ± 3.2%) and 5HT (451.4 ± 20.4%). These changes were significantly different ($P > 0.01$ level) from baseline levels. These findings suggest differences in the released regulation of NE and 5HT nerve terminals of the dorsal horn in their modulation of nociceptive neurotransmission.

222.2

THE ANTINOCICEPTIVE EFFECT OF NUCLEUS RAPHE MAGNUS (NRM) STIMULATION ARE MEDIATED THROUGH A 5-HT_1-LIKE RECEPTOR. N. El-Yassir* and S.M. Fleetwood-Walker* (SPON: W.A.MacKay) Dept. of Physiology, Univ. of Toronto, Canada and Dept. of Preclinical Vet. Sciences, R(D)SVS, Univ. of Edinburgh, U.K.

Serotonin (5-HT) is one of several endogenous compounds implicated to play a role in analgesia. The medullary NRM is thought to be the major source of the descending 5-HT-containing terminals in the spinal dorsal horn and stimulating NRM has been reported to result in antinociception. Recent studies have established the existence of several types of 5-HT receptors. Our aim was to investigate the type of 5-HT receptor which mediates the antinociceptive effect of NRM stimulation in the dorsal horn. Experiments were carried out on rats anaesthetized with a mixture of chlorolose and urethane. Extracellular recordings were made from multireceptive dorsal horn neurones through the central barrel of a 7-barreled electrode which was also used for ionophoresis of compounds. The effect of NRM stimulation (0.4 ms, 33-100 Hz) on both nociceptive and non-nociceptive responses was assessed. Stimulation of the NRM and adjacent reticular formation resulted in selective inhibition of the nociceptive responses of 14/20 cells, but caused non-selective inhibition of both nociceptive and non-nociceptive responses in 6/20 cases. Cyanopindolol, a selective 5-HT_1 receptor antagonist, reversed the effects of brainstem stimulation on 6/6 cells. Ketanserin, a selective 5-HT_2 antagonist on the other hand, failed to antagonize the effects of brainstem stimulation in 4/4 cases. The results of this study strongly suggest that the antinociceptive effect of 5-HT at the level of the dorsal horn is mediated through a 5-HT_1-like receptor.

222.3

N-ACETYLSEROTONIN INDUCES ANALGESIA ON THE HOT PLATE TEST. G.M. Brown, M.C. Brown* and D. Grossi*. McMaster University, Hamilton, Ontario L8N 3Z5

In a previous study (Psarak, S., et al, Life Sci., 42:1109, 1988.) we reported that 10 nM N-acetylserotonin (NAS) infused in the lateral ventricle (IVC) elicited an analgesic response in the rat tail flick test. This response was not elicited by equivalent doses of serotonin or melatonin.

In order to determine whether this was not simply due to an effect on nonresponsive spinal reflexes we have examined effects of IVC NAS on the hot plate test using paw licking as an end point. An analgesic effect was seen 3 hours after NAS administration (NAS: 37.45 ± 12.6 seconds vs VEHICLE: 20.7 ± 11.4; Mean ± SD). No animals displayed gross sensory or motor disturbances, catalepsy or sedation. These findings provide further evidence that NAS has an analgesic effect on pain induced by thermal stimuli. Taken together with previous findings employing IVC antiserum to NAS this study supports the concept that endogenous NAS plays a role in pain regulation.

222.4

PATTERNS OF ANALGESIA PRODUCED BY THE NOVEL ANXIOLYTIC BUSPIRONE IN THREE PAIN TESTS IN RATS. L.Rogers* and J.Giordano*(SPON:P.LACY) Drake Univ.Coll.Pharm.Health Sci. Des Moines IA 50311

The novel anxiolytic buspirone is an agonist at serotonin 5-HT1a receptors. Other 5-HT1a agonists have been shown to produce analgesia. Therefore, the present study examined temporal and dose-related patterns of analgesia produced by buspirone in tests of acute thermal,mechanical and formalin-induced chemical inflammatory pain. Long Evans rats were injected with 1,3,5 mg/kg buspirone i.p.; controls received saline injections. Analgesia was assessed at 15-30 min. intervals post-injection to 240 min. Buspirone produced time- and dose-dependent analgesia in all nociceptive tests, with greatest effect against chemical and mechanical pain; buspirone was less potent in the thermal pain test. At maximal dose, peak analgesia occurred at 30 min. post-injection, with significant effects persisting to 60-90min., dependent upon the test. Locomotor ability and overt activity (eg-grooming,rearing) were unaffected at any dose. These data demonstrate differential analgesic efficacy of buspirone against specific types of pain. Ongoing studies in our laboratory are characterizing the neuropharmacologic substrates mediating these effects.

222.5

PERIPHERALLY ADMINISTERED SEROTONIN 5-HT3 RECEPTOR ANTAGONISTS PRODUCE ANALGESIA AGAINST ACUTE AND CHRONIC INFLAMMATORY PAIN. J.Giordano* and L.Rogers*(SPON:T.SOWA) Drake Univ.Coll.Pharm.Health Sci., Des Moines IA 50311

Systemic, but not centrally administered serotonin 5-HT3 receptor antagonists attenuated acute chemical-inflammatory pain (Giordano & Dyche, Neuropharmacol.28, 1989). In light of the mechanisms of the inflammatory pain response and the localization of 5-HT3 sites on C-fiber afferents, these findings suggested a role for peripheral 5-HT3 sites in inflammatory pain. The present study examined analgesic effects of peripherally administered 5-HT3 antagonists against acute and chronic inflammatory pain in rats.

ICS-205-930 or MDL-72222(1-100 µg/50µl) were intraplantarly injected; acute inflammatory pain was assessed by the formalin test. Chronic inflammation was produced by hindpaw inoculation with Freunds complete adjuvant; analgesia was assessed by drug-induced changes in pressure sensitivity in the affected paw. ICS-205-930 and MDL-72222 produced dose-related analgesia against acute and chronic inflammatory pain. 5-HT3 antagonists had greater effect in the acute pain test than in the chronic paradigm. In both tests, ICS-205-930 was more potent than MDL-72222. These data further support the involvement of peripheral 5-HT3 sites in inflammatory pain, and suggest the utility of specific 5-HT3 receptor antagonists as peripheral analgesics.

222.6

L-TRYPTOPHAN POTENTIATES ANALGESIC EFFECT OF LOW CURRENT TRANSCRANIAL ELECTROSTIMULATION (TE). M.H. Skolnick, R.F. Hamilton*, J.R. Lake* and D.H. Malin. Univ. of Texas Grad. School of Biomedical Sciences, Houston, TX 77030 and Univ. of Houston - Clear Lake, Houston, TX 77058.

TE involves ultralow amplitude, charge-balanced current pulses applied across the head through low impedance sites in the external ears. TE produces analgesic effects which are blocked by pretreatment with the 5-HT synthesis inhibtor pCPA, suggesting serotonergic mediation. Forty rats were pretested for latency with a 50°C wet tail-flick test and injected with either 5-HT precursor L-tryptophan (200 mg/kg i.p.) or with vehicle alone. Forty minutes after injection, half of each group received TE (10µA, 10Hz, 2msec pulse width), while the other group received "sham stimulation". After 30 minutes of stimulation, each rat was retested for tail flick latency. Analgesia scores were the % increase in latency from pretest to posttest. Anova revealed significant stimulation and drug effects. Post-hoc analysis revealed that rats receiving both TE and L-tryptophan (* in table) had higher analgesic scores, $p<.05$, than any other group.

ANALGESIA SCORES (% Change Pretest To Posttest) M± SEM	TE	SHAM STIMULATION
L-TRYPTOPHAN	65.4 ± 16.6*	-2.1 ± 4.2
VEHICLE	33.2 ± 9.8	-5.9 ± 7.5

222.7

THE EFFECT OF CHRONIC TRYPTOPHAN TREATMENT ON AUTOTOMY INDUCED BY NERVE LESIONS. F.V. Abbott, and S.N. Young. Dept. of Psychiatry, and School of Nursing, McGill Univ., Montreal, Canada H3A 1A1.

Serotonin is one of the neurotransmitters in the spinal cord that gates nociceptive afferents, and its precursor, tryptophan (TRP), has been tested in clinical pain. However, a serotonin system in the brain can also antagonize morphine analgesia. Thus, it is important to determine which types of pain might be helped or exacerbated by TRP. We have now tested the effect of chronic tryptophan treatment on autotomy induced by nerve lesions in the rat, a potential model of phantom limb pain. Rats underwent unilateral sciatic and saphenous neurotomy and were placed on diets containing either 0.15% or 0.75% TRP. Animals were observed for autotomy of the paw on the lesioned side and were killed when the digits were removed. The high TRP diet caused a significant decrease in autotomy and significant elevations in brain and spinal cord serotonin even during the daytime, when food intake is at its lowest. Chronic tryptophan did not alter the response to noxious heat or formalin. Our data suggest that tryptophan will be a useful treatment of phantom limb pain. (Supported by the MRC of Canada).

222.8

AUTORADIOGRAPHIC MAPPING OF SEROTONIN RECEPTORS IN RAT SPINAL CORD. R.M. Murphy, A.M. Zieleniewski and F.P. Zemlan. Dept. of Psychiatry, Univ. of Cincinnati College of Medicine, Cincinnati, OH 45267-0559.

In vitro autoradiography was used to study the distribution and types of serotonin (5-HT) receptors in rat spinal cord. Tissue sections (8 uM) from cervical, thoracic and lumbar spinal cord were cut at -15°C with a cryostat and thaw-mounted onto gelatin-coated slides. Following preincubation, sections were incubated for 60 min with 2 nM ^{3}H-5-HT in a 0.17M Tris-HCl buffer (pH 7.6), in the presence and absence of 10 uM cold 5-HT, washed twice with ice-cold (4°C) buffer, and dried with cool air. To identify serotonin receptor subtypes, competition studies were performed with several ligands (100pM-1uM) including 8-OHDPAT, RU24969 and mesulergine. Slides were opposed against ^{3}H-Ultrofilm, exposed for 2 months, and analyzed by micro-densitometry.

Across all three spinal cord levels, the total number of 5-HT receptors was 2-3 times higher in the dorsal horn than in the ventral horn, with the highest densities in laminae 1-4 and the lowest densities in laminae 7 and 8. Receptors characteristic of the 5-HT$_{1A}$ binding site were distributed throughout all layers of the dorsal horn. Receptors characteristic of the 5-HT$_{1B}$ site were distributed with nearly equal densities throughout layers 4-10 of the cord. The presence of 5-HT$_{1C}$ binding (^{3}H-mesulergine) was marginally detected in layers 1-4 of the cord. The remaining receptors appear to be a novel 5-HT$_1$ binding site similar to the one recently identified in spinal cord synaptosomes.

222.9

DOMINANT SPINAL CORD 5-HT RECEPTOR, 5-HT$_{1F}$: RELATION TO PAIN. E.F. Schwab, F.P. Zemlan and R.M. Murphy. Dept. of Psychiatry, Univ. of Cincinnati College of Medicine, Cincinnati, OH 45267-0559.

We have previously described that in spinal cord synaptosomes about 25% of ^{3}H-5-HT labeled receptors are 5-HT$_{1A}$ receptors, while 26% are 5-HT$_{1B}$. The remaining 49%, the dominant spinal cord 5-HT receptor subtype, is a novel binding site, the 5-HT$_{1F}$ site. The pharmacology of the 5-HT$_{1F}$ receptor has been defined in ^{3}H-5-HT (2nM) competition studies ([competitor] = 0.1 nM to 1 mM) employing a 100 nM 8-OH-DPAT and 10 nM RU24969 mask for 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors. The 5-HT$_{1F}$ receptor had >10,000 nM affinity for 5-HT$_{1C}$ (mesulergine), 5-HT$_{1D}$ and 5-HT$_{1E}$ (methysergide), 5-HT$_2$ (ketanserin) and 5-HT$_3$ (MDL7222, ICS205-930) selective drugs, demonstrating a unique pharmacology. The 5-HT agonist, 5-MeO-DMT demonstrated a high affinity for 5-HT$_{1F}$ receptors (K_i=27 nM) while methysergide demonstrated low affinity for 5-HT$_{1F}$ receptors (>10,000 nM) but high affinity for other 5-HT spinal cord receptors (K_i 10 to 100 nM). Therefore, behavioral pain studies employing the 5-HT agonist 5-MeO-DMT in the presence of a methysergide mask for non-5-HT$_{1F}$ receptors were conducted indicating a potent 5-MeO-DMT induced increase in tail-flick latency. The present study pharmacologically characterizes the 5-HT$_{1F}$ receptor and demonstrates its involvement in spinal pain processing.

222.10

BEHAVIORAL EFFECTS OF 5-HT$_1$ AGONIST ADMINISTRATION ON SPINAL REFLEXES. A.M. Zieleniewski, R.M. Murphy and F.P.Zemlan. Dept. of Psychiatry, Univ. of Cincinnati College of Medicine, Cincinnati, OH 45267-0559.

The purpose of the present study was to determine if 5-HT$_1$ receptor subtypes differentially mediate the spinal processing of nociceptive input. Specifically, the effect of drugs selective for the 5-HT$_{1A}$ and 5-HT$_{1B}$ receptor subtypes were examined in the spinal animal on tail flick latencies (TFLs) and on three spinal withdrawal reflexes.

Three days after spinal transection at T_{10}, animals were injected i.p. at 20 min. intervals with increasing equimolar doses of either a 5-HT$_{1A}$ selective agonist (8-OH-DPAT, ipsapirone) or a 5-HT$_{1B}$ selective agonist (mCPP, TFMPP). The nociceptive sensitivity of the ventroflexion, dorsiflexion, and lateral flexion reflexes was quantified as size of the receptive field (RF) area of the reflex in cm^2. Tail flick latencies were determined ten days post-transection.

Significant dose-dependent increases in the sensitivity of all three spinal reflexes were observed following administration of the 5-HT$_{1A}$ agonists. A corresponding increase in sensitivity on TFL's was also observed. The predominant effect of 5-HT$_{1B}$ agonist administration was a dose-dependent decrease in RF area. A decrease in sensitivity was also observed in the tail flick test, with TFL's increased by 35% from baseline.

The results of the present study support the heterogeneous effects of 5-HT at the spinal level. Specifically, 5-HT$_{1A}$ agonist administration results in increased nociceptive sensitivity, while the 5-HT$_{1B}$ receptor subtype mediates an opposite effect.

223.1

THE PROJECTION OF LOCUS COERULEUS NEURONS TO THE SPINAL CORD IN THE RAT. FM Clark and HK Proudfit (SPON:H Hamm). Dept. of Pharmacol., Univ. of Ill. at Chicago, Chicago, Il 60680.

Although locus coeruleus (LC) neurons project to the spinal cord, the specific terminations of these neurons is not clear. The following studies were done to more clearly determine these projections. In the first experiment a unilateral iontophoretic injection of the anterograde tracer, phaseolus vulgaris leucoagglutinin (PHA-L), was made into the LC. A strong ipsilateral projection to the ventral horn and lamina X was seen in cervical, thoracic, and lumbar spinal cord segments. Very few axon terminals were seen in the ipsilateral dorsal horn or contralateral spinal cord. Over 90% of these axon terminals contained dopamine beta hydroxylase-like immunoreactivity (DBH) and were, therefore, assumed to contain catecholamines. In the second experiment, unilateral electrolytic lesions of the LC were made. Lesions produced a marked depletion of DBH positive axon terminals in the ventral horn and lamina X. These results indicate that LC neurons have a strong ipsilateral projection to ventral horn and lamina X and only a minor projection to the ipsilateral dorsal horn. The contralateral spinal projection was very minor. (Supported by USPHS Grant DA 03980.)

223.2

ST-91 MICROINJECTED INTO THE MEDULLARY DORSAL HORN ALTERS A MONKEY'S PERCEIVED INTENSITY OF NOXIOUS THERMAL STIMULATION. D.A. Thomas*, R. Dubner, F. Anton*, and D.R. Kenshalo, Jr. NAB, NIDR, NIH, Bethesda, MD 20892

We examined the effect of the α-2 adrenergic receptor agonist ST-91 microinjected into the medullary dorsal horn (MDH) of a monkey trained in a psychophysical task. The task required the monkey to detect temperature changes of 0.4, 0.6 or 1.0°C (T2) from a 3-9 sec noxious heat level of 46°C. We have previously shown that T2 detection latency is a measure of the perceived intensity of stimulation. ST-91 (0, 1, 3, 10, and 30 μg/0.2μl) dissolved in saline produced a dose-dependent and stimulus intensity-dependent increase in T2 detection latency. The α-2 receptor specificity of this effect was assessed by the injection of the α-2 receptor antagonist idazoxan during ST-91's peak effect on detection latencies. Idazoxan (2 mg/0.1 ml/kg of body weight; i.m.) in comparison to saline, significantly attenuated (p <0.01) the effects of ST-91 on detection latencies of the 0.4 and 1.0°C stimulus changes for a period of approximately 10 minutes. The effect of ST-91 on attentional, motivational and motoric aspects of the monkey's behavior was assessed by having the animal detect innocuous cooling and visual stimuli of similar difficulty. ST-91 had no effect on the detection of cooling or visual stimuli. These data suggest a role for α-2 adrenergic receptors in altering the perceived intensity of noxious heat stimuli at the level of the MDH, the earliest central relay pathway transmitting noxious information.

223.3

Effects of the alpha-2 agonists Dexmedetomidine and ST-91 on the Tail-Flick and Cardiovascular Responses to a Noxious Thermal Stimulus *H. Nagasaka, and T.L. Yaksh. Dept of Anesthes., Univ. of Calif., San Diego, La Jolla, CA 92093

The effects of intrathecally (IT) administered alpha-2 adrenoceptor agonists on the nociceptive threshold as measured by tail-flick (TF) latency and cardiovascular changes as measured by blood pressure (BP), and heart rate (HR) were investigated in 0.75% halothane-anesthetized and artificially ventilated male rats prepared with chronic spinal catheters. The TF and autonomic response BP and HR were evoked by the immersion of the tail for a maximum of 15 sec. in a 53° C waterbath. IT administration of Dexmedetomidine (DMET: 3.3ug, 0.33ug, and 0.033ug) and ST-91 (30ug, 3ug), and intravenous administration (IV) of DMET (0.33ug) was carried out with each dose being given in 3 or more rats. Administration of IT DMET or ST-91 produced a dose dependent block of the TF response and reduction in the BP and HR response evoked by the thermal stimulus. Maximum effects after DMET were observed in 5 min. and within 30 min. after ST-91. While IT alpha-2 agonist yielded a fall in resting BP and HR, the effect on these measures were more profound after IV administration, though IV agent had little effect on TF latency nor blocked completely the thermally evoked autonomic responses. These effects of IT DMET (3.3 ug) and ST-91(30ug) were dose dependently antagonized by the alpha-2 adrenoceptor antagonist Idazoxan IT (100 - 300 ug) pretreatment. These data suggest a potent spinal alpha-2 modulation of somatosensory and autonomic response to pain. (Grant: DA02110: T.Y.)

223.4

SPINAL ALPHA-TWO NORADRENERGIC RECEPTORS MEDIATE THE ANTINOCICEPTION PRODUCED BY MICROINJECTION OF PHYSOSTIGMINE INTO THE NUCLEUS RAPHE MAGNUS. **M.A. McCartney and H.K. Proudfit, Dept. Pharmacology, University of IL Coll. Med., Chicago, IL 60612.**

Activation of nucleus raphe magnus (NRM) neurons by local injection of carbachol produces antinociception which appears to be mediated by bulbospinal noradrenergic neurons, but not by raphe-spinal serotonergic neurons. The present study was designed to determine whether the antinociception induced by the microinjection of the cholinesterase inhibitor, physostigmine into the NRM of rats is mediated by serotonergic or noradrenergic bulbospinal neurons, and to determine the noradrenergic receptor subtypes involved.

Microinjection of physostigmine into the NRM produced antinociception that was reversed by intrathecal injection of the non-selective noradrenergic antagonist (ANT) phentolamine (30 ug), or yohimbine (alpha-two ANT-38 ug) using the tail flick test. Methysergide (serotonergic ANT-30 ug) had no effect, while intrathecal administration of the alpha-one ANT WB4101 (37 ug) enhanced the antinociceptive action of physostigmine. These antagonists had no effect on hot plate values, indicating the possible involvement of supraspinal sites in the mediation of physostigmine-induced antinociception.

These data provide further evidence that the antinociception induced by microinjection of cholinergic agonists in the NRM is produced by activation of bulbospinal noradrenergic neurons and is independent of raphe-spinal serotonergic involvement. (Supported by USPHS Grant DA03980).

223.5

ADENOSINE MEDIATES CALCIUM-INDUCED ANTINOCICEPTION AND POTENTIATION OF NORADRENERGIC ANTINOCICEPTION IN THE SPINAL CORD. J. Sawynok, A. Reid* and R. Isbrucker*, Dept. Pharmacol., Dalhousie Univ., Halifax N.S. B3H 4H7

Spinal administration of Ca^{2+} produces antinociception and potentiates spinal antinociception by morphine (J.P.E.T. 246: 500, 1988), but the mechanism of these actions is not known. Recently, we have demonstrated that adenosine release may mediate spinal antinociception by morphine (J.P.E.T. 243: 657, 1987). In this study, we have examined the action of Ca^{2+} on spinal antinociception by adenosine analogs, noradrenaline (NA) and 5-hydroxytryptamine (5-HT), and of methylxanthine adenosine receptor antagonists on Ca^{2+}-induced antinociception. Intrathecal coadministration of Ca^{2+} 50μg, which has no effect alone, potentiates the spinal action of morphine but not the analogs of adenosine CHA or NECA, using the rat tail flick test. This dose also potentiates the action of NA but not 5-HT. At higher doses (100-350μg), Ca^{2+} produces antinociception which is eliminated by pretreatment with theophylline 50μg or 8-phenyltheophylline (8-PT) 3μg. 8-PT also eliminates potentiation of the action of NA by the lower dose of Ca^{2+}. These observations suggest that Ca^{2+} can release adenosine from the spinal cord, and this both mediates antinociception and the interaction with NA and possibly morphine. (Supported by MRC Canada)

223.6

CHRONIC CAFFEINE AFFECTS MONOAMINE SYSTEMS SUBSERVING TRICYCLIC ANTIDEPRESSANT-INDUCED ANALGESIA IN RATS. S. Brock*,[1], J. Blajek[3], C. J. Drebing*,[1], G.A. Gerhardt[1,2] and J. Giordano[3] (SPON: E.P. Finnerty). Depts. of Psychiatry[1] and Pharmacology[2], Univ. of Colorado Health Sci. Ctr., Denver, CO 80262 and Coll. of Pharmacy and Health Sci.[3], Drake Univ., Des Moines, Iowa 50311.

The present study examined the effects of chronic caffeine administration on patterns of amitriptyline (AMI)- and desipramine (DMI)-induced analgesia, and CNS monoamine levels in Long-Evans rats. Animals were injected with caffeine (10-30 mg/kg i.p.) for 28 days; control animals were injected with saline. Twenty-four hours following the final injections, animals were injected with AMI or DMI (5-20 mg/kg i.p.). Thermal and mechanical analgesic tests were performed according to the methods of Giordano and Barr (Devel. Brain Res. 32: 1987). Twelve hours following analgesic tests, rats were sacrificed and their brains and spinal cords were rapidly removed and frozen at -70°C. Tissue levels of monoamines and monoamine metabolites were determined using HPLC-EC. Chronic caffeine administration produced dose-dependent decreases in AMI- and DMI-induced analgesia in both thermal and mechanical tests. Significant neurochemical changes in the serotonin systems of the spinal cord, brain stem and cortex of the rats were also observed. In addition, caffeine produced dose-related hyperanalgesia as reflected by decreased baseline responses.

223.7

DESIPRAMINE AND ZIMELIDINE INCREASE TAIL FLICK LATENCIES MAINLY BY REDUCING SKIN TEMPERATURE.
A. Tjølsen*, A. Lund* and K. Hole.
Dept. of Physiology, University of Bergen, Bergen, N-5009 Norway.

A constant, reproducable negative correlation between tail skin temperature and tail flick latency to radiant heat has been demonstrated in rats and mice. Experiments involving manipulations of serotonergic systems have shown that changes of tail flick latency due to alterations of skin temperature may be misinterpreted as changes in nociception. On this basis, we have concluded that effects on temperature regulation must be taken into consideration when performing tests of nociception involving thermal stimuli.

The effect of the antidepressant drugs desipramine (5, 15 and 25 mg/kg intraperitoneally (i.p.)) and zimelidine (5, 20 and 30 mg/kg i.p.) in the tail flick test was investigated. A dose-dependent increase in the tail flick latency was found for both drugs, with maximal effect after 2-3 h. The tail skin temperature showed a similar dose-dependent reduction compared to control animals. This explained most of the increase of tail flick latency (ANCOVA), and only the highest dose of desipramine showed antinociceptive effect. In the increasing temperature hot plate test, however, low doses (2 and 5 mg/kg) of desipramine showed a dose-dependent antinociceptive effect.

223.8

EFFECT OF AMITRIPTYLINE ON INHIBITORY MECHANISMS IN THE TRIGEMINAL COMPLEX. G.H. Fromm, M. Nakata*, and T. Kondo*. Dept. of Neurology, Univ. of Pittsburgh Sch. of Med. Pittsburgh, PA 15261

Neuropathic pain is relieved by amitriptyline (AMI) in about half the patients. The antineuralgic action of AMI has been confirmed by several controlled trials, but AMI's mechanism of action has not been established. In previous experiments we found that AMI enhances the segmental inhibition and periventricular inhibition of some wide dynamic range (WDR) and nociceptive specific (NS) neurons but not low threshold mechanoceptive (LTM) neurons.

We have now further investigated the effect of AMI on inhibitory mechanisms in the trigeminal complex of cats anesthetized with alpha-chloralose. The i.v. administration of 1.0-4.0 mg/kg AMI facilitated diffuse noxious inhibitory control (DNIC) of WDR neurons in half the experiments. On the other hand, AMI usually depressed nucleus raphe magnus inhibition of these neurons.

Opioid analgesics are ineffective against neuropathic pain and have been reported to depress DNIC. The fact that AMI enhanced the action of this inhibitory mechanism therefore suggests that AMI's effect on neuralgic pain may be due to its ability to facilitate the action of DNIC on WDR neurons. (Supported by NS-19889)

223.9

SUPRASPINAL MEDIATION OF COCAINE ANALGESIA IN THE RAT. C.R. Belczynski Jr., Y. Lin., S. Neumann, T.J. Morrow and K.L. Casey. VA Medical Center and Depts. of Neurology, Physiology and Neuroscience Program, University of Michigan, Ann Arbor, MI 48105.

We have shown that cocaine (25 mg/Kg, i.p.) is a potent, centrally acting analgesic in the rat as measured using the formalin and hot-plate tests (Brain Res., 479:306, 1989). The study examines whether cocaine analgesia is due to direct spinal action or the result of descending supraspinal control. Twenty-six adult male rats were anaesthetized with chloral hydrate (300mg/Kg, i.p.) and allowed to recover spinal reflexes. The animals were then given cocaine (25 mg/Kg, i.p.) or saline and tested for analgesia using the tail flick test. Significant increases ($p<.01,.05$) in response latencies were observed within 10 minutes, and lasting beyond 60 minutes, as compared to control. Seven of these animals were spinally transected at thoracic levels (T4-T8) and tail flick was allowed 24 hours to recover. Surgical transection of the spinal cord at thoracic levels eliminated cocaine dependent increases in response latency. These results suggest that cocaine's analgesic effect in the rat is supraspinally mediated and not the result of direct action on spinal neurons. To determine possible locale for this descending control, localized R/F lesions were made and rats were tested with cocaine using the formalin test. 75% (6/8) of rats with lesions in Nucleus Raphe Magnus (NRM) and 100% (4/4) of rats lesioned in the Nucleus Accumbens (N.Acc) showed no reduction in cocaine analgesia. In addition, 100% (11/11) of rats microinjected with lidocaine(2%) into NRM showed no attenuation of cocaine analgesia. 90% (9/10) of rats with lesions in Locus Coeruleus (LC) and 100% (7/7) of rats with combined lesions in Ventral Tegmental Area (VTA) and N.Acc demonstrated a marked reduction in cocaine analgesia. **These results suggest that descending supraspinal mechanisms originating in the brainstem are necessary for cocaine analgesia in the rat. Further experimentation is necessary to determine whether these nuclei are sufficient for cocaine analgesia.**

(Supported by the Dept. of V.A. and a Bristol-Myers award)

223.10

THE ROLE OF SPINAL NORADRENERGIC, SEROTONERGIC AND OPIOID RECEPTORS IN DESCENDING MODULATION FROM THE A5 CELL GROUP. A. Burnett and G.F. Gebhart. Department of Pharmacology, College of Medicine, The University of Iowa, Iowa City, Iowa, 52242.

Previous studies have shown that the A5 noradrenergic cell group plays a role in both descending modulation of nociception and regulation of cardiovascular and other autonomic functions. The objective of the present study was to determine to what extent noradrenergic, serotonergic and opioid pathways are involved in mediating descending inhibition and changes in blood pressure and heart rate.

Experiments were conducted in lightly pentobarbital-anesthetized rats using the tail-flick reflex as a model of nociception. A5 cells were electrically stimulated and the effects of intrathecal injections of adrenergic, serotonergic and opioid antagonists were evaluated. Changes in blood presure and heart rate were recorded via a femoral arterial catheter.

Phentolamine produced a dose-dependent increase in the stimulation threshold for inhibition of the tail-flick reflex in the rostral A5, but did not produce significant increases in threshold in the caudal A5. Methysergide and naloxone produced no significant increases in threshold. Stimulation-produced pressor effects and increases/decreases in heart rate were not affected by the administration of these antagonists. These experiments suggest that spinal noradrenergic, but not serotonergic or opioid, receptors are involved in descending modulation of nociception from the A5.

223.11

THE INVOLVEMENT OF NOREPINEPHRINE (NE) AND SEROTONIN (5-HT) IN MORPHINE AND FENTANYL-INDUCED SPINAL ANALGESIA. T. Crisp, J.L. Stafinsky*, M. Uram* and V.C. Perni*. Dept. of Pharmacology, N.E. Ohio Universities College of Medicine, Rootstown, OH 44272.

Male Sprague-Dawley rats were injected intrathecally (i.t.) with morphine sulfate (MS) or fentanyl. Both drugs dose-dependently elevated tail-flick latency in a naltrexone-reversible manner. To determine if MS or fentanyl interact with spinopetal monoaminergic nerves to produce spinal antinociception, NE or 5-HT receptor antagonists were co-administered with the two μ agonists and tested for an ability to alter opiate-induced analgesia. The α-adrenoceptor antagonist phentolamine attenuated the effects of fentanyl, but did not alter MS-induced analgesia. Conversely, the 5-HT receptor antagonists spiroxatrine (5-HT_1), ritanserin (5-HT_2) and ICS 205-930 (5-HT_3) diminished the spinal effects of MS, but did not alter fentanyl-induced analgesia. Apparently, endogenous NE contributes to the analgesic effects of fentanyl, whereas 5-HT is involved in MS-induced spinal analgesia.

223.12

PAIN MODULATION PRODUCED BY GLUTAMINERGIC INTERACTION BETWEEN N. CUNEIFORMIS AND N. RAPHE MAGNUS . R.C. Richter and M.M. Behbehani. Dept. of Physiology and Biophysics, U. Cincinnati College of Med. Cincinnati, OH 45267-0576.

We examined the role of glutamic acid in the interaction between n. cuneiformis and the n. raphe magnus (NRM) in light of Fields' et.al. classification system of NRM neurons (as on-cells, off-cells, and neutral cells).

In the lightly anesthetized rat model, the response properties of 112 NRM neurons to electrical stimulation of NCF at 1 Hz demonstrated that 53% were excited, 20% were inhibited, and 28% were unaffected. Almost all excitatory responses exhibited very short latencies of 2-4 msec. In contrast, examination of the responses of 92 NRM neurons to NCF electrical stimulation at 100 Hz showed that 41% were inhibited while only 22% were excited and 37% were unaffected. No significant difference in response pattern was seen among the 3 classes of NRM neurons at either stimulation frequency. The response of 36% of NRM cells to electrical stimulation and microinjection of glutamic acid into the NCF could be blocked by microinjection of kynurenic acid into the NRM.

Thus the differential responses of NRM cells to NCF electrical stimulation do not appear to be attributable to their physiological classification. The short latency excitatory responses seen at 1 Hz are suggestive of a fast excitatory amino acid transmitter while the inhibitory responses seen with train stimulation would be consistent with a slower acting, possibly peptidergic, transmitter, or with a local inhibitory collateral network within NRM. Supported by PHS Grant #NS20643.

223.13

AN INTRACELLULAR STUDY OF THE EFFECT OF EPINEPHRINE ON PAG NEURONS AND ITS ROLE IN PAIN MODULATION. M. Jiang, S.D. Chandler and M.M. Behbehani. Dept. of Physiology and Biophysics. Univ. of Cincinnati College of Medicine. Cincinnati OH, 45267-0576.

The basic electrical and membrane properties of 16 PAG neurons and their response to epinephrine (EPI) were studied in an in vitro slice preparation. Neurons were recorded throughout the PAG, but due to size and density, the majority were located in dorsolateral PAG. The majority of cells were spontaneously active, but some were not. In 60% of the neurons epinephrine caused an inhibitory effect that was associated with hyperpolarization of the membrane. This effect of EPI was dose dependent and had a duration of action that varied from 3 to more than 20 minutes. In 6% of cells EPI did not produce any effect. In the remaining cells EPI caused depolarization of the membrane and an increase in the firing rate. In 75% of neurons that were depolarized by EPI, no change in the membrane resistance was detected. On the other hand, in neurons that were hyperpolarized, EPI consistently caused a decrease in membrane resistance. There was no correlation between resting membrane potential or the location of the neurons and their response to EPI.

Our observation that the inhibitory effect of EPI is associated with a decrease in the membrane resistance suggests that this effect of EPI is mediated by a post synaptic mechanism possibly by an increase in potassium conductance. On the other hand, lack of consistent change in the membrane resistance in neurons excited by EPI suggests that this effect of EPI may be due to a presynaptic mechanism. Supported by PHS grant NS20643.

223.14

RELEASE OF ASPARTATE AND GLUTAMATE IN THE DORSAL HORN OF THE CAT LUMBAR SPINAL CORD IN RESPONSE TO INTRADERMAL CAPSAICIN INJECTION. D. Liu*, L.S. Sorkin, M.G. Hughes* and D.J. McAdoo Marine Biomedical Institute, University of Texas Medical Branch, Galveston, Texas 77550.

Glutamate and aspartate are putative neurotransmitters found in a significant percentage of myelinated and unmyelinated primary afferent fibers. Intrinsic spinal cord neurons immunoreactive to glutamate and aspartate have also been observed. To explore the role of excitatory amino acids in pain transmission, we stimulated cutaneous unmyelinated fibers by intradermal capsaicin (0.5 cc, 3%) injection and measured release of amino acids within the spinal cord of anesthetized cats. Perfusates were collected following placement of a microdialysis probe in the dorsal horn and the perfusates analyzed by HPLC with fluorimetric detection. Surprisingly, as much or more aspartate was released when capsaicin was injected into the contralateral leg as when injection was in the ipsilateral leg. Glutamate was also released in response to contralateral stimulation. This suggests that, after noxious peripheral stimulation, excitatory amino acids may be released by intrinsic spinal neurons as well as primary afferent fibers. (Supported by NIH NS11255, the Spinal Cord Research Foundation and Bristol Myers.)

223.15

BILATERAL RELEASE OF 5HT INTO THE DORSAL HORN OF THE CAT LUMBAR SPINAL CORD ELICITED BY INTRADERMAL CAPSAICIN(CAP) INJECTION. L.S. Sorkin and M.G. Hughes*. Marine Biomed. Inst., Univ. Texas Medical Branch, Galveston, Tx. 77550.

Activation of the raphe spinal system should produce bilateral release of 5HT in the spinal cord. This study measures 5HT levels on one side of the dorsal horn after noxious stimulation of either the ipsi- or contralateral hindlimb.

Cats were anesthetized with α-chloralose and a dialysis probe was inserted through the lumbosacral enlargement of the spinal cord. The dialysis zone was confined to one side of the dorsal horn. CSF was perfused (4 µl/min) through the probe and samples collected for consecutive 20 min periods. Samples were injected without pretreatment into an HPLC with EC detection. After stable levels of 5HT were achieved, 3% CAP was injected intradermally at several sites on either the ipsilateral or contralateral leg at the start of a collection period. Some animals were pretreated with 8-OH DPAT, a $5HT_1$ agonist.

Ipsilateral CAP produced a significant increase in 5HT levels for 1 or 2 sampling periods. Contralateral injections also increased 5HT levels; both were totally blocked by the prior administration of 8-OH DPAT.

These results suggest that ipsilateral activation of C-fibers induces a bilateral release of 5HT and presumably a bilateral hypoalgesia. (Supported by the Forman Research Foundation and Bristol Myers Co.)

223.16

RELEASE OF CALCITONIN GENE-RELATED PEPTIDE (CGRP) FROM RAT SPINAL CORD IN VITRO. R.E. Solomon, L.D. Aimone, T.L. Yaksh and G.F. Gebhart. Dept. Pharmacol., Univ. Iowa, Iowa City, IA and Dept. Anesth., Univ. Calif., La Jolla, CA.

The release of CGRP from superfusates of rat spinal cord slices in vitro was studied using methods described for studying substance P (sP) release (Soc. Neurosci. Abs. 14: 562, 1988). CGRP was measured in perfusates and post-perfusion tissue extracts by radioimmunoassay. Elevation of the potassium concentration from 2 to 50 mM or addition of 10.0 µM capsaicin evoked release of CGRP from dorsal, but not from ventral, spinal cord at rates 4.9- and 22.3-fold, respectively, above the basal rate of CGRP release. The effects of potassium and capsaicin were concentration-dependent and were abolished by depletion of calcium from the perfusion buffer. CGRP release evoked by potassium or capsaicin was inhibited by clonidine or morphine (10 - 100 µM), but not by serotonin (1 mM). Tissue content of CGRP was 4307 ± 442 and 317 ± 45 pg/mg in dorsal and ventral spinal cord, respectively. These results indicate that CGRP is released from primary afferent nociceptive nerve terminals in the rat dorsal spinal cord and that the modulation of such release may mediate the spinal actions of certain analgesic drugs. Comparison of these results with those that we reported on the release of sP suggests that CGRP and sP may be co-released from primary afferent neurons, and that CGRP is present in the spinal cord and released by capsaicin in amounts much greater than is sP. Supported by T32 GM 07069, DA 05329, NS 16541 and DA 02879.

223.17

NORADRENERGIC REGULATION OF CALCITONIN GENE-RELATED PEPTIDE (CGRP) AND SUBSTANCE P (SP) RELEASE FROM RAT DORSAL HORN SLICES. L.D.Aimone,C.L.Hagaman* and T.L. Yaksh, Dept. of Anesthesiology, Univ. of Calif., San Diego, La Jolla, CA

Noradrenergic terminals from bulbospinal pathways are thought to modulate spinal nociceptive processing in part by a reduction of neurotransmitter release from primary afferent neurons. Using an *in vitro* model described previously (Soc. Neurosci. Abst. 14: 562, 1988), the present study examined the effect of increasing endogenous norepinephrine (NE) levels on the K^+-evoked release of CGRP and SP. NE was measured by HPLC with electrical chemical detection; SP and CGRP were measured by radioimmunoassay. The release of all three neurotransmitters was evoked in a dose-dependent manner by the addition of K^+. Basal levels of NE (0.06 ng/ml) were dose-dependently increased by the addition of desipramine (1-100 µM) or pargyline (100 µM). The addition of both compounds produced an additive effect, resulting in basal NE levels of 490 ng/ml. Desipramine had no effect on basal peptide secretion, but produced a dose-dependent decrease in the amount of CGRP and SP evoked by the addition of 70 mM K^+. The evoked release of these peptides was also inhibited by the addition of pargyline. Thus, the increase in NE secretion was correlated with a decrease in the release of CGRP and SP. These data indicate that NE can act presynaptically to inhibit the release of CGRP and SP from primary afferent neurons. This mechanism could account for the antinociceptive actions of spinally administered noradrenergic agonists.
Supported by DA 05329 and NS 16541

224.1

EFFECTS OF ETHANOL ON EXTRACELLULAR DOPAMINE AND ITS CALCIUM DEPENDENCY AS MEASURED BY IN VIVO MICRODIALYSIS. K.M. Wozniak*, A. Mele*, A. Pert and M. Linnoila. (Spon: T. Nakajima). LCS/NIAAA, and BPB/NIMH, Bethesda, MD 20892.

Dopaminergic systems are thought to play a major role in the stimulant and reinforcing properties of drugs of abuse, including ethanol. The present study describes the effects of direct local infusion of ethanol on extracellular dopamine levels using the microdialysis technique. Animals were anesthetized with chloral hydrate, stereotaxically positioned and a 3mm microdialysis probe inserted into the corpus striatum. Following the establishment of basal dopamine extracellular levels (2-3h), various concentrations of ethanol in artificial csf (0.01-10% v/v) were slowly infused through the probe. Ethanol was found to increase dopamine levels at each dose used. This increase appeared to be dose-related at the higher concentrations (1-10%). The inclusion of 12.5 mM magnesium in the perfusion medium was found to significantly lower basal dopamine levels and also prevented, or significantly attenuated, the ethanol-induced dopamine release. This provides evidence that the release of dopamine by ethanol may involve calcium dependent processes.

224.2

BINDING OF [^{3}H]MUSCIMOL AND [^{3}H]RO 15-4513 IN THE CEREBELLUM OF RAT LINES WITH DIFFERING ALCOHOL SENSITIVITIES. E.R. Korpi and M. Uusi-Oukari*. Res. Labs, Alko Ltd, POB 350, SF-00101 Helsinki, Finland.

The binding of a $GABA_A$ agonist muscimol and of a benzodiazepine partial inverse agonist Ro 15-4513 was studied in brain membranes of two rat lines developed for high (ANT) and low (AT) sensitivity to moderate doses of alcohol.

The binding of [^{3}H]muscimol in cerebral cortical and hippocampal membranes was similar in both lines, whereas the B_{max} in cerebellar membranes was greater in the AT's than ANT's. The total binding of [^{3}H]Ro 15-4513 was fairly similar in both lines in all these brain areas. However, micromolar diazepam displaced all the specific cerebellar binding defined by 10 µM Ro 15-1788 in most ANT rats, while leaving about 25% non-displaced in most AT rats.

Since the sites for both the muscimol and the diazepam-insensitive Ro 15-4513 binding are localized in the cerebellar granular layer, the present results suggest a possible target of genetic modulation in the granule cells or in the cerebellar circuitry affecting them in the rat lines with differing alcohol sensitivities.

224.3

GABA TURNOVER IN THE BRAIN OF RAT LINES SELECTED FOR DIFFERENTIAL SENSITIVITY TO ETHANOL. K. Kiianmaa* and K. Hellevuo* (SPON: K.Nieminen). Res. Labs, Alko Ltd., 00101 Helsinki, Finland.

The effect of ethanol on GABA turnover was studied in different regions of the brain in the AT (Alcohol Tolerant) and ANT (Alcohol Nontolerant) rat lines developed for low and high degree of motor impairment from ethanol. The rate of GABA turnover was estimated from the accumulation of GABA after inhibition of GABA-T with aminooxyacetic acid (AOAA, 50 mg/kg, IP) given 10 min after administration of ethanol (2 or 4 g/kg, IP). The rats were killed two hours after the AOAA treatment with focused microwaves. GABA was analyzed by HPLC with fluorescence detection. The saline-treated ANT rats had a higher concentration of GABA in the striatum and a higher rate of GABA accumulation in the cerebellum than the AT rats. Ethanol suppressed the accumulation of GABA in both lines, but the suppression was significantly greater in the AT rats than in the ANT rats. In specific regions, this line difference was significant in the cerebral cortex and cerebellum with the higher ethanol dose. The results suggest that GABAergic mechanisms are involved in the differential sensitivity to the motor impairing effects of ethanol between the AT and ANT rats.

224.4

IN VITRO EFFECTS OF ETHANOL ON THE BRAIN ß-ENDORPHIN SYSTEM OF MICE WITH VARIABLE PREFERENCE FOR ETHANOL SOLUTION. J.-P. De Waele and C. Gianoulakis, Douglas Hospital Research Center and Department of Physiology, McGill University, Montréal, Québec.

Genetic factors may modify the effects of ethanol on neuronal and endocrine systems and, as a result, may influence the sensitivity of animals to ethanol as well as their consumption of ethanol solutions. The objective of this study is to examine the response to ethanol of the brain ß-endorphin system in two inbred strains of mice (C57BL/6 and DBA/2) by investigating the release of ß-endorphin by the hypothalamus following the In Vitro exposure to various doses of ethanol. This type of stimulation induced an increase in the release of ß-endorphin-like-peptides (ß-EPLPs) of both strains of mice. However, it was observed that both the spontaneous release and the ethanol stimulated release of ß-EPLPs was more pronounced by the hypothalamus of the C57BL/6 strain (voluntary drinker) than by that of the DBA/2 strain (showing aversion to ethanol). Furthermore, analysis by Sephadex G-75 chromatography indicated the release of ß-endorphin sized peptides by the hypothalamus at basal conditions as well as following ethanol exposure.

224.5

ETHANOL EFFECTS ON LIPID- AND PROTEIN-BOUND SIALIC ACID IN MICE OF DIFFERENT AGES. L. Cherian* and W. R. Klemm. Dept. Veterinary Anatomy, Texas A&M Univ., College Station, TX. 77843.

Mice of different ages were tested for changes in brain lipid- and protein-bound sialic acid (SA) two hours after ethanol (2 gm/kg, IP), either as a single dose or after five repeated doses of ethanol spaced 2 hours apart.

Ethanol generally decreased both gangliosidic and glycoprotein SA, but the effect varied with number of doses and age. Single-dose ethanol decreased both lipid- and protein-bound SA in young adults and aged mice, but there was no effect on lipid-bound SA in weanlings. With repeated injections, the 23% decrease in SA seen with young adults did not occur. Repeated injections did decrease both lipid- and protein-bound SA in aged mice, by 49% and 48%. Ethanol increased free SA in singly-dosed young adults and in multiply-dosed aged adults, while causing a distinct decrease in singly-dosed weanlings.

Comparison of single, repeated, or no saline injection in young adult controls indicated that the injection handling procedures themselves also affected both gangliosidic and glycoprotein SA. Supported by grant AA 06920, NIAAA.

224.6

ALCOHOL INDUCED ALTERATIONS IN ADENYLATE CYCLASE ACTIVITY IN CULTURED ASTROCYTES. T. Ritchie and E.P. Noble*. Neuropsychiatric Institute and Brain Research Institute, University of California, Los Angeles, CA 90024.

Primary astrocyte cultures prepared from neonatal rat brains were used to study the effects of ethanol (EtOH) on adenylate cyclase (AC) activity. Cultures grown in 24-well plates were prelabeled with ^{3}H-adenine followed by stimulation of AC activity in the presence of acute doses of EtOH (25-500 mM). A dose-dependent enhancement of 10^{-5} M norepinephrine (NE)-stimulated AC activity was observed with significant enhancement occurring at EtOH doses as low as 50 mM. Virtually identical dose-dependent enhancements were seen with stimulation by 10^{-5} M isoproterenol and 10^{-5} M adenosine suggesting that EtOH may be exerting its effects through these two receptor systems via a common mechanism. Similar EC_{50} values for stimulation of AC in the presence and absence of 100 mM EtOH by both NE and isoproterenol indicate that EtOH has little effect on the sensitivity to these agonists. When astrocytes were maintained in serum free medium, EtOH enhancement of NE-stimulated AC activity was sharply attenuated as opposed to cells maintained in serum supplemented medium. These findings suggest that acute exposure of astrocytes to EtOH may enhance AC activity by producing alterations at a site distal to the receptor, and that these alterations may be subject to hormonal influence. (Supported in part by USPHS grant AA07653.)

224.7

DIFFERENTIAL EFFECTS OF PRENATAL ETHANOL EXPOSURE ON CORTICOSTERONE RESPONSES TO RESTRAINT STRESS IN MALE AND FEMALE RATS. J. Weinberg. Dept. of Anatomy, Faculty of Medicine, The University of British Columbia, Vancouver, B.C. V6T 1W5, Canada.

Hyperresponsiveness to stress (enhanced pituitary-adrenal activation and/or delayed recovery) has been reported to occur primarily in fetal ethanol exposed (FEE) females and not in FEE males. However, the stressors studied have typically been acute and/or of short duration. The present study examined the corticosterone time course to prolonged (4 hr) restraint in adult males and females from prenatal ethanol (E), pair-fed (PF) and ad libitum-fed (C) treatment groups.

All animals showed a significant corticoid increase over the first hr of testing and a gradual return toward basal levels over the next 3 hr, although corticoids never completely returned to basal levels during the 4 hr test. In addition, in contrast to previous studies, E females were found to be similar to PF and C females in their pattern of corticoid responsiveness; E males, however, were similar to PF and C males in their initial response to stress, but showed a prolonged corticoid activation over the 4 hr test period. These data suggest that under conditions of prolonged rather than short duration stress, adrenocortical hyperresponsiveness may be demonstrated in E males, but not females.

Supported by grant #AA07789 from NIAAA.

224.8

CHRONIC ETHANOL EFFECTS ON THE ACIDIC PHOSPHOLIPIDS IN MOUSE BRAIN REGIONS. M. Navidi*, F.G. Yao* and G.Y. Sun (SPON: D. O'Brien). Sinclair Research Farm and Biochemistry Department, University of Missouri, Columbia, MO 65203.

We have reported previously that chronic ethanol administration resulted in an increase in the level of acidic phospholipids in brain. The increases in levels of phosphatidylserines (PS) as well as phosphoinositides (PI, PIP and PIP_2) are best shown in the synaptosomal membrane fraction, reflecting that these alterations are associated with synaptic mechanisms. Recently, the response of brain acidic phospholipids towards chronic ethanol treatment can be more effectively probed by prelabeling the phospholipids with ^{32}Pi through intracerebral ventricular injections. In this study, C57b16J mice were pair-fed a Sustacal (chocolate flavor) liquid diet containing 5% ethanol or a control diet containing an equal amount of glucose for 8 weeks. ^{32}Pi (20 uCi per brain) was injected to each brain 4 hr prior to killing and subsequent dissection of brain regions on ice. Cerebral cortex, hypothalamus, hippocampus as well as a synaptosome fraction from the cerebral cortex were obtained for analysis. the procedure for lipid analysis (Sun and Lin, Life Sci. 44: 689, 1989) allowed separation of all phospholipids including the poly-PI and PA in one single high performance thin-layer plate. Results from this study indicated an increase in labeling of poly-PI in cortex and hippocampus but not in hypothalamus. Among these brain regions, hippocampus from ethanol-treated group (n=8) exhibited most dramatic increases in labeling of the acidic phospholipids (PA, PS and poly-PI) as compared to the pair-fed controls (n=8). These results confirm other studies indicating that the hippocampus is more sensitive to the effects of ethanol and further emphasize the importance of this brain region in manifestation of the physiological effects of chronic ethanol administration. (Supported in part by AA 06661 from NIAAA).

224.9

INTERACTIONS WITH ADENOSINE (ADO) AND ETHANOL (ET) OF ENDOGENOUS GLUTAMATE (GLU) RELEASED FROM RAT CEREBELLAR SYNAPTOSOMES. M. Clark* and M. S. Dar. BPB, NIMH, Bldg 10, Rm 3N212, Bethesda, MD 20892 and Department of Pharmacology, School of Medicine, East Carolina University, Greenville, NC 27858.

Effects of ET and ADO receptor agonist R-PIA and antagonist theophylline (TH) on release of endogenous GLU were tested in rat cerebellar synaptosomes. GLU release for 5-60 s was followed by its separation by rapid filtration. Released GLU was measured by an enzyme-linked fluorometric assay. Basal GLU release was 3.7±0.3 nmol/mg protein/5 s and was stimulated by high K^+. GLU release consisted of an initial rapid phase for the first 10 s followed by a relatively slower phase. Observation of Ca^{2+}-dependent and -independent GLU release suggested the role of neuronal and glial constituents of the synaptosomal preparation, respectively. ET (25-100 mM) caused a trend toward a dose-dependent inhibition of GLU release. R-PIA and TH inhibited and stimulated, respectively, basal release of GLU and R-PIA-inhibited release was blocked by TH. ET (25 mM) blocked the stimulatory effect of TH and the data with ADO deaminase suggested ADO role in this effect of ET. Results suggest ET inhibits GLU release in the cerebellar cortex via an ADO-sensitive mechanism and support our findings of cerebellar ADO modulation of ET-induced motor disturbances. [Funded in part by NC Alcoholism Res. Authority Grant #8605].

224.10

TEMPERATURE-RELATED CHANGES IN SENSITIVITY TO ETHANOL-INDUCED FLUIDIZATION OF C57 MOUSE BRAIN SYNAPTOSOMAL* PLASMA MEMBRANES (SPMs). R.L. Alkana, K. von Hungen*, C.F. Baxter, P.J. Syapin, and M. Bejanian. Alcohol and Brain Research Laboratory, School of Pharmacy, Univ. of Southern Calif., Los Angeles, CA 90033.

The present study tested the hypothesis that temperature-related changes in ethanol's fluidizing effects on brain membranes might underlie the effects of temperature on brain sensitivity to ethanol in C57 mice. SPMs were prepared from the pooled forebrains of 10 C57BL/6 mice. Fluidity measurements were made at 25, 32, and 37°C using 1,6-diphenyl-1,3,5-hexatriene (DPH). As previously reported, baseline fluidity increased significantly with increases in temperature. Further, increasing the temperature from 25 to 37°C significantly increased the fluidizing effect (% change in polarization) of ethanol on SPMs. Increasing the temperature from 32 to 37°C caused a nonsignificant increase in the fluidizing effect of ethanol. The minimum concentration of ethanol required to cause a significant increase in SPM fluidity was higher at 25°C (170.7 mM) than at 32 and 37°C (85.3 mM). Taken with previous behavioral studies, these results indicate that sensitivity to the fluidizing and behavioral effects of ethanol both increase with temperature. (Supported by NIAAA grants AA05234 & AA03972 & VA Medical Res. Serv.).

224.11

GANGLIOSIDES AND CHOLESTEROL DIFFERENTIALLY AFFECT MEMBRANE SELECTIVITY AND SENSITIVITY. R. Hitzemann, J. Lin* and K. Dains*. Psychiatry Service-VAMC Northport, NY 11768; Dept. Psychiatry, SUNY: Stony Brook, NY 11794

The selectivity and sensitivity of two different membranes (x and y) to perturbation (P) by non-electrolyte anesthetics (i), such as the n-alcohols, are related by $\log P_{i,y} = \log P_{i,x} * S_{x,y} + R_{x,y}$, where S is the selectivity constant and R is a measure of the difference in membrane sensitivity (Hitzemann, 1988). When $S_{x,y}$ is > 1, membrane "x" will show differentially greater perturbation by more hydrophobic molecules. We have examined, using fluorescence polarization techniques, the effects of ganglioside GM1 and cholesterol to alter the selectivity and sensitivity of DMPC liposomes to perturbation by n-alcohols (C1 to C8). The incorporation of GM1 (30 mole %) increases (> x 2) membrane sensitivity to perturbation (DPH as probe) without affecting membrane selectivity. In contrast, the incorporation of cholesterol (50 mole %) increases both membrane selectivity and sensitivity (> x 4). Because of the increase in selectivity (S = 1.30) the C-1 to C-3 alcohols are less potent to perturb structure in the presence of cholesterol. The addition of GM1 to the cholesterol containing liposomes has no effect on selectivity but markedly attenuates (> 50%) the increase in sensitivity. The data will be discussed in terms of the structure of anesthetic sensitive domains.

224.12

AFFERENT FIBER DISTRIBUTION IN RAT HIPPOCAMPUS AFTER CHRONIC ETHANOL EXPOSURE. C.T. Smothers and B.E. Hunter. Dept. of Neuroscience, University of Florida, Gainesville, FL 32610.

Chronic ethanol treatment (CET) has been shown to increase the amplitude of hippocampal population spike responses in the ventral hippocampus. These studies were designed to determine whether this regional variation results from underlying changes in afferent fiber distribution. Ethanol-treated (E) and control (S) groups were fed a liquid diet containing either ethanol or sucrose for 20 weeks followed by an 8 week abstinence period. Hippocampal slices were prepared from septal and temporal poles and maintained in a slice chamber. Stimulating electrodes were placed in stratum radiatum (SR) and a recording micropipette was placed in SR (afferent volley; dendritic EPSP) or in stratum pyramidale (population spike) of CA1. Spatial mapping studies were performed by generating input/output functions with stimulating/recording electrode distances of 100,250 and 500 microns.

CET resulted in significantly greater population spike responses in slices from the ventral pole as compared to the dorsal hippocampus. Population spike amplitude tended to decrease with increasing inter-electrode distance in S as compared to E animals in the ventral hippocampus. When population spike amplitude was plotted as a function of dendritic EPSP amplitude, a sigmoid function was obtained which exhibited a greater rate of rise with increasing electrode separation. Such results suggest that local circuit inhibitory interneurons are more effectively activated with decreasing distance between stimulating and recording electrodes. Together with previous studies, these results indicate that CET increases excitability in the ventral hippocampus by producing an enduring reduction in inhibitory synaptic transmission.

Supported by the Veterans Administration and NIAAA AA00200.

224.13

ETHANOL-RESPONSIVE PROMOTER ELEMENTS. M. F. Miles and J. Sturdivant*. Dept. of Neurology and The Ernest Gallo Research Center, Univ. Calif. San Fran., San Francisco, CA 94110.

We have recently shown that chronic ethanol exposure induces a specific pattern of changes in mRNA abundance in the NG108-15 neuroblastoma-glioma hybrid cell line. Included in those genes that are induced by ethanol is the constitutive member of the 70 kDa stress protein family, Hsc70. This protein is thought to play an important role in membrane protein trafficking and thus could mediate many of the pleiotropic effects of ethanol.

We have investigated the mechanism of ethanol regulation of Hsc70 by using transfection analyses with a Hsc70 promoter region fused to the coding region for bacterial chloramphenicol acctyltransferase (CAT). Ethanol causes a dose dependent increase in CAT activity in NG108-15 cells transfected with this construct. Control cultures (using viral promoters) show no change or decreased CAT activity.

These results suggest that ethanol regulates Hsc70 at the transcriptional level and that ethanol-responsive cis-acting sequences are present in the Hsc70 promoter. Further characterization of these will be presented.

224.14

LIVER DISEASE WITH OR WITHOUT VITAMINS A AND E DEFICIENCY AND BRAIN FUNCTION. A.M. Arria*, M.A. Kabene*, R.E. Tarter*, V. Warty*, D.H. Van Thiel* (SPON: J.R. JENNINGS). Dept. of Psychiatry, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213.

Vitamin A and E deficiency are a common consequence of cirrhotic liver disease. A deficiency of vitamin E has been postulated to produce a specific neurologic syndrome characterized primarily by disturbances in motor function. The purpose of the study was to assess the combined effect of vitamin A and E deficiency in adults with chronic liver disease who were not acutely encephalopathic. The association between vitamins A and E deficiency and neurologic status related to liver disease was assessed using a battery of neurocognitive tests. Controlling for liver injury status, it was found that vitamin A and E deficient patients (N=70) performed more poorly on tests of psychomotor capacity than vitamin A and E sufficient patients (N=51). The results suggest that nutritional deficiency may underlie, in part, the neurologic syndrome of hepatic encephalopathy.

224.15

COMPUTER ANALYSIS AND THREE-DIMENSIONAL RECONSTRUCTION OF SERIAL-SECTIONED BRAINS FOLLOWING CHRONIC ALCOHOL ADMINISTRATION: TOPOGRAPHIC DISTRIBUTION OF VASOPRESSIN- AND OXYTOCIN-CONTAINING NEURONS. GP Kozlowski, NC Smith*, WK Smith, JH deSchweinitz* and DJ Woodward. Departments of Physiology and Cell Biology, University of Texas Southwestern Medical Center, Dallas, TX 75235-9040 and Biographics Inc, 1950 Stemmons Freeway, Dallas, TX 75207.

The aim of this study was to determine the effects of chronic ethanol (ETOH) on the numbers and distributions of vasopressin (VP) and oxytocin (OT) neurons in the rat hypothalamus. The CARP/386 (Computer Aided Reconstruction Package for 80386 microcomputers) System was used to plot, quantitate and visualize cell population data. Male, Long-Evans rats were simultaneously pair-fed either a control liquid diet or one containing 5% ETOH for 15 da. They were perfuse-fixed and 40 μm serial-sections of their brains were processed for immunocytochemistry using either anti-VP or anti-OT sera. The positions of VP and OT neurons in traced outlines of sections were plotted using a computer/microscope. Neurons within particular areas of interest, e.g. the paraventricular nucleus (PVN), were color-coded for comparison with neurons in other areas of the reconstructed brains. The images can be rotated and viewed from multiple directions. Chronic ETOH appears to diminish the population of VP neurons in the PVN, but to augment OT neuronal number in the PVN. Supported by NIAAA AA-06014 and NINDS NS-25321.

224.16

PENTOBARBITAL RESPONSE IN MALE VS. FEMALE WLE SEGREGATING INBRED STRAIN RATS: MUTANT ALBINO VS. PIGMENTED LITTER-MATES. J.R.Taylor* and I.S.Westenberg (SPON: D.F.Wooley-Mc-Kay). Psychology Dep't., Glendale Com.Col., Glendale, AZ 85302.

With repeated pentobarbital (PB) dosing albino rat strains have been more PB-sensitive than pigmented strains, but within a strain albino (c/c) and pigmented (c/+) rats of the same strain have not differed in PB sensitivity. We made a within-strain comparison of c/c-vs.-c/+ PB sensitivity to single PB doses. Littermate quadruplets (c/c male, c/+ male, c/c female, c/+ female) of Westenberg-Long-Evans (WLE) segregating inbred strain rats were c/c or c/+ but otherwise genetically nearly identical. 80 mg/kg ip PB killed 5/10 females (2 c/c, 3 c/+) and 1/10 males (1 c/+). The lethal-dose-to-50% (LD50) for the females was 80 mg/kg, which was significantly below the LD50 for the males (sign test, $p < 0.05$). There were no significant c/c-vs.-c/+ differences in mortality for males or females. Of the surviving rats males slept 4-7 h and females slept 17-47 h. Male-vs.-female differences in sleeptimes were statistically significant ($U=0$, $p < 0.01$); c/c-vs.-c/+ differences in sleeptimes were not significant for males or females. These results parallel those of the within-strain, repeated-dosing study. They reinforce the conclusion that the PB-sensitivity difference between albino rat strains and pigmented strains is due to genetic factors other than just the albino mutation. Analysis of the between-strain difference may elucidate factors in PB's mode(s) of action.

224.17

MK-801 ENHANCES THE EFFECTIVENESS OF GENERAL ANESTHETICS. L. C. Daniell. Dept. Pharmacol. and Toxicol., Med. Coll. of Ga., Augusta, GA. 30912-2300.

The effect of MK-801 on potency or duration of anesthesia was tested for anesthetics from several chemical classes. Male ICR mice were pretreated with various doses of MK-801 ((+)-isomer, 0.3 to 3.0 mg/kg, i.p.) 30 min before administration of ethanol (4.2 g/kg, i.p.) or pentobarbital (50 mg/kg, i.p.) or 15 min before adminstration of various concentrations of halothane or diethyl ether. MK-801 alone did not produce anesthesia at any of the doses used in this study. The duration of loss of righting reflex induced by ethanol and pentobarbital administration was increased by MK-801 in a dose dependent manner. The concentration of halothane or diethyl ether required to produce non-responsiveness in 50% of subjects (the minimum alveolar concentration) was reduced by pretreatment with MK-801. These results show that MK-801 increases the effectiveness of general anesthetics and may provide information about neurochemical mechanisms of anesthesia.

224.18

DRUG DISCRIMINATION LEARNING: EFFECTS OF REPEATED DOSE-RESPONSE ASSESSMENTS.
S. Pournaghash, D. Owens*, R. Jeffreys and A. Riley. Psychopharmacology Laboratory, The American University, Washington, D.C. 20016.

Recently, Riley and his colleagues (Riley et al., Drug Dev. Res., 16:229, 1989) reported rapid drug discrimination learning within a conditioned taste aversion paradigm. Specifically, rats avoided a taste paired with a toxin when the taste was preceded by pentobarbital and drank the solution when preceded by distilled water. Because of this sensitivity, a confound may arise when different (nonpoisoned) doses of the training drug are given as probes to assess generalization. Specifically, the animals may rapidly learn that probes are not poisoned. Repeated assessments may result in the learning of another discrimination (training vs. probe dose). This possibility was addressed in the present experiment in which following the acquisition of a pentobarbital drug discrimination (10 mg/kg) a dose generalization was repeatedly determined. Although 3.2 and 5.6 mg/kg initially produced dose-related decreases in consumption, with repeated assessments consumption gradually increased following these doses. These data suggest that repeated dose-response assessments can affect dose-response functions within the conditioned taste aversion procedure.

225.1

SEROTONIN 5-HT_{1C} RECEPTOR MEDIATES CYCLIC GMP FORMATION VIA ARACHIDONIC ACID METABOLITES. M.J. Kaufman[1], F. Hirata[1]*, P.R. Hartig[1,2] and B.J. Hoffman[1,3]. [1]The Johns Hopkins Medical Institutions, Balto., MD, 21205, [2]Neurogenetic Corp., Paramus, NJ, 07652, and [3]Lab of Cell Biology, NIMH, Bethesda, MD, 20892.

The serotonin 5-HT_{1C} receptor mediates both phosphoinositide (PI) hydrolysis and formation of cGMP in choroid plexus. The pathway of cGMP formation is not known; however we have demonstrated that cGMP formation requires extracellular calcium. Cytosolic calcium elevation induced by PI turnover might stimulate calcium dependent enzymes such as phospholipase A_2 (PLA_2) to produce arachidonate. Then arachidonate metabolites could promote increases in cGMP. Therefore we have examined the role of PLA_2, cyclooxygenase and lipoxygenase in 5-HT stimulated cGMP formation.

Inhibition of PLA_2 with p-bromophenacyl bromide (10uM) reduced 1uM 5-HT stimulated cGMP formation by 40%. Inhibition of the cyclooxygenase pathway with 10uM indomethacin enhanced 1uM 5-HT stimulated cGMP formation by nearly 50%. Blockade of the lipoxygenase pathway with BW 755C (100uM) abolishes serotonin stimulated cGMP formation. Thus it appears that 5-HT_{1C} receptor stimulation leads to increases in arachidonic acid, and metabolites of the cyclooxygenase pathway inhibit while those of the lipoxygenase pathway stimulate cGMP formation.

225.2

SEROTONERGIC MODULATION OF LOCAL INHIBITION IN THE HIPPOCAMPAL SLICE IS MEDIATED BY cAMP. S. SPRINGFIELD, E. NEIL*, M. TILLMAN*. Dept. of Biology, City College of CUNY, New York, N.Y. 10031

Previous studies from our laboratory have demonstrated that serotonin (5-HT) attenuates local inhibition in the hippocampus and this modulatory action occurs through the 5-HT_{1A} receptor. This receptor subtype was previously established to be coupled to the adenylate cyclase/cAMP system. The present experiments address the hypothesis that cAMP may be the second messenger in 5-HT's modulatory action.

Hippocampal slices (350-450 µm) were obtained from adult male Sprague-Dawley rats and constantly superfused with a bicarbonate-buffered balanced salt solution. Slices were allowed to equilibrate at 32° C and aerated with 95%O_2/5%CO_2. Evoked population spikes were recorded with a single glass micropipette (2-10MOhms) filled with 3M NaCl. Twin stimuli (200-1000 µsec; 1-10V; 1/35-1/60 Hz) of interstimulus intervals of between 5 and 20 msec were presented to the stratum radiatum at the border of the CA1-CA2 region. Local inhibition occurred when the amplitude of the test (second) population spike is smaller than the conditioning (first) spike. The reduction has been attributed to the action of GABA-mediated inhibition superimposed upon orthodromic excitation.

Application of cAMP (10^{-9}-10^{-8}M) resulted in an increase in the amplitude of the test spike. In addition adenine (10^{-9}M), a metabolite of cAMP, attenuated local inhibition. The similarity of actions of these agents suggest that 5-HT's modulatory action in the hippocampus may involve cAMP as a second messenger. (NSF grant BNS 8606419, PSC-CUNY award and MBRS program).

225.3

NEUROCHEMICAL, ELECTROPHYSIOLOGICAL AND BEHAVIOURAL EVIDENCE THAT 8-OH-DPAT STIMULATES CENTRAL DOPAMINE SYSTEMS. M. Davies*, B.D. Sloley* and P.J.Fletcher* (SPON: A.A. Boulton). Neuropsychiatric Research Unit, Medical Research Bldg, University of Saskatchewan, Saskatoon, Sask., Canada.

We have used neurochemical, electrophysiological and behavioural techniques to investigate a potential interaction between the 5-HT receptor agonist 8-OH-DPAT and central DA systems. Striatal concentrations of DA and DOPAC were obtained from aliquots of homogenized rat striata using HPLC-ED techniques. Peripheral administration of 8-OH-DPAT (15 to 240 µg/kg IP) or the administration of 8-OH-DPAT (1µg) into the dorsal raphé nucleus (DRN) 30 mins prior to sacrifice increased the ratio of DOPAC:DA in the striatum. Electrophysiological studies in anaesthetized rats showed that IV administration of 8-OH-DPAT (0.3 to 10 µg/kg) increased the spontaneous activity of striatal neurones. This effect was completely reversed by haloperidol (0.1 mg/kg IV). Food intakes in free-feeding rats were measured over 1 hour following the microinjection of 8-OH-DPAT (1µg) into the DRN. 8-OH-DPAT consistently elicited an increase in food intake which was completely blocked by pretreatment with haloperidol (0.1 mg/kg SC) or spiperone (0.05 mg/kg SC). Spiperone (20 µg) was ineffective, however, when microinjected into the DRN suggesting that DA is involved downstream from the DRN. Taken together these results provide strong evidence that 8-OH-DPAT stimulates central DA systems which is probably due, at least in part, to a reduction of the inhibitory influence which 5-HT systems exert over central DA transmission. (Supported by Sask Health & Sask Health Res. Board).

225.4

ROLE OF SOMATODENDRITIC AUTORECEPTORS IN THE REGULATION OF SEROTONERGIC NEURONAL ACTIVITY IN BEHAVING CATS. C.A. Fornal, W.J. Litto, L.E. Ribeiro-do-Valle, and B.L. Jacobs. Prog. Neurosci., Dept. of Psychol., Princeton Univ., Princeton, NJ 08544.

Somatodendritic autoreceptors (presumably $5HT_{1A}$) may play an important role in regulating the firing rate of central serotonergic neurons. The present study examined the effects of spiperone, a presumed $5HT_{1A}$ antagonist, on a) the spontaneous firing rate of serotonergic dorsal raphe nucleus (DRN) neurons, and b) the suppression of serotonergic DRN neuronal activity produced by $5HT_{1A}$ agonists. Serotonergic neurons were recorded and identified using methods previously described (Fornal et al., Exp. Neurol. 98:388-403, 1987). Drugs were administered remotely via an indwelling jugular catheter. The spontaneous activity of serotonergic DRN neurons varied in association with behavioral state. Spiperone (1 mg/kg) increased unit activity when cats were aroused or alert, but not when cats were drowsy or asleep, as indicated by polygraphic criteria. Spiperone pretreatment (1 mg/kg) blocked the effects of 8-OH-DPAT (5 µg/kg), buspirone (20 µg/kg), and 5-MeODMT (20 µg/kg), for 2-3 hrs. Similar effects were not observed following haloperidol (1 mg/kg). We conclude that serotonin autoreceptors exert a tonic inhibitory influence on the firing rate of serotonergic DRN neurons during periods associated with high activity, such as active waking, and not during periods associated with low activity, such as sleep.

225.5

NERVE TERMINAL AUTORECEPTOR REGULATION OF SEROTONIN RELEASE. J.J. RUTTER* and S.B. AUERBACH. Dept. of Biol. Sci., Rutgers Univ., New Brunswick, NJ 08903.

Stimulated serotonin (5-HT) release from synaptosomes is enhanced by the 5-HT_1 antagonist methiothepin. This suggests the presence of 5-HT nerve terminal autoreceptors. We have used microdialysis in unanesthetized rats to further test the possibility that terminal autoreceptors regulate 5-HT release *in vivo*. Concentric-type dialysis probes were implanted in the ventromedial hypothalamus. Serotonin in the dialysate was measured by HPLC-EC. The synaptic origin of 5-HT was verified by 50% or greater inhibition with local administration of TTX or systemic administration of the somatodendritic autoreceptor agonist, 8-OH-DPAT.

Methiothepin, 20 µM, was locally perfused for two h in the dialysate, with or without pre-perfusion with fluoxetine, 10 µM, a selective 5-HT reuptake inhibitor. Fluoxetine pre-perfusion alone produced a fivefold increase above baseline; with the addition of methiothepin, 5-HT release was significantly increased at 30 min to 219 ± 51.4% of fluoxetine baseline levels (n=14). In contrast, without fluoxetine pre-perfusion, 5-HT was slightly, but non-significantly increased at 30 min to 39 ± 31% above baseline levels (mean ± s.e.m., n=6). This suggests that the nerve terminal autoreceptor is preferentially activated at supraphysiological levels of synaptic 5-HT. Preliminary data suggest that methiothepin also preferentially enhances 5-HT release during 2 h of restraint stress as compared to 2 h of undisturbed behavior.

Supported by NSF grant BNS-8708014.

225.6

EFFECTS OF ELECTRICAL STIMULATION OF THE DORSAL RAPHE NUCLEUS ON 5-HYDROXYTRYPTAMINE (5-HT) IN MEDIAL PREFRONTAL CORTEX (MPC) AND DORSAL HIPPOCAMPUS (DH) BY IN VIVO MICRODIALYSIS D.J. Mokler and J.H. Johnson. Dept. of Pharmacol., Univ. New England, Biddeford ME 04005 and Dept. Anatomy, Vir. Comm. Univ., Richmond VA 23298.

Previous experiments have shown that electrical stimulation (ES) of the dorsal raphe nucleus results in an increase in the levels of the major metabolite of 5-HT, 5-hydroxyindoleacetic acid (5-HIAA) in forebrain areas. The purpose of the present experiment was to examine the effects of ES of the dorsal raphe on the levels of 5-HT and 5-HIAA simultaneously in the DH and MPC by in vivo microdialysis. Male Sprague-Dawley rats were implanted with a bipolar electrode into the dorsal raphe nucleus and guide cannulae into the DH and MPC. After one week for recovery, microdialysis probes were placed into the DH and MPC under light ether anesthesia. Microdialysis was started immediately at a rate of 5 µl/min for 10 min periods. Samples were collected for one hour for baseline determinations. ES (200 µA, bipolar, 100 µsec duration, 20 Hz) produced an increase in 5-HIAA in the DH and MPC to a maximum of 120% of control after 20 min. The 5-HT_{1a} agonist 8-OH-DPAT, infused in the microdialysis fluid into the DH, produced a slight decrease in levels of 5-HIAA in the DH. Therefore, in vivo microdialysis can be used to detect changes in 5-HT activity following ES of the dorsal raphe. (Supported by NIH grant HD-12165)

225.7

REGULATION OF SEROTONIN RELEASE IN RAT STRIATUM: 5-HT$_1$ RECEPTOR AGONISTS INTERACT WITH THE 5-HT UPTAKE CARRIER. S.L. Garber and M.H. Makman *. Albert Einstein College of Medicine, Bronx, NY 10461.

The possible roles for serotonin (5-HT) release, receptor activation and intraneuronal 5-HT levels in mediating autoinhibition of 5-HT synthesis were studied in rat striatal synaptosomes. 5-HT itself inhibited basal formation of 5-HT from ^{14}C-tryptophan. Zimelidine, which inhibits 5-HT uptake, diminished the effect of 5-HT. 5-HT also blocked the stimulatory effect of forskolin, an activator of adenylate cyclase shown previously to stimulate 5-HT synthesis (Molec. Brain Res. 3:1-11,1987). Basal formation of 5-HT was not inhibited by the 5-HT$_1$ receptor agonists 5-methoxy-3(1,2,3,6-tetrahydropyridin-4-yl)1-H-indole (RU-24969), trimethylphenylpiperazine (TFMPP) or 8-hydroxy-2-(di-n-propylamino) tetralin (DPAT). However, efflux of prelabeled ^{3}H-5-HT was enhanced not only by 5-HT but also by TFMPP, DPAT and RU-24969; chlorimipramine, an uptake carrier blocker, reversed these effects on efflux. Specific uptake of ^{3}H-5-HT (Km=143nM) was inhibited by DPAT (Ki=345nM) or RU-24969 (Ki=1040nM), as well as by chlorimipramine. However, specific uptake of ^{3}H-DPAT itself did not occur. It is proposed that the 5-HT$_1$ agonists RU-24969, DPAT and TFMPP bind to the outer surface of the uptake carrier to promote exchange or release of 5-HT as well as to block uptake. Thus, certain 5-HT$_1$ agonists may act to regulate 5-HT release and uptake, independent of 5-HT synthesis, similar to the action of tricyclic and related antidepressants.

225.8

ACTIVATION OF 5-HT-2 RECEPTORS ALTERS UNIT ACTIVITY OF CORTICAL NEURONES. R.S. Neuman and G. Zebrowski*. Faculty of Medicine, Memorial University, St. John's, Nfld., Canada A1B 3V6

Noxious stimulation induces cortical desynchronization which is mediated by serotonin (Neuman, EEG and Clin Neurophysiol, 64: 546-555, 1986). In the present study we investigated the site of serotonin's action.

Unit activity recorded from layer IV neurones in the frontal-parietal cortex, in urethane anaesthetized rats, consisted of bursts separated by long interburst intervals which were synchronous with slow wave activity recorded from the same electrode. Noxious stimulation reversibly abolished the long interburst intervals resulting in continuous firing of the unit during and often outlasting the stimulus. This corresponded with cortical desynchronization. Systemic administration of ketanserin, cinanserin and cyproheptadine, but not vehicle, reversibly blocked the alteration of unit activity and accompanying desynchronization induced by noxious stimulation. The effects of low iontophoretic currents (10-20 nA) of ketanserin and cinanserin applied from adjacent electrode barrels mimicked the systemic administration of these agents, i.e. the burst-pause activity persisted despite the application of noxious stimulation.

Layer IV of the cortex contains a high concentration of 5-HT-2 receptors along with a dense plexus of 5-HT immunoreactive fibres which originate from the dorsal raphe. We conclude that noxious stimulation activates serotonergic neurones of the dorsal raphe which in turn enhance the firing rate of cortical neurones. Supported by the Canadian MRC.

225.9

SEROTONIN-DEPENDENT HIPPOCAMPAL ELECTRICAL ACTIVITY IN FREELY MOVING ANIMALS: A METHOD FOR EVALUATING SEROTONERGIC ANTAGONISTS. N.V. Watson*, E.L. Hargreaves and C.H. Vanderwolf (SPON: D.J. Stewart). Dept. of Psychology, Univ. of Western Ontario, London, Ontario. CANADA N6A 5C2.

The hippocampal Rhythmical Slow Activity (RSA) that persists following a large dose of scopolamine and that correlates with Type I motor activity (walking, struggling), appears to be dependent on central serotonergic transmission (Vanderwolf, 1988). Possible blockade of this activity by putative serotonergic antagonists was assessed in freely moving rats, pretreated with scopolamine (5 mg/kg s.c.). RSA was quantified by integrating 6-12 Hz activity during waking immobility and struggling.

Mianserin (1-50 mg/kg i.p.), and pizotifen (1-50 mg/kg i.p.) were without effect, but methiothepin (0.2-5 mg/kg i.p.) produced a dose-related suppression of scopolamine-resistant RSA. As a control for the cataleptogenic effect of methiothepin, it was shown that haloperidol (0.2-5 mg/kg i.p.) did not suppress scopolamine-resistant RSA.

Study of scopolamine-resistant electrocortical activity may provide a convenient assay of central serotonergic activity with a known relation to behavior. Supported by grants from NSERC to C.H.V.

225.10

SEROTONERGIC MODULATION IN THE HAMSTER HIPPOCAMPAL SLICE DIFFERENT THAN IN THE RAT HIPPOCAMPUS. D. J. Horrigan* and J. M. Horowitz. Dept. of Animal Physiology, Univ. of Calif., Davis, CA 95616.

The effects of serotonin on hippocampal population spikes at 34°C in the rat, a nonhibernator, have previously been described. To determine if similar effects could be observed in the hamster, a hibernator, hippocampal pyramidal cell activity was monitored in hamsters and rats following Schaffer collateral stimulation using methods described by Thomas *et. al.*, J. therm. Biol. 11:213, 1986. Population spike amplitude was measured before, during and after bath administration of 10 μM serotonin at a bath temperature of 20°C. The response consisted of a depression of the population spike during serotonin application and a recovery and overshoot of the spike amplitude following drug removal. At 20°C the average depression in the hamster was 52.4±10.1% and in the rat was 76.4±5.5% (mean ± SE). (In the hamster the depression was measured over a large temperature range and shown to persist for temperatures from 15°C to 35°C). The average overshoot in the hamster was 33.7±9.3% and was significantly ($p < 0.05$) larger than 7.4±4.8%, the overshoot seen in the rat. (In the hamster the enhancement was a minimum at 25°C and had a greater amplitude at 35°C and at 15°C). In summary, the overshoot is more pronounced in the hamster than in the rat indicating a difference between species. [Supported by NASA grant NAGW-1458].

ACETYLCHOLINE III

226.1

RECOVERY OF BIOCHEMICAL RESPONSES MEDIATED BY MUSCARINIC RECEPTORS FOLLOWING ADMINISTRATION OF AN IRREVERSIBLE CHOLINERGIC AGONIST (BM 123). V.H.Sethy, D.K.Hyslop, and T.P.Boyle. CNS Diseases Research, The Upjohn Company, Kalamazoo, MI 49001.

The irreversible cholinergic agonist BM 123 was injected to rats to investigate the role of subtypes of muscarinic receptors in the regulation of (3H)-acetylcholine [(3H)-Ach] release and phosphatidylinositol hydrolysis (PIH) which may be mediated by M2 and M1 receptors, respectively. Treatment with BM 123 significantly reduced the inhibitory effect of oxotremorine (10 uM) on presynaptic release of (3H)-Ach and oxotremorine-M (300 uM)-induced PIH at 12 hr ($p<0.002$, <0.001, respectively). Time-dependent recovery of both muscarinic receptor-mediated (3H)-Ach release and PIH was observed, and the responses returned to normal levels by 144 hr. There was a linear relationship between the recovery of (3H)-Ach release response and (3H)-oxotremorine-M (3H)-Oxt) binding sites, but the relationship was non-linear with respect to the recovery of PIH response and (3H)-Oxt binding sites. These results indicate that the presynaptic muscarinic receptor system of the hippocampus involved in the regulation of (3H)-Ach release may lack spare receptors. On the other hand, there may be a receptor reserve in the muscarinic receptor system which mediates PIH.

226.2

PHARMACOLOGICAL CHARACTERIZATION OF PD 129409: A NOVEL MUSCARINIC RECEPTOR AGONIST. R.D. Schwarz, L.L. Coughenour, R.E. Davis, D.T. Dudley, W.H. Moos, and H. Tecle*. Parke-Davis Pharm. Res. Div., Warner-Lambert Co., Ann Arbor, MI. 48105.

Muscarinic receptor agonists may alleviate some of the symptoms associated with Alzheimer's disease. Present results characterize 1,2,5,6-tetrahydro-1-methyl-3-pyridinecarboxaldehyde, O-methyloxime, HCl (PD 129409), an orally active muscarinic agonist. In vitro, PD 129409 like other known agonists, binds with high affinity to muscarinic receptors (with no binding at a number of other receptors), shows a concentration-dependent and atropine-sensitive increase in phosphatidylinositol turnover (SK-N-SH cells) and a decrease in the release of [^{3}H]-ACh from rat cortical slices, as well as having no effect on brain AChE activity. Dose-dependent effects were observed upon oral and subcutaneous administration in both rodents (0.1-2mg/kg) and non-human primates (0.03mg/kg) using models of central (EEG, body temperature, and cerebral blood flow) and peripheral (heart rate and GI motility) cholinergic activity. Thus, PD 129409 is a novel, orally active muscarinic receptor agonist which may be useful in the treatment of Alzheimer's disease.

226.3

INTERACTION OF HOE 427, A SYNTHETIC ACTH(4-9)-ANALOGUE WITH MUSCARINIC M1-RECEPTORS IN RAT BRAIN. H.J. Gerhards*, B. Jablonka*, M. Leven*, G. Wiemer*, F.J. Hock and R. Geiger* (SPON: E.T.W. Liske). HOECHST AG, P.O.B. 80 03 20, D-6230 Frankfurt/M. 80, FRG.

In a previous publication, we described that the synthetic ACTH-analogue Hoe 427 improves cognitive functions and enhances central cholinergic transmission in rodents (F.J. Hock et al., Peptides, 9: 575, 1988; G. Wiemer et al., Peptides, 9: 1081, 1988).

We now report, that Hoe 427 displaces ^{3}H-Pirenzepine, a ligand that specifically labels the M1-subtype of muscarinic receptors in neural tissues. In vitro binding studies with brain homogenates and quantitative receptor-autoradiography revealed an affinity of Hoe 427 to this site in cortex, hippocampus and striatum of the rat brain.

Although the affinity of Hoe 427 to the ^{3}H-Pirenzepine labelled M1-site is rather low (IC50: 4.7 x 10^{-6} M, the possibility, that the observed effects of this peptide on central acetylcholine metabolism are mediated by an interaction with a M1-muscarinic site has to be discussed.

226.4

DIFFERENTIAL UPREGULATION OF HIPPOCAMPAL M-1 AND M-2 MUSCARINIC RECEPTORS AFTER CHOLINOTOXIN (AF64A) LESION OF THE MEDIAL SEPTUM OR DIAGONAL BAND. V.L. Dawson and J.K. Wamsley. Dept. Psych. and Pharm., West. Inst. Neuropsych., Univ. of Utah, SLC, UT

Cholinergic cell bodies in the medial septum (MS) and vertical nucleus of the diagonal band (VDB) project axons through the fimbria-fornix to terminate in a laminated pattern in the hippocampal formation. Inorder to investigate the cellular localization of muscarinic receptors, a selective cholinergic neurotoxin, AF64A, was stereotaxically injected into MS or VDB and changes in M-1 and M-2 muscarinic receptors and choline uptake (SDHACU) sites in the hippocampus, as well as, the MS and VDB were examined.

AF64A MS and VDB lesions resulted in the significant loss of M-1 receptors labeled with [^{3}H]pirenzepine (PZ), M-2 receptors labeled with [^{3}H]QNB+100nM PZ, and SDHACU site with [^{3}H]hemicholinium-3 (HC-3) in the MS or VDB. In the hippocampus of the MS lesion [^{3}H]HC-3 binding was significantly reduced in all lamina and M-2 receptors were significantly increased in the stratum oriens (Or) of areas CA2-4. In the hippocampus of AF64A VDB lesions, [^{3}H]HC-3 binding was only significantly reduced in the stratum radiatum (Rad). In these animals M-1 receptors were significantly increased in the Rad of areas CA2-4, and M-2 receptors were significantly increased only in the Or of area CA4. These data provide evidence that the hippocampus may be differentially innervated by the MS and VDB and the MS regulates postsynaptic Or M-2 receptors and the VDB mediates activity at the Rad M-1 receptor.

226.5

SECTOR-DEPENDENT NEUROTOXICITY OF ETHYLCHOLINE AZIRIDINIUM (AF64A) IN THE RAT HIPPOCAMPUS (H). S.Laganiere*, M. Marinko*, J.Corey*, E.Wulfert*, & I.Hanin. Dept. Pharmacol., Loyola U. Chgo. Sch. Med., Maywood, IL., & UCB sa, Brussels.

ICV administration of AF64A results in an irreversible hypofunction of the hippocampal cholinergic network (Fisher and Hanin, Ann. Rev. Pharmacol. Toxicol. 1986). If this effect is due to a direct action at the site of contact with AF64A, the H adjacent to the ventricular space should show the largest effect. Our results indicate that this is not the case. Whole H from Sprague-Dawley rats [225-300 g; 6 days after bilateral ICV saline or AF64A (1, 2 or 3 nmol/ventricle); N= 6 rats/group] were vibratome-sectioned along their long axis and divided into 5 equal sectors from the ventral, to the cortical surface of H. In saline treated animals choline acetyltransferase (ChAT) activity showed a linear gradient of decreasing activity away from the ventral surface (31%; $p<0.001$). Conversely, the effect in the AF64A-treated rats was curvilinear, and dose dependent at all AF64A concentration used. For example, in 1 nmol AF64A-treated rats ChAT activity was significantly ($p<0.005$) reduced by 33% and 41% in the first sectors adjacent to the ventricle; was maximally reduced (45%) in the third sector; and attained only a 37% and a 23% decrease, respectively, in the fourth and fifth (most cortical) sectors. The neurotoxicity of ICV administered AF64A in vivo is thus dependent upon both: 1) diffusion of the compound within the ventricle; and 2) the intrinsic susceptibility of selective cholinergic terminals to AF64A.

226.6

DARK RESIN, ANGELATES (ANG), FROM PEREZIA ROOTS ACTION ON RAT INTESTINE IN VITRO. X. García, E. Gijón and G. Alcántara*. Dept. of Physiol. and Dept. of Pharmacol. Sch. of Med. Universidad Nacional Autónoma de México, Ap. P. 70-250, México, D.F. 04510. MEXICO.

The isomeric mixture of hidroxiperezone angelates, a dark viscous compound, is one of the active principles obtained from Perezia roots, known by its laxative properties as a prehispanic remedy (Alcántara, G. Res. Congr. Nacl. Farmacol. Reu. Reg. Asoc. Latinoam. Farmacol. 10:52, 1986). The purpose of this study was to know if the ANG share some of the perezone properties on Wistar male rat intestine by isometric recording in vitro (García, X., Alcántara, G. y Gijón, E. Res. Congr. Nacl. Cienc. Fisiol. 31:C220, 1988). The ANG, like perezone, interrupt spontaneous intestinal activity, they relax intestinal smooth muscle and diminish muscle tonus, they block the contractile response induced by acetylcholine (ACh) in a reversible form. The exposure to a high ACh dose prolongs the recuperation time in spite of showing no ACh response at all after the ANG, whereas small ACh doses recover their response completely. The ANG also relax the ACh-induced contraction. The ANG induced a contraction followed by a rélaxation and a later slow contraction. These results show that the ANG behave like perezone, they probably affect the smooth muscle by interfering with the calcium movement and also suggest their interaction with the ACh receptor.

226.7

PEREZONE (PZN) ANTICHOLINERGIC ACTION FROM THE MUCOSAL TO THE SEROSAL SURFACE OF EVERTED AND NON-EVERTED RAT INTESTINE. E. Gijón, X. García and G. Alcántara*. Dept. of Physiol. and Dept. of Pharmacol. Sch. of Med. Universidad Nacional Autónoma de México, Ap. P. 70-250, México, D.F. 04510. MEXICO.

Perezone is one of the active principles isolated from Perezia roots, known by its laxative properties as a prehispanic remedy (Alcántara, G. Res. Congr. Nacl. Farmacol. Reu. Reg. Asoc. Latinoam. Farmacol. 10:52, 1986). Perezone shows anticholinergic actions when applied to the serosal surface of rat intestine in vitro (García, X., Alcántara, G. y Gijón E. Res. Congr. Nacl. Cienc. Fisiol. 31:C220, 1988). It is important to know if these PZN actions are also observed when applied on the mucosal surface, since in humans it is used orally. We found negative results with the in vitro method of everted intestine (Crane, R.K. and Wilson T.H. J. Appl. Physiol. 12:145-146, 1958), due to the need of isometric recordings of Wistar male rat intestine. A later development was the use of non-everted small intestine tied to a small piece of polyethylene tube 4mm O.D. The tube is a cantilever during the isometric recording. PZN applied to the mucosal surface through the tube, by a polyethylene cateter 1.5mm O.D., shows its characteristic anticholinergic properties with a latency of 20 minutes. This suggests a slow transference of PZN from the mucosal to the serosal surface.

226.8

REACTIVATION BY 2-PAM, ICD 467, MMB-4 and HI-6 OF SOMAN-INHIBITED ACETYLCHOLINESTERASE (AChE) IN RABBITS. L.W. Harris*, D.R. Anderson*, C.L. Woodard* and W.J. Lennox* (SPON: S.B. McMaster). USAMRICD, Aberdeen Proving Ground, MD 21010-5425.

This study determined whether pralidoxime Cl (2-PAM), 2-hydroxyiminomethyl-3-methyl-1-(2-(3-methyl-3-nitrobutyl-oxymethyl))-imidazolium Cl (ICD 467), 1-1'-methylenebis[4-(hydroxyiminomethyl) pyridinium] di-Cl (MMB-4) and 1-(2-hydroxyimino methyl-1-pyridinio-3-(4-carbamoyl-1-pyridinio)-2-oxapropane di-Cl (HI-6) would reactivate soman-inhibited AChE in vivo. Rabbits were atropinized (8 mg/kg, im) and given soman (13 ug/kg, iv; 1.2 x LD_{50}) 5 min later. Three min after soman, animals received oxime (50, 100 or 150 umol/kg, im). Whole blood was collected just before soman, at 2 min after soman and at 2, 5, 10, 15, 30, and 60 min after oxime or vehicle for determination of AChE activity. After anesthetizing and perfusing the rabbits, tissues were removed for AChE assay on the cortex, medulla-pons and diaphragm. HI-6 and MMB-4 (50 umol/kg, im) reactivated ($p < 0.05$) inhibited whole blood AChE and diaphragm ChE, but not brain AChE. 2-PAM and ICD 467 were ineffective. HI-6 was better ($p < 0.05$) than MMB-4 in reactivating blood AChE. These data suggest that HI-6 or MMB-4, but not ICD 467, would be effective with atropine as therapy against soman.

226.9

ATTEMPTS TO IMPROVE AN ANIMAL MODEL FOR THE STUDY OF SOMAN TOXICITY AND ITS TREATMENT. V.R. Jimmerson, T.-M. Shih, D.M. Maxwell* and R.B. Mailman. USAMRICD, APG, MD 21010 and Curric. Toxicol., UNC, Chapel Hill, NC 27599.

Cresylbenzodioxaphosphorin oxide (CBDP) was used to preferentially block sites on carboxylesterase (CaE) that bind soman (GD) irreversibly and to increase GD availability to more critical sites of action. After sc treatment with CBDP alone or followed by selected GD doses we examined plasma and lung CaE, brain cholinesterase (ChE) and acetylcholine (ACh), and severity of intoxication in the rat. CBDP treatment (1, 2, or 16 mg/kg) inhibited ChE (by 5-12%, 15-25% and 30-40% respectively) in selected brain regions with no effect on ACh, and inhibited plasma and lung CaE completely. CBDP pretreatment (1.0 mg/kg) reduced the minimal sign-producing dose of GD from 70.8 to 10.3 ug/kg and potentiated the ED50 for GD-induced inhibition of ChE activity by 9- to 14-fold in brain regions. ChE inhibition of 90% (18.5 ug/kg GD) was accompanied by approximately 2-fold elevations of ACh in brain regions independent of the CBDP pretreatment dose. Following CBDP pretreatment, the presence and severity of toxic signs did not vary widely from animal to animal as has been reported for rats treated with GD alone [Jimmerson et al, Toxicology, in press]. These data suggest that CBDP pretreatment may increase GD availability to critical ChE sites, and offer an improved animal model for examination of GD toxicity and its treatment.

226.10

PIRENZEPINE ATTENUATES THE STIMULUS PROPERTIES OF PHYSOSTIGMINE BUT DOES NOT GENERALIZE TO THE STIMULUS PROPERTIES OF SCOPOLAMINE. R.F. Genovese, T.F. Elsmore* and C.H. Rose* Department of Medical Neurosciences, Walter Reed Army Institute of Research, Washington, DC 20307.

Two groups of rats were trained to discriminate the effects of S.C. injections of scopolamine (SCO) (0.237 mg/kg, n=12) or physostigmine (PHY) (0.237 mg/kg, n=5) from vehicle, using a two-lever, variable interval 18 sec schedule of food presentation. Scopolamine (0.013-0.750 mg/kg) produced dose-dependent generalization (i.e. responding on the scopolamine appropriate lever) in SCO rats. Physostigmine (0.024-0.422 mg/kg) produced dose-dependent generalization in PHY rats. Pirenzepine (1.78-17.80 mg/kg) attenuated the physostigmine discriminative stimulus in PHY rats, but did not generalize to the scopolamine discriminative stimulus in SCO rats. These results suggest that the physostigmine stimulus is produced by both M_1 and M_2 muscarinic receptor activity and also suggest that the drug discrimination paradigm may be valuable for assessing the behavioral effects of M_1 and M_2 muscarinic receptor activity.

226.11

CENTRALLY-ACTIVE ANTIMUSCARINIC ANALOGS OF OXOTREMORINE (Oxo) BLOCK EXPERIMENTAL HYPERTENSION. H.M. Vargas* and B. Ringdahl*(SPON: P. Lomax). Dept. of Pharmacology, UCLA-School of Medicine, Los Angeles, CA 90024

The Oxo analogs BM-5 (N-methyl-N-(1-methyl-4-pyrrolidino-2-butynyl)acetamide) and Bok-1 (4-pyrrolidino-2-butynyl)5-methyl-2-pyrrolidone) are potent tremorolytic agents in vivo. We compared these compounds to atropine (ATR; reference agent) and assessed their ability to inhibit physostigmine(PHY)-induced hypertension. In urethane anesthetized Sprague-Dawley rats, intravenous (iv) Phy (77 nmol/kg) increased AP, 40 ± 5 mmHg. After 1 hour, each rat received a single dose of antagonist, then a second bolus of Phy ten minutes later. Antagonist dose-percent inhibition curves were constructed and 50% inhibitory doses calculated (ID_{50}). The results indicate that BM-5 and Bok-1 were equipotent with ATR, all having ID_{50} values in the 1.6-1.8 umol/kg range. The Oxo analogs did differ in two ways from ATR. First, BM-5 and Bok-1 produced selective antagonism of brain muscarinic function at doses which did not inhibit the peripheral muscarinic depressor response to acetylcholine (21 nmol/kg, iv), yet ATR produced 100% inhibition of the peripheral response at its ID_{50} for Phy. Second, ATR and BM-5, but not Bok-1, produced tachycardia via the blockade of cardiac muscarinic receptors. In conclusion, BM-5 and Bok-1 may be useful probes to study cardiovascular regulation by brain cholinergic neurons. (Supported by USPHS grant GM37816 to B.R.)

226.12

CHOLINERGIC MODULATION OF STARTLE AND FIGURE-8 MAZE ACTIVITY IN FEMALE MICE. J. Buelke-Sam, J.A. Johnson*, H.E. Shannon*, J. Tizzano and J.F. White*. Lilly Research Laboratories, Eli Lilly & Co., Greenfield, IN 46140.

The role of acetylcholine in modulating startle is not clearly understood. This study evaluated the dose-response curves produced by scopolamine (SCO, 0.3, 1, 3 mg/kg), oxotremorine (OXO, 0.03, 0.1, 0.3 mg/kg), physostigmine (PHY, 0.03, 0.1, 0.3 mg/kg) and pilocarpine (PIL, 3, 10, 30 mg/kg) on auditory and tactile startle and locomotor activity. Female CD-1 mice (8/group) were injected ip immediately prior to testing. Locomotor activity was measured for 1 hr in SDI figure-8 mazes; startle was measured in automated SDI chambers. Each 50-trial startle session was initiated with a 5-min adaptation period at a background noise level of 70 dBA followed by alternating 5-trial blocks of auditory (120 dBA noise) and tactile (20 psi air puff) stimuli presented at 8-sec intervals. SCO produced a dose-related increase in activity and auditory startle amplitude, but no change in either tactile startle amplitude or rate of startle habituation. OXO and PIL produced dose-related decreases in activity and decreased auditory and tactile startle; PHY decreased activity but increased startle. The ability of each agent to reverse the hyperactivity produced by 1 mg/kg SCO, or reverse the increase in auditory startle produced by 3 mg/kg SCO was evaluated. Only PIL was effective in reversing hyperactivity; OXO and PIL reversed enhanced auditory startle produced by SCO. PHY+SCO increased both auditory and tactile startle compared to the control or SCO-only groups.

226.13

INTERACTIVE EFFECTS OF PHYSOSTIGMINE, ACUTE AND TRAINED EXERCISE ON AChE ACTIVITY IN BRAIN AND MUSCLE OF RAT. SN Dube and SM Somani (SPON: D Desaiah) Dept of Pharmacol Southern IL Univ Sch of Med Springfield, IL 62708.

Physostigmine (Phy) is considered to be a potential pre-pretreatment agent against organophosphate intoxication. The effect of Phy on the time course of AChE following an acute bout of exercise (80% VO_2max) was investigated in endurance-trained rats. Male Sprague-Dawley rats (160-200 g) were exercised for 6 weeks (5 days/week) at progressively intensive levels on a motor-driven treadmill. Immediately following the acute bout of exercise, both groups (trained and untrained) rats were administered Phy (70 μg/kg,i.m. and were sacrificed at 2, 5, 10, 15, 30, 45 and 60 min. post-exercise. Blood, brain, thigh muscle, heart, and diaphragm were collected for AChE by radiometric method. Phy produced AChE activity 60 and 67% of control in brain and muscle, respectively at 15 min., was recovered to 80-85% of control within 60 min. Acute exercise plus Phy increased AChE activity to 69 and 74% of control in brain and muscle at 15 min. respectively. Trained rats showed AChE activity 93 and 86% of control in brain and muscle, respectively at 15 min. Trained exercise plus Phy further decreases the AChE activity to 48 and 62% of control in brain and muscle at 15 min. respectively. Similar pattern of AChE activity was also seen in RBC, heart, and diaphragm. It seems that acute exercise plus Phy increases and training plus Phy decreases the AChE activity in RBC brain, muscle, heart and diaphragm as compared to Phy alone. (Supported by U.S. Army contract No. DAMD 88-C-8024.)

226.14

CNS EFFECTS OF NEW ANTICHOLINESTERASES. P. DeSarno*, E. Giacobini, X.C. Tang*, M. Pomponi* and K. Sugaya* (SPON: B. Clark). Dept. Pharmacology, Southern Ill. Univ. Sch. Med., Springfield, IL 62794

For the experimental therapy of Alzheimer disease (AD), there is a need to develop new cholinesterase inhibitors (ChEI) that produce fewer side effects and have greater therapeutic action than presently tested drugs such as physostigmine (Phy) and THA (tacrine). We have examined the effect of two new reversible ChEI on the central cholinergic system of the rat and compared it with that of Phy, THA and metrifonate. Heptyl-physostigmine [(heptyl-Phy), MF-201, Mediolanum] is a carbamate derivative of Phy, Huperzine A (HUP-A) is a natural substance isolated from a Lycopodium plant. Our results show that both drugs can produce long-term inhibition (6 hrs) of ChE activity and a substantial increase of acetylcholine (ACh) levels in brain (up to 125%) following single dose i.m. administration. Side effects are mild for both drugs. Release of ACh from cortical slices is inhibited (19-26%) only at the highest concentration (10^{-5}-10^{-4}M). Our results demonstrate that both heptyl-Phy and HUP-A compare favorably to other reversible ChEI, in particular to Phy, as far as producing a more long-lasting inhibition of ChE activity and a more prolonged increase in ACh levels in brain with less severe side effects. Both drugs are interesting candidates for a cholinomimetic therapy directed to reverse cognitive deficits in AD patients. (Supported by NIA AG05416 and State of Ill. Dept. Pub. Health to E.G.)

226.15

HEMICHOLINIUM MUSTARD: AN IRREVERSIBLE INHIBITOR OF CHOLINE TRANSPORT IN RAT BRAIN SYNAPTOSOMES. K.H.Gylys, B.Ringdahl* and D.J.Jenden. Dept. of Pharmacology and BRI, UCLA School of Medicine, Los Angeles, CA 90024.

Hemicholinium mustard (2,2'-(4,4'-biphenylene)bis[2-hydroxy-4-(2-bromoethyl)-morpholine]),(HCM), is a mustard analog of hemicholinium 3 (HC3); like other mustard compounds, HCM cyclizes in aqueous solution to form a reactive aziridinium ion with intrinsic alkylating ability. The potency, reversibility, and time course of HCM effects in synaptosomes were examined and compared with HC3 effects. HCM inhibits 50% of high affinity choline transport (HAChT) at concentrations of 7nM (incub.7 min., 37°C); this activity is completely abolished by pre-incubation of the cyclized HCM with 0.1 mM Na thiosulphate(20 min., 20°C). However, thiosulphate added after a 12 min. incubation with HCM (50nM) failed to reverse HCM effects: 70% inhibition of HAChT remains even after up to 24 min. in the presence of thiosulphate (0.1mM). This suggestion of irreversibility is reinforced by experiments in which HCM preincubation (1-8 min) was followed by two thorough washes in fresh Krebs before the initiation of choline uptake measurement. In these experiments, choline transport remained inhibited by up to 40% following HCM incubation; transport in synaptosomes preincubated in HC3 returned to control values following the washes. HCM effects are rapid: if thiosulphate is added to synaptosomes 2 min. before HCM is added a 76% inhibition of choline transport still occurs; moreover, a 1 min. preincubation with HCM followed by 2 washes still results in a 17% inhibition. These results indicate that HCM is a potent and irreversible inhibitor of HAChT. (Supported by MH 17691)

226.16

ACTIVATION AND ION CHANNEL BLOCKADE BY THE NICOTINIC AGONIST (+)ANATOXIN AND DERIVATIVES. P. Kofuji[1,2*], Y. Aracava[1,2*], K.L. Swanson[1] and E.X. Albuquerque[1,2] (SPON: E.M. Glaser). [1]Dept. Pharmacol. Exp. Ther., U. Md Sch. Med., Baltimore, MD 21201 & [2]Lab. Mol. Pharm. II, UFRJ, Brazil.

(+)Anatoxin-a [(+)AnTX] is a potent agonist for the nicotinic acetylcholine receptor (AChR) and is toxic at the peripheral and central nervous system (CNS)(Spivak *et al.*, *Mol. Pharmacol.* 18: 384, 1980; Aracava *et al.*, *Neurotox'88*, Ed. G.G. Lunt, Elsevier Publ., 1988). In CNS, using micromolar concentrations of (+)AnTX (1-2 μM), some effects consistent with channel block have been observed. Previous data have not shown blocking effects of up to 200 nM (+)AnTX at peripheral nicotinic receptors. We have examined the effects of higher concentrations of the drug using the patch-clamp technique (cell-attached configuration) in dissociated muscle fibers. Channel activation was apparent with (+)AnTX in the nanomolar range; lengthening of the open state as a function of membrane potential hyperpolarization without an increase in number of brief closures was as expected for a pure agonist. By contrast, with further increases in drug concentration in the micromolar range there was a gradual loss of voltage dependence of the open state in association with a marked decrease in the duration of single channel currents. These blocking effects did not display stereoselectivity, as the blocking and unblocking rates were very similar for (+)AnTX and its enantiomer (-)AnTX. Methylation of (+)AnTX's amine moiety evoked a large decrease of agonist potency without disturbing the blocking effects. In summary, the nicotinic receptor blocking site recognized by (+)AnTX and some of its derivatives is much less structurally selective than the agonist binding site. (Support: CNPq and FINEP, Brazil; NIH Grant NS25296).

226.17

NICOTINIC POTENCY OF ANATOXIN ANALOGUES WITH MODIFICATIONS OF THE ACETYL MOIETY. K.L. Swanson[1], R.S. Aronstam[2], S. Wonnacott[3*], H. Rapoport[4*], and E.X. Albuquerque[1,5]. Depts. of [1]Pharmacol. & Exp. Therap., Univ. MD Sch. Med., Baltimore, MD 21201; [2]Pharmacol. & Toxicol., Med. Coll. GA, Augusta, GA 30912; [3]Biochem., Univ. Bath, Bath BA2 7AY U.K.; [4]Chemistry, Univ. CA, Berkeley CA 94720; and [5]Lab. Mol. Pharmacol. II, UFRJ, Brazil.

The high stereoselectivity and potency of (+)-anatoxin-a (AnTX) at nicotinic acetylcholine receptors (AChR) of the neuromuscular junction (Spivak *et al.*, *Mol. Pharmacol.* 18:384, 1980; Swanson *et al.*, *Mol. Pharmacol.* 29:250, 1986) and of CNS synapses (Aracava *et al.*, *Neurotox '88*, Ed. G.G. Lunt, Elsevier Publ., 1988) makes this semirigid agonist an ideal focal compound for structure-activity studies. In earlier studies, N-methylation(s) caused a reduction in potency and revealed ion channel blocking activity. The present study includes and interrelates high affinity binding studies of the AChR sites for [^{125}I]α-bungarotoxin and [^{3}H]per-hydrohistrionicotoxin in *Torpedo* electroplaque and [^{125}I]αBGT binding, contracture and patch clamp studies in *Rana pipiens* skeletal muscle. (For binding to neuronal sites, see Wonnacott *et al.*, this meeting). In this series, the blocking activity was relatively weak in most cases and was unrelated to agonist potency. Modification of the acetyl moiety by reduction to an alcohol, alteration in the number of methyl groups, or a combination of these reduced the agonist binding affinity and the nicotinic efficacy of AnTX. The loss of potency in AnTXal, ahydroxyAnTX and AnTX-dimethylamide and the difference in potency between 10S and 10R AnTXols suggest that the methyl group has a specific role in definition of the pharmacophore. Supported by NIH NS25296.

226.18

COMPARATIVE EFFECTS OF ANTHELMINTICS ON POSTJUNCTIONAL MEMBRANE POTENTIAL OF RATS AND NEMATODES. D.P. Thompson and W.D. Atchison. Parasitol. Res./Devel. Labs, The Upjohn Co., Kalamazoo, MI 49001 and Dept. Pharmacol./Toxicol., Mich. State Univ., E. Lansing, MI 48824.

The anthelmintics, levamisole, pyrantel, and several of their structural analogs block neuromuscular transmission of the rat hemidiaphragm preparation and isolated axial muscle of the parasite Haemonchus contortus. Intracellular recordings of postjunctional membrane potential (E_M) were made during application of these drugs to ascertain whether the agents caused depolarizing neuromuscular block in mammals or nematodes. In H. contortus, application of levamisole and pyrantel caused depolarization of the axial muscle to 58% and 68% of control, respectively. In contrast, only pyrantel depolarized the end-plate membrane at the rat diaphragm, and neither levamisole nor pyrantel affected muscle E_M in non-endplate regions. At a concentration of 100 μM pyrantel depolarized E_M to 75% of control. Preadministration of 2 μM d-tubocurarine (d-TC) prevented the depolarizing action of pyrantel at the rat neuromuscular junction. While levamisole did not depolarize the rat end-plate region, 3-NH_3-levamisole depolarized E_M by approximately 20% after 15 min exposure at 100 μM. This effect was blocked by prior application of d-TC. Thus, a differential pattern of neuromuscular blocking action is produced by these anthelmintics at mammalian and nematode neuromuscular junctions. (Supported by a collaborative research grant from the Upjohn Co. to Michigan State Univ.)

MONOAMINES AND BEHAVIOR II

227.1

NEUROCHEMICAL CORRELATES OF PUNISHMENT. J.E. Barrett, S.N. Olmstead* M.A. Nader* and S. Gleeson* (SPON: G.T. BOLGER). Department of Psychiatry, Uniformed Services University of the Health Sciences, Bethesda, MD 20814.

A number of drugs such as the benzodiazepines and barbiturates have specific effects on punished behavior. These drugs increase punished responding at doses that do not increase or increase to a lesser extent low rates of responding that is not punished. This study examined neurochemical correlates of punished and non-punished responding of pigeons through the use of cerebrospinal fluid (CSF) analyses collected from lateral ventricular guide cannulae. Pigeons' key pecks were reinforced under a fixed-interval 3-min schedule (FI) of food presentation. When the keylight was blue, pecks were not punished; when the keylight was red, however, every 30th peck during the FI produced shock. Keylight colors alternated irregularly on a daily or weekly basis. CSF was collected after certain sessions during which responding was punished and after other sessions when it was not. Control procedures included response-independent food and response-independent shock delivery. CSF was analyzed using HPLC-EC techniques. Punishment increased levels of dopamine and serotonin metabolites compared metabolite levels of the same pigeons under non-punishment or control conditions. Environmental consequences that control behavior are reflected specifically and dynamically in distinctive neurochemical changes. Supported by DA-02873.

227.2

DOPAMINE TRANSMISSION IN THE RAT STRIATUM, NUCLEUS ACCUMBENS AND PRE-FRONTAL CORTEX IS DIFFERENTLY AFFECTED BY FEEDING, TAIL PINCH, AND IMMOBILIZATION. G.Damsma, M.Yoshida*, D.Wenkstern*, G.G.Nomikos, A.G.Phillips@, and H.C.Fibiger, Div. of Neurol. Sci., Depts. of Psychiatry and @Psychology, Univ. of British Columbia, Vancouver, Canada, V6T 2A1.

Dopaminergic (DA) neurons in the midbrain are the origin of discrete projections to the forebrain. To examine the functional properties of some of these projections, microdialysis probes were implanted in the striatum (STR; 4mm exposed membrane), nucleus accumbens (NAC; 2mm) or prefrontal cortex (PFC; 4.7mm) of male Wistar rats (n=3, for each brain area). The day following probe implantation DA and metabolites were recordered on-line (Westerink et al., *Life Sci.*, 41:1763; 1987) at 20 minute intervals. All rats were deprived of food 36 hours prior to three conditions: feeding for 20 minutes, mild tail pinch for 10 minutes and immobilization for 10 minutes; each procedure was followed by the collection of six samples. Baseline values were: STR 6.9+2.2 fmol/min (mean+S.E.M), NAC 2.0+0.5, and for PFC 0.7+0.2. Feeding increased extracellular DA markedly in NAC and PFC while a minor increase in the STR was observed. Tail pinch induced a small increase of extracellular DA in the NAC and STR and a marked increase in the PFC. Immobilization stress increased DA profoundly in the PFC but did not change extracellular DA in NAC or STR. The changes in acid DA metabolites were similar, although less pronounced, supporting the conclusion that DA transmission differs regionally in reponse to various stimuli.

227.3

STRESS-INDUCED DOPAMINE RELEASE AND MOTOR IMPAIRMENTS IN 6-HYDROXYDOPAMINE-TREATED RATS.
K.A. Keefe, E.M. Stricker, M.J. Zigmond, E.D. Abercrombie. *Department of Behavioral Neuroscience and Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260.*

Using *in vivo* microdialysis, we examined the relation between stress-induced dopamine (DA) release in striatum and the appearance of stress-induced akinesia in recovered 6-hydroxydopamine- (6-HDA) treated rats. DOPAC, HVA, and 5-HIAA levels in extracellular fluid (ECF) also were measured. The striatal tissue DA content was reduced by 98% in these animals, whereas basal DA in ECF (13.9 pg / 20 ul) was reduced by only 57% relative to untreated rats. Intermittent tail-shock stress increased DA in the ECF of the DA-depleted striatum by 10.9 pg / 20 ul (79%), while DOPAC and HVA concentrations both increased by 52% and 5-HIAA levels remained relatively constant (116%). The stress-induced increase in DA efflux was observed despite the presence of akinesia and catalepsy 15 min after the termination of the stress. We recently have shown that striatal DA in dialysates is increased by 9.5 pg/20 ul during stress in intact rats (Abercrombie et al., J. Neurochem, 1989). We propose that the relatively normal absolute increase in ECF-DA in the DA-depleted striatum after stress reflects the decreased density of innervation and consequent decrease in high affinity DA re-uptake sites in 6-HDA treated rats. Thus, it seems that the residual presynaptic dopaminergic function of 6-HDA-treated rats does not fail in response to stress and thereby underlie the induced akinesia, as has been hypothesized. (Supported by Grant NS 19608, MH 18273, and the National Alliance for Research on Schizophrenia and Depression.)

227.4

ASSESSMENT OF AMPHETAMINE- AND HALOPERIDOL-INDUCED CHANGES IN DOPAMINE NEUROTRANSMISSION IN THE PRESENCE OF NOVEL AND FAMILIAR STIMULUS ENVIRONMENTS.
M. T. Bardo, S. A. Bowling*, R. C. Pierce* and R. B. Ennis*. Dept. of Psychology, Univ. Kentucky, Lexington, KY 40506.

Rats were administered amphetamine (0, 0.5 or 2.0 mg/kg) or haloperidol (0, 0.05 or 0.2 mg/kg) in the presence of either novel or familiar environmental stimuli. An inhibitor of the enzyme dihydroxyphenalanine (DOPA) decarboxylase, NSD-1015 (100 mg/kg), was also administered in order estimate synthesis of dopamine (DA) within these environments. Following injections, behavioral measures of both horizontal and vertical activity were obtainecd within each environment. Animals were then sacrificed for assaying brain DOPA, dihyroxyphenylacetic acid (DOPAC) and DA by high-pressure liquid chromatography.

As expected, the behavioral measures indicated that amphetamine increased and haloperidol decreased locomotor activity. Locomotor activity was also enhanced in the novel environment relative to the familiar environment. The neurochemical measures revealed that amphetamine produced a dose-dependent increase in DOPA accumulation in the striatum and dose-dependent decrease in DOPAC levels in the striatum and nucleus accumbens. Exposure to novelty had no significant effect on these amphetamine-induced neurochemical changes. In contrast to amphetamine, administration of haloperidol produced a dose-dependent increase in DOPA accumulation in the striatum, nucleus accumbens, and olfactory tubercle, as well as a dose-dependent increase in DOPAC in the striatum, nucleus accumbens and olfactory tubercle. More important, haloperidol-treated animals exposed to the novel environment had significantly higher levels of DOPAC in the striatum and nucleus accumbens relative to haloperidol-treated animals exposed to the familiar environment, thus indicating that novel stimulation alters DA metabolism. (Supported by USPHS grant DA05312.)

227.5

MEASUREMENT OF FOOTSHOCK-INDUCED CHANGES IN DOPAMINE AND METABOLITES BY IN VIVO DIALYSIS IN RAT PREFRONTAL CORTEX.
B.A. Sorg* and P.W. Kalivas (SPON: A.T. Campagnoni). Dept. of VCAPP, Washington State University, Pullman, WA 99164-6520.

We used in vivo dialysis to measure the effect of a 20 min intermittent footshock (0.35 mA) on DA levels and its metabolites in regions of the medial prefrontal cortex of male Sprague-Dawley rats. Samples were collected every 20 min and the levels of DA, DOPAC, and HVA were quantified by HPLC-EC. We assigned placement of cannulae into one of three rostro-caudal subareas of the prefrontal cortex: caudal = 2.2 - 3.1 mm, central = 3.2 - 4.1 mm, and rostral $\geq$ 4.2 mm from bregma (Paxinos and Watson, 1986), and into two medio-lateral regions: 0.1-0.9 mm, and $\geq$ 1.0 mm from the midline. While no consistent changes in DA levels were found after footshock, DOPAC levels increased after the 20 min shock in all regions and were maximal 20 min after the shock ended. In the medial portion of each rostro-caudal subdivision, the DOPAC increase was most pronounced in the central region (72% above baseline), and lower in the rostral (28%) and caudal (38%) regions. In the lateral portion, DOPAC increases were 38% in the central and 11% in the caudal regions. HVA levels paralleled DOPAC increases in all brain regions, although maximal levels were obtained 20 min later and were approximately 20% lower.

227.6

BRAIN DOPAMINE CONTENT AND THE INDUCTION OF SEXUAL BEHAVIOR IN THE FEMALE MUSK SHREW.
E.F. Rissman*, K.J. Darney*, A.L. Clendenon* and J.G. Vandenbergh* (SPON: D.J. Hudson). Depts. Psychol. and Biol., Univ. of Virginia, Charlottesville, VA 22903 and Dept. of Zoology, N.C. State Univ., Raleigh, N.C. 27695.

The female musk shrew (Suncus murinus), in striking contrast to other laboratory mammals, does not have a behavioral estrous cycle. Females are continually sexual receptive. When virgins are paired with males an initial period of aggressive contact, typically lasting for 45 minutes, ends when the female demonstrates receptive behavior. When females are paired with another female, for one hour prior to introduction to a male, receptive behavior occurs more rapidly than under control conditions. To explore the role of neurotransmitters in this rapid shift from aggressive to sexual behavior we sacrificed females at this transitional time and dissected chunks of brain tissue for HPLC analysis of catecholamine content. Higher DA content was found in MOB and MBH when females were paired either with a male or another female as compared with DA levels in females allowed to explore a novel cage for an equivalent length of time. In addition, DA activity was higher in AOB in females paired with males than in isolated controls. Taken together these data suggest a relationship between aggressive behavior, DA and the induction of receptivity. Supported by NSF 8706770, USPHS MH18411 and HD21632.

227.7

ELECTROCHEMICAL DETECTION OF CENTRAL DOPAMINE EFFLUX DURING SEXUAL ACTIVITY IN MALE RATS.
J.G. Pfaus[1], T.N. Newton*[1], C.D. Blaha[1,2], H.C. Fibiger[2], and A.G. Phillips[1]. Depts. of Psychology[1] and Psychiatry[2], Univ. British Columbia, Vancouver, BC, Canada, V6T 1Y7.

Pharmacological evidence suggests that central dopamine (DA) systems facilitate sexual behavior in male rats. Ex vivo studies confirm an increase in DA synthesis and release within mesotelencephalic and hypothalamic DA terminal regions following periods of copulation. However, this approach does not permit a detailed analysis of DA activity during sexual behavior.

In the present study, sexually active male rats were implanted with stearate-modified electrodes aimed at the nucleus accumbens. Chronoamperometry was used to examine extracellular DA concentrations before, during, and after 30-min tests of copulation. DA signals increased in each male when the female was placed into the chamber and this rise preceded the initiation of mounts and intromissions. Most ejaculations were followed by a sharp decline in the DA signal. DA levels began to rise before reinitiation of copulation. The DA signal remained elevated for at least 40 min after the female was removed from the chamber. These data indicate that mesolimbic DA levels increase before and during bouts of copulatory behavior. Studies are currently underway to examine DA efflux in other regions, (e.g. MPOA and caudate) and to define the precise stimulus that leads to the initial increase in DA levels.

227.8

IN VIVO VOLTAMMETRY IN THE NEOSTRIATUM OF FREELY MOVING RATS: MONITORING EXTRACELLULAR ASCORBATE AND DOPAC DURING ACUTE AND CHRONIC AMPHETAMINE.
G.V. Rebec, M.E. Kraft*, P.E. Langley*, and M.T. Ciancone*. Prog. Neural Science, Dept. Psychology, Indiana Univ., Bloomington, IN 47405.

Amphetamine, a well-known dopamine agonist, increases neostriatal ascorbate (AA) release, which may modulate both neostriatal function and the behavioral response to this drug. To study this issue further, we monitored extracellular AA and DOPAC, a dopamine metabolite, in the neostriatum of freely moving animals that received amphetamine at doses and treatment schedules known to produce different behavioral effects. Rats received single, daily injections of saline or D-amphetamine (1.0 or 5.0 mg/kg) for up to 15 days. Voltammetric recordings and corresponding behavioral measures were obtained at the beginning (Day 1), middle (Days 6-8), and end (Days 13-15) of treatment. On each recording day, an electrochemically modified, carbon-fiber electrode, which provides distinct waves for AA and DOPAC, was lowered into the neostriatum and, following the drug response, was removed and tested in a post-calibration step. Although acute amphetamine increased extracellular AA by up to 200 uM, this effect was not dose-dependent despite clear dose-dependent differences in the behavioral response. Multiple amphetamine injections produced biphasic changes in AA release: treatment for 6-8 days enhanced the amphetamine-induced rise in extracellular AA, but this effect declined as treatment continued for 13-15 days. Simultaneous behavioral measures indicated a uniform sensitization throughout the treatment period. DOPAC showed a monophasic decline with repeated amphetamine. Thus, amphetamine-induced changes in neostriatal AA release do not bear a simple relationship to the corresponding behavioral changes produced by this drug. Voltammetric recordings from other AA-rich forebrain sites, including the nucleus accumbens, may shed light on the role of AA in the amphetamine-induced behavioral response.

Supported by NSF (BNS 87-11240) and NIDA (DA 02451).

227.9

CHANGES IN EXTRACELLULAR DOPAMINE CONCENTRATIONS MEASURED BY IN VIVO INTRACEREBRAL MICRODIALYSIS AFTER DOPAMINE DEPLETION. E. Castaneda, I. Q. Whishaw and T. E. Robinson, Depts. of Psychology, Univ. Lethbridge, Lethbridge, Alberta, Canada T1K 3M4 & Univ. Michigan, Ann Arbor, Michigan, 48109.

Intracerebral microdialysis was used to measure extracellular dopamine (DA) from the corpus of striatum in rats depleted of DA. Rats received 6-hydroxydopamine (6-OHDA) in bilateral intraventricular infusions as 3-day old neonates or in bilateral infusions into the substantia nigra pars compacta as adults. After recovery, dialysate samples were collected from awake subjects during resting conditions and after 1.5 mg/kg amphetamine (AMPH), s.c. At least 3 days later tissue DA concentrations were determined. All samples were assayed by HPLC-EC. In adult-depleted rats extracellular DA concentrations decreased only when tissue DA levels were extremely low (<10%). Similarly, extracellular levels of DA were decreased to 12-54% in neonatally-depleted rats, all of which sustained greater than 99% tissue DA depletions. The response to an AMPH challenge was attenuated but all animals were able to increase extracellular catecholamines. Surviving mesostriatal DA cells may contribute towards normalizing extracellular DA levels following DA depletion. This in turn may contribute to recovery of behavioral function.

227.10

DOPAMINE RELEASE IS REDUCED IN THE NUCLEUS ACCUMBENS OF UNDERWEIGHT RATS. E. Pothos*, G.P. Mark and B.G. Hoebel, Department of Psychology, Princeton University, Princeton, NJ 08544-1010

Prior microdialysis experiments showed that feeding can increase extracellular dopamine (DA) in the nucleus accumbens (NAC) of freely moving, food deprived rats. In the present study, we tested the hypothesis that food deprivation leading to reduced body weight would alter basal DA or serotonin release in the nucleus accumbens. *In vivo* microdialysis and high performance liquid chromatography coupled with electrochemical detection (HPLC-EC) were used at 1-hour intervals for 3 hrs during the dark cycle on 3 test days each 10 days apart. Nine male rats were reduced to 80% body weight for the second of the three tests. Results showed that mean basal extracellular DA decreased 40-50% in the NAC of male rats reduced to 80% body weight ($p<.05$). A similar trend was observed in DOPAC levels, but not in HVA or 5HIAA output. DA release was restored by returning the animals to their pre-test body weight. Extracellular serotonin levels did not appear to change with weight reduction. As a control, DA and serotonin output remained unaltered over a 3-week period in non-deprived subjects. These results indicate that DA release in the nucleus accumbens is reduced in underweight rats.

Supported by USPHS grant DA-03597.

227.11

A METHOD FOR REPEATED INTRACEREBRAL MICRODIALYSIS. Dianne M. Camp* and Terry E. Robinson. (SPON. E.S. Valenstein). Department of Psychology and Neuroscience Program, The University of Michigan, Ann Arbor, MI, 48109.

High performance liquid chromatography (HPLC) coupled to series oxidative-reductive electrochemical detection with three flow-through coulometric electrodes was used to assay dopamine (DA), dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) in dialysate. The purity of DA in dialysate was analytically verified by series reduction at two different points on DA's current/voltage curve. Using this HPLC method a new removeable concentric style (250 µm O.D.) dialysis probe was characterized over two days following insertion of the probe into the striatum of freely moving rats, and again following a second insertion into the same rats two weeks later. It was found that: (1) the basal concentration of DA was stable by the second 20 min sample obtained after insertion of the probe and declined only slightly 24 hrs later; (2) in contrast, the basal metabolite concentrations increased dramatically over the first 3 hrs after probe insertion and declined markedly 24 hrs later; (3) tetrodotoxin (TTX, 0.5 µM) administered via the probe decreased DA concentrations to a much greater extent 24 hrs than 3-6 hrs after probe insertion; (4) the basal concentration of DA following the second probe insertion was similar to that seen after the first insertion; (5) in contrast, the metabolite concentrations were lower following the second insertion; and (6) the sensitivity of DA to TTX following the second probe insertion was comparable to that seen following the first insertion.

227.12

LONG TERM BEHAVIORAL EFFECTS OF CHRONIC AMPHETAMINE USE. P. E. Paulson*, D. M. Camp* and T.E. Robinson (SPON: D. Green). Dept. Psychology & Neuroscience Program, University of Michigan, Ann Arbor, MI 48109.

This experiment characterized the behavioral response to an amphetamine (AMPH) challenge (2.6 mg/kg) given at various times (3, 7, 14, 30, 90 or 180 days) following pretreatment with saline or escalating doses (1→10 mg/kg over 6 weeks) of *d*-AMPH. The more rapid onset of stereotypy typical of sensitized animals, and measured by a marked decrease in locomotion about 40 min after the challenge, was not present at 3 or 7 days after the cessation of AMPH pretreatment, was present by 14 days, and then persisted undiminished for at least 180 days. In contrast, post-stereotypy hyperactivity was not seen until 30 days after the discontinuation of AMPH pretreatment and disappeared by 90-180 days. In summary: 1) Sensitization of stereotypy showed a delayed onset after the cessation of AMPH pretreatment, but persisted indefinitely thereafter. 2) Sensitization of post-stereotypy hyperactivity showed an even more delayed onset, but did not persist. These data suggest that the neural systems responsible for behavioral sensitization undergo dynamic changes for a prolonged period of time following the cessation of repeated AMPH treatment. Furthermore, different behaviors sensitized by AMPH (eg., onset of stereotypy vs post-stereotypy hyperactivity) must be mediated by different neural systems, and each of these neural systems is changed somewhat differently by AMPH pretreatment. Studies are in progress to identify neural correlates of these behavioral phenomena.

227.13

DOES AMPHETAMINE PREFERENTIALLY INCREASE EXTRACELLULAR DOPAMINE IN THE MESOLIMBIC SYSTEM? Terry E. Robinson and Dianne M. Camp*. Dept. of Psychology and Neuroscience Program, University of Michigan, Ann Arbor, MI

Intracerebral microdialysis in freely-moving rats was used to test the hypothesis that drugs of abuse have in common an ability to preferentially increase the extracellular fluid (ECF) concentrations of dopamine (DA) in the mesolimbic system (DiChiara & Imperato, *PNAS* 85:1988, 5274). One dialysis probe (250 µm O.D., concentric style) was placed chronically into the nucleus accumbens (ACC) and one into the contralateral dorsolateral caudate nucleus (STR). The next day at least 3 baseline samples were collected (1.5 µl/min; 20 min intervals) and then each animal received either 0.75, 1.5 or 3.0 mg/kg of *d*-amphetamine sulfate (AMPH, wt. of salt). The basal concentration of DA in ECF was significantly higher in the STR than ACC (25.6 vs 17.7 fmol/min-corrected). There were also regional differences in DA metabolism, with HVA (but not DOPAC) concentrations being 2-fold greater in STR than ACC. AMPH produced a dose-dependent increase in the ECF concentrations of DA in both structures, but 1.5 and 3.0 mg/kg produced a significantly greater increase in the STR than the ACC (data expressed as fmol/min). When AMPH-stimulated DA release was expressed as a percent of baseline there was no statistically significant regional difference in the effect of any dose of AMPH. These data do not support the hypothesis that drugs of abuse preferentially increase the synaptic concentration of DA in mesolimbic structures; although they may preferentially influence mesolimbic DA activity in other ways.

227.14

EFFECTS OF DOPAMINE DEPLETIONS ON DAYS 15 AND 20 AFTER BIRTH ON INTRACRANIAL SELF-STIMULATION AND NEUROLEPTIC SENSITIVITY IN ADULT RATS. K.S. Sidhu*[1], J.R. Stellar[1], and J.P. Bruno[2]. 1-Northeastern Univ. Boston, MA 02115; and 2-Ohio State Univ. Columbus, OH 43210.

Stellar et al. (Pharm.Biochem.Beh. 30:365, 1988) showed that rats receiving 6-OHDA lesions on neonatal day 3 and undergoing lateral hypothalamic self-stimulation (LHSS) as adults were 1) normal in LHSS reward and 2) depressed in LHSS operant motor capacity when compared to controls. We now extend these conclusions to rats given 6-OHDA lesions at day 15 or day 20 after birth.

Stellar et al. (1988) also showed from dose-response pimozide studies that LHSS in these day 3 lesioned rats was markedly subsensitive to dopamine receptor blockade. In preliminary form, we now report that same result for the day 15 lesion, but show normal-range sensitivity to pimozide for day 20. All LHSS testing was done with the rate-frequency curve method to measure reward and motor performance capacities independently.

Supported by the Whitehall Foundation.

227.15

THE EFFECTS OF UNILATERAL 6-HYDROXYDOPAMINE LESIONS ON INPLACE TURNING IN RATS. K.E. Sabol, J.B. Richards, and C.R. Freed. Univ. Colo. Health Sci. Ctr., Denver,CO 80262.

Rats trained to rotate show a strong contra- and a milder ipsilateral turning deficit after unilateral 6-hydroxydopamine (6-OHDA) lesions. Here, we looked at the effects of unilateral 6-OHDA lesions on an alternate turning paradigm: rats were trained to run inplace for water with an asymmetric body posture, on a circular treadmill. After CRF training (6 days each direction), 7 rats were given unilateral 6-OHDA lesions (8 ug free base in 2 ul ascorbic acid/saline vehicle), and 5 rats received vehicle injections. The coordinates were A: 4.4 mm post. to Bregma; L: 0.9 mm right of midline; and V: 7.5 mm below dura, nosebar -2.3 mm. The lesioned rats had dopamine depletions >95%. Turn rate was reduced in both directions 6-8 wks post-lesion. Two control and 2 lesioned rats were looked at in detail: The lesioned rats, when turning contralateral, ran longer before stopping to drink, and paused longer before starting to run again. This indicates that in the contralateral direction, the lesioned rats had difficulty making the transition between running and drinking, and vice versa. After CRF testing, rats were trained on an FR5 schedule. The lesioned rats had a reduced turn rate only in the contralateral direction. To summarize, rats with unilateral 6-OHDA lesions have bilateral inplace turning deficits with a more severe impairment contralateral to the lesion.

227.16

"MODERATE" NEONATAL DOPAMINE (DA) DEPLETION: SPATIAL MEMORY IMPAIRMENT BUT RESPONSIVENESS TO ENRICHED ENVIRONMENT. S.J.E. Murtha, B.A. Pappas, G.A.S. Park*, R.M. Szirtes*, K. Condon* and J.N. Armstrong. Dept. of Psychol., Carleton University, Ottawa, Canada K1S 5B6.

Within 36 h of birth, rats received bilateral intraventricular 6-hydroxydopamine (15 ug free base total) injections with or without desmethylimipramine pretreatment to protect noradrenergic terminals. This resulted in moderate, persisting loss of frontal cortical (63% loss) and caudate (75%) DA. Half of the rats were raised in enriched environments and the other half as dyads in standard (impoverished) cages. Behavior was assessed at 80 days. Both enriched rearing and neonatal DA depletion caused increased exploration of an elevated plus maze. DA depletion did not alter the effect of enrichment. Enriched rearing enhanced the spatial memory of control and of DA depleted rats as assessed by the Morris water maze procedure. The DA depleted rats rapidly learned the platform location, but showed impaired 48 h retention of the location. This impairment was partly ameliorated by enriched rearing. Thus, moderate neonatal DA depletion does not affect the response to enriched rearing but does cause an enduring hyperactivity and impaired spatial memory. The latter may be analogous to the impaired effortful processing which occurs with DA lesions in humans (i.e., Parkinson's disease). Moderate neonatal DA depletion causes unique, severe and permanent behavioral changes.

227.17

DOPAMINE DEPLETIONS IN DEVELOPING RATS: AGE-DEPENDENT EFFECTS OF D1 AND D2 RECEPTORS IN THE CONTROL OF SENSORIMOTOR BEHAVIOR. **B.J. Johnson* and J.P. Bruno. (SPON: G. Berntson). Dept. of Psychology, The Ohio State University, Columbus, Ohio 43210.**

Depletions of brain dopamine (DA) in adult rats produce sensorimotor deficits followed by eventual recovery. This recovery is mediated by residual DA neurons since these animals regress back to their initial deficits when challenged with doses of DA antagonists that have no effect on normal rats. In contrast, rats depleted of DA during the first 3 weeks of development are spared from the initial deficits seen in rats depleted as adults.

To assess whether residual DA neurons mediate this sparing phenomen we measured the effects of the antagonists haloperidol (D1/D2), SCH 2390 (D1), or sulpiride (D2) on the sensorimotor behavior of adult rats given 6-HDA (100-250 µg, ivt) or its vehicle at 3, 15, or 20 days of age. Vehicle-treated animals displayed sensorimotor deficits after receiving (ip) each of the DA antagonists. Rats depleted at 15-20 days also exhibited these deficits but they were less sensitive than controls. Rats depleted at 3 days of age were insensitive to any DA antagonists. These results suggest 2 different mechanisms underlying behavioral sparing, depending upon the age at the time of depletion.

227.18

MEDIAL PREFRONTAL CORTICAL LESIONS INCREASE STRESS SENSITIVITY IN THE RAT. G.E. Jaskiw, A. Braun, F. Karoum, N. Breslin and D.R. Weinberger. CBDB, NIMH, Neurosciences Center at St. Elizabeths Hospital.

Destruction of efferents from the medial prefrontal cortex (MPFC) of the rat induces transient disturbances in subcortical catecholaminergic indices and in exploratory behavior which attenuate by the fourth posotperative week. Since the MPFC may play a unique role in the rat's response to stress, we hypothesized that later disturbances in MPFC lesioned animals could be elicited by stress. Cohorts of rats received bilateral sham (S) or ibotenic acid (IA) (5$\mu g/\mu l$ AP $\pm$ 3.5, ML $\pm$ 0.7, VD - 3.5 mm) lesions of the MPFC and were evaluated after a minimum 6 week recovery period. In cohort 1 either a 24 hour or a 48 hour food deprivation stress reduced the exploratory activity of IA lesioned rats in a novel environment. Cohort 2 animals received an injection of the anxiogenic β-carboline FG-7142 (15mg/kg/ip) or vehicle (V) 15 minutes before introduction to photocell monitors. The IA/FG rats show a marked attenuation of locomotion. Moreover, a significant but smaller attenuation was seen in IA/V rats compared to the the S/V group, suggesting that MPFC lesioned rats had an altered response to the stress of a V injection alone. The latter was consistent with results from the third cohort in which each of DA, DOPAC and HVA were elevated by 20% within the nucleus accumbens of MPFC lesioned animals 7d after one week of daily V injections. These and other results suggest that loss of the modulation provided by the MPFC markedly augments sensitivity to a variety of stressful stimuli and in particular alters the effects of stress on limbic dopamine turnover.

227.19

6-HYDROXYDOPAMINE INJECTIONS IN POSTERIOR GLOBUS PALLIDUS: DIMINISHED BRAIN STIMULATION REWARD OR MOTOR FUNCTION? **J. C. Hall, C. J. Corral-Yagnam* and D. B. Neill. Dept. of Psychology, Emory University, Atlanta, GA 30322.**

The effect of striatal dopamine depletion on self-stimulation of lateral hypothalamus in rats was assessed using the autotitration method. In our procedure, stimulation intensity decreased 3 uA every 5th bar press; the rat could reset the intensity back to maximum by responding on a second lever. Bilateral injection of 5.6 ug 6-hydroxydopamine (6-OHDA) into posterior globus pallidus produced a decrease in response rate and resetting at higher intensities, i.e., the rats would not perform at the low currents previously accepted. These injections also reduced the reset-lowering (reward-enhancing) effect of intraperitoneally administered amphetamine. Although we conclude that these data indicate posterior pallidal 6-OHDA injections lower the reward of hypothalamic stimulation, we also consider some motoric hypotheses, due to sensorimotor impairments exhibited by these animals.

228.1

COEXISTENCE OF NADPH-DIAPHORASE ACTIVITY WITH CRF ANTI-IDIOTYPE IMMUNOREACTIVITY. G. J. Michael, S. A. Joseph and K. M. Knigge. Neuroendocrine Unit, Univ. Rochester Med. Ctr., Rochester, NY 14642.

A corticotropin-releasing factor anti-idiotypic antibody (CRF-AIA) made in this laboratory is hypothesized to recognize neurons that are postsynaptic to CRF neurotransmission in the central nervous system. Several immunochemical studies were performed to elucidate the relationship between CRF anti-idiotype immunoreactivity and reduced nicotinamide adenine dinucleotide phosphate- (NADPH-) diaphorase in the rat brain.

In order to determine the histologic relationship between these two neuronal markers, dual staining histochemistry was performed. After fixation with 4% paraformaldehyde/ 0.2% picric acid and cutting on a vibratome, serial rat brain sections were processed for CRF-AIA immunocytochemistry using the avidin-biotin complex method (Vector Labs.), NADPH-diaphorase histochemistry (Scherer-Singler, U.,*et al.*J. Neurosci. Meth., 9:229, 1983) or dual staining. All neurons found stained with one of the activities in the dual stained preparations also contained the other activity.

Biochemical analysis was conducted of rat neural membranes solubilized and electrophoresed on nondenaturing polyacrylamide gels as described by Kuonen, *et al.*(J. Neurochem., 50:1017, 1988). Western blot analyses of neural proteins revealed that a high molecular weight complex (> 400 kD) possessed both NADPH-diaphorase activity and CRF-AIA immunoreactivity. The 180 kD band of diaphorase activity did not crossreact with the CRF-AIA.

Dual staining of brain sections with NADPH-diaphorase histochemistry and CRF immunocytochemistry was also conducted. Examination at the light microscopic level revealed that CRF-like immunoreactive processes often could be found associated with NADPH-diaphorase cells and processes.

We hypothesize that NADPH-diaphorase is a marker for central neurons that are postsynaptic to CRF neurotransmission.

228.2

Coexistence of Corticotropin-Releasing Factor (CRF) and Enkephalin (ENK) in the Paraventricular Nucleus (PVN) of the Rat Hypothalamus. S Pretel and D Piekut, Neuroendocrine Unit, University of Rochester, Rochester, N.Y. 14642.

Coexistence of two or more putative neurotransmitters has become a prominent feature in the CNS. It usually is demonstrated using immunocytochemical labeling of antibodies against the neurochemicals of interest. In the present study the coexistence of two peptides, CRF and ENK is demonstrated using a) immunocytochemical labeling of antibodies and b) immunocytochemical labeling of antibodies to one peptide and the mRNA of the other peptide. Double-label immunocytochemistry (ICC) was obtained by the sequential labeling of two antigens in the same tissue section, yielding a brown and a blue reaction product respectively (Piekut, '87). Immunocytochemical labeling of the antibodies and labeling of the mRNA was also performed on the same tissue section; the slide was first processed for ICC and second for in situ hybridization (ISH) of synthetic oligonucleotides, complemenary to the mRNA for either CRF or ENK (generously provided by Drs. Young and Brownstein, NIH). ISH was performed by 3' endlabeling of the oligonucleotides (48 bases) with ^{35}S-deoxy ATP, using terminal deoxynucleotidyl transferase. Prehybridization and hybridization solutions were the same, except the latter did not contain any labeled probe. They consisted of 50% deionized formamide, 20% 20XSSC, 20%$H_2$0, 4% denatured salmon testes DNA, 1% t-RNA (25mg/ml), 2% 50XDEN, 10% dextran sulfate. The ISH solution also contained 2µl of labeled probe. After ICC processing, the slides were prehybridized for 3-4 hrs at 37°C, followed by application of the hybridization solution and incubation overnight at 37°C. They were exposed to NTB2 emulsion for 25 (CRF) or 34 (ENK) days. PVN neurons which contained CRF and ENK are observed throughout the rostro-caudal extent of the parvocellular (pc) component of the PVN. The majority of these double-labeled neurons was found in the medial and posterior lateral pcPVN. Most of the ENK labeled neurons (>75%) contained the genetic appartus to synthesize CRF, whereas a smaller portion of the CRF neurons (<30%) contained the genetic apparatus to synthesize ENK. We conclude that CRF and ENK coexist in subpopulations of PVN neurons. Both peptides are involved in the regulation of stress behavior. They could potentially initiate the involvement of different groups of CNS neurons in different stress responses. Supported by Grant #NS 18626.

228.3

LOCALIZATION OF CORTICOTROPIN-RELEASING FACTOR (CRF)-LIKE IMMUNOREACTIVITY IN MONKEY (Saimiri sciureus) OLFACTORY BULB AND ITS EFFERENTS. J.L. Bassett, S.L. Foote and M.T. Shipley[1]. Dept. Psychiatry, Univ. Calif. San Diego, La Jolla CA 92093; [1]Dept. Anat. & Cell Biol., Univ. Cincinnati 45267.

Several lines of evidence suggest that olfactory bulb projection neurons utilize a glutamate-like moiety as a neurotransmitter. Recently, Imaki et. al. (in press) have shown that many mitral and tufted cells in the rat olfactory bulb are immunoreactive for CRF and contain the mRNA for this peptide, raising the possibility of a cotransmitter within this system. The distribution of CRF has not been examined in the primate olfactory bulb or its efferents.

In the present study, sections through the monkey olfactory bulb and its target areas were processed for immunohistochemistry using a polyclonal antiserum directed against the human form of CRF (donated by J. Rivier & W. Vale). Within the olfactory bulb, nearly all mitral and many tufted cells contained CRF-like immunoreactivity. CRF-positive fibers were seen within the olfactory tract and olfactory stria. Immunoreactivity with a fine, particulate appearance, as well as numerous immunoreactive coarse, varicose fibers and isolated puncta were seen within the anterior olfactory nucleus and layer Ia of the olfactory tubercle and pyriform cortex. CRF-positive cells were seen within layer III of the latter two regions. Immunoreactive fibers and varicosities also were seen within olfactory recipient regions of the amygdala.

228.4

NOVEL BRAINSTEM LOCALIZATIONS OF CORTICOTROPIN-RELEASING FACTOR (CRF) mRNA BY HYBRIDIZATION HISTOCHEMISTRY. J. Imaki*, T. Imaki*, P.E. Sawchenko and W. Vale, Peptide Biology Laboratories, The Salk Institute, La Jolla, CA 92037.

Though best known for its role in the hypothalamo-adenohypophyseal system, CRF is broadly distributed in the CNS. Current knowledge is based largely on immunocytochemical (ICC) findings, but the sensitivity afforded by *in situ* hybridization (ISH) histochemistry has provided new insights. We have used both approaches to compare in the brainstem the distributions of CRF peptide and preproCRF mRNA, the latter achieved using a ^{35}[S]-labeled 1.2 kb antisense cRNA probe. Localizations afforded by ICC and ISH data were congruent in many regions of the brainstem, including the parabrachial, laterodorsal tegmental, lateral reticular and medial vestibular nuclei, the inferior olive and the nucleus of the solitary tract. Other cell groups, including the nucleus prepositus hypoglossi, the nucleus of Roller, the ventral nucleus of the lateral lemniscus, and discrete parts of the medial geniculate complex, contained a substantial number of cells showing a clear mRNA signal but contained few, if any, CRF-immunoreactive cells. None of the localizations suggested by ISH were evident using sense strand cRNA probes. In suggesting that CRF mRNA is prominently expressed in some precerebellar and auditory relay nuclei, the results contribute to recent indications that preproCRF-derived peptide(s) may serve as transmitters or modulators in arenas beyond the neuroendocrine and autonomic systems with which CRF has been most commonly associated.

228.5

COMPARISON OF DISTRIBUTION OF PEPTIDERGIC NEURONS BETWEEN THE BED NUCLEUS OF STRIA TERMINALIS AND AMYGDALA. X.Bao, S.Y.Shu. Dept. of Neurobiology, Fourth Military Medical University, Xi'an, People's Republic of China.

To investigate relationships of peptidergic distribution between the bed nucleus of stria terminalis(BST) and amygdala(AMYG), immunohistochemical studies for the CCK,VIP,NT,CRF,GAL,SOM, CGRP,alpha-MSH,ANG II,L-ENK,B-END and SP were performed in the BST and AMYG of the rat.

In the oval nucleus of BST and lateral central nucleus of AMYG, a dense immunoreactivity of VIP, CRF,NT,CGRP,L-ENK,SP and SOM was observed in both parties. In the anterodorsal nucleus of BST and medial central nucleus of AMYG, all the immunoreactive positive fibers and CRF,NT,SOM and SP positive perikarya were found. VIP,CCK,CRF,NT, GAL,SOM,L-ENK and SP positive cell bodies were observed in the principal nucleus of posterior BST and in the lateral or basal nucleus of AMYG as well. In addition, all the immunoreactive positive fibers and VIP,CRF,SOM,NT,GAL,CGRP,L-ENK and SP positive cell bodies were demonstrated in the ventral part of BST and medial AMYG.

The results indicate that there are some mediolateral and anteroposterior correlations between the BST and AMYG.

228.6

MORPHOLOGICAL EVIDENCE FOR A SUBSTANCE P PROJECTION FROM MEDIAL SEPTUM TO HIPPOCAMPUS. C.L. Shurlow* and G.M. Peterson, Department of Anatomy and Cell Biology, East Carolina University School of Medicine, Greenville, N.C. 27858-4354.

Substance P-immunoreactive (SP-ir) perikarya are present at the lateral border of medial septum (MS) and SP-ir fibers are present in the hippocampal formation. SP levels within the hippocampus decrease following ablation of the MS. Together these data suggest that SP-ir neurons in the MS project to the hippocampus. However, the reduction of hippocampal SP levels could result from destruction of SP fibers passing through the MS. To determine if the SP-ir perikarya in MS project to the hippocampus we have combined anterograde tracing and immunocytochemistry. Sprague-Dawley rats were iontophoretically injected with *Phaseolus vulgaris* leucoagglutinin (PHA-L) through a glass micropipet (tip 10-15 µm in diameter). Following survival of one week rats were transcardially perfused with paraformaldehyde and five sets of adjacent sections of MS and hippocampus were cut at 40 µm. Sections were incubated in a cocktail of rabbit anti-SP (1:4000) and goat anti-PHA-L (1:1000), followed by fluorescein isothiocyanate (FITC) labeled anti-goat IgG (1:50) to label the PHA-L and by biotinylated anti-rabbit IgG (1:200) and avidin-conjugated Texas Red (1:50) to label the SP-ir fibers. Adjacent sections were processed for either SP or PHA-L alone or by omitting primary antibodies. Sections were observed under a fluorescent microscope using band pass filters designed specifically to discriminate the emission spectra of FITC from Texas Red. In the double-labeled sections, numerous fibers were observed to contain both SP-ir and PHA-L-ir in the MS and in the CA-2 region of the hippocampus. This co-localization of SP in PHA-L labeled fibers in the MS and hippocampus indicate that one source of SP in the hippocampus is from the MS.

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228.7

IDENTIFICATION OF SUBSTANCE P-CONTAINING PROJECTIONS TO THE VENTRAL RESPIRATORY GROUP IN THE RAT. J.R. Holtman, Jr., D. F. Speck and L. J. Marion. Departments of Pharmacol. and Physiol., Coll. of Med., Univ. of Kentucky, Lexington, KY 40536.

The procedure of retrograde tracing with rhodamine-labelled microspheres combined with immunohistochemistry was used to identify substance P afferents to the VRG in the rat. Four rats were anesthetized with pentobarbital (50mg/kg, i.p.). A micropipette containing rhodamine bead solution in normal saline was positioned at the site of maximal inspiratory activity in the VRG. A pressure injection of 50-100nl was made at this site. Rats were allowed to recover for 3-5 days. The rats were reanesthetized and colchicine (150ug) was administered into the lateral ventricle. Twenty-four hours later the animals were perfused and the brain was removed and processed using the indirect immunohistochemical procedure. Double-labelled neurons (rhodamine beads plus substance P) were found in the caudal raphe nuclei (obscurus, pallidus and magnus). In addition, neurons were found more laterally near the ventral surface of the medulla in the paraolivary/parapyramidal nuclei. Neurons containing only rhodamine beads were found in classical respiratory nuclei with known projections to VRG. Results of this study suggest that substance P afferents may influence the activity of inspiratory neurons in the VRG. (Support by HL-36050 to JRH).

228.8

LOCALIZATION OF SUBSTANCE P mRNA IN CHOLINERGIC CELLS OF THE RAT LATERODORSAL TEGMENTAL NUCLEUS: IN SITU HYBRIDIZATION HISTOCHEMISTRY AND IMMUNOCYTOCHEMISTRY. E.L. Sutin and D.M. Jacobowitz. Lab. of Clin. Sci., NIMH, Bethesda, MD 20892 and University of California San Diego, La Jolla CA 92093.

Immunocytochemical studies have shown that the neuropeptide, substance P (SP) is colocalized within cholinergic neurons of the laterodorsal tegmental nucleus (LDT) of the rat brain. In this study, in situ hybridization histochemical techniques in combination with immunocytochemistry and acetylcholinesterase (AChE) histochemistry were used to study the colocalization of messenger RNA (mRNA) encoding for SP in LDT cells. To demonstrate that SP is synthesized within cholinergic cells, alternate serial sections were hybridized with a ^{35}S-labeled 33-mer synthetic oligonucleotide probe encoding for SP using in situ hybridization histochemistry and processed either histochemically for AChE, or immunocytochemically for choline acetyltransferase (ChAT). Serial section analysis was also used to demonstrate the correlation between SP neuropeptide and SP mRNA in the same cells of the LDT. The methodology of in situ hybridization histochemistry complements the ability of standard immunocytochemical procedures to detect cellular peptides and protein because the combined use of these techniques provides a cross check of their specificity. This study definitively reveal that the cholinergic neurons of the LDT contain the genetic message to synthesize SP.

228.9

CO-LOCALIZATION OF SUBSTANCE K AND SUBSTANCE P IN THE MATING PATHWAY OF THE MALE GOLDEN HAMSTER M. Damlama and J.M. Swann. Institute of Animal Behavior, and Department of Biological Sciences, Rutgers University, Newark, NJ 07102.

In the male Golden hamster the medial nucleus of the amygdala (M), the bed nucleus of the stria terminalis (BNST), and the medial preoptic area (MPOA) have been shown to be critically involved in normal mating behavior. These areas contain steroid concentrating neurons, and in many species, all three areas contain numerous neuropeptides. In the Golden hamster, these areas contain substance P (SP) immunoreactivity which is steroid hormone dependent such that, castration decreases the number of immunoreactive neurons, and testosterone treatment restores it to normal levels. In these areas the neuronal distribution of substance K (SK), a related neuropeptide, seems to be similar to that of SP. We now report preliminary data suggesting that these two peptides co-exist within single neurons in M, BNST, and MPOA.

Adult male Golden hamsters were injected with colchicine into the lateral ventricle and perfused with 2% paraformaldehyde 48 hours later. SP and SK immunoreactivity was visualized simultaneously with immunofluorescence techniques, or individually in DAB reacted material.

Extensive cell body and fiber immunoreactivity for both neuropeptides was observed in M, BNST, and MPOA. In all immunoreactive neurons and their processes, SP and SK immunoreactivity appeared to be co-localized. In the BNST, the strongest immunofluorescent labeling was found in the medial division throughout its caudal extent. Maximal labeling in the MPOA was seen in the lateral and dorsal parts of post commissural median preoptic nucleus (MPN). Here it appeared on a continuum with caudal medial BNST as the latter extends ventrally. Labeled neurons in MPN and BNST were of small size (9-16 μm) emitting two to three primary dendrites. Immunoreactivity in M was confined to its caudal extent. However, due to intense fiber labeling in M, individual cell morphology could not be precisely characterized at this time.

The physiological and behavioral significance of the co-occurrence of these tachykinins in the mating pathway of the male hamster remain to be elucidated.

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228.10

SUBSTANCE P IMMUNOREACTIVITY IN FROG MOTOR NERVE TERMINALS. M. Matteoli*, P. De Camilli§ and C. Haimann*#. CNR Ist.Fis.Centri Nervosi, #CNR Center of Cytopharmacol. Dept. of Pharmacol. and Center for the Study of Periph. Neuropathies, Milano, Italy and §Dept. of Cell Biol., Yale Univ. School of Medicine, New Haven, USA (Spon. M. Vitadello)

Substance P (SP) is an undecapeptide widely distributed in the central and peripheral nervous system, where it functions as a peptide neurotransmitter. It has been reported that SP may exert a modulatory role on the function of neuronal and muscle nicotinic Acetylcholine receptors. In addition, SP has been found to induce an increase in quantal release of ACh at the frog neuromuscular junction. However, whereas SP-like immunoreactivity has been shown to be present in a variety of different cholinergic synapses, no evidence has been so far reported for the presence of SP in motor nerve terminals. We have now shown by immunohistochemical methods the presence of SP in frog motor neurons and neuromuscular junctions. The pattern of immunoreactivity is similar to that observed for calcitonin-gene related peptide-immunoreactivity and suggests a localization of SP in large dense core vesicles. These results, together with previous findings, suggest a physiological role for SP at the frog neuromuscular junction.

228.11

CHOLECYSTOKININ AND SOMATOSTATIN GENES ARE EXPRESSED IN THE HUMAN THALAMUS. J.-M. Burgunder* and W.S. Young III, Neurologic Clinic, University of Berne, CH-3010 Berne, Switzerland and Laboratory of Cell Biology, NIMH, Bethesda MD 20892.

Cholecystokinin (CCK) gene is expressed in thalamocortical and thalamostriatal neurons of the rat (Mol Brain Res 4:179,1988). In the cat, this peptide is found in some intralaminar and midline nuclei whereas somatostatin (SS) is expressed in the reticular nucleus of the same species but not in the rat. Since the putative neurotransmitters used by thalamic neurons are still incompletely known, especially in humans, we investigated the expression of the CCK and SS genes in the human thalamus using hybridization histochemistry. Serial, 12μm cryostat sections from two thalami from humans without known neurological disease were probed with synthetic, 48-base oligonucleotides against human CCK or human SS mRNAs. They were labeled using [^{35}S]dATP and terminal transferase. A vasopressin sense probe served as control and did not show any specific hybridization pattern.

CCK mRNA was expressed by many neurons located in several nuclei of the dorsal thalamus. They were especially numerous and widespread in the intralaminar nuclei (e.g., centrolateral) but smaller numbers were found in other nuclei as well (e.g., in the ventral group). SS mRNA was found in many neurons of the reticular nucleus and in a few of the zona incerta, but not in the dorsal thalamus. Neurochemical features of the human thalamus, for the genes studied here, resemble features found in the cat. SS may play a role in modulating dorsal thalamic impulses which may be conveyed to the striatum and, partly, to the cortex through CCK innervation.

228.12

TRH AND SOMATOSTATIN RECEPTORS MAPPING IN ADULT HUMAN HYPOTHALAMUS. D. Jordan*, M. Najimi*, J. Champier*, F. Chigr*, N. Kopp*, A. Slama* J. Bertherat* and J. Epelbaum. Lab. Pathol. Anatomy. A. Carrel Univ., Lyon, France and INSERM U 159, Paul Broca Center., Paris, France.

Eight adult human hypothalami (from 22 to 82 year old subjects, postmortem delay : 12 to 48 hours) were processed for both ^{3}H-Me TRH and ^{125}I Tyr0-Dtrp 8 Somatostatin 14 quantitative in vitro autoradiography. Each peptide displayed a specific mapping within the hypothalamus as revealed by a rostro-caudal analysis of 20 μm frozen serial sections.

A high density (> 600 fmol/mg protein) of Somatostatin binding sites is present in the anterior preoptic region and the posterior infundibular nucleus, while a moderate density (300 to 500) occurs in the anterior area, dorsomedial, ventromedial, and lateral mammillary nuclei and a low one (< 200) in the paraventricular and periventricular nuclei.

The highest density of TRH binding sites is observed in the diagonal band of Broca, the lateral preoptic area, and the infundibular nucleus (> 120 fmol/mg protein), while a moderate binding is quantified in the ventromedial nucleus (< 100) and a low one in the paraventricular nucleus (< 70).

In conclusion, one might point out the low density of TRH and Somatostatin binding sites in the paraventricular and periventricular nuclei in which both peptides are synthetized. In contrast, the high density of TRH and Somatostatin binding sites in the infundibular nucleus indicate that most interactions between both peptides occur at the terminal level.

228.13

IMMUNOREACTIVE ANP (IR-ANP) IN RAT AND RABBIT RETINAS.
D.E. Palm*, L.C. Keil*, J.W. Sassani* and W.B. Severs.
Depts. Pharmacol. & Ophthalmol., Col. Med., Penn State Univ. Hershey, PA 17033

IR-ANP was found in rat retina by RIA, but "receptors" have not been detected. Here, we report results of retinas from 6 S.D. rats and 6 albino rabbits. Tissue was obtained after pentobarbital anesthesia, and cardiac perfusion with neutral buffered formalin, then fixative. Sections (8 µ) were incubated with anti-rat (1-28)1°Ab 1:500 (with or without pre-absorbtion with 30 µg/ml synthetic rat (1-28)-ANP at 4°C overnight. After applying goat anti-rabbit 2° Ab, slides were processed with the Vectostain ABC kit, using diaminobenzidine as the chromogen. The anti-rat ANP had less than 0.01% cross-reactivity with oxytocin, arg-8-vasopressin, angiotensins I, II and NPY did not cross-react. Human ANP showed 7% cross-reactivity; atriopeptins I, II, III - 45-55%, and auriculin B, 100%. Only the outer and inner plexiform (OPL, IPL) layers of the rat and rabbit retinas showed IR-ANP, which was abolished by pre-absorbed 1° Ab. OPL staining was more intense, and appeared to be localized in photoreceptor terminals, although horizontal and/or bipolar cell processes could not be excluded. The border between the IPL and ganglion cell layer was demarcated by IR-ANP. Although the role of ANP in the retina is unknown, intraocular pressure control emerges as a possibility.
Supported by NASA NCC-2-127 and NGT-70035

228.14

ATRIAL NATRIURETIC PEPTIDE-LIKE IMMUNOREACTIVITY (ANP-LIR) IN GLIAL CELLS OF THE PARENCHYMA AND GLIA LIMITANS OF THE CANINE BRAIN.
J.C. McKenzie* and R.J. Cowie. SPON: (B.H. Turner). Dept of Anatomy, Coll. of Medicine, Howard University, Washington, DC, 20059.

Atrial natriuretic peptide has been localized in areas of the rodent brain which regulate cardiovascular function and fluid/electrolyte balance. We are currently investigating the distribution of ANP-LIR in the more cytoarchitecturally complex canine brain. Dogs (4) were deeply anesthetized with sodium pentobarbital and perfused with saline followed by 4% formaldehyde. Brains were removed, blocked, immersed in sucrose and sectioned at 50 um on a freezing microtome. Sections were incubated 72 h at 4 deg C in primary antiserum (anti-rat ANF IV; 1:10,000) and processed by the ABC technique. Small numbers of astroglia-like cells were intensely stained in many regions of the brain including neocortex and brain stem. In gray matter, positive glial cells presented as dense ovoid masses of processes from which longer processes often emerged to contact local vessels. In white matter, positive cells more closely resembled fibrous astrocytes with long filamentous processes. Isolated astrocytes of the glia limitans also displayed intense ANP-LIR, often sending long processes into the parenchyma to contact microvessels. The localization of ANP in astroglia represents the 2nd putative peptide neurotransmitter/neuromodulator to be identified in glial cells. Interestingly, the first was angiotensin II, an endogenous antagonist of ANP. We suggest that these two systems may interact in the control of local blood flow.
Supported by grants from the American Heart Association (Nation's Capital Affiliate) and Howard University.

228.15

ATRIAL NATRIURETIC PEPTIDE-LIKE IMMUNOREACTIVITY (ANP-LIR) IN BASAL FOREBRAIN-RELATED NEURONS AND PATHWAYS OF DOG AND CAT.
R.J. Cowie and J.C. McKenzie*. Dept. of Anatomy, Col. of Medicine, Howard University, Washington, DC, 20059.

Atrial natriuretic peptide has been localized in areas of the rat brain, some of which regulate cardiovascular function and fluid-electrolyte balance. We are currently investigating ANP-LIR in the more cytoarchitecturally complex carnivore brain. Three dogs and a cat were anesthetized with pentobarbital and perfused with saline, followed by 4% formaline. Brains were removed, blocked, immersed in sucrose and frozen sectioned at 50 um, incubated 72 h in primary antiserum (anti-rat ANF IV; 1:10,000), and processed by the ABC technique. Substitution of normal rabbit serum for primary antibody provided controls. Preliminary results indicated that both species possessed medium-to-large sized cells extending dorsolaterally in bridges from each of the highly vascularized core/caps of the Islands of Calleja (ICC) into the entopeduncular n., and from the bed n. of the stria terminalis to the amygdala. Additional cells of various sizes were labeled in the septal-accumbens region, lateral hypothalamus (LH), Forel's field H (FfH), lat. habenular n., substantia nigra/ventral tegmental area, and the PAG/dorsal raphe. Many ANP-LIR fibers coursed within the LH, FfH, and parts of the PAG. Minor differences were noted in labeling patterns between species. Findings of an ANP-LIR system extend the known peptidergic neuromodulators of the striatopallidal complex and support suggestions of a neuroendocrine function for the ICC.
Supported by grants from the American Heart Association (Nation's Capital Affiliate) and Howard University.

PEPTIDES: RECEPTORS II

229.1

CHARACTERIZATION OF NEUROKININ-1 AND -3 BINDING SITES IN RAT BRAIN USING SELECTIVE RADIOLIGANDS
T.V. Dam, B. Martinelli*, E. Escher* and R. Quirion. Douglas Hosp. Res. Ctr. McGill Univ. Montréal, Québec, Canada; Dépt. de pharmacol., Fac. de Méd. Univ. de Sherbrooke, Sherbrooke, Québec, Canada and F. Angelini Res. Inst., Rome, Italy.

The existence of three brain neurokinin (NK) receptors namely the NK-1, NK-2 and NK-3 subtypes has been proposed with substance P (SP), neurokinin A (NKA) and neurokinin B (NKB) acting as their preferred endogenous ligands, respectively. However, the selectivity of these NKs is limited since each one shows appreciable affinity for the other receptor classes. Therefore, the development of better radioligands is crucial in order to selectively label a given receptor subtype. In this study, we use two selective tritiated radioligands, [^{3}H]-Sar^9 Met(O$_2$)-SP ([^{3}H]-SM-SP) and [^{3}H]-Suc-(Asp^6, MePhe^0)-SP (6-11) ([^{3}H]-senktide) to characterize brain NK-1 and NK-3 binding sites respectively. Rat brain sections and membrane binding assays were prepared and performed as described before (Quirion, R. and Dam, T.V. J. Neurosci., 6: 2187-2199, 1986). The results show that [^{3}H]-senktide binds to a single class of high affinity (Kd=4.1nM), low capacity (54.56 fmol/mg protein) sites which are mostly concentrated in layers IV and V of the cortex and in the hippocampus, this distribution being reminiscent to that reported for the NK-3 receptor. On the other hand, [^{3}H]-SM-SP appears to label NK-1 sites since high densities of binding is seen in the striatum. Thus, these two new ligands should be most useful to selectively characterize NK-1 and NK-3 receptors. (Supported by Medical Research Council of Canada).

229.2

INHIBITION OF NEUROKININ-INDUCED PLASMA EXTRAVASATION AND BRONCHOCONSTRICTION BY THE NK-1 ANTAGONIST, GR71251.
C.C. Jordan*, I.J.M. Beresford*, G.B. Ewan*, R.M. Hagan*, S.J. Ireland*, M.L. Stephens-Smith* & P.Ward* (SPON: Brain Research Assn). Glaxo Group Research Ltd, Ware, Herts, SG12 0DP, U.K.

Tachykinins may mediate neurogenic inflammation and bronchoconstriction in respiratory disease. It is thus of interest to characterize the neurokinin receptors involved. Plasma extravasation (PEV), measured as accumulation of Evans blue-tagged plasma proteins, was induced by intradermal injection of tachykinins in the skin of the anaesthetized rat. This effect was mimicked by the NK-1 selective agonists substance P methylester (SPOMe) and δ-aminovaleryl[Pro^9,MeLeu^{10}]-SP(7-11) (GR73632). In animals pretreated with mepyramine (10mg/kg, i.v.), the novel NK-1 selective antagonist, GR71251, (0.1-0.5µmol/kg, i.v.), attenuated PEV induced by GR73632 (1-1000 pmol, i.d.).

In the anaesthetized guinea-pig, insufflation pressure was markedly increased by SPOMe (1-10nmol/kg, i.v.) and GR73632 (0.1-1.0nmol/kg, i.v.). The response to SPOMe was unaffected by mepyramine (5mg/kg i.v.) but was inhibited by GR71251 (3µmol/kg, i.v.).

229.3

HAMSTER URINARY BLADDER (HUB) AND GUINEA-PIG TRACHEA (GPT) MAY EACH CONTAIN A DIFFERENT SUBTYPE OF THE TACHYKININ PEPTIDE NK-2 RECEPTOR. H.C. Cheng; S.L. Harbeson; S.A. Shatzer* and S.H. Buck (SPON: W.E. Heydorn). Merrell Dow Research Institute, Cincinnati, OH 45215.

Neurokinin A (NKA) is the most potent mammalian tachykinin peptide in producing contraction of the HUB and the GPT (pD_2 approximately 8.0 in both tissues). NKA is at least 10X more potent than substance P or neurokinin B in both tissues (Dion et al., Life Sci. 41:2269, 1987). Based on the high potency of septide (slightly NK-1 selective) and DiMeC7 (NK-2 inactive) in GPT, McKnight et al. (Reg. Peptides 22:126, 1988) have suggested that GPT contains a unique tachykinin receptor rather than NK-2 or NK-1. We have investigated the tachykinin receptors in HUB and GPT using the new peptide Leu^9-CH_2NH-Leu^{10}-NKA(4-10) (MDL 28,564). MDL 28,564 inhibited iodinated NKA binding in HUB membranes with a K_I of 200 nM (NKA K_I = 1 nM). NKA stimulated phosphatidylinositol (PI) turnover with EC_{50} of 3 nM in chopped HUB and in chopped GPT muscle. In HUB, MDL 28,564 stimulated PI turnover with EC_{50} of 100 nM and maximum efficacy only 10% of that of NKA. In GPT, however, MDL 28,564 stimulated PI turnover with EC_{50} of 1 uM and maximum efficacy 100% of that of NKA. In contraction assays (in the presence of enkephalinase inhibition), MDL 28,564 up to 10 uM did not produce any contraction in HUB whereas it produced a full contraction at 10 uM in GPT. These results indicate that HUB and GPT contain NK-2 receptors that can be distinguished by C-terminal modifications in NKA. These receptors are most likely NK-2 subtypes.

229.4

CROSS-LINKING OF ^{125}I-IODOHISTIDYL^1-NEUROKININ A (INKA) TO TACHYKININ PEPTIDE NK-2 RECEPTORS IN HAMSTER URINARY BLADDER. S.H. Buck, B.O. Fanger; A.C. Wade* and S.A. Shatzer; Merrell Dow Research Institute, Cincinnati, OH 45215.

The tachykinin peptide NK-2 receptor from bovine stomach has been cloned and functionally expressed in Xenopus oocytes. It is a G-protein linked receptor with molecular weight of 43,000 (Masu et al., Nature 329:836, 1987). The hamster urinary bladder contains a high number of INKA binding sites that appear to be NK-2 receptors linked to phosphatidylinositol turnover. We have used BIS(sulfosuccinimidyl) suberate (BS^3) to investigate the INKA binding protein in this tissue. 1 nM INKA and 25 mg bladder membranes were incubated in PBS buffer at 25°C for 2 hr. The membranes were pelleted by centrifugation and resuspended in buffer containing 5 mM BS? The pellets (+ dithiothreitol) from this step were separated by SDS-PAGE with 10-20% gels which were then exposed to film. A single major band of 43,000 was observed. Unlabeled NKA blocked labeling of this band with an IC_{50} of 6 nM. 1 uM NKA blocked at least 95% of labeling of the band. Substance P, senktide, bombesin, bradykinin, α-MSH, somatostatin, or VIP did not compete for labeling. Neurokinin B competed with less potency than NKA. Under nonreducing conditions (- DTT), a second labeled band (86,000) was seen that contained most of the specific binding (blocked by 1 uM NKA). These results indicate that hamster urinary bladder contains an NK-2 receptor protein that is similar to the reported cloned receptor. This protein may be associated with another similarly sized molecule.

229.5

CENTRAL BINDING SITES FOR MELANOTROPIC PEPTIDES W. Lichtensteiger, M. Schlumpf and *A. Eberle, Inst. of Pharmacology, Univ. of Zürich, Zürich, and *Zentrum für Lehre u. Forschung, Basel, Switzerland

Melanotropic peptides affect brain function, but the site of action is uncertain. We studied binding of 3H(Nva 13)-alpha-MSH to membranes of Long Evans rat hippocampus and by in vitro autoradiography. Scatchard and competition data indicate the existence of high (Kd 4-8 nM) and low affinity sites (Kd 5 uM) for alpha-MSH. Slightly lower affinities are found with desacetyl-alpha-MSH which also occurs in brain, and gamma-1-MSH which displays only week activity on melanocytes. The fragment Lys-Pro-Val NH2 containing the C-terminal message sequence had a comparatively high affinity. ACTH 4-10 with the central message sequence was virtually inactive at the high affinity site but bound to the low affinity site. A particular activity of the C-terminal sequence has been reported for certain central actions. Quantitative autoradiography revealed high densities of the high affinity site in hippocampus, dentate gyrus, cortical areas, cerebellum and several brainstem areas. Our data provide a basis for central actions of MSH peptides and support the idea of a possible central role.

229.6

MOUSE VAS DEFERENS (MVD) AND RAT BRAIN BINDING STUDIES SUGGEST TWO DELTA OPIOID RECEPTORS. L.K.Vaughn, W.S.Wire, Y.Shimohigashi, G.Toth, V.J.Hruby, T.F.Burks, H.I. Yamamura. U. of Arizona, Tucson, AZ 85724, Kyushu U. Fukuoka, Japan.

Certain enkephalin analogs which contain the conformationally restricted amino acid E-(2R,3S)-cyclopropylphenylalanine ((2R,3S)-V^EPhe) have been shown to be active at brain but not MVD δ-opioid receptors (Shimohigashi et al., FEBS Lett. 222:71,1987). We compared the ability of a selective δ-opioid compound, [D-Pen^2,pCl-Phe^4,D-Pen^5]-enkephalin (pCl-DPDPE), and [D-Ala^2,(2R,3S)-V^EPhe^4,Leu^5]-enkephalin methyl ester (CP-OMe), to inhibit [^{3}H]pCl-DPDPE binding in both rat brain and MVD. 500 μg protein from rat brain homogenate or 1000 μg protein from MVD homogenate were incubated with 800 pM [^{3}H]pCl-DPDPE and with varying concentrations of unlabelled pCl-DPDPE or CP-OMe in 50 mM Tris buffer with 5 mM $MgCl_2$, 100 μM PMSF, 1 mg/ml BSA, pH 7.4 for 4 h at 25°C. Specific binding was defined with addition of 10 μM naltrexone. pCl-DPDPE recognized brain and MVD binding sites with equal affinity, however, CP-OMe showed 33 fold lower affinity in MVD compared to brain.

	BRAIN		MVD	
	IC_{50} (nM)	(Hill slope)	IC_{50} (nM)	(Hill slope)
pCl-DPDPE	1.24	0.99	1.30	1.06
CP-OMe	2.18	0.86	73.84	1.04

This suggests that MVD δ-opioid receptors may be distinct from brain δ-opioid receptors. Supported by USPHS grants.

229.7

EVIDENCE FOR TWO SUBTYPES OF U50,488-SENSITIVE KAPPA$_1$ RECEPTORS. L. Liu*, J.A. Clark, M. Edelson* and G.W. Pasternak. (SPON: J. Posner). The Cotzias Laboratory of Neuro-Oncology, Memorial Sloan-Kettering Cancer Center, New York, NY 10021.

In the presence of high concentrations of mu and delta ligands, ^{3}H-ethylketocyclazocine selectively labels kappa receptors in the guinea pig cerebellum. Opiates with kappa actions, including U50,488, tifluadom, Mr2034, Mr2266, and Win44,441, all compete binding with K_i values under 10 nM and Hill coefficients of approximately one. Dynorphin A also potently lowers binding with a K_i of 0.27 nM and a Hill coefficient of 0.83 ± 0.20 (n=4). However, competition studies with dynorphin B yield a Hill coefficient of 0.46 ± 0.03 (n=5) and nonlinear regression analysis gives a best fit for two sites (K_i 0.13 nM and 40 nM). Similarly, α-neoendorphin also produced a biphasic competition curve best fit with two components (K_i 0.12 nM and 33 nM). Competition studies with α-neoendorphin in the presence of a fixed concentration of dynorphin B suggest that both compounds label the same site (kappa$_{1b}$) with high affinity. These data suggest that ^{3}H-ethylketocyclazocine labels two U50,488-sensitive subtypes of kappa receptors in the guinea pig cerebellum, which we have termed kappa$_{1a}$ and kappa$_{1b}$. Classical kappa opiates have high affinity for both kappa receptor subtypes, as does dynorphin A. In contrast, both dynorphin B and α-neoendorphin selectively label kappa$_{1b}$ receptors. We find similar results with ^{3}H-U69,593.

229.8

KAPPA$_3$ BINDING: DEVELOPMENT OF A SELECTIVE BINDING ASSAY. E. Huang,* B. Hersh,* L. Liu,* and G.W Pasternak (SPON: K. Foley). The Cotzias Laboratory of Neuro-Oncology, Memorial Sloan-Kettering Cancer Center, New York, NY 10021

We have characterized the binding of ^{3}H-NalBzoH to κ_3 receptors in calf striatal membranes and compared it to the U50,488-sensitive κ_1 receptors in the guinea pig cerebellum. ^{3}H-NalBzoH labeled κ_3 sites with high affinity (K_D 0.74 ± 0.04 nM) and high density (Bmax 14.6 ± 0.69 fmoles/mg wet weight tissue), approximately twice levels of μ receptors and far greater than κ_1 receptors. This site correlates with neither μ nor δ receptors. Although a large number of κ ligands examined competed binding quite potently with K_i values under 10 nM, U50,488 (1 μM) did not significantly inhibit ^{3}H-NalBzoH binding in calf striatal membranes despite its potent inhibition of ^{3}H-NalBzoH binding to κ_1 receptors in the guinea pig cerebellum (K_i 6.1 nM). ^{3}H-NalBzoH binding in calf striatal membranes was highly stereospecific and selective for opioids. Thus, in calf striatal membranes ^{3}H-NalBzoH labels a novel subtype of κ receptor, termed κ_3. Differences in sensitivity to U50,488 clearly differentiate κ_3 from κ_1 sites present in the guinea pig cerebellum. The high affinity of ethylketocyclazocine, tifluadom and cyclazocine for κ_3 sites distinguish them from κ_2 sites reported in rat brain homogenates. We have also identified κ_3 receptors with similar characteristics in rat and mouse brain.

229.9

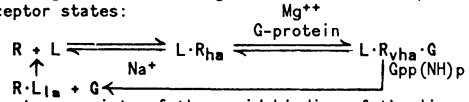
DIFFERENTIAL EFFECT OF DIVALENT CATIONS ON MU_1, MU_2 AND DELTA OPIATE RECEPTOR BINDING. K. Kinouchi,* K.M. Standifer and G.W. Pasternak. (SPON: W. Shapiro). Memorial Sloan-Kettering Cancer Center, New York, NY 10021 USA.

Recent work has suggested the existence of a variety of opiate receptor types and subtypes. Mu receptors, with their high affinity for morphine, were easily distinguished from delta receptors which bound the enkephalins selectively. Evidence now indicates the presence of two subtypes of mu receptors, termed mu_1 and mu_2, which can be differentiated on the basis of their binding selectivity and pharmacological actions. In the present study we have examined the actions of three divalent cations on the binding of mu_1, mu_2 and delta receptors. Magnesium, manganese and calcium all increased mu_1 binding. Of the three, magnesium was the most effective, elevating binding 3-fold. Manganese increased binding 2-fold and calcium increased binding only 70%. The divalent cations also increased mu_2 binding, but far less than mu_1: 50% for magnesium, 35% for manganese and 15% for calcium. Against delta binding magnesium increased binding 70%, manganese by 60%, and calcium produced no effect. Saturation studies revealed a mixed effect on K_D and Bmax for the mu receptors. The increased mu_1 and mu_2 binding resulted from an increase in both affinity and Bmax. The modest effects on delta binding appeared to be predominently a change in Bmax. The differential sensitivity of mu_1 and mu_2 binding towards divalent cations supports the concept of multiple mu receptors.

229.10

EVIDENCE AGAINST A SIMPLE BIMOLECULAR MODEL FOR MU_1 BINDING. K.M. Standifer, J.A. Clark, and G.W. Pasternak. The Cotzias Laboratory of Neuro-Oncology, Memorial Sloan-Kettering Cancer Center, NY, NY 10021.

In thalamic membranes ^{3}H-DADL in the presence of DPDPE labels mu_1 sites almost exclusively. Our data suggest a complex binding model consisting of at least two steps and three receptor states:



The first step consists of the rapid binding of the ligand to the receptor to form a high affinity complex ($L{\cdot}R_{ha}$; $\tau_{1/2}$ ~10 min) followed by a slower conversion of this bound receptor in the presence of Mg^{++} to yield a very high affinity state ($L{\cdot}R_{vha}$; $\tau_{1/2}$ ~75 min). EDTA prevents the conversion of $L{\cdot}R_{ha}$ to $L{\cdot}R_{vha}$. Gpp(NH)p rapidly dissociates the $L{\cdot}R_{vha}$ complex ($\tau_{1/2}$ ~2 min), suggesting the possibility that the $L{\cdot}R_{vha}$ complex includes a G-protein, but has little effect on the dissociation of the $L{\cdot}R_{ha}$ state. In contrast, the $L{\cdot}R_{ha}$ state is more sensitive to Na^+ than the $L{\cdot}R_{vha}$ state. These results illustrate the complexity of opiate binding mechanisms and raise serious questions regarding the use of computer models based upon simple bimolecular interactions between a ligand and its receptor.

229.11

PHOTOAFFINITY LABELING MULTIPLE MU AND KAPPA RECEPTORS. G.W. Pasternak, K.M. Standifer,* L.L. Steele* The Cotzias Laboratory of Neuro-Oncology, Memorial Sloan-Kettering Cancer Center and Departments of Neurology and Pharmacology, Cornell U. Medical College, New York, NY 10021

The benzoylhydrazone derivative of naloxone (NalBzoH) labels both mu and kappa receptors in standard homogenate binding assays. We now report that ^{3}H-NalBzoH is an effective photoaffinity label for multiple classes of mu and kappa receptors. Using differing binding conditions, we can selectively label mu_1, mu_2, $kappa_1$ or $kappa_3$ receptors. In homogenates from the guinea pig cerebellum, ^{3}H-NalBzoH labels U50,488-sensitive $kappa_1$ receptors. In homogenates from calf striatum, ^{3}H-NalBzoH selectively labels a U50,488-insensitive kappa receptor ($kappa_3$). Membranes can be labeled with ^{3}H-NalBzoH using selective binding conditions, washed to remove unbound radioligand and the ^{3}H-NalBzoH covalently linked to proteins with exposure to UV light. ^{3}H-NalBzoH dissociates from mu receptors quite slowly ($\tau_{1/2}$ 24 hr at 25°C and 96 hr at 0°C), but Gpp(NH)p (100 μM) or lowering the pH to 5 dissociates ^{3}H-NalBzoH quite rapidly, with a $\tau_{1/2}$ under 15 min. Following exposure to UV ^{3}H-NalBzoH is stable to both Gpp(NH)p and low pH, suggesting the formation of covalent bonding. Binding in all assays is stable to solubilization of membranes with SDS. This approach may be a useful method to affinity label, characterize and purify opiate receptor subtypes.

229.12

CROSS-LINKING OF 125I-BETA-ENDORPHIN TO A MU-DELTA OPIOID RECEPTOR COMPLEX IN RAT NEOSTRIATUM. A.N.M. Schoffelmeer*, Y-H. Yao* and E.J. Simon. Dept. of Psychiatry, NYU Medical Center, New York, NY 10016 and Dept. of Pharmacol., Free Univ., Med. Faculty, Amsterdam, 1081 BT, The Netherlands (A.N.M.S.).

In a modified Krebs-HEPES buffer at 37°C ^{3}H-DAGO and human ^{125}I-beta-endorphin bound to rat striatal homogenates with Kd values of 7.5 and 4.6 nM, respectively. The Bmax values were 97 and 261 fmole/mg protein in agreement with a selective labelling of mu receptors by ^{3}H-DAGO and that of both mu and delta receptors by ^{125}I-beta-endorphin. Displacement curves of DAGO and the delta agonist DSTBULET against 2 nM ^{125}I-beta-endorphin were monophasic with half maximal inhibition at 30 and 500 nM, respectively. Saturation curves of ^{3}H-DAGO showed that 500 nM DSTBULET caused a non-competitive inhibition of mu receptor binding. ^{125}I-beta-endorphin (2 nM) was cross-linked to mu and delta receptors with 1 mM BSCOES. SDS-PAGE followed by autoradiography of the gels revealed a major band of about 80 kDa that was diminished by 10/uM naloxone and strongly reduced not only by 30 nM DAGO but also by 500 nM DSTBULET. These data provide strong evidence for the existence in rat striatum of a mu-delta opioid receptor complex with non-competitively interacting mu and delta binding sites and indicate that under physiological conditions striatal mu- and delta-opioid receptors represent physically associated glycoproteins.

229.13

TYR-MIF-1 BINDING SITES ARE PRESENT IN SH-SY5Y HUMAN NEUROBLASTOMA CELLS: THEIR REGULATION MAY DIFFER FROM THAT OF MU OPIATE RECEPTORS. J.E. Zadina, S.L. Chang, L-J. Ge*, A.J. Kastin and R.E. Harlan. VA Medical Center and Tulane Univ. Sch. of Med. New Orleans, LA 70146

Human neuroblastoma SH-SY5Y cells express mu and delta opiate receptors and develop tolerance to the effects of opiates (Yu and Sadee, JPET, 240:350-355, 1988). We tested these cells for the presence of binding sites for Tyr-MIF-1 (Tyr-Pro-Leu-Gly-NH_2), a recently isolated brain peptide with antiopiate activity. In rat brain, Tyr-MIF-1 binding sites decrease after chronic morphine, while opiate receptors increase (Zadina et al., Life Sci. 44: 555, 1989). SH-SY5Y cells were grown and synaptic plasma membranes (SPM) prepared from them according to the methods of Yu and Sadee, 1988 and Zadina et al., 1989. Specific binding (per mg protein) of Tyr-MIF-1 to SPM from SH-SY5Y cells was comparable to that of SPM from both Charles River and Zivic-Miller rat whole brain. Treatment of SH-SY5Y cells with retinoic acid significantly ($p < 0.05$) increased the binding of both ^{3}H-DAGO, in good agreement with Yu and Sadee (1988), and ^{125}I-DAGO. In contrast, Tyr-MIF-1 binding was significantly ($p < 0.05$) decreased in one experiment and did not change in another. SH-SY5Y cells thus provide a second model in which opiate receptors and Tyr-MIF-1 binding sites may be differentially regulated.

229.14

NON-MATURE OPIOID RECEPTORS IN DEVELOPING STRIATUM AND HIPPOCAMPUS J.Barg*, V.Hatini* and R.Simantov (SPON:M.Devor) Dep. of Genetics, Weizmann Ins. of Sci., Rehovot, Israel

The developmental pattern of the three opioid receptor subtypes μ, δ and k was determined in the rat and guinea pig hippocampus and striatum. In the guinea pig the total receptor density in the two brain regions reached a peak value at the embryonic days 45 and 55, respectively, and then decreased one day after birth. A similar decrease in total receptor density before birth was evident also in the rat, indicating therefore that during the embryonic period the receptors undergo, at least in these two brain structures, an apparent regressive developmental pattern. Various opioids were used to characterize the receptors that participate in this process, but the results were surprising; DAGO, DPDPE and U-50,488 that are widely used to characterize the three receptor subtypes in adult tissues were non-selective during the early developmental period. These and additional experiments indicate that opioid receptors in embryonic hippocampus and striatum cannot be classified to μ, δ and k receptors, possibly because the protein is yet 'non-mature'. This finding supports therefore the notion that a common gene may code for more than one opioid receptor subtype, and the final opioid selectivity of the receptor depends on post-translational modifications. Alternatively, the receptors in embryonic tissues may not possess the mature interaction with other membrane-bound components like the GTP-binding proteins.

229.15

PRESENCE OF ZETA AND OTHER OPIOID BINDING SITES IN CEREBELLUM OF ADULT AND DEVELOPING HUMANS. D.M. Gibo*, P.J. McLaughlin and I.S. Zagon (SPON: R. Hamilton). Dept. Anatomy, Penn. State Univ. Coll. Med., Hershey, PA 17033.

The presence of at least 3 types of opioid binding sites have been documented in adult and developing mammalian CNS. Recently, a new opioid receptor, zeta (ζ), related to cell replication, has been identified in abnormal neural tissue. [Met^5]-enkephalin, which exhibits high affinity for the zeta receptor has been immunocytochemically localized in germinative cells of the developing rat cerebellum; neurons derived from these germinative cells are not immunoreactive. In this study, zeta and other opioid binding sites, and the developmental patterns of their expression, were determined in human cerebellum. Binding assays to [Met^5]-enkephalin, DAGO, DADLE, and EKC were used to probe for ζ, μ, δ, and κ sites, respectively, in developing (embryonic to 90 days) and adult human cerebellum. Specific and saturable binding to radiolabeled [Met^5]-enkephalin, DADLE, and EKC, but not DAGO, were recorded for both developing and adult cerebellum. Bmax values were significantly greater (3-6 fold increase) in developing tissue than adult cerebellum; Kd values did not differ with age. These results indicate that opioid binding sites, including those related to growth, are present in the human cerebellum, and in greatest number during ontogeny.

Supported by NIH Grant NS-20500.

229.16

AUTORADIOGRAPHIC CHARACTERIZATION OF BINDING SITES FOR THE ANTIPSYCHOTIC PEPTIDE DES-ENKEPHALIN-γ-ENDORPHIN (ORG-5878) IN THE RAT BRAIN.
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The non-opioid effects of the endogenous neuropeptide γ-endorphin resemble those of classical neuroleptic drugs in rat behavioural models. These properties are shared by the non-opioid congeners of γ-endorphin, of which des-enkephalin-γ-endorphin (β-endorphin(6-17); DEγE; Org 5878) is the shortest and most potent peptide. In addition, DEγE has antipsychotic properties in certain schizophrenic patients. We studied the binding of [^{35}S]Methionine-DEγE ([^{35}S]β-endorphin(5-17); 1400 Ci/mmol) to coronal rat brain cryosections using in vitro autoradiography. Incubations were done with 500 pM [^{35}S]Met-DEγE and specificity was assessed by coincubation of adjacent sections with 1 μM DEγE. Displaceable binding was found in a number of anatomically defined brain areas which constitute a major part of the mesolimbic feedback circuitry. This specific topography of DEγE binding may be relevant with respect to the antipsychotic properties of the peptide. Structure-activity relationship studies suggest that the binding is selective for DEγE-related peptides and does not involve dopamine D_2 receptors.

229.17

CHARACTERIZATION AND ANALYSIS OF SUBTYPES OF RECEPTORS FOR SUBSTANCE P. E. Burcher*, D.P. Geraghty*, D. Regoli*[1], G. Drapeau*[1] and B.G. Livett[2]. Dept. of Biological Sciences, Deakin Univ., Vic 3217, Australia, [1]Dept. of Pharmacology, Sherbrooke Univ., Canada JIH 5N4 and [2] Dept. of Biochemistry, Melbourne Univ., Vic. 3052, Australia.

We have investigated the binding characteristics of the radioligands [^{125}I]-Bolton-Hunter SP (BHSP), BH-[Sar^9 Met-$(O_2)^{11}$]-SP (BH-SarSP, Lew, R. et al., Regul. Pept. 22:113, 1988) and [^{3}H]-SP in tissue homogenates. Membranes were prepared as described previously (Burcher, E. et al., JPET, 236:819, 1986) and incubated with 0.01-1 nM BH-SarSP (rat brain, rat submandibular gland, SG) and 0.01-3 nM BHSP and 0.5-20 nM [^{3}H-SP] (bovine adrenal medulla, BAM). In brain and SG, BH-SarSP binding was of high affinity (K_D's 0.26, 0.10 nM respectively). Rank potency orders for inhibition of BH-SarSP binding were similar in both tissues, suggesting binding to an NK1 receptor, but most competitors were 4-60 times less potent as inhibitors in brain compared with SG. In BAM, no specific binding of BHSP occurred but binding of [^{3}H]-SP was saturable and reversible with K_D 1.46 ± 0.73 nM. Specific binding of [^{3}H]-SP was inhibited by SP > neurokinin A ≈ SP(3-11) > SP(1-9) > SP(1-6) > neurokinin B, with Lys^3 apparently essential for effective competition. These data suggest (1) that SP receptors in rat brain and SG have different characteristics indicating possible subtypes of the NK1 receptor, and (2) that SP receptors in BAM are not of the NK1 type but represent a novel SP receptor subtype.

229.18

SUBSTANCE P-INDUCED REDUCTION IN CYTOSOLIC ^{3}H-*myo*-INOSITOL IN RAT PAROTID ACINAR CELLS. M.M. Dietl*, Y. Torrens*, J.-C. Beaujouan* and J. Glowinski*. (SPON: L. Di Giamberardino) Collège de France, INSERM U.114, Chaire de Neuropharmacologie, 75005 Paris, France.

Coincubation of rat parotid acinar cells with substance P (SP) and ^{3}H-*myo*-inositol resulted in a significant reduction of labeled cytosolic inositol. This effect was rapid (30sec), the maximal reduction (about 45%) being observed at 15min. The response to SP was temperature-dependent since at 4°C no reduction was observed. The SP-induced reduction of cytosolic ^{3}H-*myo*-inositol was dose-dependent (EC_{50}=5.7±2.4 nM). Spantide, a SP antagonist, at 10^{-5}M, competitively shifted the effect of SP (EC_{50}=30.0±5.1 nM). [L-Pro^9]SP and SP methyl ester, two selective agonists of NK1 receptors, reduced the accumulation of ^{3}H-*myo*-inositol. Long SP C-terminal fragments were more potent than shorter ones. SP N-terminal fragments and SP free acid were without effect. [Pro^7]NKB, a selective neurokinin (NK)B analog, had no effect. The rank order potency of tachykinins was SP>NKA>NKB. These findings and the close correlation between EC_{50} values and IC_{50} values obtained in binding studies implicate the NK1 receptor. Activation of muscarinic receptors by carbachol also resulted in a reduction of cytosolic ^{3}H-*myo*-inositol in rat parotid acinar cells, an effect reversed by atropine. Atropine alone had no effect. Moreover, atropine was unable to alter SP-induced reduction of cytosolic ^{3}H-*myo*-inositol. Other neurotransmitters (5HT, glutamate, CCK, NT, BK) were without effect on cytosolic ^{3}H-*myo*-inositol accumulation. In conclusion, NK1 and muscarinic receptors seem to be implicated in the regulation of membrane transport of inositol in rat parotid gland. The measurement of cytosolic ^{3}H-*myo*-inositol in rat parotid glands could be adopted as a simple, sensitive, and specific method for screening the biological activity of potential agonists and antagonists at NK1 receptors.

229.19

PHOTOAFFINITY LABELLING THE SUBSTANCE P RECEPTOR USING A p-BENZOYLPHENYLALANINE-CONTAINING SUBSTANCE P ANALOG. N.D.Boyd*, C.F. White*, S.E. Leeman, R. Cerpa*+, E.T. Kaiser*+ Dept. of Physiol., Univ. Mass. Med. Sch., Worcester, MA 01655 and +Lab. of Biorg. Chem/ Biochem.,Rockefeller Univ., New York, NY 10021

A photoactive analog of substance P (SP), containing L-p-benzoylphenylalanine in place of Phe^8, was prepared as a probe for the SP receptor by solid-phase peptide synthesis methodology. The ^{125}I-Bolton-Hunter conjugate of Phe^8(pBz)-SP bound, in the absence of light, in a saturable and reversible manner to an apparently homogeneous population of binding sites on rat submaxillary gland membranes (B_{max}=0.5 pmoles/mg, K_d=1.2 nM; parameters similar to those characterizing the binding of SP to the same membrane preparation.) ^{125}I-Phe^8(pBz)-SP binding was competitively inhibited by SP and other tachykinin peptides with a specificity appropriate for SP/NK-1 receptors. Upon irradiation at 350nm, about 70% of the ^{125}I-Phe^8(pBz)-SP bound to the SP receptor became covalenty attached yielding radiolabelled bands of Mr=53000 and Mr=46000. Labelling of both bands occurs with characteristic SP receptor specificity and both bands are equally sensitive to the inhibitory effects of GppNHp. The 46kDa receptor peptide is most likely derived from the higher molecular weight species by proteolytic nicking. The high affinity of ^{125}I-Phe^8(pBz)-SP for SP receptors and remarkable photoincorporation efficiency suggest that this photoaffinity ligand will be of considerable value in the molecular characterization of SP receptors.

229.20

IMMUNOHISTOCHEMISTRY OF SUBSTANCE P BINDING SITES IN THE GUINEA PIG TRACHEA. W.Kummer*, A. Fischer*, U.Preissler*, J.Y.Couraud*[2] and Ch. Heym*. (SPON: J.Y. Jew) Inst. f. Anatomie und Zellbiologie, Univ., D-6900 Heidelberg, FRG; [2] Dept. de Biologie, CEN/SACLAY, France.

Recently, anti-substance P (SP) anti-idiotypic antibodies (anti-Id ab) have been demonstrated as a tool for cytochemical identification of SP receptors in the central nervous system (Couraud et al., J. Histochem. Cytochem., 36:1397-1401, 1988). We used this approach for studying the localization of SP binding sites in the guinea pig trachea. Immunoreaction was considered to be specific when it could be abolished by preabsorption of the anti-Id ab with a C-terminal specific monoclonal SP-antibody.

Specific labelling was seen at the lateral membranes of the epithelial cells, over the trachealis muscle and on singly lying cells in the lamina propria mucosae. The results are in general agreement with previous autoradiographic studies (Hoover and Hancock, J.Auton.Nerv.System, 19:171-174, 1987), but provide information on cellular localization of SP binding sites due to the higher resolution of immunohistochemistry.

230.1

GANGLIOSIDE EXPRESSION DURING SYNAPTOGENESIS *IN VITRO*.

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Two model systems that undergo synapse formation, *in vitro*, were used in these studies; NG108-15 cells in coculture with fetal rat myotubes and murine dorsal root ganglia cocultured with spinal cord. Thin layer chromatograms of glycolipid extracts from various tissues were incubated with A2B5, a monoclonal antibody that recognizes a family of gangliosides. A2B5-positive bands, identified by autoradiography, were scraped from the TLC plates and quantitated by liquid scintillation spectrometry. In NG108-15 x muscle cocultures compared to N18TG2 x muscle cocultures (which do not make synapses) there was a marked decrease (approx. 50%) in a ganglioside that comigrated with GM2. Both NG108-15 x muscle and N18TG2 x muscle cocultures showed a 1.5 to 2-fold increase in two gangliosides-one comigrating with GD1a and the other with GT1b-compared to muscle, NG108-15 or N18TG2 cells cultured alone. In murine spinal cord (SC) cultures a ganglioside was identified that migrated between GD1a and GT1b. This ganglioside was not found in dorsal root ganglia (DRG) cultured alone or in SC x DRG cocultures. Further, a ganglioside that comigrated with GT1b was found to be reduced in SC x DRG cocultures by 70% compared to SC or DRG cells cultured alone. These results demonstrate that ganglioside expression undergoes significant modulation in cocultures capable of making synapses and that it may be possible to identify specific gangliosides that play a key role in synaptogenesis.

230.2

COLD ACCLIMATION INCREASES POLARITY OF GANGLIOSIDES IN SALAMANDERS. L.Irwin, K.Talentino*, J.Cohen*, C.Nelson*. Dept. Biology, Simmons Col., Boston, MA 02115

While the biological importance of ganglioside heterogeneity remains unexplained, growing evidence suggests that ganglioside patterns may be subject to ecophysiological adaptation within phylogenetically prescribed genetic limits. To test the influence of chronic temperature shifts on ganglioside patterns axolotls (Ambystoma mexicanum) were randomly assigned to groups acclimated for 3 weeks at either 15 C or 23 C. Metabolic rates were determined using closed-system dissolved oxygen analysis. Brain gangliosides were extracted with chloroform: methanol (2:1), isolated on a bed of silicic acid, purified by ion exchange and reverse-phase chromatography, and chromatographed on thin-layer plates of silica gel in chloroform:methanol: 0.2% aqueous calcium chloride. Individual gangliosides were visualized with resorcinol reagent and quantified by scanning laser densitometry. Gangliosides from the cold acclimated animals showed a consistent shift toward a higher concentration of more polar (tri- and tetrasialo-) molecules. Metabolic rates were significantly depressed in the cold acclimated salamanders as well. Studies are continuing to determine if ganglioside composition and metabolic rate are causally or coincidentally related. (We thank Biomed Instruments, Inc., for the loan of densitometry equipment and software.)

230.3

EXPRESSION AND SYNTHESIS OF GANGLIOSIDE 9-O-ACETYL-GD3 IN MOUSE GLIOMA C26 AND SUBCLONES. D.M. Bonafede, A.C. Missias* and M. Constantine-Paton. Dept. of Biology, Yale University, New Haven, CT 06511.

We are using the mouse glioma line C26 as a model for the synthesis and expression of ganglioside 9-O-acetyl-GD3, which has been implicated in migratory events in neural development (Mendez-Otero et al.,1988, J.Neurosci. 6: 564; Schlosshauer et al.,1988, J.Neurosci. 6: 580), as well as in malignancy (Cheresh et al.,1984, JBC 259: 7453). The JONES mAb (Constantine-Paton et al.,1986, Nature 324: 459) is used for detection of 9-O-acetyl-GD3 and mAb R24 (Pukel et al.,1982, J.Exp. Med. 155: 1133) for detection of GD3.

Approximately 30% of C26 glioma cells carry the JONES epitope on their surface. By limiting dilution subcloning we generated four lines, with expression ranging from 0 to 85%. Ganglioside extraction and HPTLC analysis showed the ganglioside profile of this tumor to be restricted to the GM3 synthetic pathway. C26 and the 3 positive subclones have abundant GM3, and varying levels of GD3 and 9-O-acetyl-GD3; the negative line has a synthetic block in the conversion of GM3 to GD3, which leaves GM3 as its only ganglioside.

Preliminary results from experiments in which negative cells, supplied with exogenous pure GD3 in their tissue culture medium (20 μM), incorporated GD3 in their plasma membranes (a 12 hour incubation was sufficient) and started expressing 9-O-acetyl GD3 (after 3 days), suggest that 9-O-acetyl-GD3 is synthesized from GD3 by acetylation of the terminal sialic acid, rather than by direct addition of an acetylated sialic acid to GM3. GD3 incorporation and conversion to 9-O-acetyl-GD3 were monitored by immunostaining with mAbs R24 and JONES, respectively.

We characterized the cell morphologies of the 4 subclones, and, because the different subclones seem to have different proliferation rates, we are using BrdU/FdU to detect any correlations between 9-O-acetyl-GD3/GD3 expression and cell division times.

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230.4

CHRONIC OPIATE ADMINISTRATION INCREASES POLYSIALYLGANGLIOSIDES IN RAT BRAIN. J. Fishman*, M. Cahill*, and K. Carlson. Dept. of Pharmacology, U. Mass. Med. Sch., Worcester, MA 01655

Gangliosides and other sphingolipids have been shown to modulate a variety of cellular functions (Hannun and Bell [1989] Science 243, 500-507). Utilizing high performance thin-layer chromatography (HPTLC), we have observed that animals which have become physically dependent to the opiate agonist etonitazene (ET) by oral self-adminstration have significantly elevated polysialylgangliosides in synaptic vesicles from striatum, with no change in the monosialylgangliosides GM_1 or GM_3. In hippocampus, we observed decreases in GM_1, GD_{1a}, and GD_{1b}, but increased GT_{1b}. Small but variable changes in gangliosides were observed in cerebellum but no changes were observed in lower brainstem or cortex. Further observations with animals allowed to withdraw from opiate dependency for 2 days showed a potentiation of the ganglioside changes in striatum. Since chronic opiate administration alters monoamine metabolism and receptor binding kinetics in rat brain, the above changes in gangliosides may be responsible, at least in part, for the manifestation of physical dependency. (Supported by DK 39328)

230.5

ISOLATION AND PARTIAL CHARACTERIZATION OF A LAMININ/COLLAGEN RECEPTOR ON ASTROCYTES.

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We have reported (Turner et al., 1989, J.Neurosci. in press) the isolation of a monoclonal antibody (Mab 3A3) that inhibits neurite outgrowth by PC-12 cells on laminin (LN) and collagen (CN) as well as nerve regeneration in vivo (Toyota et al.,1988, Soc. Neurosci. Abstr.,14:498). Mab 3A3 detaches rat astrocytes in culture from LN and CN, but not from fibronectin or polylysine-coated substrata. Mab 3A3 does not bind to its electroblotted antigen but it does immunoprecipitate proteins of 180 and 125 kDa from detergent extracts of astrocytes. Antisera that recognize the β_1 subunit of the integrin family of heterodimeric, adhesive receptors crossreact in immunoblots with the 125 kDa protein immunoprecipitated by Mab 3A3. These and other data suggest strongly that Mab 3A3 recognizes a dual LN/CN receptor ($\alpha \approx 180$ kDa;$\beta_1 \approx 125$ kDa) of the integrin family. Immunocytochemical studies indicate that Mab 3A3 binds to some regions of the astrocyte surface in a pattern distinct from other β_1 integrins. We have immunoaffinity purified the LN/CN integrin from neonatal rats and have generated α and β_1 specific antisera. These reagents together with microsequencing of the protein will be valuable in molecular cloning of this integrin alpha subunit.

230.6

AVIAN NEURAL CREST CELL ADHESION TO LAMININ: CHARACTERIZATION OF A NOVEL INTEGRIN HETERODIMER.

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Neural crest cells adhere to and migrate avidly on laminin. *In vivo*, antibodies to a laminin-heparan sulfate proteoglycan complex, to the HNK-1 epitope, or to the β_1 subunit of integrin (CSAT) perturb cranial neural crest cell migration. *In vitro*, CSAT and HNK-1 antibodies block attachment of neural crest cells to laminin, suggesting that their proper migration requires interactions with a laminin-rich matrix.

Here, we describe two distinct mechanisms of neural crest cell adhesion to laminin using the quantitative cell adhesion assay of McClay. At low laminin coating concentrations (1 ug/ml), neural crest cell adhesion is inhibited by the HNK-1 and CSAT antibodies. This adhesion is unaltered over a range of divalent cation concentrations from 1 uM to 1 mM for Ca^{++}, Mg^{++} or Mn^{++}. At high laminin coating concentrations (10 ug/ml), neural crest cell adhesion is inhibited by the CSAT antibody, but not by the HNK-1 antibody. Adhesion at this coating concentration of laminin requires 1 mM Ca^{++} or Mn^{++} for binding, and these cations cannot be substituted by Mg^{++}. Neural crest cell adhesion to laminin at all coating concentrations is unaffected by heparin and sensitive to trypsin proteolysis. The E8 fragment of laminin acts similarly to intact laminin at high and low coating concentrations. In immunoprecipitates using the CSAT antibody, the HNK-1 antibody immunoblots a 105 kD glycoprotein under non-reducing conditions, possibly representing a new integrin subunit on the surface of neural crest cells. We conclude that neural crest cells possess multiple receptors for laminin which may function independently. (Supported by USPHS HD15527).

230.7

CHARACTERISTICS OF THE BINDING OF CRANIN TO LAMININ.
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Cranin is a 120 kDa laminin-binding membrane glycoprotein, found on the cell surfaces of neural and certain non-neural cells (N.S. and N. Schwartz, PNAS 84, 6457, '87). Binding of cranin to laminin is of very high apparent affinity; requires divalent cations; is destroyed by gentle proteolysis of laminin or cranin; and is blocked by polyclonal anti-laminin antiserum, but not by an antiserum against the cross-arm (E1 fragment) of laminin.

More recently, thermolability studies were performed. When laminin was heated for 20 min to 57-62°, laminin remained soluble, and structurally and antigenically intact, but binding was sharply lost. Heating to ~60° is known to denature the coiled-coil alpha helices in the long arm. Moreover, an antiserum to the end of the long arm of laminin (E3 fragment; gift of D. Hall, UCSF) blocked binding to cranin in 14 day embryonic chick brain, although an antiserum to the denatured, reduced distal portion (domain G) of the laminin A chain had no effect. Protease-, heat-, and antiserum-sensitivity all suggest that cranin binds within the long arm, though these treatments might exert effects on some other site within laminin. The role of N-linked carbohydrates was then investigated by treating cranin with N-glycanase or endo F. This did not affect its ability to bind laminin; its apparent MW shifted by ~10 kDa, but still appeared as a broad, fuzzy band. Cranin may play a role in mediating neurite growth, since conditions which abolish the ability of laminin to bind cranin also destroy the major neurite-promoting activity of intact laminin. Supported by March of Dimes, Dysautonomia Foundation, Schweppe Foundation, and NIH HD09402, HD04583, NS26055.

230.8

ASSAY OF CELL ADHESION RECEPTOR INVOLVMENT IN NEURON-GLIA INTERACTION.
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Neuron-glia interactions are important to glial-guided neuronal migration, to the neuronal regulation of astroglial differentiation and to glial support of axon extension. To provide a rapid, specific assay for receptor systems involved in cell-cell contacts between granule neurons and astroglial cells, cerebellar granule cells, purified from early postnatal mice, were metabolically labelled with 35S-methionine, plasma membranes were prepared and the kinetics of binding of radiolabelled material to either primary glial cells or to the mouse G26-24 astrocytoma cell line (Stitt and Hatten, 1987) was measured in the presence or absence of antibodies against astrotactin, N-CAM, NILE, integrin, N-cadherin and the peptides RGD and YIGSR. Addition of astrotactin antiserum reduced granule cell membrane binding by 60% below control levels. N-CAM , NILE and N-cadherin, and integrin antisera had no effect. Inclusion of the fibronectin cell attachment peptide RGD (1 mg/ml) in the assay also did not reduce membrane binding, suggesting that integrin alone does mediate initial neuron-glial binding. Similarly addition of the laminin cell attachment peptide YIGSR (1 mg/ml) failed to block membrane binding. These results are consistent with results from *in vitro* blocking assays with a microculture system (Edmondson *et al*, J. Cell Biol. 106:505, 1988). Experiments are in progress to analyze the effects of combinations of anti- N-CAM, NILE and integrin antibodies with RGD and YIGSR peptides.

230.9

BIOSYNTHESIS OF NEURAL CELL ADHESION MOLECULE IN CHICK MYOTUBES.
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The neural cell adhesion molecule (NCAM) is expressed in skeletal muscle as several Mr forms: 120K and 150K which are attached to the external cell surface via phosphoinisitol tails and 140K which is membrane inserted. We have examined NCAM biosynthesis by S-35 methionine labelling in cultured chick myotubes. The main polypeptide initially synthesized has an Mr of 172K; lesser amounts of 146K and 134K forms are also synthesized. Their size range broadens during chase periods, indicating glycosylation, including polysialation, and processing, to yield forms of: 165-172K, 144-150K and 125-138K, which correspond to the desialated 150K, 140K and 120K forms. Secreted NCAM was detected with an Mr of 200K and is not appearently highly glycosylated. Degradation studies indicate two populations of NCAM: one with a short half life of circa 4 hours and a longer lived species. Hence, the production of different forms with different cellular localizations and half-lifes may modulate the role of NCAM in cell adhesion in vivo.

230.10

STRUCTURE AND CHARACTERIZATION OF NG-CAM AND A CLOSELY-RELATED MOLECULE.
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The neuron-glia cell adhesion molecule, Ng-CAM, is a glycoprotein found only on neurons and Schwann cells which binds specifically to the surfaces of neurons and astroglial cells. The molecule is comprised of components of Mr 200,000 and 135,000 which have identical amino acid sequences for at least the first 20 residues and an Mr 80,000 which is immunologically related to the Mr 200,000 but not to the Mr 135,000. To study the structure of Ng-CAM in detail, we searched for cDNA clones in libraries prepared from chick embryo brain mRNA. A 105 bp cDNA probe for Ng-CAM was synthesized in a polymerase chain reaction using as primers oligonucleotides based on amino acid sequences within the Mr 135,000 polypeptide. This probe recognized a cDNA clone with a 1.2 kb insert which in Northern blots of embryonic chick brain hybridized to at least one mRNA species $\geq$6 kb which is long enough to code for the Mr 200,000 polypeptide. Sequence analysis of this and related clones confirmed that they correspond to Ng-CAM by extensive matches to amino acid sequences of fragments within the Mr 135,000 polypeptide. Moreover, several repeated domains were identified which are homologous to C2 domains of immunoglobulins indicating that, like N-CAM and other CAMs, Ng-CAM is a member of the immunoglobulin superfamily. We have also identified cDNA clones which are homologous to but distinct from Ng-CAM and other CAMs suggesting that they may represent a new molecule, possibly a CAM. Supported by NS21629 & NS22789.

230.11

ADHESION MOLECULE AMOG IS HOMOLOGOUS TO A Na,K-ATPase BETA SUBUNIT.
K.J. Sweadner, H. Antonicek*, and M. Schachner. Mass. General Hospital, Boston, MA 02114, and U. Heidelberg, Heidelberg, FRG.

AMOG (adhesion molecule on glia) was implicated in neuron-astrocyte adhesion by the blocking effects of a specific monoclonal antibody (Antonicek et al., 1987. J. Cell Biol. 104, 1587-1595); purified AMOG in liposomes binds to subclasses of neurons (Antonicek & Schachner, 1988. J. Neurosci. 8, 2961-2966). Immunoaffinity-purified AMOG antigen has subunits resembling the α (catalytic) and β (glycoprotein) subunits of the Na,K-ATPase, but the smaller subunit alone is sufficient for adhesion. A cDNA coding for AMOG was cloned (Pagliusi et al., 1988. J. Neurosci. Res. 22, 113-120), and subsequently sequenced (Gloor et al., submitted). It is the same as an independently-cloned protein (tentatively named β2) that is 35% identical to the β subunit of the Na,K-ATPase (Martin-Vasallo et al. 1989. J. Biol. Chem. 264, 4613-4618). Here we show that AMOG/β2 specifically associates with certain Na,K-ATPase α subunit isoforms.

Purified Na,K-ATPase from brain and immunoaffinity-purified AMOG have subunits with indistinguishable electrophoretic mobilities in SDS. Polyclonal antisera raised against AMOG reacted with both subunits of Na,K-ATPases purified from brain and kidney, and antisera raised against purified Na,K-ATPase reacted with immunoaffinity-purified AMOG subunits. Epitope differences were revealed, however, by the relative intensity of the cross-reactivity. Monoclonal antibodies raised against the α subunit of AMOG reacted preferentially with the α2 and α3 isoforms of Na,K-ATPase, while isoform-specific Na,K-ATPase monoclonal antibodies detected α2 and a small amount of α3 in purified AMOG. AMOG/β2 distribution in the brain, like that of the α isoforms, is widespread but heterogeneous at the cellular level. Adhesion is a new role postulated for the β subunit of the Na,K-ATPase, which is well-known to face the cell exterior.

230.12

HIGH AND LOW AFFINITY OUABAIN-BINDING SITES OF NA,K-ATPASE IN RAT BRAIN DEMONSTRATED BY QUANTITATIVE AUTORADIOGRAPHY.
A.A. Maki*, P.E. Filuk*, D.G. Baskin and W.L. Stahl (Spon: D. Farrell). V.A. Medical Center and Univ. Washington Sch. Med., Seattle, WA 98108.

Quantitation of the number of ouabain binding sites in identified regions of brain at high anatomical resolution is desirable in order to understand long-term regulation of the Na,K-pump and its modification in disease states. The cellular distribution of high and low affinity ouabain-binding sites in brain is largely unknown. Sites with high affinity for ouabain can be demonstrated in tissue sections by quantitative autoradiography (QAR)(Antonelli et al., J. Neurochem. 52:193-200, 1989). However, it was unclear if low affinity sites could be identified. In order to answer this question equilibrium binding studies were carried out with 1-5000 nM [^{3}H]ouabain. Binding kinetics were studied at 50 nM and 1500 nM. Specific low affinity sites were found in rat somatosensory cortex(SSC) and both kinetic and equilibrium studies yielded similar K_d values (589-728 nM). Binding at these sites was blocked by K^+ (IC_{50}=4 mM). Specific high affinity sites in lamina 3-4 of SSC also had similar K_d values (8-17 nM). These high affinity binding sites were blocked by erythrosine B (IC_{50}=10 μM) and K^+(IC_{50}=1.4 mM). Therefore, the QAR method permits discrimination and quantitation of both high and low affinity [^{3}H]ouabain-binding sites in nervous tissue.

230.13

PRODUCTION AND CHARACTERIZATION OF POLYCLONAL ANTIBODIES TO NA,K-ATPASE. J.H. Peng and J.C. Parker, Jr.* Dept. of Pathology, Univ. of Missouri-Kansas City Sch. of Med., Truman Med. Ctr., Kansas City, MO 64108.

We have recently reported the purification of Na,K-ATPases from human and rat brainstem tissues. In order to characterize Na,K-ATPase immunochemically, we have further prepared polyclonal antisera to these antigens. Each rabbit was immunized bi-weekly with 100 ug of the highly purified preparations initially emulsified with an equal volume of Freund's complete adjuvant, and subsequently boosted with antigen emulsified with incomplete adjuvant by subcutaneous injection at many sites of the neck and scapular regions. Ten days after the last immunization, antisera were obtained through cutting of the marginal ear vein. Antisera were checked for their immunoreactivities by Western blotting using peroxidase-anti-peroxidase method and chloronaphthol as a chromogen. All sera showed strong immunoreactivity with titers of 8,000 for anti-rat, and 4,000 for anti-human Na,K-ATPase sera. All sera cross-reacted well with alpha 2 bands, but less with alpha 1 bands. Anti-human sera cross-reacted strongly with human enzyme, but weakly with rat enzyme, and vice versa. The patterns of immunoreactive fragments were found to be different, when blots of the protease-digested peptide fragments of the alpha 1 and alpha subunits were stained with these specific antisera. These antisera will be a valuable reagents for immunocytochemical localization.

230.14

CHARACTERIZATION OF A Na, K - DEPENDENT ADENOSINE TRIPHOSPHATASE FROM A PLANARIAN. L. G. Hammerland* and J. F. Ash*. (SPON: V. Yip). Dept of Anatomy, Univ. of Utah Sch. of Med., Salt Lake City, UT 84132.

Although the Na,K-ATPase is assumed to be a ubiquitous and vital component of animal cell membranes, biochemical and molecular analyses have been focused on a few examples among vertebrates and crustacea. A wider evolutionary study of this enzyme might help elucidate important features, such as structure/activity relationships, the role of the inhibitory ouabain binding site and the significance of different isoforms seen, for instance, in neurons versus glia. We have surveyed a number of primitive invertebrates for ouabain toxicity and found that ouabain killed freshwater planaria, but not hydra.

In this study we have obtained a subcellular membrane fraction from the brown planaria, *Dugesia dorotocephala*, and initiated a characterization of associated ATPase(s). A Na^+ and K^+ stimulated, ouabain inhibited activity is the major ATPase in this fraction. The apparent K_ms for Na^+ (7.7 mM) and K^+ (1.2 mM) are similar to those vertebrate enzymes. The apparent K_i for ouabain is <10 μm. As seen with purified vertebrate Na,K-ATPase, the planaria enzyme appears to have a high affinity FITC binding site which has allowed us to estimate the MW of the catalytic α subunit (110-115 kD) by electrophoretic mobility. This is significantly larger than the value obtained for a vertebrate α subunit. (Supported by NSF Grant DCB 8517961)

230.15

FOLATE TRANSPORT BY BRAIN SLICES AND CNS CELL LINES. S.R. Snodgrass, Z.H. Zhang and M.H. Morita* (SPON:M.C. Citron). Neurology Research Lab., Childrens Hospital Los Angeles and Dept. Neurol., Univ. So. Calif. Schl. of Med., Los Angeles, CA 90027.

Distribution and uptake of folates within the CNS may explain their selective neurotoxicity. The only published study of brain folate transport reported that uptake of 5-methytetrahydrofolate (MTHF) was not found (J. Neurochem. 29:121,1977). We studied the uptake of 3H folic acid (FA) and 3H MTHF by rat brain slices, cultured mouse astrocyte PC12 cells, and C6 rat glioma cells. All showed saturable uptake of both FA and MTHF. Transport of each is inhibited by the other and by methotrexate. Transport of each was inhibited by pterin, reported to have no effect on peripheral folate uptake. Differences between preparations included absence of high affinity MTHF uptake in C6 cells, stimulation of MTHF uptake in NGF treated PC12 cells, and stimulation of FA but not MTHF uptake in PC12 cells by growth in folate-depleted media. Our data suggest but do not prove that at least two different folate uptake systems exist in the CNS. Autoradiographic studies of uptake in brain slices are an important part of this analysis and will be shown. CNS and peripheral folate transport differs.

PROCESS OUTGROWTH, GROWTH CONES AND GUIDANCE MECHANISMS II

231.1

DYNAMICS OF SENSORY NEURON DEVELOPMENT IN *DROSOPHILA MELANOGASTER*. K.E. Whitlock* and J. Palka. Dept. of Zoology, University of Washington, Seattle, WA 98105.

The arrival time of sensory axons in the central nervous system and their behavior prior to the establishment of the definitive adult projection pattern has not previously been described in *D. melanogaster*. We have studied sensory neurons arising in the wing by staining them in fixed tissue with the fluorescent dye 1,1'-dioctadecyl-3,3,3',3 -tetramethylindo-carbocyanine perchlorate (di I), (Godement, P., et al., Development 101, 1987). We find sensory axons in the CNS as early as 9-12 hours after pupariation (25°C), and all components of the adult pattern are present by 36 hours. The sequence of axon arrival in the CNS parallels the development of their corresponding sensory populations in the periphery. Growth cones, numerous in the entering nerve bundle, become concentrated at the leading edge of growing axon projections. Solitary, apparently exploratory fibers with prominent growth cones are often seen but are usually confined to the area that will contain the final adult wing sensory projections. The developing sensory tracts have a fuzzy appearance due to the presence of abundant short branches, possibly reflecting growth cone exploration. With the passage of time these are lost and the mature tracts look smooth except in areas of terminal axon arborization, suggesting the opperation of a selective pruning process during the early development of the sensory projections.

231.2

PUTATIVE MUSCLE PIONEERS IN THE DEVELOPMENT OF THE *DROSOPHILA* EMBRYONIC MUSCULATURE. M.E. Halpern, J. Johansen and H. Keshishian. Biology Dept., Yale Univ., New Haven, CT.

In *Drosophila* embryos the bodywall muscle fibers are arranged in the final segmental pattern by 13 hours post-fertilization (stage 16), and are contractile by 16 hours. Previously we discovered that precise features of motoneuronal outgrowth and growth cone exploration lead to highly stereotypic muscle fiber-specific axonal projections. We now describe the development of the target muscle fibers in relation to the timing of neurite outgrowth and neuromuscular innervation, from studies at the light microscope level and from Lucifer yellow dye-filling of presumptive precursor cells in staged embryonic filets. At 9-10 hours (stage 13), when the first axons exit the CNS, the mesodermal somatopleura consists of segmented clusters of unfused spheroidal cells. However, intracellular dye-fills reveal larger, segmentally repeated cells that share several properties with grasshopper muscle pioneers, including a bipolar shape, extended growth cone-like processes at each end, and an early appearance among the developing myoblasts. Within an hour (stage 14), mesodermal cells aggregate and fuse to form muscle fibers. Lucifer yellow fills at this stage indicate transient dye-coupling between developing longitudinal muscle fibers. By stage 15, coupling is lost and each muscle fiber is an independent syncytial cell. The onset of phalloidin staining at late stage 15 indicates the presence of filamentous actin in the muscle sarcomeres. Shortly afterwards (stage 16), as a result of the coordinated program of axonal outgrowth, the first neuromuscular contacts are made synchronously throughout the bodywall, and the neurotransmitter glutamate is detectable in motoneuronal endings. Time lapse video microscopy and the use of muscle-specific markers will enable us to examine further the role of the *Drosophila* putative muscle pioneers in the development of the musculature. These observations indicate that the mechanisms underlying the organization of embryonic muscle patterns are broadly conserved among insects.

231.3

GROWTH CONES OF MONOPOLAR AND MULTIPOLAR NEURONS IN *DROSOPHILA* LARVAL CNS CULTURE DIFFER IN MORPHOLOGY AND MOTILITY Yun-Taik Kim* & Chun-Fang Wu (Spon: M. Burg). Dept. of Biology, University of Iowa, Iowa City, IA 52242.

Growth cones play a central role in axonal guidance and branching and establishing synaptic connections. Thus, morphological differences between neuronal types might in part result from intrinsic differences among their growth cones. We examined the growth cones of monopolar and multipolar neurons in dissociated larval *Drosophila* CNS cultures (Wu *et al.*, *J. Neurosci.* 3:1888, 1983) with phase-contrast optics. Analysis of contrast-enhanced video images indicated that growth cone morphology can be well characterized by several morphometric parameters including the number and length of filopodia, and area, roundness, convexity and concavity of lamellipodium boundary. In addition, the growth cone dynamics is best described by the motility index and boundary flow plots.

We found that the overall composition of nerve cell types was strikingly different among cultures prepared from different CNS regions. Monopolar neurons were the major cell type in brain cultures while multipolar neurons were predominant in ventral ganglion cultures. Moreover, the growth cones of monopolar and multipolar neurons show intrinsic differences in their morphology and motility. Compared to multipolar neurons, growth cones in monopolar neurons have larger lamellipodia of less erratic shape accompanied with fewer and shorter filopodia, which are correlated with higher motility and less directionality in motion. These morphological and behavioral distinctions may result from differences in adhesion and transport properties that may reflect intrinsic differences in axonal and dendritic growth cones.

Supported by NIH grants NS 26528 and HD 18577.

231.4

PROJECTION OF MOTOR AXONS IN HYPEREXCITABLE *DROSOPHILA* MUTANTS. V. Budnik, Y. Zhong*, and C.-F. Wu. Dept. of Biology, University of Iowa, Iowa City, IA 52242.

There is considerable evidence in vertebrates that nerve connectivity can be influenced by electrical activity. The body wall muscles of *Drosophila* larvae provide a promising invertebrate preparation in which to study the role of excitability in the development and maintenance of muscle innervation. The ventral longitudinal muscles have been the focus of extensive electrophysiological investigation. In addition, mutations are available which either eliminate nerve activity, or increase excitability and enhance neuromuscular transmission in these muscles.

We have initiated an anatomical study of neuromuscular junctions in mutants which affect electrical activity by analyzing mutants which induce hyperexcitability. To visualize nerve terminals we have used anti-HRP immunocytochemistry.

Wild-type ventral longitudinal muscles are innervated in a relatively stereotypic fashion. In muscles 12 and 13, for example, a projection from the segmental nerve branches off over the muscles giving rise to sparsely branched neurites which run approximately parallel to the muscle fiber. We found that in *ether a go-go* (*eag*) mutants, and more dramatically in *eag Shaker* double mutants, this basic pattern of innervation is altered. The alterations can be described as substantial ramification of neurites throughout the muscle fiber, an increase in the density of varicosities, and, as a result, an increase in the total number of varicosities. Preliminary results indicate that these alterations can be reversed by introducing a mutation which reduces nerve excitability in the double mutant background. Supported by NIH grant NS 26528.

231.5

NAVIGATION DECISIONS BY PIONEER NEURON GROWTH CONES *IN VIVO*. **J.S.Duerr, T.P.O'Connor, and D. Bentley, Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720.**

In grasshopper limb buds, the Til pioneer neurons establish a stereotyped pathway from the periphery to the central nervous system. DiI was used to label living Til neurons in a semi-intact preparation. The behavior and morphology of extending growth cones and filopodia were observed for several hours with computer-enhanced fluorescent video microscopy. Growth cone morphologies and filopodial arrays differed in different regions of the limb, correlating well with morphologies observed in fixed tissue. Growth cone behavior during two steering events was observed. The normal pathway contains turns at two presumptive navigation cues, the trochanter/coxa segment border and the Cx1 guidepost neurons. Growth cone behavior differs at these two decision points. At the segment border, the growth cones usually send branches in opposite directions along the presumptive border; the 'incorrect' branch is eventually withdrawn. At the decision point for the turn to the Cx1 neurons, the first major branch to leave the trochanter/coxa border usually forms in the correct direction. Examination of living pioneer neurons reveals different behaviors by growth cones and filopodia in regions hypothesized to differ in the type of guidance cues which they provide.

231.6

DEVELOPMENTAL EXPRESSION AND PURIFICATION OF A NERVOUS SYSTEM SPECIFIC ANTIGEN FROM GRASSHOPPER. Rolf O. Karlstrom* and Michael J. Bastiani (Spon: J. Pollock), Dept. of Biology, Univ. of Utah, Salt Lake City, UT 84112.

Surface molecules specific to the nervous system during embryogenesis may play a role in the cellular interactions necessary for nervous system development. The 1C10 MAb recognizes an antigen specific to the nervous system in grasshopper embryos. The antigen was determined to be on cell surfaces by labelling live embryos.

The 1C10 antigen is first expressed on presumptive ectodermal cells and at the gastrulating midline early in development (10-20%). Later, neural ectoderm cells, some neuroblasts, and developing sensory structures in the legs and body wall express the 1C10 antigen. During the period of rapid neuron generation from 35-50% of development, neuroblasts and their families of ganglion mother cells and neurons express high levels of the antigen. Axons express the antigen from the time of early axon formation (30-33% of development) to approximately 60% of development, when axonal expression is no longer seen. This window of axonal expression coincides with the developmental period when complex axonal pathfinding establishes the embryonic nervous system. Antigen expression is seen on nerve cell bodies and a subset of glial cells into the adult.

The 1C10 MAb immunoprecipitates a 38 kD doublet from embryonic membranes. Using a MAb affinity column microgram quantities of the antigen have been purified. Sequencing attempts show the amino terminal is blocked. We are continuing to purify the 1C10 antigen in order to generate polyclonal serum and for the generation of peptide fragments for sequencing.

(Supported by NIH grant NS25387, McKnight Scholars Award, and NSF Graduate Fellowship to R.O.K.)

231.7

RESTRICTED SPATIAL AND TEMPORAL EXPRESSION OF A NERVOUS SYSTEM SPECIFIC ANTIEN DURING DEVELOPMENT OF THE GRASSHOPPER. E.C.Seaver*, R.O.Karlstrom*,andM.J.Bastiani(SPON: D. Yoshikami). Dept. of Biology, Univ. of Utah, SLC, UT 84112.

In an attempt to identify molecules important for pathfinding of growing axons, monoclonal antibodies(MAb) have been generated against embryonic grasshopper tissue. One MAb, 2B2, specifically recognizes developing neurons in the grasshopper. The expression pattern of the antigen(s) recognized by the 2B2 MAb was examined over the entire course of embryonic development. Initial labeling occurs at 30% of embryonic development when the first growth cones are extended. During early neurogenesis the MAb appears to recognize all axons in the CNS. In contrast to fasciclin I, II, and III, the antigen(s) recognized by the Mab is not seen outside the nervous system. As embryonic development proceeds (70%) the labeling pattern becomes restricted to a subset of axons within each ganglion in a segmentally repeated pattern. This pattern continues throughout the remaining period of embryonic development. Preliminary evidence suggests that the 2B2 MAb does not label adult grasshopper ganglia.

Immunoprecipitation of the antigen(s) recognized by the 2B2 MAb from grasshopper embryonic tissue yields a band of 160kD and possibly of 38kD when analyzed by SDS PAGE. Labeling of live embryos and electron microscopic studies indicate that the antigen recognized by the 2B2 MAb is a cell surface molecule.

The restricted spatial and temporal pattern of expression of the antigen(s) recognized by this MAb during embryogenesis suggests a specific role in the development of the nervous system.(Supported by the McKnight Foundation and NIH grant #NS25387.)

231.8

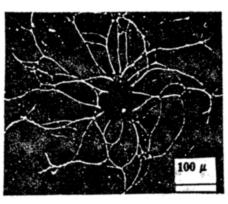
A SIMPLE METHOD FOR GROWING AND MAINTAINING DISSOCIATED *HERMISSENDA* CNS NEURONS IN CULTURE. S. Sombati, R. Forman, R. Lee* and R. DeLorenzo, Dept. Neurology, Medical College of Virginia - VCU, Richmond, Va. 23298.

The mollusc *Hermissenda* is a popular experimental preparation for the study of cellular physiology and neuronal plasticity. Analysis of some biophysical, pharmacological and biochemical properties of CNS neurons, including synaptogenesis, have until now been limited by the unavailability of neurons in culture. We report here a simple method for culturing *Hermissenda* neurons.

The brain of *Hermissenda* was treated with protease (Sigma type XXIV, 2mg/ml) for 40 min at 22°C. After wash, neurons of interest, including the identified neuron LP1, were dissociated in a 35 mm culture dish containing 8-10 drops of the culture medium (isotonic L-15 solution plus 10% FCS) on a poly-lysine coated glass cover slip (PCGS). The cultures were maintained at 15-18°C and fed twice weekly.

Three factors seemed to be critical to obtaining successful cultures: 1) the degree of enzymatic treatment; 2) the temperature at which the cells are maintained during culture; and 3) use of PCGS as the substrate for cell attachment.

Most large cells (soma >50μ) were multipolar. These neurons had numerous, long (1 mm at day 3) and highly branched processes emerging from the soma (see figure). Some smaller neurons exhibited either monopolar or bipolar morphology. Often, many neurons appeared to have their processes intermingled.



Cultured neurons could be maintained up to 3 weeks and are suitable for physiological, pharmacological, biophysical and biochemical investigations, some of which are currently being undertaken in our laboratory.

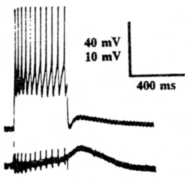
231.9

MEMBRANE PROPERTIES AND CONNECTIVITY OF *HERMISSENDA* NEURONS IN CULTURE. R. Forman, S. Sombati, R. Lee*, and R. DeLorenzo, Dept. Neurology, Medical College of Virginia - VCU, Richmond, VA. 23298.

Individual neurons were isolated from the CNS of the nudibranch mollusc *Hermissenda* and maintained in culture for periods of up to 21 days. Within 24h following dissociation, these cells initiated neurite outgrowth, typically from multiple sites on the soma. Cells with no closely neighboring cells often showed symmetrical patterns of neurite outgrowth, while cells plated at higher density showed selective orientation of their processes with respect to one another.

Conventional electrophysiological recording techniques applied to 30 neurons ranging in age from 1 to 7 days revealed resting potentials between -40 and -70 mV, overshooting action potentials in response to depolarizing current stimuli, varying degrees of spike frequency adaptation during long depolarizations and prolonged afterhyperpolarizations following spike trains. All these observations were within the range of similar phenomena recorded from *Hermissenda* neurons *in situ*.

Pairwise recording from cells with intermingled processes showed evidence for both rectifying and non-rectifying electrical coupling and for chemically mediated transmission (see figure). A cultured identified neuron (LP1) formed rectifying electrical junctions with neighboring cells. Evidence for chemical transmission included synaptic delay and changes in the amplitude of post-synaptic potentials as a function of external [Ca^{2+}] and of postsynaptic membrane potential.



231.10

GROWTH CONES OF APLYSIA NEURON L10 EXHIBIT TARGET-SPECIFIC BEHAVIORS. D. Hawver and S. Schacher, Dept. Pharmacology & Ctr. Neurobiol. & Behav., Columbia CPS & NYS Psych. inst., New York, NY 10032.

We have previously shown that selective synapse formation in vitro by neuron L10 of Aplysia is associated with specific patterns of neurite outgrowth (Hawver and Schacher, Soc. Neorosci. Abstr. 14:749, 1988). L10 neurites adhere to and extend along appropriate targets (RB neurons), while crossing over or turning away from inappropriate targets (RUQ neurons). To explore the mechanisms responsible for the generation of these patterns, we have used phase and fluorescence microscopy to examine the early interactions of L10 growth cones with both RB and RUQ growth cones and neurites. While the majority of L10 interactions with either RB or RUQ resulted in L10 crossing over the target (RB:58% n=19, RUQ:45% n=22), a large fraction of L10-RB contacts resulted in adhesion and overlapping of L10 along the target neurite (37%), compared to only 9% of L10-RUQ contacts. In contrast, L10-RUQ interactions were often characterized by either retraction of contacts (23%) or by edge-skirting behavior(23%) -- turning to follow along the edge of the target by repeatedly establishing and retracting contacts without overlapping. Only 5% of L10-RB interactions resulted in retraction of contacts, and none exhibited edge-skirting behavior. When a single L10 cell was cultured with both RB and RUQ cells, multiple types of interactions were observed. This suggests that the growth cone behaviors which lead to the formation of target-specific patterns are mediated by signals acting locally, at the level of individual growth cones, rather than cell-wide.

231.11

REQUIREMENT OF CALMODULIN FOR CALCIUM MEDIATED CHANGES IN GROWTH CONE MOTILITY. K.A. Polak*, A.M. Edelman*, M.H. Xia* and C.S. Cohan (SPON: D.M. Higgins). Dept. of Pharm. & Ther., Dept. of Anat. Sci., SUNY Buffalo, Buffalo NY 14214.

In neuron B19, isolated from the buccal ganglion of *Helisoma trivolvis*, it has been demonstrated that changes of intracellular calcium levels influence growth cone motility and neurite elongation. Neurotransmitters such as serotonin (5-HT), which increase intracellular calcium levels presumably through receptor mediated activation of membrane calcium channels, suppress neurite elongation. The mechanism(s) by which changes in intracellular calcium regulate neurite elongation have not yet been characterized. As some of the effects of calcium in a variety of systems are mediated by calmodulin, we tested the possibility of such an interaction in the regulation of neurite outgrowth. In these experiments a new calmodulin antagonist with increased selectivity, CGS9343B (CGS), was used to inhibit calmodulin activity during the application of serotonin to B19. Subsequent effects on growth cone movements and neurite elongation were then analyzed.

Identified neurons B19 were isolated and cultured in conditioned medium. Rates of growth cone advance were measured over a 120 minute period of which the first 45 minutes served as a control period. In addition, total cell outgrowth was determined after approximately 48 hours in culture.

The addition of 100μM 5-HT to the culture medium resulted in a significant decrease in rate of growth cone advance ($p<.001$), as reported previously. However, administration of 1.8μM CGS to the culture medium prior to 5-HT blocked inhibitory effects of 100μM 5-HT, both on rate of growth cone advance and total outgrowth. CGS alone had no significant effect on either parameter. Preliminary experiments indicate that CGS is not acting at membrane calcium channels as a competetive antagonist. These observations suggest that calmodulin may play a regulatory role in mediating the effects of calcium on growth cone movements.

(Supported by NIH grants NS24738 and NS25789)

231.12

ELECTRICAL STIMULATION DIFFERENTIALLY EFFECTS GROWTH CONES OF DIFFERENT IDENTIFIED NEURONS. C.S. Cohan and M.H. Xia*. Dept. of Anatomical Sciences, SUNY Buffalo, Buffalo, New York 14214.

A variety of signals have recently been shown to alter neurite outgrowth by influencing growth cone movements. One signal, action potentials, when generated in the cell bodies of *Helisoma* neurons, reversibly inhibits growth cone motility. The quantitative features of electrical activity may play an important role in determining neuronal growth responses. In the present experiments, changes in stimulation frequency were used to assess 1) how the parameters of electrical activity influence growth cone movements and 2) whether different identified neurons respond differently to the effects of electrical activity.

Identified *Helisoma* neurons B4 and B19 were isolated from buccal ganglia and cultured in conditioned medium. Twelve hr after plating, neurons were stimulated by an intracellular microelectrode within the cell body while growth cone movements were measured. During experiments neurons were observed for a 45 min control period, followed by a 45 min stimulation period, followed by a 45 min recovery period.

Growth cones of neurons B4 and B19 exhibited a decrease in growth rate at a threshold frequency of 2/sec. Higher stimulation frequencies caused greater inhibition of growth rates indicating that stimulus frequency had a graded effect on growth cone movements. However, B4 was significantly more sensitive to stimulation than B19. For example, at 2/sec B4 growth rates were inhibited by 58% whereas B19 growth rates were inhibited only by 29%. Moreover, B4 displayed a unique effect; growth rates *increased* during the recovery period for stimulation frequencies of 1 and 2 per sec. These data indicate that growth cones of different *Helisoma* neurons respond differentially to electrical activity.

(Supported by NIH grant NS25789)

231.13

CADMIUM MIMICS THE EFFECTS OF AXOTOMY ON THE ACTION POTENTIAL IN AN IDENTIFIED NEURON OF HELISOMA. P.J. Kruk and A.G.M. Bulloch. Department of Medical Physiology, University of Calgary, Calgary, Alberta, Canada, T2N 4N1.

After axotomy, the action potential (AP) of buccal neuron 4 (B4) of the freshwater snail Helisoma shows a loss of pseudoplateau on the declining phase. In principle, this could be due to the axotomy-induced elevation of intracellular calcium, which can block calcium inward current and/or activate calcium dependent potassium outward current. This study was designed to determine which of these two currents, if either, is actually affected by axotomy.

The AP of B4 was recorded in an acute preparation that did not alter morphology of the neuron. The AP waveform recorded both from (1) B4 in normal saline two days after in situ axotomy, and (2) from intact B4 in saline containing 0.1 mM cadmium sulfate, exhibited identical changes (loss of pseudoplateau) when compared to the normal waveform. Neither resting potential nor input resistance of B4 were significantly affected by axotomy or cadmium.

These results suggest that the axotomy-induced changes in the AP waveform result from a modulation of calcium conductance rather than from a modulation of calcium-dependent potassium conductance.

Supported by Alberta Heritage Foundation for Medical Research, and by Medical Research Council (Canada).

231.14

LAMININ IMMUNOREACTIVITY IN THE CNS OF THE SNAIL, *HELISOMA TRIVOLVIS*. J. D. Miller* and R. D. Hadley. Dept. Anat. & Cell Biol., Med. Univ. S. C., Charleston, S.C. 29425.

The extracellular matrix (ECM) is involved in many morphogenetic processes including neuroblast migration and synaptogenesis. In this study, evidence is presented supporting the hypothesis that a **laminin-like** molecule is present in the ECM of *Helisoma* brain tissue. We suggest that this molecule may function as a neurite outgrowth promoting factor (NOPF) in *Helisoma* conditioned media (CM).

Immunofluorescence of snail brain utilizing an antibody directed against mouse EHS tumor laminin (EHS-LAM) revealed a basement membrane and intensely labeled cells within the ganglionic sheath. Identical (though less intense) fluorescence was obtained with an antibody directed against the B chains of EHS-LAM, whereas an antibody directed against the A chain showed no specific reactivity.

On Western blots of Triton-extracted brain tissue, antibodies directed against whole EHS-LAM recognized multiple proteins of ~400kD under reducing conditions and one protein of ~120kD under both reducing and non-reducing conditions. The same antibody showed no reactivity on blots of CM. On blots of both reduced brain tissue and reduced CM, antibodies directed against EHS-LAM B chains showed specific recognition of a protein between ~500-700kD and multiple proteins between ~200-450kD. This antibody showed no specific reactivity on blots of gels run under non-reducing conditions.

Triton-extracted brain tissue (but not EHS-LAM) supported the outgrowth of *Helisoma* neuron B5 in culture. We are presently investigating the possibility that the laminin-immunoreactive proteins described above are related to and/or function as the NOPF in *Helisoma* CM.

This work was supported by a Fellowship from the Klingenstein Foundation to RDH. We thank Dr. Roy Ogle for his generous gift of antibodies.

231.15

ACTIVITY DEPENDENT LOCALIZED CALCIUM ENTRY IN ISOLATED LEECH NEURONS IN CULTURE. N. Lasser-Ross*, S.R. Young and W.N. Ross. (SPON: F. Horvath) Dept. of Physiology, N.Y. Medical College, Valhalla, NY 10595.

Individual leech neurons, including their axon stumps, can be removed from segmental ganglia and will sprout new processes in culture (Dietzel et al. (1986), J. Physiol. 372, 191-205). Selected cells were grown on Con A and injected with fura-2 free acid. A sequence of high spatial resolution fluorescence images (excitation at 380 nm) was acquired with a CCD camera while the cell was intrasomatically stimulated to fire single action potentials. The location and magnitude of calcium entry were determined from the normalized change in fluorescence intensity following the action potential. In Retzius cells low level changes were detected in most of the soma. A larger signal was detected in the axon stump with the highest levels found where the processes sprouted from the tip. Calcium entry into new processes was detectable but at a much lower level than into the rest of the cell. In cells without an obvious stump the highest level of calcium entry was found where the new processes sprouted from the soma. Since these cells have been shown to be isopotential (Ross et al. (1987) J. Neurosci. 7, 3877-3887) the location of calcium entry also indicates the location of voltage dependent calcium channels.

Supported by the NIH and the Whitaker Foundation.

231.16

GROWTH-INHIBITING INTERACTIONS BETWEEN GROWTH CONES OF SEGMENTAL HOMOLOGUES IN THE LEECH CNS. W.-Q. Gao and E.R. Macagno. Dept. of Biological Sciences, Columbia University, New York, NY 10027.

In vitro studies have documented inhibitory interactions that result in growth cones stopping or slowing their growth. We have been examining this phenomenon *in vivo* in the leech CNS, where we have found that several neurons extend transient longitudinal projections (*J. Neurobiol.*, 18:295, 1987). Neighboring AP neurons, for example, extend oppositely-directed projections along the same paths in the interganglionic connectives. Their growth cones meet one other at about halfway between the segmental ganglia and grow past each other only a short distance before they stop. They remain near each other for a few days before the projections disappear altogether. Ablation of one of the homologues before the projections begin to atrophy, however, induces the remaining stopped growth cone to extend again (see above reference). When we examined the growing tips of these projections in their initial stages in the light microscope at high magnification, we observed typical growth cone features, including enlarged flattened regions as well as numerous filopodia, suggesting that they were growing vigorously. Soon after meeting each other, however, these growth cones became smaller and increasingly simpler, with fewer filopodia. Examination of restarted growth cones following ablation of the homologue shows that they do not reestablish their initial morphologies (i.e., large size and many filopodia), though they grow rapidly towards the adjacent ganglia and on to the periphery. Detailed study of the stopped axons filled with different dyes suggests, within the limit of resolution of the light microscope, that they are in direct contact at several locations, although no indication of dye-coupling was found. We are currently examining these putative contacts at the ultrastructural level.

231.17

IN THE LEECH NERVOUS SYSTEM, 130 AND 260 KD GLYCOPROTEINS UNIQUE TO SENSORY, GLIAL, MUSCLE AND CONNECTIVE TISSUE CELLS ARE DISTINGUISHED BY THEIR CARBOHYDRATE MOIETIES. R.N. Cole, I.S. Thorey, M.L. Bajt, and B. Zipser. Neuroscience Program, Dept. of Physiology, Michigan State Univ., East Lansing, MI 48824.

Four different 130 kD surface glycoproteins are found on the full set and nested subsets of sensory afferents (Peinado et al., Brain Res., 410:335, 1987). A fifth 130 kD surface glycoprotein is expressed on both peripheral and central glial cells. In addition, connective tissue and muscle cells in the nervous system are identified by still other 130 and 260 kD surface proteins. We are obtaining evidence that the cell-type specificity of these glycoproteins is due to their different carbohydrate domains. The carbohydrate domains may be recognition sites during the formation of the nervous system as suggested by antibody perturbation data (Zipser et al., Neurosci. Abst. 1989).

The neuronal and glial cell surface glycoproteins are of the high mannose/hybrid type with a fucosylated invariant core since they bind to Con A and to lentil lectin. The full set sensory protein is recognized by mab Lan3-2 binding to a specific mannose epitope, that can be cleaved by N-glycanase. Coprecipitation of the full set and the 3 subset sensory proteins with mab Lan3-2 suggests that the sensory proteins possess closely related carbohydrate epitopes. Sensory and glial proteins are not coprecipitated by glial or sensory mabs, demonstrating that the sensory glycans are not shared by glia.

On immunoblots, the 130 kD connective and 260 kD muscle surface proteins run as broad bands suggestive of glycosylated proteins. These connective tissue and muscle proteins do not bind to Con A or lentil lectin, and therefore, are not of the high mannose/hybrid type.

231.18

ROLE OF 130 KD SENSORY PROTEINS IN AXONAL PATHFINDING BY FUNCTION IN THE LEECH GERMINAL PLATE. B. Zipser, R. J. Morell, and M. L. Bajt. Dept. of Physiology, Neuroscience, Program. Michigan State University, East Lansing, MI 48824.

Sensory afferents are "color-coded" through unique 130 kD surface glycoproteins of the high mannose type. A given sensory afferent is labeled with the main sensory protein (Lan3-2) and at least one of the subsensory proteins (Laz2-369, Laz6-212 and Laz7-79). The peripheral cell bodies of sensory afferents project their axons as common tightly fasciculated tracts through the body wall into the central ganglia. The entry of the central neuropile is the decision point where afferents separate from their joint tract and choose either dorsal or ventral tracts. The majority of afferents with subsensory proteins Laz6-212/Laz7-79 prefer the dorsal tracts (Peinado et al., Brain Res., 410: 335, 1987).

We tested the role of the main sensory protein on sensory afferent growth by organ-culturing the germinal plate in Fab fragments of mab Lan3-2. The 9-10 day (20C) germinal plate is fully antibody permeable. Control embryos, grown in Fab fragments of a mab directed against an internal sensory protein (Lan3-6), exhibited the normal pattern of sensory afferent tracking in the central ganglion. In experimental embryos, the central tracking of sensory afferents was dramatically modified.

Thus, sensory afferent growth is under the control of the main sensory protein. The main sensory protein appears to function in axonal self-self recognition and in axon-matrix interactions while the subsensory proteins may function in modality-specific recognition.

231.19

ROLE OF EMBRYONIC GLIA IN DEVELOPMENT OF THE LEECH NERVOUS SYSTEM. R.J. Morell, R.N. Cole, and B. Zipser. Dept. of Zoology, Neuroscience Program, MSU, E. Lansing, MI 48824.

We are studying the role of embryonic glial processes in the development of the leech nervous system. Using monoclonal antibodies (Mabs Laz6-297 and Laz6-56) specific to a 130 kD surface glycoprotein that is expressed on macroglia in the adult, we have documented the embryonic expression of this glial antigen at the sites of neurogenesis, migration, and neurite extension.

In order to determine whether the glial processes prefigure axon tract formation, we double labelled embryos with the glial Mabs and neuron-specific Mabs. In the CNS we stained the first detected axonal processes, which belong to the Bipolar cells, with Mab Laz1-1; and in the PNS we stained the pioneering afferents with Mab Lan3-2. In both cases, the earliest neuronal processes were seen to extend along established glial tracts.

To investigate whether developing axons utilize the 130 kD surface glycoproteins expressed along glial processes, we performed antibody pertubation experiments. When germinal plates are organ-cultured in the presence of Laz6-297, we detect aberrant projections by the Bipolar cells, suggesting that they pioneer CNS tracts using glial surface antigens as their guide. In these same experiments we also observed a greater degree of neuronal differentiation in experimental groups over controls, as measured by the segmental level at which peripheral and central neurons are seen to differentiate. It appears that the glial Mab stimulates a trophic response.

231.20

SEGMENTATION OF THE LEECH GERMINAL PLATE PROCEEDS IN REGISTER WITH A CHAIN OF MYOGENIC MIDLINE CELLS. I.S. Thorey and B. Zipser. Dept. of Physiology, Neuroscience Program, Michigan State University, East Lansing, MI 48824.

The leech germinal plate is derived from 5 pairs of teloblasts producing bilateral bandlets of blast cells. Primary blasts cells divide and then aggregate into segmentally reiterated somites and central ganglia. The progress of segmentation in the leech germinal plate can be visualized by staining myogenic cells with monoclonal antibody Laz10-1 which recognizes a unique mesoderm/muscle surface protein.

The future segmentation of the leech germinal plate is prefigured by a chain of myogenic cells expressing the Laz10-1 protein at the primary axis of embryonic symmetry. These cells and their interconnecting processes form a myogenic fascicle which is already present on the midline during the production of primary mesoblast cells. The 32 somites and central ganglia aggregate in register with the myogenic cell bodies. The somites form laterally halfway between 2 consecutive myogenic cell bodies. The ganglionic primordia aggregate at the midline just posterior of the myogenic cell bodies. The myogenic cell bodies differentiate into the CNS muscles.

In the mesoderm, the expression of Laz10-1 protein fluctuates during the development of the germinal plate. Laz10-1 protein expression is elevated when mesodermal cells aggregate into cohesive tissues, e.g. in the nascent mesoderm and in the forming somites.

232.1

PALMITOYLATION OF GAP-43 IN INTACT GROWTH CONES AND ISOLATED GROWTH CONE MEMBRANES IN VITRO. Sean I.Patterson* and J.H.Pate Skene, Dept. Neurobiology, Stanford University, CA 94305.

Covalent binding of palmitic acid to proteins has been shown to confer subcellular localization by membane attachment, and may regulate the function of some proteins. In intact growth cones incubated with 3H-palmitate in vitro, the major acylated protein has previously been identified as the neuronal growth-associated protein, GAP-43 (J.Cell Biol. 108, 613-624). In order to study the regulation of palmitoylation we have developed an in vitro acylating system using membranes from lysed growth cones. Lysed growth cones incorporated 3H-palmitoyl coenzyme A into GAP-43 more rapidly than 3H-palmitate, and this process was more efficiently competed by excess unlabelled palmitoyl-CoA than unlabelled palmitate, suggesting that the slower labelling observed with the free fatty acid may be partially due to its conversion to the precursor for acylation. Preliminary results indicate that the incorporation of 3H-palmitate into lysed growth cone membranes may be blocked by homologs of tunicamycin, an antibiotic that contains a fatty acyl chain. Separation of non-acylated GAP-43 from GAP-43 attached to membranes could be achieved by pelleting growth cone membranes (100,000x*g*, 60min). Washed growth cone membranes rapidly incorporated 3H-palmitate into GAP-43, implicating an endogenous membrane associated deacylating activity that may play a role in the regulation of proteins attached to membranes through acylation. Growth cone membranes therefore provide a simple and experimentally amenable model for the characterization of protein acylation and deacylation, and for exploring acylation as a potential regulatory system.

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232.2

Phosphorylation Sites on the Growth-Associated Protein GAP-43. S.M. Schuh, S.A. Spencer, and M. Willard. Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

GAP-43, a neuronal phosphoprotein, is a major protein of growth cones; in addition, it is a component of presynaptic terminals, where its phosphorylation has been reported to be associated with changes in synaptic efficacy. To identify the phosphorylation sites on GAP-43, we phosphorylated purified GAP-43 with purified protein kinase C and ^{32}P-ATP, and analyzed radioactive tryptic peptides by reverse phase HPLC chromatography followed by amino acid sequencing. A single site, serine 41, that is adjacent to a region reported to bind to calmodulin, was phosphorylated. To investigate whether this site is also phosphorylated in living cells, we labeled primary cultures of rat superior cervical ganglia and several established cell lines with ^{32}P-orthophosphate. Reverse phase chromatography of tryptic fragments of GAP-43 from these cultures yielded 6 peaks of radioactivity. One of these coeluted with a hexapeptide containing phosphorylated serine 41. When the superior cervical ganglia cultures were treated with phorbol ester to stimulate protein kinase C, the radioactivity in this peak was increased 100%, whereas no change was detected in the other five peaks. These results demonstrate that serine 41 is the major phosphorylation site of protein kinase C in vitro. They suggest that, in living cells, GAP-43 is phosphorylated at multiple sites, of which only serine 41 is a substrate for protein kinase C. Supported by NIH grant EYO 2682.

232.3

NGF-STIMULATED IN SITU PHOSPHORYLATION OF GAP-43 IN ISOLATED GROWTH CONES. K.F. Meiri and D.A. Burdick*, Dept. Pharmacology, SUNY Health Science Center Syracuse N.Y. 13210.

Little is known about the intracellular signal transduction processes that regulate growth cone motility and pathfinding functions. In PC12 cells the action of NGF involves stimulation of the A & C kinases, but the consequences of the subsequent protein phosphorylations are not clear. In certain non-neuronal cells, activation of signal transduction mechanisms **has** been definitively linked to both shape changes and motility: In platelets, activation of the α2 receptor and then kinase C alters the association of actin with membranes; migration of leucocytes towards NGF involves kinase C. Investigations in growth cones have been hampered by the difficulties of isolating events in the growth cone from those occurring elsewhere. Subcellular fractions of isolated growth cones (IGCs) facilitate such studies, and we report here that intact IGCs can synthesize radiolabelled ATP from ^{32}P orthophosphate, and utilize it to phosphorylate proteins in situ. We have used this preparation to demonstrate the phosphorylation of specific IGC proteins by both direct stimulation of kinase C and by exposing IGCs to extracellular ligands. We have shown stimulation of GAP-43 phosphorylation when IGCs are incubated with NGF, both in vitro and in situ using this intact IGC model. GAP-43 phosphorylation is also stimulated when IGC membranes of were depolarized with potassium. In both cases rapid dephosphorylation of GAP-43 occurs, demonstrating that GAP-43 phosphorylation in response to extracellular stimuli is dynamically regulated. Supported by NS26091 and March of Dimes.

232.4

SPECIFIC PROTEOLYSIS OF B-50 (GAP-43) IS INHIBITED BY ACTH. P.J. Coggins* and H. Zwiers* (SPON: M.A. Bisby). Depts. of Medical Physiology and Medical Biochemistry, University of Calgary, Calgary, Alberta T2N 4N1 Canada

The neuronal protein B-50 (GAP-43) was originally identified as a rat synaptosomal plasma membrane protein whose PKC mediated phosphorylation was specifically inhibited in the presence of ACTH. Extraction of the membrane fraction under mild, non-denaturing conditions yielded a protein complex highly enriched in B-50, B-50 kinase (i.e. PKC) and a protease that specifically cleaved B-50 at Ser41, the single site of PKC mediated phosphorylation. The short sequence adjacent to Ser41 (i.e. B-50 35-51) contains the calmodulin binding domain of B-50 and phosphorylation prevents association of the two proteins. In the present study we have used the same protein complex to investigate the proteolysis of B-50 to B-60 (B-50 41-226) in the presence and absence of ACTH 1-24 and fragments derived from it. In common with its action on kinase activity the results demonstrated a time, dose and structurally dependent inhibition of B-50 proteolysis by ACTH (IC50 of approx. 2 μM). ACTH 1-10, ACTH 11-24 and a combination of both fragments were ineffective. In addition, calmodulin (0.2 and 20 μM) also reduced the amount of B-60 detected. Therefore, B-50 and ACTH both of which have been implicated in diverse aspects of neuronal plasticity interact on two biochemical levels, phosphorylation and now, proteolysis.

232.5

CHICKEN GAP-43 AND PROTEIN KINASE C ARE CLOSELY ASSOCIATED WITH THE NEURONAL MEMBRANE SKELETON. P.Fernyhough and D.J. Moss, MRC Cell Biophysics Unit, Kings College, London.

The membrane associated cytoskeleton, termed membrane skeleton, has been isolated from chick brain and cultured sympathetic neurons (Moss, D. J., Eur. J. Biochem. 135:291, 1983). This complex contains an approximately equal amount of actin and a similar complement of actin filaments when compared with the erythrocyte membrane skeleton. In addition, we have shown that the chick brain membrane skeleton is a major location of GAP-43, protein kinase C (PKC), and the neuron-specific glycoprotein contactin (Ranscht, B., J. Cell Biol. 107:1561, 1988). Chick GAP-43 and rat GAP-43 are biochemically similar, and endogenous phosphorylation of chick GAP-43 within the membrane skeleton is enhanced upon treatment with phorbol esters. PKC is highly enriched in the membrane skeleton relative to detergent-soluble fractions as shown by immune blot using antibodies specific for PKC types II and III. Membrane skeleton prepared from a human neuroblastoma cell line is also a source of PKC and its abundance may be regulated by growth factors, such as NGF and insulin.

232.6

CARBACHOL-INDUCED CHANGES IN THE PHOSPHORYLATION OF GROWTH-ASSOCIATED PROTEIN KINASE C SUBSTRATE B-50 (GAP-43) IN NEURONAL GROWTH CONES. C.O.M. VanHooff, P.N.E. DeGraan, L.H. Schrama, M. DeWit, A.B. Oestreicher and W.H. Gispen (SPON: E.E. Codd). Div. Mol. Neurobiol., Rudolf Magnus Inst., Inst. Mol. Biol. & Med. Biotechnol., and Lab. Physiol. Chem., Univ. of Utrecht, Padualaan 8, 3584 CH Utrecht, NL.

Neuronal growth cones, the highly motile tips of neurites, contain high levels of the growth-associated protein B-50 (GAP-43). Using growth cone membranes we recently provided evidence for a role of this neuron-specific protein kinase C (PKC) substrate in signal transduction (VanHooff et al., *J. Neurosci.* 8:1789, 1988). In the present study we investigated whether external stimuli, such as K^+ depolarization and muscarinic receptor activation, could modulate B-50 phosphorylation in the intact growth cone. B-50 phosphorylation was monitored by quantitative immunoprecipitation of B-50 from $^{32}P_i$ prelabeled intact growth cones, isolated from 5 day old rat forebrain. Depolarization with 30 mM K^+ induced a transient Ca^{2+}-dependent increase in B-50 phosphorylation, which is maximal after 15 sec and declines to basal level within 5 min. This increase, as well as that induced by 100 nM 4β-phorbol 12,13-dibutyrate (PDB), can be inhibited by the PKC inhibitor staurosporin. Carbachol enhanced B-50 phosphorylation in a concentration-dependent manner (50% at 10^{-3} M). Since the effect of carbachol could be inhibited by atropine (10^{-7} M) and staurosporin, we conclude that activation of muscarinic receptors on the growth cones stimulated PKC-mediated B-50 phosphorylation. The carbachol-induced increase in B-50 phosphorylation was parallelled by an increase in [^{3}H]-inositol phosphate accumulation, which could be attenuated by PDB. Our data support the hypothesis that PKC-mediated B-50 phosphorylation exerts a negative feedback on receptor-mediated (poly)-phosphoinositide hydrolysis.

232.7

DISTRIBUTION OF GAP-43 IN RELATION TO DEVELOPMENT OF THE RAT MESENCEPHALIC DOPAMINERGIC SYSTEM. C.W. Shults,†* D.M. Armstrong,* L.I. Benowitz,§ †Neurology Service, VA Med. Ctr., San Diego, *Dept. Neuroci., UCSD, La Jolla, CA, §Dept. Psychiatry, Harvard Med. Sch., McLean Hosp., Belmont, MA

Adjacent sagittal sections of embryonic rat brain were immunolabelled for growth-associated protein-43 (GAP-43) and for tyrosine hydroxylase (TH). At E14 thick GAP-43 immunoreactive (GAP-43-IR) fibers ran from the ventricular zone toward, but did not reach, the ventral pial surface in certain regions of the mesencephalon. Previous studies of the developing mesencephalon demonstrated that radial glia follow a similar pattern and TH-IR cells align along radial glia. GAP-43-IR fibers ran to the developing striatum and along the course of the fasciculus retroflexus. TH immunoreactive (TH-IR) cells were noted at the mesencephalic flexure with some TH-IR fibers running rostrally toward, but not yet reaching, the developing striatum. At E15 and E16, GAP-43-IR fibers continued to course from the ventricular zone toward the ventral pial surface in certain regions of the mesencephalon. At E15, 16, and 18, GAP-43-IR fibers appeared to precede and be more widely distributed than TH-IR fibers in the developing dopaminergic nigrostriatal tract and fasciculus retroflexus. Our observations suggest that GAP-43 may play a role in developmental organization of the substantia nigra and the ventral tegmental area and their respective projections to the striatum and habenula.

232.8

GAP-43 EXPRESSION WITHOUT NEURITE EXTENSION
J. Thomas Megerian, James P. Sullivan* and William L. Klein Department of Neurobiology & Physiology, Northwestern University, Evanston Il. 60208

Previous studies from other laboratories have shown that introducing the gene for neuronal Growth Associated Protein-43 (GAP-43) into non-neuronal cells results in the formation of neurite-like processes. Here, in order to determine whether its expression is sufficient for neurite outgrowth, GAP-43 was visualized by immunoflourescence in both primary and cloned cell cultures. In N1E-115 mouse neuroblastoma and SH-SY5Y human neuroblastoma, we detected strong labeling in cells induced to undergo neuritogenesis. However, in non-induced cultures GAP-43 was expressed as robustly. Thus in both lines, dividing cells that lacked neurites continuously expressed GAP-43. Primary cultures of embryonic rat basal forebrain also expressed GAP-43 but in a much more selective fashion. In both primary cells and differentiated cell lines, labeling was present in cell bodies, neurites and growth cones. For cells containing several processes, labeling was not restricted to one neurite. Our results, showing GAP-43 expression without neurite extension, demonstrate that GAP-43 function in neuritogenesis depends on the induction of other critical components. Supported in part by NIH grants NS 21088, 23348, & 21234 to WLK.

232.9

GAP-43 IMMUNOCYTOCHEMICAL DISTRIBUTION IN DEVELOPING CULTURED NEURITES AND GROWTH CONES
R.W. Burry[1], D. Hayes[1,*], N. Perrone-Bizzozero[2], L. Benowitz[2] and J.J. Lah[1]. [1]Dept. Anat., & Neurosci. Prog, Ohio State Univ, Columbus, OH, [2]McLean Hosp., Harvard Med. Sch., Belmont, MA

The developmentally regulated protein GAP-43 is present at high levels in the cerebellum during neuritic outgrowth and synapse formation. To determine the cellular distribution of GAP-43, cell cultures of granule neurons were studied with light microscopic immunocytochemistry. Anti-GAP-43 labeled the neuritic outgrowth at 1 day after plating. By 10 days in culture most neurites labeled heavily, and some isolated neurites have lost labeling for GAP-43. By 14 days, the majority of neurites labeled lightly, while many isolated neurites had no GAP-43 labeling. In cultures at 45 days, the majority of neurites had lost GAP-43 label, with only a few still intensely labeled. We conclude that the pattern of GAP-43 distribution in cultured granule neurons parallels developmental changes in vivo, and these cultures can be used to effectively study subcellular distribution of GAP-43.
Research Support: Bremer Foundation, The College of Medicine, The Ohio State University.

232.10

DOES NCAM DISTRIBUTION DEPEND ON UNDERLYING CYTOSKELETAL COMPONENTS? H. Safferstein* and F.J. Roisen (Spon: R. Dagirmanjian). Dept. of Anatomical Sciences & Neurobiology, University of Louisville, School of Medicine, Louisville, KY 40292.

The distribution of NCAM during differentiation of the Neuro-2a cell line is dependent on the underlying cytoskeleton. Our previous studies demonstrated that the level of differentiation can be regulated by exposure to unique neuritogenic signals. Neuro-2a cells grown in media with limited serum, dibutyryl cyclic AMP, or ganglioside GM1 exhibit increased levels of neuritogenesis, respectively. TEM of neurites formed in response to these stimuli revealed a correlation between the level of differentiation and the cytoskeletal organization. We have shown, previously, that each of these signals produces a characteristic NCAM pattern. In this study, we utilized cytoskeletal disruptive agents to probe the role of structural organelles in NCAM distribution. Exposure of Neuro-2a to cytochalasin D (microfilaments), colcemid (microtubules), or taxol (microtubule stabilization and promotion) disrupted the NCAM pattern produced by GM1. However, the simultaneous addition of cytochalasin D and taxol augmented the linear distribution of NCAM-positive material seen in cultures grown in GM1. Immunoelectron localization studies are in progress to visualize NCAM's relationship to cytoskeletal organelles. Supported by NIH grant NS24524 to FJR.

232.11

EXPRESSION OF NCAM ANTIGEN IN THE NORMAL AND REGENERATING GOLDFISH RETINOTECTAL SYSTEM
M.Bastmeyer,B.Schlosshauer* and C.A.O.Stuermer, Friedrich-Miescher-Lab. der Max-Planck-Ges. und Max-Planck-Inst. für Entwicklungsbiol.,Tübingen, FRG.

The goldfish visual system is unique in that it grows throughout life. The retina adds neurons circumferentially at its peripheral margin, the tectum in a crescent shaped zone around its caudal pole. The axons from the newborn ganglion cells travel as a bundle through distinct aspects of the nerve and tract and along the dorsal and ventral periphery of the tectum. The neural cell adhesion molecule (NCAM) is well known to mediate adhesion between neurons and neurites in the developing nervous system. Using the monoclonal antibody D3, which recognizes the intracellular domain of the 180 kD form of NCAM in the chick (Schloßhauer, J.Neurochem.52: 82-92, 1989) we analyzed the distribution of this antigen in the retinotectal system of adult goldfish.

On cryostat sections of normal fish, Mab D3 labels cells exclusively in the marginal growth zones of the eyes and the tecta. In the eyes, nerves and tecta it stains the paths of the youngest, still growing axons. Following optic nerve section (ONS) retinal axons in fish regenerate and reinnervate the tecta from rostral to caudal. Correspondingly, in tecta at 2 weeks after ONS the retinorecipient layers exibit staining only rostrally but throughout the rostrocaudal extent by 5 weeks. Regenerating optic nerves are also intensively stained. After eye enucleation the optic nerves and the retinorecipient layers of the tecta are unlabeled, suggesting that regenerating axons express the antigen whereas nerve glia and tectal cells do not, except those in the caudal growth zone. Accordingly, retinal axons and their growth cones growing *in vitro* are Mab D3 positive.

Thus, NCAM antigen is expressed by newly proliferated cells in the eyes and tecta and by growing retinal axons in normal adult goldfish. The antigen reappears on regenerating retinal axons after ONS.

232.12

A UNIQUE NCAM GLYCOFORM PRESENT ON A SUBSET OF AXONS IN FROG BRAIN. B. Key* and R.A. Akeson. Basic Research, Children's Hospital Medical Center, Cincinnati, Ohio, 45229.

We have previously described the expression of a unique glycosylated form of NCAM in the olfactory bulb of frogs (Neurosci. Abstr., 14:919, 1988). This NCAM glycoform was detected with a monoclonal antibody (Mab) called 9OE. In the present study we have analysed in detail the distribution of this unique NCAM in the brain of the adult bullfrog. Serial transverse sections of frog brain were reacted with Mab 9OE and visualized by the ABC immunochemical technique. Mab 9OE labelled a discrete ventrolateral quadrant of the telencephalon. In addition subsets of axons in several nerve fiber tracts were also labelled. These pathways included the ventrolateral olfactory tract (VLOT), the medial olfactory tract (MOT) and the accessory olfactory habenular tract (AOHT). The VLOT projects from the accessory olfactory bulb to the amygdala. Both of these regions were labelled by Mab 9OE and have previously been implicated in the control of reproductive behaviour. Both the MOT and AOHT have also been shown previously to contain a subset of LHRH immunoreactive axons that are believed to have a similar function. Interestingly, the distribution of these fibers resembles that of Mab 9OE labelled axons. No labelling was observed on any structures in the spinal cord, brain stem, cerebellum, tectum and pallium. The presence of a unique NCAM glycoform on a subset of axons may represent a novel mechanism for the development of specific functionally related axon pathways in the vertebrate central nervous system. (Supported by NIH grants NS23348 and HD21065).

232.13

NEURITE OUTGROWTH OVER MONOLAYERS OF CELLS THAT EXPRESS TRANSFECTED HUMAN N-CAM.

P. Doherty, C.H. Barton*, G. Dickson*, and F.S. Walsh. Department of Neurochemistry, Institute of Neurology, London WC1N 3BG.

We have cultured both human and rat sensory neurons on monolayers of 3T3 and/or L-cells that have been stably transfected with full length cDNAs for a variety of human N-CAM isoforms. Neurons grown on monolayers expressing transmembrane or GPI-linked N-CAM isoforms exhibited a greater degree of morphological differentiation than neurons grown on monolayers of control cells or cells transfected with a cDNA encoding a secreted N-CAM isoform. The increased morphological differentiation was associated with significant increases in immunoreactive neurofilament protein. Modification of the extracellular structure of N-CAM, consequent to the expression of a glycosylated 37 amino acid sequence normally found expressed exclusively in muscle N-CAM isoforms, did not obviously affect the ability of transfected cells to support increased neuronal differentiation. These data provide direct evidence for both transmembrane and lipid-linked N-CAM isoforms being components of the regulatory machinery that determine process outgrowth.

232.14

IMMUNO-ELECTRONMICROSCOPIC LOCALIZATION OF CELL ADHESION MOLECULE L1 IN DEVELOPING RAT PYRAMIDAL TRACT. E.Joosten, A.Gribnau and T.Gorgels (SPON:P.Apkarian). Dept.Anat.and Embryol.,Univ.of Nijmegen,6500HB Nijmegen,The Netherlands.

The glycoprotein L1 has been proposed to function in the PNS as an adhesion molecule involved in fasciculation and onset of myelination. In this study the localization of L1 was studied during the development of a major central path way: the pyramidal tract (PT).The (sub)-cellular localization of L1 was determined both by pre-embedding staining on vibratome-sections and by immunogoldlabelling on ultracryosections in developing rat PT at C5. Polyclonal antibodies to mouse L1 were a generous gift of Dr.F.Rathjen (Dept.Mol. Neurobiol.,Hamburg,FRG). On arrival at C5,i.e. between P1 and P4, PT growth cones of pioneer fibres did not exhibit L1-ir, but L1-ir was noted when situated adjacent to other small unmyelinated axons. L1-ir was observed on later arriving,i.e. between P4 and P10, small unmyelinated fasciculating PT axons. During PT myelination,i.e. between P10 and P21,a slight L1-ir was noted in between unmyelinated PT-axons but L1-ir was also observed within the axoplasma of (un)-myelinated axons. Axons ensheated by oligodendrocytic processes did not express the L1 antigen. Furthermore,both compact myelin as well as glial processes were L1-neg. From these results it may be deduced that:1. L1 is only involved in guidance of outgrowing PT fibres by means of axon-fasciculation, 2. In contrast to developing PNS L1 is not involved in the onset of myelination in this central tract.

232.15

EVIDENCE FOR THE INVOLVEMENT OF C KINASE IN LAMININ INDUCTION OF NEURITE OUTGROWTH. John L. Bixby and Perseus Jhabvala*, Dept. of Pharmacol., Univ. of Miami Sch. of Med., Miami, FL 33136.

We are interested in the intracellular events underlying the induction of neurite outgrowth by ECM/integrin interactions, and particularly the involvement of specific protein kinases. We have begun to study these events by culturing chick ciliary ganglion (CG) neurons on various ECM and non-ECM substrates, in the presence and absence of drugs that affect C kinase activity. The tumor promoter TPA, an activator of C kinase, potentiates process outgrowth from CG neurons cultured on suboptimal laminin (LN) concentrations, but not on optimal LN concentrations. TPA also strongly potentiates growth on fibronectin and collagens that is similar in extent to that observed on LN in control conditions. TPA does not, however, induce CG neuron process outgrowth on non-permissive substrates (poly-D-lysine or tissue culture plastic). Manipulations that elevate intracellular cAMP levels (expected to activate A kinase) reduce process extension on LN. The C kinase inhibitors H7 and sphingosine reversibly block LN-induced neurite outgrowth in a dose-dependent fashion. H7 does not inhibit the neurite outgrowth induced by concanavalin A in the same neurons. Our results suggest that 1) activation of C kinase is an important step in the neurite outgrowth induced by LN binding to its integrin receptor(s), 2) there are other important functions of these receptors involved in neurite outgrowth, and 3) the "neurite" growth induced by concanavalin A in CG neurons occurs through a distinct mechanism from that induced by LN. Supported by the MDA, the PMAF, and a BRSG from the NIH.

232.16

A NEURONAL CELL LINE (PC12) EXPRESSES TWO β_1-CLASS INTEGRINS THAT RECOGNIZE DIFFERENT CELL ATTACHMENT SITES IN LAMININ. K.J.Tomaselli*, D.E.Hall, L.F.Reichardt, L.A.Flier*, D.C.Turner*, and S.Carbonetto. HHMI/UCSF, SF CA 94143; Biochem.& Mol.Biol.,SUNY, Syracuse NY; Montreal Gen.Hosp.Res.Inst.,Montreal, Quebec.

The role of β_1-class integrins in attachment of PC12 cells has been studied with specific antibodies. PC12 cells express two α/β_1 heterodimers: α_1/β_1 and α_3/β_1. A monoclonal antibody (3A3) recognizes the α_1/β_1 heterodimer and precipitates two PC12 cell surface proteins of M_r 180,000 (α_1) and 120,000 (β_1). An antiserum to the cytoplasmic domain of the chicken integrin α_3 subunit (gift of Dr. R.O. Hynes) recognizes the α_3/β_1 dimer and precipitates two proteins of M_r 140,000 (α_3) and 120,000 (β_1). The α_1/β_1 dimer functions as a receptor for laminin and collagens I and IV. Function-blocking studies with antibody 3A3 indicate that α_1/β_1 is the major receptor for collagen on PC12 cells, but is not the only β_1 integrin receptor for laminin. Attachment of PC12 cells to proteolytic fragments of laminin confirms this hypothesis. PC12 cells attach to two different laminin elastase fragments: fragment E1-4, derived from the intersection of the long and short arms of laminin, and fragment E8, which consists of the long arm and the terminal heparin-binding globular domain E3. Attachment to either fragment is blocked by β_1 integrin antibodies. Antibody 3A3 inhibits attachment to fragment E1-4 but has no effect on attachment to fragment E8. These results indicate that PC12 cells express two β_1-class integrins that bind to separate sites on laminin: α_1/β_1, binds to a site at the top of the laminin cross, and α_3/β_1 recognizes a site in E8, most likely near the end of the long arm.

232.17

EVIDENCE FOR INVOLVEMENT OF THE INTEGRIN ALPHA 3 SUBUNIT IN PROCESS OUTGROWTH ON LAMININ. D.O. Clegg, E.A. Wayner*, J. Snyder*, D.B. Gervin*, A. Bradshaw*, G. Cann*, E.S. Choi*, P.D. Sullivan*, D.M. Eckley*. Neuroscience Research Institute, University of California, Santa Barbara, California 93106; and Department of Biochemical Oncology, fred Hutchinson Cancer Research Center and Pacific Northwest Research Foundation, Seattle, Washington 98104.

The beta$_1$ subunit of the integrin family of cell surface receptors has been implicated in mediating neurite extension on laminin, but the identity of the alpha subunit(s) that combine with beta$_1$ to mediate outgrowth on laminin have not been identified. At least three different alpha subunits can combine with beta$_1$ to form heterodimers that interact with laminin: alpha$_1$, alpha$_3$, and alpha$_6$. The alpha$_3$-beta$_1$ heterodimer appears to be a promiscuous matrix receptor that can mediate cell attachment to laminin, collagen, and fibronectin.

We have analyzed the effects of function blocking anti-alpha$_3$ monoclonal antibodies on neurite extension by the human neuroblastoma cell line SY-5Y. We have found that these antibodies will bring about rapid retraction of neurites on laminin substrates. Control anti-alpha$_2$ antibodies had no effect. These results suggest that the alpha$_3$-beta$_1$ heterodimer can mediate neurite outgrowth on laminin and may be present on neurons *in vivo*. Experiments are in progress to determine the effects of other function blocking monoclonal antibodies on neurite outgrowth properties of this cell line. Our goal is to determine the roles of different integrin alpha subunits in the development of the nervous system.

232.18

NEURITE OUTGROWTH OF RAT FETAL SEPTAL CELLS IN CULTURE IS STIMULATED BY MATRIGEL, LAMININ, AND LAMININ-DERIVED SYNTHETIC PEPTIDES. *M. Jucker[1]*, H.K. Kleinman[2]*, H. Kametani[1]*, E.L. Bresnahan[1], G.R. Martin[1]*, and D.K. Ingram[1]* (SPON: M. Talan). [1]Gerontol. Res. Ctr., Natl. Instit. on Aging, NIH, Baltimore, MD 21224; [2]Lab. Dev. Biol. Anom., Natl. Instit. of Dental Res., NIH, Bethesda, MD 20892

Laminin, a major glycoprotein of basement membrane, is a potent stimulator of neurite outgrowth *in vitro* for a variety of cells. Using fetal septal cell cultures, we examined cell development as affected by laminin, Matrigel (a mixture of basement membrane proteins), or synthetic peptides corresponding to sequences in laminin chains. Cell suspensions were made from septal tissue dissected from fetal rat brains (E16-17). Aliquots of 0.3-0.6 x 10^5 viable cells were grown in 12 mm wells containing 0.5 ml DMEM and either laminin (0-50 μg), Matrigel (0-50 μl), or laminin derived synthetic peptides (0-100 μg) as coated onto plastic or added to medium. Cell attachment and process formation were quantified by automated image analysis at 3, 7, 10 days. Uncoated and untreated dishes showed no significant adhesion or outgrowth. Compared to controls, cultures with laminin or Matrigel showed greatly enhanced survival, and neurite outgrowth was also stimulated in a dose-dependent manner by these materials. A synthetic peptide derived from the laminin A-chain sequence also promoted survival and neurite outgrowth although processes were shorter than using the native protein. A laminin B1-chain peptide (YIGSR: Graf J. *et al., Cell* 48:989, 1987) did not promote survival or process formation. Preliminary *in vivo* results also showed that fetal septal cells suspended in Matrigel exhibited viability and neurite outgrowth when transplanted to hippocampus and lateral ventricle of rats. These studies indicate that fetal septal cell development is stimulated by laminin and that a synthetic peptide can partially duplicate this activity to suggest *in vivo* applications.

232.19

LAMININ: IS IT THE YELLOW BRICK ROAD OF THE DEVELOPING AUDITORY RECEPTOR? T. R. Van De Water[1], V. Galinovic-Schwartz*[1] and G.S. Swanson[1,2], [1]Laboratory of Developmental Otobiology, Depts. of Otolaryngology and Neuroscience, [2]Developmental Biology Unit, Imperial Cancer Research Fund, Oxford University, Oxford, England.

Laminin is an extracellular matrix glycoprotein that has been implicated as a possible hapten for the guidance of neurites both in vitro (Hammerback, etal., Dev. Biol. 126:29-39, 1988) and in the developing optic tract (Cohen, etal., Dev. Biol. 122:407-18, 1987). The pattern of laminin distribution in embryonic mouse and chick auditory receptors was examined by immunohistochemical localization using species specific polyclonal antibodies. Mouse embryos from 13 to 18 gestation days and chick embryos from Hamilton & Hamburger Stages 18 to 40 were used in this study. The relationship between neuritic ingrowth and the basement membranes of the sensory portions of the cochlear duct was examined. Where nerve fibers are first seen in close association with the basement membrane of the presumptive sensory areas (but not yet penetrated into these areas) we failed to detect any tracts of laminin staining that might act to guide these dendrites. Laminin staining of basement membranes underlying auditory sensory areas becomes intermittent at the sites of nerve fiber penetrations. Other regions of the basement membrane underlying sensory areas not penetrated by neurites remain intact and show continuous staining for the presence of laminin. No inter-epithelial staining for laminin was found within the epithelium in the regions of ingrowing auditory nerve fibers. This observation is in contrast to that found by Cohen, etal. 1987 in the developing optic tract where the pattern of laminin distribution correlates with inter-epithelial spaces along which the axons of the retinal ganglion cells grow. In the nerve bundles themselves, laminin staining was predominantly associated with the perineural sheath and often contiguous with that of the epithelial basement membrane. In the non-sensory areas of epithelium staining was detected, in heaviest concentration, in the apical portions of the prospective stria vascularis epithelium. Our observations are incompatible with the hypothesis that laminin plays an active role in directed neurite ingrowth to the sensory areas of the auditory receptor in chick and mouse embryos. Rather, in the developing murine cochlear duct, the presence of an intact basement membrane, as shown by laminin staining, appears to correlate with the absence of neurite ingrowth.

(This work was supported by NIH Grant NSO8365 to TRV and Imperial Cancer Research Funds to GJS.)

ENDOCRINE CONTROL AND DEVELOPMENT III

233.1

CREMASTER NUCLEUS SUBSTANCE P PATHWAY CONTAINS A SEXUALLY DIMORPHIC POPULATION OF INTRASPINAL NEURONS. B.W. Newton. Dept. of Anatomy, U. of Arkansas for Med. Sci., Little Rock, AR 72205

The cremaster nucleus (CN) is sexually dimorphic, with males containing a greater number of motoneurons than females. The substance P (SP) innervation of the CN is also sexually dimorphic: males have a massive SP pathway which females lack. Previous studies (Brain Res. 301:243; Neurosci. Lett. 57:185) suggest the SP pathway to the male CN is of intraspinal origin. Adult rats were given subarachnoid colchicine and 36 hrs later SP-immunoreactivity (IR) was visualized using the PAP technique. The male lumbar spinal cord (laminae V, VII) contains a large number of SP-IR neurons immediately dorsal to and within the SP pathway to CN. This population of SP-IR neurons is sparse in females. Previous studies (Soc. Neurosci. 14:285) show that exogeneous neonatal androgens induce the formation of a male-like SP pathway to the female CN. Investigations are underway to determine if this androgen-induced SP pathway in females also contains SP-IR intraspinal neurons. Supported by BRSG RR05350 and the Arkansas Caduceus Club.

233.2

ANDROGEN-REGULATED PROTEIN EXPRESSION DURING DEVELOPMENT OF A SEXUALLY DIMORPHIC NEUROMUSCULAR SYSTEM. Nancy G. Forger, G.L. Firestone* & S.M. Breedlove, Depts. Psychology & Physiology-Anatomy, University of California, Berkeley, CA 94720.

Survival of the bulbocavernosus/levator ani (BC/LA) muscles of rats beyond the neonatal period is dependent on androgen and, as a consequence, the BC/LA normally degenerates in females. Treatment of perinatal females with androgen permanently rescues the BC/LA muscles and their innervating motoneurons in the spinal nucleus of the bulbocavernosus (SNB; Breedlove & Arnold '83). To identify the molecular events mediating androgenic rescue of this neuromuscular system, we examined androgen-regulated protein expression in the BC/LA using 2-dimensional gel electrophoresis. BC/LA muscle tissues were removed from perinatal Sprague-Dawley rats and incubated at 37 °C for 4 h in serum-free media supplemented with [^{35}S]methionine. Labeled proteins were separated by isoelectric focusing in the first dimension, SDS-PAGE in the second, and visualized by autoradiography.

BC/LA tissue of untreated females lacked an acidic ~26 kDa protein doublet which was abundantly expressed in the BC/LA of males and in other striated muscles from both sexes. Perinatal androgen treatment for 96 h induced expression of the 26 kDa doublet in the BC/LA of females. Based on position in 2-D gels, we tentatively identified the doublet proteins as isoforms of fast muscle myosin light chain 1 ($LC1_f$); immunoprecipitation of the doublet proteins with polyclonal antibody to $LC1_f$ confirmed this identification. Expression of other identified muscle-specific proteins (e.g. myosin LC3) was not sexually dimorphic in the BC/LA. Therefore, $LC1_f$ expression may play a role in the androgenic sparing of the BC/LA-SNB neuromuscular system.

Supported by the Muscular Dystrophy Association and NSF #BNS8451367.

233.3

CHANGES IN MOTONEURON SIZE INDEPENDENT OF CHANGES IN TARGET MUSCLE SIZE WITH ANDROGEN EXPOSURE IN ADULTHOOD. Marianne Leslie, N.G.Forger, S.M.Breedlove, Department of Psychology, University of California, Berkeley, CA 94720

Last year we reported significant sex differences in size of motoneurons of the retrodorsolateral nucleus (RDLN) and one of the muscles it innervates, the flexor digitorum brevis (FDB); motoneuron size was larger in males than in females. In the present study, we examined the influence of adult exposure to testosterone (T) on this neuromuscular system.

At 60 days of age, male Sprague-Dawley rats were castrated (GdX, N=21) or sham-castrated (Sham, N=17); females were ovariectomized and implanted with blank (OVX, N=14) or T-filled silastic capsules (OVX+T, N=17). All animals were sacrificed 45-47 days later; spinal cords were frozen sectioned at 50μ and thionin stained. Mean soma and nuclear areas were determined from camera lucida drawings of 20 RDLN cells from each animal. FDB muscles were removed and weighed.

RDLN	SHAM	GDX	OVX+T	OVX
-Soma(μ^2)	1013 ± 24*	979 ± 23	1038 ± 28*	937 ± 20
-Nucleus(μ^2)	244 ± 10	240 ± 6	259 ± 10	240 ± 13
FDB mass(mg)	48 ± 1.3*	48 ± 0.8*	39 ± 1.0	39 ± 1.3

*Significantly different from untreated OVX females $p<.025$.

We again found a significant sex difference in RDLN motoneuron size. Androgen treatment of adult OVX females significantly increased soma area over that in control females, thus eliminating the sex difference. Castration did not significantly reduce motoneuron size compared to that of control males. We found no significant differences in nuclear area. FDB mass was found to be strikingly sexually dimorphic, but no change in FDB mass was observed following T manipulation in either males or females. This neuromuscular system may provide a useful model of general sex differences in motoneurons and body size.

Supported by PHS NS19790.

233.4

DIMINISHED IMMUNOREACTIVITY IN A SEXUALLY DIMORPHIC ENKEPHALINERGIC FIBER SYSTEM IN THE PREOPTIC AREA OF THE AGED FEMALE RAT. R. E. Watson, Jr., M.C. Langub, Jr.*, and J. W. Landis*. Dept. Anatomy and Neurobiology, Univ. of Kentucky, Lexington, KY 40536

A sexually dimorphic enkephalin (ENK) fiber system, in which expression is governed by the gonadal steroids in both an organizational and activational manner, is present in the periventricular preoptic nucleus (pePOA) of the rat in only the female. This system is not expressed until puberty (Devl. Br. Res. 44: 49-58). In this study, the effects of aging and associated reproductive senescence upon expression of this dimorphic system were investigated. Estrous cycles of young adult (12 week) and aged (19 month) female F344 rats were followed for 3-8 weeks. Young adult rats had normal 4-5 day estrous cycles. Aged females had repeated pseudopregnancy-like cycles typified by 10-12 day periods of leucocytic smears interrupted by 1-2 days of smears with nucleated and cornified cells. Animals were perfused and the brains prepared for immunocytochemistry (ICC) using either a polyclonal antiserum to met-ENK or a monoclonal antiserum to leu-ENK. The female-typical dense ENK-immunoreactive (ir) fiber plexus in the pePOA was present in all female young adult rats. In contrast, in 5 of 7 aged females analyzed to date, the female-typical dense ENK-ir band was absent (3) or greatly attenuated (2). In an effort to determine whether normal estrogen responsivity of this system is retained in the aged female, young and old rats were ovariectomized. Two weeks later, empty or 17ß-estradiol (E2) containing silastic capsules were implanted subcutaneously, and 4 or 14 days later the brains were prepared for ENK ICC. In young rats the female-typical ENK-ir plexus was re-expressed following E2 exposure. However, in the aged females E2 did not result in enhanced ENK-ir in the pePOA. These data indicate that the aging process is associated with diminished expression of the female-typical ENK-ir fiber plexus in the pePOA, and that with age this system may become increasingly refractory to the activational actions of E2. These observations may aid in identifying the physiological significance of this sexually dimorphic system. {Supported by the University of Kentucky Medical Center Research Fund}.

233.5

INTERACTIONS OF A SEXUALLY DIMORPHIC ENKEPHALIN FIBER SYSTEM WITH TARGET TYROSINE HYDROXYLASE NEURONS IN THE PERIVENTRICULAR PREOPTIC AREA: ELECTRON MICROSCOPY.

M. C. Langub, Jr.*, B. E. Maley, and R. E. Watson, Jr. Department of Anatomy and Neurobiology, Univ. of Kentucky Medical Center, Lexington, KY 40536.

A dense plexus of enkephalin (ENK) immunoreactive (ir) fibers that responds powerfully to the steroid environment during development and adulthood is present in the periventricular preoptic area (pePOA) of the female rat. To elucidate the functional significance of this sexually dimorphic system, it is important that the neurochemical identity of target neurons in the pePOA that receive afferent input be identified. Using light-level double labelling techniques (Soc. Neurosci. Abstr. 14: 1181) a close relationship has been observed between tyrosine hydroxylase (TH) -ir cells and ENK-ir fibers in the pePOA. In the present study, female Sprague-Dawley rats were perfused with buffered 4% paraformaldehyde and 0.3% glutaraldehyde. ENK-ir was localized using a rat monoclonal antiserum to leu-ENK (Sera-Lab) with diaminobenzidine (DAB) as a substrate. Sections were then re-incubated in rabbit polyclonal antiserum to TH (Pel-Freeze) and reacted using tetramethylbenzidine (TMB) as a substrate and stabilized using the technique of Horn and Hoffman (Br. Res. 409: 133-138). Ultrastructurally, TH-ir neurons in the pePOA were identified by the presence of the distinct spicule-like TMB reaction product. In contrast, ENK-ir elements were identified by the more dispersed DAB reaction product. Abundant DAB-containing ENK-ir terminals in the pePOA were observed in apposition to single TH-ir neurons. Many of the ENK-ir terminals contained a mixture of small, clear vesicles and large granular vesicles. In addition, many ENK-ir terminals were in apposition to unlabelled perikarya and dendrites. These data provide morphological evidence for synaptic interactions between enkephalinergic and catecholaminergic systems in the pePOA. This circuitry may be important to the control of sexually differentiated function. (Supported by the University of Kentucky Medical Center Research Fund)

233.6

SEXUAL DIFFERENTIATION OF ADULT LORDOTIC RESPONSE TO CHOLECYSTOKININ IN RATS.

P.E Micevych, C.Ulibarri, & P.Popper. Department of Anatomy & Cell Biology, Laboratory of Neuroendocrinology, Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

The neuropeptide cholecystokinin (CCK) has a sexually dimorphic distribution in the limbic-hypothalamic system of the rat. The number of cell bodies and the levels of CCK are much higher in males than in females. CCK also has a sexually dimorphic effect on lordotic behavior. Infusions of the biologically active form of CCK, sCCK-8, into the ventromedial nucleus of the hypothalamus (VMH) of estradiol-treated female rats inhibit lordosis. Similar infusions in males have no effect.

On the day of birth (postnatal day 1 = PND 1), males were either castrated or received sham surgeries under cold anesthesia. On PND 2, females received either 0 or 100 ug testosterone propionate (TP). Treatments were counterbalanced across litters. On PND 90 all animals were implanted with unilateral cannulae directed at the VMH. After one week the rats were tested for female sexual behavior following estradiol benzoate (EB) injections. The animals were tested either after progesterone (P) injection or sCCK-8 infusion. P injections were 4 h before tests; sCCK-8 infusions were 10 min before tests. Rats were placed with vigorous males and their response to 10 mounts assessed. The experimental animals received either 0, 5, 50, or 100 ug CCK/0.3 ul artificial CSF in a Latin Square design. After the last test, the rats were injected with 5 ug EB/0.1 ml safflower oil, and decapitated 48 h later. The brains were removed, frozen, sectioned, and processed for ^{125}I-sCCK-8 binding.

As expected, control males did not respond to sCCK-8 infusions, however control females, TP-treated females, and neonatal castrates showed clear inhibition of lordosis behavior after infusions of sCCK-8. The degree of inhibition increased with increased dose of sCCK-8. Control females showed the highest levels of sCCK-8 binding in the VMH. Control males, TP-treated females and neonatal castrates had similar levels of binding. Supported by NS 21220.

233.7

DISTRIBUTION OF PROGESTERONE RECEPTOR-LIKE IMMUNOREACTIVITY IN THE BRAIN OF THE RAT.

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Several laboratories have published characterizations of antibodies directed against progesterone receptors (PR). Although these antibodies crossreact with PR in fowl, guinea pigs, and humans, as yet there are no reports of immunohistochemical visualization of PR in rats. We were interested in determining if an antibody (PR6; gift from David Toft, MAYO, MN) that recognizes PR in other species could also be used to visualize PR in rat brain. PR-6 was generated against chick oviduct. The rat uterus was used as a positive control tissue since the PR concentration in the rat uterus is similar to that in the chick oviduct.

Female Long Evans rats were ovariectomized and allowed to recover for one week. Forty-eight and twenty-four hours before perfusion, the females received either 25ug estradiol benzoate (EB)/0.1ml safflower oil, or vehicle. One to four hours before perfusion, the females received 0, 500, 1000, or 1500 ug progesterone (P)/0.1ml safflower oil. Under deep anesthesia, the females and intact males were perfused transcardially with either paraformaldehyde, acrolein, picric acid, Bouin's fixative, mercuric formaldehyde, buffered acetone, parabenzoquinone, or paraformaldehyde and glutaraldehyde. The brains and uteri were removed and postfixed with paraformaldehyde for 2-4 hours. They were then cryoprotected in 25% sucrose in paraformaldehyde for 24 hours. Serial sections were cut on a sliding microtome and processed for localization of PR6-immunoreactivity.

Intense PR6-immunoreactivity was observed in uteri from EB and EB + P-treated females. In both male and female brains, we observed PR6-immunoreactivity in the medial preoptic area, arcuate nucleus, ventromedial nucleus, paraventricular nucleus, and midbrain central gray. Cells in the hippocampus, subfornical organ, septohypothalamic nucleus, median preoptic area, and bed nucleus of the stria terminalis were intensely immunoreactive. The most intense staining was seen in rats treated with both EB + P. Supported by NS 21220.

233.8

ONTOGENY OF CHOLECYSTOKININ mRNA AND RECEPTORS IN MALE AND FEMALE RATS.

L.A. Abelson*, P.Popper, C. Ulibarri, & P.E Micevych. (SPON: C.H. Sawyer). Dept Anatomy & Cell Biology, Lab. of Neuroendocrinology, Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

The sexually dimorphic distribution of the neuropeptide cholecystokinin (CCK) in adults rats may reflect a sexually dimorphic distribution of both message and receptors. The purpose of this research was to delineate the pattern of CCK mRNA and receptors in developing animals and to determine if CCK mRNA and receptors for CCK are distributed dimorphically in developing pups. On postnatal day 1 (day of birth = PND 1), 5, 10, 15, and 30, male and female pups were killed and processed for either [^{125}I]-sCCK$_{26-33}$ receptor autoradiography or in situ hybridization.

The CCK riboprobe was a ^{35}S-labelled single-stranded cRNA transcribed from a genomic probe for the entire coding sequence for the CCK gene (gift from Dr. J. Dixon, Purdue University, IN). The CCK oligoprobe (Operon Tech., Inc., CA) was directed against the RNA that codes for CCK$_{26-33}$. For in situ hybridization pups were perfused with 4% paraformaldehyde/0.2% glutaraldehyde in 0.1M Sorensen's phosphate buffer. The riboprobe was hybridized according to Angerer et al in In Situ Hybridization; oligoprobe hybridization was according to the protocol supplied by Genofit (Geneva, Switzerland). Autoradiograms were prepared by dipping slides in emulsion. The distribution of prepro-CCK mRNA was mapped.

For receptor autoradiography, the pups were decapitated, the brains removed, frozen at -70°C, and cut at 10um on a cryostat. Sections were processed for ^{125}I-sCCK$_{26-33}$ binding as described previously (Akesson, et al, Neuroendo. 45:257,'87). The sections were opposed to Hyperfilm (Amersham) and analyzed for specific binding using a PDP-11 Spatial Data Analysis system. On PND 1 and 5, there were very low levels of CCK binding. On PND 10 binding was apparent in the claustrum and cingulate cortex. By PND 15, CCK receptors were apparent in the ventromedial nucleus (VMH). On PND 30, the pattern of CCK binding in the VMH was similar to that seen in the adult; highest binding was seen in VMHvl and VMHdm with the relatively low levels in the VMHc. Supported by NS 21220.

233.9

ENKEPHALIN-IMMUNOREACTIVE NEURONS OF THE HYPOTHALAMIC VENTROMEDIAL NUCLEUS CONCENTRATE ESTROGEN IN MALE AND FEMALE RATS.

T.R. Akesson and P. E Micevych. Department of Anatomy and Cell Biology, Laboratory of Neuroendocrinology, and Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

The ventromedial nucleus (VMH) and its projections to the preoptic area and midbrain represent essential components of a circuity that regulates estrogen-dependent reproductive behavior. A role for endogenous opiates in this circuitry is indicated by the findings that; 1) the distribution of met-enkephalin immunoreactive (mENKir) cells precisely overlaps the distribution of estrogen-concentrating (E-conc.) cells in the ventrolateral subdivision of the VMH (VMHvl), 2) mENKir fiber density is steroid sensitive in preoptic regions (Watson, et al., Dev. Brain Res., 44:49, '88), 3) mENK injections in the periaqueductal central grey inhibit lordosis (Bednar, et al., Neurosci. Lett., 79:341, '87), and 4)messenger RNA coding for preproenkephalin in the VMHvl is stimulated by estrogen (Romano, et al., Neurosci. Abstr., 12:692, '86). Whether estrogen activates expression of mRNA's coding for preproenkephalin by a direct genomic effect or via a second population of neurons has yet to be determined. Since the morphological signature of an estrogen receptive cell is concentration of labeled estrogen in its nucleus, we combined steroid autoradiography with immunohistochemistry for mENK.

Males were found to have a population of 5114 ± 577 mENKir cells, 3098 ± 303 E-conc. cells and 1481 ± 228 cases of coexistence in the VMHvl whereas in females, there were 4432 ± 211 mENKir cells, 4754 ± 696 E-conc. cells, and 1251 ± 25 cases of coexistence. Thus, there was no apparent sex difference in the numbers of mENKir cells, however differences in the size of the E-conc. population resulted in differential proportions of doubly labeled cells. In males, 48% of the E-conc. cells contained mENK and only 26% of the E-conc. cells were mENKir in females. These findings identify a subset of mENKir neurons that are directly activated by estrogen and a sex difference which may be importantly involved in the regulation of reproductive behavior. (Supported by HD 22869 to TRA and NS 21220 to PEM).

233.10

ESTROGEN-CONCENTRATING CELLS WITHIN THE MEDIAL PREOPTIC AREA: SEX DIFFERENCES AND COLOCALIZATION WITH GALANIN-IMMUNOREACTIVE (GAL-I) CELLS.

GJ BLoch*, RA Gorski, PE Micevych and TR Akesson, Dept. of Anatomy and Cell Biology, Lab. of Neuroendocrinology, UCLA School of Medicine, Los Angeles, CA 90024.

The distribution of GAL-I cells in the medial preoptic area (MPOA) overlaps with the distribution of estrogen-concentrating cells, and because estrogen (E) is also known to increase anterior pituitary GAL mRNA and plasma GAL levels [Kaplan et al., Proc. Nat. Acad. Sci., 85:7408, '88], we looked for evidence of E-concentration by GAL-I cells within the rat MPOA. In addition, because of the larger size of the anteroventral periventricular nucleus (AVPv) in females [Bloch and Gorski, J. Comp. Neurol., 275:613, '88], we compared the number of E-concentrating, GAL-containing cells within the AVPv of the female with that in the male. Approximately 33% of the E-concentrating cells within the central portion of the medial preoptic nucleus (MPNc), its medial portion (MPNm), lateral portion (MPNl), and the AVPv were GAL-I in males and females, which represented 47% of the GAL-I cells. The number of estrogen-concentrating and doubly labeled cells was greater within the AVPv of females than males. Within the MPN of each sex, the density of doubly labeled cells was highest in the MPNc and decreased progressively in the MPNm and MPNl. The high percentage of doubly labeled cells suggests that GAL within the MPOA may participate in the regulation of gonadal steroid-sensitive reproductive function. (Supported by HD 01182 to RAG, NS 21220 to PEM and HD 22869 to TRA).

233.11

POSSIBLE AFFERENT PROJECTIONS TO THE SPINAL NUCLEUS OF THE BULBOCAVERNOSUS IN ADULT MALE RATS.
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The spinal nucleus of the bulbocavernosus (SNB) consists of androgen-sensitive motoneurons in the rat spinal cord. The SNB motoneurons and their target muscles are present in adult male rats but reduced or absent in adult females. To begin to define an androgen-sensitive neural pathway from the brain to the periphery, we attempted to identify supraspinal afferents to the SNB. Fluorogold (5% in distilled water) was iontophoresed (5uA, 20min, 30um electrode tip) into the SNB region of adult male rats. After approximately a four-week survival time, the rats were sacrificed, perfused, and their brains and spinal cords isolated. The spinal cords were cut into 50um sections and viewed under a fluorescence microscope. Brains from animals with good injection sites in the spinal cords were cut at 80um and viewed under fluorescence. The brain sites which contained a significant number of fluorescently labeled cells were the gigantocellular reticular nucleus (including the ventral and alpha nuclei) and the lateral vestibular nucleus. Many labeled cells were found in the ventral medullary reticular nucleus, raphe obscurus nucleus, raphe magnus nucleus, and the caudal pontine reticular nucleus. Labeled cells were also observed in the paramedian reticular nucleus, the spinal vestibular nucleus and the paraventricular nucleus of the hypothalamus. Supported by NIH grants NS23468, GM07185 and HD15021.

233.12

CALCITONIN GENE-RELATED PEPTIDE mRNA IN MOTONEURONS: EFFECT OF CASTRATION. P. Popper and P.E Micevych. Dept Anatomy & Cell Biology, Laboratory of Neuroendocrinology, Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

Calcitonin gene-related peptide (CGRP)-like immunoreactivity is widely distributed in motoneurons of the spinal cord and brainstem. Within the spinal nucleus of the bulbocavernosus (SNB) the number of CGRP-positive motoneurons increases following castration, and testosterone reduces the number to intact levels. In the present study we used in situ hybridization to investigate the effect of testosterone on steady state levels of CGRP mRNA in adult male rat SNB. Eighteen adult male Long Evans rats were used. Six were intact, six were castrated for four weeks, and six were castrated and implanted with testosterone-containing Silastic tubing. Rats were killed by intracardiac perfusion of 4% paraformaldehyde in 0.1M phosphate buffer and the spinal cords removed. The spinal cord were postfixed for 2 hours and then cryoprotected with 10% sucrose in phosphate-buffered saline. Serial sections (20 um) were cut at -15 °C on a cryostat. The sections were stored at -70°C until hybridization. Tissue sections were processed for in situ hybridization using a ^{35}S-labelled single-stranded cRNA transcribed from a genomic probe from the 3' end of the calcitonin gene (gift from Dr. Rosenfeld, UCSD, La Jolla) and a ^{35}S-oligonucleotide probe complementary to the mRNA segment coding for residues 7-21 of CGRP. Motoneurons containing message for CGRP were identified by overlying silver grains. At the level of L5 and L6, motoneurons in the retrodorsolateral nucleus, dorsolateral nucleus, ventral motor pool, and SNB were observed to contain CGRP mRNA. Comparisons across treatment groups were analyzed using ANOVA. Supported by NS23468 to PEM.

233.13

CALCITONIN GENE-RELATED PEPTIDE LIKE-IMMUNOREACTIVITY IN MOUSE SPINAL NUCLEUS OF THE BULBOCAVERNOSUS IS AFFECTED BY CASTRATION. L.Clemens, C.Wagner, P.Popper, C.Ulibarri, & P.E Micevych. Dept Zoology & Neuroscience Program, Michigan State University, East Lansing, MI 48824, Dept Anatomy & Cell Biology, Laboratory of Neuroendocrinology, Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

The mouse spinal nucleus of the bulbocavernosus (SNB) is located at levels L5-L6. It contains approximately 100 motoneurons that innervate primarily the bulbocavernosus muscle. In rats calcitonin gene-related peptide like-immunoreactivity (CGRP-LI) in SNB motoneurons is dependent on circulating testosterone levels. In this study immunohistochemistry was used to investigate the changes in CGRP-LI in SNB motoneurons in male B6D2F1 mice that were tested for male sex behavior. Comparisons were made between sham castrated males, castrated males that continued to exhibit male sex behavior eleven weeks after castration (continuers), castrated males that did not (non-continuers), and males that were castrated for four weeks and not tested for male sexual behavior. Mice were tested for male sexual behavior and then either castrated or sham-castrated (N = 10). Five weeks later, they were retested for six weeks. Castrated males were divided into continuers (N = 10) and non-continuers (N = 7). Mice were perfused with 4% paraformaldehyde in 0.1M Sorenson's buffer. The spinal cords were removed, postfixed for 2-3 hours and cryoprotected. Serial sections (30 um) were taken through the SNB. Alternate sections were processed for CGRP-LI according to avidin-biotin peroxidase-antiperoxidase method using polyclonal anitbodies raised in rabbits (gift from Dr. Sternini, CURE, UCLA). The nuclei of CGRP-LI containing neurons and unstained were counted. In sham-castrated males 45.3$\pm$2.8% of the SNB neurons had CGRP-LI. There was a significant decrease in CGRP-LI neurons after castration [ANOVA $F(2,24) = 7.10$, $p < 0.01$)]. In continuers, 25.3$\pm$4.3% and in non-continuers, 20.2$\pm$3.2% of the SNB neurons had CGRP-LI. There were no differences between continuers and non-continuers, [Orthogonal comparison $F(1,24) = 0.73$, ns]. Supported by NS 23468 and HD 06760.

ENDOCRINE CONTROL AND DEVELOPMENT IV

234.1

MATCHING OF PRE- AND POSTSYNAPTIC SIZE IN NEUROMUSCULAR JUNCTIONS OF ANDROGEN-SENSITIVE MUSCLES. N. Nagaya and A.A. Herrera, Dept. of Biological Sciences, University of Southern California, Los Angeles, CA 90089.

The development and maintenance of synapses involves mechanisms which match pre- and postsynaptic structure and function. In vertebrate skeletal muscles, such mechanisms may account for the well-documented correlation between neuromuscular junction (NMJ) and muscle fiber size. We are using histology to examine this relationship in the forearm flexors of male Xenopus laevis. These muscles are sexually dimorphic, mixed in fiber type, and undergo seasonal hypertrophy or atrophy as plasma androgen levels increase or decrease, respectively. Experimental manipulation of androgen levels mimics these seasonal changes. In non-sexually dimorphic muscles, we found that the correlation between NMJ and fiber size is maintained throughout life and tends to maintain synaptic efficacy during growth. In androgen-sensitive muscles, however, there is no overall correlation between NMJ and fiber size. Histology also reveals more abandoned synaptic sites than in non-sexually dimorphic muscles, suggesting extensive remodelling. Our inability to detect a correlation may be due to androgen-induced synaptic remodelling. In addition, the relation between NMJ and fiber size may vary between fiber types. Observations to test these possibilities are in progress.

234.2

HORMONE MANIPULATION AND THE DEVELOPMENT OF LARYNGEAL MUSCLE FIBER NUMBER IN *XENOPUS LAEVIS*. Melanie Marin*, Martha Tobias, and Darcy Kelley, Dept. of Biol. Sci., Columbia Univ., N.Y., N.Y. 10027.

Sex differences in the larynx of *Xenopus laevis* contribute substantially to sex specific vocal behavior. Adult male larynges have more muscle fibers than females. Antiandrogen treatment prevents fiber addition in juvenile males (Sassoon and Kelley, Am. J. Anat., 1986). When during development does this sexual difference first appear? Does androgen secretion affect muscle fiber addition, and if so, when?

At metamorphosis, both males and females average 7,000 laryngeal muscle fibers. Throughout the first year of post-metamorphic (PM) development males add fibers at a much faster rate than females. At 3 mos (PM), fiber numbers are 20,000 for males and 12,000 for females; by 6 mos PM, males average 31,000 and females 11,000 fibers. Adult males have 35,000 and females 20,000 fibers. Testosterone (T) treatment for 5 wks at metamorphosis does not increase fiber number in intact males or females. At 6 mos PM, T treatment significantly decreases fiber number in males (to 24,000) but does not affect females. Castration at metamorphosis retards muscle fiber addition, resulting in only 12,500 (38% of expected) at 9 mos PM. Castration at 6 mos PM, however, does not block further fiber addition. Subsequent T treatment does not affect fiber number. Females ovariectomized and given testes at metamorphosis have significantly increased fiber numbers (26,000) at 9 mos PM.

These results suggest that gonadal hormones play a significant role in the development of laryngeal muscle fiber number. The castration experiments place this period of hormonal control between metamorphosis and nine months PM. That any muscle fibers at all are added after castration raises the possibility that events before metamorphosis set the stage for later myogenesis. Supported by NS 19949.

234.3

SLOW MUSCLE FIBERS WITH MULTIPLE TERMINALS IN THE SONGBIRD SYRINX W. Bleisch, C. Scharff* and F. Nottebohm Laboratory of Animal Behavior, Rockefeller University, NY, NY 10021

The muscles of the syrinx, the vocal organ of birds, contain several types of fibers. The female syrinx has a higher proportion of slow fibers than the male syrinx and testosterone treatment causes females to exhibit a male-like pattern. Most syringeal fibers are innervated at single focal terminals, but some slow fibers receive multiple terminals. To study this heterogeneity of fiber type and innervation, we combined immunocytochemistry to classify zebra finch muscle fibers with techniques to visualize neuromuscular junctions. One monoclonal antibody, mAb S58, directed against a slow class of myosin, labels only fibers which have multiple terminals. We also examined the distribution of immunoreactivity for neural cell adhesion molecule (NCAM). S58 positive fibers have elevated NCAM immunoreactivity, indicating that most, and perhaps all, fibers which have multiple terminals express high levels of NCAM. This suggests that NCAM may play a role in the maintenance of multi-terminal innervation in adult muscle.

234.4

ANDROGENIC REGULATION OF MOTOR UNITS IN SEXUALLY DIMORPHIC MUSCLES OF FROGS. M. Regnier and A.A. Herrera, Dept. Biol. Sci., Univ. of Southern Calif., Los Angeles, CA 90089.

Male frogs use their forearm flexors to clasp females during mating. Muscle size and contractility have been shown to vary in response to seasonal fluctuations in circulating androgens, with muscles being larger and more slowly contracting during the breeding season. Castration and androgen treatment cause changes that mimic these seasonal differences. In confirmation of previous work we found that castration reduces fiber size by 20-40% in two androgen sensitive forearm flexors (sternoradialis & flexor carpi radialis of Xenopus & Rana). The non sexually dimorphic sartorius muscle was not affected significantly by castration. To determine the cellular basis of these androgen effects we are correlating changes in muscle size and contractility with the histochemical properties of fibers. Preliminary evidence suggests that the capacity for oxidative metabolism is reduced by castration. Work is in progress to confirm this finding and measure the fatigue resistance and contraction kinetics of these muscles. We are also examining the possible roles of myogenesis, fiber death, and fiber type conversion in these changes, as well as testing whether motor units are equally or differentially sensitive to androgens.

234.5

ANDROGENIC REGULATION OF MOTONEURONAL DENRITIC MORPHOLOGY IN THE ADULT RAT. M. Sasaki* and A.P. Arnold, Dept. Psychology, UCLA, Los Angeles CA 90024-1563.

Previous reports indicated that androgens regulate the soma size and dendritic length of motoneurons in the spinal nucleus of the bulbocavernosus (SNB) of the adult rat. To replicate and extend these findings, we made quantitative measurements of the morphology of SNB dendrites after intracellular iontophoresis of HRP. Adult male rats were castrated and implanted with Silastic tubes containing testosterone (T) or nothing. One month later, bulbocavernosus motoneurons were identified by antidromic activation, and then stained by intracellular iontophoresis of HRP for reconstruction and measurement of individual dendritic trees.

As previously reported, soma size was significantly larger in T-treated castrates (1173 ± 43 um^2, n=31) than in blank-treated castrates (894 ± 53 um^2, n=28). However, there was no significant difference in overall dendritic length per neuron between groups (T-treated castrates: 22,170 $\pm$1048 um, n=10; blank-treated castrates: 21,676 $\pm$ 988 um, n=11). However, there were significant positive correlations between the cross-sectional area of the soma and the following parameters: total dendritic length, dendritic membrane surface area, and dendritic volume. Because soma size differs between groups, a modest effect of androgen on dendritic length would be expected in a larger sample of dendrites. Nevertheless, these results suggest that the effect of androgen on dendritic length is less than previously reported. Interestingly, dendritic growth cones were found at the distal ends of dendrites, most prominently in T-treated castrates and in the larger motoneurons of castrates, suggesting that these dendrites grow during adulthood. Supported by NIH grant HD15021.

234.6

EFFECT OF SEX AND ANDROGEN TREATMENT ON DENDRITIC DIMENSIONS IN THE FERRET PREOPTIC AREA. J.A. Cherry, M.J. Baum and T.J. DeVoogd, Dept. Biology, Boston Univ., Boston MA 02215 and Dept. Psychology, Cornell Univ., Ithaca, NY 14853.

In male ferrets a nucleus exists dorsally at the border of the preoptic area (POA) and anterior hypothalamus (AH) which is not present in females. Dendritic characteristics of neurons in the dorsal POA/AH were studied using Golgi-Cox stained brains from adult male and female (n=4/group) ferrets which had been gonadectomized and treated daily for 5 weeks with either testosterone propionate (TP, 5 mg/kg) or oil vehicle. Dendrites from multipolar neurons (n$\geq$15 neurons/group) were traced 3-dimensionally from 120 um sections with the aid of a semi-automated computer and microscope system; adjacent sections were used when necessary to follow cut-off dendrites. Somal areas were measured on a digitizing tablet.

Overall effects of Sex (2-way ANOVA, $p<0.05$) were found in measures of total dendritic length, dendritic density (the number of intersections of dendrites with concentric spheres of increasing radii from the cell body), and median radial distance (the distance at which there are an equal number of intersections proximal and distal to the cell body). There were no overall effects of Hormone on any measure, but significant Sex X Hormone interactions were observed for all three variables. These interactions were due to an apparently inhibitory effect of TP on dendritic dimensions in females together with a smaller trophic effect of TP in male dendrites. In contrast, dendritic measures were similar for oil-treated males and females. Mean somal area of male cells was significantly greater than females, irrespective of adult treatment. These results suggest that in the dorsal POA/AH of ferrets, dendrites of male and female multipolar neurons respond differently to androgen exposure in adulthood.

234.7

DEVELOPMENT OF SEX DIFFERENCES IN DENDRITIC LENGTH IN *XENOPUS LAEVIS* LARYNGEAL MOTOR NEURONS . James Watson and Darcy Kelley. Dept. Biol. Sci., Columbia Univ., N.Y., N.Y. 10027

In adult *X. laevis*, laryngeal motor neurons are more numerous and have longer dendrites in males than in females. The number of axons innervating the larynx becomes dimorphic before metamorphosis (Dennison and Kelley, Abstr. Soc. Neurosci., 1989), and laryngeal muscle dimorphism is established by postmetamorphic fiber addition (Sassoon and Kelley, Am. J. Anat., 1986). To determine how and when CNS sex differences are established, laryngeal motor neuron morphology was examined using the fluorescent dye, DiI. Metamorphic male (n=2) and female (n=3) froglets were fixed, their brains were embedded in 15% gelatin, and the 4th root of the IX-X cranial nerve complex was injected with DiI in ethanol. After 4-5 days at 37°C, 50 µm sections were prepared with a vibratome and micrographed. Primary apical dendrites were measured on type I and II laryngeal motor neurons (males, n=15; females, n=29; terminology of Kelley et al., J. Neurobiol., 1988). Males were found to have longer dendrites than females (Mean $\pm$ SD: 28.6 ± 5.2 µm vs. 23.7 ± 8 µm; $p<0.05$). This sex difference was due to a greater percentage of female neurons having short dendrites, male dendrites were uniformly long. Previous Golgi impregnations of n. IX-X neurons in adults revealed that dendrites were longer in both sexes (30-60µm), the variability in female dendritic length was small, and the sex difference in primary dendritic length was greater than was found here (meta: 1.2:1; adult: 2:1). Thus, while a sex difference in dendritic length is apparent by metamorphosis, a different adult pattern may be established by post-metamorphic dendritic remodeling in either sex or cell loss in females. Supported by NS23684 and NS08403.

234.8

DELAYED EXPOSURE TO GONADAL STEROIDS ENABLES VOCAL LEARNING IN ADULT ZEBRA FINCHES. S.W. Bottjer & S.J. Hewer. Department of Biology, USC, Los Angeles, CA 90089-0371.

Male zebra finches normally learn a specific song pattern during development. By the time they reach adulthood (>90 days), the song pattern has "crystallized"; this stereotyped song pattern is maintained without change throughout adulthood, and zebra finches are normally incapable of learning new song patterns as adults. We have previously reported that the development of learned vocal behavior in juvenile birds is dependent on circulating gonadal steroids: castration of juvenile male zebra finches combined with systemic exposure to anti-steroid drugs disrupts normal development of song production (Bottjer & Hewer, 1988). We wondered if delayed exposure to steroid hormones would result in any improvement in vocal behavior of such birds as adults; such an outcome might indicate that lack of exposure to steroids during development could prolong the so-called critical period for vocal learning.

We addressed this question as follows: males were anesthetized and castrated at 20 days of age. They received Silastic implants of an anti-androgen (flutamide) and/or an anti-estrogen (tamoxifen) from 20 to 95 days, during which time their vocal behavior was recorded periodically. At 95 days the anti-steroid implants were replaced with one containing testosterone, and song behavior was recorded for an additional 6 weeks. The vocal behavior of adult birds improved substantially in response to steroid exposure. This result suggests that normal exposure to steroids contributes to the curtailment of the capacity for song learning, and that the critical period for vocal learning can be extended by preventing such exposure.

234.9

CHRONIC TESTOSTERONE TREATMENT IMPAIRS VOCAL LEARNING IN MALE ZEBRA FINCHES DURING A RESTRICTED PERIOD OF DEVELOPMENT. S. Korsia & S.W. Bottjer. Dept. of Biology, USC, Los Angeles, CA 90089-0371.

Juvenile male zebra finches learn their song during a specific period of development. We have previously reported that development of learned vocal behavior in juvenile males is impaired by chronic administration of testosterone (T) from the day of birth until adulthood. T-treated males produce a very limited number of syllables and tend to repeat a single syllable in a pattern lacking the phrasing characteristic of normal song (Korsia & Bottjer, 1988). We investigated whether chronic testosterone administration later in development has a similar disruptive effect on the process of song learning. Normal development of song behavior in the case of delayed treatment might indicate the existence of a restricted sensitive period for susceptibility to chronic testosterone exposure.

Male birds were divided into three groups: one group received subcutaneous implants of T from the day of birth until day 20, at which age they received 1 or 2 Silastic implants packed with 10 mm of crystalline T; the second group received 1 or 2 Silastic T implants at day 20; the third group received 1 or 2 Silastic T implants at day 25. Song behavior was recorded until day 90, when stereotyped song has crystallized. Chronic T administration at day 25 failed to disrupt vocal learning in about 50% of the birds, whereas earlier treatment always impaired song development. This result suggests that chronic T treatment disrupts song acquisition only when administered during early life.

234.10

THE DEVELOPMENT OF AFFERENT PROJECTIONS TO RA IN MALE ZEBRA FINCHES: AN EM STUDY. K. Herrmann and A.P. Arnold. Dept. of Psychology, UCLA, Los Angeles, CA 90024-1563.

We used quantitative electron microscopic techniques to measure the development of synaptic input to the neuropil of nucleus RA in young zebra finches, since the ontogeny of these projections figures prominently in current theories of song learning and sexual differentiation of the brain. We lesioned HVc or MAN in male zebra finches of different ages: 25 days (d), 53d, and adulthood. After a survival time of 60-80 hours (HVc-lesion) or 80-120 hours (MAN-lesion), the animals were perfused, and tissue blocks including RA were dissected out and processed according to conventional electron microscopic techniques. Lesion-induced electron-dense degeneration was used to identify synapses from either HVc or MAN occurring in the neuropil of RA. Untreated control animals of the same age were also analyzed.

In control animals the density of synapses/100 um^2 increased slightly between day 25 and 53 and remained constant thereafter (25d:12.69; 53d: 15.42; adult:14.85). The occurrence of naturally degenerating synapses was fairly low in controls (25d:1%; 53d:0.8%; adult:0.6%). The incidence of terminals of HVc origin declined slightly with age (25d:10.53%; 53d:6.01%; adult:8.32%), whereas the terminals from MAN axons decreased more drastically (25d: 22.32%; 53d:4.55%; adult:1.41%). The ratio of axospinous to axodendritic terminals was 3:2 for HVc-originating terminals and 1:1 for those from MAN, irrespective of age. These values can be used to calculate the total number of synapses of each type in RA during development. These results imply that song learning is correlated with a reorganization of HVc and MAN synapses in RA. Supported by DFG grant He1539/1-1 and NIH grant NS19645.

234.11

TAMOXIFEN FAILS TO BLOCK ESTRADIOL-INDUCED MASCULINIZATION OF THE ZEBRA FINCH SONG SYSTEM. G.A.Mathews* & A.P.Arnold. (SPON:H.-J.Bischof). Department of Psychology, UCLA, Los Angeles, CA 90024-1563.

Estradiol masculinizes the female zebra finch song system while antiestrogens masculinize the female and hypermasculinize the male song systems. Although antiestrogens can be estrogenic when administered alone, they are typically antiestrogenic when administered with estradiol. We asked whether tamoxifen was capable of being an antiestrogen in the song system by antagonizing the effects of exogenous estrogen.

Zebra finch chicks received daily subcutaneous injections of 100ug tamoxifen (TAM), 20ug estradiol benzoate (EB), 100ug TAM combined with 20ug EB (TAM+EB), or vehicle, for the first 20 days after hatching. At 60 days the animals were killed and their brains were sectioned at 50um and stained with thionin for light microscopic analysis.

In females neuronal soma area (n.s.a.) in HVc and HVc volume were increased by EB alone (by 100% & 54% respectively, over control) and TAM alone (68% & 71%). Both compounds induced Area Xs in females. In addition, TAM increased n.s.a. in female RA (51%). In males n.s.a. in MAN and HVc were increased by EB alone (15% & 81%) and TAM alone (22% & 40%). TAM alone in males also increased n.s.a. in RA and Area X volume. TAM potentiated the EB effects: In females, TAM+EB increased volumes of Area X (92%), HVc (43%), and RA (64%), over EB alone. In males, n.s.a. in MAN was increased by TAM+EB (18%) over EB alone. These results show that simultaneous treatment with tamoxifen and estradiol does not prevent the masculinization caused by estradiol alone, suggesting an estrogenic role for tamoxifen in its effects on the song system. Supported by NIH grant NS19645.

234.12

NEURAL PLASTICITY IN HVC RELATED TO SONG ACQUISITION IN THE DEVELOPING ZEBRA FINCH. R.P. Clower and T.J. DeVoogd. Dept. of Psychology, Cornell University, Ithaca, NY 14853.

The principal song control nuclei of adult male zebra finches are much larger than those of adult females. These differences arise during development, when males are acquiring their permanent repetoires. Since females normally never sing, there may be specific anatomical requirements for learning to sing which are only met in young males. It is during the second month after hatching that males learn most of their adult repetoires, but closure of the sensitive period for acquisition of specific syllables appears to be delayed when young males are deprived of an appropriate song model. The present research studies the structural plasticity necessary for song learning by using multi-level analyses of male and female song control areas in young and adult normal animals and in animals deprived of exposure to song.

RA and HVc in normal male and female zebra finches at 10, 20, 30, 55 and 100+ days of age, as well as in males raised to 30 and 55 days by females only, were evaluated using an array of light and electron microscopic measures. In HVc of normally raised males, there is a peak at day 55 in total neuron number; the nucleus contains 33% more neurons at this age than at 100+ days (adulthood) ($p<.02$). Together with trends toward a 55 day peak in HVc volume and total synapses, this peak may represent an overproliferated state that disappears as song patterns stabilize. In HVc, there appear to be more synapses/neuron in females than in males across development ($p<.02$), possibly due to the presence in male HVc of many small, newly generated neurons that support relatively few contacts. At day 30, PST's in HVc of males raised by females only are 14% shorter than in HVc of normally raised males ($p<.02$), and neuronal density tends to be lower in HVc of song deprived males. Since these differences do not exist at day 55, it appears that some aspects of song system development are delayed when an appropriate song model is unavailable.

Overall, these results suggest that multi-level plasticity in HVc is important for early song acquisition in male zebra finches, and in particular that the continuation of neurogenesis in male HVc through the second month of development may play a critical role in permitting the actual production of song in males.

234.13

HORMONAL CONTROL OF TESTOSTERONE-METABOLIZING ENZYMES IN THE BRAIN OF THE ZEBRA FINCH.

A. Vockel* and J. Balthazart, Lab. General. and Comparative Biochemistry, University of Liège, Belgium. (SPON: ENA)

We recently described the distribution of testosterone-metabolizing enzymes (aromatase, 5α- and 5β-reductases) in the zebra finch (*Taeniopygia guttata*) brain using radioenzyme assays combined to the Palkovits punch method. A number of sex-differences in the activity of these enzymes were observed especially in nuclei of the song-control system. The hormonal controls of these differences have now been analyzed by gonadectomizing birds of both sexes and giving them a replacement therapy with silastic implants of testosterone (T). Five nuclei of the song system (*X, MAN, RA, ICo, HVc,*) and 3 preoptic-hypothalamic areas (preoptic anterior [POA], periventricular magnocellular nucleus [PVM] and posterior medial hypothalamic nucleus [PMH]) were studied. Data were analyzed by two-way ANOVAS with sex (male/ female) and treatments (intact/ gonadectomized/ gonadectomized+T) as factors. The activity of the 5α-reductase was higher in males than females for the 5 song control nuclei and was not affected by the treatments. On the contrary, the activity of this enzyme was not sexually dimorphic but was enhanced by T in POA, PVM and PMH. The activity of the 5β-reductase was higher in females than in males in all nuclei of the song system and in POA but was not influenced by the changes in T level in any of these nuclei. Both sex and treatment effects were observed in the control of the aromatase. The production of estrogens was dimorphic (females>males) in RA and PMH (and also in HVc of intact birds). It was increased by T in POA, PVM and PMH and also in RA but not in HVc. These data show that some of the sex differences in T-metabolizing enzymes result from the exposure to different levels of T while others persist even if birds are exposed to the same hormonal conditions. These are presumably the result of organizational effects of steroids.

234.14

ENDOCRINE AND NEUROENDOCRINE SYSTEMS DURING SEXUAL DIFFERENTIATION IN JAPANESE QUAIL. M.A. Ottinger, M.A. Abdelnabi*, Quichang Li* and B. Alston-Mills*. Depts. of Poultry Science and Animal Sciences, Univ. of Maryland, College Park, MD 20742.

Gonadal steroids stimulate permanent effects on central neuroendocrine systems during sexual differentiation. Experiments were conducted to monitor changes in gonadal steroids in blood, gonads and adrenal glands and hypothalamic levels of monoamines and luteinizing hormone releasing hormone (LHRH) in male and female quail between day 10, posthatch through day 7, posthatch. In males, levels of testosterone (T) peaked late in embryonic development; estradiol (E) levels remained relatively lower. In females, E was elevated relative to T in all samples, increased throughout development and declined posthatch. Hypothalamic LHRH concentrations also increased late in the embryonic development. Similarly, hypothalamic monoamines (norepinephrine, dopamine, epinephrine and serotonin) peaked late in embryonic development with a minor peak at day 1, posthatch. These data give support for early function of the endocrine system with involvement of the monoaminergic systems, particularly during the time of sexual differentiation.

235.1

DORSAL RAPHE STIMULATION MODIFIES DENDRITIC SPIKING OF NIGRAL DOPAMINERGIC NEURONS *IN VIVO* F. Trent*, and J.M. Tepper (SPON: I. Creese)
Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark, NJ 07102

A serotonergic projection from the dorsal raphé nucleus (DR) to dopaminergic (DA) neurons of the substantia nigra, pars compacta has been identified at both the light and electron microscopic levels. *In vitro* intracellular recordings from nigral DA neurons have shown that application of serotonergic agonists results in an increase in the amplitude of dendritic calcium spikes evoked by direct intracellular depolarization, suggesting a possible role for serotonergic afferents in the modulation of dendritic dopamine release in substantia nigra. The present study was designed to examine the effects of DR stimulation on the excitability of the somadendritic region of nigral DA neurons *in vivo*, in order to test the physiological significance of the *in vitro* studies.

Adult male Sprague-Dawley rats were anesthetized with urethane, and bipolar stimulating electrodes were implanted in the anterior-lateral neostriatum and DR. Extracellular single unit activity of nigral dopaminergic neurons was recorded by conventional means, and antidromic responses were elicited from the neostriatal stimulating site. Antidromic responses of nigral DA neurons consist of either an initial segment (IS) spike only, or a full IS-somadendritic (SD) spike, with IS-only responses predominating. The proportion of full IS-SD antidromic responses to total antidromic responses was used as an index of somadendritic excitability. Only neurons exhibiting at least 15% IS-SD antidromic responses were studied.

In the control condition, antidromic responses to neostriatal stimuli at the antidromic threshold consisted of 33.1±2.7% IS-SD spikes. Electrical stimulation of the DR with a train of 1-5 pulses (250 μA, 250 μs) delivered 2-7 ms prior to the antidromic response significantly and reversibly reduced this proportion to 24.4±3.0% (t=5.7, df=22, p<.0001), without significantly altering the neostriatal-evoked post-stimulus inhibitory period. Admininstration of the serotonergic receptor antagonist, metergoline (0.5 - 1.0 mg/kg, i.v., n=4), eliminated the DR-induced reduction in IS-SD spikes, suggesting that DA dendrites are subject to modulation by serotonergic afferents *in vivo*. Further studies are needed to determine whether this effect is mediated by direct DR-nigral afferents or indirectly, through DR-striatal projections.

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235.2

SEROTONERGIC INVOLVEMENT IN THE CLASSICAL DOPAMINE MEDIATED STEREOTYPY AND CATALEPSY. E.S. Onaivi*,B. Costall* and R. J. Naylor*, (SPON: D.A. Brase) Dept. Pharmacology and Toxicology, MCV/VCU, Richmond, VA 23298, and Postgraduate School of Studies in Pharmacology, University of Bradford, Bradford, BD7 1DP, England.

Dopamine (DA) agonists induce stereotypy while the classical antagonists produce catalepsy in laboratory animals. In this study we assessed the nature of serotonergic (5-HT) influence on dopamine mediated behaviors and the neurochemical correlates using HPLC with electrochemical detection. The stereotype behavior of BKW mice in the following groups: (a) Vehicle, (b) apomorpine (0.25-5 mg/kg), (c) 5-hydroxytrptophan (5-HTP; 200-400 mg/kg) and (d) 5-HTP plus apomorphine were assessed in individual perspex observation cages using a modification of the rating system of Naylor and Olley. Haloperidol-induced catalepsy in naive and following dorsal or medial raphe nucleus (DRN or MRN) lesions were evaluated. 5-HTP maximally potentiated the apomorphine stereotypic response at doses which caused significant changes in striatal and limbic brain neurochemistry implicating raised levels of DA, and 5-HT in animals treated with 5-HTP plus apomorphine when compared to mice treated with vehicle, apomorphine or 5-HTP alone. The electrolesion of DRN but not the MRN reduced the intensity of haloperidol-induced catalepsy whereas the MRN lesions actually potentiated this response. The results suggests an interaction of serotonergic mechanisms on these classical DA dependent behaviors. Although, the neuronal and molecular events leading to serotonergic and dopaminergic interactions are not fully understood, the control of dopamine neurotransmission by serotonergic mechanisms are being exploited.

235.3

SEROTONIN (5-HT) REGULATES CHOLINE ACETYLTRANSFERASE (ChAT) ACTIVITY AND SUBSTANCE P IN THE DEVELOPING STRIATUM. L. Ni*, S. Schotland*, and G.M. Jonakait (SPON: R.P. Hart), Dept. of Biological Sciences, Rutgers University, Newark, N.J. 07102

To assess the effects of serotonergic innervation on cholinergic and substance P (SP)-containing neurons of the developing striatum, 3-day-old rat pups received 5,7-dihydroxytryptamine intracisternally to destroy serotonergic neurons. Tryptophan hydroxylase activity in the cortex fell 76-82%, confirming a successful lesion. Four days after the lesion, ChAT activity in the striatum fell 34±4.0% ($p<.01$), but recovered after 4 weeks to control levels. By contrast, SP levels remained normal during the first week after surgery, but fell to 56±3.1% ($p<.02$) of control thereafter. SP levels had not recovered 4 weeks after surgery.

To determine whether the direct action of 5-HT on striatal cells might account for maintenance of ChAT activity, organotypic tissue cultures of E16 rat striata were grown for 10 days. Cultured striata lack all afferent input. In the presence of 5-HT agonists 8-OH-DPAT (10uM) and alpha-methyl-5-HT (2uM), ChAT activity increased 150-166%. Agonist-induced increases in ChAT were blocked by mianserin (1 or 10 uM) and ketanserin (2 uM) respectively. These data suggest that the direct activation of 5-HT receptors within the developing striatum can facilitate the restoration of ChAT activity in denervated striata.

Supported by MH43365 and RR07059-22. GMJ is a Johnson & Johnson Discovery Research Fellow.

235.4

NEOSTRIATAL PREPROTACHYKININ (PPT) mRNA FALLS FOLLOWING INHIBITION OF SEROTONIN (5-HT) SYNTHESIS. P. D. Walker, L. Ni*,S. Schotland*, R.P. Hart and G. M. Jonakait. Dept. of Biological Sciences, Rutgers University, Newark, NJ 07102.

Substance P is a neurotransmitter in projection neurons of the neostriatum (NS). Following blockade of afferent dopamine neurotransmission, striatal SP peptide and PPT (the peptide precursor of SP) mRNA levels are decreased (Bannon et al., Brain Res. 3:31, 1987). Since the NS also receives a substantial serotonergic projection from the midbrain raphe, we sought to determine whether 5-HT inhibition alters NS PPT mRNA levels.

Adult female rats received Alzet minipumps (Model 2ML2) containing *p*-chlorophenylalanine (*p*CPA). *p*CPA suppresses 5-HT formation by inhibiting tryptophan hydroxylase (TPH). Each pump delivered 100 mg/kg/day of *p*CPA for 14 days. Control animals received sham surgery but no pump. TPH activity in dorsal raphe nuclei from *p*CPA-treated animals was inhibited by 70-80% after 1, 2, and 3 weeks. At these times total NS RNA was subjected to Northern blot analysis using a radioactive RNA probe for rat PPT. PPT mRNA levels were normalized to levels of 28S ribosomal RNA. One week after pump installation, striatal PPT mRNA had fallen to 18%, but recovered gradually over the next two weeks to 65% of control.

These results suggest that serotonin plays a role in the regulation of SP biosynthesis in the NS. Because the midbrain raphe projects to substantia nigra as well as NS, further studies are required to determine whether the effects of 5-HT inhibition on SP biosynthesis in the NS are mediated by the nigrostriatal pathway. Funded by MH43365, and AHA-NJ. GMJ and RPH are Johnson & Johnson Discovery Research Fellows.

235.5

DOPAMINERGIC REGULATION OF STRIATAL NEUROPEPTIDE Y SYSTEMS. L. Midgley*, K.Merchant*, L. Bush*, J.W. Gibb, G.R. Hanson. (SPON:F.Matsuo). Univ. of Utah, Salt Lake City, UT 84112.

Recent reports have shown that the number of striatal neuropeptide Y (NPY) neurons, as well as their labeling intensity, are decreased by treatment with multiple daily doses of the dopamine (DA) antagonist, haloperidol (Neurosci. 26 [1988] 819). This finding suggests that the striatal NPY system is regulated by dopaminergic mechanisms. To study this possibility further, we administered the potent indirect DA agonist, methamphetamine (METH), and assessed the response of the striatal NPY system by measuring striatal content of NPY-like immunoreactivity. Eighteen hours after METH treatment (5 doses, 10 mg/kg/dose given every 6 hr), NPY levels were significantly decreased to 73% of control. In order to characterize the nature of this response, selective D-1 (SCH 23390) and D-2 (sulpiride) antagonists were given alone and coadministered with METH. Treatment with sulpiride alone decreased striatal NPY levels to 52% of control and when combined with METH, NPY content decreased further to 42% of control. In contrast, SCH 23390 administered alone had no effect on striatal NPY content, but may have attenuated the METH effect as NPY levels decreased to only 85% in animals which received both drugs. These findings suggest that D-2, and possibly D-1, receptors have a role in the regulation of striatal NPY projections. (Supported by USPHS grants DA 00869 and DA 04222).

235.6

NEUROTENSIN REGULATES ENDOGENOUS ACETYLCHOLINE RELEASE FROM RAT BRAIN SLICES: REGIONAL SPECIFICITY. A. Beaudet, D.M. Araujo, R. Quirion and P.A. Lapchak. Neuroanat. Lab, Mtl. Neurol. Inst. and McGill Univ., Dept. of Psychiatry, Montreal, Quebec, Canada.

Neurotensin (NT) receptors were recently shown to be selectively associated with the perikarya and dendrites of basal forebrain cholinergic (ACh) neurons (Neurosci. Lett. 83, 47-52, 1987). Several lines of evidence suggest that NT receptors might also be associated with ACh axons in several brain areas, thus providing a basis for NT-ACh interactions in the brain. In the present study we tested whether NT is involved in the regulation of ACh release from areas enriched in cholinergic markers and NT receptors. NT (10 μM) increased evoked (25 mM K^+) ACh release from slices of parietal cortex (36.2 ± 8.8%) and striatum (50.1 ± 10.5%) and decreased evoked ACh release from slices of frontal cortex (28.7 ± 5.3%): these effects were concentration-dependent. Spontaneous ACh release was not altered by NT in these three brain areas. In contrast, in slices of the basal forebrain, diagonal band and hippocampus, NT (10 μm) did not significantly alter either spontaneous or potassium-evoked ACh release. These results indicate that NT modulates ACh release from both corticopetal and intrinsic neostriatal neurons and may therefore exert a widespread influence on the regulation of cholinergic neuronal activity in the brain. (Supported by MRC, Canada and FRSQ, Quebec).

235.7

TONIC REGULATION OF THE NEUROTENSIN SYSTEMS BY BASALLY RELEASED DOPAMINE IN THE BASAL GANGLIA OF THE RAT. K.M. Merchant*, J.W. Gibb and G.R. Hanson, Department of Pharmacology and Toxicology, University of Utah, Salt Lake City, UT 84112.

Elimination of central dopaminergic activity by treatments with reserpine causes a substantial increase in the content of neurotensin (NT)-like immunoreactivity (NTLI) in the striatum but decreases the nigral concentration of this peptide by about 30% of control. These data suggest that basal dopaminergic activity tonically but differentially regulates the NT systems in these basal ganglia structures. The present study characterizes the roles of dopamine D-1 and D-2 receptors in mediating this tonic regulation. Administration of 4 doses (every 3h) of the D-2 selective agonist, LY 171555 (5 mg/kg), completely blocked the reserpine mediated increase in striatal NTLI level but did not affect the nigral response. On the other hand, an identical treatment with the D-1 selective agonist, SKF 38393 (18 mg/kg), prevented the decrease in nigral NTLI content induced by reserpine but only slightly attenuated the striatal response. These data indicate that tonic dopaminergic regulation of striatal NT systems is mediated chiefly by the D-2 receptors whereas that in the substantia nigra is mediated by the D-1 receptors. This hypothesis was confirmed when treatments with the D-2 selective antagonist, sulpiride, increased only the striatal NTLI content whereas treatment with the D-1 antagonist, SCH 23390, decreased the NTLI content only in the substantia nigra. (Supported by USPHS Grants DA 00869 and DA 04222)

235.8

ROLE OF GLUTAMATE IN DOPAMINE-MEDIATED NEUROTENSIN CHANGES. N.A.Singh*, K.M.Merchant*, J.W. Gibb and G.R.Hanson. Dept. Pharmacol. and Toxicol., Univ of Utah, Salt Lake City, UT 84112.

Previous studies have demonstrated that methamphetamine (METH) induced neurotensin (NT) increases in the striatum, nucleus accumbens and substantia nigra are primarily mediated by dopamine D1 receptors. To determine if other neurotransmitter systems play a role in DA-mediated peptide responses, we coadministered selective glutamatergic and cholinergic antagonists with METH. Firstly, animals were given a combination of MK-801 (2.5mg/kg, a selective noncompetitive NMDA antagonist) fifteen minutes prior to METH (10mg/kg) for five doses at 6-hr intervals and sacrificed 18hr after the last dose. MK-801 alone did not alter NT levels in the striatum, n. accumbens or s. nigra; however MK-801 completely blocked METH-induced increases in NT in all structures examined. MK-801 was found to be a very potent blocker of these METH effects since doses of this NMDA antagonist as low as 0.1mg/kg completely prevented the METH-induced NT changes. Secondly, the muscarinic antagonist, atropine, was coadministered with multiple doses of METH. Unlike MK-801, atropine had no significant impact on METH-induced changes in NT levels. These findings suggest that glutamate projections (related to NMDA systems), but not cholinergic neurons, are involved in the D1 regulation of extrapyramidal and limbic NT systems.(Supported by grants DA 00869 and DA 04222).

235.9

NEUROTENSIN-EVOKED OVERFLOW OF ENDOGENOUS DIHYDROXY-PHENYLACETIC ACID FROM SUPERFUSED RAT STRIATAL SLICES IS GREATLY DIMINISHED BY STRESS. L.P. Dwoskin and S.T. Buxton*. Univ. KY Col. Pharmacy, Lexington, KY 40536.

Neurotensin (NT) has been suggested to be an endogenous neuroleptic (Nemeroff, Biol. Psych. 15:283, 1980) and is predicted to influence the release of DA. Single striatal slices (6 mg) were superfused (1 ml/min) with Kreb's buffer. After 1 hr, NT (0.3-30 uM) was added to the buffer and 15 one-ml samples collected. Samples were assayed by HPLC-EC (Gerhardt et. al., J. Neurosci. Meth. 26:217, 1989). NT in a dose-related manner increased the amount of DOPAC collected in superfusate from slices from Sprague-Dawley rats from both Sasco and Harlan suppliers. Although the maximal amount of NT-evoked overflow of DOPAC was not different (1660 ±490 and 1930 ±320 pg/ml/mg tissue, Sasco and Harlan, respectively), the shapes of the dose-response curves were very different. The results from the Sasco rats revealed an S-shaped curve (EC50 3 uM), and that from the Harlan rats an inverted-U (apex at 1 uM). Stress was induced by subjecting the rat (and the experimenter) to loud, irritating, vacuum pump noise for a 5 min period prior to sacrifice. This unquantitated stressful event greatly diminished the amount of DOPAC collected in experiments using rats from both suppliers. This serendipitious finding will be followed-up in future experiments. Supported by NIMH Grant MH42934.

235.10

NEUROTENSIN REVERSES THE EFFECTS OF AMPHETAMINE ON IN VIVO DOPAMINE METABOLISM IN THE ACCUMBENS BUT NOT THE STRIATUM. R. Rivest[1], F.B. Jolicoeur[1], F. Crespi*[2] and C.A. Marsden*[2]. [1]Dept. psychiatry, Fac. of Med., Univ. of Sherbrooke, PQ, Canada J1H 5N4 and [2]Dept. of Physiol. and Pharmacol., Queen's Med. Centre, Nottingham, UK, NG7 2UH.

The antagonistic properties of neurotensin (NT) on the behavioural hyperactivity induced by dopaminergic agonists are thought to be mediated via the mesolimbic system rather than the nigrostriatal pathway (Ervin et *al.* Nature. 291, 73-76, 1981; Jolicoeur et *al.* Neurosc. Biobehav. Rev. 7, 385-390, 1983). In the present study, we examine the effect of intracerebroventricular (ICV) administration of NT in combination with amphetamine, on the extracellular level of DOPAC measured by *in vivo* differential pulse voltammetry in both the nucleus accumbens and the striatum. Male Wistar rats (250-280 g) were anaesthetised using chloral hydrate (400 mg/kg i.p.) and stereotaxically implanted with a guide cannula in the left lateral ventricle and with an electrically-pretreated 12 µm carbon fiber electrodes in the nucleus accumbens and the striatum. Saline 1 ml/kg or amphetamine 2mg/kg/ml s.c. were administered, followed five minutes later with an ICV injection with saline 10 µl or NT 10 µg/10µl and the extracellular DOPAC was recorded every five minutes for two hours. Compared with baseline value, amphetamine produced similar decrease in extracellular DOPAC in the nucleus accumbens (52%) and the striatum (47%). NT produced a significant increase in the DOPAC peak in the accumbens (30%) and the striatum (25%). However, when administered with amphetamine, NT partly reversed the decrease induced by the drug in the accumbens but not in the striatum. Together, these results support previous neurobehavioural data indicating that NT reduce the hyperactivity induced by amphetamine by a specific action on the mesolimbic dopaminergic system.

235.11

REGULATION OF MESOLIMBIC DOPAMINE/CHOLECYSTOKININ NEURONS AND THEIR POST-SYNAPTIC CELLS; EFFECTS OF AMPHETAMINE. Y.L. Hurd, N. Lindefors*, S. Brené*, E. Brodin*, W. O'Connor*, T. Hökfelt, U. Ungerstedt* and H. Persson*. Depts. of Pharmacology, Histology and Medicinal Chemistry, Karolinska Institute, Box 60400, S-10401 Stockholm, Sweden.

Activation of dopamine (DA) neurotransmission in the nucleus accumbens has been frequently theorized to mediate the reinforcing effects of psychomotor stimulants, e.g. amphetamine (AMP) and cocaine. However,it has been observed that repeated self-administration of cocaine attenuates the enhanced overflow of DA previously evident during first-time exposure to the drug. In the present study the effect of acute and repeated (1.5mg/kg; twice daily for 7 days) AMP administration on extracellular levels of DA, cholecyctokinin (CCK),GABA and acetylcholine in the nucleus accumbens of halothane-anesthetized rats was assessed by in vivo microdialysis. Acute AMP increased DA overflow in saline pretreated rats, concomitant with a reduction of DOPAC and HVA levels. However, following repeated AMP administration, DA overflow was attenuated although the reduction of the DA metabolites was maintained. Extracellular basal and K^+ stimulated CCK levels were slightly elevated following repeated treatment of AMP. Regulation of DA/CCK release and characterization of the cells types were assessed by in situ hybridization and immunocytochemistry. Presynaptic modulation of mesolimbic DA/CCK neurons comcomitant with supersensitive post-synaptic receptors are proposed.

235.12

DIRECT GABAERGIC-DOPAMINERGIC SYNAPTIC INTERACTIONS IN THE RAT SUBSTANTIA NIGRA: AN ULTRASTRUCTURAL DOUBLE LABELLING IMMUNOCYTOCHEMICAL STUDY. B.A. Flumerfelt, I. Mendez* and K. Elisevich. Depts. of Anatomy and Clinical Neurological Sciences, University of Western Ontario, London, Canada, N6A 5C1.

The substantia nigra contains a prominent intrinsic GABAergic system, and a striato-nigral GABAergic projection, and there is evidence of physiological and pharmacological effects of GABA on dopaminergic neurons in the substantia nigra. However, the structural nature of the GABAergic-dopaminergic interactions in the substantia nigra is not clear. Simultaneous localization of GABA immunoreactive terminals and tyrosine hydroxylase (TH) immunoreactive neurons was achieved with the PAP method, using two different chromogens with distinct reaction products that are easily differentiated at the light and electron microscope levels. GABA-positive neurons and terminals were first localized with 3,3'- diaminobenzidine, then TH-positive neurons were localized using benzidine dihydrochloride. GABA immunoreactive terminals made symmetrical synaptic contacts with TH-positive dendrites in the substantia nigra pars compacta and pars reticulata. The present results demonstrate a direct GABAergic input to dopaminergic neurons in the substantia nigra, supporting the view that GABA modulates dopaminergic neurons within the substantia nigra and likely originates within the striato-nigral system.
(Supported by the MRC and Parkinson's Foundation of Canada)

235.13

GABA RELEASE IN SUBSTANTIA NIGRA MEASURED BY MICRODIALYSIS: MODULATION BY DOPAMINE. B. K. Yamamoto. Dept. of Pharmacology, Northeastern Ohio Univs. College of Med., Rootstown, OH 44272

Previous findings indicate that the effects of locally applied GABA on substantia nigra-reticulata (SN-R) neuronal activity is inhibited by dopamine (DA). To test the hypothesis that DA modulates GABA neurotransmission, the present study used microdialysis to examine the effect of the locally applied D1 antagonist, SCH 23390, on in vivo GABA release in the SN-R of awake-behaving rats. Male Sprague-Dawley rats (n=6) were chronically implanted with guide cannulae. The tip of the cannula was positioned dorsal to the SN-R. Three days after surgery, a microdialysis probe (210 µm O.D., 1.0 mm length) was inserted through the guide cannula to extend into the SN-R. The probes were perfused with artificial CSF (pH 6.5) at a flow rate of 2.0 µl/min. The amino acid content of each 25 µl dialysate sample was assayed by pre-column derivatization and HPLC/EC. Basal levels were allowed to stabilize for at least 3 hrs. Following this baseline stabilization period, SCH 23390 (500 µM) was infused directly into the SN-R via the probe for 40 min while simultaneously measuring GABA release. SCH 23390 increased basal GABA release in SN-R by 54%. These increases returned to baseline within 1 hr after the end of the drug infusion. The effects of a D-2 antagonist on GABA release will also be presented. These data may indicate that dendritic DA release in SN-R inhibits striatonigral GABA transmission via the D-1 receptor and thus may regulate motor output commands from the striatum.

235.14

MODULATION OF GABA RELEASE IN VIVO BY GABA-B RECEPTORS IN THE RAT STRIATUM. R.Singh* and R.G.Fariello (SPON: F.A.Davis), Dept.of Neuro. Sci. Rush Med. Ctr., Chicago, IL 60612.

GABA containing neurons and terminals in the rat striatum(ST) are targets of local GABAergic interneurons. In this study, we have examined if GABA release in ST is under feedback control by GABA autoreceptors, specifically GABA-B. Male rats (250-300 g) were anaesthetized with halothane/O_2 and dialysis probes were implanted into the anterior caudate. Lactate-Ringer buffer (pH-7.4) was perfused through the probes at 1 ul/min. Dialysates were collected at 20 min intervals and analyzed by OPA derivatization and HPLC with fluorescence detection. After 3 baseline samples, a specific GABA-B agonist, baclofen (100uM) was added to the perfusate for 40 min and sampling continued for 2 h. Baclofen decreased GABA release by 22±6 % from the basal levels. This decrease was maximal within 20 min and returned to base line by 40 min. Glutamate release was also decreased by 30±12 % from the basal levels and this effect was present up till 40 min after which the levels returned to baseline. These results indicate that GABA-B receptors inhibit GABA release in ST and may also affect glutamatergic cortical input.

235.15

DOPAMINE MODULATION OF THE GABA PATHWAY PROJECTING FROM THE NUCLEUS ACCUMBENS TO THE VENTRAL PALLIDUM IN CONCIOUS RATS. A. Bourdelais* and P.W. Kalivas (SPON: R. Kuczenski). Dept. of VCAPP, Washington State University, Pullman, WA 99164-6520.

Locomotor activity induced by amphetamine (AMP) and cocaine has been shown to be associated with a rise in extracellular dopamine (DA) levels in the nucleus accumbens (NA). An increase in spontaneous locomotion produced by DA agonists in the NA is prevented by pretreatment with an injection of a GABA agonist in the ventral pallidum (VP). This has led to the postulate that DA may be acting in the NA to inhibit GABA release in the VP. In the present experiments, intracranial dialysis was used to directly compare the effect of cocaine and AMP on extracellular GABA concentration in the VP with changes in locomotor behavior in concious rats. Rats were chronically implanted with a guide cannula 3mm above the VP. At 6-8 days postsurgery the rat was placed in a photocell apparatus and a dialysis probe was inserted into the VP via the guide cannula. After a 2 hr equilibration period, 4 x 20 min baseline samples were collected and the rat was then injected with one of the following: saline, 15.0 mg/kg cocaine (i.p.), or 2.0 mg/kg AMP (s.c.). 20 min samples were collected for another 180 min. Artificial CSF, at a flow rate of 2.31 ul/min was used for dialysis. HPLC-EC was used to measure GABA levels, with OPA/t-butylthiol precolumn derivatization. Baseline concentration for GABA was 0.15 pM/ul. After cocaine administration a maximal GABA decrease of (30%) was seen by 80 min. After AMP administration a significant decrease in GABA concentration (36%) was seen by 40 min.

235.16

DOPAMINE-GABA INTERACTIONS IN THE LIMBIC FOREBRAIN FOLLOWING LOCAL APPLICATION OF K+, AMPHETAMINE AND FLUPENTHIXOL. J. Kehr*, F. Weiss, A. Carlsson*, G.F. Koob, and U. Ungerstedt*. (SPON: S. Foote) Dept. of Pharmacology, Karolinska Institute, Stockholm, Sweden; Research Institute of Scripps Clinic, La Jolla, CA.

Recent evidence suggests a significant role for GABA in the mediation of dopamine (DA) related functions within the basal forebrain. We have further investigated DA-GABA interactions in this brain region by measuring the effects of DA stimulation or blockade on extracellular GABA concentrations in the nucleus accumbens (NAS) and ventral pallidum (VP) using in vivo microdialysis. Perfusate was collected simultaneously from two microdialysis probes implanted into the NAS and VP of halothane anesthetized rats and assayed for both DA and GABA (NAS) or GABA only (VP). Drugs, including K+, d-amphetamine [AMP] and flupenthixol [FLU] were administered directly into the NAS via the perfusion medium at 30 min pulses. K+ (30mM) stimulated both GABA and DA release (450-800% of basal) in the NAS, followed by a late, gradual rise in VP GABA concentrations (>200%) of basal). In contrast, while AMP (100uM) produced substantial increases in DA overflow (>5000% basal), this enhancement of DA activity was not associated with changes in extracellular GABA levels in the NAS. AMP, however, elevated GABA levels in the VP and produced a sharp "spike" (300% of basal) in GABA release which occurred with a delay of approximately 20-30 min after termination of AMP administration. This effect was antagonized by concurrent administration of FLU (100uM). The same treatment (AMP+FLU) strongly enhanced GABA release in the NAS (>250% basal). The results suggest a differential regulation of GABA activity within the NAS and the GABAergic efferents by DA, and confirm an important role for the NAS-VP pathway in the effects of mesolimbic DA stimulation.

235.17

L-GLUTAMIC ACID EVOKES Ca^{2+}-INDEPENDENT RELEASE OF DOPAMINE FROM RAT STRIATUM VIA DOPAMINE UPTAKE SYSTEM. G. Lonart and M.J. Zigmond. *Department of Behavioral Neuroscience and Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260*

We have examined the effects of exogenous L-glutamate (10 mM) on the in vitro release of dopamine from rat striatum. Slices (350 um) were superfused (100 ul/min) with a Krebs-bicarbonate buffer containing a physiological concentration of Mg^{2+} (1.2 mM). L-Glutamate produced a 7.5-fold increase in endogenous dopamine efflux. The NMDA receptor antagonists, 2-amino-5-phosphonovaleric acid (0.1 mM) and MK 801 (0.01 mM), reduced this effect by 70%. The glutamate-induced release of dopamine was insensitive to the removal of Ca^{2+}, addition of tetrodotoxin (0.5 uM), or pretreatment with reserpine (5 mg/kg, s.c.). However, the inhibitor of high affinity dopamine, nomifensine (0.01 mM), completely abolished the effect. These data suggest that L-glutamate might evoke a Ca^{2+}-independent release via the high affinity dopamine uptake system in the rat striatum. (Supported in part by USPHS grants NS-19608, MH-43947, and MH-00058.)

235.18

EFFECT OF RITANSERIN ON THE INTERACTION OF AMFONELIC ACID AND NEUROLEPTIC-INDUCED STRIATAL DOPAMINE METABOLISM. P.G.Conway, L.Brougham* and D.B.Ellis* Dept. of Biological Research Hoechst-Roussel Pharmaceuticals Inc. Somerville, NJ 08876.

In the present study we evaluated the interaction of amfonelic acid (AFA) with the typical neuroleptic haloperidol (H) or the atypical antipsychotic clozapine (C) on rat striatal dopamine metabolism in the absence or presence of the 5HT2 receptor antagonist ritanserin. In the absence of ritanserin, AFA significantly enhanced H stimulated DOPAC accumulation by 36% above that produced by H alone. This effect is believed to be due to AFA's ability to facilitate dopamine release produced by the potent H-induced increase in nigralstriatal impulse flow. In contrast, AFA did not potentiate the ability of C to stimulate DOPAC. This lack of potentiation could be explained by C's known potent 5HT2 receptor blocking activity attenuating its stimulatory effects on impulse flow. To test this we introduced 5HT2 receptor blocking activity to H by combining this drug with ritanserin and again studied the interaction with AFA on dopamine metabolism. In the presence of ritanserin AFA failed to potentiate the effects of H on DOPAC accumulation; an effect similar to that seen with C. These results extend the idea that 5HT2 receptor blockade modulates nigralstriatal dopaminergic neurotransmission. Additionally, when 5HT2 receptor blockade is added to the profile of H this drug functions similarly to C in the AFA interaction test.

235.19

THE EFFECTS OF EXCITATORY AMINO ACIDS ON DOPAMINE NERVE TERMINALS IN THE NEOSTRIATUM OF THE ANESTHETIZED RAT: AN IN VIVO ELECTROCHEMICAL STUDY. *M.N. Friedemann[1] and G.A. Gerhardt[1,2] (SPON: L. Adler). Depts. of Pharmacology[1] and Psychiatry[2], Univ. of Colorado Health Sciences Center, Denver, CO 80262.

The striatum receives a substantial cortical input containing the excitatory neurotransmitter, glutamate. In order to investigate the effects of selective glutamate agonists on dopamine (DA) -containing nerve terminals, high-speed electrochemical measurements were used to study the effects of locally applied N-methyl-D-aspartate (NMDA) and kainate (KA) in the neostriatum of the anesthetized Sprague-Dawley rat. Local application of KA via pressure ejection (1-10 mM barrel concentration) resulted in long-lasting and non-recoverable DA overflow. NMDA also evoked DA overflow at barrel concentrations of 0.1-10 mM in a somewhat dose-dependent fashion. Lower concentrations of NMDA (10 μM), which did not elicit DA release alone, potentiated potassium-evoked DA overflow. This potentiation was inhibited by the non-selective antagonist, kynurenate (20 μM). Studies with more selective NMDA antagonists are underway to elucidate the receptor specificity of the NMDA effects. The effects of glycine, a potential NMDA receptor modulator, are also under investigation. Supported by USPHS AG06434 AND AG00441.

235.20

EFFECTS OF EXCITATORY AMINO ACIDS ON *IN VIVO* DOPAMINE RELEASE AND METABOLISM IN THE NUCLEUS ACCUMBENS. M. M. Payson* and B. A. Donzanti. Northeastern Ohio Univ. Col. of Med., Dept. of Pharmacology, Rootstown, OH 44272.

Excitatory amino acids (EAA) induce a marked increase in spontaneous locomotor activity when injected into the nucleus accumbens (NA). Although pharmacological studies suggest that this hyperactivity is the result of EAA-induced dopamine (DA) release, *in vitro* experiments have failed to show any effect of these neuroexcitants on endogenous DA release. The present study assessed the effects of EAA on endogenous DA release from the NA of the awake rat using intracranial microdialysis. Rats were implanted with guide tubes and allowed 2 days to recovery from surgery prior to insertion of a microdialysis probe into the NA. Dialysate samples were collected for 20 minutes at a flow rate of 1.5 μl/min and subsequently analyzed for DA, DOPAC, and HVA content using HPLC-ECD. Following baseline stabilization, either N-methyl-D-aspartate (NMDA), kainate (KA), MK-801, or high K^+ was infused through the probe for 20 minutes. The infusion of NMDA (2 mM) and KA (2 mM) produced a marked increase in extracellular DA, a marked decrease in DOPAC and HVA, and a noticable increase in locomotion. High K^+ (80 mM) produced the same neurochemical profile but did not increase spontaneous movement. A lower concentration of KA (0.2 mM) did not alter locomotion or DA release. MK-801 (0.1 mM) did not alter spontaneous DA efflux. Thus, EAA apparently release newly synthesized DA from the NA and the glutamate input to the NA appears to be non-tonic.

235.21

DOPAMINE RECEPTORS MODULATE NORADRENALINE RELEASE FROM RAT FRONTAL CORTEX. Z.L. Rossetti*, L. Pani, C. Portas* and G.L. Gessa (SPON: S.A. Tjioe) Department of Neuroscience, University of Cagliari, 09124 Cagliari, Italy.

Dopamine (DA) neurons with cell bodies located in the ventral tegmental area provide a diffuse innervation to the frontal cortex. Brain microdialysis in conscious animals was used to investigate whether DA receptors, other than α_2-adrenoceptors, might control the release of noradrenaline (NA) from nerve terminals in the rat frontal cortex. NA in perfusates (50 μL/30 min) was quantitated by HPLC-EC. The selective α_2-adrenoceptor agonist clonidine (0.3 mg/kg, ip) reduced NA release (-50%), whereas yohimbine (5 mg/kg, ip), a selective α_2-adrenoceptor antagonist, increased cortical NA release (about 250% of baseline). Quinpirole, a selective DA D_2 receptor agonist, caused a dose-dependent decrease in NA release. A maximal inhibition of about 50% of baseline was observed at the dose of 150 μg/kg, sc. (-)Sulpiride, a selective DA D_2 receptor antagonist, at the dose of 100 mg/kg, ip, produced a 180% increase in NA release, the output remaining elevated for over 2 hr. Yohimbine (5 mg/kg ip), which prevented the inhibitory effect of clonidine, failed to prevent the response to quinpirole (50 μg/kg sc). Vice versa, the response to quinpirole (50 μg/kg sc) was prevented by (-)sulpiride (150 mg/kg ip). These results indicate the existence of DA D_2 receptors modulating the release of NA in the frontal cortex.

235.22

MODIFICATION OF EXCITABILITY OF CORTICOSTRIATAL AFFERENTS: EVIDENCE FOR AUTO AND HETERORECEPTOR REGULATION. M. Garcia-Munoz, S.J. Young* and P.M. Groves. University of California, San Diego. La Jolla, CA 92093

Considerable evidence suggests that synaptic release of transmitters may be regulated by neuroactive substances via non-synaptic interactions. Thus, interactions involving nigral dopamine(DA) and cortical glutamate-containing terminals in the striatum have been suggested using several different methods. For instance, decreased electrical excitability of the nigrostriatal terminal fields has been observed after frontal cortex stimulation. The experiments reported here were designed to address the following questions: Does the release of DA alter the excitability of corticostriatal terminals? Is the excitability of cortical terminals affected by glutamate release? Male Sprague-Dawley rats were anesthetized with urethane. Bipolar stimulating electrodes and cannulae for local drug infusion were placed in the striatum. Single unit extracellular recordings were obtained from the contralateral medial prefrontal cortex. Terminal excitability was measured by determining the threshold for antidromic activation of a contralateral cortical cell on 100% of non-collision trials. The mean threshold was 1.8 ±0.1 mA and the antidromic latency 15.4 ±0.6 ms. Excitabilty of the cortical terminal field decreased following striatal administration of amphetamine (1 μM/ 300nl/ 5min) or electrical stimulation of mesencephalic DA cells (1.5mA/ 1Hz/ 2min) The decrease in excitability could be blocked or reversed by local application of sulpiride (10nM) or haloperidol (1μM, 300nl/ 5min) . In a subset of cortical neurons, increases in firing rate were associated with increased excitability and vice-versa. This relation was abolished by local administration of NMDA-receptor antagonists. Experiments examing these excitabilty effects following striatal kainic acid lesions will also be reported. These results suggest that glutamate and dopamine exert opposing influences on the excitability of the corticostriatal terminal field.

The work was partially supported by a grant from the National Institute on Drug Abuse .

REGIONAL LOCALIZATION OF RECEPTORS AND NEUROTRANSMITTERS II

236.1

ENDOGENOUS HOMOVANILLIC ACID LEVELS DIFFER BETWEEN RAT AND RABBIT CAUDATE, HIPPOCAMPUS AND CORTICAL REGIONS. L. Grondin*, T.A. Reader, and K.M. Dewar (SPON: H.H. Jasper). CRSN, Département de physiologie, Université de Montréal, Montréal (Qué.) Canada.

Endogenous dopamine (DA) and its metabolites 3,4-dihydroxyphenylacetic acid (DOPAC), 3-methoxy-tyramine (3MT) and homovanillic acid (HVA) were measured by HPLC-ED in the entorhinal-piriform (EnPi), cingulate (CIN), sensorimotor (SSM) and visual (VIS) cortices as well as in the caudate (CAU) and hippocampus (HIP) of Sprague-Dawley (SD) rats and New-Zealand rabbits. The DA, DOPAC and 3MT contents were similar in both species. The HVA levels however, although they followed the DA distribution, were several-fold higher in rabbits than in SD-rats for all cortices, HIP and CAU. In addition, total metabolite contents and DA turnover (DA metabolites/DA ratios) were significantly higher in the rabbit than in the rat, suggesting an increased release and/or metabolism in the former species. The HVA/DA ratios were much greater for rabbit regions than for SD-rats, indicating an increased DA release in the former species since the DOPAC/DA ratios (index of intraneuronal degradation) were similar. It can be proposed that the elevated DA turnover documented for the rabbit is a consequence of an increased release of DA as reflected by the larger HVA/DA indexes.
[Supported by the MRC (Canada) and the FRSQ (Québec)].

236.2

AUTORADIOGRAPHIC LOCALIZATION OF TWO SUBTYPES OF THE CANINE ALPHA-1 ADRENERGIC RECEPTOR R.R. Dean*, E. Mignot* and W.C. Dement Stanford Sleep Disorders Center, Stanford Univ. Sch. of Med, Palo Alto, CA 94304

The two subtypes of the alpha-1 adrenoceptor (α-1a, high-affinity and α-1b , low-affinity), previously demonstrated in the rat on the basis of different pharmacological properties, were shown to be present in the canine CNS using Scatchard analysis and drug competition studies (Mignot et al. Brain Res.in press). In the present study, the α-1a and α-1b receptor subtypes were localized autoradiographically in the canine CNS (n=6). 15 μ thick frozen sections were thaw-mounted onto gelatin coated slides. Slides were incubated for 40 min. at 30° C in 1nM [^{3}H]-Prazosin; 80 nM WB 4101 was used to mask the a-1a sites (50 mM Tris, pH. 7.4, 1.25×10^{-4}M ascorbate). Incubation was followed by a 30 min rinse, then a 15 min rinse in ice-cold buffer and then dipped in dH_2O to remove residual buffer salts. Anatomically adjacent tissue sections were incubated under the same conditions but with BE 2245 (100 μM), which has a binding profile similar to prazosin, to determine the nonspecific binding. After thorough drying, slides were apposed to tritium-sensitive film. Following an appropriate exposure period the resulting autoradiograms were developed using standard photographic techniques and analyzed using an Imaging Research Inc. computerized microdensitometric image-analysis system. α-1b receptors were localized directly in the presence of WB 4101, a compound with much higher affinity for the α-1a receptors. The relative distribution of α-1a was obtained by subtraction of the α-1b from the total α-1 (prazosin) binding.The relative distribution of the two receptor subtypes in a number of brain structures was studied. Structures exhibiting a higher density of the α-1a subtype included nuc. lemnisci lateralis, nuc. pontis oralis, nuc. tr. mesenceph. n. trigemini,nuclei pontis, substantia nigra, central grey, putamen, nuc. parataenealis, and nuc. ruber. Structures possessing a larger percentage of the α-1b subtype included the caudate, lateral and medial geniculate, several amygdala nuclei, nuc. medialis dorsalis, stria medullaris thalami, and centromedial nuc. of the thalamus.

236.3

COMPARATIVE DISTRIBUTION OF ALPHA-1 AND ALPHA-2 ADRENERGIC BINDING SITES IN THE LOCUS COERULEUS AND THE NUCLEUS TRACTUS SOLITARII OF THE RAT BRAIN BY QUANTITATIVE AUTORADIOGRAPHY

G. Chamba*, D. Weissmann*, C. Rousset*, B. Renaud and J. F. Pujol*. Laboratoire de Neuropharmacologie, CNRS UMR 12, Université C Bernard, 8 Av. Rockefeller, 69373 LYON and CERMEP, 59 Bvd Pinel, 69003 LYON, FRANCE.

Many studies have shown that noradrenergic neurons of the locus coeruleus (LC) and of the nucleus tractus solitarii (NTS) play a major role in various physiological processes including control of blood pressure, neuroendocrine regulation and behavioral mechanisms.

The present study was performed to compare the precise rostro-caudal distribution of alpha-1 and alpha-2 adrenergic binding sites within the LC and the NTS. Distribution of the density of [3H] prazosin and [3H] idazoxan binding sites were analyzed by quantitative autoradiography on 20 µm frontal sections, every 200 µm throughout these two regions. Binding of each ligand to their respective adrenoceptors was specific and saturable. The results obtained show: i) that the density of alpha-1 binding sites was 3 to 4 times higher than the density of alpha-2 binding sites in the two regions analyzed, ii) that the rostro-caudal distribution of the two types of adrenergic binding sites was differential and heterogenous in the LC and in the NTS.

These results will be analyzed in relation with the cellular localization of alpha-1 and alpha-2 binding sites on noradrenaline-containing elements.

236.4

QUANTITATIVE AUTORADIOGRAPHY OF CORTICAL α_1-ADRENERGIC RECEPTORS IN SUICIDE VICTIMS. *V. Arango, L. Hoffman, P. Ernsberger, D.J. Reis and J.J. Mann.* Div of Neurobiology and Lab of Psychopharmacol, Cornell Univ. Med. Coll., New York, NY 10021.

We previously reported increased β-adrenergic receptors in the prefrontal cortex (PFC) of suicide victims by both membrane binding (Mann et al. *Arch Gen Psych 43:954, '86)* and autoradiography (Arango et al. *Soc Neurosci Abstr 14:171, '88).* Other adrenergic receptor subtypes have not been examined in suicide. Using quantitative autoradiography, we sought to determine the normal distribution of α_1-adrenergic receptors in PFC (area 9) and temporal cortex (TC, area 38) and whether α_1 receptors are altered in suicide. Suicide victims and controls were paired for assay by matching for postmortem delay, age and gender (PFC: 10 pairs, TC: 8 pairs). Toxicology was negative for centrally active drugs. Slide-mounted sections were preincubated in Kreb's buffer then incubated with 2 nM ^{3}H-prazosin to label α_1-adrenergic receptors. Non-specific binding was defined by 10 µM phentolamine. Sections and ^{3}H-standards were exposed to Ultrofilm for 7 weeks and developed in GBX. The resulting autoradiograms were quantified using a PC-based image analysis system. In both cortical areas α_1 receptors formed a distinct laminar pattern, with 4 clear bands for which receptor density was: layers I-II > VI = III > IV-V (identified by overlapping with adjacent Nissl-stained section; ANOVA, $p < .01$). TC had more binding in all layers than PFC (ANOVA, $p < .001$). In layers IV-V of PFC, suicide victims had more α_1 sites than controls (ANOVA, $p < .03$). In summary: a) α_1 receptors in both PFC and TC are distributed in a distinct laminar pattern; b) α_1 receptors are selectively increased in layer IV-V of PFC in suicide victims; c) α_1 receptor density is higher in TC than in PFC; d) there are no differences in α_1 receptor binding in TC of suicide victims compared to controls. We conclude that in contrast to β-receptors, the alteration in α_1-receptors in suicide selectively involves some cortical layers.

236.5

SELECTIVE LOCALIZATION OF D1 RECEPTORS TO STRIATONIGRAL NEURONS IN THE RAT. M.B. Harrison, R.G. Wiley* and G.F. Wooten. Dept. of Neurology, UVA School of Medicine, Charlottesville, VA 22908 and *Depts. of Neurology and Pharmacology, Vanderbilt Univ., Nashville, TN 37212.

To investigate the cellular localization of dopaminergic receptor subtypes in the striatum, we have studied the autoradiographic distribution of D1 and D2 receptors after a lesion of striatonigral neurons produced by micro-injection of volkensin into the substantia nigra. Volkensin is taken up by terminals and retrogradely transported to the soma where it is cytotoxic (Wiley and Stirpe, Brain Res., 438(1988)145-154). Six rats received injections of volkensin (1.3-10.8 ng); after 17-21 day survival, the brains were processed for autoradiography, with adjacent sections examined for binding of ^{3}H-SCH23390 (D1 antagonist), ^{3}H-spiperone (D2 antagonist), and in 2 animals, ^{3}H-hemicholinium (^{3}H-Hch) (a presynaptic cholinergic marker). The resultant lesion localized to the dorsomedial quadrant of the rostral striatum. Specific binding in the striatum, both contralateral and ipsilateral to the injection site was compared, with the following results: D1 decreased from 171 fmol/mg± 15 (mean ± S.E.) to 24± 6 (mean decrease 86%**); D2, from 127± 8 to 77± 4 (mean decrease 39%**); ^{3}H-Hch, from 117± 20 to 52± 18 (mean decrease 56%*). When the percent change was compared among the three groups, a significant difference was found between the decreases in D1 and D2 binding** and D1 and ^{3}H-Hch binding*, but not between D2 and ^{3}H-Hch. These data suggest that striatonigral neurons express D1 receptors selectively. The reduction in D2 binding may reflect the presence of a population of D2 receptors on striatonigral neurons or alternatively may reflect transsynaptic effects of the toxin or secondary changes on cholinergic interneurons following the loss of striatonigral neurons ($^{**}p<.01$; $^{*}p<.05$).

236.6

PRE AND POST SYNAPTIC DOPAMINE D2 RECEPTORS OF THE NIGROSTRIATAL, MESOLIMBIC, AND MESOCORTICAL PATHWAYS ARE ENCODED BY THE SAME mRNA. D.M. Weiner and M.R.Brann (SPON: P. Gardner), Research Scholars Program, HHMI,and Laboratory of Molecular Biology, NINDS, Bethesda MD 20892.

Based on the recently reported sequence of a D2 receptor cloned from rat brain (Bunzow, et al., Nature. 336, 783), we prepared a series of cDNA probes to determine the distribution of mRNA encoding this receptor. Three probes were generated corresponding to regions encoding the n-terminal, c-terminal, and large third cytoplasmic loop of the receptor. The specificity of the probes was verified by two criteriae. First when *in situ* hybridizations of rat brain were performed each probe used individually showed an identical pattern of labeling as did a mixture of the three probes. Secondly, the mixture of probes hybridized to a single mRNA of 2.9 kb on northern blots of total RNA from rat brain. Within the forebrain, D2 receptor mRNA was abundant throughout the caudate-putamen, nucleus accumbens and olfactory tubercle. Moderate to low levels of mRNA were observed in the diagonal band, various septal nuclei, medial habenular nucleus, claustrum, dorsal endopiriform nucleus, and entorhinal cortex. Within the mesencephalon, D2 receptor mRNA was abundant within the substantia nigra pars compacta and ventral tegmental area. Comparison of the distribution of the mRNA and ligand binding indicates that both the pre- and post-synaptic D2 receptors of the nigrostriatal, mesolimbic and mesocortical pathways are derived from the same mRNA.

236.7

AUTORADIOGRAPHIC EVALUATION OF D_1 AND D_2 DOPAMINE RECEPTORS FOLLOWING CENTRAL DOPAMINERGIC DENERVATION B. E. Mileson and R. B. Mailman University of North Carolina Curriculum in Toxicology and Biological Research Center, Chapel Hill, NC 27599

Chemical denervation of greater than 90 % of central dopamine neurons by intracisternal (IC) injection of 6–hydroxydopamine results in a behavioral supersensitivity to agonist challenge. The mechanism responsible for mediating this locomotor supersensitivity remains unknown. Compensatory changes in striatal dopamine receptor density or ligand affinity have not been found using homogenate binding techniques. In the present study, film autoradiographic techniques were used to examine D_1 and D_2 dopamine receptor density in local striatal regions and in the terminal areas of the mesolimbic dopaminergic tracts, the nucleus accumbens and the olfactory tubercles. Single concentrations of the [^{125}I] iodinated ligands, SCH23982 (D_1) and iodosulpride (D_2) were used to compare slide–mounted lesioned and control brain sections. No differences in D_1 receptor binding was seen in the rostral, central or caudal regions of the striata. In addition, no localized changes in D_1 receptors were seen in either the nucleus accumbens or olfactory tubercles. No significant increase in D_2 receptor binding was seen in the striata, nucleus accumbens or olfactory tubercles of lesioned rats compared to controls. This information indicates the behavioral supersensitivity exhibited by IC–lesioned rats is not due to increases in dopamine receptor number or ligand affinity in any brain region. Supported by ES01104, MH40537, and Training or Center Grants ES07126, HD03110 and MH33127.

236.8

GENETIC SELECTION FOR AGGRESSION IN MICE: AUTORADIOGRAPHIC ANALYSIS OF ALTERATIONS IN DOPAMINE RECEPTOR DENSITIES. L.L. Devaud, R.B. Cairns*, J.L. Gariepy*, R.B. Mailman, and M.H. Lewis. Biological Sciences Research Center and Departments of Pharmacology, Psychiatry and Psychology, University of North Carolina, Chapel Hill, NC 27599.

ICR mice have been selectively bred over 19 generations for high or low levels of isolation–induced aggression. The low aggressive (NC100) line has decreased concentrations of dopamine, DOPAC, and HVA in the nucleus accumbens and, to a lesser extent, in the caudate nucleus. Quantitative receptor autoradiography was employed to determine if these neurochemical changes were associated with compensatory changes in dopamine receptor density. D_1 and D_2 dopamine receptor densities were estimated by quantifying ^{125}I–SCH23982 and ^{125}I–iodosulpride binding sites, respectively, in brains of high (NC900) and low (NC100) aggressive mice. Slight (5–10%), but consistent, increases in the density of D_1 sites were observed in the rostral caudate of NC100 mice; larger increases (ca. 20%) in D_1 sites were observed in the nucleus accumbens. NC100 mice also exhibited an increased density of D_2 sites in the same regions. However, the line difference was more pronounced in the caudate nucleus (ca. 20%) than in the nucleus accumbuns (10%). Both the line differences and regional differences are consistent with the neurochemical data. The apparent increase in dopamine receptor density in a mouse line displaying low levels of aggression provides additional support for the hypothesis that dopamine systems play an important role in mediating genetic selection effects on behavior. (Supported by PHS Grants HD07201 and HD03110)

236.9

SIMULTANEOUS LOCALIZATION OF DOPAMINERGIC RECEPTOR BINDING SITES IN RAT NEURAL TISSUES. M.A. Ariano, F.J. Monsma, Jr.[1], A.C. Barton[1], H.C. Kang,[2*] R.P. Haugland,[2*] & D.R. Sibley.[1] Anatomy & Neurobiology, UVM College of Medicine, Burlington, VT 05405; [1]ETB, NINDS-NIH, Bethesda, MD 20892, & [2]Molecular Probes, Eugene, OR 97402.

Specific antagonist ligands for the D_1 and D_2 dopamine receptors have been chemically derivatized to the fluorescent compounds, fluorescein, rhodamine, Texas Red®, and Bodipy®. This has allowed the simultaneous ***regional*** and ***cellular*** visualization of these dopamine receptor populations in slices of rat forebrain, pituitary, retina, and superior cervical ganglion (SCG). The regional localizations of the two dopamine receptors reflect previous work ascertained using *in vitro* receptor autoradiographic methods. The most robust regional localization of the D_1 receptor type was found in the striatum and nucleus accumbens, displaying a membranous neuropil distribution. Medium sized neurons were the most prominent cell type that exhibited D_1 binding sites. Also, large cortical pyramidal neurons displayed this type of dopamine receptor population. The pituitary gland and the SCG did not show D_1 binding sites, however the outer nuclear layer of the retina exhibited some fluorescence for this receptor subtype. The D_2 receptor was also prevalent within the striatum and nucleus accumbens, but did not show as much staining intensity as the D_1 receptor subtype. Medium sized and an occasional large neuron demonstrated fluorescent binding sites for D_2 receptors in these forebrain regions. The anterior pituitary showed that about 30% of the cells were reactive for the D_2 subtype, with little fluorescence present in the posterior lobe. The retinal photoreceptor outer segments were highly fluorescent for the D_2 antagonist probe, as was the outer nuclear layer. The postganglionic neurons of the SCG and a fine rete within the parenchyma showed D_2 receptor binding. The fluorescent probes enable concurrent detection of the two subtypes of dopaminergic receptors in various rat neural tissues.

236.10

Corticothalamic neurons in rat cingulate cortex contain DARPP-32. C.C. Ouimet. Psychology Dept., Florida State Univ., Tallahassee, FL 32306.

DARPP-32 is a protein whose phosphorylation is regulated by dopamine and by cyclic AMP (Walaas et al., Nature 301: 69,1983). DARPP-32 is enriched in neurons that receive a dopamine input and contain the D_1 dopamine receptor, and is most highly concentrated in dopaminoceptive regions of the basal ganglia (Ouimet et al., J. Neurosci., 4: 111, 1984; Walaas and Greengard, J. Neurosci., 4: 84, 1984). In addition, DARPP-32 is enriched in a subset of layer VI neurons in cerebral cortex. In the present study, retrograde tracers were used to determine if the DARPP-32- immunoreactive neurons in layer VI of cingulate cortex project to the thalamus. Small injections of fluorgold or fluorescent latex microspheres were placed in the mediodorsal and anterior nuclei. After post-injection survival times of 2-10 days, animals were sacrificed and processed for immunocytochemistry. Layer VI neurons containing DARPP-32 immunoreactivity were consistently labeled with tracer. Additional tracer injections into thalamic relay nuclei such as the lateral geniculate and ventral posterior nuclei also retrogradely labeled DARPP-32-containing cells in layer VI in appropriate neocortical regions. These data suggest that cyclic AMP and possibly dopamine regulate DARPP-32 in some corticothalamic neurons, and raise the possibility that the DARPP-32-containing layer VI cells are targets for cortical dopamine fibers.

236.11

LOCALIZATION OF SEROTONIN$_{1B}$ AND SUBSTANCE P BINDING SITES IN THE FACIAL NUCLEUS FOLLOWING MOTONEURON DEGENERATION. S.J. Tallaksen-Greene, R.P. Elde and V.S. Scybold Dept. of Cell Biology and Neuroanatomy, University of Minnesota, Minneapolis, MN 55455.

Serotonin (5-HT) and substance P (SP) have been shown to coexist within nerve fibers in the facial nucleus of the rat. Iontophoretically applied 5-HT and SP have both been reported to have an excitatory effect on somatic motoneurons. To examine the question of whether facial motoneurons have receptors for 5-HT and/or SP we used *in vitro* autoradiography combined with chemical ablation of facial motoneurons. Animals received unilateral injections of the suicidal transport agent, ricin (0.5µg in 0.5µl phosphate-buffered saline, with 0.01% Fast Green dye), into the facial nerve, just distal to the exit of the nerve from the skull. The extent of facial motoneuron loss was assessed using a Thionin stain and cholinesterase histochemistry. [^{125}I]-iodocyanopindolol (^{125}I-ICYP) was used to label 5-HT$_{1B}$ binding sites, and [^{125}I]-labeled Bolton-Hunter substance P (^{125}I-SP) was used to label SP binding sites. A computerized densitometry system was used to quantify the density of autoradiographic grains representing radiolabeled ligand binding sites.

A nearly complete ipsilateral loss of facial motoneurons was observed 7-10 days following unilateral ricin injection into the facial nerve. In all cases, the medial subnucleus was spared because injections were made distal to the point where the posterior auricular nerve leaves the facial nerve. The number of facial neurons was reduced by 80-100% in the ipsilateral subnuclei of the intermediate and lateral divisions of the nucleus. Associated with the loss of motoneurons was an ipsilateral reduction in the density of both 5-HT$_{1B}$ and SP binding sites compared to values obtained from either the contralateral facial nucleus or control animals. These data suggest that there may be 5-HT$_{1B}$ and SP binding sites on the dendrites and perikarya facial motoneurons.

236.12

AUTORADIOGRAPHIC LOCALIZATION OF THE 5HT$_3$ ANTAGONIST ^{3}H-BRL43694 IN THE DORSAL VAGAL COMPLEX OF THE FERRET. R.A. LESLIE, D.J.M. REYNOLDS*, P.M. GRASBY* and D.G. GRAHAME-SMITH*. M.R.C. Unit and University Department of Clinical Pharmacology, Radcliffe Infirmary, Oxford, U.K. OX2 6HE.

5HT$_3$ antagonists are effective anti-emetics in cytotoxic- and radiation-induced emesis. Radioligand binding studies in brain membrane homogenates have identified binding sites for these compounds (eg., Kilpatrick, G.J. et al. Eur. J. Pharmocol. 159:157, 1989). We used autoradiography to localize binding sites for ^{3}H-BRL43694 (granisetron) in ferret brain. In addition, we removed one nodose ganglion in some animals to see the effect of vagal lesion on binding. Anesthetized ferrets were perfused with saline and their brains removed. Cryostat sections were incubated in 10nM ligand in HEPES buffer with or without the addition of 100 µM unlabelled GR38032F. Sections were washed, dried and exposed to Amersham Hyperfilm for six weeks. Tritium standards were exposed along with sections for quantitative analysis. Specific binding (128±4.2 fmol/mg wet wt.; ±SEM) was seen in the solitary nucleus (NTS) and area postrema (AP; 71±4.0 fmol/mg). Nodosectomy reduced binding in the NTS by 65%. Our results suggest that 5HT$_3$ binding sites are concentrated in a medullary nucleus involved in mediating vomiting and that some of the binding sites in the NTS may occur on vagal afferents.

We thank Beechams Pharmaceuticals for ^{3}H-BRL43694 and Glaxo Group Research for GR38032F.

236.13

CELLULAR ORIGIN OF THE DEPOLARIZATION-INDUCED RELEASE OF TAURINE FROM CEREBRAL CORTEX SLICES FROM DEVELOPING MICE. S.S. Oja, M. Pelto-Huikko* and Pirjo Kontro. Tampere Brain Research Center, Department of Biomedical Sciences, University of Tampere, Box 607, SF-33101 Tampere, Finland.

Potassium stimulation evokes a massive release of pre-loaded taurine, an inhibitory neuromodulator, from cerebral cortex slices from developing mice. It is not known which tissue pools accumulate the label and which cellular structures this release originates from. We have assessed the release of endogenous taurine from cerebral cortex slices from adult and developing mice by high-performance liquid chromatography and studied changes in taurine-like immunoreactivity by peroxidase-antiperoxidase reaction using rabbit taurine antiserum. The potassium-stimulated release was more than six times greater and considerably more prolonged in 3-day-old than in adult mice. In the adults taurine-like immunoreactivity was localized in astrocytes and oligodendroglia. Neurons were generally not stained. Potassium stimulation did not change this pattern. In 7-day-old mice immunoreactivity was most intense in neurons and nerve endings in the outermost cortical layers. Immunoreactivity clearly decreased in neurons and nerve endings after potassium stimulation. The results show differences in the cellular origin of the stimulated release of taurine in the developing and adult cerebral cortex and focus attention to the role of taurine in the regulation of neuronal activity in the immature brain.

236.14

MOLECULAR WEIGHT OF THE MELATONIN RECEPTOR. D.S. Pickering and L.P. Niles. Dept. of Biomedical Sciences, Division of Neuroscience, McMaster University, Hamilton, Ontario, CANADA L8N 3Z5.

It is only within the past three years that the pharmacological characterisation of melatonin receptors has been achieved by use of the radioligand 2-[^{125}I]iodo-melatonin. Differences in the pharmacological profile of the receptor are apparent between hamster brain and chick retina. It was the goal of this study to further characterise the melatonin receptor at the molecular level by using the technique of target-size analysis.

Radiation inactivation is a method whereby the in situ functional molecular mass of a receptor can be determined. We exposed frozen (-45°C) whole tissue homogenates to high doses of ionising radiation (0-30 Mrad, using 1.5 Mev electrons). Simultaneous irradiation of a series of enzyme standards of known molecular mass permitted creation of a calibration curve (mass vs. dose) from which the size of the melatonin receptor was determined: hamster hypothalamus, 43.7 ± 1.2 kDa (n=4); chick retina, 53.3 ± 3.4 kDa (n=3). As an internal control, the size of the β-adrenergic receptor was calculated to be 55.9 ± 2.4 kDa (n=4).

In summary, the melatonin receptor from chick retina was found to be considerably larger (22%) than that from hamster hypothalamus. This is consistent with an interspecies difference in the receptor.

This work was supported by the OMHF and MRC of Canada.

236.15

MELATONIN RECEPTOR SITES IN THE CHICKEN BRAIN: LOCALIZATION BY QUANTITATIVE RECEPTOR AUTORADIOGRAPHY. J. A. Siuciak and M. L. Dubocovich. Dept. of Pharmacol., Northwestern Univ. Med. Sch., Chicago, IL. 60611.

The avian pineal gland, through the secretion of its primary neurohormone melatonin, regulates circadian rhythms of activity, sleep like behavior and serotonin levels in different brain regions. The aim of this study was to localize melatonin receptor sites in chicken brain using in vitro autoradiography. Coronal brain sections were thaw-mounted on slides, incubated with 2-[^{125}I]-iodomelatonin in 170 mM Tris-HCl buffer (pH 7.4, 25 °C), rinsed in Tris-HCl buffer (0 °C), and allowed to air dry. Slides were apposed to X-ray film for one week. Specific binding, defined with 3uM melatonin, was unevenly distributed through the chicken brain. Autoradiographs were analyzed with an image analysis system. The highest density of binding sites was found in areas of the midbrain (optic lobe tectum, Edinger-Westphal nuclei, dorsal and dorsolateral oculomotor nuclei) and in the diencephalon (nucleus rotundus, tectothalamic tract, and the ventrolateral geniculate and dorsolateral and lateral thalamic nuclei). Lower density of binding sites was observed in the optic chiasm, hypothalamus, cerebellum and striatum. This regional distribution of 2-[^{125}I]-iodo-melatonin binding corresponds closely to that found in chicken brain homogenates (Dubocovich et al., 1989). Supported by grants MH42922 and DK38607 to MLD and NS07140-06 to JAS.

236.16

TYROSINE HYDROXYLASE (TH) IMMUNOREACTIVE NEURONS IN THE HYPOTHALAMUS OF CATTLE AND PIGS. L. S. Leshin, R. D. Kineman*, C. R. Barb*, T. E. Kiser*, G. B. Rampacek* and R. R. Kraeling*. USDA-ARS, Athens, GA 30613 and Animal and Dairy Sci. Dept., Univ. of Georgia, Athens, GA 30602.

Hypothalamic catecholaminergic neurons were examined in cattle (n=3) and pig (n=4) brains to identify anatomical sites possibly involved in the regulation of gonadotropin and prolactin secretion. Coronal and sagittal frozen sections (60 μm) of Zamboni's fixed hypothalamic-preoptic area tissue were incubated with TH primary antisera (Eugene Tech) for 24 hr, then visualized by biotin-avidin D-peroxidase immunocytochemistry. In both species, TH immunoreactive perikarya were observed in the lateral and caudal aspects of the arcuate nucleus (ncl), in the periventricular ncl, anteriorly within ncl periventricularis preoptica, in the dorsal-lateral region of the paraventricular ncl, suprachiasmatic ncl and supramammillary ncl. Some scattered cells were observed surrounding the ventromedial ncl and along the ventral hypothalamic surface between the supraoptic ncl and median eminence. Perikarya tended to be either bipolar, fusiform or multipolar, 3-5 processes, with numerous branched arborizations. Immunoreactive fibers were observed throughout these areas and within the median eminence. The location of TH immunoreactivity coincides with some areas previously reported to contain ß-endorphin and luteinizing hormone releasing hormone.

236.17

LOCALIZATION OF HISTAMINERGIC NEURONS IN THE FROG BRAIN. W. Briner, P. Guth, & C. Norris. Tulane University Medical Center, Depts. of Pharmacology and Otolaryngology, New Orleans, LA 70112.

Histamine (HA) is a suspected neuromodulator and has been found in the brains of several species (Panula, 1986). We examined the CNS of the frog using a well characterized antibody to HA with immunocytochemistry (ICC).

Rana pipiens were pretreated with colchicine, histidine which is the precursor of HA, and quinacrine which blocks histamine-methyl transferase, and perfused with a 3% 1-ethyl-3-(3-dimethylamino-propyl)carbodiimide and then by 3% paraformaldehyde in buffer (Panula et al., 1988). Frozen sections were taken and ICC performed using the ABC method.

Histaminergic neurons were found in many of the same areas as other species including; hypothalamus, periventricular area, optic tectum, thalamic regions, and cortex.

We report preliminary findings of light labelling in the cerebellum and its nuclei as well as in the vestibular nuclei. These regions are of special interest because of the effects of antihistamines on vestibular function.

236.18

BRAIN NEUROTRANSMITTER DISTRIBUTION IN THE WINTER FLOUNDER. L.W. Crim* and D.M. Evans* (SPON: N.M. Sherwood). Marine Sciences Research Laboratory/Ocean Sciences Centre, Memorial University of Nfld., St. John's, Nfld., A1C 5S7,

Biogenic amine neurotransmitters were determined in winter flounder brains collected from fish in prespawning or postspawned condition. Whole brains were dissected under ice-cold conditions into 7 regions including 1) olfactory lobes, 2) telencephalon, 3) optic tectum, 4) cerebellum, 5) mid-brain, 6) rhombencephalon and 7) the hypothalamus. After sonication of brain pieces in 0.2M perchloric acid, the supernatant was directly submitted to HPLC/EC analysis of norepinephrine (NE), dopamine (DA) and serotonin (5-HT). The greatest NE concentrations were found in the flounder telencephalon, mid-brain and hypothalamus while the rhombencephalon, olfactory lobe and optic tectum contained relatively lower NE levels. The olfactory bulbs were the richest source of DA in the flounder brain; smaller, yet detectable amounts of DA were localized in the mid-brain, telencephalon, rhombencephalon and the flounder hypothalamus. Detectable amounts of 5-HT were obtained in the mid-brain, hypothalamus, telencephalon and flounder rhombencephalon.

Although flounder brain neurotransmitter levels were generally similar irrespective of fish reproductive condition, significantly greater levels of NE ($P<0.05$) were found in the optic tectum of postspawned flounder compared with fish in prespawning condition.

DIFFERENTIATION, MORPHOGENESIS AND DEVELOPMENT: NEUROGENESIS AND CONTROL OF NEURON NUMBER

237.1

NEUROGENESIS AND HODOGENESIS IN THE OLFACTORY SYSTEM OF RAT EMBRYOS. M.-C. Bélanger*, S. Miloševič*, L. Bertrand* and R. Marchand, Lab. Neurobiol., Hôp. Enfant-Jésus, 1401, 18e Rue, Québec, (QC) G1J 1Z4.

Recent studies show that the olfactory system is functional early during foetal life. We wanted to verify the anatomical bases of this phenomenon by searching a correlation with the neurogenesis and the hodogenesis of the cell groups of this system. Neurogenesis was studied by injecting ^{3}H-thymidine to precisely-timed (E10, E11 and E12) gestating rats and, to trace early tracts, crystal of DiI (D282, 1,1'-dioctadecyl-3,3,3'3'-tetramethylindocarbocyanine perchlorate) were implanted into the olfactory epithelium, the vomeronasal organ and the olfactory bulb of precisely-timed fixed embryos.

The autoradiographic data show that olfactory neurons are among the first to be generated in the rostral telencephalon. In the adult, they are found among the mitral cells of the accessory olfactory bulb, the periglomerular cells of the special glomerular complex, the primary olfactory cortex, the bed nucleus of the accessory olfactory tract, the medial amygdaloid nucleus and the bed nucleus of the stria terminalis. In very young embryos (E13, E14), all these neurons constitute a longitudinal column (the rostral portion of the basal forebrain cell column) that extends along the ventrolateral edge of the telencephalic vesicles. At these early stages, a well-differentiated marginal zone is associated to the cell column. Under epifluorescence, we could trace orthogonally, the lateral olfactory tract from the olfactory bulb to the region of the bed nucleus of the accessory olfactory tract and retrogradely, the olfactory nerve to the cell bodies of the olfactory epithelium that showed a bright fluorescence. Therefore, we suggest that the rostral portion of the basal forebrain cell column serves as a template for guiding the first axons that emerge from the primordial mitral neurons en route to their target structures. The coupling of these two techniques opens a wide field for the study of the strategies involved in the morphogenesis of the nervous system. (Supported by MRC, FRSQ and FCAR).

237.2

QUANTITATIVE RELATIONSHIPS BETWEEN THE NOSE AND THE FOREBRAIN IN XENOPUS LAEVIS TADPOLES. G. A. Monti Graziadei and P. P. C. Graziadei. Dept. of Biological Science, Florida State University, Tallahassee, FL 32306.

Previous experiments in Xenopus laevis have shown that removal of the olfactory anlagen impairs the development of the telencephalon. Here we report the results obtained from those cases in which asymmetrical regeneration of the olfactory organs occurs. The olfactory anlagen were removed from 22-24 stage Xenopus embryos. The animals, sacrificed at stage 50, were embedded in paraffin, serially sectioned, and stained with hematoxylin. The nasal organs and brains were reconstructed with an IBM PC-based 3-D reconstruction program, and the respective volumes calculated. Our results indicate that the asymmetric development of the nasal organs is always accompanied by asymmetric development of the two hemispheres, and this relationship is linear. Although the forebrain in amphibians receives inputs from other sensory modalities, the olfactory input seems to be a major influence in determining its full development. (NSF Grant BNS 86 17022.)

237.3

DIFFERENTIAL DISTRIBUTION OF DIVIDING CELLS IN THE DEVELOPING CNS OF THE LEECH *HIRUDO MEDICINALIS* REVEALED BY BRDU INCORPORATION. C.A. Baptista*, T. Gershon* and E.R. Macagno. (SPON: M.-M. Poo). Dept. of Biological Sciences, Columbia University, New York, NY 10027.

The sex ganglia of *Hirudo* contain several hundred PE neurons which are not found in other segmental ganglia (SG). These cells can first be identified by the end of embryogenesis (30 days after egg-laying), with the majority appearing postembryonically. Early ablation of the male genitalia results in the complete absence of these cells (Baptista and Macagno, *J. Neurobiol.* 19:707, 1988). To shed some light on the nature of this interaction, we have begun to examine the frequency and spatial distribution of terminal cell division by exposing animals to pulses of bromodeoxyuridine (BrdU) and visualizing the incorporated nucleotide with anti-BrdU. We have detected a large population of labeled cells in the CNS of 20-30 day embryos. In contrast with younger animals, animals exposed to BrdU at these stages and examined within a few days show a dramatic difference between the sex and neighboring SG, with many more labeled cells in the former. When these animals are examined postembryonically, they still exhibit a large number of labeled cells, with more in the sex SG. However, if postembryonic animals are exposed to a pulse of BrdU between 30 and 65 days of development, when the number of PE neurons is rising sharply, the frequency of cell division appears to be very low in all SG. Ablation of the male organs in 10-day-old animals caused a reduction in the number of new cells seen in the sex SG, eliminating in most cases the differential distribution of cell division. We are currently using morphological criteria and immunochemical properties to test whether any of the cells labeled with BrdU correspond to the PE neurons.

237.4

CORRELATIONS BETWEEN TARGET-ORGAN GROWTH AND ADDITION OF CELLS IN THE PeIb CLUSTER OF LYMNAEA STAGNALIS. K.G. Bina* and R.P. Croll. Dept. of Psychol. and Dept. of Physiol. Biophys., Dalhousie University, Halifax, NS Canada B3H 4J1.

Recent studies demonstrate that most serotonergic cell clusters add to their cell numbers at different rates post-embryonically in Lymnaea stagnalis. For e.g. cells in the PeIb cluster show a sudden increase in number and size at about the time of sexual maturity (Croll & Chiasson, J. Comp. Neurol., 280:122-142, 1989). This study focuses on the relationship between the growth of the penis (the target organ of the neurons in the PeIb cluster) and the addition and/or differentiation of the cells in the PeIb cluster. Correlations drawn between penis size and shell length suggest that at about the time of sexual maturity the penis length also shows a dramatic increase. Nickel-lysine back fills performed on the penis nerve to determine the addition of cells innervating the penis in the PeIb cluster with age, suggests that cells are added at the time of sexual maturity. This was supported by the lack of serotonin-like immunoreactivity in sexually immature penis and the appearance of serotonergic projections in the sexually mature penis. Baptista and Macagno (J. Neurobiol.,19:707-726,1988)suggest that in leech the penis may have a trophic influence resulting in postembryonic addition of cells in the CNS. Our studies on the effect of penile ablation on addition of cells in the PeIb cluster, the effect of serotonin depletion in the CNS on the growth of penis and penis transplantation will be discussed.

237.5

PERSISTING HIPPOCAMPAL PRIMORDIUM FOLLOWING PRENATAL ADMINISTRATION OF METHYLAZOXYMETHANOL. S. Chen and D. Hillman. Dept. Physiol. & Biophys. NYU Med. Ctr. NY 10016.

In Nissl stained coronal sections of adult rats injected with methylazoxymethanol (MAM) at gestational day 15 (G15), an abnormal cellular nodule was found located on the ventricular wall between the caudate and corpus callosum. This aberrant nodule was negative for acetylcholinesterase as compared to the intense staining in the adjacent striatum. Serial reconstruction through the region revealed that the nodule continued along the ventricle extending caudally onto the hippocampal region adjacent to the fornix. The hippocampal formation was markedly reduced in size and ectopic cells extended from the nodule and appeared to be streaming into the dispersed CA2 layer near the junction of CA1 and CA2. These cells were clustered in columns that dispersed through the external plexiform layer. Following G16, or G17 days injections, the cellular nodule was much smaller in size while the hippocampus was larger. Interruptions were also commonly found in the CA1 layer of these animals, but located more medially. This study suggests that the cellular nodule may represent a persisting primordial anlage of the subventricular stem cells which normally would have proliferated and migrated to their final destination. The topographical distribution of interruptions may be either a direct toxic effect of MAM on developing neurons and glia, or a secondary effect due to a deficit of selected afferents projecting into this region. [Supported by USPH grants NS13742 & HD20349]

237.6

MIGRATION OF NEURONS TO THE TRIGEMINAL PRINCIPAL SENSORY NUCLEUS OF THE RAT. W.M. Al-Ghoul* and M.W. Miller. UMDNJ- Sch. Osteopathic Med., UMDNJ- R.W. Johnson Med. Sch., and Rutgers Univ., Piscataway, NJ 08854.

During their migration, post-mitotic neurons move from their site of generation to their final destination. We examined neuronal migration in the trigeminal principal sensory nucleus (PSN) with autoradiographic and immunohistochemical techniques. The subjects for this study were fetuses and pups from timed pregnancies. Three experiments were performed. **(1)** Neurons generated on gestational day (G) 12 and G14 were identified using [^{3}H]thymidine autoradiography. It took 3-8 days for neurons born on G12 and 4-10 days for neurons born on G14 to complete their migration to the PSN. **(2)** Changes in the positions of these two neuronal subpopulations were determined using a combined autoradiographic-immunohistochemical technique; one subpopulation was labeled with [^{3}H]thymidine and the other with bromodeoxyuridine. Early-generated neurons ended up in the medial PSN, whereas late-generated neurons ended up in the lateral PSN. **(3)** Radial glial fibers were examined using the antibody Rat 401. These fibers that extended from the fourth ventricle through the PSN were identified throughout the third week of gestation; no immunoreactivity was detected by postnatal day 5. Thus, the results show that neurons initially arrive at the PSN in the order of their time of generation but are distributed by an inside-to-outside pattern. This migration is organized by a transient array of radial glial fibers. Funded by DE 07734, AA 06916, AA 07568.

237.7

CHRONOLOGY OF NEURON ORIGIN IN FEMALE RAT PARACERVICAL (PG) AND INFERIOR MESENTERIC (IMG) GANGLIA. H.H. Traurig and R.E. Papka. Anatomy & Neurobiology, Univ. of Kentucky, Lexington, KY 40502 & Anatomical Sciences, Univ. of Oklahoma, Oklahoma City, OK 73190.

PG and IMG provide autonomic innervation to the female reproductive tract and other pelvic organs. We have previously observed the development of phenotypic expressions of neurotransmitter markers by PG neurons during fetal and neonatal life. The present study determined the pattern of prenatal neurogenesis of autonomic neurons in the PG and IMG. Timed-pregnant rats were injected with tritiated-thymidine to label fetal PG and IMG neuroblasts proliferating on a particular gestational day beginning on day 14 (E14). PGs and IMGs were subsequently obtained from the adult female offspring (50-60 days) of these injected rats, serially sectioned and processed for autoradiography. The proportions of PG or IMG neurons arising on a particular gestational day were estimated from the daily declines in neurons previously labeled as neuroblasts on each gestational day. Results showed that virtually all neurons were labeled in rats exposed to tritiated-thymidine on E14 or E15 (93%). A few PG and IMG neurons arose on E14 or earlier, most neurons were generated on E15-E16 and 80% originated by E17. A few neurons arose as late as E21 or later. Thus, the majority of autonomic neurons innervating the female reproductive tract are generated on E15 through E17. (Supported by NIH grant NS23354 and UKRF4-31293.)

237.8

GENERATION PATTERNS OF SPINAL SYMPATHETIC AND PARASYMPATHETIC PREGANGLIONIC NEURONS IN THE RAT SPINAL CORD. R.P.Barber,* P.E.Phelps and J.E.Vaughn. Div. of Neurosciences, City of Hope, Duarte, CA 91010.

We have studied the embryonic generation of spinal cholinergic preganglionic autonomic neurons in the rat using a monoclonal antibody against choline acetyltransferase combined with tritiated thymidine autoradiography to label dividing cells. The thoracic and upper lumbar segments contain four subgroups of cholinergic sympathetic neurons that span the intermediate gray from the lateral funiculus to the dorsal gray commissure (Barber et al., 1984). Sympathetic preganglionic neurons in upper thoracic and upper lumbar spinal cord were generated on days E11-12, with peak generation in these two regions being E11 and E12, respectively. The single group of parasympathetic neurons in the first sacral segment was generated between E11-13, with the peak generation on E13. At all spinal levels, the range and peak generation of the autonomic motor neurons coincided with that of somatic motor neurons at the same rostrocaudal spinal level. Findings that these neurons are generated synchronously raise the possibility that visceral and somatic motor neurons may be derived from the same region of the germinal zone. In addition, they indicate that visceral efferent neurons do not obey the general ventrodorsal gradient of spinal neurogenesis since, despite the simultaneous time of origin, such cells are located substantially more dorsally than somatic motor neurons. Supported by NIH grant NS25784.

237.9

DEVELOPMENT OF CHOLINERGIC PREGANGLIONIC NEURONS IN UPPER THORACIC RAT SPINAL CORD. P.E. Phelps, R.P. Barber*, and J.E. Vaughn. Division of Neurosciences, Beckman Res. Inst. of the City of Hope, Duarte, CA 91010.

A monoclonal antibody against choline acetyltransferase (ChAT) was used to study the embryonic development of ChAT expression within sympathetic preganglionic neurons. On E21, all four subgroups of sympathetic neurons were immunoreactive and distributed in their characteristic pattern across the intermediate zone, forming striking arrays of transverse dendritic bundles. In contrast, on E15-16, ChAT+ sympathetic neurons appeared in laterally located, triangular shaped groups, with their processes extending 50-75% of the distance across the intermediate zone. Our findings suggest that the transverse dendritic bundles are formed between E15 & 19. In addition, the ChAT+ sympathetic neurons in both E15 & 16 specimens are found in the ventral spinal cord, whereas at later ages they have achieved their more dorsal location. These observations, combined with our other findings that thoracic somatic and autonomic motor neurons have identical generation times, suggest that both populations may be derived from the same, or adjacent parts of the ventral ventricular zone, despite their different final positions. Younger stages will be studied for differences in migratory pathways of these two types of spinal cholinergic motor neurons.

Supported by NIH grant NS25784.

237.10

CORRELATION OF NEURONAL BIRTHDATE AND TRANSMITTER PHENOTYPE FEMALE RAT PG NEURONS. K.A. Sullivan R.E.Papka and H.H.Traurig Dept. of Anat. and Neurobiol., Univ. of Kentucky,Lexington KY., Dept. of Anatomical Sciences, Univ. of Oklahoma, Oklahoma City, OK.

We have previously reported that approximately 80% of PG neurons are generated by E15 and E16 while a significant proportion arise on E19. In the present study, timed pregnant rats received tritiated thymidine (^{3}HT) to label proliferating fetal neuroblasts. When these offspring reached adulthood, immunohistochemistry or histochemistry for peptide/transmitter localization was combined with autoradiography on sections of the PG to correlate phenotypic expression of transmitter markers with neurogenesis. Preliminary results indicate that NPY containing neurons are generated as early as E15 and as late as E20. TH-containing neurons appear to be generated later in development. Very few neurons generated on E14 and E15 contain both ^{3}HT and TH labels, while double-label is more commonly observed on E18-E20. (Supported by NIH Grant NS-22526).

237.11

BRAIN/BODY GROWTH IN ALLIED RATS. M.D. Mann and A.L. Towe, Departments of Physiology and Biophysics, Univ. Neb. Sch. Med., Omaha, NE 68105 and Univ. Wash. Sch. Med., Seattle, WA 98195

Of the specimens used by J.M. Taylor in her study of the reproductive biology of *Rattus assimilis* (*Univ. Calif. Pub. Zool.*, 1961, 60:1-66), the skulls and skins of 89 wild-caught and 82 laboratory-born/reared specimens are available for study in the Museum of Vertebrate Zoology at the University of California, Berkeley. The lab-reared group spans ages at death of 16 days to 3.5 yr pp, and, therefore, is one of the most complete developmental studies available. We measured all cranial volumes (E), and related them to body weight (P), body length (BL) and tail length (TL). In the lab-reared group, we also considered these variables as functions of age (A) at death.

Comparing E with P in the wild-caught group, we obtained a reduced major axis with slope, b = 0.247, comparable with those for other rats, $\bar{b} = 0.280$ (± 0.054) for 10 species (Mann *et al.*, *Brain Behav. Evol.*, 1988, 31:111-124). In this sense *R. assimilis* is not an unusual rat. Comparing E with BL, b = 0.748, and comparing BL with P, b = 0.332. Thus, the three slopes calculated for E:P, E:BL and BL:P bear the appropriate relations $0.247 = (0.748)(0.332)$ [$b_{E:P} = b_{E:BL} \times b_{BL:P}$]. Comparing BL with TL, b = 0.715.

For lab-reared animals, all variables were fitted by two linear regressions on age, one in the rapid growth of youth and one in the slow growth of adulthood. The slope ratios showed that $E \propto BL$ and $E \propto P^{1/3}$ throughout life, but TL stabilized at the end of rapid growth. Therefore, $E(A) = kBL(A)$. The simplest explanation is that for every fiber that is added to the spinal cord from brain, the brain itself increases by the volume of one cell body. Whatever the reason, brain and body growth in rodents differ sharply from those in primates and carnivores. The mean E:P slope of 1/3 for different species of rodents (Mann *et al.*) may simply reflect a normal growth pattern of rodents, rather than some more obscure constraint on brain/body relations.

237.12

PATTERNS OF GROWTH IN GOLDFISH OLFACTORY BULB AND SENSORY EPITHELIUM. J. Silveira and P.C. Brunjes. Dept. of Psychology, Univ. of Virginia, Charlottesville, VA 22903.

Goldfish exhibit slow growth throughout much of their life, making them a useful model for developmental processes. We have demonstrated that goldfish olfactory receptor sheets (rosettes) and olfactory bulbs grow in proportion to the rest of the fish. Rosettes expand by 1) adding lamellae (fleshy radiations containing sensory epithelium) medially and 2) elongating existing lamellae. Laminar volume analysis of olfactory bulbs showed that proportionately less volume is represented by the granule cell layer in large versus small fish, suggesting addition of neuropil instead of cells during growth. The present study investigated patterns of mitotic activity in growing rosettes and bulbs using ^{3}H-thymidine autoradiography. Olfactory bulbs of fish exposed to ^{3}H-thymidine in aquarium water (100μCi/l) had only a few labeled cells at the granule cell layer periphery ($< 1/100\mu m^2$), suggesting that the increase in bulb size seen with fish growth is due to the addition of neuropil, rather than the addition of new cells. Rosettes exhibited substantial cellular proliferation, but labeled cells were distributed evenly throughout the structure suggesting no foci of cellular production.

Supported by NS 23154 and MH 18411

237.13

ANGIOGENESIS IN NORMAL AND DEPRIVED OLFACTORY BULBS. D.L. Korol and P.C. Brunjes. University of Virginia, Charlottesville, VA 22903.

Permanently blocking stimulus access to olfactory receptors on the day after birth (P1) results in a series of changes within the olfactory bulb, including rapid decreases in metabolism and protein synthesis and later reductions in bulb cell number and laminar volume. Perhaps decreased cellular activity resulting from deprivation reduces regional blood flow, subsequently altering vascular development. Altered angiogenesis might in turn impair bulb growth. To address this hypothesis we examined the development of vasculature in olfactory bulbs of rats that underwent unilateral naris closure or sham surgery on P1. At several postnatal ages pups were perfused with either a WGA-HRP or an india ink/gelatin solution. Computerized imaging was used to determine area fractions of blood vessels across the extent of the bulb. In normal bulbs considerable increases in vascular area and complexity were observed in nearly all laminae between P10 and P20. After 30 days of deprivation 10-20% reductions in vessel area in the glomerular and external plexiform layers were observed. Deprived bulbs, therefore, are not merely miniaturized versions of normal bulbs: they contain less vasculature per unit area. The possibility that deprivation-induced changes in the vasculature arise prior to reductions in bulb size is presently being evaluated. Supported by NS23154 and HD07323

237.14

PRENATAL CELLULAR PROLIFERATION IN THE FOREBRAIN OF THE PRECOCIAL MOUSE *Acomys cahirinus* P. C. Brunjes, D. L. Korol and K. G. Stern*. Univ. of Virginia, Charlottesville, VA 22903.

Acomys, like the laboratory rat and mouse, is a member of the rodent subfamily muridae. However, unlike its altricial cousins, *Acomys* is born after a long (39 day) gestation period with functional ears and eyes and quite sophisticated locomotor capabilities. Widespread differences in the development of the olfactory bulb, hippocampal formation and visual cortex have been reported between the species in both prenatal and postnatal life. We have examined the time of cell production in *Acomys* with ^{3}H-thymdine techniques to specify patterns of early growth. Substantial differences were observed between *Acomys* and the rat, with *Acomys* exhibiting much later and more protracted periods of proliferation. For example, mitral cell production was observed to occur near day E17 in *Acomys*, while most proliferation is completed in the rat 2-3 days earlier. Hippocampal pyramidal cells were generated in *Acomys* between E19-21, and dentate granule cells between E21 and birth. Labelled cells were first observed in the visual cortex on E17, with production ending by E35. The protracted development in *Acomys* suggests it is quite suitable for detailed examinations of early growth, while the differences between the species suggest they are ideal for examining phylogenetic differences in brain development. Supported by NS23154 and HD07323

238.1

EXPRESSION OF JONES GANGLIOSIDES IN EXPLANTS OF RAT CEREBELLUM. R. Méndez-Otero and M. Constantine-Paton. Instituto de Biofísica Carlos Chagas Filho, UFRJ, Rio de Janeiro, RJ, 21944 and Dept. of Biology, Yale University, New Haven, CT. 06511.

In dissociated cell cultures of postnatal rat cerebellum, JONES gangliosides are expressed by neuronal and glial sub-populations (Méndez-Otero et al., Soc. Neurosci. Abstr., 13: 1636, 1987). To test the hypothesis that JONES expression is associated with migrating granule cells, we have used cerebellar explant cultures where the temporal relationship between granule cell migration and differentiation has recently been described (Hockberger et al., J. Neurosci. 7: 1370, 1987). Explants were prepared from P2-P6 postnatal rats, fixed after different intervals in culture and immunostained. After 1 to 3 days in vitro (young cultures), JONES gangliosides are expressed by the vast majority of granule cells and by the radiating glial processes. JONES immunoreactivity decreased progressively with distance from the explant thus paralleling the maturation of granule cells. The results confirm our hypothesis that expression of JONES gangliosides is correlated with migrating behavior in vitro.

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238.2

MONOCLONAL ANTIBODIES TO CARBOHYDRATE ANTIGENS IDENTIFY SUBSETS OF OLFACTORY AND VOMERONASAL AXONS IN THE RAT. G.A. Schwarting and J.E. Crandall. Depts. of Biochemistry and Developmental Neurobiology, E.K. Shriver Center, Waltham, MA 02254.

Cell surface glycoconjugates are thought to play an important role in cell-cell interactions during development of CNS pathways. Monoclonal antibodies were raised against PC12 cells in order to obtain stage and region specific carbohydrate antigens in the nervous system. Two of these monoclonal antibodies, CC1 and CC2, were used to examine the expression of glycoconjugates in the forebrain of postnatal rats. CC2 reacts with complex glycolipids which contain terminal α-fucose residues. In the forebrain of 15 day old rats, CC2 reacts with surfaces of axons in both the olfactory nerve and vomeronasal nerve. No immunoreactivity is seen in the glomeruli of the main olfactory bulb. In contrast, CC2 immunoreactivity is seen throughout the accessory olfactory bulb. CC1 monoclonal antibody reacts specifically with the glycolipid globoside, which contains a terminal N-acetylgalactosamine residue. In the forebrain, CC1 reacts with the axons of the vomeronasal nerve. CC1 immunoreactivity is also distributed in the glomeruli and external plexiform layer of a restricted portion of the accessory olfactory bulb. Thus CC1 reacts with a subset of the neuropil that is stained with CC2. (Supported by NIH grants NS25580 and NS24386).

238.3

COMPOSITION OF THE EXTRACELLULAR MATRIX AT THE GLIAL SEPTUM LEVEL IN THE EMBRYONIC RAT BRAIN. A. Nadeau, S. Woerly and R. Marchand. Lab. Neurobiol., Hôp. Enfant-Jésus, 1401, 18e Rue, Québec, (QC) Canada, G1J 1Z4.

Van Hartesveldt et al. (1986) described, within the midline raphe, a transitory glial structure that appears to extend within the midbrain, hindbrain and cervical spinal cord. This structure would be present from at least embryonic day 15 to postnatal day 7 or 8. More recently, the same structure which is closely associated to the floor plate has been shown to promote and orient commissural axonal growth through the secretion of a diffusible factor (Tessier-Lavigne et al., 1988). To better circumscribe the biochemical nature, the origin and extent of the glial septum in the developing brain, we have aimed our study at two main aspects of the development of this structure. First and based upon the important implication of various extracellular matrix (ECM) components in the growth and orientation of axons towards their target structures, we have focused our attention upon the identification of different molecular elements of the glial septum ECM. The second aspect of the study was oriented towards the morphological development and organization of the glial septum. Precisely timed embryos of various ages were fixed in 10% formalin, embedded in paraffin and cut at 10µm. Specific histochemical techniques known to reveal different ECM elements were applied to these sections. Other embryos were also injected with ^{3}H-thymidine to determine the time of origin of the floor plate cells. The results show the presence of collagen, possibly of types I, III and IV, carboxylated and sulfated glycosaminoglycans and reticulin fibers that are associated to basement membranes. The glial septum is rich in several ECM molecules that are known to provide a favorable environment for axonal migration. Our data also indicate that the cells of the floor plate are generated as precociously as day 11 of gestation, that the glial septum is formed rapidly thereafter, and that it does not extend rostrally farther than the fovea isthmi. (Supported by MRC, FRSQ and FCAR).

238.4

TRANSIENT EXPRESSION OF CYTOTACTIN AND ITS PROTEOGLYCAN LIGAND IN THE BARREL FIELD OF DEVELOPING MOUSE SOMATOSENSORY CORTEX. K.L. Crossin*, S. Hoffman, and G.M. Edelman. Laboratory of Developmental and Molecular Biology. The Rockefeller University. New York, New York 10021

The expression of cytotactin, a high molecular weight glycoprotein, and cytotactin-binding (CTB) proteoglycan, a chondroitin sulfate proteoglycan with which cytotactin interacts, was examined in the developing barrel cortex of postnatal mice. At PN6-7, both molecules and their common HNK-1 antigenic determinant were expressed in the barrel walls and were excluded from the hollows. This molecular exclusion began at about PN2.5-3 concurrent with the entry of thalamocortical axons. This pattern was maintained until PN12, but by PN13 the expression of both molecules was low and uniform throughout the barrel field. The expression of both molecules was perturbed after removal of a row of whiskers and electrocauterization of the whisker follicle, consistent with previous studies showing altered morphology after such treatment. In cellular studies, glia synthesized cytotactin but not CTB proteoglycan, whereas mixed cultures of neurons and glia synthesized both molecules, suggesting that CTB proteoglycan was synthesized by neurons in these cultures. The results are consistent with the idea that there exist molecular correlates of CNS pattern formation and that both glia and neurons contribute to the molecular pattern.

238.5

GLIAL DISTRIBUTION IN THE RODENT BARREL FIELD FOLLOWING MECHANICAL LESIONS OF THE SOMATOSENSORY CORTEX. Eric D. Laywell* and Dennis A. Steindler. Department of Anatomy and Neurobiology, College of Medicine, The University of Tennessee, Memphis.

The unique geometry of the mouse somatosensory cortical barrel field, and its transient cordoning off during development by certain glia and glycoconjugates including the J1/tenascin adhesion molecule (Steindler et al., Dev. Biol., 131:243, 1989), provide an opportunity to study neuron-glia interactions in the developing normal and lesioned cortex. During normal development, by postnatal day 11 there is a transition from predominantly boundary glia (i.e. in the future interbarrel septae) to non-boundary glia (i.e. in barrel hollows). To see if there is a subpopulation of glia that maintains a boundary distribution in adult barrels, but evaded detection in our previous immunocytochemical (ICC) studies due to the reduced expression of the glial fibrillary acidic protein marker (GFAP) during later stages of normal cortical maturation, the following lesion study was performed in adult mice using a similar labeling approach. Stab wounds were made in the somatosensory cortices of adult ICR mice, and following different survival times of up to 14 days, the brains were processed for GFAP-ICC. It appears that there is not a subpopulation of reactive glia that demarcates barrels in the adult, and the pattern of GFAP labeling is similar to that seen in the second postnatal week in normal animals. In the lesioned adult barrel cortex, GFAP-positive astrocytes reside in both boundary and non-boundary positions. Lesion studies are currently underway during and just after the critical period in barrel development, but it is tempting to speculate now that the disintegration of glial boundaries following the stabilization of cortical patterns may be one reason why there is a failure to achieve complete functional reorganization following damage in the adult. Supported by NIH/NINCDS grant NS 20856.

238.6

PLASTICITY OF ADHESION MOLELCULE BOUNDARIES IN THE CORTICAL BARREL FIELD OF NORMAL AND REELER MUTANT MICE. T.F. O'Brien*, K. Harrington*, A. Faissner*, M. Schachner, and D.A. Steindler. (SPON: J. Schweitzer). Dept. of Anat. & Neurobiol., Univ. of Tenn., Memphis, and Dept. of Neurobiol., Univ. of Heidelberg.

We have previously shown glial and glycoconjugate boundaries that delineate developing normal and aberrant vibrissae barrels in normal and reeler mutant mice. The aim of the present study was to define changes in the reeler and normal barrel field boundaries following vibrissae lesions in the first postnatal week. Monoclonal antibodies to the J1/tenascin adhesion molecule, and the 473 proteoglycan expressed by glia (Faissner, Neurosci. Abs. 14:920, 1988), were used in immunocytochemistry studies following lesions of the C row of whiskers on P2-4. In normal (+/+,+/rl) animals, both antibodies revealed altered C row boundaries. This consisted of an attenuated C row with a corresponding increase in size of the adjacent D row of barrels, and a loss of interbarrel boundaries within row C that was not always accompanied by a detectable loss of barrel area. Both antibodies revealed the reeler barrel field in the control hemisphere similar to that previously reported using lectin binding. In the lesioned hemisphere in reeler, the presence of an attenuated band with altered staining adjacent to a row of enlarged barrels suggests that barrel boundaries in reeler are malleable in a way that is comparable to that seen in normal animals. Furthermore, this lesion/boundary study in reeler affords a barrel row assignment that is not so easily accomplished using other approaches. The findings suggest that the reeler defect, although resulting in altered morphogenesis, may not affect neocortical plasticity during development as witnessed by the reorganization of transient adhesion molecule boundaries. Supported by USPHS grant NS 20856, and the Deutsche Forshungsgemeinschaft.

238.7

TENASCIN IN SECONDARY NEURULATION. L-H.J. Liu*, K.S. O'Shea, and R. Chiquet-Ehrismann*(SPON: C.J. D'Amato). Dept. Anatomy & Cell Biol., Univ. of Mich., Ann Arbor, MI.

The distribution of tenascin, an extracellular matrix glycoprotein associated both with mesenchymal-epithelial interactions during morphogenesis as well as with oncogenesis, was examined in secondary neurulation in the mouse embryo. In this process, mesenchyme derived from the primitive streak aggregates, "epithelializes", forms a lumen and merges with the more anterior neuroepithelium, extending the length of the neural tube. In the current investigation, sections through the forming secondary neural tube from day 11 mouse embryos were processed for immunocytochemical localization of tenascin. Briefly, tenascin was initially found throughout the mesenchyme, particularly in the lateral mesenchyme bordering the surface ectoderm. With initial aggregation of mesenchymal cells near the dorsal surface of the embryo, anti-tenascin staining was lost from between forming epithelial cells and was displaced into the region of developing basement membrane. With consolidation of the aggregate, tenascin was densely deposited in the lateral region of the neuroepithelial basement membrane and in basement membranes of notochord and gut. Mesenchymal staining, except at the dorsolateral aspect of the neuroepithelium, was considerably reduced. As in the morphogenesis of other organs, tenascin may play a role in the mesenchymal-epithelial conversion involved in secondary neurulation.

238.8

TOPOGRAPHY OF N-CADHERIN EXPRESSION DURING CNS DEVELOPMENT SUGGESTS BOTH STRUCTURAL AND INFORMATIONAL ROLES. Laura A. Lagunowich* and Gerald B. Grunwald, Department of Anatomy and Training Program in Developmental Biology/Teratology, Thomas Jefferson University, Philadelphia, PA 19107.

N-cadherin is the major calcium-dependent cell adhesion protein expressed in the developing nervous system. Our previous studies have indicated that N-cadherin is expressed throughout the developing chick retina but becomes highly localized in the mature tissue. We now report similar biochemical and immunohistochemical studies of N-cadherin in the developing chick brain and spinal cord. Rostrally, localization of N-cadherin was seen in the ependymal lining of all ventricles and in the choroid plexus. Staining was also seen in the external granule cells of the cerebellum of the hatched chick while staining of other cellular layers of the brain was much reduced by this time. Caudally, specific staining was seen in the raphe of the midbrain and the floorplate of the spinal cord. Parallel biochemical experiments indicate that changes in the topographic expression of N-cadherin are accompanied by molecular modifications as well. 2-D gel autoradiograms of labelled neural retina indicate that N-cadherin is both a phosphoprotein and a sulfoprotein and that these post-translational modifications are developmentally regulated. Thus N-cadherin appears to be generally expressed at high levels throughout the CNS during early developmental stages but becomes restricted in mature tissues. The topographic distribution of N-cadherin in the hatched chick indicates that it has a continuous structural role in the maintenance of supportive components such as the ependyma, choroid plexus and retinal outer limiting membrane and also may have an informational role in specific regions such as the raphe and floor plate where N-cadherin may provide cues for directed axonal growth. Supported by NIH grants NRSA-EY-06067 to LAL and EY-06658 to GBG and March of Dimes grant 5-569 to GBG.

238.9

TEMPORAL AND SPATIAL DISTRIBUTION OF NEURAL CELL ADHESION MOLECULE (NCAM) IN THE EMBRYONIC RAT TRIGEMINAL SYSTEM. I.A. Scarisbrick* and E.G. Jones (Spon: Y. Torigoe) Dept. of Anatomy and Neurobiology, Univ. of California, Irvine, CA 92717.

Differential expression of cell adhesion molecules plays an important role in morphogenesis. To investigate the expression of NCAM during the development of the rat Trigeminal (V) system, paraffin embedded Wistar rat embryos of known gestational ages were sectioned and processed for NCAM immunoreactivity (I) using a rabbit antiserum to NCAM and the avidin-biotin technique.

Trigeminal ganglion cells were NCAM-I at their first appearance on embryonic day 10 (E10). Ganglion cells of placodal origin formed small clusters and strands of NCAM-I cell bodies extending medially from the level of the epithelium of the maxillo-mandibular arch, which was itself NCAM-I. The most intense immunoreactivity was seen at the cell surface. Centrally projecting fibers approaching the neural tube and peripherally projecting fibers beginning to sprout at the lateral aspect of the primordial ganglion were intensely NCAM-I. By E12, the neural crest derived basal cap cells, at the base of the first rhombomere, were also NCAM-I. The developing infraorbital nerve was not readily distinguished on E12, but the motor root was evident within the mandibular arch and tufts of centrally projecting fibers extended into the first rhombomere. The infraorbital nerve became intensely NCAM-I on E13 and approached the skin epithelium which was intensely NCAM-I basally. Developing vibrissae follicles became apparent on E14 but were only lightly NCAM-I. Both centrally and peripherally projecting fibers remained intensely NCAM-I, but immunoreactivity at the surface of V ganglion cells was greatly reduced. By E15 the infraorbital nerve reached the developing vibrissae follicles which showed increased levels of NCAM-I.

These results suggest that the differentiating whisker pads may express NCAM as they are contacted by the infraorbital nerve. The further development of this system will be described.
Supported by NIH Grant 21377.

238.10

THE PRODUCT OF THE NEUROGENIC NOTCH GENE IS A CELL SURFACE GLYCOPROTEIN. K.M. Johansen*, R.F. Fehon*, J.Johansen[1], & S. Artavanis-Tsakonas. (SPON: K. Frederiksen) Dept. of Biology, Yale Univ., New Haven, CT 06510 and [1]Dept. of Zoology, Iowa State Univ. Ames, IA 50011.

The *Notch* locus of *Drosophila melanogaster* is one of a small number of zygotically acting "neurogenic" genes involved in the correct segregation of neural from epidermal lineages during embryogenesis as well as in other postembryonic developmental events. The *Notch* gene product is predicted by DNA sequence analysis to be a large transmembrane protein with several striking features, most notably an array of 36 EGF-like repeats in the extracellular domain. We show that *Notch* encodes a large, glycosylated surface protein with an apparent molecular weight of 300 kD: 1) antibodies raised against three regions of the putative protein detect *Notch* on Western blots as a high molecular weight, primarily full-length product; 2) immunoelectron microscopy localizes the *Notch* protein to the cell membrane; and 3) lentil lectin column binding demonstrates that the protein is glycosylated, indicative of its surface protein nature. Early labelling in the blastoderm appears ubiquitous except for the pole cells, but as development proceeds some distinctive features emerge: stronger staining is seen within the germ band layer where neuroblast delamination occurs, and the developing embryonic nervous system shows pronounced axonal staining. The eye and wing discs are two other tissues showing a defined localization of *Notch* product: a set of cells within each ommatidial cluster in the eye disc and the presumptive wing veins are selectively labelled.

238.11

EFFECT OF PURIFIED EXTRACELLULAR MATRIX COMPONENTS ON THE BEHAVIOR OF NEUROEPITHELIAL CELLS IN VITRO. V. Nurcombe*, J. Drago* and P.F. Bartlett* (SPON: L. Hemmendinger). Walter and Eliza Hall Institute of Medical Research, Royal Melbourne Hospital, Australia 3050.

We have investigated in vitro the role of laminin, fibronectin and type IV collagen in influencing the growth and differentiation of neuroepithelial cells obtained from the telencephalon of embryonic day 10 mice. It was found using thymidine assay that both laminin and type IV collagen were mitogenic. Laminin also promoted neural differentiation, when applied to the culture substrate at concentrations greater than 50 µg/ml, as evidenced by cell flattening and profuse neurite outgrowth. Fibronectin was not mitogenic and its only observable effect was a transient increase in cell attachment and spreading. Both the phenotypic and mitogenic response of neuroepithelium to laminin could be reproduced by the laminin E8 fragment, whereas the E1-4 crossarm had no effect. Evidence will be presented that laminin can markedly potentiate the response of neuroepithelial cells to the soluble family of fibroblast growth factors, and that a particular precursor subpopulation can be stimulated to upregulate its own synthesis of extracellular matrix components.

238.12

EVIDENCE FOR THE IN VIVO POLYMERIZATION OF EPENDYMIN: A PROCESS IMPLICATED IN LONG-TERM MEMORY FORMATION. V.E. Shashoua G.W. Hesse* and B. Milinazzo* (SPON: T.O. Fox) McLean Hospital, Harvard Medical School, Belmont, MA 02178

Ependymin, a glycoprotein of the brain extracellular fluid, has been implicated in synaptic changes associated with the consolidation process of long-term memory formation and the activity-dependent sharpening of connections of regenerating optic nerve. In vitro experiments have demonstrated that ependymin has the capacity to polymerize into fibrous insoluble proteins (FIP) when the Ca^{2+} concentration is reduced by the addition of EGTA. Such products, once formed, do not dissolve in 2% SDS in 5 M urea. This property was used to develop an assay for detecting the presence of FIP in Brain. The results show that a reproducible quantity of FIP aggregates is present in goldfish and mouse brain. This was highly concentrated in the synaptosomal fraction and had identical immunoreactivity properties to FIP aggregates obtained by the polymerization of pure ependymin in vitro as well as a cross-reactivity to other protein components of the extracellular matrix such as fibronectin and laminin. Labeling studies with ^{35}S-methionine showed that labeled FIP aggregates associated with the synaptosomal fraction can be obtained in vivo. Comparisons of the amino acid sequence of ependymin with proteins of the extracellular matrix showed that domains with epitopes recognizable by antibodies to fibronectin, laminin and tubulin exist in the ependymin molecule. These results suggest that ependymin can polymerize to form FIP aggregates in vivo, and that they have immunoreactivity properties to major components of the brain extracellular matrix. Supported by NIH Grant No. NS25748.

239.1

VAGAL AND ACCESSORY NERVE PROJECTIONS OF A SENSORY GANGLION LOCATED IN THE ACCESSORY NERVE. J.P.O'Reilly* and L.P.Schramm (SPON: J.W. Osborn). THE JOHNS HOPKINS SCHOOL OF MEDICINE. BALTIMORE,MD. 21205.

The accessory (XI) cranial nerve is generally considered a purely motor nerve. However, small ganglia have been described in it. In the present study, retrograde tracing techniques were used to determine the peripheral projections of a ganglion found consistently in XI of the rat. Male Sprague-Dawley rats weighing 250-300 grams were anesthetized, and a ventral incision was made to allow access to the glossopharyngeal (IX), vagus (X), hypoglossal (XII) and XIth nerves. In initial experiments we labeled (fluoro-gold, true blue, or diamidino yellow) individual nerves. In subsequent experiments we labeled (true blue, diamidino yellow) both X and XI. After appropriate survival times, the animals were perfused, and the brainstem and upper cervical spinal cord were prepared for fluorescence microscopy. The presence of labeled soma in XI was recorded. As a precaution against potential peripheral mixing of pairs of dyes, we required that there be no double labeling of neurons in the dorsal motor nucleus of the vagus or the accessory nucleus. Both the Xth and the XIth nerves were found to contain peripheral projections from the accessory ganglion. However, the IXth and XIIth nerves did not show projections from this ganglion. In addition to single-labeled neurons (from both X and XI) in each ganglion, double-labeled neurons were also evident. We conclude that the sensory neurons in the accessory nerve subserve both accessory and vagal functions. Further, a restricted population of these neurons, those with peripheral branches in both the vagus and the accessory nerves, may represent a site of peripheral somato-visceral integration. Supported by NIH Grant HL161315.

239.2

BILATERAL INJECTION OF MUSCIMOL INTO THE RAT ROSTRAL VENTROLATERAL MEDULLA FAILS TO DECREASE MEAN RENAL NERVE ACTIVITY. L.R. Poree* and L.P. Schramm, Department of Biomedical Engineering, Johns Hopkins University School of Medicine, Baltimore, MD 21205

Muscimol, a GABA agonist, has previously been shown to decrease mean arterial pressure (MAP) when injected into the rostral ventrolateral medulla (RVLM). In the present study, MAP and renal nerve activity (RNA) were recorded before and after injections of muscimol (125 ng) into the RVLM of anesthetized, paralyzed, and artificially respirated rats. Microinjection (100 nl) of muscimol into the RVLM caused MAP to fall 55 ± 5 mmHg, but it failed to decrease mean RNA. Although mean RNA was unaffected by muscimol treatment, the frequency and amplitude of the discharges were significantly altered. Prior to muscimol treatment, low frequency (0.5-1.5 Hz), high-amplitude bursting activity was superimposed over the higher 6 Hz cardiac-linked activity. Following a train of these low frequency bursts, an extended silent period (1-2 sec) was often observed. After muscimol treatment the activity became non-bursting with only small changes in amplitude. Baroreceptor modulation of RNA was also abolished. These changes in RNA were similar to those observed after transection of the rat spinal cord. Further, after injection of muscimol, electrical stimulation of the superficial, dorsolateral cervical spinal cord elicited substantial inhibitions of RNA. Similar inhibitions had previously been elicited only after full, C1, spinal transection. Supported by NIH Grant HL 16315.

239.3

TONIC BRAINSTEM INFLUENCES ON SYMPATHETIC PATHWAYS TO THE KIDNEY AND SPLEEN IN RATS. K. Hayes*, C.P. Yardley* and L.C. Weaver. The John.P. Robarts Research Institute and Dept. of Physiol., Univ. of Western Ontario, London, Ontario, Canada.

Selective brainstem influences on pre- and postganglionic nerves controlling the kidney and spleen were investigated in fifty-two urethane-anaesthetized rats. Changes in renal (RNA), splenic (SNA) and preganglionic greater splanchnic (GSPNA) or white ramus (T13WR) nerve activity were compared after cervical spinal cord transection or blockade of the rostral ventrolateral medulla (RVLM) by unilateral microinjection of glycine. Although cord transection reduced RNA by 27±2%, RVLM blockade decreased RNA by a greater extent (-45±4%). In contrast, cord transection caused a greater decrease in SNA (-48±8%) than did RVLM blockade (-30±2%). This indicates that the decreased RNA after RVLM blockade was caused, in part, by sympathoinhibition. selective for renal nerves, that was unmasked by blockade of excitatory inputs. Similar to splenic nerve responses, cord transection decreased GSPNA by 32±6% while RVLM blockade decreased it by only 16±4%. Activity of T13WR increased by 12±36% after cord transection and decreased by 34±6% following RVLM blockade, responses comparable to those of renal nerves. These results suggest that the different balance of tonic supraspinal excitatory and inhibitory influences on two populations of lower thoracic preganglionic neurons leads to selective control of renal and splenic sympathetic nerves.(Support: Rick Hansen Legacy Fund and Canadian Heart Foundation)

239.4

COMPARISON OF THE DISTRIBUTIONS OF RENAL AND SPLENIC NEURONS IN SYMPATHETIC GANGLIA IN RATS. V. Chevendra* and L. C. Weaver (SPON: S. Sims). The John P. Robarts Research Institute and Dept. of Physiology, University of Western Ontario, London, Ontario, Canada.

Selective connections between pre- and postganglionic sympathetic neurons may depend upon the distribution of postganglionic neurons in different sympathetic ganglia. The distributions of postganglionic neurons innervating the spleen and kidney were compared in rats using retrograde transport of fluorescent dyes True Blue and Fluoro Gold. Crushed splenic and left renal nerves were simultaneously exposed to 1 μl of the dyes (4%) for 45 min. Four days after surgery, sympathetic prevertebral (greater splanchnic and solar plexus) and left paravertebral ganglia (T6 - L4) were removed, cut at 15 μM and labelled neurons were counted (n = 4). The following table illustrates the distributions of 7078 splenic and 6768 renal neurons.

	T6-T9	T10-T13	L1-L4	Splanchnic	Sol plex
Splenic	< 1%	13%	< 1%	64%	22%
Renal	< 1%	66%	14%	14%	5%

Within the sympathetic chain, splenic neurons were located mostly in T11 and renal neurons mostly in T13. In the prevertebral ganglia, renal and splenic neurons were found in different locations. The preferential distribution of renal neurons in the paravertebral ganglia and splenic neurons in the prevertebral ganglia provides an anatomical basis for selective preganglionic innervation. (Research supported by the Medical Research Council of Canada)

239.5

PARASYMPATHETIC AND SYMPATHETIC INNERVATION OF THE HEART IN THE DEVELOPING PIGLET. D.A. Hopkins, J.A. Armour, N. Gootman* and P.M. Gootman. Depts. of Anatomy and Physiology and Biophysics, Dalhousie University, Halifax, NS B3H 4H7, Dept. of Physiology, SUNY-Hlth Sci Ctr, Brooklyn, NY 11203 and Schneider Child. Hosp.-LIJMC, New Hyde Park, NY 11042.

The neonatal piglet is an important animal model of cardiovascular function but relatively little is known about the organization and development of nerves and ganglia innervating the piglet heart. In the present study seven piglets ranging in age from 4-46 days received injections of retrogradely transported fluorescent tracers (True Blue, Diamidino Yellow) into the heart. After 3-7 days, the thoracic ganglia and nerves were identified by microdissection. The brain stem and ganglia were processed for fluorescence microscopy. The results show that the gross anatomy of the thoracic ganglia and cardiac nerves of the piglet are more similar to those in the primate than those in cat and dog. Retrogradely labeled perikarya were observed in the major sympathetic and sensory ganglia, bilaterally. Extremely few double labeled cells were observed. The stellate ganglia were most consistently labeled but when middle cervical ganglia could be identified they also contained labeled perikarya. The brain stem contained labeled perikarya bilaterally in the ventrolateral nucleus ambiguus. Supported by MRC, N.S. Heart Foundation, NIH (HL20864) and LIJMC.

239.6

CONNECTIONIST ANALYSIS OF THE NEURAL PATHWAYS INVOLVED IN THE CONTROL OF THE CARDIOVASCULAR FUNCTION IN CATS AND RATS. M.A.L.Nicolelis*, C.H.Yu*, L.A.Baccala* (SPON:I.M.Patel). Dept. Physiol. Biophys., Hahnemann Univ., Philadelphia, PA 19102

A microcomputer-based program was created for storage and modelling of neuroanatomical circuits. As its first application the system was filled with information collected from the literature on the neural control of the cardiovascular system function in cats and rats. A matrix representation of these data was built by defining 39 structures involved in this control process and 123 direct connections between them. These neural structures and their connections were interpreted as a network and its topological properties were analyzed through the employment of graph theory. First, each nucleus of this network was individually classified according to its number of afferents and efferents. Afterwards the network was analyzed as a whole with procedures derived from graph theory. These emphasized distributed features of the network by transforming the original complex system of separated nuclei into a condensed representation formed by clusters of nuclei. This condensed representation and the interactions between the clusters it contains are in accordance with the physiological processes that the cardiovascular control network is known to carry out. The results derived from this biological based network were compared with 20 randomly built matrices containing the same number of nodes and connections. None of the structural properties found in the biological matrix could be reproduced with these latter networks. These results reflect the possibility that structural analysis of neural systems can be used to identify functionally important clusters. Also, these data suggest that different categories of physiological functions may be directly related to the patterns of connectivity of the networks which support them.

239.7

PROJECTIONS FROM THE LATERAL PARABRACHIAL NUCLEUS OF RAT. D. ZENG* AND S.L. STUESSE. NEUROBIOLOGY DEPT., N.E. OHIO COLLEGE OF MED., ROOTSTOWN, OH 44272

The parabrachial complex (PBC) is a visceral integrative area involved in taste, pulmonary, and cardiovascular function. The lateral parabrachial nucleus, especially, has been implicated in cardiac control. We injected a sensitive anterograde tracer, Phaseolus vulgaris leucoagglutinin, in the lateral portion of the PBC to see if it projected primarily to "cardiac autonomic" regions of the brain. Rats were anesthetized with Nembutal and PHA-L was iontophoresed into the PBC. The rats survived a minimum of 5 days. Immunohistochemistry was used to detect the presence of PHA-L. The major projection from the lateral PBC was, as expected, the central nucleus of the amygdala. Other rostral areas receiving projections included the hypothalamus (paraventricular, median preoptic, and dorsal nuclei) and the bed nuclei of the stria terminalis. Few thalamic projections were seen. Some fibers projected to the central gray, dorsal raphe, and pedunculopontine tegmental nucleus. Caudal projections were much sparser than rostral but targeted the presumptive A1/C1 area and nucleus ambiguus. Although these areas may be involved cardiac control, some of the projections probably subserve other functions as well.

239.8

A 3-DIMENSIONAL DIGITAL BRAIN ATLAS FOR ANATOMICAL DATA COMPARISON AND INTEGRATION. W.T. Rogers, A. R. Moser, and J.S.Schwaber. E.I. duPont Co., Wilmington, DE 19898

A 3-dimensional digital brain atlas has been developed which allows the comparison, integration, and manipulation of neuroanatomical information. This implementation overcomes some of the limitations of standard paper atlases by providing the means for high-resolution matching of experimental data into the 3-dimensional atlas reference frame. In addition to providing the ability to correct for plane of section, atlas software allows limited correction for the inevitable distortion of tissue due to sectioning, mounting, and histological manipulation. The primary advantage of the 3-dimensional digital atlas is that arbitrary planes of sections can be displayed. This, combined with the ability to warp data into the reference frame defined by the atlas, allows accurate comparison of data from different experiments.

The atlas is constructed from gray level electronic images of tissue sections. These images are brought into registration and stacked in a voxel format so that the complete set of images forms a 3-dimensional, electronically-stored representation of the brain. A special purpose image computer combined with a Unix workstation is used to manipulate the atlas data, display experimental mapped tissue sections, and project mapped anatomical data into the coordinate system defined by the atlas. Computer graphics and statistical techniques are used to analyse the composite data stored in the atlas.

While the atlas technology could be used for any species, we have used it for the rat brain. Specifically, it has been used by us to quantitatively compare the vagal motor neuronal populations in two individuals. We have found that the nuclei are highly homologous in both number and distribution of cells.

239.9

MAPPING OF CAROTID SINUS INPUTS AND VAGAL CARDIAC OUTPUTS IN THE RAT. J. Bradd*, J. Dubin*, B. Due*, R.R.Miselis, S. Montor*, W.T.Rogers*, K.M. Spyer and J.S. Schwaber [SPON: R.O. Davies]. E.I. duPont Co., Wilmington, DE 19898

Towards the goal of establishing the brainstem architecture mediating cardiovascular control and specifically the baroreceptor vagal reflex, carotid sinus afferents (CS) were examined using transganglionic transport of cholera toxin-horseradish peroxidase conjugate (CT-HRP). Sections were taken in the transverse and horizontal planes. Cable-like filling of fibers permitted detailed mapping of fibers, branching patterns and terminal fields. Labeled CS fibers ipsilateral to the injected sinus coursed caudally in the tractus solitarius (ts) and terminated in three distinct subnuclei of the nucleus of the tractus solitarius (NTS): dorsal cap of the ts, lateral interstitial and commissural (caudal to the area postrema). Fibers typically leave the ts before branching and then may give rise to multiple branches while traversing a subnucleus of the NTS. Cardiac injections of CT-HRP were made into locations containing vagal postganglionic neurons. Cardiac afferent labeling is prominent in the commissural subnucleus of the NTS, and extends more caudally in the nucleus than does baroreceptor input. Golgi-like labeling was achieved in three populations: two in the nucleus ambiguus (NA) and one in the dorsal vagal nucleus (DVM). A rostral NA population appears to be coextensive with the loose formation of the NA. These are large, multipolar, stellate cells with dendrites oriented in palisades. At the level of the obex, labeled DMV cells are present. These cells are smaller, with less extensive dendrites, typically confined to the DMV. More caudally a scattered population of cardiomotor cells with tortuous dendrites extends from the DMV ventrolaterally through the reticular formation to the NA. All data is integrated into a 3-dimensional digital brain atlas for modeling. Support: E.I. duPont and GM27739

239.10

DIENCEPHALIC PROJECTIONS TO THE NUCLEUS TRACTUS SOLITARIUS; INVOLVEMENT IN CARDIOVASCULAR CONTROL. P.N.Izzo* and K.M. Spyer. Department of Physiology, Royal Free Hospital Sch. Med., Rowland Hill St., London NW3 2PF, England.

Previous electrophysiological studies in the cat have demonstrated that electrical stimulation of the hypothalamus can evoke profound cardiovascular changes. Furthermore, it has been demonstrated that some of these effects are mediated via inhibition of the baroreceptor reflex at the level of the nucleus tractus solitarius (NTS, Mifflin et al., J. Physiol 399: 369,1988). This study seeks to investigate the location of neurones in the hypothalamus that may contribute directly to the integration of cardiovascular control at the level of the NTS.

Injections of horseradish peroxidase (HRP: 10-50nL) were made into the regions of the NTS that receive afferent input from the cardiovascular and respiratory receptors. The majority of neurones retrogradely labelled were found in the hypothalamus and the central nucleus of the amygdala (CNA). In the hypothalamus almost all neurones labelled were found in the paraventricular nucleus (PVN), a small number of neurones were also observed lying ventral to the PVN and close to the third ventrical. This projection to the NTS is bilateral with an ipsilateral predominence. The results obtained in these experiments suggest that inhibition of the baroreceptor reflex by hypothalamic stimulation in the cat occurs either by stimulation of neurones in the paraventricular nuclei and/or by stimulation of fibres of passage originating from the CNA.

239.11

TOPOGRAPHIC PROJECTIONS FROM THE PERIAQUEDUCTAL GRAY (PAG) TO THE VENTROLATERAL MEDULLA (VLM) IN THE RAT. Gary Aston-Jones, E.J. Van Bockstaele, V.A. Pieribone and M.T. Shipley[1] Div Behav Neurobiol, Dept Mental Hlth Sci, Hahnemann Univ, Phila., PA 19102. [1]Dept Cell Bio Anat, U Cincinnati, OH 45267.

Previous studies have demonstrated projections from PAG to VLM in rodent (Beitz, '83). In the present study using WGA-HRP retrograde transport, we find that distinct subregions of PAG project to VLM. At the level of the dorsal raphe, labeled neurons are located ipsilaterally, immediately lateral to the cerebral aqueduct. Further rostrally, a second group of labeled neurons appear dorsally to the cerebral aqueduct. A third group, not reported previously, is found densely labeled just dorsal to the oculomotor nucleus, in the contralateral supraoculomotor nucleus of the central gray (SOM).

Topography of PAG terminations in VLM was examined using discrete injections of Phaseolus vulgaris-leucoagglutinin (PHA-L) into various subregions of PAG. Following injections into lateral PAG, dense anterograde fiber labeling was found above the inferior olive and in the vicinity of B3 serotonin neurons while labeling in VLM proper was less dense. Anterograde labeling from dorsal PAG yielded fibers in caudal VLM. With injections in SOM, a large number of fibers were seen directly beneath the nucleus ambiguus, in nucleus rostral ventrolateral medulla, RVL. These fibers were highly varicose and appeared to be restricted to RVL.

These results also clarified PAG projections to other regions for example, recent studies have found that afferents to locus coeruleus (LC) are surprisingly restricted but were unable to further analyze nearby areas including PAG as possible afferents. We found scattered PHA-L fibers from PAG in LC indicating that this nearby structure may be a minor afferent to the LC nucleus (Ennis et al., this meeting).

Stimulation in PAG has been reported to affect cardiovascular and pain mechanisms, functions also prominently represented in VLM. It is possible that the various subregions in PAG afferent to VLM are functionally distinct in nature, and substantially contribute to the broad integrative properties of the VLM. Supported by PHS grant NS24698 and ONR/AFOSR Contract N00014-86-K-0493.

239.12

Axonal projections from caudal ventrolateral medulla to rostral ventrolateral medulla, nucleus tractus solitarius and spinal cord in rabbit. Y.W. Li* and W.W. Blessing. (SPON: C. Straznicky) Centre for Neuroscience, Flinders University of South Australia, Bedford Park, SA 5042.

The caudal ventrolateral medulla (CVLM) contains vasodepressor neurons whose activity inhibits sympathetic vasomotor tone. The present study has investigated the efferent projections of the neurons of the CVLM in rabbit using the Phaseolus Vulgaris Leucoagglutinin (PHA-L) anterograde tracing technique. Twenty N.Z.W. rabbits were anesthetized with Halothane and PHA-L (Vector) was iontophoretically deposited into the CVLM. After 5-14 days of survival rabbits were re-anesthetized and the brains were perfused with aldehyde fixative and sectioned on a Vibratome. PHA-L was localized using anti-PHA-L antibody (diluted 1/5000) combined with the avidin-biotin-peroxidase procedure. Neurons in the CVLM projected in three main directions. 1) Ascending projections coursed rostrally through the ventrolateral reticular formation up to the rostral medulla. Large numbers of PHA-L labeled terminals were seen in a restricted region of the rostral ventrolateral medulla (RVLM) ventromedial to the nucleus ambiguus where sympathetic excitatory neurons are located (Dampney, R.A.L., et al, Brain Res., 249:223, 1982). Some ascending axons continued rostrally through the RVLM, passing ventromedially to the facial nucleus to pontine, midbrain and forebrain regions. 2) PHA-L labeled axons could be followed from the CVLM to the dorsomedial medulla, including the nucleus tractus solitarius. 3) Descending fibres took a straight course through the lateral reticular formation in the medulla. They entered the lateral funiculus of the spinal cord, with the majority terminating in the grey matter at the cervical level. No terminals were observed in the intermediolateral region at the thoracic level. The anatomical finding supports the idea that the vasodepressor neurons affect sympathetic preganglionic neurons by an indirect route, probably by a short inhibitory projection to the RVLM (Blessing, W.W., Am. J. Physiol., 254 : H686, 1988).

239.13

EVIDENCE OF A MONOSYNAPTIC PATHWAY BETWEEN THE CAUDAL RAPHE NUCLEI AND IDENTIFIED SYMPATHETIC PREGANGLIONIC NEURONS IN THE RAT. A. Zagon*, S.J. Bacon* & A.D. Smith Department of Pharmacology, South Parks Road, Oxford, U.K.

Electrophysiological and anatomical studies have suggested the existence of a pathway between the caudal raphe nuclei and the sympathetic preganglionic neurons (SPNs). However monosynaptic connection between them has not yet been shown. We combined anterograde tracing using Phaseolus vulgaris leucoagglutinin (PhAL), retrograde tracing using a conjugate of cholera B chain and HRP (CB-HRP) and electron microscopy to look for such a pathway in rats. Iontophoretic injections of PhAL were made into the region containing the raphe pallidus (RPa), caudal raphe magnus (RMg) and obscurus (ROb) of 5 rats. 12 days later the same rats were given injections of CB-HRP into their left adrenal gland. Fixed tissue from the brainstem and spinal cord (T2-T12) was sectioned and reacted to reveal CB-HRP then incubated with an antibody to PhAL and processed using immunohistochemistry. Serial ultrathin sections from the spinal cord were examined in the electron microscope. When PhAL had been injected into the regions mainly restricted to the RPa and RMg, synaptic contacts were found between PhAL-containing terminals and retrogradely labelled SPNs. Out of the 43 synaptic contacts analysed, 26 were onto somata and 14 onto dendrites. 75% of the total appeared to have symmetric membrane specifications, 20% asymmetric and the remainder could not be classified. Synaptic contacts were not seen when the PhAL injection site involved cells only in the ventral ROb and surrounding gigantocellular reticular formation.

These findings provide evidence of a direct monosynaptic pathway between cells in the RPa and caudal RMg and identified SPNs.

239.14

SEGMENTAL ORGANIZATION OF THE C1 PROJECTION TO THE INTERMEDIOLATERAL CELL COLUMN. *I. Jeske and K.E. McKenna.* Dept. of Physiology, Northwestern Univ. Med. Sch. Chicago IL 60611.

An anatomical substrate for selectivity in sympathetic motor outflow was examined by comparing quantitative data from ketamine-xylazine anesthetized male Sprague Dawley rats that were injected with Fluorogold dye (50-80 nl, 4% v/v, FG) into upper (T3-T5) middle (T7-T9) and lower (T11-T13) levels of the intermediolateral cell column (IML). After a survival period of 2 weeks they received colchicine (10μg/10μl) injections into the ipsilateral lateral ventricle to enhance immunofluorescent detection of phenylethanolamine N-methyltransferase containing (PNMT+) neurons in the rostral ventrolateral medulla (RVLM). The number of cells PNMT+; PNMT+ and labeled with FG (FG+); and PNMT- and FG+ were counted under epifluorescent illumination within the C1 area from 1 to 1.7 mm rostral to obex. Bulbospinal neurons projecting to different rostrocaudal extremes of the IML column arose from comparable or intermingled sites in the RVLM, suggesting that the location of a bulbospinal neuron in the RVLM is not anatomically correlated with the spinal segment it innervates. The C1 bulbospinal innervation was segmentally directed to upper thoracic levels of the IML column, since 56±3%, 35±6%, 25±8%, of the PNMT+ population was labeled after upper, middle, and lower level injections, respectively. In comparison, the number of PNMT- bulbospinal neurons, contained in the same area and closely intermingled with the PNMT+ bulbospinal population, remained relatively constant, regardless of which thoracic levels had been injected. These non-catecholaminergic neurons comprised 62±8% of the total bulbospinal population labeled in the C1 area. If selectivity in sympathetic motor outflow at the level of the IML is neurochemically encoded, the finding that the RVLM adrenergic input is directed to upper thoracic segments, which contain functionally identified baroreceptive (Seller H., Pflugers Arch. 343:317-330,1973) and cardioacceleratory (Calaresu et al., Am. J. Physiol. 222(3):700-704, 1972) sympathetic preganglionic neurons, is consistent with a role for C1 neurons in influencing sympathetic outflow preferentially to cardiovascular structures.

239.15

CAUDAL VENTROLATERAL MEDULLA (CVL): A SOURCE OF TONIC AND BARORECEPTOR-MEDIATED SYMPATHOINHIBITION. S.L.Cravo*, S.F.Morrison, D.J.Reis. Division of Neurobiology, Cornell Univ.Med.Coll., New York, NY10021.

The caudal ventrolateral medulla (CVL) is a major vasodepressor center. CVL stimulation produces hypotension and bradycardia, while lesion in this area causes hypertension of neurogenic origin. Whether the CVL also mediates the baroreceptor reflex (BR) is not certain since lesions of CVL elevating arterial pressure (AP) do not invariably abolish the reflex. We sought to determine whether the CVL is functionally heterogeneous. Rats were anesthetized with urethane, paralysed and ventilated and AP and splanchnic sympathetic nerve activity (SNA) were recorded. The BR was elicited by electrical stimulation of the aortic depressor nerve (ADN) or infusion of norepinephrine. Local neuronal blockade was produced bilaterally in subareas of CVL by microinjections of kainic acid (KA, 10 mM, 10-20 nl). Injection sites were anatomically reconstructed. KA injected into rostral CVL (CVLr, between 0.8 and 1.8 rostral to the calamus) elicited: a) an increase in MAP (34 ± 6.1 mmHg, n=10), and SNA (to 214% of control), b) abolition of the BR; c) elimination of the cardiac-related rhythm in SNA. KA restricted to caudal CVL (CVLc, between 0.5 rostral and 1 mm caudal to the calamus) also elevated AP (20 ± 4.3 mmHg) and SNA (to 196% of control) but had no effect on the BR or the cardiac rhythm in SNA. We conclude that: (a) the CVL is functionally heterogeneous, (b) neurons in CVLr mediate tonic and baroreceptor-evoked inhibition of SNA, (c) CVLc contains a tonic, baroreceptor-insensitive sympathoinhibitory system, d) dysfunction within the CVL could result in hypertension of neurogenic origin.
Supported by FAPESP 86/2985-5, NIH 18974.

239.16

DESCENDING PROJECTIONS OF HYPOTHALAMIC (HYP) SYMPATHOEXCITATORY (SE) NEURONS IN THE CAT. Susan M. Barman. Dept. Pharm. & Tox., Mich. State Univ., E. Lansing, MI 48824.

This study was designed to test the hypothesis that the major pathway mediating HYP-induced increases in sympathetic nerve discharge (SND) is a direct projection to the rostral ventrolateral medulla (RVLM). Posterior or lateral HYP stimulation increased inferior cardiac SND (latency, 68-94 ms) and synaptically activated (modal latency, 7-64 ms) 10 RVLM-SE neurons. An SE neuron is one whose activity is correlated to SND and whose firing rate is decreased by baroreceptor reflex activation. HYP-SE neurons (n=14) were antidromically activated by RVLM stimulation. However, antidromic mapping suggested that the axons of 11 of these neurons passed through rather than terminated in the RVLM. These data do not support the hypothesis under consideration. The possibility that HYP-SE neurons project to the periaqueductal gray (PAG) was then tested. HYP-SE (n=15) neurons were antidromically activated by PAG stimulation. Antidromic mapping suggested that these neurons terminated in this region since their longest latency antidromic responses (9-35 ms) were elicited with the least stimulus current when stimulating sites in the PAG (A2.5-3; LO.7-1.3; H-0.6 to +1.5). Antidromic latencies of the same neurons were shortened by 1-18 ms when stimulating other sites at this level or by raising stimulus current at the presumed site of termination. These studies suggest that HYP-induced SE responses are mediated in part via a synapse in the PAG. (Supported by NIH grant HL33266.)

239.17

LESIONS OF THE KOLLIKER-FUSE / LATERAL PARABRACHIAL NUCLEI INCREASE ARTERIAL PRESSURE AND WATER INTAKE. D.G. Ward and J.H. Ward*. H.M. Ward Memorial Lab., Valley Home, CA 95384

The role of the parabrachial complex (PBN) in control of basal arterial pressure and water intake was examined in 13 conscious freely-behaving male rats. On Day-0, under ketamine anesthesia (150 mg/kg), a femoral arterial catheter was exteriorized to a spring tether attached between the scapulae, and lesions directed toward the Kolliker-Fuse nucleus were placed bilaterally in one group of animals (lesion) or unilaterally in a second group of animals (control). Mean arterial presure (MAP, mmHg), heart rate (HR, beats/min), basal water intake (H2O, ml) and urine volume (Urn, ml) were measured daily for 8 to 11 days. The average differences in measurements between lesion and control animals (* $p < 0.05$) were as follows:

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
MAP	3.1	19.8*	10.5	12.8	13.3	20.1*	22.3*	27.1*
HR	-21.2	-17.5	-3.4	-9.2	-3.9	-11.9	-81.0*	-14.3
H2O	-3.3	4.4	4.0	15.3*	19.7*	20.8*	8.6	5.2
Urn	-4.9	2.3	3.7	15.1*	20.3*	17.8*	3.6	8.0

Thus, destruction of the Kolliker-Fuse nucleus leads, in the unanesthetized rat, to a slowly developing hypertension, to a transient and parallel hyperdipsia and polyuria preceeding the hypertension, and to a transient bradycardia following the hyperdipsia. These findings suggest the presence of autonomic pathways within the parabrachial complex that are in opposition to known sympatho-excitatory pathways. (supported by USPHS HL36034 and MH43989)

239.18

POSTERIOR HYPOTHALAMIC RECEPTORS INVOLVED IN THE PRESSOR RESPONSE PRODUCED BY STIMULATION OF THE C1 REGION. H. Bachelard* and C.A. Marsden* (spon. by Brain Research Association). Dept. Physiology & Pharmacology, Medical School, Queen's Medical Centre, Nottingham, England, NG7 2UH

The increase in the blood pressure (BP) induced by the stimulation of the C1 region is partly mediated via the adrenergic pathway projecting toward the hypothalamus. In this study we have further examined the receptors into the posterior hypothalamus (PH) involved in the pressor response elicited by stimulation of the C1 neurones. Under anaesthesia male Wistar rats were implanted with a guide cannula in the PH. The C1 area was electrically stimulated ipsilateral to the cannula and the BP was continously monitored. Fifteen minutes after the stimulation, saline or drugs were injected i.v. or into the PH followed 15 min later by a second stimulation. The increase in BP produced by C1 stimulation was significantly attenuated by pentolinium (1mg/kg; i.v.), or by the injection into the PH of propranolol (20μg), clonidine (8μg), atropine (8μg) and methysergide (10μg). No significant reduction in the pressor effect have been found after intra-PH administration of d-propranonol (20μg), idazoxan (66μg), chlorphenydramine (12μg), cimetidine (11μg) or naloxone (10μg). Together these results confirm previous observations indicating that C1 stimulation provides excitatory drive to the preganglionic sympathetic neurones of the spinal cord and to the PH that results in increased BP . This response is in part centrally modulated by α2 and ß adrenoceptors but with central cholinergic and serotonergic components involvement.
We thank the FRSQ and the Wellcome Trust for their financial support.

239.19

THE VENTRAL LATERAL MEDULLA MEDIATES INSULAR CORTEX SYMPATHETIC RESPONSES. D.F.Cechetto and S.J. Chen*. Robarts Research Inst./Dept. of Physiology, Univ. of Western Ontario, London, Ont. N6A 5K8.

Previously we have demonstrated that the efferent pathway for sympathetic responses originating in the insular cortex (IC) involves a synapse in the lateral hypothalamic area (LHA). This site projects to both the ventral lateral medulla (VLM) and directly to the thoracic spinal cord. To determine the role of the VLM in mediating sympathetic responses from the IC and LHA, in chloralose anesthetized rats, renal nerve responses were recorded, following electrical stimulation of these two forebrain sites before and after bilateral injection (300 nl) of cobalt (a synaptic blocking agent) into the VLM. The results demonstrated that a complete block of the sympathetic nerve response following stimulation of the IC or the LHA could be obtained with cobalt injections into the VLM. The effective injection sites were coincident with the A1 group of neurons. Injections into the rostral VLM in the region of the C1 neurons were much less effective. Chemical stimulation with D,L-homocysteic acid to activate only cell bodies evoked a decrease in arterial blood pressure and sympathetic nerve activity. These responses were also blocked by cobalt injection into the caudal ventral lateral medulla. These results indicate that the efferent pathway for sympathetic responses from the IC through the LHA are mediated by a mandatory synapse in the caudal VLM.

(Supported by the Heart and Stroke Foundation of Ontario).

239.20

CARDIAC CHRONOTROPIC ORGANIZATION OF THE RAT INSULAR CORTEX. S.M. Oppenheimer*, V.C. Hachinski and D.F. Cechetto. Robarts Research Inst./Dept. of Physiology, Univ. of Western Ontario, London, Ont. N6A 5K8 Canada.

Clinical studies suggest that cortical mechanisms may be involved in arrythmogenesis. Cortical sites mediating only cardiac effects have been scantily identified in the past. The insular cortex has been shown to have extensive autonomic inputs and autonomic effects are elicitable on microstimulation. This area was accordingly chosen as a likely site for cortical representation of heart rate. In 31 rats anesthetized with chloralose the organization of changes in ECG and heart rate were examined in response to cortical microstimulation. In order to evoke only cardiac responses from the rat insula, a new technique was used in which the discriminated R wave of the ECG served as a trigger for the stimulator. Single or paired pulses were delivered to the insula coincident with the P wave every second or fourth PQRT cycle. A map of bradycardia and tachycardia sites was obtained. Increases in heart rate occurred from the rostral part of the posterior insular cortex, while decreases were obtained from the caudal region of the posterior insula. Pharmacological studies suggest these effects are mediated by the sympathetic nervous system. This is the first report of pure chronotropic effects being elicited from a cortical area in a systematic manner, and suggests a possible foundation for the heart rate changes commonly seen during temporal lobe seizures and a cortical site for arrythmogenesis.

(Supported by the Canadian Heart Foundation).

239.21

L-GLUTAMATE MAPPING OF CARDIOREACTIVE AREAS IN THE POSTERIOR HYPOTHALAMUS. W.B. Sawyer, S.E. Spencer and A.D. Loewy, Departments of Neurology and Anatomy & Neurobiology, Washington University School of Medicine, St. Louis, MO 63110 (Sponsor: S.G. Eliasson)

The posterior hypothalamus has long been regarded as a CNS region that provides a sympatho-excitatory influence on the cardiovascular system and thermoregulation. This idea is largely based on electrical stimulation and lesion experiments. We have used microinjection of the excitatory amino acid L-glutamate to activate cell bodies in the posterior hypothalamus and to test the hypothesis that this is a sympatho-excitatory center.

Fifteen nl microinjections of a 500 mM L-glutamate (7.5 nmole) and ^{3}H-glutamate (1.5 μCi) solution were stereotaxically delivered into the posterior hypothalamic region of pentobarbital anesthetized rats. Animals were ventilated and blood pressure, temperature, and heart rate monitored. The injection sites were subsequently determined by the autoradiographic method. Injections in the posterior hypothalamic nucleus elicited small reductions in blood pressure (-10±1mmHg; mean±SEM) and heart rate (-11±2 bpm). Injections in the dorsal hypothalamic area produced a similar response (BP=-12±3mmHg; HR=-3±4bpm). The most responsive area was in the periventricular zone caudal to the posterior hypothalamic nucleus and centered around the fasciculus retroflexus (ΔBP=-26±4mmHg; ΔHR=-27±8bpm). Injections ventral to this zone involving the supramammillary nuclei were less reactive (ΔBP=-11±1; ΔHR=-6±2), while experiments rostral and involving the periventricular nucleus were similar (ΔBP=-25±3; ΔHR=-10±0).

Minor elevations in rectal temperature were seen after injections in the dorsal hypothalamic area and the supramammillary region.

Chemical stimulation of the posterior hypothalamus elicits hypotension and bradycardia. No pressor responses were seen and the critical area was the periventricular zone, not the posterior hypothalamic nucleus.

239.22

INTERACTION BETWEEN PRESSOR AND DEPRESSOR AREAS IN CAT VENTROLATERAL MEDULLA. Philip J. Gatti* and Richard A. Gillis* (SPON: S.N. Pradhan) Depts. Pharm.; Howard Univ. Coll. of Med. & Georgetown Univ. Schl. Med., Washington, D.C. 20059.

Although application of gamma-aminobutyric acid (GABA) to the intermediate area (IA) of the cat ventrolateral medulla produces a profound decrease in arterial blood pressure (BP), application of the GABA antagonist bicuculline (BI) by itself to this area raises BP only when very high doses are used. These data suggest that although there are GABA receptors at the IA which are involved in the control of BP, the GABA tone is very low at this site in the chloralose anesthetized cat. We investigated whether stimulation of the caudal depressor area (CA) in the medulla lowered BP by enhancing this GABA inhibitory tone at the IA. In 4 cats, application of BI (10 ug/5 ul bilaterally) to the IA raised BP by 24 ± 8.2 mmHg. In another group of animals (n=7) the neuroexcitant kainic acid (KA) was first applied to the CA and resulted in a decrease in BP. Thirty minutes later, the same dose of BI was applied to the IA and BP increased by 74 ± 8.7 mmHg. This effect was significantly different from the control BI response ($p < .05$, unpaired Student's t-test). These results suggest either a direct or indirect GABAergic projection from the CA to the IA which when stimulated, will enhance GABA release at the IA to influence cardiovascular function.

CARDIOVASCULAR REGULATION: HYPERTENSION

240.1

MORPHOLOGICAL DIFFERENCES IN STELLATE GANGLION CELLS OF NORMOTENSIVE AND HYPERTENSIVE RATS. D. Peruzzi[1], E.D. Hendley[2], and C.J. Forehand[1] (SPON: E. Ezerman). [1]Dept. of Anatomy and Neurobiology, and [2]Dept. of Physiology and Biophysics, University of Vermont, Burlington, VT 05405.

The size of postganglionic autonomic neurons has been positively correlated with the size of the targets innervated (Voyvodic, J.T., Soc. Neurosci. Abstr., 13:574, 1987). Hypertension is accompanied by cardiac hypertrophy and an increase in the thickness of the smooth muscle wall of resistance vessels (Folkow, B., Clin. Sci. Mol. Med. 55:3, 1987). We have thus asked whether sympathetic ganglion cells in the stellate ganglion of hypertensive rats reflect this change in target size. We compared the cell body size and total dendritic length (TDL) of stellate ganglion cells from spontaneously hypertensive rats (SHR) and the normotensive Wistar-Kyoto rats (WKY) from which they were derived. To do this, we filled individual ganglion cells by intracellular injection with horseradish peroxidase.

The average TDL of SHR stellate neurons (2082 um) was 21% longer than that of WKY neurons (1645 um). Moreover, about half of the SHR neurons had TDL's larger than the largest WKY cells. The range of cell body sizes observed for SHR and WKY overlapped, but the biggest cell bodies were from the SHR and the smallest from the WKY.

We are currently exploring whether the increase in TDL seen in hypertensive rats is specific to the hypertension per se, and/or the associated behavioral hyperactivity. To do this we are using inbred strains derived from the SHR that are hyperactive (HA) but not hypertensive (HT), or vice-versa (Hendley et al., Hypertension, 5:211, 1983). The average TDL of HT stellate neurons is equivalent to that of the SHR and therefore greater than that of the WKY. Studies on the HA cells are now in progress.

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240.2

NEURONAL ACTIVITIES OF THE ROSTROVENTROLATERAL MEDULLARY NEURONS IN SPONTANEOUSLY HYPERTENSIVE RATS. T.M. Wong*, R.K.W. Chan* and Y.S. Chan. Dept. of Physiology, University of Hong Kong, Hong Kong.

It is known that the rostroventrolateral medulla (RVL) plays an important role in the control of circulation. It was, therefore, hypothesized that altered neuronal activities of RVL may lead to abnormalities in cardiovascular functions. In support of the hypothesis, we found in a preliminary study that pentobarbitone-anaesthetized spontaneously hypertensive rats (SHR) exhibited exaggerated responses in arterial pressure upon electrical stimulation of RVL neurons compared with the normotensive Wister Kyoto rats (WKY), indicating an enhanced cardiovascular responsiveness in SHR. In this study, we further tested the hypothesis by comparing the extracellular activities of RVL neurons of WKY and SHR. The RVL neurons exhibited single and double spikes and the mean interspike intervals between single spikes or double spikes were similar in the two types of rats. The relative proportion of neurons recorded in WKY exhibiting single and double spikes were 92% and 8%, respectively, whereas the corresponding values in SHR were 45% and 55%, respectively. The differences were statistically significant. In addition, the firing of the RVL neurons was more regular in SHR than in WKY. The results of the present study indicate that the neurons of the RVL in SHR exhibit a higher spontaneous discharge rate than that of normotensive WKY. This together with the enhanced cardiovascular responsiveness upon electrical stimulation of RVL are probably responsible for the development of hypertension in SHR. (Supported by HKU research grants, Sun Yat Sen Foundation Fund and Lee Wing Tat Medical Research Fund).

240.3

EFFECTS OF DIETARY TRYPTOPHAN ON DAHL SALT INDUCED HYPERTENSION. L. Lark, K. Becker*, R. Park*, and J. A. Weyhenmeyer. Neural and Behavioral Biology Program and College of Medicine, Univ. of Ill., Urbana, IL 61801.

The present study expands on our previous findings that a trp supplemented diet attenuates the development of hypertension in inbred Dahl salt-sensitive (DS/JR) rats (Lark et al., Neurosci. Abstr. 14:974, 1988).

DS/JR and their inbred control Dahl salt-resistant (DR/JR) rats were placed on an 8% salt diet with or without trp supplementation (25 g/kg food) from 4-9 wks of age. Supplemental trp attenuated the development of hypertension in DS/JR, while DS/JR animals on the control diet showed a steady elevation in blood pressure (185 ± 9 vs. 127 ± 5 mm Hg, respectively, $p < .01$). Although the DR/JR rats on the high trp diet had significantly lower blood pressures at the end of the study (127 ± 3 vs. 146 ± 5 mm Hg, respectively, $p < .05$), a consistent developmental trend was not observed. DS/JR rats on control diets drank significantly more water than all other groups, however, no significant differences in urine output were found. Trp supplementation had no effect upon urinary Na^+ output in either strain, but DS/JR rats were found to excrete significantly less Na^+ than DR/JR rats. No significant differences in food consumption were found. High trp diets also had no effect on heart weights, however, DS/JR hearts were significantly larger than DR/JR hearts. We conclude that elevated dietary trp blocks the development of Dahl salt induced hypertension.

Supported by NSF grant BNS 17117.

240.4

MICROINJECTION OF L-GLUTAMATE AND TETRODOTOXIN INTO THE ROSTRAL AND CAUDAL VENTROLATERAL MEDULLA IN ADULT SPONTANEOUSLY HYPERTENSIVE RATS. J.K. Smith* and K.W. Barron. Dept. of Physiology, Univ. of KY, Lexington, KY 40536

The purpose of this study was to compare the responsiveness of the rostral (RVLM) and caudal (CVLM) ventrolateral medulla in 12-15 week old spontaneously hypertensive (SH) and normotensive Wistar-Kyoto (WKY) rats to microinjection of L-glutamate (L-glu), and to estimate tonic output of the CVLM and RVLM by microinjecting the neurotoxin tetrodotoxin. Rats were anesthetized with 1.25 g/kg urethane s.q., implanted with arterial (femoral) and venous (femoral) catheters, artificially ventilated and paralyzed with gallamine triethiodide (10 mg/kg). Using a ventral approach to the brainstem, the mean arterial pressure (MAP) and heart rate (HR) responses to microinjection (30 nl) of L-glu (1, 10 and 100 mM) and tetrodotoxin (10 μM) into the RVLM and CVLM were compared in SH (n=7) and WKY (n=7) groups. Microinjection of L-glu into the RVLM produced equivalent dose-dependent increases in MAP (maximum +33 ± 3 and +36 ± 6 mmHg, SH and WKY groups, respectively) and minimal changes in HR. Similar administration of L-glu into the CVLM caused dose-dependent decreases in MAP and HR; changes in MAP were significantly greater in the SH group than in the WKY group (-52.3 ± 2.9 mmHg for SH, -22.6 ± 2.6 mmHg for WKY). Bilateral microinjection of tetrodotoxin into the CVLM produced significantly larger increases of MAP in WKY rats (+8 ± 4 vs +46 ± 8 mmHg for SH vs WKY). These data indicate that SH rats have a lower tonic activity of neurons in the CVLM, resulting in a lower restraining influence on sympathetic outflow in the SH rat. (Supported by: HL 36552, NIH T32-07632 & KY Heart Assoc).

240.5

INTRACRANIAL PRESSURE MONITORING IN SPONTANEOUSLY HYPERTENSIVE RATS. M.L.LEAVITT*, D.V.LOESCH* and J.C.MAROON*(SPON:N.Mason). Allegheny-Singer Research Inst.,Pittsburgh, PA 15212.

This study monitored cerebrospinal fluid pressure (CSF-p) in conscious adult spontaneously hypertensive rats(S,N=5)and control Wistar rats (W,N=6). Following implantation of a lateral ventricular guide cannula, a push-pull internal cannula was used to infuse art.CSF (2μl/min.) and measure CSF-p once per second over two 5 hr. runs separated by 2 days. During run 2, CSF-p for the two groups was significantly different ($p<.03$) from 270 through 300 min. Starting at 140 min(S) or 190 min(W), CSF-p was consistently and significantly ($p<.04$) increased compared to baseline. Run 1 and 2 measurements were not significantly different from each other for either group. CSF-p in cmH_2O:

	10min	80min	140min	230min	300min
S:	8.2±2.0*	9.3±1.0*	12.8±1.2$^+$	19.8±4.4$^+$	21.8±3.8*$^+$
W:	14.1±0.6	14.2±1.2	17.6±3.0	31.2±5.7$^+$	37.9±4.6$^+$

*$p<.03$,non-paired t,S vs W;$^+p<.03$,paired t,vs 10 min. Thus CSF-p is lower in S compared to W under baseline conditions as well as following ivt art CSF infusion which elevates the pressure of both groups.

240.6

LESIONS OF THE PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS (PVH) ALTER THE DEVELOPMENT OF DOCA-SALT HYPERTENSION IN THE RAT. T. X. Zhang and J. Ciriello. Dept. of Physiology, Univ. of Western Ontario, London, Canada, N6A 5C1.

The PVH has been shown to be involved in the hypertensive process in several different experimental models of hypertension. Recently, we have demonstrated increased metabolic activity in PVH of deoxycorticosterone acetate (DOCA)-salt hypertensive rats. The present study was done to investigate the contribution of PVH to the development of DOCA-salt hypertension in the rat. Arterial pressure (AP) was measured using the indirect tail cuff method. The rats were randomly assigned to 4-groups and the left kidney was removed in all animals. After a 1-wk recovery period the rats were subjected to bilateral PVH lesions or sham PVH lesions followed by the administration of DOCA-salt or sham DOCA-salt. PVH lesions delayed the development and attenuated the level of the hypertension in the DOCA-salt hypertensive rats compared to sham PVH lesioned DOCA-salt hypertensive rats. At the end of the study, the average AP was 178 ± 8, 204 ± 4, 120 ± 5, and 125 ± 6 mmHg in PVH lesioned-DOCA-salt, sham PVH lesioned-DOCA-salt, PVH lesioned-sham DOCA-salt and sham PVH lesioned-sham DOCA-salt animals, respectively. The heart rates among the 4 groups were not significantly different during the period of the experiment. These data suggest that PVH plays an important role in the initial phase of development and in the full expression of DOCA-salt hypertension. (Supported by HSFO).

240.7

DIFFERENCES IN SALT APPETITE IN SALT-SENSITIVE (SHR-S) AND SALT-RESISTANT SHR (SHR-R). M.J. Meldrum, C. Eich*, R. Dawson. Dept. Pharmacodynamics, Coll of Pharmacy, Univ. of Florida, Gainesville, Fl. 32610.

Increased dietary salt exacerbates blood pressure in SHR-S (Taconic Farms) while similar increases in salt intake have no effect in SHR-R (Charles Rivers). We therefore measured salt appetite in SHR-S and SHR-R to determine if it may play a role in the differential response to dietary salt. Animals were placed in metabolism cages and the following 24hr parameters were measured: fluid intake; urine volume; food intake;and urinary Na^+ excretion. Urinary catecholamines (NE,DA) were also assayed by HPLC-EC after alumina extraction. After 2 days of basal measurements (tap water), various concentrations (0.5,1.0,1.5,2.0,2.5,3.0%)of NaCl given randomly were presented as the only drinking fluid, followed by 2 basal days of measurements. SHR-S drank significantly greater quantities of 1.5 and 2.0% NaCl than SHR-R. Urine volume and sodium excretion paralleled differences in fluid intake. Urinary NE excretion was not different between SHR-S and SHR-R however levels were significantly elevated in a concentration dependent manner by NaCl. These differences in salt appetite may reflect differences in sodium chloride regulation between SHR-S and SHR-R which may affect regulation of blood pressure. (Supported by the American Heart Association- Florida Affiliate).

240.8

BAROREFLEX CONTROL IN HYPERTENSION-SENSITIVE (SS/Jr) AND HYPERTENSION-RESISTANT (SR/Jr) RATS. C. A. Murphy* and R. McCarty* (SPON: N. Desmond) Department of Psychology, University of Virginia, Charlottesville, VA 22903.

Baroreflex control of heart rate was examined in adult male inbred SS/Jr and SR/Jr rats. Animals were surgically prepared with catheters in the jugular vein to permit graded infusions of both a pressor agent, phenylephrine, and a depressor agent, sodium nitroprusside. A third catheter was fitted in the ventral tail artery through which recordings of mean arterial pressure and heart rate were obtained. Basal blood pressures were clearly higher in SS/Jr rats compared to SR/Jr rats. In contrast to previous reports of impaired baroreceptor sensitivity in the prehypertensive DS rat, no significant difference in the total range of the heart rate response to blood pressure change was observed in the adult hypertensive SS/Jr. Average gain of the baroreflex was in fact significantly greater in the SS/Jr rat, indicating slightly enhanced baroreceptor sensitivity over the range of heart rate change. The absence of baroreflex impairment in this model of hypertension may be a secondary effect of the development of hypertension and play a compensatory role in the presence of established elevations in blood pressure.

240.9

THE EFFECTS OF STRESS AND DIETARY COPPER DEFICIENCY ON BLOOD PRESSURE IN RATS. E.S. Halas and L.M. Klevay*. Dept. of Psychology, Univ. of North Dakota and USDA, ARS, Human Nutr. Res. Ctr., Grand Forks, ND 58202.

The etiology of most hypertension is unknown; stress is thought to elevate blood pressure. In two experiments, male weanling Sprague-Dawley rats were fed an adequate diet. Systolic blood pressure (BP) was measured weekly without anesthesia. After being matched by mean weight (c280g) and BP into 4 groups of 15, groups 1 and 2 received a diet without copper. After 24 days, rats in groups 2 and 4 were restrained for 45 min. daily for 23 days in a small cage. In Exp. 1, ten of 15 rats in Group 2 and one in each other Group died prematurely; there were no striking differences in mortality in Exp. 2. There was a progressive increase in mean BP for Groups 2, 3, and 4. In Exp. 1 mean BP were highest in Group 2 (131mm) and lowest in Group 3 (114mm). Both stress and copper deficiency independently caused some elevation of BP as shown by Groups 4 (123mm) and 1 (119mm). Results were similar in Exp. 2, but responses were delayed 1 week. Deficiency and stress caused small but significant increases in cardiac sodium and decreases in cardiac potassium, the latter only in Exp. 1. Stress and copper deficiency can cooperate to produce adverse effects on health.

240.10

CNS REGULATION OF THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS IN SHR RATS. S. Bhatnagar and M. J. Meaney. Douglas Hosp. Res. Ctr, Dept. Psychiatry, McGill Univ., Montreal, Quebec, H4H 1R3.

Hypothalamic-pituitary-adrenal (HPA) dysfunction has been implicated in the etiology of hypertension in the Spontaneously Hypertensive Rat (SHR). We sought to characterize the involvement of the HPA axis by examining AM and PM basal levels of ACTH in conscious adult SHRs (n=9) as well as their normotensive counterparts, the WKY rat (n=15). We found that SHRs had lower plasma levels of ACTH at both a.m. and p.m. samples (28.3 and 42.3 pg/ml, respectively), as compared to to WKYs (45.4 and 83.6 pg/ml, respectively), although ACTH levels increased for both groups in the p.m. samples. We found that SHR's (n=15) had significantly lower levels of CBG than did WKYs (n=15) at both a.m. and p.m. samples, suggesting more free corticosterone in circulation in SHRs than in WKYs.

Previous research has suggested an impaired negative feedback response in the HPA of SHRs. We, therefore, examined glucocorticoid receptor binding in the hippocampus, pituitary, and hypothalamus of 6 mo old SHRs and WKYs using the specific Type II, glucocorticoid receptor ligand [3H]RU-28362. Preliminary data suggests that hippocampal receptor concentrations were lower in the SHRs than in the WKYs (30% lower), while no such differences were found in either pituitary or hypothalamus. These results provide further evidence for impairment in HPA functioning in the SHR, and implicate hippocampal glucocorticoid receptors in this impairment.

240.11

EFFECTS OF CALCIUM CHLORIDE ON BLOOD PRESSURE AND VASOPRESSIN mRNA IN THE HYPOTHALAMUS OF SPONTANEOUSLY HYPERTENSIVE RATS. B. H. Hwang. Terre Haute Ctr. for Medical Education, Indiana Univ. Sch. of Med., Terre Haute, IN 47809.

Salt (sodium chloride) can cause hypertension. However, recent literature has also indicated that another kind of salt, calcium chloride can lower blood pressure. But it is unknown whether or not the vasopressin (VP) system in the hypothalamus is involved. Spontaneously hypertensive (SHR) rats and Wistar-Kyoto (WKY) rats at 4 weeks of age were used. The experiment consists of 4 groups of animals: (a) WKY-water group - allowed to drink deionized water; (b) WKY-$CaCl_2$ group - allowed to drink 0.15M $CaCl_2$; (c) SHR-water group; and (d) SHR-$CaCl_2$ group. The VP mRNAs were determined by quantitative autoradiography after in situ hybridization with ^{35}S-VP oligonucleotide probe. Results showed that $CaCl_2$ was able to lower blood pressure and prevent cardiac hypertrophy in SHR rats without altering VP mRNAs in the paraventricular hypothalamic nucleus (PVN) and supraoptic nucleus (SON). However, $CaCl_2$ did not change blood pressure significantly in WKY rats, whereas it greatly induced VP mRNA gene expressin in the PVN and SON of WKY rats. In conclusion, calcium chloride's ability to lower blood pressure in SHR rats is not closely associated with the hypothalamic VP system. (supported by NIH grant NS25087).

240.12

MEDIAL FRONTAL CORTEX LESIONS ELIMINATE CARDIAC BAROREFLEX GAIN CHANGES DURING STRESS IN THE RAT.
R.J. Frysztak & E.J. Neafsey, Department of Anatomy, Loyola Univ Med Ctr, 2160 S. First Ave, Maywood, IL 60153

Bilateral medial frontal cortex (MFC) lesions were made in 15 male Sprague-Dawley rats with .4 ul injections of N-Methyl-D-aspartate. Controls (N=15) received isotonic saline. Animals were conditioned to a 10 sec. tone paired with a mild footshock. During trials, the rat received the tone alone. Baroreflex gain was measured at 4 periods during the conditioned emotional response (CER): 1-rest, 2-during tone, 3-early post-tone, 4-late post-tone. Blood pressure was recorded via the brachial a. and activation of the cardiac baroreflex was produced via an intra-aortic balloon catheter (Fogarty-2F). Increases in BP produced by the balloon averaged 15 mm Hg, and lasted approximately 2 sec. Cardiac baroreflex gain was calculated by dividing the change in HR by the change in BP (Δbpm/ΔmmHg). Results are shown in the following table:

	PERIOD 1	PERIOD 2	PERIOD 3	PERIOD 4
CONTROL	-5.37	-2.33	-2.35	-4.31
MFC LESION	-2.54	-2.03	-2.27	-2.44

The gain at rest in controls was similar to that described by Verberne (BR 426,'87). Gain values during the CER (per 2,3,4) were significantly less then period 1 ($p<.05$). In MFC lesioned animals, the gain at rest was less than that of controls ($p<.01$), but no significant changes occurred during the CER (per. 2,3,4). (Supp by Loyola BRSG funds)

240.13

ACTIVATION OF A SPINO-MEDULLARY CHOLINERGIC PRESSOR PATHWAY DOES NOT INVOLVE MEDULLARY RESPIRATORY CENTERS. H. Takahashi* and J.J. Buccafusco. Dept. Pharmacology & Toxicology Medical College of Georgia & Veterans Administration, Medical Center, Augusta, GA 30912.

Intrathecal (IT) injection of neostigmine (NEO) produced a marked increase in blood pressure (BP) and heart rate (HR) in freely-moving rats (J Autonom Ner Sys 25:69, 1988). It was suggested that this spinal cholinergic system may play a role in the cardiovascular reflex response to a painful stimulus. It is known, however, that stimulation of sensory afferents produce hyperventilation as well as increases in BP and HR. The purpose of this study is to determine whether 1) the cholinergic system activated by NEO also affects respiration, and 2) this system is intrinsic or ascending to higher centers. NEO (10μg, IT) inhibited acetylcholinesterase activity of the thoracic and lumbar cord, but not the cervical cord or medulla, indicating a local site of action. In anesthetized spontaneously breathing rats, NEO increased BP and HR, but not tidal volume (TV) or respiratory rate (RR) and minute volume (MV). The increases in BP and HR were inhibited by IT pretreatment with 10μg atropine. Intracisternal (IC) injection of NEO also increased BP and HR, but in addition, it increased TV, and decreased RR and MV. In contrast to IT NEO, these responses to IC, NEO were not altered by IT pretreatment with atropine. The increases in BP and HR to IT NEO were observed in decerebrate but not in spinal C7-8 transected preparations. These results support the presence of an ascending cholinergic pressor pathway which terminates in the medulla and is independent of the medullary respiratory pathway. Suppted: NIH, HL&B and the Veterans Administration.

240.14

EXTRACELLULAR NOREPINEPHRINE (NE) IN SPECIFIC HYPOTHALAMIC NUCLEI AND MEAN ARTERIAL PRESSURE (MAP) ARE INCREASED BY SODIUM CHLORIDE (NaCl): APPLICATION OF SMALL-GAUGE MICRODIALYSIS PROBE IN AWAKE RATS. T. Nakata*,W. Berard*, E. Kogosov* and N. Alexander. (SPON:B.Newman). Departments of Med. and Anat. Univ. of So. Calif. Sch. of Med., Los Angeles CA 90033.

We compared the effect of NaCl on MAP and extracellular NE in 3 hypothalamic nuclei known to affect cardiovascular and metabolic function. Microdialysis probes were contructed with dialysis bags (1.0 mm long, 0.18 mm diam., 8000 mw cutoff) inserted into a 27 g. needle. The probe tip was inserted through a guide tube into lateral (LH), ventromedial (VMH) or posterior (PH) hypothalamus of awake, male, Wistar rats with a femoral artery catheter. Artificial CSF was perfused (2ul/min) through the probe and dialysate samples (10 ul) were collected for radioenzymatic assay of NE. Generally, rats rested or slept during perfusions. Baseline dialysate (extracellular) NE values were 54±8, 61±8 and 66±14 pg/ml for LH, VMH and PH. resp.; (not corrected for recovery). NaCl (3.2%) added to CSF perfusate produced similar changes in all 3 nuclei, namely, a 6-10 fold increase in dialysate NE and a 15% increase in MAP. Mannitol (20%), added to perfusate as an osmotic control, increased NE 3-6 fold in the 3 nuclei without changing MAP. We conclude that:1) NaCl added directly to LH, VMH or PH will produce a rise in MAP and increase local NE release and, 2) hypertonic perfusate may have a non-specific osmotic effect on dialysate NE independent of neuronal release.

240.15

ANTISTRESS EFFECT OF SUBSTANCE P (SP) ON THE CARDIOVASCULAR SYSTEM IN NON-HUMAN PRIMATES. M.Colditz+,G.Stechmesser+,G.Martin+ and C.Gurk+ Dept. of Neuroregulation Res., Central Institute for Cardiovascular Research and WHO Collaborat. Centre,Academy of Sciences of the G.D.R.,Berlin, G.D.R.

The hypothesis that only the peripheral application of the regulide SP,combined with a renewed activation of systemic mechanisms of psychoemotional stress, induces a long-term blood pressure decrease in individuals with manifest hypertension, caused by psychoemotional stress, was examined and confirmed in 36 primates (rhesus monkeys, baboons). A pilot study demonstrated that SP (2,5 µg/kg body weight x 4 days), intravenously injected under psychoemotional stress, reduced the prestimulus EEG frequency and abolished the disturbances of the evoked potentials related information processing in the frontal cortex, locus coeruleus and nucleus raphe, and reduced the heart rate compared with the respective stress reactions.

It might be suggested that under psychoemotional stress SP induces such frontocorticofugale mechanisms which increase the sympatho - inhibition and, thus, contribute to the discovered antihypertensive long-term effect of SP.

CELL LINEAGE AND DETERMINATION II

241.1

NEUROBLASTS EXPRESS GLIA-ASSOCIATED ANTIGENS DURING INSECT EMBRYONIC DEVELOPMENT. M.R. Meyer and J.S. Edwards, Dept. Zoology, University of Washington, Seattle, WA 98195.

Observations in a variety of developing insects suggest that some glial cells may arise from neuroblasts (NBs). This notion is supported here by results obtained with selective immunological probes which recognize distinct glia-related antigens in the cricket *Acheta domesticus* (Meyer et al., 1987, J. Neurosci. 7:512).

Antigen 3G6 is associated with glia which invest the adult CNS neuropil (but not central tracts). Early in development, 3G6 is expressed throughout ventral neuroepithelium, but NB labelling becomes progessively more pronounced; by later stages (ca. 50-60%) many NBs strongly express 3G6. NB expression then subsides as neuropil expression increases, but some late 3G6+ NB nests in brain persist. Similar NB-selective labelling occurs in larval Drosophila CNS. Striking reactivity is also associated with crystalline cone cells in the insect retina.

Antigen 5B12 is a glia-associated glycoprotein that delineates nerve tracts in the adult insect. In the early embryonic CNS, 5B12 is associated with basal lamina overlying developing neuropil. Later (ca.45-55%), antigen is expressed upon a discrete set of posteriolateral NBs and along subsets of commissural axon fascicles.

Two distinct glia-selective markers thus support the hypothesis that NBs are likely to be involved in producing glial elements as well as neurons in insects, with NB-related gliogenesis following neurogenesis. Supported by NIH #NS07778-20.

241.2

NEURONS AND GLIA ARISE FROM A COMMON PROGENITOR IN CHICK OPTIC TECTUM: DEMONSTRATION WITH TWO RETROVIRUSES AND CELL TYPE-SPECIFIC ANTIBODIES. D.S. Galileo*, G.E. Gray , G.C. Owens*, J. Majors[1] and J.R. Sanes, Departments of Anatomy & Neurobiology, and [1]Biochemistry, Washington University School of Medicine, St. Louis, Missouri 63110

We have been using a recombinant retrovirus to study cell lineage in the chick optic tectum. The virus inserts the lacZ (ß-galactosidase) gene into the genome of an infected cell; a histochemical stain fills the progeny of infected cells with a blue precipitate. By observing the shapes and positions of blue cells we found that individual clones frequently contain diverse neuronal types (Gray et al., PNAS 85: 7356, 1988). We next wanted to ask whether individual clones contain glia as well as neurons. However, identification of cells as glia by shape and size was often ambiguous, and identification by immunostaining was hampered by the blue precipitate. Furthermore, putative glia were sometimes dispersed, leading to occasional ambiguity in the definition of clonal boundaries.

To solve both of these problems, we constructed a new recombinant virus in which lacZ is fused to the nuclear localization signal of the SV40 T antigen. Cells infected with this virus are marked with blue nuclei instead of blue somata. Following infection of embryos with a mixture of the two retroviruses, individual clusters contained cells with only one type of label (nuclear or cytoplasmic), showing them to be clones. We were also able to immunostain the somata of cells that had blue nuclei, and thereby to show that neurons (neurofilament*) and glia (glutamine synthetase*) are found within a single clone. This demonstrates unambiguously that neurons and glia can develop from a common precursor in optic tectum. (Support: NIH, MS, and McKnight)

241.3

MIGRATORY PATTERNS OF CLONALLY RELATED CELLS DIFFER IN CHICKEN TECTUM AND FOREBRAIN. G.E. Gray and J.R. Sanes, Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, Missouri 63110

We previously used a retroviral tracing method to show that clonally related cells form radial arrays in chick optic tectum (Gray et al., PNAS, 85: 7356, 1988). Because the optic tectum has a radial functional organization, clonally related cells may frequently be functionally related. In the present study, we compared the spatial arrangement of clones in the tectum with clones in the forebrain, much of which has no known radial organization.

Recombinant virus bearing the lacZ gene (whose product can be marked histochemically in the progeny of infected cells) was injected into the neural tube on E3 (stage 17-19). Subsequently, brains were fixed, sectioned, and reacted for lacZ. Until E8, most clones in the forebrain were radial. However, by E10 many clones showed some dispersal, and by E19 most clones comprised loose clusters of cells, with little radial organization. One basis for these migratory differences might be that tectum and forebrain differ in the organization of their radial glia, which are thought to guide migration. To examine this possibility, we labeled tissue from the two areas either with an antibody to radial glia or with DiI. Both stains showed that radial glia remain present in the hatchling (E20) in the optic tectum (confirming Vanselow et al., Dev. Br. Res. 45:15, 1989), but largely disappear by ca. E12 in the forebrain. These experiments show 1) that the different arrangement of clonally related cells in tectum and forebrain reflects (and may influence) a difference in the functional organization of these two areas; and 2) that loss of radial glia is correlated with (and may underly) a loss of radial migratory constraint as the forebrain develops. (Support: NIH & McKnight)

241.4

Retroviral Vectors Reveal Compartment Specific Cell Lineages In The Developing Rat Forebrain. L.A. Krushel, J.G. Johnston, G. Fishell, and D. van der Kooy Neurobiology Research Group, Dept. of Anatomy, University of Toronto, Toronto, Ontario, Canada, M5S 1A8.

The two major structures of the rat forebrain, the cortex and striatum, are each developmentally and functionally compartmentalized. The cortex can be separated into deep and superficial layers, while the striatum can be divided into the patch and matrix compartments. Do these compartments arise from separate progenitor populations? To investigate the role of cell lineage in this compartmentalization of the forebrain, we injected a replicant-deficient retrovirus (containing a lac-Z marker gene) into the lateral ventricle of embryonic pups *in utero*. The virus was obtained from the supernatant of a psi 2 BAG alpha cell line (ATCC #CRL-9560). At birth, true blue was injected into the mesencephalon of the pups. This retrogradely labeled the striatal patch and deep layer corticospinal neurons. The pups were sacrificed at postnatal day 7 and processed histochemically for B-galactosidase. Embryonic day (E) 17 injections produced small neuronal clones (<3 cells) generally restricted to either the cortical superficial lamina or striatal matrix compartments. In contrast, E14 injections produced three classes of neuronal clones, 1) clones restricted to either the superficial cortical layers or matrix compartment, 2) single cell clones located in the deep layers, and 3) large clones (up to 11 cells) distributed across the deep and superficial layers. This suggests there may be three distinct neuronal progenitor populations, two of which give rise to cells that respect the boundaries of forebrain compartments, and thus may be directly involved in the formation of these compartments.

241.5

CELL LINEAGE IN THE MOUSE SOMATOSENSORY CORTEX: ANALYSIS OF CHIMERAS. K.Herrup and J.E. Crandall. E.K. Shriver Center, Waltham, MA 02254.

Experimental aggregation chimeric mice offer a perspective on cell lineage relationships complementary to that seen with recombinant retroviruses. Previous studies of cerebellar cortex in such chimeras indicate that there is a non-random spatial distribution of clonally related Purkinje cells (Herrup & Bower, *Neurosci. Abst.* **12**,769). Whereas the Purkinje cells are found topologically in only two dimensions, the cerebral cortex is a multilayered structure and the analysis of the spatial distribution of cell lineages must be approached in three dimensions. We have analyzed radial probes of somatosensory cortex in $Gus^{h}/Gus^{h} \leftrightarrow Gus^{b}/Gus^{b}$ chimeras. The position and genotype (determined with ß-glucuronidase) of all cortical cells were plotted. Preliminary analyses of the distribution of these cells indicate that there are significant differences in genotype ratios among: 1) adjacent radial probes in the tangential dimension and 2) cortical laminae. Retroviral studies indicate that different individual cortical cell clones are found in a variety of spatial distriburions ranging from predominantly radial to widely spread in the tangential plane. The chimera studies suggest a significant non-random spatial distribution of the entire set of cortical cell lineages.

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241.6

LINEAGE ANALYSIS OF THE MOUSE CEREBRAL CORTEX USING RETROVIRUS VECTORS. C. Austin* and C. Cepko* (SPON: D. Fekete) Dept. of Genetics, Harvard Medical School, Boston, MA 02115.

The development of the cerebral cortex is being investigated using retrovirus vectors to introduce a stable, genetic marker into mitotic cells of the lateral ventricle of mice. Embryonic day 12 and 13 fetuses are infected with a small number of replication-incompetent retrovirus particles (BAG) which transduce and express the marker gene encoding E. coli ß-galactosidase (Price et al., PNAS 84:1345, 1987; Walsh and Cepko, Science 241:1342, 1988). Animals are then harvested after a variety of survival times, ranging from 2 days post infection to greater than 3 weeks. Xgal histochemistry is then performed on serial frozen sections to reveal the location of infected cells. By using a small number of particles to infect a large series of fetuses (over 200), we are able to reproducibly generate approximately 50-100 labelled cells distributed throughout the brain. Analysis of the distribution of labelled cells with respect to each other and with respect to defined anatomical structures is being pursued through 3-dimensional reconstruction of complete cortices. By using a series of harvest dates, patterns of migration and proliferation of labelled cells are being defined.

241.7

MIGRATING NEURONS OF THE MURINE CEREBRUM ASCEND IN PARALLEL TO RADIAL FIBERS: ANALYSIS BASED UPON DOUBLE-LABELING OF MIGRATING NEURONS AND RADIAL FIBERS. J.P.Misson*, C.Austin*, T.Takahashi*,C.Cepko*,V.S. Caviness Jr.,(SPON: J. Nathanson) Dept. Neurology, Mass. Gen. Hosp, & Dept. Genetics, Harvard Med. Sch., Boston, MA

We consider here the relationship of the pathway of neuron migration to the course of ascent of local Radial glial fibers(RGF) in the late embryonic cerebral wall. Migrating neurons, carrying a viral-inserted gene for E-Coli B-galactosidase, are identified histochemically at intervals of 3-8 days following intraventricular injection of the viral vector on E12-14 (Walsh et al, Science, 1988, 241, 1342). The entire population of RGF is delineated in the same histological preparation by immunostaining with RC2 antibody (Misson et al, Dev. Brain Res., 1988, 44,95). The majority of the migrating cells, as delineated, are simple in configuration: radially elongated with leading but no other processes. At all subcortical levels identified migrating cell is contacted by multiple fascicles of RGF and at cortical levels by at least one fascicle or single RGF. The long axis of migrating cell is invariably parallel to the path of ascent of adjacent RGF. Thus, in the dorsal convexity of the hemisphere where fiber ascent is radial, the migrating cell is radially aligned. Laterally in the hemisphere fiber alignment is more nearly tangential across the Intermediate Zone, becoming inflected sharply to radial alignment in its cortical span. Even in this circumstance migrating cell alignment is that of the adjacent fibers, tangential in crossing the IZ, but radial through cortical strata. The constant parallelism of alignment of migrating cells and adjacent fibers is consistent with the hypothesis that the fibers ' surface serves as a guide to migration. This parallel relationship holds for the presumed monoclonally derived single or paired migrating cells as well as for larger clusters of presumed polyclonally derived migrating cells arising from the same region in the Ventricular Zone.(supported by NIH grants NS12005 (V.S.C.) and NS23021 (C.C.) and by a C.A.King trust fellowship(J.-P.M.)

241.8

PROLIFERATIVE KINETICS OF CELLS IN THE SUBEPENDYMAL ZONE OF THE ADULT FOREBRAIN C.M. Morshead and D. van der Kooy. Neurobiology Research Group, Department of Anatomy, University of Toronto, Toronto, Ontario, Canada M5S 1A8

In the adult brain mitotic figures are rare, except in the subependymal layer which lines the lateral ventricles. Proliferation is most pronounced in the dorsolateral portion of this region where a single pulse of Brdu (a DNA synthesis marker) in the adult mouse labels 5% of the cells. In order to ask if the entire population in this region divides, Brdu was made continuously available for 3 days (injecting 165mg/kg every 8 hours). The population of labeled cells plateaued at 16% after 2 days. This suggests that only a minority of cells in the subependymal zone make up the proliferative population. Moreover, the plateau of Brdu labeled cells suggests that cell death is occurring in balance with continued proliferation. As an independent test of these suggestions, we injected a recombinant retrovirus into the lateral ventricle to mark clones of proliferating cells. Although multicell clones could be seen 1 and 2 days post-injection, we observed primarily single clones after survival times of 1 week. These results support the existence of a stem cell type of proliferation with survival of the self-renewing cell and death of the progeny. Additional experiments suggest some plasticity within this region as stress (exposure to anaesthetics) increases the number of dividing cells pulse labeled with a DNA synthesis marker. We are investigating whether stress decreases the cell cycle time of the stem cells or recruits cells from the non-dividing population.

241.9

FATE MAPS AND THE EARLY DETERMINATION OF THE FOREBRAIN IN ZEBRAFISH EMBRYOS. L.S. Ross, T.J. Parrett*, R.C. Marcus*, and S.S. Easter, Jr. Dept. of Biology, University of Michigan, Ann Arbor, MI 48109.

Lineage tracer molecules (HRP and fluorescent dextrans) were injected into single blastomeres at the animal pole of zebrafish embryos. At 24h postfertilization, the embryos were fixed and skinned, and their brains examined as whole mounts. Injections prior to 3h produced labeled cells, scattered throughout the brain, most concentrated in the forebrain. Injections at 3-4h gave a more restricted distribution, with large compact clones in the forebrain, most concentrated in the telencephalon. Injections after 4h (late blastula) produced small compact clones restricted to the telencephalon. We conclude that the dispersion of cells relative to their clonal neighbors ends quite early for those cells that will form the telencephalon.

In addition to mapping cell fates, we assessed differentiation by other methods. HNK immunoreactivity (MAb supplied by C. Stern) delineated the two major afferent pathways of the telencephalon as early as 20h. BRdU labeling indicated that the telencephalon has more postmitotic cells than other regions of the brain at 24h. Acetylcholinesterase staining showed differentiated cells concentrated in the telencephalon at 24h. These results support the idea that the telencephalon develops early relative to other regions of the brain.

(Supported by HD-07274 and EY-00168.)

241.10

PROGRESSIVE CHANGES IN CELL CYCLE AND DNA-SYNTHETIC PHASE LENGTHS DURING THE DEVELOPMENT OF THE DENTATE GYRUS OF C57Bl/6J MOUSE. R.R Gulay*, M.W. Miller and R.S. Nowakowski (Spon: N.L. Hayes). Dept. of Anatomy, UMDNJ-Robert Wood Johnson Medical School and School of Osteopathic Medicine, Piscataway, NJ 08854.

We have used a cumulative bromodeoxyuridine (BUdR) labeling technique (Nowakowski et al., J. Neurocytol., 1989) to determine the lengths of the cell cycle (T_C) and the DNA-synthetic phase (T_S) of proliferating cells in the developing hilus of the dentate gyrus. On postnatal days 0, 5, 10 and 20, C57BL/6J mice were injected with BUdR at two hour intervals for a total period of 10-12 hours. An iterative computer method was used to determine the lengths of T_C and T_S from counts of BUdR labelled and unlabelled cells.

The results (see Table) show that the lengths of T_C and T_S for the dentate gyrus change significantly during the postnatal period. Moreover, the ratio T_S/T_C increases progressively during development. Most widely used estimates of neuronal production based on single injections of ^{3}H-thymidine or BUdR have the implicit assumption that the T_S/T_C ratio is constant at different developmental stages. Consequently, previous studies should be re-evaluated in the context of the T_S/T_C ratio for each specified region and age analyzed.

	T_C	T_S	T_S/T_C
P0	9.9±0.6	2.0±0.2	0.20
P5	17.2±0.8	4.9±0.2	0.28
P10	15.3±0.7	6.1±0.3	0.39
P20	16.1±0.8	8.0±0.4	0.49

Supported by NIH Grants NS23647, AA07568, AA06916, DE07734 and a grant from the Schizophrenia Research Foundation.

241.11

REGULATION OF NEURON NUMBERS IN THE VERTEBRATE CNS: A COMPUTER SIMULATION. Richard Wetts & Scott E. Fraser. Dept of Physiology & Biophysics and the Developmental Biology Center; Univ Calif, Irvine, 92717.

What parameter tells a neuroblast to become postmitotic? Analyses of cell numbers in chimeric mice suggest that small groups of progenitors are committed in the neurula (for example, 10 cells per side in C57BL/6 give rise to the cerebellar Purkinje cells); each progenitor gives rise to a specific number of descendants (9200 Purkinje cells; Range ± 3%). [Herrup & Sunter, 1986, *Dev Biol.* 117: 417.] Analyses of the terminal lineages (last 5 generations) in the frog retina suggest variability in the numbers of descendants that are produced by precursors labeled at the same developmental stage [Wetts & Fraser, 1988, *Science*, 239: 1142].

To understand which events are important for controlling the number of descendants in a clone, we compared these experimental observations to the output of a neural proliferation model. In this model, a single progenitor cell proliferates until all of its descendants become postmitotic; the decision to stop dividing is modeled as a stochastic process. We have examined the effects of making this probability a function of 1) Time, 2) total Cell Number in the population, or 3) Number of Generations. In each case, parameters can be specified such that the model replicates many of the experimental observations. The most realistic fit to the experimental data occurs when CNS neuroblasts use the number of divisions to decide when to stop dividing.

241.12

A CASE STUDY OF A +/LC CHIMERA WITH PURKINJE CELLS UNILATERALLY DISTRIBUTED IN THE LEFT CEREBELLAR HEMISPHERE. M.W. Vogel and K. Herrup. Maryland Psychiatric Research Center, Baltimore, MD 21228 and E.K. Shriver Center, Waltham, MA. 02254.

We recently discovered a +/*Lc* ↔ C3H chimera with its cerebellar Purkinje cells unilaterally distributed in the left hemisphere. Preliminary Purkinje cell counts indicate that this animal contains on the order of 7 to 10 thousand Purkinje cells, and of these, less than 5% are positioned in the right cerebellar hemisphere, all within 300 μm of the midline. Wetts and Herrup (1982, J. Neurosci.,2:1494) report a Purkinje cell clone size of 10,200 neurons for C3H mice. We have analyzed four additional +/*Lc* ↔ C3H chimeras, and the combined data is best fit by a clone size of 10,350 Purkinje cells. Our interpretation of the +/*Lc* chimera described here is that all of its Purkinje cells are descended from a single wild type progenitor cell. The unilateral distribution of Purkinje cells suggests that progenitor selection events on the right and left sides are independent and that there is little crossover of Purkinje cells between the right and left cerebellar hemispheres Furthermore, the ratio of granule to Purkinje cells is increased in +/*Lc* chimeras, and we have suggested that the deafferentation of wild type Purkinje cells is responsible for the increased granule cell survival. The granule cells of this chimera will be analyzed to determine if the increase in granule cell survival is a global property of +/*Lc* chimeras or due to local interactions. Qualitatively, the granule cell layer in the Purkinje cell-less hemisphere is greatly reduced in size favoring the later hypothesis.

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241.13

LINEAGE OF MOTONEURONS IN CHICK SPINAL CORD STUDIED WITH A RETROVIRAL MARKER. S.M. Leber, S.M. Breedlove, and J.R. Sanes, Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, Missouri 63110

To define the range of cell types to which spinal motoneuron (MN) progenitors give rise, we injected replication-incompetent retroviruses into the neural tubes of stage (st.) 13-18 chick embryos. The progeny of infected cells expressed virally-encoded ß-galactosidase and were stained histochemically at st. 38-41 (E15). (MNs are born ~st. 15-23 and many die ~st. 29-36; V. Hamburger.) Isolated clusters of labeled cells were identified as clones by the methods of Gray et al. (PNAS 85: 7356, 1988) and Galileo et al. (see abstract). Cell types were identified by morphology, size, location, and the presence or absence of Nissl substance. Clones were usually radial or planar in shape, with rostro-caudal spread of <150 μm (a segment is ≥ 600 μm long at E15). As expected, injections at later stages gave rise to clones containing fewer MNs. Clonally related MNs were not restricted to a single column or motor pool, indicating that single progenitors can produce MNs that project to different muscles.

Multicellular clones containing only MNs were rare. Even after late (st. 17-18) injections, relatives of MNs were found in both the gray and white matter and included a variety of other neurons, as well as glia (probably both astrocytes and oligodendrocytes) and ventricular zone cells. Although most non-MNs in MN-containing clones were in the ventral part of the cord, some were found dorsally, and some were contralateral to the labeled MNs. We conclude that the close relatives of MNs are not necessarily other MNs, but include a variety of cell types. (Support: MDA, NIH, and McKnight)

241.14

CELL LINEAGE IN CHICK SENSORY GANGLIA STUDIED WITH A RETROVIRAL MARKER. E. Frank and J.R. Sanes[1], Dept. of Neurobiology, Anatomy and Cell Science, U. Pittsburgh School of Medicine, Pittsburgh, PA 15261, and [1]Dept. of Anatomy and Neurobiology, Washington U. School of Medicine, St. Louis, MO 63110

We have used retroviruses to trace the lineage of cells that populate dorsal root ganglia in chick embryos. Replication-deficient virions bearing the lacZ (ß-galactosidase) gene were injected into the neural tube at stages (St.) 13-18, when crest cells are migrating from the tube. At St. 35-49, embryos were fixed and reacted with X-gal to visualize individual labeled cells in sensory and sympathetic ganglia and their nerves. Many sensory ganglia bearing lacZ+ cells were isolated --i.e., adjacent and contralateral ganglia were lacZ-. Simultaneous injections with 2 different retroviruses (see abstract by Galileo et al.) indicated that labeled cells within such ganglia were likely to be clonal relatives: all labeled cells within each ganglion had a single viral phenotype. Individual clones sometimes contained labeled neurons, satellite cells, and Schwann cells, demonstrating that some ganglionic progenitors are pluripotent. Other clones contained sensory neurons or glia, but not both, suggesting that some crest cells are restricted in the range of cell types they normally produce. Clonally related sensory neurons were usually tightly clustered in a restricted part of the ganglion. In contrast, sensory neurons containing a specific peptide (e.g., CGRP) or projecting to a particular target (e.g., triceps muscles) were not obviously clustered. Thus, although neural crest cells may become committed in terms of major cell phenotypes (neurons or glia), single neuroblasts apparently produce a variety of sensory neuronal types. (Support: NIH, MDA and McKnight)

UPTAKE, STORAGE, SECRETION AND METABOLISM II

242.1

IDENTIFICATION OF THE HIGH-AFFINITY CHOLINE CARRIER IN RAT BRAIN SYNAPTOSOMAL MEMBRANES USING ^{3}H-CHOLINE MUSTARD. E.H. Colhoun* and R.J. Rylett. (SPON: T.E. Feasby) Depts. Physiology and Pharmacology, University of Western Ontario, London, Canada.

Choline used as substrate for acetylcholine synthesis is taken up into cholinergic nerve terminals by a sodium-dependent, hemicholinium-sensitive transporter. We reported previously that the choline analogue ^{3}H-choline mustard aziridinium ion (^{3}H-ChM Az) binds irreversibly to polypeptides of 40-42 and 58 kDa in presynaptic plasma membranes of Torpedo electromotor organ in the presence of sodium and absence of hemicholinium-3. The objective of the present study was to compare binding of this affinity ligand to choline transporters in rat brain synaptosomal membranes. Purified striatal synaptsomes from rat brain were incubated with ^{3}H-ChM Az, lysed and the washed presynaptic plasma membranes were solubilized and analyzed by SDS-polyacrylamide gel electrophoresis. Tritium-labelled polypeptides were observed with apparent molecular masses of about 42 and 56 kDa; a minor tritium-labelled polypeptide of Mr 33 kDa was also apparent, but could represent a proteolytic fragment. In contrast to results obtained with Torpedo presynaptic membranes, the predominantly labelled polypeptide in rat striatal membranes was that with Mr of about 56 kDa compared to the 42 kDa polypeptide. Binding of the affinity ligand was blocked by hemicholinium.

(Supported by Medical Research Council of Canada.)

242.2

HIGH AFFINITY CHOLINE CARRIER FROM SYNAPTOSOMAL MEMBRANES. H. Breer, M. Knipper*, C. Kahle*. Institute of Zoophysiology, Univ. Hohenheim 7000 Stuttgart 70, F.R.G.

The accumulation of choline via a specific high affinity uptake system is the rate-limiting step for the synthesis of acetylcholine. Using insect synaptosomal ghosts with artificially imposed ion gradients the energetics of choline translocation has been explored. The transport rate was found to be regulated via second messengers and specific protein kinases. Binding studies, using hemicholinium-3 as specific probe, suggest that the number of carrier increased upon kinase activation and that occult choline transporter may be recruited via phosphorylation. Towards an identification of the carrier constituents, monoclonal antibodies were produced which block the high affinity choline uptake. These antibodies labelled specific neuropil areas in immunocytochemical approaches and recognized a specific polypeptide band in Western blots. These putative carrier polypeptides were purified to homogeneity and functionally reconstituted in liposomes.

242.3

SYNTHESIS OF AN AFFINITY LABEL FOR THE ACETYLCHOLINE TRANSPORTER AND ITS USE IN REVERSIBLE AND IRREVERSIBLE STUDIES. Stanley M. Parsons and Gary A. Rogers.* Department of Chemistry, University of California, Santa Barbara, CA 93106

A structure-activity study of acetylcholine (ACh) elucidated structural features in ACh analogs giving potent, competitive inhibition of ACh active transport by synaptic vesicles purified from Torpedo electric organ. The analog Z-cyclohexylmethyl N-(4´-azidophenacyl)-N-methylisonipecotate was synthesized in unlabeled and tritium-labeled (10.8 Ci/mmol) forms. Unlabeled analog inhibited active transport of [^{3}H]ACh(K_m = 0.3 mM) with IC (0.85± 0.15) μM, and inhibited binding of [^{3}H]analog with K_i (1.0±0.6) μM. Unlabeled analog also was a competitive inhibitor of [^{3}H]vesamicol binding with K_i (0.46±0.06) μM. Vesamicol is a noncompetitive inhibitor of ACh active transport (K_i = 20 nM). Specifically bound [^{3}H]analog dissociated at a rate of (0.19±0.02) min^{-1} at 0°, which is slow enough to carry out filter-based binding studies. [^{3}H]ACh itself dissociates from the ACh transporter (AChT) much too quickly for such studies. Binding of [^{3}H]analog to synaptic vesicles exhibits a saturable component with K_d 0.4 μM and B_{max} 800 pmol/mg, and a large nonsaturable component. ACh partially displaced bound [^{3}H]analog with IC_{50} (3.2± 1.4) mM in the absence and presence of MgATP. Choline was less effective with IC_{50} (25± 13) mM. Vesicles trapped on a glass fiber filter and incubated with nonlabeled analog lost 90 percent of their ability to bind [^{3}H]vesamicol when photolyzed. Vesicles incubated with [^{3}H]analog, photolyzed and subjected to SDS slab gel electrophoresis and fluorography exhibited labeling of a species extending from 50,000 M_r to the top of the gel (M_r > 200,000). Labeling was completely inhibited by excess nonlabeled analog, 100 mM ACh or 1μM vesamicol, and partially inhibited by 3 mM ACh. We conclude that the AChT exhibits a very heterogeneous structure in SDS and a range of affinities for ACh, and it is intimately associated with the vesamicol binding site.

242.4

EFFECTS OF DM-9384, A NEW COGNITION-ENHANCING AGENT , ON GABA RELEASE AND CHOLINE UPTAKE IN RAT CORTEX. S. Watabe*, H. Yamaguchi* and S. Ashida* (SPON: M. Yoshii). Lab. of Pharmacol., Res. Insti., Daiichi Seiyaku Co. Ltd. Tokyo 134, JAPAN.

Previous reports have shown that N-(2,6-dimethylphenyl)-2-(2-oxo-1-pyrrolidinyl)acetamide(DM-9384) inhibits the amnesia induced by GABA and ACh antagonists(Nabeshima et al., Psychopharmacol., 96:Suppl. 305,1988). DM-9384 also potentiated learning acquisition and increased choline acetyltransferase(ChAT) activity in rat cortex. These results indicated that DM-9384 related to GABA and cholinergic system. In the present study, we performed on the effects of DM-9384 and aniracetam, an analogue of DM-9384, on the K^+-evoked efflux of ^{14}C-GABA from rat cortical slices and on the uptake of ^{3}H-choline in the cortical synaptosomes. The release and uptake experiments were carried out according to the procedures of Davies(1975), Atweh(1976), respectively. In high concentrations of DM-9384, the GABA release caused by the high K^+(40mM) was remarkably decreased by approximately 30%(10^{-3}M). On the contrary, the low concentration(10^{-6}M) of DM-9384 significantly increased by about 15%. On the other hand, aniracetam was no effect in all concentrations. The Na^+-dependent choline uptake slightly increased at 10^{-5}M of DM-9384. These results suggest that in GABA release, DM-9384 possesses two differential effects by different concentrations, and the GABAergic system is facilitated by the low concentrations of DM-9384. DM-9384 also accelerates the turnover in cholinergic system.

242.5

QUISQUALATE CAUSES GLYCOGEN DEPLETION AND INCREASED GLUCOSE UTILIZATION IN PRIMARY ASTROCYTE CULTURE. R.A. Swanson and F.R. Sharp Neurol. Dept., VAMC and UCSF, San Francisco, CA.

Previous studies have shown that incubation with L-glutamate (L-GLU) causes increased glycogen accumulation and decreased glucose utilization iin primary astrocyte culture. It has now been found that several analogues of L-GLU have effects opposite to these.

Primary astrocyte cultures were prepared from neonatal rat cortices as described by Hertz et al. and used after 4-5 weeks. L-GLU and analogues were added at initial media concentrations of 1mM. Glycogen was measured after 4 hr incubations by an amyloglucosidase linked flourometric procedure. Glucose utilization was assessed by adding (^{3}H)2-deoxyglucose for 45 min followed by 3 15 min washes in fresh media. The ^{3}H retained in the cells provided a relative index of the glucose utilization rates

L-GLU increased glycogen content 87±6% and reduced glucose utilization by 53±9%. In contrast, D-GLU, quisqualate, and L-α-aminoadipate each reduced glycogen content to less than 25% and increased glucose utilization to greater than 150% of controls. These effects on astrocyte metabolism may contribute to CNS toxicity.

242.6

GLUTAMATE UPTAKE SYSTEMS IN CELL CULTURES FROM RAT BRAIN. *B. Flott* and W. Seifert. (SPON: F.W. Schürmann) Max-Planck-Institut für biophys. Chem., Department of Neurobiology, Göttingen (F.R.G.)*

The neurotransmitter glutamate and the NMDA-receptor have become an important area of research on both synaptic plasticity and neurotoxicity in recent years. Most studies deal with the protection of glu-sensitive neurons by glu-receptor antagonists or other agents. An other approach is the investigation of glu-uptake as the physiological mechanism to stop glu-induced excitation. In addition to the well known Na^+ dependent glu-transport and the Cl^- dependent transport we have recently described a Ca^{++} dependent uptake in synaptic plasma membrane preparations which is active in the absence of Na^+ and Cl^- ions (Hollmann, Harnecker and Seifert, 1988, FEBS-Letters 228, 74-78.). In our studies we used cultured astrocytes and hippocampal neuronal cultures. We investigated the three different uptake systems including the Ca^{++} dependent system and the modulation of glu-uptake under various conditions and--as glu is probably involved in ischemic neuronal cell death--in the absence and presence of glucose. Our results show that, as documented for synaptosomes, physiological concentrations of Ca^{++} [2 mM] indeed induce Glu-uptake into cultured astrocytes significantly (1.5 times of the basal uptake). Cl^- gives an increase of about 8 times and Na^+ about 40 times of the control value. In addition, Ca^{++} enhances the Na^+ and Cl^- dependent transport.

242.7

AVERMECTIN ENHANCES DEPOLARIZATION-EVOKED ^{3}H-GABA RELEASE FROM RAT BRAIN SYNAPTOSOMES K.Subbarao*, T.J.Turner* and S.M.Goldin. Dept.of Biol. Chem.and Mol.Pharmacology, Harvard Medical School, Boston,MA 02115

Multiple components of Ca-dependent and Ca-independent ^{3}H-GABA release from rat brain synaptosomes have been resolved using a rapid superfusion method with a resolution time of ≈60 msec (Turner & Goldin [1989] Biochem. 28,586). 22,23-dihydro avermectin B_{1a} (AVM), a broad-spectrum anti-parasitic drug, selectively enhances the rate of K^+ depolarization-stimulated, Ca-independent GABA release 6-8 fold at [1μM]; 3-fold enhancement was observed at 100 nM [AVM]. The AVM enhanced release reaches a peak with a τ of 0.4 sec and decays exponentially (τ=1.6sec). AVM modestly increases basal GABA release but its major effect is seen only with depolarization. Bicuculline and picrotoxin block AVM enhanced release. The presence of Cl^- is a requirement for AVM to enhance GABA release. AVM enhanced GABA release was not blocked by nipecotate, an inhibitor of high-affinity GABA uptake carrier, suggesting that AVM enhanced GABA release is probably not mediated through the reversal of this carrier. AVM marginally enhanced the release of ^{3}H-dopamine but had no effect on ^{86}Rb release. These results indicate that AVM is mediating its effect through binding to the $GABA_A$ receptor, and requires Cl^- entry through its associated ion channel.

242.8

EXPRESSION OF GABA-TRANSPORTER mRNA IN XENOPUS OOCYTES. J. Guastella*, N. Davidson, H.A. Lester (SPON:W. Agnew). Division of Biology, 156-29, Caltech, Pasadena, CA, 91125.

We are using Xenopus oocytes as an expression system to characterize mRNA encoding the proteins responsible for the carrier-mediated transport of γ-aminobutyric acid (GABA). Microinjection of rat brain poly(A)$^+$ RNA into oocytes results in an 8 to 10-fold increase in the accumulation of ^{3}H-GABA above that seen in uninjected or water-injected controls. This increase is roughly linear with translation time up to about 96 hours, and is linear with incubation time up to 1 hour. Uptake is saturable, indicating that it is mediated by a carrier system, and is of high affinity, with a K_m of approximately 2 μM. The accumulation of ^{3}H-GABA is dependent on the presence of sodium ions, and is reduced 50-90% by 500 μM ß-alanine, nipecotic acid and 2,4-diaminobutyric acid. These results indicate that the GABA transporters being expressed share the properties of GABA transporters studied in more conventional preparations, such as brain slices, ganglia, and synaptosomes.

Fractionation of poly(A)$^+$ RNA on sucrose gradients resulted in a well-defined peak of activity corresponding to RNA's with lengths of about 3 to 6 kb. This fraction is currently being used to construct cDNA libraries enriched for GABA transporter cDNA. These libraries may also eventually prove useful for the isolation of cDNA's encoding other neurotransmitter transporters. (Supported by NIH Grants GM-10991 and GM-29836. JG is an NIH Postdoctoral Fellow).

242.9

REGION SPECIFIC REGULATION OF THE BRAIN SODIUM-DEPENDENT HIGH AFFINITY GLUTAMATE UPTAKE PROCESS BY HYPERAMMONEMIA. M.B. Robinson and M. Hunter-Ensor*. Depts. of Pediatrics and Pharmacology, University of Pennsylvania, Philadelphia, Pa, 19104.

Extracellular levels of excitatory amino acids, glutamate and aspartate, are primarily regulated by sodium-dependent high affinity uptake processes. The purpose of this investigation was to identify a condition that would lead to regulation of this uptake process. Hyperammonemia leads to increases in the level of brain glutamine (Gln), a putative precursor of releasable glutamate. Urease-containing mini-osmotic pumps were implanted intraperitoneally to develop prolonged hyperammonemia in rats with a 3.1-fold elevation of brain Gln. At 72 h post-implantation, we observed significant increases in the V_{max} for synaptosomal uptake of L-[^{3}H]-glutamate in cerebellum (45 %) and brainstem (28 %) with no changes observed in hippocampus, striatum, cortex and midbrain. The K_m did not change in any of these brain regions. Glutamate analogues were screened for inhibition of synaptosomal L-[^{3}H]-glutamate uptake. The IC_{50}'s are: (units = μM)

Compound	Cere	Brst	Hippo	Cor	Str	Mdbr
DL-*threo*-ß-hydroxyaspartate	3.2	2.4	1.2	2.3	1.4	1.5
L-Aspartate ß-hydroxamate	3.6	3.8	8.1	10	7.5	6.4
ß-Glutamate	1.9	1.5	2.8	3.6	4.1	2.8
L-Cysteine sulfinate	1.6	0.88	1.2	1.6	1.2	1.1
α-Aminoadipate	34	130	590	760	610	540
Dihydrokainate	>3000	440	150	120	81	150

These studies suggest that the region specific regulation may, in part, be due to heterogeneity of this uptake process. Supported by Pew to MBR.

242.10

FOREBRAIN AND CEREBELLAR mRNAS INDUCE PHARMACOLOGICALLY DISTINGUISHABLE L-GLUTAMATE TRANSPORTERS IN XENOPUS LAEVIS OOCYTES. R.D.Blakely and S.G.Amara. Section of Molecular Neurobiology, Howard Hughes Medical Institute, Yale Univ. Sch. Medicine, New Haven, CT 06510.

Previously, we have described the ability of poly(A)$^+$ RNA from different regions of the rat brain to induce the Na^+-dependent transport of neurotransmitters including L-glutamate (GLU), GABA, glycine, and dopamine (Blakely et al., PNAS 85, 9846-9850,1988). Transport of [^{3}H]GLU was found to be saturable (K_M =14μM), time- and temperature-dependent, and sensitive to competitive substrates of synaptosomal GLU carriers. These studies also determined that both GLU and GABA transporters are induced by 2.5-3.0 kb mRNAs. In the present study, we have sought to determine 1) if the mRNAs encoding forebrain GLU and GABA transporters are resolved by sucrose density gradients, 2) if forebrain and cerebellar mRNAs encoding these activities are similarly sized, and 3) if pharmacological distinctions could be made between forebrain and cerebellar mRNA carriers. Transport assays with fractions from a linear 10-31% sucrose density gradient with forebrain mRNA reveal comigrating peaks of [^{3}H]GLU and[^{3}H]GABA transport activity in the same size range as previously observed for cerebellar mRNAs. Consistent with synaptosomal studies by Ferkany et al. (J. Neurosci. Res. 16, 491-503, 1986), dihydrokainate (1mM) substantially inhibits forebrain (61±5%) and spinal cord (63±5%) mRNA-induced GLU transport while having only weak effects on transport induced by cerebellar (16±5%) mRNA. Thus, while mRNAs encoding forebrain and cerebellar Na^+-dependent GLU transporters appear of similar size, pharmacological heterogeneities persist after oocyte reconstitution suggesting the presence of distinct mRNAs encoding these activities.

242.11

BEHAVIORAL AND BIOCHEMICAL EFFECTS OF CHRONIC ADMINISTRATION OF GABA UPTAKE INHIBITORS IN MICE. W. Karbon*, M.Wyza*, S. Goode*, K. Naper*, J. Ferkany, S.J. Enna. (Sponsor: L. Steranka) Nova Pharmaceutical Corp., Baltimore, MD 21224-2788.

SKF 89976A and SKF 100330A are potent GABA uptake inhibitors with anticonvulsant properties in rodent models. Chronic (14 days, b.i.d., i.p) administration of 0.46 mg/kg SKF R-89976A did not produce a change in anticonvulsant activity, although a higher dose (8.9 mg/kg) caused a 2-fold increase in the ED_{50} (0.63 mg/kg vs. 1.23 mg/kg) for protection. Chronic administration of SKF 100330A (1.8 mg/kg, 13.6 mg/kg) did not influence the anticonvulsant potency of this compound. In contrast, the catalepsy observed in response to acute administration of the higher doses of each compound was markedly reduced in animals treated for as few as 4 days, and was accompanied by a significant increase in the cataleptic ED_{50} for SKF R-89976A (8.5 mg/kg vs. 36.5 mg/kg). [^{3}H]-GABA uptake measured in mouse forebrain synaptosomes was not altered by chronic drug administration, nor was [^{3}H]-$GABA_A$ or [^{3}H]-$GABA_B$ receptor binding. Likewise, [^{3}H]-sulpiride (D2) binding in mouse striatal membranes was unaffected by chronic drug treatment. These findings suggest that following chronic administration, the anticonvulsant effects of SKF R-89976A and SKF 100330A are influenced to a lesser extent than the cataleptic effects.

242.12

QUINOLINIC ACID AND KYNURENIC ACID SYNTHESIS BY HUMAN GLIOMA. [1]M.A. Stasi*, A. Vezzani[1], J.B.P. Gramsbergen, C. Speciale and R. Schwarcz (SPON: F. Du). [1]Istituto Di Ricerche Farmacologiche "Mario Negri", Milano (Italy) and Maryland Psychiatric Research Center, Baltimore, MD 21228.

In the rat brain, the enzymes responsible for the production of the neuroactive kynurenines quinolinic acid (QUIN) and kynurenic acid (KYNA) are localized in astrocytes. We have therefore examined human gliomas, obtained during neurosurgery, for their ability to synthesize QUIN and KYNA from their respective bioprecursors.

QUIN production was assessed in homogenates of glioma tissue after incubation with either kynurenine (KYN; 500 μM), 3-hydroxykynurenine (500 μM) or 3-hydroxyanthranilic acid (2.8 μM). In all cases (N=6), QUIN synthesis could be demonstrated. However, it appeared that kynurenine hydroxylase activity was very low and may constitute a rate-limiting step in QUIN production.

KYNA neosynthesis was assessed in 7 tumors according to the method of Turski et al.(J. Neurochem., 52:1629, 1989) in slices of human astrocytomas within 60 minutes after surgical removal of the tumor. The tissue was exposed to KYN, and KYNA was measured in the medium. Dose-dependent production of KYNA could be demonstrated.

It remains to be elaborated if and to what extent gliomas produce QUIN and KYNA in situ, and how glioma-derived kynurenines may play a role in pathophysiological conditions. Supported by USPHS grant NS 16102.

242.13

ROLE OF GLUTAMINE SYNTHETASE IN GLUTAMATE METABOLISM BY CULTURED ASTROCYTES. S.Farinelli*and W.J.Nicklas (SPON:E.T.Browning). Dept. of Neurology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854.

Glutamine synthetase (GS) catalyzes the amidation of glutamate (GLU) to glutamine (GLN). In the brain, GS has been shown to be localized within the astrocyte where it plays a pivotal role in maintaining the homeostasis of GLU. Previously we and others have shown that the major pathway of GLU metabolism in cultured rat astrocytes favors GLN synthesis and not oxidation. By treating cultures with methionine sulfoximine, an irreversible inhibitor of GS, and allowing the cells to resynthesize new GS enzyme for various periods up to one week, astrocyte cultures were generated with a range of GS activities (0-3.0 μmoles GLN formed/hr/mg protein). These cultures were subsequently used in the study of glutamate metabolism in which incorporation of label from [^{14}C(U)]-GLU into GLN, ASP and deaminated metabolites was quantified. The data suggests that the flux of GLU carbon into GLN is proportional to the amount of GS activity within the astrocyte. The level of GS activity also regulates the steady-state concentration of GLU inside the cell. The results further corroborate the importance of GS in the homeostatic regulation of astrocytic GLU metabolism.

242.14

ANALGESIC EFFECT OF THE GABA UPTAKE INHIBITOR NO-328 (N-(4,4-DI(3-METHYL-2-THIENYL)BUT-3-EN-1-YL)NIPECOTIC ACID). M.J.Sheardown,J.U.Weis,L.J. Knutsen,M.Swedberg and P.D. Suzdak. Dept. of CNS Pharmacology,NOVO Industries and Depts. of Neuropharm. and Behavioral Pharm., Ferrosan, Denmark.

NO-328 has previously been shown to be both a potent inhibitor of ^{3}H-GABA uptake in-vitro (IC_{50} =67 nM),and possess potent anticonvulsant effects in both mice (ED_{50} for inhibiting DMCM induced convulsions=0.8 mg/kg i.p.) and rats (ED_{50} for inhibiting PTZ induced convulsions=6 mg/kg i.p.). The present report examines the analgesic activity of NO-328. Following i.p. administration, NO-328 produced potent analgesic effects in acetic acid writhing (ED_{50}=0.18 mg/kg), hot-plate (ED_{50}= 3.7 mg/kg) and grid-shock tests (ED_{50}=1.75 mg/kg) in mice and Randall Selitto test in rats (ED_{50}= 12 mg/kg).NO-328 also showed analgesic activity following p.o. administration in mice.The analgesic activity of NO-328 was comparable to that of morphine,and superior to that of the GABA agonist THIP.NO-328 does not bind to central opiate receptors,and its analgesic activity is not blocked by naloxone.The analgesic activity of NO-328 in mice chronically treated with either NO-328 or morphine will also be discussed.These data suggest that NO-328 possesses a good analgesic profile in both mice and rats.

242.15

FUNCTIONAL RECONSTITUTION IN PROTEOLIPOSOMES OF PRESYNAPTIC VESICLE GLUTAMATE UPTAKE ACTIVITY P. R. Maycox*, T. Deckwerth* and R. Jahn. Dept. of Neurochem., Max Planck Institute for Psychiatry, Am Klopferspitz 18A, 8033 Planegg-Martinsried, FRG.

Glutamate uptake by presynaptic vesicles is a secondary active process and is dependent on the proton electrochemical potential generated by a Mg-dependent ATPase (NEM-sensitive vacuolar proton-pump) present in the vesicle membrane. The carrier is unique for synaptic vesicles and is clearly distinguishable from the carriers of the plasma membrane and mitochondria. A detailed study of the energy dependence has shown that glutamate uptake is totally dependent on the membrane potential, with no pH component involved. Recently we have reconstituted the ATP-dependent glutamate uptake activity in proteoliposomes. Highly pure synaptic vesicle membranes are dissolved with 1% sodium cholate and after the addition of exogenous lipids the system is reconstituted by dilution directly into the assay buffer. The reconstituted uptake shows all the characteristics of the endogenous system and represents at least 60% of the activity observed in native synaptic vesicles. We have also reconstituted glutamate uptake using the monomeric form of the light-driven bacterial proton-pump, bacteriorhodopsin (BR). The BR monomers are added to the reconstitution mixture, which is diluted into assay buffer lacking ATP. Glutamate uptake activity is detected only when the proteoliposomes are stimulated with light, indicating that BR generates the membrane potential required by the glutamate transporter. This system enables us to dissect the components involved in glutamate uptake and begin fractionation studies in order to purify the glutamate transporter.

242.16

[3H]-DIMETHYLSTAUROSPORINE: A LIGAND TO STUDY THE CATALYTIC DOMAIN OF PROTEIN KINASE C. W. F. Herblin, J. L. Gross*, U. H. Do* and S. Pounds* Medical Products and Dupont-NEN Products Departments, E. I. du Pont de Nemours & Company, Wilmington, DE and Boston, MA.

The microbial alkaloid staurosporine is the most potent and selective inhibitor of PKC thus far described. The interaction of [N,N-dimethyl-3H]staurosporine ([3H]DMS) with PKC was examined using an automated soluble binding assay. Total [3H]DMS binding to mouse brain PKC was stable over a broad pH range and was not dependent on calcium or phospholipid. The binding was high affinity (Kd=3.8 nM), reached equilibrium within 15 min. on ice, was reversible, with a t 1/2 of 69 min. and could be displaced by the PKC catalytic antagonists staurosporine, K252a, and the isoquinoline sulfonamide H-7. Staurosporine and dimethylstaurosporine were equipotent inhibitors of [3H]DMS binding to PKC and of PKC catalytic activity. [3H]DMS bound to the catalytic domain of PKC since high concentrations of phorbol dibutyrate had no inhibitory effect. In addition, rat brain PKC which had been proteolyzed to generate a phospholipid and calcium independent enzyme (PK-M), retained the ability to bind [3H]DMS but not [3H]phorbol dibutyrate. MgATP inhibited [3H]DMS binding to PKC, but only at high concentrations (2.5 mM). At concentrations of 12-50 uM, ATP partially reversed the inhibition of [3H]DMS binding by H-7, suggesting that ATP and H-7 compete with each other, but not at the [3H]DMS binding site. [3H]DMS is thus a useful ligand to study the mechanism of interaction of catalytic inhibitors with PKC.

242.17

ASCORBATE TRANSPORT BY RODENT ASTROGLIAL CELLS. J.X. Wilson*, A. Kulaga*, E.J. Jaworska* and S.J. Dixon* (SPON: J. Kraicer). Dept. of Physiol. and Div. of Oral Biol., Univ. of Western Ontario, London N6A 5C1, Canada.

Uptake of L-ascorbate (vitamin C) by astrocytes was studied in primary cultures prepared from the neopallium of newborn Swiss CD-1 mice or Sprague-Dawley rats. Exposure of cultures to dibutyryl cyclic AMP changed cell morphology from polygonal to stellate and stimulated ascorbate uptake, with the greatest stimulation occurring in mouse astrocytes. Uptake was specific for ascorbate since it was not diminished by the presence of other organic anions including acetate, formate, lactate, malonate, oxalate, p-aminohippurate, pyruvate and succinate. Astroglial ascorbate uptake was Na^+-dependent, but it did not have a specific requirement for external Cl^-. Ascorbate transport was rapidly ($\leq$1 min) and reversibly inhibited by the anion transport inhibitors furosemide, 4-acetamido-4'-isothiocyanostilbene-2,2'-disulfonic acid (SITS) and 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid (DIDS). The rapid and reversible effects of the impermeant inhibitors are consistent with direct inhibition of Na^+-ascorbate cotransporters located in the astroglial plasma membrane.

242.18

CHARACTERIZATION OF THE ATPASES FROM SYNAPTIC VESICLES OF TORPEDO CALIFORNICA. Barry W. Hicks*, Stanley M. Parsons (SPON: R. Refinetti). Dept. of Chemistry, Neuroscience Research Program, Univ. of Calironia, Santa Barbara, CA 93106.

Cholinergic synaptic vesicles from the electric organ of Torpedo Californica contain a V-type and a P-type ATPase. The two ATPase activities can be distinguished by assaying in the absence and presence of vanadate. Using immunobeads coupled to SV2 monoclonal antibody both types of activity can be pelleted. Neither activity is significantly affected by common anions. The P-type has an absolute requirement for magnesium ion over calcium ion whereas the V-type is less specific. The P-type is slightly stimulated by sodium and potassium ions. Both types of activity were examined in the presence of the uncouplers FCCP, nigericin, valinomycin, gramicidin and A23187. Stimulation of the ATPase activities occurred, but at concentrations higher than required to inhibit active transport of acetylcholine. Tributyltin chloride inhibits both activities. Profiles of individual activities versus pH were generally bell shaped between pH 5 and 9 but also showed some heterogeneity. Lineweaver-Burke plots showed biphasicity for both activities. The P-type has an apparent subunit structure similiar to the sodium-potassium ATPase. Western blots of whole synaptic vesicles showed faint staining at 98K, but no cross reactivity with the vesicle P-type ATPase using polyclonal antibodies to sodium-potassium ATPase.

242.19

AN IMPROVED INVERTEBRATE SYNAPTOSOMAL PREPARATION WITH CHOLINERGIC PROPERTIES. C. Torrence - Campbell*, S. Sweet*, H. Gates* and J.G. Townsel. Department of Physiology, School of Medicine, Meharry Medical College, Nashville, TN 37208

Our laboratory has previously characterized a high affinity choline uptake system (HAChUS) in **Limulus** tissues (Maleque **et al., Biochem. Pharmac. 28**: 985-990, 1979; Ivy, M. and Townsel, J.G. **Comp. Biochem. Physiol.86(C)**:103-110, 1987) and synaptosomes (Newkirk, R.F. **et al., Comp. Biochem. Physiol. 70(C)**:177 - 184; 1981) We report here on the characterization of the HAChUS in synaptosomes prepared selectively from central nervous system tissues shown to be enriched for presumed cholinergic functions; namely the protocerebrum, corpora pedunculata and abdominal ganglia. Synaptosomes were prepared from these tissues by means of a modification of the subfractionation procedure developed by Dowdall and Whittaker (**J. Neurochem. 20**:921-935, 1973). In our modification, we harvested a PP_2L fraction exclusively from the S_2 fraction. Compared to the P_2L fraction, the PP_2L was approximately four times more efficient in (^3H) choline uptake and was significantly more sensitive to inhibition with micromolar concentrations of hemicholinium -3. The PP_2L fraction HAChUS was shown to have characteristics common to the HAChUS of identified cholinergic tissues. We also report on the kinetic isolation of the HAChUS from the low affinity transport of choline in these synaptosomes. (This project was supported in part by the Surdna Foundation, MBRS Grant RR08037 and the NSF/MRCE Grant 871485.)

242.20

PHOSPHATIDYLINOSITOL-SPECIFIC PHOSPHOLIPASE C SENSITIVITY OF ACETYLCHOLINESTERASE FROM THE CENTRAL NERVOUS SYSTEM OF MANDUCA SEXTA. D. J. Prescott* and C. G. MacLean*. (SPON: P. J. Stephens) Dept. of Bio., Bryn Mawr College, Bryn Mawr, PA 19010.

Acetylcholinesterase (AChE) can be extracted from central nervous system tissue of Manduca sexta by buffers containing detergent and 0.5 M NaCl. Approximately 20% of the enzyme is soluble in the absence of these agents, while 0.5 M NaCl alone releases 30 to 40% of total enzyme, and detergent (Triton X-100, 1%) releases 50% of the total enzyme in crude homogenates. Treatment of the crude brain homogenate with phosphatidylinositol-specific phospholipase C (PIPLC) from Bacillus thurengiensis results in substantial (70 to 80%) solubilization of AChE. Greatest response was achieved with PIPLC from Bacillus thurengiensis, while PIPLC from Bacillus cereus and from Trypanosoma brucei have the effectiveness of detergent, solubilizing about half the total AChE activity. Non-denaturing polyacrylamide gel electrophoresis indicates that detergent-isolated AChE, like the AChE which is natively soluble, occurs primarily in two molecular weight forms with residual large forms which do not enter 6% gels. AChE solubilized by B. thurengiensis PIPLC and by B. cereus PIPLC occurs only in one molecular form, corresponding to the lowest molecular weight form of detergent-isolated enzyme indicating that PIPLC treatment abolishes the ability of AChE to aggregate.

Preliminary studies indicate no evidence of autolysis. If an endogenous phospholipase is present either the amount is small or its activity somehow inhibited by tissue homogenization conditions.

The AChE in this invertebrate system demonstrates sensitivity to PIPLC similar to other invertebrate and vertebrate AChE's, thus joining a disparate, growing family of membrane-associated proteins attached to the membrane by covalent bonding to phosphatidylinositol.

243.1

ANALYSIS OF TREMOR WITH METHODS OF NONLINEAR DYNAMICS: ATTRACTOR DIMENSIONS. R.J. Elble. Southern Illinois Univ. School of Medicine, Springfield, IL 62794-9230.

Physiologic tremor and essential tremor exhibit irregularities in amplitude and frequency that could be attributed to stochastic noise or to nonlinear dynamics of the neural oscillators that are responsible for tremor. The spatial correlation method of Grassberger and Procaccia (Physica 1983; 9D:189-208) was used to estimate the functional nonlinear complexity (i.e., attractor dimension) of wrist tremor that was recorded from normal adult volunteers and from patients with essential tremor. The attractor dimension of essential tremor was 1.0-1.5 during times of greatest tremor and was 4-6 during times of relative tremor quiescence. Mild essential tremor and physiologic tremor exhibited large amplitude fluctuations, and the attractor dimension was often indeterminate (i.e., indistinguishable from infinity) for these forms of tremor. These results suggest that essential tremor fluctuates between chaotic and limit-cycle modes of oscillation and that essential tremor is most symptomatic when it behaves as a limit-cycle oscillator. By contrast, mild essential tremor and physiologic tremor tend to persist in either a chaotic, stochastic, or nonstationary mode of oscillation. These data are consistent with the notion that essential tremor results from a reduction in the functional degrees of freedom of the involved neural pathways. (Supported by NINDS NS20973).

243.2

FRACTALS IN KINESIOLOGY. JB Myklebust, BM Myklebust. Marquette University, College of Engineering, Laboratory of Sensory-Motor Performance, Zablocki VA Medical Center, Medical College of Wisconsin, Milwaukee, WI 53295.

Deviations of the center of pressure during quiet standing have been characterized for normal adults and in aging. These measurements have been used to assess neurological disorders of the vestibular and cerebellar systems, and lesions of the pyramidal and extrapyramidal tracts. The deviations have been quantified in terms of the area encompassed by the deviations of the center of pressure in the X-Y plane, as well as by spectral methods.

A method of characterizing planar curves which has had considerable success is the determination of the fractal dimension, D, of a curve[1]. In this context, D is a measure of the degree to which a curve fills the available space. D is calculated from the equation $D = \log(n)/[\log(n)+\log(d/L)]$; n is the number of connected line segments comprising the curve, d is the planar diameter of the curve (the largest distance between 2 points), and L is the cumulative length of the curve. A straight line has a dimension of 1; a curve which completely fills the space has a dimension of 2 (the Euclidean dimension). A curve which traces over itself multiple times could exceed 2.

Data was collected from 11 normal subjects in good general health ranging in age from 22 to 75 years and from 3 elderly patients with Alzheimer's disease with impairments of gait and stability, by clinical examination. The subjects were asked to stand quietly on a Kistler force plate for 1 minute with eyes open, followed by 1 minute with eyes closed. The 3 forces and 3 moments were sampled at 100 Hz and the positions of the center of pressure were computed. The middle 20 second period was analyzed for each trial. The mean value of D was 2.09 (sd=0.33) for the normal subjects and 1.67 (sd=0.11) for the Alzheimer's patients. These values were significantly different ($p<.03$); no statistical difference was observed between the 5 normal young adult subjects (22-40 yr) and the 6 healthy aging subjects (45-75 yr).

These preliminary studies suggest that measurement of the fractal dimension may be a simple and practical method to assess standing balance and characerize pathology.

1. Katz MJ, George EB: Bull Math Biol 17:273, 1985.

This work has been supported by research funds from VA Rehabilitation R&D.

243.3

SYNCHRONIZING HAND AND FOOT MOVEMENT IN A PROJECTIVE OR REACTIVE MODE: TWO CONTRASTING SCHEDULES OF MOTOR COMMAND. J.Paillard, CNRS-LNF2, Marseilles, C. Bard & M. Fleury, Human Motor Performance Lab., Laval Un., QC, Canada.

The accuracy with which the motor system may achieve temporal goal is surprisingly precise. Earlier study (Paillard 1948), however, showed contrasting motor commands schedules depending on whether the movement is triggered by an external or a self-induced signal. To further evaluate this hypothesis, two experimental conditions were presented. In a reactive condition, subjects were required to react simultaneously with the two hands or with the homolateral hand and foot to an auditive stimulus as quickly as possible. In a projective condition, subjects were instructed to simultaneously self-activate the two hands or the hand and foot. Seven subjects participated to the study. Temporal difference between the two limbs motor response was calculated on each trial (25 trials/condition). In the reactive condition, the hand significantly preceded foot by 30 ms. This delay corresponds to the difference observed in the reaction time of the two limbs when measured independently. Such a result reflects the observed difference in the efferent pathways conduction time assuming a quasi-simultaneous activation of the descending pathways at the central level. However, in the projective condition the serial order paradoxically inverted. Indeed, the foot preceded hand initiation by 20 ms, as if the temporal target used for scheduling the onset of the two motor commands was the synchronous return to the cortex of the reafferent information generated by each movement initiation. The results support the assumption that commands for goal-directed movement (temporal goal) are planned, organized and scheduled in accordance with the anticipated sensory consequences of the action. Fast reactions to an external signal, result from a direct synchronous motor commands coactivation.

243.4

SENSORIMOTOR TRANSFORMATIONS FOR ARM MOVEMENTS: SERIAL AND PARALLEL PROCESSES. J.F.Soechting, M.Flanders and S.I.Tillery*. Department of Physiology, University of Minnesota, Minneapolis, MN 55455.

We have used errors in pointing movements to gain insight into the processes whereby visual information about target location is transformed into a representation of joint angles.

We asked subjects to make movements in the absence of possible visual corrections, that is to point to the remembered location of the target or to match the target's direction. Based on the results of a variety of such experiments we suggest that information about target location undergoes at least two serial transformations. Initially, target location is represented in a retinotopic frame of reference. It is then transformed into a shoulder-centered representation in terms of distance and direction of the target relative to the shoulder. A second transformation is used to compute orientation angles of the shoulder and elbow which would correspond to target location in shoulder-centered coordinates.

These two serial transformations also involve parallel processes. Target distance and direction (azimuth and elevation) appear to be represented separately. Target distance and elevation are used to compute joint elevation while, in parallel, target azimuth is used to compute the joints' yaw angles.

243.5

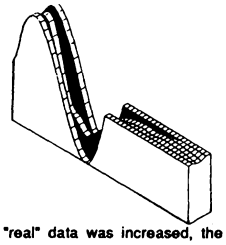
DONDER'S AND LISTING'S LAW FOR REACHING AND GRASPING ARM SYNERGIES. K. Hepp and M.-C. Hepp-Reymond, Physics Dept., ETHZ and Brain Research Institute, University of Zurich, Switzerland

In a large part of its workspace the arm has 4 rotatory degrees of freedom: 3 at the shoulder and 1 at the elbow joint. For pointing or grasping movements the grip (g) and elbow (e) vector from the shoulder defines as torsion (t) the rotation of the g-e plane around g. As for the eye, Donder's law requires t to depend only on g and Listing's law predicts that all rotations of the g-e plane from a forward reference position should have their axes in a fixed plane. We have investigated these hypotheses in 7 healthy humans. The subjects, lying on the floor to fixate the shoulder, had to reach or grasp targets in various positions of the workspace, in which scapula movements could be neglected. The starting position was varied systematically. For a fixed task Donder's law is violated in rapid unconstrained movements with a "hysteresis" which varies systematically in well-coordinated subjects. The influence of the starting position on the torsion of the arm can be "erased" by requiring a spiraling approach. In this case Donder's law holds with small fluctuations around a curved surface of rotation (quaternion) vectors through which a Listing plane can be fitted with an error of 5° to 10° standard deviation. This plane is tilted mostly downward up to 30° relative to the foreward position and varies for different subjects and experimental days. These arm synergies in repetitive pointing and grasping movements allow to interprete findings by Georgopoulos et al. (1988) of a directional representation in 3-D space in the firing patterns of motor cortical neuron populations.

243.6

IDENTIFYING MOVEMENT CORRECTIONS BY FORECASTING. A.M.Wing*, J.Wann*, I.Nimmo-Smith*, E.Miller* and B.Abernethy*, (SPON: D.Kennedy). MRC Applied Psychology Unit and Addenbrookes Hospital, Cambridge, CB2 2EF, UK and U Queensland, Australia.

To determine the nature of corrective adjustments during rapid positioning movements, a technique for identifying their presence and timing is required. We propose a method in which the time-course of a kinematic variable for an individual trial is evaluated using a forecasting approach. Data for hand movements to a target 20-cm in front of the body were obtained using an accelerometer. Acceleration waveforms for control trials were described by a mean and covariance matrix for the coefficients of a third-order polynomial consistent with a minimum jerk criterion. Illustrative data from a trial with an unexpected change in target position (that required the subject to increase movement amplitude) may be seen to diverge from flanking cubic polynomials. Cubic polynomials were fit to progressively increasing proportions of the acceleration waveform of individual trials. Fitting was constrained by using an estimate for the "missing" end portion based on control trial coefficients, ie a "forecast". As the proportion of "real" data was increased, the behaviour of the coefficients and of the residuals from the forecast was diagnostic of corrective adjustment to movement.



243.7

EFFECT OF IMPOSED AUDITORY RHYTHMS ON HUMAN INTERLIMB COORDINATION. Z. Beheshti and J.R. Higgins.* Teachers College, Columbia University, N.Y., N.Y. 10027.

We investigated the effect of imposed auditory rhythms on the coordination of rhythmic upper and lower limb movements. Seven seated subjects performed clapping and alternate foot tapping in 3 sessions. Data were collected on period, variability and phase-linkage of clap and foot tap cycles. In session 1 (control), when subjects performed at their preferred rate, 43% exhibited tight phase-linkage of the clap and foot tap cycles, while 57% manifested loose phase-linkage. In session 2, subjects foot tapped with the metronome (1, 2, 3 & 4Hz), while clapping at the preferred rate. In session 3, subjects clapped with the metronome and foot tapped at preferred rate. The results of sessions 2 and 3 indicated that both tight and loose phase-linked subjects retained their preferred rate phase-linkage, suggesting that externally-paced or self-paced rhythmic limb movements may be coordinated in a similar fashion. Our findings also suggest that interlimb coordination may be controlled in a manner similar to that of coupled oscillators.

243.8

DYNAMIC JOINT STIFFNESS VARIES SYSTEMATICALLY WITH THE PATTERN OF BIMANUAL COORDINATION DURING THE SAME MOTOR TASK. J. P. Scholz, School of Life & Health Sci., Univ. of Delaware, Newark, DE 19716.

A preliminary study of the neurophysiological correlates of differences in coordinative pattern stability, often associated with movement pattern transitions, is described. Dynamic stiffness of the second metacarpo-phalangeal joint (MCPJ) was measured to test the hypothesis that joint stiffness would be lower when moving in a more stable in-phase than in a less stable anti-phase coordination pattern (Kelso, J. A. S, et al., Physica Scr., 35:79, 1987). Subjects (N=3) placed their index fingers into two manipulanda and performed alternating in-phase or anti-phase flexion/extension movements. Movement frequency was increased from 1-2 Hz in 0.2 Hz steps using a metronome. A 75 Ncm torque was delivered randomly to extend the right MCPJ during flexion. Stiffness was estimated by dividing torque magnitude by the amplitude of finger displacement from torque onset to offset (50 ms). MCPJ stiffness was significantly ($p<.0001$) greater in the anti-phase (K=145.5 Ncm/rad) than in the in-phase (K=124 Ncm/rad) pattern for all frequencies studied. Results suggest that differences in coordinative pattern stability are associated with systematic differnces in the mechanical properties of the movement components.

243.9

IS HUMAN ARM TRAJECTORY FORMATION SIMPLIFIED BY VISCO-ELASTIC TUNING? S. A. Ellias* (SPON: H. B. Stanton). Mass. Gen. Hosp. & Dept. of Brain & Cognitive Sci., MIT, Cambridge, MA 02139.

A major issue in the study of human arm movements is how to generate multi-joint movements. Many investigators have suggested that multi-joint movements are obtained by using the net elastic (or stiffness) properties of the human arm, and not by solving the complete inverse dynamics problem. Experiments on multi-joint arm posture have demonstrated invariant spatial properties of the stiffness measured at the hand both at rest and under external loads (Mussa-Ivaldi, et al., 1985, 1987). Recently, a model assuming compliant motion about a *virtual* trajectory and incorporating these invariant stiffness properties has predicted the form of human planar arm movements (Flash, 1987).

The objective of the present computational study was to examine how the relationship between arm visco-elastic properties and inertia could influence trajectory formation. Simulations of planar arm movements were conducted using a model governed by visco-elastic properties and a *virtual* trajectory. The stiffness was systematically altered from the values measured in humans. The viscosity was either covariant or varied independently. The multi-joint arm inertia was computed along the arm paths.

The results of this study indicate that 1) visco-elastic values which mimic the invariant static stiffness properties measured in humans create better tracking of the *virtual* trajectory while using less stiffness; 2) these same values of stiffness appear to match inertial factors: i.e., they minimize some differences between the multi-joint inertia and stiffness when averaged over many paths in workspace. Thus, if particular visco-elastic properties during motion resemble those measured at rest, trajectory control is simpler. This suggests definite functional advantages of neuro-muscular visco-elastic tuning for the execution of movement. This work is supported by NIH Grants K07-NS00883 and NS09343.

243.10

PATTERN FORMATION IN A MULTISTABLE COORDINATIVE SYSTEM. J.J. Jeka and J.A.S. Kelso (SPON: A.J. Nash). Center for Complex Systems and Department of Psychology, Florida Atlantic University, Boca Raton, FL 33431.

We study the coordination among multiple, anatomically different components and their (multistable) patterns within the context of dynamic pattern theory which uses the concepts and tools of nonlinear dynamical systems (especially Haken's [1983] synergetics) as an operational framework for understanding coordinated behavior at several levels of description (e.g. Kelso, Schoner, Scholz and Haken, Physica Scripta, 35:79, 1987; Schoner and Kelso, Science, 239:1513, 1988).

An apparatus specifically designed for this purpose, allows us to measure the kinematics of (up to) four rhythmically moving limbs in the sagittal plane of a seated subject. Frequency of motion is increased by an auditory metronome to which the subject must synchronize his/her actions. Patterns are differentiated by qualitative changes in behavior, observed through the collective variable, relative phase. Several different phase transition phenomena were discovered in a set of four experiments: 1) When the system is prepared in the D mode (i.e., an arm and a leg cycling in Different directions) and frequency is increased, a spontaneous switch to the S mode (i.e., an arm and a leg cycling in the Same direction) occurs at a critical frequency. However, no such transition occurs when the system is prepared in the S mode; 2) At higher frequencies, further spontaneous transitions are observed to a variety of frequency- and phase-locked states, that can be quantitatively differentiated by their stability; 3) The time taken to *intentionally* switch from one 4-limb pattern to another is demonstrated to depend upon the relative stability of the different patterns, opening the way to experimentally explore switching routes among multiple attractors. These results may be understood in terms of dynamic pattern theory and its recent extensions to multi-component (>2) coordination.

This research was supported by NIMH (Neurosciences Research Branch) grant MH42900-01 and ONR contract N00014-88-J-1191.

243.11

SPATIALLY DEPENDENT PHASE TRANSITIONS IN SINGLE MULTI-JOINT LIMB MOVEMENTS. S.A. Wallace[1], J.J. Buchanan, and J.A.S. Kelso (SPON: I.B. Johanson). Program in Complex Systems and Brain Science, Center for Complex Systems, Florida Atlantic Univ., Boca Raton, FL 33431, and Department of Kinesiology, Univ. of Colorado-Boulder[1].

The coordination of multiple joints within a single limb was examined within the theoretical context of nonlinear dynamical systems. Six human subjects simultaneously flexed and extended the elbow and wrist joints of the right arm in synchrony with a metronome under the following conditions: 1) elbow flexion with wrist flexion and vice versa; and 2) elbow flexion with wrist extension and vice versa. Subjects began each trial with the forearm pronated or supinated under instructions to maintain synchrony among the joints for as long as comfortably possible as metronome frequency increased. With the forearm supinated, transitions from the elbow flexion-wrist extension pattern to the flexion-flexion pattern were observed as frequency of oscillation increased, accompanied by enhancement of fluctuations in the relative phasing between the joints. Fewer transitions in the pronated elbow flexion-wrist flexion pattern occurred, but phase fluctuations were greatly amplified relative to the pronated elbow flexion-wrist extension condition. To assess possible inertial contributions to these transitions, two subjects performed the supinated elbow flexion-wrist flexion and pronated elbow flexion-wrist extension patterns with a 'relaxed' wrist. Resultant kinematic and EMG data suggest that the transitions are neurally based and not due to inertial coupling alone. Switching between behavioral patterns within a single limb (as in multi-limb coordination) can be understood in detail as a nonequilibrium phase transition of the collective variable (relative phase). The stability of elbow and wrist joint patterns is dependent upon the spatial orientation of the forearm, and not upon the underlying muscle pairing across the relevant joints.

Work supported by NIMH Grant MH 42900-01 and ONR Contract N00014-88-J-1191.

243.12

CYCLIC VOLUNTARY MOTION: KINEMATIC SMOOTHNESS. R. N. Stiles. Dept. Physiol. and Biophysics, Univ. Tenn., Memphis, Memphis, TN 38163.

While considered in terms of minimum-jerk, smoothness of rhythmic motion may be better considered in terms of compactness of its spectrum (Bracewell, R., The Fourier Transform and Its Applications. McGraw-Hill, 1965), with a single sinusoid being maximally smooth. On this basis, smoothness of self-paced, cyclic motion was analyzed in the frequency domain for hand, finger, forearm, and jaw. Each subject was asked to oscillate the body part in a smooth fashion without pauses, at a frequency between 1-7 Hz. Acceleration records were digitized at 64/s. For intended frequencies of 1-2 Hz, calculated displacement records appeared maximally smooth, while acceleration spectra indicated higher, usually odd, harmonics of the intended frequency, as well as a tremor. The tremor frequency typically was at, or near, one of the higher odd harmonics. Jaw motion was the least smooth, with several odd (and certain even) harmonics. Higher harmonics reflect biphasic acceleration pulses that followed brief pauses near the peak positions, indicating quick start-ups of this sinusoidal motion following such pauses.

243.13

ORGANIZING PRINCIPLES FOR SINGLE JOINT MOVEMENTS: THE SPEED-INSENSITIVE STRATEGY AS DEFAULT. D. M. Corcos, G.L.Gottlieb, G. C. Agarwal and M.L.Latash. University of Illinois at Chicago, IL 60680 and Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL 60612.

The dual-strategy hypothesis of motor control suggests that certain classes of movement are controlled by regulating the duration of an "excitation pulse" to the motoneuron pool. The defining characteristic of such movements is that the task can be made at any speed and as such are "speed insensitive". Other classes of movements are generated by regulating the intensity of the excitation pulse and are classified as "speed sensitive". Examples of such movements are those that must be generated in a specific movement time. One interpretation of the speed-insensitive strategy is that it is a control strategy that is only employed for movements which are made at maximal performance levels and that duration increases because intensity is at a maximum. Another interpretation is that duration can be regulated across a wide variety of intensity values. To differentiate these two possible interpretations, human subjects made discrete elbow flexions in a horizontal plane over different distances, from a stationary initial position to a visually defined stationary target, 9° wide. We measured joint angle, acceleration and electromyograms from two agonist and two antagonist muscles. Subjects made movements over four different distances following one of four different instructions. The instructions produced a wide range of movement velocities. The initial rise of the inertial torque and consequently of the acceleration, as well as the initial slope of the agonist EMG, were all invariant over changes in the target distance for any single instruction. These results are interpreted to suggest that the speed-insensitive strategy chooses the intensity of motoneuron excitation and uses the duration of excitation as the primary control variable to adjust muscle forces to a level sufficient to accomplish the movement task. The results demonstrate that the uniform intensity of the excitation pulse is not a by-product of moving at maximal speed but is a selected feature of the pattern of movement control. (Supported by NIH grants AR 33189 and NS 23593)

243.14

EFFECT OF LOCALIZED FATIGUE ON COORDINATION PATTERN INVARIANCE AS DEFINED BY THE HOMOGENOUS ELEMENTS MODEL. T. B. Hoshizaki*, V. Vardaxis*, A. Matsuo*, and T. Fukunaga* (SPON:P Gardiner) Biomechanics Laboratory McGill University, Montreal, Quebec, Canada and University of Tokyo, Tokyo, Japan.

Movement pattern and underlying strategies employed for their optimization have been shown to be influenced by muscle properties or neural circuitry (Stein, Oguztoreli & Capaday, Human Muscle Power, 1982). While human movements may be consciously optimized the characteristics of the movements in part reflect the properties of the motors used and their limitations (Brady, Hollerback, Johnson, Lozano-Perez & Mason, MIT Press, 1982). Therefore the purpose of this study was to investigate changes in movement patterns in response to a muscle specific, fatigue stimulus. Five sprinters were required to perform a maximal velocity sprint under the following three conditions control (no fatigue), quadriceps fatigue and hamstring fatigue (60% of max.). Movement patterns were analyzed using inverse Newtonian equations to calculate power flow at the hip and knee joints during the recovery portions of the sprint stride (Robertson & Winter, J. of Biomech. 13: 845-854, 1980). This information was then used to develop a homogenous elements model for identification of the functional elements (Hoshizaki and Vardaxis, Proceedings Canadian Biomech. Society, 1988.) Analysis of the data identified five distinct phases at the hip and seven at the knee joint. The phase components remained invariant when comparing the three levels of the muscle condition supporting the existance of a generalized pattern. However significant differences were observed when the duration of each phase was compared across fatigue conditions.

243.15

HUMAN POSTURAL SYNERGIES: A GEOMETRIC ANALYSIS M.F. Gordon and F.E. Zajac. Rehab. Res. & Dev. Ctr. (153), VA Medical Center, Palo Alto, CA 94304, and Mechanical Engineering Dept., Design Division, Stanford University, Stanford, CA 94305-4021.

It is hypothesized that human upright posture is maintained using combinations of only a few stereotyped responses, called synergies. If the same synergy, appropriately scaled, were used for both strong and weak disturbances, CNS control could be further simplified. Therefore, we hypothesize that synergies activate muscles necessary for maximal acceleration of the body; thus, we propose that sub-maximal and maximal responses activate the same sets of muscles with a similar relative activation pattern. Our computational methodology finds all the maximal-acceleration synergies that correspond to a particular perturbation. We believe the identification of such synergies provides insight into the neural control of posture, and is also useful in the design of functional neuromuscular stimulation systems for paraplegics.

We modeled standing posture with sagittal-plane motion about the ankle and hip joints. For a given postural perturbation, possible body accelerations were points in the hip-ankle acceleration plane. Body acceleration was limited by finite muscle strengths, and by requirements that the feet remain flat and on the ground. When graphed in the hip-ankle plane, the feet constraints defined a V-shaped (open) region, and finite muscle strengths defined a closed polygon. Thus, possible body acceleration were those within the intersection of these regions. All synergies along the boundary of the muscle-strength polygon were identified. For any desired ratio of ankle and hip accelerations, a direction in the hip-ankle plane was specified. Maximal body acceleration and the corresponding synergy could then be determined graphically. Supported by the Veterans Administration and NIH grant NS 177662.

243.16

A SOLUTION FOR THE PROBLEM OF MULTI-JOINT MOVEMENT REDUNDANCY BY MINIMIZING JOINT ANGLE INCREMENTS. S.R.Gulman*, G.L.Gottlieb (SPON: G.Agarwal) Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL 60612

The problem of redundancy of the motor control system requires imposing additional constraints for specifying parameters. Different constraints have been introduced in models of multi-joint movements, explicit (Mussa Ivaldi et al, *Biol. Cyber.* 1988) or implicit (Berkinblit et al, *Biofizika* 1986.). Here we suggest a solution for the redundancy problem by minimizing the sum of the joint angle increments during a movement. This leads to the following expression:

$$dp=J^*(JJ^*)^{-1}dx,$$

where J is a Jacobian matrix of the mapping of parametrical space W (with coordinates p, corresponding to joint angles) into the physical space V (with coordinates x).

In spite of the simplicity of this equation, the simulated behavior of the multi-joint limb demonstrates some features similar to those of real movements. This behavior has been modeled on a computer, using some additional assumptions concerning real movement tactics.

Computer simulation give the following results:

1. Postur and movement are both accompanied by oscillation of the working point resembling the tremor.
2. The working point of the limb can track any trajectory in V with some relative accuracy.
3. Cyclic reproduction of a given trajactory leads to variable actual trajectories in both the V and W with much higher variability in W.
4. Modifying the model parameters leads to movements of different accuracy and speed.
5. Joint fixation may not prevent a task execution although accuracy can suffer.

The study was supported in part by NIH grant AR 33189.

243.17

EXPLOITING DEGREES OF FREEDOM IN A BIARTICULAR PHASE-TRANSITION. P. V. McDonald*, and R. E. A. van Emmerik*. (SPON:E. Donchin). Department of Kinesiology, University of Illinois at Urbana-Champaign, Urbana, IL 61801

A phase-transition was examined occurring in a multi-segmental, bimanual drawing task. Although similar transitions have been modeled in terms of a 2nd order phase-transition (Haken, Kelso & Bunz, 1985) little attention has been paid to the exploitation of available degrees of freedom as the control parameter is varied. Subjects drew circular patterns with both limbs, starting in an out-of-phase mode involving nonhomologous muscle groups (both circles are drawn in the same direction). Increasing the frequency of movement resulted in a dramatic transition to an in-phase mode where one limb changed direction of movement, resulting in homologous muscle action. We report on the relationship between differences in the utilization of available degrees of freedom in the dominant and non-dominant limbs. Specifically, the impact this has on the order parameter (relative phase) dynamics before, during and after the phase transition. These results are considered in light of the physical theory of phase transitions in which common behavioral patterns are observed at many different scales of length as the critical point is approached (Wilson, 1971).

243.18

A MATHEMATICAL SIMULATION OF NEURAL CONTROL IN A MOTONEURON POOL. J.A.Hodgson*, A.Garfinkel*, R.R.Roy, R.G.Gregor and V.R.Edgerton. (SPON: R.F. Zernicke) Department of Kinesiology and Brain Research Institute, UCLA, Los Angeles, CA 90024.

A mathematical model of a motoneuron pool was created to investigate changes of motoneuron activity patterns in response to activity in Renshaw cells and in pathways found to reverse normal recruitment order (e.g. Rubrospinal pathway in cats). The input-output characteristics of the pool were tested by computer simulation. Results show that combinations of activities in several pathways generate a broader range of responses than is possible with activity in a single path. The results indicated mechanisms which may be responsible for the reduction of motor unit firing rate with increased recruitment and changes in the relative activities of slow and fast muscles in movements at different speeds. Renshaw cells may reduce the 'gain' of active neurons as excitation to the pool increases, limiting firing rates of excitable motoneurons by a mechanism intrinsic to the motoneuron pool. Pathways which in isolation reverse the normal order of motoneuron recruitment may be used to adjust threshold and gain of motoneurons, modifying their response to the Size Principle. Such pathways, peripheral or central may be used to 'set up' a motoneuron pool in preparation for activity in addition to modifying responses once activity begins. This provides a mechanism where properties of a motoneuron pool may be modified by other areas of the nervous system in anticipation of an activity rather than relying on mechanisms responding to motoneuron activity after movement has been initiated. SUPPORTED BY NIH GRANT NS 16333.

243.19

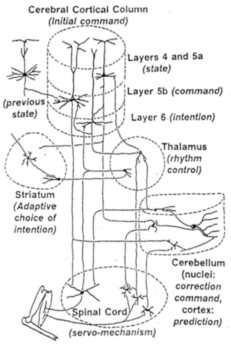
APPLICATION OF THE BACKPROPAGATION MODEL TO THE MUSCLE EMG TO TORQUE RELATION OF THE ANKLE JOINT UNDER ISOMETRIC, SUPINE CONDITION. L.M.Kent[1], A.Guez[2] and W. Freedman*[1,2] (SPON: J.P.Walsh). [1]Dept of Biomedical Engineering and [2]Dept of Electrical Engineering, Drexel University, Philadelphia, PA 19104.

The application of a neural network approach to solution of the muscle EMG -joint torque relation in the ankle joint under isometric, supine conditions is addressed in this study. The backpropagation neural network model (BEP) was used in this analysis. The experiment was performed at the FNS laboratory of the Philadelphia Unit of Shriners Hospitals. A KinCom Robotic Dynamometer, 6 surface electrodes, EMG amplifier system, and MicroVax II minicomputer were utilized. Six sites of muscle activity, monitoring the agonist/antagonist muscle activity about the ankle joint, were recorded on the subject's leg. The BEP was trained by being shown examples of the muscle EMG signals (full-wave rectified, bandpass filtered (100-1000 Hz)) and joint torque at many discrete torque levels (ranging from -1200 (plantarflexion) through +1000 (dorsiflexion) N-m)). The training signal was repeatedly presented to the network until the error between the calculated model torque and measured torque was minimized (<5%). The calculated and desired outputs were normalized between -0.9 and +0.9. The inputs were not scaled and ranged in value from 0 through 8.0 V. The architecture of the BEP, the learning rate and momentum were important parameters for prediction of torque. The relation between the muscle signals and joint torque is embedded within the trained network; i.e., within the hidden units and connections (weights). The muscle signals applied to the trained network may be considered the "intent" of the system, while the joint torque predicted is the "controlled" variable. It is expected that neural network models may be incorporated into controllers of intent recognition systems such as in functional electrical stimulators, myoelectric control of prosthetic devices, and robotic applications.

243.20

SIMULATION OF GOAL-DRIVEN BEHAVIOR LEARNED BY EXPERIENCE IN A NEURAL NETWORK MODELLING CEREBRUM AND CEREBELLUM, **A. de Callataÿ***, *IBM IEC, La Hulpe, B-1310 Belgium*

A computer simulation has shown the feasibility of a robot controller (brain model) learning to reach a target with an android arm. A sensation, e.g. a body position, is neurally *represented* when its activity in a CNS layer is created in another way. This representation is an *intention* (a problem) when the body movements needed to reproduce this sensation can be generated from it. The robot's behavior is controlled by two cognitivist formulas: *1) "stimulus & context → intention."* Any intention can be tried and the best ones are adaptively learned by perceptrons in basal ganglia. *2) "intention → action."* The action solving the goal is deduced from the most similar invariant rules (repeatable events) redundantly stored in the cerebral cortex after each action: *"a sensation S is followed by a sensation I* (a potential intention) *after an interval T (e.g. 260 ms) when a command M is applied."* The initial muscle contraction M is subsequently counteracted by a command of a fast servo-mechanism (located in the cerebellar internal nuclei) to prevent the vertebrate's serious risks of breaking joints or losing equilibrium. To offset the delay of the feedback signal, the cerebellar cortex predicts the limb position, using the rules redundantly stored, when known, after each event: *"when a command C is given with the limb in position A, it goes into a state B after a time V (e.g. 130ms)."* The neural mapping predicts unlikely physiological functions such as *"epilepsy as memory model"* (Goddard, 1969) and dendritic spine resorption (A. de Callataÿ, 1986, *Natural and Artificial Intelligence: Processor Systems Compared to the Human Brain*. North-Holland, Elsevier).



LEARNING AND MEMORY: ANATOMY III

244.1

LESIONS OF THE RAT POSTSUBICULUM IMPAIR PERFORMANCE ON SPATIAL TASKS. J.S. Taube, J.P. Kesslak, and C.W. Cotman. Department of Psychobiology, University of California, Irvine, CA 92717.

Previous studies have identified a population of neurons in the post-subiculum which discharge as a function of the rat's head direction in the horizontal plane, independent of the animal's location and on-going behavior (Taube et al., Neurosci. Abstr. 13:1332). The directional selectivity of these cells suggests that they may be critical components of a neural network mediating spatial abilities. To assess the contribution of these cells to learning and memory, we tested male Long-Evans rats in a variety of spatial and non-spatial tasks following bilateral electrolytic lesions of the postsubiculum.

The performance of animals on an 8 arm radial maze was evaluated on the basis of the percentage of correct arms entered (number of arms correct/total arms entered). Compared to unlesioned control animals, lesioned animals were impaired on the 8 arm maze, although they improved over the course of the experiment. Animals were then evaluated in the Morris water maze task where the platform location was fixed and the animal's entry into the tank was randomly varied over 28 trials. Preliminary results suggest that animals with complete postsubicular lesions showed longer escape latencies compared to controls, although they showed improvement over the course of the experiment. Lesioned animals were unimpaired in a cued version of the water maze task when the platform was marked with a small flag and moved around to different locations. In a conditioned taste aversion paradigm, no difference was observed in the performance of lesioned animals compared to controls. All postsubicular lesioned animals showed significant hyperactivity in an open field apparatus throughout the series of experiments.

These results suggest that the postsubiculum may play a role in behavioral tasks involving the utilization of spatial information.

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244.2

DISSOCIATION BETWEEN THE BEHAVIORAL EFFECTS OF HIPPOCAMPAL- AND CAUDATE-LESIONED RATS TESTED IN A 6-ARM RADIAL TUNNEL MAZE. T. Steckler[1]* and M. Sarter[2]* (SPON: B. Lee).
[1]Dept. Neuropsychopharmacology, Schering AG, P.O.B. 650311, D-1000 Berlin 65, FRG. [2]Dept. Psychology, The Ohio State University, 142 Townshend Hall, 1885 Neil Avenue, USA - Columbus, OH 43210.

In order to test the influence of hippocampal-and caudate-lesions on maze-exploration of rats guided by cue- and praxis-strategies, animals were tested in an automated 6-arm radial tunnel maze which provides neither visible nor auditory extramaze cues. In this maze, each arm is divided into a short blind arm and a long main arm. Rats explored the maze on the basis of spontaneous exploration, i.e., neither positive nor negative reward were provided. Following the lesion, rats were allowed to recover for 2 weeks. Acquisition was tested for 7 days, then the maze configuration was reversed to its mirror image from day to day for 5 days and, following 2 sessions using the same configuration as used during the last reversal session, rats were re-tested in the maze for 3 days after a 7-day break. From each session, several behavioral measures were obtained, i.e., number of blind arm entries, number of repetitive arm entries, locomotor activity, incomplete arm entries. From the beginning of the experiment, rats with hippocampal-lesions showed major behavioral changes in entering blind arms more frequently than sham operated animals. In contrast, caudate-lesioned rats showed no behavioral changes during the acquisition session, and only slight impairments during the reversal and re-test sessions.

The results will be discussed in terms of the ability of lesioned rats to use different strategies to explore a complex spatial task.

244.3

NEUROBIOLOGICAL ANALYSIS OF COGNITIVE MEMORY: ROLE OF THE HIPPOCAMPUS IN SPATIAL AND CUED DISCRIMINATION. S.Golski-Brennan, D.S. Olton, M. Mishkin, J.L. Olds, D.L. McPhie, and D.L. Alkon. Dept. of Psych., Johns Hopkins University, Baltimore, MD 21218.

The functional role of the hippocampus was examined by testing rats in a spatial and cued discrimination with equivalent conditions, except for the relevant discriminative stimuli. In Exp.1, acquisition (ACQ) was tested following control (CON, n=6) operations and complete hippocampal (HPC, n=6) lesions. HPC lesions in both the spatial and cued discrimination impaired ACQ (p's<0.01). During the 15-18th blocks of 12 trials, the mean choice accuracy (and standard error) was: spatial discrimination, CON=11(0.23), HPC=8(0.77); cued discrimination, CON=11(0.35), HPC=7(0.56). In Exp.2, protein kinase C (PKC) distribution was determined by autoradiography of 3 groups of rats (n=4 each group): tested in the spatial discrimination, tested in the cued discrimination, and untested (cage controls). [3H]PDBu binding in the CA1 region was consistent with the lesion data, as well as with the results of previous investigations suggesting that learning alters PKC localization. [3H]PDBu binding was normalized to each rat's global mean. The normalized value (and the standard error) for the CA1 region for each group was: cage=1.63(0.12); spatial discrimination=1.82(0.10); cued discrimination=1.89(0.09). Together, these data suggest that the hippocampus is involved in acquisition of both the cued and the spatial discriminations.

244.4

DISSOCIATION OF PREFRONTAL, POSTERIOR PARIETAL, AND TEMPORAL CORTICAL REGIONS TO SPATIAL NAVIGATION AND RECOGNITION MEMORY IN THE RAT. B. Kolb, K. Buhrman*, and R. McDonald* Dept of Psychology, University of Lethbridge, Lethbridge, Canada, T1K 3M4.

Rats with lesions of the medial prefrontal (Zilles Cg1, Cg3, Fr2), posterior parietal (Zilles anterior Oc2M; Krieg's 7), temporal (Zilles Te2, Te3), or visual (Zilles Oc1) cortex were trained on several learning tests designed to assess: (1) spatial navigation using distal cues; (2) spatial navigation using a landmark cue; (3) egocentric-directed spatial navigation; (4) visual recognition memory; and (5) visual pattern discrimination. Rats with prefrontal lesions were impaired at spatial navigation in all tests, especially the egocentric test, and were impaired at delayed but not immediate recognition memory. The rats with parietal lesions were mildly impaired at the first two spatial navigation tasks but performed normally on the visual discrimination and visual recognition tasks. The rats with temporal lesions were impaired only on recognition memory, even with 0 sec delay. These results: (1) dissociate the prefrontal, parietal and temporal regions; (2) are parallel to those observed in primates; and, (3) in view of the mild deficits from parietal lesions suggest that this area is poorly developed in the rat relative to the primate and may play a minor role in spatial navigation in rodents.

244.5

THE ROLE OF THE HIPPOCAMPUS AND THE MEDIAL PREFRONTAL CORTEX IN THE TEMPORAL CODING OF SPATIAL LOCATION. A. Chiba* and R.P. Kesner(SPON: S. Stensaas). Department of Psychology, University of Utah, Salt Lake City, Utah 84112.

Research suggests that the hippocampus and the medial prefrontal cortex play important roles in memory for the temporal order of events. As an extension of this research, memory for the temporal order of spatial locations across lags was examined.

Rats with medial prefrontal cortex, hippocampal lesions, or sham operated controls were tested on an eight arm radial maze. During the study phase of each trial, animals were allowed to visit each of eight arms once in an order that was randomly selected for that trial. The test phase required the animals to choose which of two arms occurred first in the running sequence of the study phase. The arms to be selected varied according to lag (0-6), or number of items in the running sequence that occurred between the two test arms.

The control animals performed at chance at a lag of zero, but their performance was above chance for the remaining lags, improving as the lag increased. The hippocampal lesioned animals showed a marked deficit, performing at chance for all lags, whereas the medial prefrontal animals showed a less severe deficit. The results of these data support the notion that both the hippocampus and the medial prefrontal cortex play significant roles in the temporal ordering of spatial locations.

244.6

DOES THE HIPPOCAMPUS PLAY A ROLE IN MEDIATING SPATIAL AND TEMPORAL CONFIGURATIONS? P. Jackson-Smith* and R.P. Kesner. Department of Psychology, University of Utah, Salt Lake City, Utah 84112.

Rudy and Sutherland (1988) have shown that the hippocampus is critical for acquisition and retention of configural associations, but not simple discriminations. A configural association is one in which a subject learns to respond to a compound stimulus as a separate entity that is distinct from the compound's components. In their study, the presentation of the compound stimulus (light and tone) confounded spatial (emanated from different spatial locations) and temporal (presented sequentially) components. The present experiment attempted to avoid this confound by using 3-dimensional cues that were presented singly or as a temporal configuration (one stimulus presentation immediately following the other) for the other half of the rats. A food reward was located beneath the stimulus on S+ trials and latency to move the stimulus on S+ and S- trials was measured. When performance on the compound S+ stimulus was significantly better than performance on both S- stimuli, the rats received control or hippocampal lesions. Contrary to Rudy et al.'s study, there was no postoperative change in performance of the hippocampal lesioned rats on the spatial configuration task, and little change in performance on the temporal configuration task.

244.7

HIPPOCAMPAL FORMATION AND CONFIGURAL LEARNING AND MEMORY. R. J. Sutherland, R. J. McDonald*, J. M. Hoesing* and J. W. Rudy. Dept. of Psychology, Univ. of Lethbridge, Lethbridge, Canada, T1K 3M4 and Univ. of Colorado, Boulder, CO 80309.

We have hypothesized that the hippocampal formation (HPC) is necessary for normal performance in memory tasks if, and only if, animals must learn a configural discrimination (Sutherland & Rudy, Psychobiol., 1989). We now present a series of four experiments designed to test and extend this notion. Experiment 1 examined the retention of a preoperatively acquired configural discrimination by rats with bilateral HPC damage induced by intraHPC microinjections of kainic acid + colchicine. The rats were trained in a negative patterning discrimination 4 or 12 wk before receiving HPC damage or sham lesions. Control rats displayed good retention, but HPC damaged rats neither retained nor relearned the discrimination. Experiment 2 compared acquisition of the negative patterning discrimination by rats with either kainate-induced CA field damage or colchicine-induced dentate gyrus damage. The two types of lesion produce similar, severe impairments in acquisition. Experiment 3 compared acquisition of the negative patterning discrimination and acquisition of a context-based, conditioned defecation response by rats with electrolytic amygdala lesions, or kainate+colchicine HPC lesions, or combined lesions. Only the rats with HPC or HPC + amygdala lesions were impaired in both tasks. Rats with amygdala damage exhibited a specific deficit in responding to auditory cues. Experiment 4 demonstrated that HPC damage disrupted retention of a conditional black/white discrimination based upon time of day.

The results extend the empirical support for the idea that the HPC is essential for memory processes underlying configural associations.

244.8

EFFECTS OF NEUROTOXIC LESIONS OF THE HIPPOCAMPUS ON ACQUISITION OF A MODIFIED T-MAZE. D.L.Whittington*, R.H.Baisden and M.L. Woodruff. Dept. of Anatomy, Quillen-Dishner Coll. of Med., East Tennessee State Univ., Johnson City, TN 37614.

The effects of selective destruction of hippocampal subfields on performance in T-mazes requiring trial independent discrimination in the stem and a trial dependent alternation discrimination in the arms were studied. Rats in Group 1 were untreated. Groups 2 and 3 received either an intradentate injection of colchicine (CO) or an intraammonic injecton of kainic acid (KA). Group 4 rats were gavaged with TMT (6 mg/kg). Two identical split-stem T-mazes were placed in the same room with opposite sides of the stem blocked. All rats were given 160 trials of discrimination training. Each trial had a forced run and a choice run into the reinforced arm of one of the mazes. The treated groups were significantly impaired in both the stem and arm components of the task. Rats given TMT were more impaired in the stem choice component than either the CO- or KA-treated rats. Damage to the hippocampus in rats may cause deficits in spatial memory whether working or reference memory is involved. Although CA3a,b, CA1 and the dentate are relatively spared by TMT, it caused a greater defect than KA or CO. This may be due to entorhinal cortex damage produced by TMT. (Supported by NIH grant ES04070-03 to MLW).

244.9

EARLY OLFACTORY LEARNING INCREASES THE NUMBER OF NEURONS WITHIN ENHANCED 2-DG UPTAKE FOCI. C. Woo* and M. Leon. (SPON: D. Aswad) Department of Psychobiology, University of California, Irvine CA 92717

Early odor training given to rat pups induces a behavioral preference and an increased uptake of 14-C 2-deoxyglucose (2-DG) in focal regions of the glomerular layer of their olfactory bulbs. The glomeruli underlying these high uptake foci are larger in conditioned pups than in control pups. To determine whether this increase was in part due to an increased number of neurons, we characterized the distribution of cells within these high uptake foci.

Pups were exposed to either peppermint-scented or clean air with concurrent reinforcing tactile stimulation for 10 min/day on postnatal days 1-18. On day 19 all pups were injected with 2-DG followed by exposure to peppermint odor. Autoradiographs and Nissl-stained sections of olfactory bulbs were aligned and analyzed using a computer-based digital image processor and a light microscope camera lucida.

A 21% increase in total neuronal number was observed in conditioned pups relative to their controls. Since these neurons arborize in nearby glomeruli, increases in cell number could account for the increased area observed within the glomerular layer. The packing density of these cells does not change, but rather, the glomerular layer expands in width to accommodate the increases in cell number. Early olfactory learning in young rats, therefore, induces an increase in neuronal number in responsive regions of their olfactory bulbs.

244.10

HIPPOCAMPUS AND OLFACTION: "WORKING MEMORY" AND PAIRED-ASSOCIATE LEARNING. U.Staubli, T.Le & G.Lynch. Center for the Neurobiology of Learning and Memory, University of California, Irvine, CA 92717.

From a neurobiological perspective, the olfactory system is well suited to study the properties of hippocampal interactions with memory. It has most direct access to the hippocampus of all modalities, and the hippocampus has been implicated in some forms of olfactory discrimination learning (Staubli et al., 1984, 1986; Eichenbaum et al., 1986). Here we present results from studies in which novel forms of olfactory memory were sampled to provide a more complete characterization of the hippocampal role in memory.

In the first experiment, rats were trained in a radial maze to discriminate between several simultaneously present odors, a subset of which denoted food rewards. Bilateral entorhinal lesions did not affect memory of pre-operatively learned positive and negative odors, a result in agreement with earlier findings (Staubli et al., 1986), but dramatically interfered with "working memory", i.e., the ability to finish a trial by choosing each positive odor only once. In contrast, intact animals rarely re-entered positive odor-arms, even in trials with 6 positive odors and a delay interposed between the 3rd and 4th choice, during which the maze was either rotated by 90 degrees or all odors were relocated to different arms. In the second study, the same animals were trained on a paired-associate learning task to test the hypothesis, derived from anatomical arguments (Lynch, 1986), that the hippocampus plays an essential role in the forming of odor-object associations. Each of 6 positive odors was assigned an object that differed in shape and color. To obtain a reward, the animal had to displace the correct one of two objects located at the distal end of the chosen odor-arm. Intact animals learned to displace the correct object to a given odor (4-6 correct decisions per trial), while animals with entorhinal lesions performed at about chance level even after extensive training.

The results demonstrate that rats are capable of a type of paired associate learning and provide evidence that the hippocampus is essential for the formation of memories that place minimal demands on spatial information.

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244.11

HIPPOCAMPAL REPRESENTATION IN VISUALLY-GUIDED DISCRIMINATION LEARNING PARALLELS THAT IN ODOR-GUIDED DISCRIMINATION LEARNING. H.Eichenbaum & K.Russo , Dept. Biol. Sci., Wellesley College, Wellesley MA 02181.

Rats with fornix lesions(FX) are impaired in learning a simultaneous odor discrimination. Even after achieving criterion performance levels, they base performance on an abnormal stimulus sampling strategy and representation of cues (Eichenbaum, etal, Beh. Nsci. 103:331,1988). The present study extends these findings to visually-guided discrimination.

Animals were trained in a cross-maze to approach one of three two-dimensional patterns, each located above the end of a maze arm. The spatial relationships among the cues and reward were constant, but the arms associated with the cues and reward, the maze's orientation within the room, and the start arm varied. As in our odor task, FX rats learned the discrimination slowly; even after reaching criterion performance, they demonstrated abnormal stimulus sampling strategies and stimulus representation as revealed by their performance in probe tests. In probe trials where one cue was deleted, FX rats performed poorly upon removal of the rewarded cue, and were less affected by removal of other cues. In probe trials where cues were transposed, performance deteriorated slightly in both normal and FX rats; furthermore, in normal, but not FX rats, response latencies were substantially increased.

Combined, these findings confirm our results in odor-guided discrimination learning: Normal rats sample each cue individually and guide performance by a representation of the relations among cues, while FX rats acquire approach responses guided by the stimulus compound. This interpretation is compatible with O'Keefe and Nadel's cognitive map theory of hippocampal representation, but applies as well to both spatial and non-spatial discrimination learning.

244.12

SERIAL OLFACTORY DISCRIMINATION LEARNING IN RATS - A 'PRIMATE-LIKE' LEARNING CAPACITY? IC Reid*, RGM Morris* (SPON:Brain Research Association) Dept of Pharmacol. University of Edinburgh, EDINBURGH EH8 9JZ, Scotland.

To investigate claims of a "primate-like" learning capacity in rats trained on tasks using olfactory rather than visual or auditory cues, hooded rats were trained in an automated olfactory maze designed to present simultaneous 2-odour discrimination problems. Three groups were trained on: (1) serial novel discriminations, or (2) serial reversals of a single discrimination, or (3) a single discrimination continuously. Each group was then transferred to a novel discrimination problem and their performance compared.

Prior to transfer, group 1 showed progressive improvement across problems, consistent with apparent "learning set" acquisition. Animals in group 3 eventually performed with greater than 95% accuracy on the single discrimination. Group 2 performed inconsistently, only some subjects showing progressive improvement across reversals. All groups, however, transferred to the novel problem with few errors, and no significant difference was found between groups. The results suggest that exposure to a series of discriminations or reversals is not necessary for the rapid learning of novel discriminations. This in turn suggests that although rats show rapid learning of olfactory discrimination problems, this kind of learning may differ substantially from that seen in analogous primate studies.

244.13

DISSOCIATION BETWEEN COMPONENTS OF SPATIAL MEMORY AFTER IBOTENATE LESIONS OF THE HIPPOCAMPUS. R.G.M.Morris*, F.Schenk*, F.Tweedie* and L.E.Jarrard (SPON: H.E.King) Dept.Pharmacol., Univ.Edinburgh, Edinburgh EH8 9JZ, Universite de Lausanne and Washington and Lee University.

Rats with ibotenate (IBO) lesions of the hippocampus show an impairment in learning a radial-maze but performance reaches control levels if the task is first learned preoperatively (Bouffard and Jarrard, 1988). We have followed up these findings using a spatial water-maze.

Rats were given IBO lesions of the hippocampus (HPC) or subiculum (SUB) but classified as HPC1, SUB or HPC + SUB groups on the basis of histology. These and controls (C) were trained (hidden platform, 28 trials) and then given a transfer test. The C group was superior to the IBO groups on all measures. However, in a 2nd transfer test after a further 48 trials, the HPC1 and SUB groups did not differ from C's in their search bias, while HPC + SUB's remained impaired. The HPC1 group was still impaired in annulus crossings. The C rats were then given an HPC lesion or control surgery and 28 retraining trials. In a final transfer test, performance showed the opposite pattern to the earlier one, ie. HPC2 rats were now as accurate as C2 rats in annulus crossings but impaired in search bias.

These findings may be explained if spatial information is viewed as being stored outside the hippocampus, and if rats without a hippocampus have to learn ad hoc strategies for encoding or utilising spatial information.

244.14

ON THE HIPPOCAMPUS AND LEARNED CONDITIONAL RESPONDING: EFFECTS OF ASPIRATION VS. IBOTENATE LESIONS. L.E. Jarrard and T.L. Davidson*, Dept. Psychol., Washington and Lee Univ., and Dept. Psychol., Virginia Military Institute, Lexington, VA 24450.

Rats with either aspiration or ibotenate (IBO) lesions of the hippocampus, together with controls, were trained on concurrent conditional and nonconditional discriminations similar to those used by Ross et al. (Behav. Neurosci 98:211, 1984). In agreement with the earlier findings, rats with aspiration lesions of hippocampus were impaired in learning the conditional but not the nonconditional discrimination. Rats with IBO hippocampal lesions learned both discriminations as well as controls. Data obtained from transfer trials yielded results compatible with the idea that both IBO hippocampal and control rats solved the conditional discrimination using "if-then" rules.

Since rats that had the hippocampus removed with IBO were similar to controls in learning both conditional and nonconditional discriminations, while rats with aspiration lesions were unable to learn the conditional discrimination, it would appear that added damage to extrahippocampal neural elements and/or the vasculature found in rats with aspiration lesions may account for the differences.

Our results indicate that the hippocampus is not necessary for the acquisition of complex Pavlovian conditional discriminations, and thus do not support the view that the structure plays a crucial role in the control of conditional behaviors.

244.15

HIPPOCAMPAL LESIONS DISRUPT ACQUISITION BUT NOT RETENTION OF NAVIGATIONAL BEHAVIOR IN A HIGHLY FAMILIAR ENVIRONMENT. J.L. Kubie, S. Davvani,* R.J. Sutherland and R.U. Muller*. S.U.N.Y. Health Science Center Brooklyn NY and Univ. of Lethbridge, Alberta, CAN

A recent finding by Sutherland *et al* suggests a synthesis of the apparently disparate effects of hippocampal lesions in rats and primates. Among other results, it was shown that rats trained in the water maze to navigate to a specific location and given hippocampal lesions 14 weeks later have a sparing of this navigational ability (*Soc for Neuro*, 1988). The present study is aimed at replicating this observation. It also asks whether rats that exhibit spared function are capable of learning to navigate to a novel location within the familiar apparatus. The navigation procedure employed here is an appetitive task run in a dry, cylindrical enclosure (Kubie *et al*, *Soc for Neuro.*, 1987).

Twelve rats were trained for 30 trials within a two week interval, each to a particular location. Fourteen weeks later rats were subjected either to bilateral injections of colchicine and kainic acid into the hippocampus and dentate gyrus, or sham injections. After recovery, rats were given a 30-trial block of *familiar-location testing* where a rat was rewarded for digging in the the same location as 16 weeks earlier. A block of *novel-location testing* was then initiated, where the rewarded location for each rat was changed.

Rats with hippocampal lesions were able to quickly and accurately find buried food in the familiar-location test block. The same rats were unable to learn to find food buried in a novel location. Sham rats readily learned to find food in both test blocks. These results indicate that rats with hippocampal lesions can perform a navigational task if it is learned well before surgery, but cannot learn to navigate to new locations, even in a highly familiar environment. (supported by NIH grant RO1-NS20686)

244.16

DEVELOPMENTAL DISSOCIATION OF FACILITATION AND DISAMBIGUATION FUNCTIONS OF CONTEXT M. B. Carew and J. W. Rudy, Dept of Psych, Univ of Colorado, Boulder, CO 80309

Contextual stimuli may influence conditioned behavior in at least two ways (e.g. Bouton and Bolles, 1985). They may serve a **facilitating** function (**FF**) by acquiring excitatory strength through association with the US that can summate with the excitatory strength of phasic CSs also associated with that US, or a **disambiguating** function (**DF**) by specifying the meaning of an otherwise ambiguous CS (e.g. a CS that has undergone acquisition and extinction). Using an appetitive Pavlovian conditioning paradigm, we found that the processes mediating these two functions are dissociated during development. Rat pups 17-20 days old or 20-24 days old were trained using a vibratory CS paired with 2% sugar water delivered through a cheek cannula which elicited a conditioned mouthing response. A **FF** was observed in rats 17-20 days old, however no evidence for a **DF** was found until pups were 20-24 days old. Evidence for a **FF** was obtained by demonstrating that US alone presentations in the training context restored conditioned responding to an extinguished CS. Evidence for a **DF** was obtained by demonstrating that a context shift, concurrent with extinction of responding to a phasic CS, preserved responding to the CS when subsequently tested in the training context.

The comparatively late emergence of the **DF** may be related to the development of the hippocampus; the age at which the **DF** emerges corresponds to the age at which another hippocampal-dependent behavior, spatial learning, emerges. We suggest that the **DF** may be hippocampal-dependent because it may require the rat pup to construct a configural representation of contextual stimuli and phasic CSs, which Sutherland and Rudy (1989) have hypothesized requires a functioning hippocampal formation.

244.17

THE TRANSVERSE PATTERNING PROBLEM, CONFIGURAL PROCESSES AND THE HIPPOCAMPUS. M. C. Alvarado and J. W. Rudy. Psychology Department, University of Colorado, Boulder, CO 80309.

The transverse patterning problem (TPP), (Spence, *Psych. Rev.*, 59, 1952), requires that an animal concurrently solve three visual discrimination problems constructed from only three stimulus cards: [B+ vs W-; W+ vs V-; V+ vs B-; where B,W and V represent a black card, a white card and a card with black, vertical stripes]. An interesting property of the TPP is that it requires a non-linear solution. The animal cannot solve the problem simply on the basis of the excitatory strengths of the individual elements (B,W&V). Rather, solution requires the animal to utilize a configural representation of each stimulus pair. Sutherland and Rudy (*Psychobiol.*, in press) have proposed that a functioning hippocampal formation (HF) is essential to the construction of configural representations. If so, animals with damage to the HF should be unable to solve the TPP. We have studied the TPP in a water-escape version of the Lashley jump stand (Rudy and Castro, *Psychobiol.*,15:1, 1987). We report the following: **A)** Long-Evans rats solve the TPP when trained over three successive phases: Phase I: B+ vs W-; Phase II: Concurrent training on two problems, B+ vs W- and W+ vs V- ; Phase III: Concurrent training on three problems, B+ vs W-/ W+ vs V- and V+ vs B-. **B)** Normal animals begin to employ a configural strategy in Phase II, even though an elementistic strategy, in principle, will allow solution of two concurrent discriminations. **C)** Consistent with Sutherland and Rudy's hypothesis, preliminary data suggest that animals with damage to the HF are unable to solve the TPP.

244.18

HIPPOCAMPAL DAMAGE IMPAIRS RETENTION OF A CONFIGURAL CHOICE DISCRIMINATION. J. W. Rudy and R. J. Sutherland. Departments. of Psychology, University of Colorado, Boulder 80309 and University of Lethbridge, Lethbridge, Alberta, Canada T1K-3M4

We have proposed that the hippocampal formation makes a contribution to learning and memory by providing circuitry for the construction of new functional units or configurations that represent the joint occurrence of two or more elementary stimulus events (Psychobiol. in press). In support of our position, we have reported that rats cannot solve or retain a go/no go negative patterning discrimination (Behav. Br. Res. in press). In this problem the animal was reinforced for bar pressing when either a tone or light alone was presented and not rewarded whenever the tone and light were presented in compound (T+, L+ vs TL-). Although this problem requires a functioning configural representation system for its solution, it also requires the animal to withhold responding to the compound stimulus. Consequently, impaired performance may reflect a general inability of the animal to withhold responding rather than an inability to construct a configural representation of the compound. In order to address the response inhibition interpretation, we trained animals to solve a novel stimulus-patterning choice discrimination problem that requires a configural representation system for its solution but does not require the animal to withhold responding to either the compound or its elements. Specifically, animals were trained to bar press (BP) in the presence of either a light or tone alone but to chain pull (CP) when the compound stimulus was presented (T->BP, L->BP vs TL->CP). Subsequent damage to the hippocampus severely impaired performance on this problem, a result that supports the configural process view of hippocampal function.

244.19

ATTENTION TO NONSPATIAL VS. SPATIAL CUES FOLLOWING DORSAL NORADRENERGIC BUNDLE (DNAB) LESIONS. D.A. Warren*, C.A. Castro, L.R. Watkins, M. Fleshner*, W.W. Woodmansee & S.F. Maier. Department of Psychology, University of Colorado, Boulder, CO 80309.

The DNAB, which projects from locus coeruleus to diverse forebrain areas, has been implicated in attention. We used the Morris water maze (Morris, 1981) to test if DNAB lesions would influence attention to spatial and non-spatial cues. Either the neurotoxin 6-hydroxydopamine (6OHDA, 12 ug per site) or equivolume (1 ul) 0.1% ascorbic acid-saline vehicle was injected bilaterally into DNAB or ventral NAB (VNAB) (specificity of neurochemical disruption to be determined by HPLC). Animals were trained 1-2 wk later to swim to a visible platform (nonspatial cue) located in the same quadrant of the circular maze on each trial. A probe trial, in which the platform was removed, was then conducted. Animals given vehicle injections into DNAB or VNAB and those given 6OHDA into VNAB spent more time in the training quadrant than in nontraining quadrants, indicating the learning and use of spatial cues. Rats given 6OHDA into DNAB spent equivalent time in training and nontraining quadrants, indicating failure to use spatial cues. Later tests showed that DNAB lesioned rats were not impaired in learning to swim to a submerged invisible platform. Thus, DNAB lesions do not impair spatial navigation, but produce a bias toward attending to nonspatial cues. Supported by NSF grant BNS-8809527.

244.20

APPARATUS CONFIGURATION, CIRCADIAN EFFECTS AND HIPPOCAMPAL ANATOMY IN GENETICALLY DEFINED MICE. D. F. Peeler, Neurosurgery, Univ. Mississippi Med. Ctr., Jackson, MS 39216

It has been reported that the extent of mossy fiber innervation of pyramidal cells and shuttle-box performance are negatively correlated, and that the former is genetically determined. Both shuttle-box performance and circadian effects have been demonstrated to reflect genetic determinants. Seven recombinant inbred strains and their progenitors (C57BL/6ByJ & BALB/cByJ) which have a genetically determined difference in hippocampal mossy fiber distribution, were used to further examine these variables as a function of apparatus constraints. Male mice (Mus musculus) of the 2 progenitor and 7 recombinant strains were tested in 1 of 2 apparatus configurations (different barriers) at 1 of 3 times of day. Six mice were in each group. Strain was a strong and pervasive effect under all conditions, but the third-order interaction was significant. Number of avoidance responses and rate of acquisition changed with strain, time of day and type of barrier. The data support findings of genetic determinants for shuttle-box performance and circadian effects, and indicate that mode of acquistion and coping with environmental constraints are also subject to major genetic influence. Avoidance performance, circadian effects and mode of acquisition do not share genetic determinants with the mossy fiber distribution in the hippocampus, nor are they related in any simple fashion with one another.

244.21

LONG-TERM POTENTIATION ALTERS GLIAL PROCESSES IN THE DENTATE GYRUS. K. R. Isaacs, A.Marks*, A. M. Sirevaag, F.-L. Chang, W. Greenough. Neural & Behav. Biol. Prog., Depts. of Psych. & Cell & Struct. Biol., U. Illinois, Champaign IL 61820.

Ten adult rats received long-term potentiation (LTP) or equivalent low frequency control stimulation 30-70 days after the implantation of electrodes into the dentate hilus (recording) and angular bundle (stim.). 24 hours after the last LTP train the rats were perfused and 10um frozen sections were processed for immunocytochemistry using monoclonal anti-GFAP (ICN). A stereologically unbiased estimate of astrocyte surface density (Sv; Braendgaard and Gundersen, 1986) was calculated from astroycte ramifications in the molecular layer of the upper blade of the dentate gyrus. Tissue was sampled from 100 to 400um distal to the recording electrode to monitor effects due to gliosis and treatment. Gliosis was evident at 100um but not at 400um from the electrode, and at 400um LTP astrocytic Sv was approximately 7% greater than the control group Sv. In further analyses the LTP group had thicker astrocytic processes than the control group. (Supported by NIMH 35321 and ONR N00014-89-J-1556).

244.22

RATS IN COMPLEX, SOCIAL AND INDIVIDUAL ENVIRONMENTS EXHIBIT ASTROCYTIC PLASTICITY AFTER 30 AND 60 DAYS OF ENVIRONMENTAL EXPOSURE. A M. Sirevaag, O. Bell, and W.T Greenough. Depts., Psych & Cell & Struct. Biol., & Neur. & Beh. Biol. Prog., Univ. IL, Champaign, IL 61820.

In the upper half of the occipital cortex rats raised in complex environments (EC) have larger neurons with more synapses than rats raised in social (SC) or individual cage (IC) environments. Astrocytes are indicators of cellular metabolic and ionic environments and may also exhibit plasticity. Preliminary examinations of astrocytes labeled with anti-GFAP showed that after 30 days of EC, astrocytes had a greater surface density (Sv) but lower numerical density (Nv) than ICs. Recent examinations of anti-GFAP labeled astrocytes in young adult rats exposed to their environments for 10, 30 or 60 days have shown that the duration of the animal's exposure to its environment determines astrocytic response. After 10 days of environmental exposure EC and IC rats did not differ on Sv, or mean astrocytic size but they had 14% lower Nv than ICs. An estimate of the total number of astrocytes within Area 17 (N) showed no difference between EC and ICs. After 30 days of exposure EC rats had a 16% greater Sv, 40% greater mean astrocytic size and 20% lower Nv than ICs. The estimate of N within Area 17 showed no difference between EC and ICs. After 60 of environmental exposure EC rats had a 10% greater Sv than ICs. There was no difference in mean astrocytic size and Nv between ECs and ICs. However, the estimate of N within Area 17 showed that ECs had 16% more astrocytes than IC rats and that gliogenesis had probably occurred. Thus, astrocytes do not show plasticity after only 10 days of exposure to a complex environment and the initial response of astrocytes after 30 days of exposure appears to be an increase in astrocytic processes. However, after 60 days of exposure astrocytes appear to have proliferated. Supported by MH 35321.

244.23

ASTROCYTE HYPERTROPHY IN DENTATE GYRUS OF YOUNG MALE RATS IS MODULATED BY INDIVIDUAL STRESS RATHER THAN COMPLEX EXPERIENCE. J.E. Black, A.M. Sirevaag, A.C. Katz*, & W.T. Greenough. College of Medicine, Depts of Psychology, and Cell & Structural Biology, Neural & Behavioral Biology, Beckman Institute, Univ of Illinois, Champaign, IL 61820.

Glial hypertrophy has been associated with stress-induced damage in the hippocampus and with synaptogenesis in visual cortex of male rats given complex experience. This study examines GFAP immunoreactive astrocytes in a brain region where the synaptogenesis response to complex experience in male rats is minimal and under conditions of minimal environmental stress.

The 21 weanling male rats used in this study (also used in Sirevaag, et al, adjacent) were placed in three conditions for 30 days: a large cage with many toys and other rats to interact with (EC), and standard caging with rats kept in pairs (SC) or individually (IC). Astrocyte surface densities (Sv) in the upper and lower blades of dentate gyrus were estimated by counting the intersections of GFAP processes with a cycloid grid. No group differences were found for adrenal weight or Sv of upper or lower blades. Upper blade Sv and adrenal weight were positively correlated ($r=.54$, $p<.02$).

This study indirectly supports the idea that astrocyte hypertrophy is associated with synaptogenesis, for in this case of the hippocampus the glial effect is absent where the synaptogenesis effect is absent. The role of astrocytes in stress- induced damage is also supported by this study. The lack of group differences in adrenal weight and Sv suggests that the groups experienced similar levels of stress. However, adrenal weight was closely related to upper blade Sv, suggesting that within this paradigm an individual rat's experience of stress may have contributed to hippocampal damage.

Supported by MH 35321.

CEREBELLUM III

245.1

IDENTIFICATION OF A, X AND B CORTICAL ZONES AND WHITE MATTER COMPARTMENTS IN THE ANTERIOR VERMIS OF THE CEREBELLUM OF THE MONKEY (*Macacca Fascicularis*). J. Voogd* and D.T. Hess*. (SPON: D. Schreyer) Dept. of Anatomy, Erasmus Univ. Rotterdam, 3000 DR Rotterdam, Netherlands (JV) and Dept. of Neurobiology, Stanford Univ. Sch. Med., Stanford, CA 94305 (DTH).

In the cat, the cortex of the anterior vermis of the cerebellum has been divided on the basis of its afferent climbing fiber (CF) and efferent Purkinje cell (PC) connections into a medial A zone (CF: from caudal medial accessory olive [MAO]; PC: to fastigial and medial vestibular nuclei), an intervening X zone present only in lobules IV-VI (CF: from transition between caudal and rostral MAO; PC: to junction of fastigial and posterior interposed nuclei) and a lateral B zone (CF: from caudal dorsal accessory olive [DAO]; PC: to Deiter's nucleus). In the monkey, acetylcholinesterase (AChE) histochemistry reveals a compartmentalization of the cerebellar white matter (Brain Res., *369*:385, 1986). In *Macacca fascicularis,* injections of WGA-HRP into the vestibular nuclei resulted in retrograde labeling of PCs and their axons in AChE-identified A and B cortical zones and white matter compartments, sparing the X zone and compartment. After injection of ^{3}H-leucine into caudal MAO and DAO, labeled olivocerebellar fibers were confined to the A and B zones and compartments, while the X zone and compartment were labeled by injections of the rostral MAO. These observations allow the identification of A, X and B white matter compartments and cortical zones in the monkey, and demonstrate the precise correspondence between the modular organization of the primate cerebellum as revealed by afferent and efferent connectivity and by AChE histochemistry.

245.2

ELECTRON MICROSCOPIC AND IMMUNOCHEMICAL STUDIES CONCERNING THE INTRINSIC SYNAPTIC CIRCUITRY OF THE MONKEY BASILAR PONS. G.A. Mihailoff and B.G. Border. Dept. of Cell Biology and Anatomy, Southwestern Medical School, Dallas TX 75235.

Two important questions regarding the synaptic circuitry of the basilar pontine nuclei (BPN) are: (1) do the axons of pontocerebellar projection neurons give rise to collateral branches within the pontine gray and (2) are BPN interneurons present and if so, how do they participate in the synaptic circuits of the pontine gray? Regarding the interneuron, recent studies in the rat, cat, and baboon have documented the presence of GABA-immunoreactive neurons and axon terminals within the BPN. Additionally in the rat, double-labeling studies have shown that certain BPN afferent projections contain GABA-ergic components thus indicating that some GABA boutons in the BPN must originate from afferent axons while others presumably arise from the population of BPN GABA neurons. Using a rabbit anti-GABA antibody and the post-embedding immunogold method, we report here the presence of GABA boutons within the monkey BPN. Such boutons participate in serial synaptic complexes where they are postsynaptic to a round vesicle-containing bouton and presynaptic to a dendritic element. GABA boutons also form non-glomerular axodendritic synapses. The involvement of GABA boutons in serial synaptic arrangements is consistent with the organization of other brain regions such as the dorsal lateral geniculate and supports the suggestion that such boutons might arise from a GABA interneuron. Concerning projection neuron collaterals, in control BPN neuropil, certain profiles including dendrites and synaptic boutons are readily distinguished in electron micrographs by their content of a dense background matrix. Such profiles do not exhibit GABA labelling and are tentatively identified as a class of BPN projection neurons that gives rise to intrapontine collaterals. Supported by NIH NS-12644.

245.3

DEVELOPMENT OF GLUTAMATE IMMUNOREACTIVITY IN THE RAT BASILAR PONS: LIGHT AND ELECTRON MICROSCOPIC IMMUNOCHEMISTRY B.G. Border and G.A. Mihailoff. Dept. of Cell Biology, Univ. of Texas Southwestern Medical Center, Dallas, TX 75235.

The postnatal development of glutaminergic neural elements was studied in the rat basilar pontine nuclei (BPN) using a hemocyanin-conjugated glutamate (GLU) antiserum. Light microscopic immunoperoxidase techniques were applied to perfusion-fixed neonatal rat brains ranging from a few hours (PN 0) to 30 days (PN 30) of age, while post-embedding immunogold procedures were utilized to visualize the ultrastructural details of glutamate immunoreactive (GLU-IR) structures. Basilar pontine neuronal somata, fibers and axon terminals first exhibited GLU-immunoreactivity at PN 3, and the relative numbers of GLU-IR structures gradually increased with age with an adult-like pattern of immunostaining achieved by PN 18. Electron microscopic observations revealed specific, albeit light, immunogold labeling over neuronal somata of the PN 0 BPN, while GLU-IR vesicle-containing profiles were first observed at PN 3. BY PN 5, neuronal somata, dendrites, and myelinated axons were immunogold labeled. In addition at this stage, immunolabeled presynaptic boutons formed clearly defined asymmetric membrane specializations with both GLU-IR dendrites and neuronal somata. The sequence of events which characterizes the initial appearance and subsequent proliferation of GLU-IR structures within the BPN coincides with the ingrowth and maturation of cortical and cerebellar afferent projections as well as a time when substantial dendritic growth, synaptogenesis and dendritic spine elimination take place in the BPN.

Supported by NIH NS-12644

245.4

HIGH TIME RESOLUTION CALCIUM IMAGING OF GUINEA PIG PURKINJE CELL OSCILLATIONS IN VITRO. H. Miyakawa, V. Lev-Ram, N. Lasser-Ross and W.N. Ross. Dept. of Physiology, N.Y. Medical College, Valhalla, NY 10595.

The correlation of intracellular calcium changes with somatically recorded electrical activity during spontaneous oscillations was measured simultaneously from many locations in Purkinje cells using a photodiode array and the calcium indicator fura-2. Beginning with the first complex action potential calcium increased simultaneously all over the cell. Immediately following the end of the burst calcium began to decline at all locations, usually levelling off by the end of the 10-15 second interburst interval. However, the time courses of the calcium changes at different cellular locations were very different. In the fine dendrites calcium peaked within one second, remained almost constant during the rest of the burst, and declined rapidly at the end. In the soma calcium increased slowly all during the burst, declined slowly during the silent period, and the peak level was much lower than over the fine dendrites. The time course and the peak level of calcium over the mid-dendritic regions of the cell were intermediate between these extremes. In many cells the burst of complex spikes was preceded by a shortlasting plateau supporting fast sodium spikes. This plateau was eliminated by TTX and there was very little calcium change associated with this event. These results show that at all locations the calcium increase during oscillations is dominated by the contribution due to calcium spikes.

Supported by the NIH, NSF and the Whitaker Foundation.

245.5

MODIFICATIONS OF PARALLEL FIBER-PURKINJE CELL SPATIAL ACTIVITY IN THE RAT CEREBELLAR CORTEX, IN VIVO.
S.A. Elias, H. Yae*, T.J. Ebner. Depts. Neurosurgery and Physiology, Neuroscience Grad. Prog., Univ. of Minnesota, Mpls., MN 55455.

Electrical stimulation of the molecular layer of the cerebellar cortex activates parallel fibers which in turn excite Purkinje cells. The pattern of activity has been shown to be beam-like in a transverse direction by both electrophysiological recording and imaging with voltage sensitive dyes. Using the latter approach we examined the spatial pattern after neuropharmacological modification. The cerebellar cortex of anesthetized rats was stained with RH795. Optical images were recorded using an epi-fluorescence microscope and a CCD video camera. Following filtering and windowing the averaged (up to 600 times) control and stimulation images were subtracted. When Ca^{2+} free Ringers was perfused across the cerebellar surface (0.5 ml/min), the synaptically generated field potentials disappeared, leaving the pre-synaptic volley. The imaged beam was also reduced in intensity and spread, but recovered partially with normal Ringers, and increased in magnitude with Ringers containing 40 uM picrotoxin. The recorded field potentials followed similarly. These experiments show that optical recording can follow spatial pattern changes in parallel fiber-Purkinje cell excitability. Supported by NIH grants NS-18338 and NS-27210.

245.6

ACTIVITY PATTERNS IN THE CEREBELLORUBROSPINAL PATHWAY OF THE *IN VITRO* TURTLE HINDBRAIN REVEALED WITH ACTIVITY-DEPENDENT UPTAKE OF FLUORESCENT DYE.
J.C. Houk, J. Keifer and D. Vyas. Dept. of Physiology, Northwestern University Medical School, Chicago, IL 60611.

The cerebellorubrospinal circuit is believed to include recurrent excitatory connections between the cerebellum, red nucleus and medullary reticular formation. Burst activity in this positive feedback pathway is postulated to be important in the production of central motor programs. Recently we developed an *in vitro* turtle brainstem-cerebellum preparation in order to study bursting (Keifer & Houk, Neurosci. Lett., 97: 123, 1989). Here we describe the use of soluble, small molecular weight fluorescent probes to demonstrate loop activity. These probes, introduced by Lichtman et al. (Nature, 314: 357, 1985), appear to be internalized exclusively by active neurons.

The isolated brainstem-cerebellum was placed in a recording chamber and the cerebellorubrospinal pathway was activated by single pulse stimulation of the contralateral dorsolateral spinal cord. The preparation was bathed in a 0.1% solution of sulforhodamine 101 (Molecular Probes) during stimulation (4 mA, 0.1 ms, 0.5 Hz) for 1 hr. Stimulation was discontinued, the preparation was washed with physiological saline for 40 min, and the tissue was then immersion fixed in 4% paraformaldehyde. Well labeled cell bodies and proximal dendrites were observed in the contralateral red nucleus, ipsilateral lateral cerebellar nucleus, magnocellular reticular formation, nucleus of flm, raphe, perihypoglossal nucleus, and cerebellar Purkinje cells. When the bathing solution containing the dye was changed to low Ca-high Mg, which blocks synaptic activation, no label was seen.

The use of fluorescent probes to identify functionally active regions in the *in vitro* preparation should facilitate our electrophysiological studies of the cerebellorubrospinal pathway of the turtle. Furthermore, we are presently exploring the use of sequential application of different colored dyes to examine topography of this circuit. These preliminary results suggest that positive feedback in the rubro-reticulo-cerebellar loop may contribute to the production of central motor programs.

245.7

CORRELATION OF LOCAL CYTOSOLIC FREE CALCIUM DYNAMICS WITH ELECTRICAL ACTVITY OF PURKINJE CELLS IN CEREBELLAR SLICE CULTURES
T. Knöpfel and B.H. Gähwiler. Brain Research Institute, University of Zürich, CH-8029 Zürich (Switzerland)

Monolayered slice cultures grown on glass coverslips offer an unique opportunity to apply optical recording techniques to organotypically organized neuronal networks.

Fura-2 was injected through the recording electrodes into cerebellar Purkinje cells in slice cultures. Optical mapping was achieved using a combination of an 10 x 10 array of photodiodes and a charge coupled device. Electrical activity and intracellular free Ca^{2+} ($[Ca^{2+}]_i$) were simultaneously recorded by means of single electrode current-/voltage-clamp and microfluorometric techniques.

Bursts of action potentials induced by intracellular current injections, evoked synaptic activity as well as spontaneous activity were all paralleled by a transient rise in $[Ca^{2+}]_i$, which was most pronounced in dendritic regions.

These results demonstrate a dendritic segregation of Ca^{2+} conductances in slice cultured Purkinje cells as has been previously demonstrated in acute slice preparations. In more general terms, measurements of local $[Ca^{2+}]_i$ dynamics, in particular in combination with multi-site recordings of membrane potentials using voltage sensitive dyes appear to provide a promising approach for studying dendritic information processing.

245.8

PHARMACOLOGICAL CHARACTERIZATION OF EXCITATORY POSTSYNAPTIC POTENTIALS OF PURKINJE CELLS IN ORGANOTYPIC CO-CULTURES OF CEREBELLUM AND INFERIOR OLIVE.
E. Audinat, T. Knöpfel and B.H. Gähwiler; Brain Research Institute, University of Zürich, CH-8029 Zürich (Switzerland)

Both the climbing fiber and the parallel fiber inputs to cerebellar Purkinje cells (PCs) have been proposed to use an excitatory amino acid (EAA) as neurotransmitter. We have, therefore, used specific EAA receptor antagonists to compare the pharmacology of excitatory postsynaptic potentials (EPSPs) with that of EAA induced responses of PCs. Slices from cerebellum and inferior olive (IO) were obtained from newborn rats and co-cultured for 2-4 weeks. PCs were then current- or voltage-clamped at a potential of -55 to -65 mV. Postsynaptic potentials were evoked by field stimulation applied either within the cerebellar tissue or within the IO. Individual PCs were identified by their morphology, their location within the cultured slices, calbindin immunostaining and by intracellular injection of Lucifer yellow. In the presence of 1 µM TTX, inward currents were induced in PCs by L-Glutamate (500 µM), L-Aspartate (500 µM) and L-Homocysteate (500 uM), but not by NMDA (50 µM to 1 mM). These agonist-currents, the locally evoked EPSPs and the EPSPs evoked by IO-stimulation were all reversibly antagonized by CNQX (10 µM). D-APV (10 µM) had no affect on any of these responses.

These observations demonstrate that in mature PCs which apparently do not respond to NMDA, EPSPs and responses induced by EAA are mediated by non-NMDA EAA receptors.

245.9

VARIABILITY IN AND COMPARISONS OF: 1) TACTILE PROJECTIONS TO THE GRANULE CELL LAYERS OF CEREBELLAR CORTEX; AND 2) THE SPATIAL DISTRIBUTION OF ZEBRIN-I LABELED PURKINJE CELLS.
Giri Gundappa-Sulur*, Hadi Shojaeian, Mike Paulin, Leila Posakony*, Richard Hawkes(t) and James M. Bower. Div. Biol. 216-76, Caltech, Pasadena, CA. 91125 (t-Centre Res. Neurobiol., Quebec, CA. G1J 124).

Tactile responses in the granule cell layers of the cerebellar hemispheres of rats and mice are topographically arranged in patches (Shambes et al., Brain Behav. Evol. 15, 94-140, 1978). Further, casual observation suggests that specific patches recur in similar folial positions in different individuals. We have modified a statistical sequence comparison algorithm used in molecular biology, to demonstrate quantitative similarities between maps found in several folia in both rats and mice.

Certain monoclonal antibodies also display consistent patterns of labeling in the cerebellum. We were interested in comparing one of these, Zebrin-I which selectively labels bands of Purkinje cells (Hawkes et al., Brain Res., 333: 359, 1985) to the underlying tactile maps. To do so, we first electrophysiologically determined and anatomically marked tactile patch boundaries. Then, the brain tissue was sectioned and processed immunocytochemically with the positions of stained Purkinje cells eventually being reconstructed using a computer-linked microscope.

Results showed that the pattern of monoclonal staining in the cortex is highly reproducible from animal to animal. Second, superimposition of this anatomical pattern on the tactile maps revealed that some physiological patch boundaries coincided quite precisely with boundaries between regions of labeled and unlabeled Purkinje cells. In other cases, however, several transitions from labeled to unlabeled regions occurred in Purkinje cells overlying single patches. The significance of this result must await a better understanding of the function of the protein labeled by the antibody. (supported by NIH grant NS- 222205).

245.10

FUNCTIONAL PROPERTIES OF DORSAL HORN NEURONS THAT PROJECT TO THE DORSAL ACCESSORY OLIVE.
H.H. Molinari, N. El-Yassir*, and J.O. Dostrovsky. Department of Anatomy, Albany Medical College, Albany, NY 12208 and Department of Physiology, University of Toronto, Toronto, Ontario, Canada M5S 1A8.

The major sources of input from the hindlimb to the rostral dorsal accessory subdivision of the inferior olive (DAO) are dorsal horn lamina V, the ventromedial ventral horn, and the gracile nucleus. This study investigated the physiological properties of neurons in the dorsal horn that project to DAO. Single neurons in cats anesthetized with pentobarbitone were antidromically activated by microstimulation of contralateral DAO.

All of the neurons examined were excited by some form of peripheral stimulation; 87% responded to cutaneous stimuli. About one third could be excited by light cutaneous stimuli, such as air puffs or von Frey hairs less than 0.3 grams. Half required moderate stimuli such as tap or brisk brush. The receptive fields were located on the foot and/or toes; many displayed gradients of sensitivity. Conduction velocities from the dorsal horn to DAO averaged 26.7 m/s.

The population of DAO projection neurons in the dorsal horn was much more sensitive to cutaneous stimulation than that in the gracile nucleus (studied with the same techniques by Molinari and Dostrovsky, 1987). Thus, the cutaneous sensitivity of neurons in DAO (Gellman et al., 1983) may largely reflect the influence of inputs from the dorsal horn.

Supported by NSF grant BNS-8809840 and Canadian MRC.

245.11

SOMATOSENSORY MAPPING OF ANTIDROMICALLY IDENTIFIED PURKINJE CELLS IN LOBULES V AND VI IN CAT CEREBELLUM. J.F. Brons, L.T. Robertson, G. Tong*. Dept. Anatomy,SD, Oregon Health Sciences Univ., Portland, OR 97201

The projections of somatosensory fields to lateral cerebellar nucleus in cats were determined for a population of Purkinje cells in the lateral areas of lobules V and VI. Under Nembutal anesthesia, the receptive fields of Purkinje cells were determined by extracellular unit reponses to mechanical stimulation of the body surface. The cells were antidromically activated by bipolar stimulation of the lateral cerebellar nucleus. Of 330 cells, 70% in lob V and 51% in lob VI responded to tactile input; the rest were unresponsive. Cells in both lobules responded primarily to facial or forelimb input with 10% responding to both. 63% of all responsive cells could be antidromically activated. Local areas within the lateral nucleus received input from relatively well circumscribed areas of cerebellar cortex, but were not specific to body area represented. These results indicate that somatosensory input projects to the most lateral areas of deep cerebellar nuclei.

245.12

VAGAL REPRESENTATION BY THE CLIMBING AND MOSSY FIBER SYSTEMS IN THE CEREBELLUM OF THE CAT. G. Tong*, L.T. Robertson, J.F. Brons. Dept. Anatomy, SD, Oregon Health Sciences Univ., Portland, OR 97201

The role of autonomic input to cerebellum and its relation to somatosensory input is not well understood. This study investigates the organization of vagal input in the somatosensory region of lobules V and VI in cat cerebellum. The distribution of convergence of vagal and somatosensory input by the mossy and climbing fiber systems was examined. Using alpha chloralose (100 mg/kg) anesthesia, extracellular activity of single Purkinje cells in the vermal and intermediate zones of the lobules V and VI were recorded with microelectrode. The ipsilateral cervical vagus nerve was stimulated with bipolar electrodes. The somatosensory receptive fields were defined with hand delivered stimuli. About 55% of the 155 isolated Purkinje cells were activated by mossy and/or climbing fibers to low (A-fibers) or high-threshold (C-fibers) vagal stimulation. All of these units also receive convergent input from the body surface, mainly from the face or forelimb.

245.13

RESPONSES OF PURKINJE CELLS IN ANTERIOR AND POSTERIOR CEREBELLAR LOBULES TO HINDLIMB ROTATION. V. Perciavalle*, R. Poppele, L. Shen* Deptartment of Physiology, Univ. of Minnesota, Minneapolis MN 55455

Spinocerebellar pathways from hindlimb areas project separately to the anterior and posterior lobes of the cerebellum. We investigated these projections areas by recording the responses of Purkinje cells to small rotations of the foot about the ankle joint (5^{o}- 8^{o} in extension or flexion). Recordings were made from 120 Purkinje cells in pentobarbital anesthetized cats. Post-stimulus spike densities showed significant changes with respect to background discharge in 64% of the cells recorded both anterior and posterior. The highest percentages of responses were found in anterior lobules II and III and posterior lobules VIIIa and VIIIb. Responses were mostly transient increases in spike density although decreases were also observed, alone or in combination with excitation. Anterior lobe responses had a relatively short latency to peak increase in density (25 - 40 ms) and responses of a given cell to extension and flexion were usually different. Posterior lobe responses were generally larger, had a much longer latency (60 - 85 ms) and the responses to flexion and extension were usually the same. Differences among responses were also noted for cells located either medially or laterally in the paravermal areas in both the anterior and posterior areas.

Supported by NIH grant NS 21143.

245.14

CHANGES IN SIMPLE AND COMPLEX SPIKE ACTIVITY IN PURKINJE CELLS INDUCED BY SLIP OF AN OBJECT HELD BETWEEN THE THUMB AND FOREFINGER. Claude Dugas, Nathalie Picard*, & Allan M. Smith Centre de recherche en sciences neurologiques, Univ. de Montréal, Québec, Canada

Two fascicularis monkeys were trained to grasp, lift and hold an object within a narrow position window for 1.0 sec. On certain trial-blocks a predictable perturbation was introduced during the static holding which caused the object to slip between the fingers. The perturbation produced a reflex-like increase in grip force at a latency between 60 and 100 ms. In addition, anticipatory responses, characterized by gradual increases in the grip safety margin (defined as an increase in the grip force/load force ratio) and increases in the rate of grip force application, appeared increasingly within blocks of consistently perturbed trials. Together, these anticipatory and compensatory adjustments to grip force dynamics allowed the animal to reliably overcome the effects of the perturbation.

In the paravermal hand area of the cerebellar anterior lobe, twenty seven Purkinje cells (PCs), identified by their climbing fiber discharge (CF), demonstrated activity changes related to the grasping task. In 19 PCs, simple spike (SS) responses to the perturbation occurred at a latency of 37 (± 7 ms). Of these 19 PCs, 7 demonstrated an additional activation of the CF discharge. Adaptive changes in SS discharge correlated with the anticipatory responses were observed in five PCs only three of which also responded to the perturbation. Fifty of 78 non-identified cerebellar neurons responded to the perturbation and 17 cells demonstrated adaptive anticipatory changes in activity. The short latency reflex-like responses to the perturbations were locked to the stimulus and did not persist in the absence of the perturbation. In contrast, the adaptive anticipatory changes carried on for 5 or 10 trials after the cessation of the perturbation. These adaptive changes in cerebellar activity appear to be related to the alterations in the motor strategy which is continually revised as a function of both the expectancies, and real stimulus conditions encountered by the animal. Supported by the MRC and NSERC of Canada, and le Fonds FCAR du Québec.

245.15

DIRECTION TUNING OF FLOCCULAR PURKINJE CELLS IN MONKEY. R.J. Krauzlis* and S.G. Lisberger. Dept. of Physiology, Neurosci. Graduate Prog., Univ. of California, San Francisco, CA 94143

Visual tracking of moving targets requires a change in coordinate systems. All directions of motion are represented in visual areas specialized for processing of visual motion, yet the motor commands must ultimately pull the eyes in the directions of the six pairs of extraocular muscles. Therefore, the omnidirectional representation of visual motion must be converted to a more restricted coordinate system compatible with the oculomotor system. Because the cerebellar flocculus is critical for generating pursuit tracking, we asked if the output of the flocculus reflects this transformation.

We recorded the simple spike activity of 107 Purkinje cells (P-cells) in two flocculi and examined their directional preferences during tracking of moving visual targets. We assessed direction tuning using tracking of sine wave and step-ramp target motion. For sine wave tracking, we computed a tuning curve that described eye velocity sensitivity as a function of tracking direction. For step-ramp tracking, we computed separate tuning curves for the visually driven transient response and the eye velocity driven sustained response. Best directions were determined by fitting a cosine function to the data. P-cells showed broad tuning for both eye velocity and visual responses and their best directions clustered around two primary directions. Horizontal P-cells have an average best direction that is ipsilateral and a few degrees above exactly horizontal. Vertical P-cells have a best direction that is about 10 degrees oblique and contralateral of exactly down. Best directions for the visual response generally coincided with best directions for eye velocity. Our data show that both the visual and oculomotor output of the flocculus is in a coordinate frame consistent with the pulling directions of the extraocular muscles. (Supported by NSF grant BNS8616509)

245.16

EYE MOVEMENTS EVOKED FROM HISTOCHEMICALLY DISTINGUISHED COMPARTMENTS OF THE RABBIT FLOCCULUS. I. Van der Steen*, I. I Simpson and I. Tan*. Depts. Physiol. 1 and Neuroanatomy, Erasmus University, Rotterdam, Holland, and Dept. Physiol. & Biophys., NYU Med. Ctr., New York, NY 10016.

Division of the cerebellar white matter into compartments containing axons of distinct sets of climbing fibers and their associated Purkinje cells has been shown with a variety of anatomical methods, but a direct demonstration of the significance of these compartments in relation to behavior has been wanting. One method of revealing the compartments uses acetylcholinesterase histochemistry (Hess and Voogd, 1986). In the rabbit this method reveals five compartments within the floccular white matter (Tan, Voogd and Gerrits, personal communication). The present study investigated the possibility of a correspondence between the individual compartments and the particular classes of eye movements previously found (Van der Steen et al., 1987) with electrical stimulation of the floccular white matter in the alert rabbit. The three largest compartments in the flocculus are serially adjacent from medial to lateral. The dominant response evoked by stimulation of the middle compartment was abduction of the ipsilateral eye, usually accompanied by a smaller adduction of the contralateral eye. The dominant response evoked by stimulation of the two bordering compartments was a rotation of the ipsilateral eye produced by the vertical rectus muscles; in about half of these cases the dominant response of the contralateral eye was a rotation produced largely by the oblique muscles. The association of particular eye rotations with the anatomically distinguished compartments suggests that they may be the structural correlate of a coordinate system whose axes represent a particular subset of all possible eye rotations.

245.17

CAN THE CEREBELLUM BE STIMULATED THROUGH THE SCALP IN MAN? J.C.Rothwell,* Y.Ugawa,* B.L.Day,* P.D.Thompson,* and C.D.Marsden*. MRC Human Movement and Balance Unit, Institute of Neurology, Queen Square, London WC1N 3BG, U.K. (SPON: W. Hening)

In an attempt to stimulate the cerebellum, high voltage electric stimulation (Digitimer D180) was applied through two disc electrodes fixed 5cm lateral on a line joining the inion and the external auditory meatus. The stimulus intensity was set so that no EMG response occurred in either relaxed or active arm muscles. From 1-15ms after, motor cortex excitability was tested by giving a magnetic stimulus (Novametrix Magstim 200) at an intensity sufficient to produce responses in relaxed forearm and hand muscles with the coil centred over the vertex of the head. Trials were randomised so that the subject received stimuli either to the "cerebellum" or to the motor cortex, or both. In 7 subjects, "cerebellar" stimulation reduced the size of cortically-evoked EMG responses when the interstimulus interval was 5-8ms. Maximum inhibition was seen when the "cerebellar" anode was ipsilateral to the tested muscles. If the polarity was reversed, inhibition was reduced. H-reflexes evoked in forearm flexor muscles were not made smaller by the "cerebellar" stimulus. The effect is compatible with a cerebellar influence on contralateral motor cortex, although stimulation of other brainstem structures cannot be ruled out.

245.18

CEREBELLAR DISORDERS; FAST GOAL-DIRECTED FLEXION MOVEMENTS AT ELBOW, WRIST AND FINGER IN MAN B.Wild*[1] H.C.Diener[1] J.Dichgans[1] J.Hore[2] [1]Dept.of Neurology, Tuebingen University,7400 Tuebingen,West Germany;[2]Dept.of Physiology,Univ. of Western Ontario, London N6A 5CI, Canada.

Dysmetria is a prominent symptom in patients with lesions of the cerebellar hemispheres. Under clinical conditions dysmetria is tested in proximal joints only (e.g.finger-finger-test). Little is known about the disturbance of movements in more distal joints. We therefore tested 6 patients with unilateral cerebellar lesions comparing function of the affected and healthy sides, 3 patients with diffuse cerebellar atrophy and 10 normal subjects. Fast goal-directed flexions of varying amplitude (5°,30°,60°) were tested seperately at finger, wrist and elbow. Surface EMGs from agonist and antagonist were registered along with the position signal derived from a potentiometer attached to the manipulandum.

Normal subjects show remarkable similarities between the three joints for reaction times and kinematic parameters such as symmetry of acceleration and deceleration but significant differences for maximal acceleration, velocity, deceleration and accuracy.

Patients showed an increase in reaction time and movement duration. Acceleration, velocity and deceleration significantly differed from normals but could be either in- or decreased even at the same joint in one subject performing movements of various amplitudes. Hypermetria was most prominent for small movements. A constant feature was the increase of deceleration in relation to acceleration. This can be explained by the delayed onset of antagonist activity being thus less counteracted by an already ceasing agonist activity. These changes were found at all three joints despite their greatly varying anatomical features.

INGESTIVE BEHAVIORS I

246.1

INHIBITION OF INDEPENDENT INGESTION IN RAT PUPS BY GLUCOSE BUT NOT SUCROSE. S.E. Swithers* and W.G. Hall. Dept. of Psychology, Duke Univ., Durham, NC 27713

Rat pups, tested independent of the mother and suckling, will actively ingest a diet spread on the floor of test containers. We have previously shown that in pups 6 days of age, intake is controlled solely by pups' level of gastric fill and hydrational status. By 9 days of age, however, nutritive glucose pre-loads will inhibit ingestion by an additional mechanism. Because pups at this age lack sucrase activity, they are unable to digest and absorb sucrose. If the inhibition by glucose in 9-day-old pups is specific to its nutritive or metabolic consequences, then sucrose should be ineffective in suppressing intake of pups at this age. We tested the specificity of nutritive inhibition using gastric pre-loads of sucrose, glucose, glucose plus fructose or water. Pups were deprived for 4 hours, given pre-loads by gavage and ingestive responses were tested 2 hours later. In 9-day-olds, pre-loads of glucose or glucose plus fructose suppressed intake, while sucrose and water loads were ineffective. In 20-day-old rat pups, in which sucrase activity is present, sucrose, glucose, and glucose plus fructose had similar suppressant effects on independent ingestion. These data suggest that the inhibition by glucose seen in 9-day-olds is specific to a nutrient that can be absorbed by the pup, and thus may depend on its metabolic effects. Because this nutritive inhibition emerges so early, it may be fundamental to control of ingestion at all ages.

246.2

THE CHOLECYSTOKININ ANTAGONIST L364,718 BLOCKS THE CALMING EFFECTS OF INTRAORAL INFUSIONS OF MILK AND FAT BUT NOT SUGAR IN NEONATAL RATS. D.J. Shide* & E.M. Blass Dept. of Psychology, Johns Hopkins University, Baltimore MD 21218.

Intraoral infusions of milk, fat, and sucrose calm the infant rat, as measured by a reduction in ultrasonic distress vocalizations (DV's). The temporal qualities of milk-elicited calming differ from those for fat or sucrose calming; for milk the effect is delayed, suggesting involvement of a post-oral mechanism. Low doses of CCK also calm the infant rat, implicating this peptide in post-oral calming. Exp. 1 tested the hypothesis that CCK systems are involved in the calming effects of some orally infused substances. Day 10 pups were weighed and marked, and received either an injection of L364,718 (2.0 mg\kg\ml) or its vehicle control. Individually isolated rats received an intraoral infusion of milk, corn oil, 7.5% sucrose, distilled water or no substance, while the number of DV's was recorded for experimental and control groups. Pretreatment with L364,718 blocked the calming effects of milk and corn oil, but not sucrose. Compared to their vehicle controls, L364,718 treated pups vocalized twice (corn oil) or three times (milk) as much. In contrast, there were no differences between L364,718 or vehicle treated pups who received infusions of 7.5% sucrose or distilled water. These results suggest that an endogenous CCK system is involved in calming produced by intraoral infusions of milk and corn oil.

246.3

OVARIECTOMY INCREASES INTAKE OF SUCROSE DURING SHAM FEEDING. B. Tofel-Grehl*, G.P. Smith and D. Greenberg. E.W. Bourne Lab, N.Y. Hospital-Cornell Med. Ctr., White Plains, NY 10605.

The intake of sucrose during a sham feeding test is a monotonic function of concentration in female rats (Nissenbaum & Sclafani, 1987). To determine if the orosensory effect of sucrose is modulated by ovarian hormones, we compared the potency of sucrose (0.1-0.8M) to stimulate sham feeding in pair-fed, 17-h food deprived, ovariectomized and intact rats fitted with chronic gastric fistulas. Ovariectomized rats sham fed significantly more sucrose at all concentrations ($p<.04$, Table).

		SUCROSE CONCENTRATION (M)			
GROUP	n	0.1	0.2	0.4	0.8
OVX	6	23.2*	37.5*	56.2*	60.7*
INTACT	6	13.2	31.2	41.3	47.7

Note. Numbers are mean intake (ml) in 30 min; *$p<.05$.

The results suggest that ovarian hormones decrease the orosensory effect of sucrose during sham feeding after 17h of food deprivation.

246.4

FOOD AVERSION INDUCED BY AREA POSTREMA ABLATION (APX): RELATIONSHIP TO HYPOPHAGIA AND WEIGHT LOSS. Nancy J. Kenney Department of Psychology University of Washington, Seattle, WA 98195

Rats are hypophagic and lose weight immediately following APX. Lesioned rats develop aversions to foods ingested during the first 8 days after the ablation when weight loss and hypophagia are maximal. But, neither hypophagia nor weight loss are necessary aspects of the UCS underlying the food aversion. Intake and weight gain of APX rats recovers to control levels within 2-3 wk after the ablation. But, APX rats develop aversions to foods ingested after recovery. Weight reduction prior to APX eliminates the post-lesion hypophagia but does not preclude development of an aversion to foods ingested after the ablation. When rats are force fed during the first 10 days after APX, they maintain food intakes and body weights similiar to those of intact rats even after the cessation of force feeding. Force-fed APX rats develop aversions to foods ingested after the force feeding has ended. Thus, while the lesion-induced food aversion may play role in the hypophagia and weight loss which result from APX, the UCS underlying this aversion is present and effective in eliciting aversions when no hypophagia or weight loss are evident.

246.5

FOOD AVERSIONS INDUCED BY AREA POSTREMA ABLATION (APX): EFFECT OF DIET FAMILIARITY. M. Kathleen Burkhart* and Nancy J. Kenney (SPON: H.Samson). Dept. of Psychology, Univ. of Washington, Seattle Wa. 98195.

Rats develop aversions to foods ingested after APX. This study shows that pre-lesion familiarity with the post-APX food attenuates weight loss, hypophagia and food aversion induced by the lesion.

APX rats were fed a familiar pelleted chow (PEL), a novel food (AIN), or both AIN and PEL for the first 8 days after ablation. All 3 APX groups ate less and lost more weight than diet-matched sham-lesioned rats (SHAMs) during this time. Weight loss and hypophagia of APX rats fed AIN were greater than those of APX rats fed PEL or both PEL and AIN.

Food aversion was assessed through a series of 24-hr, 2-food choice tests. Rats fed PEL or AIN after APX showed aversions to the post-lesion food but the aversion to AIN was more reliable than that to PEL. APX rats fed both PEL and AIN showed aversion to both foods when they were paired with a totally novel food (MILK). The aversion to AIN was stronger than that to PEL. When offered a choice between AIN and PEL, these APX rats ate less AIN than diet-matched SHAMs but did not differ from SHAMs in PEL intake.

246.6

INTRAPERITONEAL ADMINISTRATION OF SEROTONIN (5-HT) INHIBITS SHAM-FEEDING WITHOUT ELICITING THE SATIETY SEQUENCE IN RATS. K. Eberle-Wang, F. C. Sisk*, A. Vaidya* and K. J. Simansky. Dept. Pharmacology, Med. Coll. of Pennsylvania, Philadelphia, PA 19129.

This study analyzed the effects of peripherally administered 5-HT on sham-feeding by rats restricted to 6 h daily access to milk. After injecting vehicle, rats equipped chronically with gastric cannulas sham-fed continuously during the 30-min test period and consumed 53 ± 4 ml of milk. Given in random order, 3 doses of 5-HT (1.6, 4 and 10 umol/kg, i.p.) inhibited sham-feeding by 12, 42 and 80%, respectively. These reductions agreed proportionately with those obtained previously in intact rats. 5-HT did not retard the onset of feeding. Sham-feeding rats consumed large volumes even after 5-HT (e.g., 31 ± 4 ml after 4 umol/kg), thereby arguing against motor debilitation producing the anorexia. Indeed, after 5-HT as after vehicle, rats fed virtually continuously for the 30-min testing period. Thus, 5-HT reduced sham-feeding by decreasing the rate of feeding rather than its duration. We used a time-sampling procedure to characterize the effects of 4 umol/kg 5-HT on the microstructure of feeding. Sham-feeding rats ingested less milk after 5-HT and occasionally groomed, sniffed or explored. However, these rats displayed the complete sequence of satiety **only when the cannulas were closed.** Thus, 5-HT requires more than oral stimuli to produce satiety. Supported by NIMH 41987 to KJS

246.7

NEONATAL DIETRY EXPERIENCES INFLUENCE ADULT RESPONSES TO MACRONUTRIENTS. J. Diaz, P. Garvie', G. Watkins* and E. McGarvey. Dept. of Psychology, University of Washington, Seattle, WA 98195.

We have shown that rat pups rendered obese by adjusting the volume or the content of the infused formula given during gastrostomy feedings defend this obesity throughout their lives. In the present study, shorter gastrostomy feeding periods were used and adult behavior examined.

At four days rat pups were: 1) mother reared in litters of eight (MR); 2) gastrostomy fed for eight days (Days 5-12) to match the growth of the MR group (WM); and 3) gastrostomy fed with a formula supplemented with 10% fat (OB). At Day 12, the gastrostomy fed groups were returned dams to form litters of eight. As adults, all the groups were given fat adulterated mash for 10 days, and later were given sucrose adulterated mash. Following these challenges the animals were tested in a radial arm maze. The OB animals ate more fat and sucrose adulterated mash than did the MR controls. The OB animals also took less time to complete the radial arm maze. These data suggest that there may be sensitive or critical periods early in life for adult responses to fat and sucrose, and for the reinforcement value of food.

246.8

THE EFFECT OF VASOPRESSIN ON CARBOHYDRATE AND PROTEIN PREFERENCES IN RATS. C.H.Wideman and H.M.Murphy. John Carroll Univ.,Cleveland,OH 44118.

Brain vasopressin may modulate the activity of serotonin-containing systems in the brain which affect carbohydrate and protein intake. Vasopressin-deficient (DI) and Long-Evans (LE) rats were presented with a sucrose solution, a saccharin solution, tap water, and regular rat chow (relatively high in protein) for a 7 day habituation period followed by a 9 day experimental period in which the animals were food-restricted for 23h each day and given ad-lib access to the fluids. DI rats drank more sucrose than LE rats during the habituation period, but no difference was evident in the experimental period. The sucrose was preferred over saccharin and water by DI and LE rats during both the habituation and experimental conditions. Food intake differed significantly during the latter part of the habituation period where LE rats consumed more food than DI animals. This phenomenon was reversed during the experimental period. DI rats follow eating and drinking patterns that are much different from LE animals. The cyclic pattern of carbohydrate and protein intake in LE rats, which is absent in DI rats, indicates a role for vasopressin in consummatory behavior. This role may involve the neurotransmitter serotonin.

246.9

DIETARY FAT INFLUENCE ON CARBOHYDRATE AND PROTEIN SELECTION MAY BE MEDIATED VIA CENTRAL SEROTONIN SYSTEM. BJ Mullen* and RJ Martin (SPON: T. Reigle) Dept. of Foods & Nutrition, University of Georgia, Athens, GA 30602

We have shown previously that type and level of dietary fat can influence an animal's subsequent selection for carbohydrate (CHO) and protein (Pro). High levels of dietary tallow result in preference for dietary protein while high levels of corn oil result in carbohydrate feeding. Present studies investigate the role of the central serotonin system in mediating this behavior. Male, Sprague-Dawley rats (75-99 g) were divided into 2 groups and fed diets containing either 34% corn oil or 34% tallow for 2 days. These diets were then replaced with 2 diets given simultaneously to test dietary selection: 1) 15% CHO/60% Pro and 2) 65% CHO/10% Pro. During this diet selection period, the corn oil and tallow groups were subdivided into 3 groups which were injected with either: 1) saline, 2) 2 mg/kg BW fenfluramine or 3) 6 mg/kg BW fenfluramine. Injections were given IP 2 hours before onset of the dark cycle. As noted earlier, the rats previously fed tallow exhibited a preference for protein while the corn oil group preferred carbohydrate. The fenfluramine depressed consumption of CHO in both corn oil and tallow groups but had a significantly greater effect in the tallow group. We propose that the central serotonin system may be mediating, in part, the behavioral effect of dietary fat on macronutrient selection.

246.10

EFFECTS OF EARLY DIETARY NACL EXPOSURE ON DIETARY OBESITY AND BLOOD PRESSURE IN ADULT RATS. R. J. Contreras. Department of Psychology, University of Alabama, Birmingham, AL 35294.

We investigated the long-term effects of early dietary NaCl exposure on body weight and blood pressure in normotensive Sprague-Dawley rats. Adult female rats were fed a diet containing either 0.12% (low) or 3% (high) NaCl throughout pregnancy and lactation. The offspring were continued on these same NaCl-containing diets until 30 days postpartum. Thereafter, all the offspring were fed Agway 1% NaCl chow. Beginning at 60 days of age, the rats raised on the low and high NaCl diets were subdivided into two groups. One group was continued on the Agway chow and the other group was fed a high fat mash and sweetened milk (HF/M) diet. Body weight and caloric intake were measured weekly as were systolic blood pressure and heart rate using tail plethysmography in awake restrained rats. After 16-wk, mean arterial pressure (MAP) and heart rate (HR) responses to the pressor effects of bolus intravenous infusions of angiotensin II (20, 40, 80, 120 ng/kg of body weight) and atriopeptin II (20, 40, 80 ug/kg) were obtained from the femoral artery in awake rats.

HF/M feeding resulted in significant elevations in body weight and in terminal brown fat pad and white fat pad weights for both the low and high salt rats. There seemed to be a group by sex interaction effect; HF/M feeding led to a greater obesity in low salt females compared to high salt females, but the obesity for the two male groups were similar. The blood pressure data are currently being analyzed.

Supported by NIH Grant HL-38630.

246.11

EFFECT OF PROLONGED PHARMACOLOGICAL STARVATION ON CALORIC INTAKE AND MACRONUTRIENT SELECTION.
J. Duhault*, F. Lacour* and Y. Rolland* (SPON: J.C.R. Randle). Institut de Recherches Servier, Suresnes, France

The present experiments were undertaken to compare the effects of complete food removal with the effects of pharmacological starvation on meal size and macronutrient selection during the refeeding period. dl-Fenfluramine and Fluoxetine were used as pharmacological tools. In self-selection experiments 18 h food-deprived adult fatty Zucker rats were offered three pure macronutrient isocaloric diets (CHO 80 %, PRO 80 %, LIP 80 %) for a 2 h period. Group 1 was maintained on the standard lab chow, group 2 was subjected to a 4 day fast, groups 3 and 4 received daily i.p. administration of dl-Fenfluramine (100 µmol/kg per day for 4 days) or Fluoxetine (100 µmol/kg per day for 4 days), respectively. On the 5th day, rats of different groups were allowed to select their food from the three separate sources of macronutrients. In group 2, the refeeding selection after fasting was characterized by a small decrease (-21 %) in total caloric intake, the percentage of total calories as carbohydrate was increased (16.5 ± 3 % versus 5.9 ± 2 %). dl-Fenfluramine and Fluoxetine had suppressant effects on overall food intake, but 6 out of 10 animals died in the Fluoxetine group before the refeeding selection trial. Total caloric intake was still reduced (-44 %) in the dl-Fenfluramine group and in the 4 surviving Fluoxetine-treated rats (-91 %). When energy stores were low, a switch in preference for carbohydrates occured in untreated food-deprived rats, whereas pharmacological activation of the 5HT system by dl-Fenfluramine or Fluoxetine administration resulted in a return to a relative preference for proteins.

246.12

RELATION BETWEEN PREFERENCE FOR "SWEET" STIMULI AND SINGLE FIBER ACTIVITY IN THE HAMSTER CHORDA TYMPANI NERVE.
B.G. Rehnberg*, T.P. Hettinger, and M.E. Frank. UCONN Health Center, Farmington, CT 06032.

Preference data and conditioned aversion studies for the hamster (Mesocricetus auratus) show that a variety of gustatory, olfactory, and trigeminal stimuli are preferred only if they are "sweet" (sucrose-like). Putative sweet stimuli at preferred concentrations (compared to water) were presented to the anterior tongue of the hamster while recording from the chorda tympani nerve. The order of stimulatory effectiveness for integrated responses of the whole nerve was Na 2-mercapto-ethanesulfonate (0.03 M) > p-phenetylurea (0.005 M) = Na 3-nitrobenzenesulfonate (0.003 M) = Ca cyclamate (0.01 M) = sucrose (0.03 M) = Na saccharin (0.001 M) > D-phenylalanine (0.01 M) = glycine (0.1 M). Single fiber recordings indicate that all are good stimuli for sucrose-sensitive (S) fibers with the exception of Ca cyclamate and Na nitrobenzenesulfonate. Ca cyclamate, instead, appears to be a good stimulus for a population of generalist (H) fibers. As expected, the sodium salts are good stimuli for both sodium-sensitive (N) and H fibers. These results suggest that sucrose-sensitive fibers carry information leading ultimately to ingestion of preferred stimuli. However, a role for other populations of fibers in the chorda tympani in initiating preferential drinking cannot currently be ruled out. (Supported by NSF BNS-8519638, NIH NS16993, and NIH NS07299).

246.13

EFFECT OF DIETARY CARBOHYDRATE SOURCES ON FEEDING BEHAVIOR, PLASMA GLUCOSE, INSULIN AND TRYPTOPHAN, AND BRAIN SEROTONINERGIC SYSTEMS IN SELF-SELECTING RATS.
L. Thibault. School of Dietetics and Human Nutrition, Macdonald Coll. of McGill Univ., Montréal, Canada, H9X 1C0.

The present study was undertaken to investigate hypothalamic and extrahypothalamic neural and hormonal mechanisms involved in the control of food intake. Sprague-Dawley rats were offered pairs of 0 and 60% casein diets using either sucrose, fructose or glucose, for an 8h daily period during 16 days. At the end of the last feeding period, arterial and venous blood samples were collected, or animals decapitated and brain dissected out. Fructose fed rats displayed slower growth rate and higher dietary energy chosen as protein (%P-E) than rats fed either sucrose and glucose. Rats fed glucose had a higher %P-E but a lower %CHO-E than those fed sucrose. Fructose feeding induced lower arterial and venous (AV) glucose levels than sucrose and glucose feeding, whereas glucose feeding induced higher AV glucose and tryptophan levels and AV difference of insulin, than sucrose feeding. The brain serotoninergic parameters showed: a fructose-induced 1) decrease in 5-HIAA/5-HT ratio and increased in tryptophan and 5-HT levels in the hypothalamus but these changes were in the opposite direction in extrahypothalamic areas, when results were compared to glucose, and 2) increase in 5-HIAA/5-HT ratio and in tryptophan and 5-HIAA levels in the raphe nuclei, and an increase in 5-HT and 5-HIAA in the brain stem, when compared to sucrose, and; a glucose-induced increase of 5-HIAA/5-HT ratio and decreased tryptophan and 5-HT content in the hypothalamus, when compared to sucrose. These results show that dietary CHO sources affect differently glycemia and insulin secretion as well as brain serotoninergic systems. Supported by NSERC Canada.

246.14

EFFECTS OF DIET AND OVARIAN STEROIDS ON BODY WEIGHT AND ADIPOSITY IN OVARIECTOMIZED (OVX) SYRIAN HAMSTERS.
A.J. Bhatia and G.N. Wade. Univ. of Massachusetts, Dept. of Psychology and Neuroscience and Behavior Program, Amherst, MA 01003.

In OVX Syrian hamsters administration of estradiol alone or with progesterone results in weight loss and reduced carcass lipid. Since it has been suggested that the weight-reducing effects of estradiol are dependent on an elevated level of body weight and estradiol treatment is reported to dictate a low weight level independent of the diet offered in OVX rats, we assessed the weight- and adiposity- reducing actions of estradiol alone and with progesterone in OVX hamsters of differing body weights and adiposity. Animals received subcutaneous Silastic implants of estradiol alone, estradiol and progesterone, or empty blanks. Hamsters given hormone implants lost body weight and lipid. The amount of weight loss depended on body weight at the time of hormone implantation, but not on the amount of dietary fat intake. Groups that had higher body weights had larger absolute losses, but the percentage of weight and carcass lipid lost among the different groups was similar. Hamsters heavier at the time of hormone implantation had higher absolute amounts of terminal carcass lipid than hamsters that were lighter at the time of hormone implantation. Thus, the weight and adiposity-reducing actions of estradiol and progesterone are enhanced in fatter animals, but an absolute low level of body weight or fatness is not dictated by the actions of these steroids.

246.15

THE PATTERN OF FOOD CHOICE FOLLOWING FOOD DEPRIVATION.
M.B. DeStefano*, T.W. Castonguay, S.K. Kramlik*, T.T. Ton*, J.J. Valeri*, and P.U. Dubuc*. Dept of Human Nutrition and Food Systems, U. Maryland, College Park, MD 20742 and Sansum Research Foundation, Santa Barbara, CA 93102.

Adult male Sprague-Dawley rats were given ad libitum access to separate protein (PRO), carbohydrate (CHO) and fat sources for 9 days. The animals were then divided into 3 groups on the basis of body weight. The first group continued to have ad libitum access to macronutrient sources and water. The second group was subjected to a 24 h food deprivation period, after which time access to all three of the food sources was restored. Intakes were measured after 1, 3, 6, 12, and 24 hours of restored access. The third group was treated similarly, except that they had had food access restricted for 48 h. During each of the 5 times that intake was measured after reinstated access, each animal was bled via tail venipuncture, and circulating levels of glucose, insulin and corticosterone were measured. Deprivation led to an increase in dietary fat during the first hour of refeeding. Controls ate 0.1 g fat during the first hour on the test day. 24 h deprived rats ate 1.67 g of fat, and 48 h deprived rats ate 2.37 g of fat during the first hour of refeeding. During this first hour of daylight, controls composed a diet that was 25% CHO, 41% PRO and 33% FAT, yielding only 2.4 kcals. During the first hour of access, 24 h deprived rats selected a diet that was 7.8% CHO, 36% PRO and 56% FAT, and yielded 23.6 kcals. 48h deprived rats selected of a diet during this first hour of access that was 9.7% CHO, 33.7% PRO and 56.5% FAT, and yielded 33.2 kcals. Deprivation played no role in altering 24 h dietary PRO. The % of calories taken from CHO, PRO and FAT by the control group was 19.8%, 42.1% and 38.1% (84.6 kcals). By comparison, on the day food access was reinstated 24 h deprived rats composed a diet made up of 2.9% CHO, 41.4% PRO, and 55.7% FAT (105.3 kcals). Similarly, 48 h deprived rats composed a diet made up of 13.2% CHO, 42.1% PRO, and 44.7% FAT (121.7 kcals). These selection patterns are consistent with the hypothesis that dietary fat intake is partially controlled by circulating corticosterone. (Supported by a grant from the Whitehall Foundation.)

246.16

ELECTRICAL STIMULATION OF HYPOTHALAMIC SITES WHICH INDUCE EATING DRIVES MASTICATORY NEURONS IN THE BRAIN STEM.
E. Murzi*, T. Baptista* & L. Hernandez* (SPON: J. Litto). Laboratorio de Fisiologia de la Conducta, Escuela de Medicina, Universidad de Los Andes, Merida, Venezuela

Previous experiments showed that electrical stimulation of the perifornical region of the hypothalamus (PFH), which induces feeding in unanesthetized rats, drives gustatory neurons in the nucleus tractus solitarii, but not in the pontine taste area in anesthetized rats[1]. This suggests that some feeding related centers in the lower brain stem are modulated by hypothalamic feeding centers. In order to further explore this functional relationship we implanted multiple electrodes in the PFH. After recovery from surgery each rat was tested for electrically induced feeding. Some electrodes induced stimulation-bound feeding (SBF) and some did not (NSBF). Then each rat was anesthetized and a glass insulated tungsten microelectrode was lowered in the masticatory nucleus of the trigeminal nerve. Masticatory neurons were identified by excitatory or inhibitory responses when the jaws of the rat were passively opened or closed. Stimulation through SBF electrodes affected the firing rate of masticatory neurons more than stimulation through NSBF electrodes. In order to produce this effect, the frequency and the duration of the stimulus train had to be at least 30 Hz and 5 seconds respectively. We conclude that a functional relationship between the lateral hypothalamic feeding center and the brain stem masticatory center exists. This relationship might underlie the increased energy biting observed in food deprived animals[2]. Additionally, this relationship might be involved in gnawing and aggression biting.

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246.17

CHARACTERIZATION OF 5-HYDROXYTRYPTAMINE (5-HT) RECEPTOR BINDING IN OBESE MALE ZUCKER RATS. Steven Rothman* and Edmund T. S. Li. Department of Nutritional Sciences, University of Toronto, Toronto, Ontario M5S 1A8, Canada.

5-HT_1 receptors which have high affinity for 5-HT are known to play a role in feeding control. 5-HT_1 receptors are composed of mainly 1A and 1B subtypes. The 1A subtype also has high affinity for the compound 8-OH-DPAT. The present study examined the binding characteristics of 5-HT to cortical membranes of the genetically obese Zucker rat which is hyperphagic. Lean (mostly fa/+) and obese (fa/fa) rats were fed lab chow for 3 to 4 weeks and killed by decapitation. Radioligand binding assays were performed using (^3H)5-HT. Total 5-HT_1, nonspecific (in the presence of unlabelled 5-HT) and 1B binding (in the presence of unlabelled 8-OH-DPAT) were determined by Scatchard analyses. 5-HT_{1A} binding was obtained by subtracting the B_{max} (pmol/g tissue) of 1B from that of the total. At 12 weeks of age, obese rats had 23% less total 5-HT_1 binding than their lean littermates (5.2 vs 6.8, t=2.70, df=22, P<0.025). 5-HT_{1B} binding was not different between genotypes (3.6 vs 3.7). Binding to 5-HT_{1A}, however, was significantly different with the obese having 48% less binding sites than the lean (1.6 vs 3.1, t=3.69, P<0.005). Affinity was not different for any of the subtypes. These preliminary results suggest a potential area of investigation in the etiology and / or maintenance of genetic obesity.

(Supported by NSERC of Canada)

246.18

THE EFFECT OF CENTRALLY ADMINISTERED ICS 205-930 ON FOOD INTAKE OF RATS FED AN AMINO ACID IMBALANCED DIET. B.J. Hrupka*, J.L. Beverly*, D.W. Gietzen, V.A. Hammer*, Q.R. Rogers. Dept. Physiol. Sci. and Food Intake Lab, Univ. Calif., Davis, CA, 95616.

These experiments were conducted to study the effect of intercerebroventricular (ICV) injections of the serotonin$_3$ antagonist ICS 205-930 (ICS) on food intake of rats fed ile imbalanced (IMB) diets. Serotonin$_3$ receptors may be involved in rat's anorectic response to amino acid imbalanced diets. Male Sprague-Dawley rats, fitted with chronic unilateral 20 gauge guide cannulae 1 mm above the lateral ventricle, were prefed a low protein basal (BAS) diet. All ICV injections were administered in 7 µl volumes over a 30-60 sec period. To verify cannulae placement, 100 ng Angiotensin II was injected, and drinking responses recorded. In the following experiments, rats received ICV injections of either ICS or saline (SAL), 30 min before onset of the dark cycle. The test diet was presented to rats at the beginning of the dark cycle, and food intake (FI) was recorded 3, 6, 12, or 24 hr after presentation. In experiment one, 26 rats (n=6-7/trt) were given ICV injections of either ICS (10 nmoles) or SAL, and fed either BAS or a severe IMB diet. ICS injected rats ate less than saline injected rats when fed a BAS diet (mean ± SE 24 hr FI=9.5±2.8 g vs 18.8±1.2 g, P<.05). ICS injections did not affect food intake of rats fed the IMB diet (mean±SE 24 hr FI=3.9±0.6 g vs 5.0±1.3 g for ICS and SAL trt respectively, P>.05). Because ICS reduced FI in rats fed the basal diet, a second experiment was conducted to investigate the effect of lower ICS doses. Twenty four rats (n=6/trt) received ICV injections of either 0, 0.01, 0.1, or 1 nmole ICS and fed a mild IMB diet. Food intake was not affected (P>.05) by ICS at any time period (mean ± SE 24 hr FI=5.2±1.3, 7.1±1.6, 5.5±1.8, 5.0±1.9 for 0, 0.01, 0.1, and 1.0 grps respectively). Additional experiments are being conducted to determine if ICS's effect is peripherally or centrally mediated. Supported by NIH AM-07355, DK-13252; USDA CRCR1-2418; CNRU DK35747-04.

246.19

LIMITED ROLE OF $5HT_{1C}$ RECEPTORS IN THE ANOREXIA AND LOCOMOTION CHANGES PRODUCED BY 5HT AGONIST ADMINISTRATION TO RODENTS. J. A. Nielsen. Pfizer Central Research, Groton, CT 06340.

An extensive literature implicates serotonin (5HT) in the regulation of food intake. It has been suggested that postsynaptic $5HT_1$ receptors (possibly of the $5HT_{1C}$ type) play an important role in the control of feeding in rats (Dourish *et al.*, *Psychopharmacol.* **97**:54, 1989) and that hypophagia induced by mCPP requires activation of both $5HT_{1C}$ and $5HT_{1B}$ receptors while that caused by RU 24,969 only requires stimulation of $5HT_{1B}$ receptors (Kennett and Curzon, *Psychopharmacol.* **96**:93, 1988). The present study extends these findings by determining the effects of the $5HT_{1C}$ antagonists mianserin and mesulergine on the anorexia and behavioral disruption caused by direct $5HT_1$ receptor agonists (RU 24,969; mCPP and TFMPP) and indirect 5HT agonists (d,l-fenfluramine; d-fenfluramine and fluoxetine) in mice and rats.

Mianserin (3.2 mg/kg, sc) and mesulergine (0.32 mg/kg, sc) antagonized the decreased food intake and hypolocomotion caused by mCPP (1.0-3.2 mg/kg, ip) in rats, but were ineffective at blocking the actions of mCPP in mice. In addition, these antagonists had no effect on the anorexia and behavioral disruption caused by TFMPP (1.0-3.2 mg/kg, ip), d,l-fenfluramine (1.0-3.2 mg/kg, ip), d-fenfluramine (1.0-3.2 mg/kg, ip), fluoxetine (5.6-17.8 mg/kg, ip) and RU 24,969 (1.8-5.6 mg/kg, ip) in rats or mice.

These data suggest that the hypophagia and behavioral disruption caused by direct and indirect 5HT agonists is not solely related to activation of $5HT_{1C}$ receptors in rodents.

NEUROETHOLOGY II

247.1

HARMONIC COMBINATION-SENSITIVE NEURONS IN THE ZEBRA FINCH. E.S. Fortune* and D. Margoliash.(SPON: J.D.Cowan) Dept. Org. Bio. and Anat., Univ. Chicago, Chicago, IL 60637.

Zebra finch song is rich in harmonics whereas white-crowned sparrow song is not. In the forebrain song system nucleus HVc of the white-crowned sparrow, auditory neurons are sensitive to temporal features of song. We explored how harmonics are represented in zebra finches by recording from single HVc neurons in urethane anesthetized birds.

In our preliminary sample, we isolated 24 'complex' neurons in 11 birds that responded well to song syllables from the individual's own (autogenous) song but poorly to tone bursts. In all pair-wise comparisons (n=61), such neurons responded better to autogenous than conspecific songs. We tested these neurons with reversed autogenous song, spectrally similar song modified to remove the natural AM, and white noise shaped with identical AM as autogenous song. Typically, these neurons did not respond to the AM-noise, AM-modified, or reverse autogenous song. Although zebra finch syllables are complex, occasionally we could produce effective artificial models of syllables. These demonstrated that complex neurons typically required 1) both 2nd and 3rd but not higher harmonics or the missing fundamental, 2) each harmonic comprising a continuous sound of one or several FM segments specified within a narrow band of starting and ending frequencies and a limited range of durations, and 3) a distinct harmonic amplitude relationship. Some neurons exhibited closed amplitude tuning curves. It appears that combination sensitivity and selectivity for autogenous song are emergent properties of HVc that may obtain widely in songbirds.

Supported by the NIH (NS25677-01), the Whitaker Foundation, and the Searle Scholars Program/Chicago Community Trust.

247.2

CYTOARCHITECTURE AND DIFFERENTIAL PROJECTIONS OF NUCLEUS OVOIDALIS IN THE ZEBRA FINCH, *Poephila guttata*. P. M. Bell, D. Margoliash and P. S. Ulinski. Dept. of Organismal Biology and Anatomy and Comm. on Neurobiology, Univ. of Chicago, Chicago, IL 60637.

Nucleus ovoidalis is a thalamic auditory nucleus that projects to Rose's field L in the telencephalon of birds. In songbirds, it may play a role in processing auditory cues associated with song recognition and production, but its internal circuitry is not understood. Consequently, we have begun analyzing the neuronal organization of ovoidalis. In zebra finch Nissl material, ovoidalis is composed of large (~18 µm) densely stained neurons with elongate, fusiform somata and a lesser number of small (~7 µm) diffusely stained neurons with round or oblong somata. Large injections of HRP into field L result in clouds of retrogradely filled neurons in ovoidalis. Large neurons all contain vesicular reaction product in the centers of such clouds, but are less consistently filled at the clouds' peripheries. Small neurons are apparently never labeled. Additionally, the range of cases indicates some degree of topography in the projection from n. ovoidalis to field L. Large neurons situated medially in the former project to the medial region of the latter, and large neurons in lateral ovoidalis project to the lateral portion of field L. We are currently using focal HRP injections within field L to further investigate the topography of the projection involving the large, fusiform cells within n. ovoidalis. If the small neurons do not project to field L, then they could represent either an interneuronal population or a parallel auditory path to a second target. Supported by grants to D.M. from the NIH (NS25677-01) and the Searle Scholars Program/Chicago Community Trust.

247.3

PLASTIC SONG IN ADULT INDIGO BUNTINGS (*Passerina cyanea*). D. Margoliash and C. A. Staicer*. Dept. of Organismal Biology and Anatomy, Univ. Chicago, Chicago, IL 60637.

Some songbirds such as the indigo bunting retain the ability to modify song into adulthood. In the field, buntings already singing a stereotyped song will alter their songs to closely match a territorial neighboring adult. Using a computer-based system, we have exhaustively examined singing behavior of individuals or pairs of males housed in IAC sound booths. Some birds sang spontaneously; others were primed with testosterone.

Over a period of months, as well as stereotyped song both yearling and older males sang bouts of what we have termed 'adult plastic' song. Characteristics of adult plastic song included standard, well-formed bunting syllables not found in the individual's stereotyped song, variable temporal pattern and bout duration (1-30s), and low amplitude singing. For 7 yearlings and 6 older birds, syllable types were distributed as follows: stereotyped $\bar{n} = 5.2$, range 3-11; plastic $\bar{n} = 12.3$, range 7-18. Adult plastic songs are therefore distinct from the short, invariant stereotyped songs. Cage mates did not exhibit song matching, but may have incorporated new syllables into their plastic songs. We are automating the analysis of this large (10^8 Kb) data base.

Buntings therefore engage in two distinct singing behaviors. These observations suggest that the mechanism for song matching in adult birds involves adult plastic song. Furthermore, buntings are clearly capable of vocal motor patterns that are normally not expressed in the stereotyped song. We are currently exploring how these alternate patterns are expressed in the (motor) song system.

Supported by the NIH (NS25677-01), the Whitaker Foundation, and the Searle Scholars Program/Chicago Community Trust.

247.4

SELECTIVE IMPAIRMENT OF SONG DEVELOPMENT IN ZEBRA FINCHES FOLLOWING LESIONS OF THE SONG CONTROL NUCLEUS, AREA X. F. Sohrabji, E.J. Nordeen and K.W. Nordeen. Psychology, U. Rochester, Rochester, NY, 14627.

Area X has been assumed to participate in the control of avian song because it connects with other vocal nuclei, and its size is positively correlated with the ability to sing. We report here that Area X specifically contributes to song development and is not needed for production of adult song.

Area X in adult and juvenile male zebra finches was bilaterally lesioned with ibotenic acid. Songs of adult birds were recorded prior to surgery, and both adult and juvenile birds were recorded weekly after surgery. Birds were sacrificed 3-5 weeks after surgery and coronal brain sections through Area X were examined to verify the location and size of the lesion.

Lesioning Area X in adult birds did not affect song quality. However, song development was severely impaired when Area X was lesioned in juvenile males. At all post-operative timepoints, these lesioned juveniles were judged to be in earlier stages of vocal development than age-matched sham lesioned controls. In fact, by 75 days, control males were producing stable adult song, yet lesioned birds were producing subsong, lacking in stereotypy and syllable definition. These results suggest that Area X is not necessary for sustaining the production of juvenile or adult song, but is important for either the acquisition of a song model, or the improvement of song through vocal practice. (Supported by USPHS, NS24862)

247.5

LESIONS IN AREA X AFFECT SONG IN JUVENILE BUT NOT ADULT MALES ZEBRA FINCHES. C. Scharff* and F.Nottebohm SPON: (A. Alvarez-Buylla). The Rockefeller University, New York, NY 10021.

The forebrain nucleus area X is implicated in the circuitry of brain nuclei controlling vocal behavior: it receives a projection from HVC and projects itself to dorsal thalamus which in turn connects to MAN, a nucleus involved in song aquisition in Zebra finches. As yet, no functional role has been ascribed to area X.

We investigated if area X plays a role in song production or development by lesioning the nucleus electrolytically. An angled approach was used so as to avoid MAN. The song of 10 adult males did not change during the two months post-operative survival. In contrast, striking impairments were seen when juveniles that had been lesioned between 28 and 45 days were recorded between 68 and 134 days. These birds did not show normal progression from plastic to crystallized song. Fewer notes (usually 1-3), poor note morphology and control of frequency modulation, unusually long notes (250+ ms) and long inter-note intervals, as well as non-stereotypic combination of notes into motifs were among the most prominent features.

These results suggest that destruction of area X interferes with the normal process of song acquisition and results in significant changes in song structure relative to normal adult song.

247.6

EFFECTS OF HVC LESIONS ON SONG SYLLABLE PRODUCTION IN ZEBRA FINCHES (POEPHILA GUTTATA). J. Cynx. Field Research Center, Rockefeller Univ., Millbrook, NY 12545.

Lesions to brain nucleus HVC cause aberrant song production in zebra finches and other songbirds. In this study, temporal properties of such aberrant song were determined. This in turn suggests how HVC might and might not control song production.

A zebra finch can be induced to stop singing in the midst of song if confronted with a brief flash of light. Song interruptions occur only between song syllables (Cynx, J., J. Comp. Psych., in press), suggesting syllables have unitary coherence, and that song is produced on a syllable-by-syllable basis. This technique was used to determine the temporal coherence of aberrant song structures.

Zebra finches were tested with the above procedure, given bilateral HVC lesions, then tested again. All birds could be induced to stop vocalizing. All interruptions occurred between syllabic structures. This suggests that HVC may not be essential to the maintenance of temporally coherent motor units. More generally, lesions to a part of the song control pathway can be shown to produce well-defined and measurable changes in song production.

247.7

EVIDENCE FOR SUBDIVISIONS WITHIN UVAEFORMIS, AN AVIAN SONG NUCLEUS. H. Williams, G.F. Ball, P.L. Faris*, Rockefeller University, Millbrook, NY 12545, Williams College, Williamstown, MA, 01267, Boston College, Chestnut Hill, MA 02167, Univ. of Minn. Sch. Med., Minneapolis, MN 55455.

Uvaeformis (Uva) is a small thalamic nucleus in the avian song system which projects to two forebrain nuclei (HVc and NIf). Using immuno-histochemical methods to stain for corticotropin releasing factor (CRF), we have identified two subregions of Uva in European starlings (*Sturnus vulgaris*), song sparrows (*Melospiza melodia*), and zebra finches (*Poephila guttata*). A dorsomedial cap of the nucleus contains CRF-reactive perikarya in males and females of all three species. This cap is least prominent in the rostral portion of the nucleus and most extensive at the caudal end of Uva.

Microstimulation in these two subdivisions of Uva while recording from the left and right tracheosyringeal nerves (NXIIts; the pathway is Uva-HVc-RA-nXIIts) of zebra finches indicates that there are differences in the connectivity of the cap and body of Uva. Stimulation of the cap region results in greater activation of the contralateral pathway while stimulation of the main body of Uva elicits larger responses in the ipsilateral NXIIts.

Reciprocal connections between the two portions of Uva may help in coordinating song production in the two hemispheres of song birds, which are acallosal. The localization of CRF to a subregion of Uva suggests that CRF may play a role in the motivation and initiation of singing.

Together, these results suggest a larger role for Uva in song production than has hitherto been hypothesized.

Supported by NIH award NS26825 to HW and RSDA (HH00595) to PLF.

247.8

FUNCTIONAL PROPERTIES OF OUTPUTS FROM NUCLEUS ROBUSTUS ARCHISTRIATALIS (RA) IN A SONGBIRD. David S. Vicario, The Rockefeller University, New York, NY 10021

The motor pathway for vocalization in songbirds includes forebrain nuclei HVc and RA, DM of ICO, and the part of the hypoglossal nucleus (nXIIts) that controls the syrinx, the bird's vocal organ. RA of the male zebra finch can be divided into subregions with different projections (Vicario & Simpson 1988). The most dorsal part projects to DM, a midbrain vocalization area. The ventral area contains zones that preferentially project to different parts of nXIIts known to control different syringeal muscles. The present study used electrical stimulation, lesions, and chronic recording to explore the functional contributions subregions of RA make to vocal behavior in the zebra finch.

Microstimulation was applied at sites throughout RA while EMG's were recorded from the largest syringeal muscles, ventralis and dorsalis, and from the abdominal respiratory musculature. Single shocks (≤10 μA) were effective at activating all three recorded muscles with a latency of 20-25 mS, indicating that RA can contribute to respiratory aspects of vocalization (perhaps via its output to DM) as well as to syringeal control. Complete bilateral **lesions** of RA abolish song production. Electrolytic lesions of dorsal RA disrupt both temporal and acoustic features of song. Lesions limited to ventral RA affect syllable morphology, but the characteristic temporal structure of song is largely spared. Microelectrode **recordings** of neural activity in RA of awake zebra finches show bursts of activity that precede song syllables and calls by 30-50 mS (cf. McCasland 1983); a regional analysis is currently in progress. In summary, these data suggest that subregions of RA may participate in the control of temporal and acoustic features of vocalization. (Supportedby NIH MH-40900)

247.9

SEASONAL EFFECTS ON BIRDSONG PRODUCTION AND ACQUISITION: II. TESTOSTERONE INDUCED SINGING BEHAVIOR IN SONG SPARROWS S. Nowicki* and G.F. Ball (SPON: P. Marler). Rockefeller University, Millbrook, NY 12545, Duke University, Durham, NC 27706, Boston College, Chestnut Hill, MA 02167.

Song sparrows (*Melospiza melodia*) show seasonal changes in the production of male-typical singing behavior, with far more song produced in the spring and early summer than in the fall and winter. The expression of song is highly influenced by the sex steroid testosterone (T), acting on sexually dimorphic telencephalic nuclei involved both in its acquisition and production. We examined the extent to which seasonal production differences are due to changes in the ability of T to activate song as opposed to changes in T titres. Song rates in 6 male sparrows were compared in the following 5 conditions: (1) birds responsive to long days (photosensitive), experiencing short days, and with low plasma titres of T; (2) photosensitive, on short days, with high T titres; (3) photosensitive, on long days, with high T titres; (4) insensitive to long days (photorefractory), on long days, with low T titres; and (5) photorefractory, on long days, and with high T titres. Plasma levels of T were monitored by radioimmunoassay; song behavior was evaluated sound spectrographically. T was equally effective in inducing song both while photosensitive and photorefractory. Thus, no seasonal change was found in the sensitivity to hormone action of the neural sites mediating this behavior in song sparrows.

247.10

SEASONAL EFFECTS ON BIRDSONG PRODUCTION AND ACQUISITION I: SONG LEARNING IN STARLINGS G.F. Ball, J Böhner*, M Chaiken*, P Marler, Rockefeller University Field Research Center, Millbrook, NY 12545; Department of Psychology, Boston College, Chestnut Hill, MA 02167,

European starlings *(Sturnus vulgaris),* like many periodically breeding songbird species, show dramatic seasonal changes in their propensity to respond to light that are associated with substantial differences in peripheral endocrine activity and hypothalamic neurochemistry. In this study we asked if this seasonal variation in physiological state had any effect on the propensity of males to acquire new songs. Such differences would presumably reflect seasonal changes in the functioning of at least a subset of the sexually dimorphic telencephalic nuclei that underly song acquisition and production.

Male starlings (N= 12) were collected as nestlings, hand-reared, and housed individually in soundproof chambers where they were tutored with recordings of adult song from 1.5 to 4.5 months of age. Photoperiod was manipulated so that at an age of 11 months, 6 of the subjects were in a photosensitive state, as naturally occurs in late winter and spring (i.e. responsive to stimulatory daylengths) and 6 of the birds were in a photorefractory state, as naturally occurs in summer and fall (i.e. non-responsive to long photoperiods). At that age both groups were exposed to a set of recordings of starling songs for 6 weeks. Analysis of singing behavior revealed that there were no significant differences between the groups in the amount of song material acquired during the learning session. Thus in male starlings the propensity to learn songs in the first breeding season does not depend on a physiological state of photosensitivity.

247.11

EFFECT OF FIELD "L" LESIONS ON AUDITORY DISCRIMINATION IN THE BUDGERIGAR (MELOPSITTACUS UNDULATUS). S. E. Brauth, S. D. Brown, T. Park, K. Okanoya and R. J. Dooling, Dept. Psychology, Univ. of Md., College Park, MD.

Operant conditioning techniques were used to assess auditory discrimination performance in budgerigars both before and after placing lesions within Field "L" (the primary telencephalic auditory area) or within portions of the adjacent neostriatum and hyperstriatum. In addition, response latencies were analyzed using multidimensional scaling (MDS) and cluster analysis procedures in order to reveal the underlying stimulus dimensions salient to the animals making discriminations among acoustic stimuli.

The results indicate that Field "L" lesions do not affect discrimination performance by several measures for either pure tones or calls (contact calls and alarm calls). However MDS and cluster analysis reveal that these lesions alter the perceptual dimensions underlying discrimination among calls. Prior to lesioning, analysis of response latency data using MDS and cluster analysis reveals that contact calls and alarm calls are grouped into separate clusters consistent with prior work showing that stimulus characteristics correlated with call type are salient to the animals. After lesioning Field "L", the structure of the stimulus groupings is altered, indicating that telencephalic auditory stuctures are involved in determining the perceptual basis on which these calls are normally discriminated.

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247.12

SEASONAL CHANGES IN AVIAN SONG CONTROL NUCLEI WITHOUT SEASONAL CHANGES IN SONG REPERTOIRES. **E. Brenowitz, D. Kroodsma, B. Nalls, and J. Wingfield, Depts Psych & Zool, Univ. Wash., Seattle, WA 98195 and Dept. Zool, Univ. of Mass., Amherst, MA 01003.**

In canaries neural song control regions (SCRs) are larger in spring than in fall (Nottebohm 1981). This may relate to development of a new song repertoire each spring. An alternative hypothesis is that SCR changes result solely from changes in androgen levels. We tested predictions of this in rufous-sided towhees since their song repertoires are constant between seasons (Ewert 1978).

Towhee males were exposed either to long (n = 10) or short days (n = 10) for 1 month. Blood samples were analyzed for testosterone (T) and estradiol (E). We measured the volumes of 5 song nuclei (HVc, RA, MAN, Area X, and nXII) and of 2 visual nuclei (Rt, Pt) in their brains.

Ratios for Long:Short day groups were: HVc=1.68*, RA=1.54*, X=1.62*, MAN=1.01, nXII=1.19*, Rt=0.96, Pt=1.07*, brain wt.=0.98, testes wt.=50*, T level=14.5, E level=1.76 (* P < .05, t-test). The results indicate that dramatic seasonal changes in SCR size may occur without associated changes in song repertoire.

247.13

THE RELATIVE CONTRIBUTIONS OF THE LEFT AND RIGHT SIDES OF THE INTACT SYRINX TO BIRD SONG. Roderick A. Suthers and Rebecca S. Hartley*, School of Medicine and Department of Biology, Indiana University, Bloomington, IN 47405.

In order to assess the degree of lateral vocal dominance when both sides of the syrinx are able to function normally, we used a pair of heated microbead thermistors to measure the rate of airflow through each side of the intact syrinx of the brown thrasher (Toxostoma rufum, family Mimidae) during song. About 80% of the song syllables studied were produced while air flowed through both sides of the syrinx. In some of these cases the bilateral pattern of airflow was similar, suggesting that both sides were making a similar contribution to the vocal output. In other cases flow was markedly different on the right vs the left side--indicating independent motor control with unequal or varying contributions from each side. About 10% of the syllables were produced only by the left and a similar proportion only by the right side of the syrinx, the contralateral side being closed as indicated by zero airflow despite a positive subsyringeal pressure. In these instances sound must be produced entirely by the side through which air is flowing. Repetition of the same syllable, as occurs in couplets, is accompanied by repetition of the same flow pattern. Although mimic thrushes such as the catbird (Dumetella carolinensis) show a greater song deficit after section of the left compared to the right tracheosyringeal nerve, data on airflow through the intact brown thrasher syrinx suggest a complex bilateral coordination in which both sides are more or less equal partners. (Supported by grant BNS 87-20192 from N.S.F.).

247.14

CHANGED DAYLENGTH CAUSES CHANGES IN DENDRITIC STRUCTURE IN A SONG-RELATED BRAIN REGION IN RED-WINGED BLACKBIRDS. K. M. Hill, D. E. Kroodsma* and T. J. DeVoogd, Depts. of Psych. and Neurobiology and Behavior, Cornell Univ., Ithaca, NY 14853

The production of birdsong is controlled by a well-demarcated system of brain nuclei including HVc, RA, nXII, MAN and Area X. Previous work has indicated that seasonal changes in singing behavior are associated with changes in the sizes of song control nuclei (Nottebohm, '81). This suggested that the capacity to modify song seasonally might be associated with adult plasticity in the neural substrate for song. In support of this hypothesis, seasonal changes in HVc and RA volume have also been observed in red-winged blackbirds (Kirn et. al, '89).

To better understand the basis for these seasonal changes in structure, microanatomical differences were quantified in RA. Male red-winged blackbirds were lab-reared and maintained under a normal circadian cycle until their second spring. Half were then sacrificed and half were placed on short days. After 4 weeks, these were also sacrificed and brain tissue from all the birds was stained using a Golgi-Cox procedure. RA volume was reduced by 26% in short-day birds ($p < .01$), replicating our earlier finding. Dendritic structure of four neurons from each animal were quantified using a computer microscope system. The density of dendritic spines was reduced by more than 20% in the short-day birds ($p < .05$). The summed length of dendritic branches was reduced by 22% ($p < .05$). Together, these results indicate a loss of at least 1/3 of the spine synapses on these neurons as a result of the altered environment.

247.15

***NUCLEUS OVOIDALIS* OF THE RING DOVE: NON-CLASSICAL PROJECTIONS AND ELECTROPHYSIOLOGICAL RESPONSES TO NEST COOS** S.E. Durand*, M.-F. Cheng, & J.M. Tepper, Institute of Animal Behavior and Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark, NJ 07102.

Studies of avian vocal communication have provided data conducive to an integrated analysis of neuronal function and behavior. Most of these studies have utilized species of vocal learners. However, the Ring Dove, *Streptopelia risoria*, provides an excellent model for analysis of neuronal discrimination of individually variable vocal patterns in a non-vocal learner. Individuals of both sexes discriminate each other on the basis of prior social interaction and it has been physiologically demonstrated that a female dove can acoustically discriminate her own nest coo vocalization from those of other females. In avian species, examination of the neural bases of discrimination of individually specific vocal features has been restricted to the telencephalon. The nucleus *Ovoidalis* (Ov) is a prime candidate for a diencephalic site contributing to vocal discrimination.

Single unit responses were recorded from Ov to multiple presentations of male and female nest coos and pure tones in urethane anesthetized doves of both sexes. Recording sites were marked by iontophoresis of Pontamine Sky Blue and verified in Nissl stained sections. A number of units in both male and female subjects have exhibited discriminatory responses that appear to correlate with the gender of the call presented; i.e., some units fired preferentially to either male or to female nest coos. The degree of response selectivity correlated with a neuron's response to pure tones ranging from 100 Hz to 1 kHz: the more highly selective units either showed no response to pure tones or exhibited a preferred frequency below the fundamental frequency of the nest coo vocalization (~500 Hz).

In conjunction with the electrophysiological studies, *Phaseolus vulgaris* leucoagglutinin was used to map the projections of Ov more precisely than has been possible with techniques used previously. Thus far, injections have been into ventral Ov, with possible spread of the tracer into immediately adjacent regions of the nucleus *Subrotundus*. Labelled fibers have been identified in the ventral hyperstriatum, parolfactory lobe and in the paleostriatum, in addition to the classic projection area, Field L. A descending projection from Ov into the region of the hypothalamus has also been tentatively identified. Supported by MH45286, NSF BNS828241, the Rutgers University Research Council, and the Busch Fund.

247.16

LIMB MOVEMENTS IN CHICK MOTOR BEHAVIORS: VARIABILITY AND CONSTRAINTS. A. Bekoff and R. M. Johnston, EPO Biology Dept., University of Colorado, Boulder, CO 80309

This study was designed to identify constraints on chick leg motor patterns to determine which features appear to be most tightly controlled. We have done this by examining variability in temporal and spatial features of both kinematic and electromyographic (EMG) records. In addition, we have compared three different locomotor behaviors to determine whether there are consistencies across behaviors.

Data were collected from 0- to 4-day old chicks during walking, swimming or airstepping. For kinematic analysis, chicks were videotaped and the hip, knee and ankle joints digitized. Cycle period, duration and latency of extension and flexion at each joint, minimum and maximum angles, joint excursions and phase of onset of flexion and extension at each joint were calculated from the digitized data. For EMG analysis, electrodes were implanted in hip, knee and ankle muscles. Records were digitized and cycle period, burst duration, latency, interburst interval and phase of burst onset were calculated for each muscle. Coefficients of variation were calculated for all variables.

Our analyses of the kinematic data indicate that temporal features (cycle period, duration, latency) are more variable than spatial features (angle, phase). Furthermore, these results are consistent across all three locomotor behaviors. Preliminary analyses of the EMG data indicate similar findings. These results indicate that aspects of form, or spatial features, are more tightly controlled than temporal aspects of the patterns.

Supported by NIH grant NS 20310.

247.17

FEMALE PREFERENCE FOR LOWER FREQUENCY MATING CALLS IN THE FROG PHYSALAEMUS PUSTULOSUS: SEXUAL SELECTION THROUGH DIFFERENTIAL SENSORY EXCITATION. **J. H. Fox, M. J. Ryan*, W. Wilczynski, and A. S. Rand*. Depts. of Psychology and Zoology, Univ. of Texas, Austin, TX 78712 and STRI, Panama.**

P. pustulosus has a mating call consisting of a whine, followed by 0-6 "chucks". Approximately 90% of the chuck energy falls within the sensitive range of the inner ear's basilar papilla (BP). Females prefer calls with lower frequency chucks. Our study examines the hypothesis that this preference results simply from greater BP excitation.

Audiograms were determined for 5 animals through multiunit recording in the torus semicircularis. Audiograms were averaged and truncated below 1.5 kHz to yield a filter function expressing average BP tuning (BF= 2.13 ± 0.07 kHz). Normal (mean dom freq=2.55 ± 0.06 kHz) and frequency-shifted FFTs from chucks of 54 males were integrated against the filter function to yield relative sensitivity. As hypothesized, sensitivity was maximized as FFT frequencies were lowered ($t=2.0$, $p<.025$) by 1.8 ± 0.9%.

Audiograms for P. coloradorum (N=7; BF=2.23 ± 0.13), which like most Physalaemus species does not produce a chuck, were compared to those of P. pustulosus. BP tuning does not differ significantly ($t=0.63$, $p>0.5$), suggesting that BP tuning characteristics preceded evolution of the chuck. We propose, therefore that males have evolved chucks so as to exploit pre-existing female sensory characteristics and that these characteristics furthermore select for lower frequency chucks. (Funded by NSF BNS 8606289)

247.18

TESTOSTERONE EFFECTS ON MOTOR BEHAVIOR AND DOPAMINE BINDING IN RANA PIPIENS. **W. Wilczynski, J. L. Knoll*, H. B. Anderson*, S. A. Castner*, and R. E. Wilcox. Depts. of Psychology and Pharmacology, Univ. of Texas, Austin, TX 78712.**

Sex steroids increase striatal dopamine D2 receptor binding in mammals, and in so doing influence motor behavior (Becker & Beer, Behav. Brain Res., 19:27-33, 1986). This has important implications for animals manifesting seasonal reproductive behavior coincident with increases in circulating sex steroids. We investigated testosterone's effects in such a seasonal breeder, the leopard frog. Adult males were anesthetized, gonadectomized, and implanted intraperitoneally either with 5 mg of testosterone propionate (T) in silastic capsules or with empty capsules. After 2-4 weeks, motor responsiveness was assessed by measuring the latency to postural recovery from a 10 min cold (3°C) challenge. T implanted frogs recovered much faster: mean latency was 99 sec (SD=11.3) vs. 231 sec (SD=36.8 sec) for the sham implanted frogs. Normal unoperated males recovered with a mean latency of 182 sec. After testing, frogs were anesthetized by cold narcosis and rapidly decapitated. Brains were removed rostral to the midtectum, homogenized, and assayed for D2 binding levels via standard techniques (Wilcox et al., Brain Res., 443:190-198, 1988) using 0.5 nM 3H-spiperone as the radioligand. Preliminary results suggest that T implants increased D2 binding by approximately 30% compared with gonadectomized sham-implanted controls. (We thank A. Hesslin and M. Bordelon for assistance. Supported by NSF grant BNS 8606289.)

247.19

CORRELATIONS BETWEEN ADVERTISEMENT CALL CHARACTERISTICS AND LARYNGEAL AND AUDITORY ANATOMY IN THREE POPULATIONS OF CRICKET FROGS (ACRIS CREPITANS). B.E. McClelland, W. Wilczynski and M.J. Ryan. Depts. of Psychology and Zoology, Univ. of Texas, Austin, TX 78712.

Previous studies indicate that the production and reception of species-typical vocalizations may have coevolved in cricket frog (Acris crepitans) populations, resulting in an acoustic match between sender and receiver which may promote local mate preference and speciation events (Sci. 240: 1786-1788). This study explores the relationships between some spectral and temporal advertisement call characteristics of individuals, and the size of the anatomical features responsible for the production and reception of vocalizations in allopatric populations from Sabine (SA), Conroe (CON) and Balmorhea (BA), Texas. Vocalizations from 30 individuals were analyzed, and serial sections of the head region enabled the snout-vent length, head width, tympanic membrane diameter, arytenoid cartilage, vocal cord, laryngeal muscle, middle ear, inner ear and the extra columellar cartilage volumes to be measured. The dominant frequencies of the three populations are significantly different from each other (Student's t-test, $p<.01$) and decrease from east to west (SA=4.03, CON=3.53, BA=2.78 kHz). Dominant frequency is negatively correlated ($p<.05$) with the laryngeal and ear anatomy which suggests that the vocal and auditory systems change in concert across the populations. Within populations, however, the relationships between morphology and dominant frequency are more complex. Although within populations some significant correlations exist between the size of laryngeal and auditory anatomy with both dominant frequency and some temporal characteristics of the final call, we observed no consistant trends. (Research supported by NSF BNS 8606289.)

247.20

SEXUAL BEHAVIOR AND 2-DEOXYGLUCOSE UPTAKE IN MALE RED-SIDED GARTER SNAKES (THAMNOPHIS SIRTALIS PARIETALIS). **E. E. Allen and D. Crews, Inst. Reproductive Biol., Zoology Dept., Univ. of Texas, Austin, TX 78712.**

The anterior hypothalamus/preoptic area (AH/POA) plays a critical role in the expression of sexual behavior in all male vertebrates studied to date. While lesions of this area disrupt courtship behavior in male garter snakes, neural activity of this region during sexual activity has not been studied. Male garter snakes court females intensely and synchronously for the first month after emergence from hibernation. In this study males recently emerged from hibernation were injected subcutaneously with ^{14}C-labeled 2-deoxyglucose (2DG) and exposed to attractive females. Patterns of 2DG accumulation throughout the brain were examined in 7 males displaying vigorous sexual activity and in 6 males exhibiting no courtship behavior. Accumulation in the AH/POA, nucleus sphericus (NS), septum (SP), and optic tract (OTR) was measured. Shown below are percent increases (+/- S.E.M.) relative to the OTR and the significance levels (T-test) of the differences between courters and non-courters.

	AH/POA	NS	SP
Courters	83.3 (30.1)	74.6 (25.1)	57.6 (19.6)
Non-courters	35.5 (10.7)	55.3 (17.1)	49.8 (35.0)
P	.003	.09	.33

These studies illustrate the utility of the 2DG technique for elucidating the neural mediation of complex, species-typical behaviors in reptiles.

Supported by NIH 41770, Research Scientist Award 00135 to D. Crews, and NICHD training grant HDO7264.

248.1

PROSEIZURE-ANTISEIZURE ACTIVITY OF CALCIUM CHANNEL AGONIST ANTAGONISTS. M.A.Moron and T.L. Yaksh. Pharmacol. Dept., Mayo Clinic, Rochester, MN 55905.

The proseizure-antiseizure relationship of calcium channel agonist-antagonists was examined in a conscious rat seizure model in which the EEG was continuously monitored and drugs were administered intracerebroventricularly (icv) This agonist-antagonist relationship is thought to be mediated via interactions at sites that regulate neuronal calcium channels. The dihydropyridine calcium channel antagonist (CCA) analogue, BAY k 8644, has calcium channel agonist activity. This agent produces convulsive-like behavior after icv administration (Shelton et al., Brain Res. 402:399,1987), however EEG evidence that this behavior is seizure activity is lacking. The present studies demonstrate agonist EEG seizure activity and reversal by CCAs via 3 approaches, 1)effects of agonist on behavior/EEG and reversal by CCAs--BAY k 8644 produced convulsive behavior, but not EEG seizure activity; 2)the stereospecific ability to lower pentylenetetrazole (PTZ) seizure threshold-- (+) BAY k 8644 has the greatest agonist activity and ability to reduce PTZ seizure threshold; 3)stereospecific reversal of 2) by CCAs--readily demonstrated. The results of this investigation show that the proseizure-antiseizure activity of calcium channel agonist-antagonists is similar to interactions at sites that requlate calcium channels, suggesting a possible site of seizure regulation.

248.2

SIMILARITIES BETWEEN DEXTROMETHORPHAN (DM) AND SIGMA SITES IN GUINEA PIG, RAT AND MOUSE BRAIN. M. Klein*, J. J. Paturzo* and J. M. Musacchio. (SPON: R. U. Margolis). Dept. of Pharmac., N.Y.U. Med. Ctr, New York, NY, 10016.

DM, a non-narcotic antitussive binds to specific high-and low-affinity sites in the guinea pig and the rat brain. In the mouse, DM binds to two sites, differentiated only by competing drugs. In all three species, several sigma ligands bind to the DM high-affinity site. Their rank order of potency, as defined by the respective Ki values, is similar to that for sites labeled with [^{3}H](+)-3-(3-hydroxyphenyl)-N-(1-propyl)piperidine ((+)-3-PPP). The (+)-isomers of benzomorphans display higher affinity than the (-)-isomers in all three species. [^{3}H](+)-3-PPP binds to high- and low-affinity sites in the guinea pig, but to one site only in the rat and the mouse with Kd values of 70 and 20 nM respectively. Moreover, in the guinea pig, DM- and (+)-3-PPP- high-affinity binding are both displaced by several drugs with identical affinities, and are both allosterically modified by the anticonvulsants ropizine and phenytoin (Musacchio, et al, Mol. Pharmac. 35:1, 1989). This suggests that σ ligands bind to a high affinity DM site. Computer-assisted analysis of cross-displacement studies confirm this hypothesis in all three species. This work was supported in part by USPHS grants NS-23926, DA-02013, MH-29591 and MH-17785.

248.3

AUTORADIOGRAPHIC VISUALIZATION OF THE DIFFERENTIAL EFFECTS OF ROPIZINE ON DEXTROMETHORPHAN (DM) BINDING IN THE GUINEA PIG BRAIN: ENHANCEMENT OF BINDING IN SOME STRUCTURES, AND DISPLACEMENT IN OTHERS. P. Canoll* and J. M. Musacchio. Pharmacol. Dept., N.Y.U. Med. Ctr., New York, NY, 10016.

DM, an antitussive with anticonvulsant activity, binds to high and low affinity sites in guinea pig brain. Autoradiographic studies demonstrated that [^{3}H]DM binds to discrete structures throughout the brain with a distribution strikingly similarity to that of sigma ligand (+)-3-(-3-hydroxyphenyl)-n-(1-propyl)piperidine ((+)-3-PPP) (Gundlach et al, J. Neurosci., 6:1757, 1986). Ropizine, an experimental anticonvulsant, allosterically enhances both (+)-3-PPP and DM binding to brain homogenate. This effect is biphasic, with an inhibitory component at higher concentrations (Musacchio et al, Mol. Pharm., 35:1, 1989). Ropizine, 10 μM, specifically enhances the [^{3}H]DM binding to the cranial motor nerve nuclei, Purkinje cell layer, granular cell layer of the dentate gyrus, pyramidal cell layer of the hippocampus, presubiculum, and other structures, but selectively displaces binding from the dorsal raphe nucleus, dorsal tegmental nucleus, substantia nigra, inferior colliculus, and other structures. These findings demonstrate that ropizine can be used to differentiate the DM sites into distinct subsites with different anatomical distributions. This work was supported in part by USPHS grants NS-23926, DA-02013, MH-29591 and MH-17785.

248.4

ANTICONVULSANT ACTIONS OF MK-801 ON THE LITHIUM-PILOCARPINE MODEL OF STATUS EPILEPTICUS IN RATS. G.C. Ormandy*, R.S. Jope and O.C. Snead. (Spon: J. Monti), Depts. of Pharmacology and Pediatrics and Neuropsychiatry Research Program, Univ. of Alabama, Birmingham, AL 35294.

MK-801, a non-competitive NMDA receptor antagonist, was tested for anticonvulsant effects in rats using two seizure models, coadministration of lithium and pilocarpine and administration of a high dose of pilocarpine. Three major results are reported. First, pretreatment with MK-801 produced a dose-dependent anticonvulsant action with the lithium-pilocarpine model but not with rats treated with pilocarpine alone suggesting that different biochemical mechanisms control seizures in these 2 models. Second, the anticonvulsant effect of MK-801 in the lithium-pilocarpine model only occurred after initial periods of seizure activity. This demonstrates in vivo that MK-801 binding requires agonist-induced opening of the NMDA receptor channel. Third, administration of MK-801 30 or 60 min after pilocarpine i.e. during status epilepticus, gradually reduced electrical and behavioral seizure activity and greatly enhanced the survival rate. These results indicate that NMDA receptor activation plays an important role in status epilepticus and brain damage in the lithium-pilocarpine model. This was further supported by results showing that non-convulsive doses of NMDA and pilocarpine were synergistic resulting in status epilepticus and subsequent mortality. Supported by USPHS grants MH-38752 and NS-26165.

248.5

PRENATAL EXPOSURE TO VALPROIC ACID REDUCES CONVULSIVE BEHAVIOR IN ADULT RATS CHALLENGED WITH PENTYLENETETRAZOL. W.J. Pizzi, L. Morris* and R. Jersey*. Department of Psychology, Northeastern Illinois Univ., Chicago, IL 60625.

The literature on antiepileptic drugs has suggested that prenatal exposure to these agents may alter seizure behavior in adult animals. In this study, we examined the effects of prenatal exposure to valproic acid (VPA) on the seizure profile in adult rats challenged with a 50 mg/kg i.p. dose of pentylenetetrazol (PTZ). Pregnant rats were dosed orally from gestational days 9-18, and allowed to proceed to term. Prenatally-exposed animals were challenged with PTZ as adults (minimal age was postnatal day 90). Convulsive behavior was quantified using a 7-point rating scale, with a score assigned every 30 seconds over a 7-minute test period. All VPA-exposed animals in both the original experiment and a replication showed a protection effect which manifested itself as fewer periods of seizure activity over the test period. The PTZ invariably caused a maximal convulsion in both VPA and control animals; however, the VPA-exposed animals appeared to be more efficient than controls in terminating the convulsive episodes.

248.6

ETHOSUXIMIDE AND VALPROIC ACID INHIBIT ACETYLCHOLINE-INDUCED EXCITATION OF SPINAL CORD NEURONS IN CELL CULTURE. A.W. Wamil*and M.J. McLean. Department of Neurology, Vanderbilt Univ. Med. Ctr., Nashville, TN 37212.

Ethosuximide (ES) and valproic acid (VPA) are useful in the treatment of generalized absence seizures. Using conventional intracellular recording techniques, we observed effects of ES and VPA on responses of spinal cord neurons in monolayer cell culture to acetylcholine (ACh, 10^{-6} to 10^{-4}M) and N-methyl-D-aspartate (NMDA, 10^{-5} to 10^{-3}M). Neurons were superfused with phosphate buffer containing 7-10 mM Mg^{++} to suppress spontaneous activity. Brief applications of ACh or NMDA by pressure ejection from a patch clamp pipette near the impaled neuron produced depolarization and firing of action potentials for up to 30 sec. Co-application of either ES (10^{-4} to 10^{-3}M) or VPA (6-120 uM) reduced or abolished responses to ACh reversibly. Phenytoin (PT, 10 uM) did not affect the responses to ACh. Responses to NMDA were not blocked by ES, VPA or PT. High frequency firing of action potentials elicited by 400 msec depolarizing current pulses applied through the recording electrode was limited to a few spikes at the onset of the current pulse by VPA and PT, but not ES. The ability to block postsynaptic responses to ACh, but not the excitatory amino acid neurotransmitter analog, NMDA, may contribute to the anticonvulsant efficacy of ES and VPA. (Supported by a Mallinckrodt Scholarship and NIH Grant NS 01194 to MJM and a Cissy Patterson Fellowship to AWW.)

248.7

CARBAMAZEPINE BLOCKS NEITHER EPILEPTOGENESIS NOR LONG-TERM POTENTIATION INDUCED IN RAT HIPPOCAMPAL SLICES. S. Clark, A.C. Bragdon and W.A. Wilson, Depts. of Pharmacology and Medicine (Neurology), Duke Univ. and V.A. Medical Centers, Durham, N. C. 27710.

We have investigated the effect of carbamazepine (CBZ) on two models of neural plasticity: (1) stimulus-induced electrographic seizures (EGSs) and (2) long-term potentiation (LTP).

EGSs are induced by a series of kindling-like stimulus trains delivered every 10 minutes to the Schaffer collateral pathway. The first few trains evoke few afterdischarges. Later trains evoke longer, biphasic activity, culminating in EGSs with a tonic-like phase followed by a clonic-like phase. We have previously reported that CBZ (≥4ug/ml) can either suppress the tonic phase of EGSs or inhibit them entirely. This action of CBZ is consistent with its anticonvulsant potential. In contrast, we now report that similar CBZ concentrations do not appear to prevent the induction of the EGSs. In some slices CBZ did mask the expression of the EGSs during induction, but after the drug was washed off, fully induced ESGs could be evoked. This implies that CBZ does not have antiepileptogenic action.

LTP was induced by a standard protocol. We found that CBZ does not prevent the induction of LTP.

Therefore, CBZ does not appear to inhibit the process of induction in either model of neural plasticity.

248.8

ANTICONVULSANT EFFECTIVENESS OF CARBAMAZEPINE IN THE COBALT-EPILEPTIC RAT. Charles R. Craig and Brenda K. Colasanti. Dept. of Pharmacol./Toxicol., WVU Health Sciences Center, Morgantown, WV 26506

Adult female Sprague-Dawley rats rendered epileptic by bilateral cerebral implantation of cobalt wire were simultaneously prepared with permanent cortical and temporalis muscle electrodes for continuous recording of EEG and EMG activities. Carbamazepine (50 or 100 mg/kg) in 10% PEG was administered intraperitoneally twice daily for 4 days commencing 8 days after cobalt placement. Cobalt-epileptic rats either not treated or administered the highest volume of vehicle and normal rats administered the highest dose of carbamazepine served as controls. Results indicated that rats receiving carbamazepine, 100 mg/kg, exhibited a reduced frequency of seizures over the 4 day period of drug administration. Although rats receiving carbamazepine, 50 mg/kg did not show a reduction in seizures over any 24 hr period, a definite reduction in seizure frequency was observed during the six-hour period following drug administration during the first two days of drug administration. (Supported by NIH NINDS Grant # NS 20226).

248.9

PHENOBARBITAL REDUCES POST-SYNAPTIC POTENTIALS AND CALCIUM CURRENTS IN RAT NEOCORTICAL NEURONS. I. Spigelman and P. L. Carlen. Playfair Neuroscience Unit, Toronto Western Hospital, Toronto, Ontario, Canada.

The actions of phenobarbital were examined using current- and voltage-clamp recording techniques on neurons in layers II & III of rat (Wistar) neocortex. In current-clamp recordings using electrodes filled with 3 M $KMeSO_4$, evoked exitatory postsynaptic potentials (EPSPs) were observed at resting membrane potentials, whereas depolarizing current injections (DC) were required to observe inhibitory postsynaptic potentials (IPSPs). Phenobarbital perfusion, at a clinically relevant concentration (100 μM), reversibly reduced the size of EPSPs (n=6) and IPSPs (n=4), measured at 35°C.

In single electrode voltage-clamp mode, calcium currents were recorded with 3 M CsCl-filled electrodes in the presence of Na^+- and K^+-channel blockers. Phenobarbital application (100 μM) consistently and reversibly reduced the amplitude of the calcium currents measured at 30°C (n=8). In addition, phenobarbital application in these neurons increased an outward current evoked by the largest depolarizing voltage commands.

These findings indicate that the reduction of calcium currents by phenobarbital may be an important component of its anticonvulsant activity in the mammalian central nervous system.

Supported by the Ontario Mental Health Foundation.

248.10

CGS 18416A: A SOLUBLE,POTENT AND NOVEL ANTICONVULSANT. P.Bernard*, R.Bowman*, M.Williams, J.Lehmann, J.Liebman and R.Robson*. Res. Dept., Pharm. Div., CIBA-GEIGY Corp., Summit, NJ 07901.

A structurally novel imidazolyl alkyl benzisoxazole, CGS 18416A, was found to have potent anticonvulsant activity. This compound prevented maximal electroshock-induced seizures in rats (ED50,8.3 mg/kg po and 2.4 mg/kg i.v.) and in mice (ED50, 17.3 mg/kg po), indicating potential efficacy against grand mal seizures. Its antagonism (ED50 = 9.8 mg/kg p.o.) of clonic seizures induced by PTZ (24 mg/kg i.v.) suggests that concurrent efficacy against absence seizures may also be present. No impairment of motor function was evident at oral doses as high as 200 mg/kg in rats or 100 mg/kg in mice, indicating relatively low neurological involvement at projected anticonvulsant doses. CGS 18416A did not bind to brain benzodiazepine receptors, indicating that this is not its mechanism of action. Aconitine-induced inhibition of [3H]L-glutamate uptake was blocked by CGS 18416A, a property shared by phenytoin and indicative of interaction with sodium channels. In marked contrast to available anticonvulsant drugs, CGS 18416A is highly water-soluble, an advantage in preparing parenteral formulations for emergency intervention in status epilepticus. The high oral efficacy, low neurological involvement, possible efficacy against absence as well as grand mal seizures and high water solubility may confer significant therapeutic advantages on CGS 18416A.

248.11

KINETIC ANALYSIS OF THE ANTIEPILEPTIFORM EFFECTS OF L-BACLOFEN DURING K⁺-INDUCED INTERICTAL DISCHARGES IN RAT HIPPOCAMPUS. F.J. Lebeda, T.H. Ton' & P.A. Rutecki. Sect. Neurophysiol., Dept. Neurol., Baylor Col. Med., Houston, TX 77030.

L-baclofen was equipotent (IC_{50} *ca.* 150 nM) in abolishing spontaneously occurring interictal discharges in the CA3 subfield that were induced *in vitro* by bethanechol, picrotoxin or soman. This effect was independent of the initial discharge frequency (f_0). In contrast, baclofen was less potent (IC_{50} values up to 1979 nM) in abolishing the discharges induced by high $[K^+]_o$. We tested the hypothesis that baclofen's potency was dependent on both $[K^+]_o$ and the f_o of these K^+-induced events. Using data from slices exposed to 7.5-10 mM K^+ and discharging at an f_o between 0.3-0.6 Hz, the [baclofen]*vs.*%(f_o) curves (plotted at a given $[K^+]_o$) were shifted in parallel to the right as $[K^+]_o$ increased. Similarly, in 10 mM K^+, the [baclofen]*vs.*%(f_o) plots were shifted in parallel to the right as f_o increased from 0.6 to 1.2 Hz. Results from Schild and Dixon analyses suggested that the changes in baclofen's potency were consistent with a coupled reaction scheme - an *apparent* non-competitive reaction between baclofen and K^+, and an *apparent* competitive reaction between baclofen and an endogenous factor that promoted discharge generation.

Supported by USAMRDC DAMD-86-C-6069, AFOSR 85-0178, NIH grants NS11535 & 01049, and the Klingenstein Fund.

248.12

ATTENUATION OF EPILEPTOGENESIS BY NONSTERIODAL ANTIINFLAMMATORY DRUGS IN RAT. M.C. Wallenstein Dept. Physiology, New York Univ., NY NY 10010

Subconvulsive doses (25 mg/kg) of pentylenetetrazol (PTZ) were administered at 4 day intervals for 20 sessions to induce kindling in conscious, free-moving rats with chronically implanted electrodes. In initial sessions, PTZ produced periods of motor arrest concurrent with bursts of spike-wave activity. Over subsequent sessions, this increased to clonic convulsions concurrent with spike activity.

Pretreatment with paracetamol (150 mg/kg) or mefenamic acid (20 mg/kg), over 20 sessions, reduced both amount of myoclonus and appearance of convulsions. The initial effects, i.e. motor arrest and spike-wave activity, were not affected by either of these pretreatments. Ibuprofen (30 mg/kg) had no significant effect on any level of the induced excitation.

The results suggest a differential activity for nonsteriodal antiinflammatory drugs in attenuating progression to convulsions. Possible explanations include tissue specificity and drug-specific actions not related to cyclooxygenase inhibition.

248.13

INFLUENCE OF NORADRENERGIC MECHANISMS ON THE ANTICONVULSANT EFFECTS OF FELBAMATE AND PHENYTOIN. R. Gordon*, W. Diamantis* and R.D. Sofia* (SPON: J.L. Perhach) Wallace Laboratories, Div. of Carter-Wallace, Inc., Cranbury, NJ 08512.

A growing body of evidence suggests that catecholamines, especially norepinephrine (NE), exert an inhibitory influence on seizure development. The purpose of this study was to determine the influence of noradrenergic mechanisms on the anticonvulsant activities of felbamate (F) and phenytoin (PH) against maximal electroshock seizures (MES) in mice. Phenoxybenzamine (30 mg/kg i.p.) and prazosin (0.15 mg/kg i.p.) significantly reduced the anticonvulsant effects of orally administered F and PH, whereas yohimbine (10 mg/kg i.p.) did not appear to influence the protective effects of F and PH. The tyrosine hydroxylase inhibitor, α-methyl-p-tyrosine (250 and 100 mg/kg i.p.) antagonized the effects of F and PH against MES. Desipramine (15 mg/kg i.p.) significantly increased the anticonvulsant activity of F but not of PH.

These results indicate that the antagonism of α_1-adrenergic receptors reduces the anticonvulsant effects of F and PH against MES, whereas blocking α_1-adrenoceptors suggests an opposite effect. Inhibiting NE synthesis also appears to reduce the anticonvulsant effects of F and PH. Finally, increasing NE availability enhances the anticonvulsant activity of F but not of PH.

248.14

ANTICONVULSIVE PROPERTIES OF TETRONIC ACID DERIVATIVES IN RAT HIPPOCAMPAL AREA CA1 AND CA3 IN VITRO.
G. Köhr* and U. Heinemann* (SPON: European Neuroscience Association) Institute for Neurophysiology, University of Cologne, Robert-Koch-Straße 39, D-5000 Köln 41, FRG

In the hippocampal slice preparation the two tetronic acid derivatives AO33 and AO78 blocked epileptiform activity which was induced by picrotoxin (PTX) or by perfusion with Mg^{2+}-free and Ca^{2+}-free medium, respectively. The slow negative field potentials and $[Ca^{2+}]_o$-decreases in PTX and in low Mg^{2+} during repetitive stimulation of either the Schaffer collateral/commissural fibers or the alveus were reduced in a dose-dependent manner. The drugs were applied in concentrations between 10 and 100 µM.

Intracellular studies showed that the resting membrane potential of CA1 cells didn't change by more than 3 mV (n=18), the input resistance was slightly decreased, but the slope conductance was reversibly reduced by AO33, especially when the membrane potential was deplarized by current injection. The threshold to evoke action potentials in CA1/CA3- and bursts in CA3-neurons seemed to be little increased. The amplitude and duration of the action potentials were unchanged. Afterhyperpolarizations which followed depolarizing current pulses were diminished by AO33. In all experiments AO33 increased the accomodation of pyramidal cell discharges. Because of missing effects on the low- and high-threshold Ca^{2+} currents, we suggest that AO33 and AO78 act as K^+ channel agonists.
(Supported by Dr. Willmar Schwabe, Karlsruhe)

248.15

EFFECTS OF ANTICONVULSANTS ON PENTYLENETETRAZOL INDUCED NEURONAL ACTIVITY OF GIANT AFRICAN SNAIL *ACHATINA FULICA* FERUSSAC. M.C.Tsai, M.L.Chen* Pharmacological Institute, College of Medicine, National Taiwan University, Taipei, Taiwan, R.O.C.

Effects of phenobarbital, succinimide and valproic acid on pentylenetetrazol (PTZ) induced neuronal activity were studied electrophysiologically on the central neuron of giant African snail *Achatina fulica* Ferussac. The neuron tested was located in the upper part of right parietal ganglion. The neuron showed a tendency of spontaneous firing of action potential. The frequency of action potential was increased if glutamic acid was added.After PTZ perfusion, the neuron produced a phasic depolarization followed by a sustained depolarization with a characteristic bursting firing pattern of action potential. The response strongly resembled the PTZ induced changes in the mammalian cerebral cortical neuron. The bursting firing pattern induced by PTZ was not affected by *d*-tubocurarine, tetrodotoxin or atropine. However, the frequency of the burst firing of neuronal action potential was decreased after phenobarbital or succinimide (2,5-pyrrolidinedione) or valproic acid administration. PTZ was a useful laboratory tool for screening anticonvulsants. Our results suggest that the decreasing of the frequency of bursting firing action potential may be associated with anticonvulsant activity.

248.16

STUDIES OF POTENTIAL ANTICONVULSANTS IN SOMAN POISONING. T.-M. Shih, T. Koviak* and B. Capacio*. U. S. Army Med. Res. Inst. Chem. Def., Aberdeen Proving Ground, Md 21010.

The acute toxic effects of the organophosphorus cholinesterase inhibitor soman include tremors, convulsions and death. The purpose of this study was to evaluate various classes of compounds for their efficacy in preventing soman-induced convulsions. They include benzodiazepines (diazepam, midazolam, clonazepam, loprazolam, alprazolam), the NMDA blocker MK-801, barbiturates (phenobarbital, pentobarbital), calcium channel blockers (flunarizine, nifedipine), anticholinergics (aprophen, atropine sulfate and methyl nitrate, azaprophen, benactyzine, benztropine, biperiden, scopolamine hydrobromide and methyl nitrate, trihexyphenidyl) and others (phenytoin, cabamazepine, valproate, caramiphen, Organon 6370 and Norwich F-721). Male rats were injected with the oxime HI-6 (125 mg/kg, ip), to increase survival time, along with various doses of these compounds (im) with or without atropine sulfate (ATS; 16 mg/kg, im) 30 minutes prior to a soman challenge dose (1.6 LD50; 180 ug/kg, sc) that produced 100% convulsions. Without ATS, only tertiary anticholinergics, MK-801 and caramiphen were effective anticonvulsants. In the presence of ATS, benzodiazepines became effective; e.g., the anticonvulsant ED50s for diazepam and midazolam were 136 and 192 ug/kg respectively. These findings indicate that certain classes of compounds are potent anticonvulsants in soman poisoning either alone or in the the presence of ATS.

VISUAL PSYCHOPHYSICS AND BEHAVIOR II

249.1

SELECTIVE ATTENTION MODULATES VISUAL PROCESSING OF FORM, COLOR AND VELOCITY: I. PSYCHOPHYSICAL OBSERVATIONS. F.M.Miezin*, M.Corbetta*,G.M.Shulman*,&S.E.Petersen. (SPON: T.O.Videen). Dept. Neurology & Neurol.Surg., McDonnell Center for Higher Brain Function, and Mallinckrodt Inst. of Radiology. Wash. Univ. Sch. of Med., St. Louis, MO. 63110.

Psychophysical data were collected during a match-to-sample task in which subjects (n=11) fixated on a small stationary spot in the center of an array of coherently moving rectangles. The rectangles all had the same shape and color and were randomly distributed against a dark background. Three of the stimulus attributes were varied during the experiment: shape, velocity & color. The moving array was displayed for 400 ms. followed by a blank black screen for 200 ms. The array reappeared for a second 400 ms. interval with 1, 2, 3 or none of the stimulus attributes changed. During the 1.5 sec. intertrial interval, the subject indicated whether the two presentations were the same or different. Stimuli were presented in blocks during which the subject was instructed to either attend to one of the attributes and report changes in it, or to attend to all three attributes and report changes in any of them. The subject's sensitivity, d', was determined for each attention condition. In each subject, before formal testing started the slight supra-threshold discriminative changes to be used for each dimension were assessed such that the subject's average performance was about 1.6 d' units (80% correct responses, 20% false alarms).

Subjects were significantly more sensitive to stimulus changes when they attended to only one attribute compared to when they simultaneously attended to all attributes (mean d' change = .9, $p < .001$). Sensitivity to velocity change was reduced when shape simultaneously changed (d' change = .6, $p < .056$) but not for color changes. Color discrimination however was not affected by variations in shape or velocity. These data suggest that selective attention modulates the visual processing of the simple visual attributes of form, color and velocity and that there is crosstalk or interference between the processing of at least 2 of the attributes.

249.2

SELECTIVE ATTENTION MODULATES VISUAL PROCESSING OF FORM, COLOR, AND VELOCITY: II. VISUAL AREAS. M.Corbetta*, F.M.Miezin*, S.M.Dobmeyer*, and S.E.Petersen. (SPON: M.I.Posner) Dept. Neurology and Neurol.Surgery, McDonnell Center for Higher Brain Function, and Mallinckrodt Inst. of Radiology. Washington University School of Medicine, St.Louis, MO 63110.

PET subtraction images of blood flow (BF) change were utilized to study normal subjects performing a match-to-sample task in which they had to attend and discriminate along one visual dimension (i.e. hue) while ignoring variations along other dimensions (i.e. shape or velocity)(Abstr. I). Two conditions were used as subtraction scans: passive presentation of test stimuli, and a divided-attention task where subjects were instructed to detect any occurring change.

Across all active conditions, BF increased in areas 17 and 18 in comparison to the passive stimulation. Area 17 (but not area 18) activation disappeared when the divided-attention scan is subtracted away, arguing for a nonspecific, arousal effect. Several extrastriate areas were modulated by attention and some of them were found only in one condition. Regions of enhanced activity along the superior temporal sulcus and area 39 were active only when attending changes in velocity. Such regions overlapped location of previously described areas related to motion detection and smooth-pursuit visual tracking. Bilaterally, the lingual and the fusiform gyri (ventro-medial area 19 and 37) and area 21 in the temporal lobe were enhanced for shape discrimination, but not for velocity discrimination. Area 21 activation was stronger on the right side, and was also present when using stationary flashing bars. Some lingual gyrus foci were also found for color-discrimination and a left lateral region in area 19 was observed only during color discrimination. There was no overlap between color- and velocity- enhanced regions.

These findings support early selective modulation in extrastriate areas during visual processing in humans.

249.3

SELECTIVE ATTENTION MODULATES VISUAL PROCESSING OF FORM, COLOR AND VELOCITY: III. AREAS RELATED TO HIGHER-ORDER SELECTIVE PROCESSES. S.E.Petersen, M.Corbetta*, F.M.Miezin*, and S.M. Dobmeyer*. Dept. Neur. and Neur. Surg., McDonnell Center for Higher Brain Function, and Mallinckrodt Inst. of Rad.. Wash. Univ. Med. Sch., St. Louis, MO.

As shown in the preceding abstracts, subjects in a divided-attention match-to-sample task are less capable of seeing a change in color, shape, or velocity than when they are attending to changes in one of these attributes. These selective changes in processing are reflected in enhanced activation of specific extrastriate areas in PET blood flow change images, e.g., different extrastriate areas are activated when attending to color change than when attending to velocity change. This enhanced activation is an effect of top-down, or higher-order, selective processes.

Two questions relating to the higher-order processes are: 1) Are there areas that are directing the early selective enhancement in the extrastriate visual areas? Candidates for such areas would be active in all three selective conditions when compared to the divided-attention condition. Several areas meet these criteria: right posterior thalamus/colliculus, right anterior thalamus/caudate nucleus, and left putamen/globus pallidus. In all cases, the activations are localized near the boundaries of two structures making a clear anatomical assignment ambiguous.

2) Are there areas that are part of a late-selection system active when an early-selective strategy along single stimulus dimensions is not useful? Such areas would be active only in the divided-attention condition, not in any of the selective-attention conditions. An anterior cingulate region fulfills these criteria, and has been active in other studies from this laboratory that demand late-selection strategies, such as verb-generation and semantic-monitoring tasks. This region appears to be a node in a general late-selection system crossing both lexical and visual tasks.

The results from this and the preceding abstracts outline brain regions that take part in both early and late selective attention processes.

249.4

TRANSPARENCY AND COHERENCE IN HUMAN MOTION PERCEPTION. G.R. Stoner*, T.D. Albright and V.S. Ramachandran* (SPON: F.H.C. Crick). Salk Institute and University of California at San Diego, La Jolla, CA 92093.

A central problem faced by motion detectors is that of integrating local motion signals. A number of criteria (e.g. relative spatial frequency, contrast, depth, speed, direction) may be used to determine whether multiple signals arise from the same moving object and should thus be combined. We have now explored the role of perceptual transparency in the integration of motion signals using the "coherence" paradigm. Subjects were required to judge whether two superimposed drifting gratings cohere, appearing as a uniformly moving plaid. We predicted that if one grating were perceived as transparent, motion signals would not be combined and we would observe a decline in coherence. We manipulated transparency by varying the relative intensities of two gratings with respect to their intersections. The "rules" of transparency dictate that optimal conditions will be achieved when the relative intensities of these different figural regions are set appropriately. Stimuli were presented at center of gaze through an 11° aperture. Grating orientation differed by 135° and both were drifted at 3°/s. Probability of coherence was greatly reduced when one grating appeared transparent. Thus the motion system has tacit knowledge of the rules of transparency, which it uses to decide whether motion signals arise from the same object. This insures that movement of transparent objects (or shadows) will not be confused with movement of objects over which they move.

249.5

OCCLUSION AND TRANSPARENCY IN MOTION PERCEPTION. V. S. Ramachandran* and D. Rogers-Ramachandran* (SPON: P. S. Churchland). Psychology Department, University of California, San Diego, La Jolla, CA 92093.

A large square-wave grating oriented 45° was moved obliquely (at right angles to its orientation) and viewed simultaneously through two narrow rectangular apertures, a vertical one and a horizontal one, cut out of an opaque circular window. Instead of seeing two independent "barber poles" (as in Wallach's experiment) subjects usually saw two holes through which they perceived a single rigidly moving grating.

Next, we cut out a single horizontal window from a transparent occluder that was superimposed on the grating. Instead of seeing horizontal motion within the window subjects always saw oblique motion. On the other hand, if the luminance contrast of different portions of the grating was adjusted so as to destroy transparency, horizontal motion was seen.

Lastly, two moving gratings of different orientations were superimposed so that they appeared to cohere and move in a single direction ("pattern motion"; Adelson & Movshon) instead of moving independently ("component motion"). We used two square wave gratings and found that if the luminance of the intersections between the two gratings was chosen appropriately the grating looked transparent and moved independently whereas if the luminance was inappropriate "pattern motion" was seen (Stoner, Albright & Ramachandran, in press).

We conclude that the solution to the aperture problem is powerfully constrained by occlusion and transparency.

249.6

MIXING DIMENSIONS IN CORTICAL VISUAL MOTION PROCESSING

Sima Shechter* and Shaul Hochstein
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Hebrew University, Jerusalem, ISRAEL.

Apparent motion is perceived when two slightly different picture frames are viewed in succession. The correspondence problem of relating the various elements in the two frames to each other is solved by the visual system with the aid of information deriving from numerous visual dimensions. Using a competitive paradigm we find that the dimensions available to the long range apparent motion system include element proximity, shape, size, luminous flux (but not luminance), and contrast polarity. Thus, information seems to be integrated in the motion processing visual areas from the form and contrast pathways, the ON and OFF pathways, and from center-surround antagonistic units. These findings restrict the classes of models of cortical parallel and hierarchical visual processing.

249.7

MOTION FIELD DETECTORS AND MOTION SEARCH-LIGHT

Jun Zhang and Siye Wu*. Neurobiology Group, University of California, Berkeley, CA 94720 and Department of Mathematics, MIT , Cambridge, MA 02139

We propose a theory of human motion perception. In this scheme, two stages of processing are involved: 1) a motion detection stage whereby motion detectors locally compute the temporal derivative of the spatial gradient of the image brightness function. 2) a motion integration stage whereby individual motion detector's responses are summed up over an area, determined by a hypothetical "motion search-light", to yield the perceived "velocity".

The local motion signal (a 2-D vector) computed by the detector is: 1) complete in that any physically extractable velocity information is preserved; 2) unique in that they are contravariant and hence independent of the particular choice of basis detector pair; 3) invariant under the addition of a background brightness pattern.

The motion search-light, reminiscent of the work by Treisman & Gelade (1980) and Crick (1984), determines the area of integration on the local motion signals. It is proved that 1) during this integration, the nearby responses cancel out so that the effective contribution is from the boundary; 2) the perceived velocity thus obtained is the correct one, so long as the search-light encompasses the entire object undergoing 2-D translational motion.

249.8

"TEMPORAL CAPTURE" AND THE ROLE OF TRANSIENCY IN VELOCITY DISCRIMINATION. S. Treue, R. J. Snowden* & R. A. Andersen. Dept. of Brain & Cog. Sci., M.I.T., Cambridge, MA 02139

Recent models of velocity perception use information about transiency in a moving stimulus. They postulate that increasing activity in a "transient" channel relative to a "sustained" channel increases perceived velocity.

This study investigated the influence of transiency on velocity perception. The stimuli consisted of dynamic random dot patterns moving at a constant velocity. Each point lasted for a preset number of frames (its lifetime) before it was randomly replotted. Decreasing the point lifetime therefore increases the transiency of the display by increasing the number of points turning on and off at any given time. Subjects were presented with two patterns (duration 400 ms) appearing left and right of a central fixation spot and were asked to indicate the faster pattern. Typically one pattern ("reference") was compared against other patterns ("comparators") moving at different speeds centered around the reference speed. These comparators were of the same dot density but in most cases the transiency was different from the reference.

The amount of transiency was varied in two ways: **I)** by changing the point lifetime and **II)** by introducing **stationary** points of very short lifetime.

The two major results were: **I)** Using lifetimes of 100 ms and longer subjects tended to perceive the patterns with **shorter** lifetimes as moving **faster**. For lifetimes lower than about 100 ms the effect reversed. At these short point lifetimes it became difficult to perceive the motion of the pattern at all. **II)** The patterns also appeared **faster** if the **moving** points of both patterns were of the same lifetime but one pattern contained a few **stationary** points of very short lifetime.

In summary our results offer support for the models mentioned above although these models cannot account for the reversal of the effect when using very short lifetimes. Interestingly the high temporal frequency of the stationary flashed dots appears to be captured by the moving dots and used in the velocity estimate, thus increasing the perceived velocity. We therefore term this effect "temporal capture".

249.9

NEURAL DYNAMICS OF VISUAL MOTION PERCEPTION: GROUP AND ELEMENT APPARENT MOTION. S. Grossberg and M. Rudd*. Center for Adaptive Systems, Boston Univ., Boston, MA 02215.

This work further develops a neural network model of motion segmentation by visual cortex that was outlined in Grossberg (1987). We illustrate model properties by simulating data concerning group and element apparent motion (Breitmeyer & Ritter, 1986; Petersik & Pantle, 1979; Ternus, 1938), including the tendency for group motion to occur at longer ISIs and under conditions of short visual persistence. These phenomena challenge recent vision models because the switch between group and element motion is determined by changing temporal but not spatial display properties. The model clarifies the dependence of short-range and long-range motion on spatial scale. Its design specifies how oriented (x cell) and unoriented (y cell) detectors cooperate and compete in successive processing stages to generate motion signals that are independent of direction-of-contrast. Ternus (1938) displays and Burt & Sperling (1981) displays generate appropriate motion signals in the circuit. The model clarifies motion after-effects and how preprocessing of motion signals is joined to long-range cooperative mechanisms to control phenomena such as induced motion and motion capture. The total model system is a motion Boundary Contour System (BCS) that is computed in parallel with the BCS of Grossberg & Mingolla (1985).

249.10

A PHYSIOLOGICAL MODEL OF BINOCULAR RIVALRY. T. J. Mueller. Dept. of Biology, *Harvey Mudd College*, Claremont CA 91711.

I present a modified reciprocal inhibition model for the temporal dynamics of binocular rivalry. The model is based on neurophysiological mechanisms and is derived from human psychophysical data. The reciprocal inhibition oscillator has long been proposed as a mechanism for binocular rivalry, but its detractors have correctly pointed out that simple reciprocal inhibition does not account for some aspects of the temporal behavior of binocular rivalry. These include the effects of contrast change on alternation rate and on the magnitudes of changes in duration of the suppressed and dominant phases.

Matsouka (1984) described a simple reciprocal inhibition oscillator with a set of four coupled differential equations having a neurophysiological interpretation. I modify those equations and their simulation to reflect three new parameters: 1) presynaptic inhibition of the reciprocal inhibition by the input, 2) the motor delays that occur when a human observer tracks rivalry and 3) a minimum threshold for each neuron's state variable. The result is a very defendable fit to psychophysically obtained data on the temporal behavior of binocular rivalry.

Finally, the model is incorporated into a larger model to suggest how rivalry can occur in a network that usually exhibits binocular fusion.

249.11

PERIPHERAL FIELD STIMULATION AFFECTS FLICKER, BUT NOT COLOR, SENSITIVITY. Z. He* and M.S. Loop. Physiological Optics, Sch. of Opt., Univ. Alabama at B'ham., Birmingham, AL 35294.

Magnocellular neurons have better temporal resolution while parvocellular neurons have better wavelength selectivity which has suggested to some that color-opponent parvocellular neurons carry the information for color vision while broadband magnocellular neurons carry the information for high frequency flicker discrimination. We have examined the effect of peripheral field stimulation upon our thresholds for color and flicker perception. Spectral increments, flickering at 25Hz, were added to a steady white background and thresholds determined for detection of stimulus color and flicker. Surrounding, but not overlapping, the background and stimulus was a peripheral field which appeared steady (70Hz) or flickering (10Hz). Surround flicker did not affect color thresholds. Surround flicker reduced flicker sensitivity when viewing was binocular or monocular but not when the flickering surround was viewed by the left eye and the background plus stimulus was viewed by the right eye. Thus neurons which carry the information for color perception are unaffected by peripheral field stimulation while neurons which carry the information for the perception of rapid flicker are affected. The effect is likely retinal in origin. Interestingly, in macaque lateral geniculate nucleus magnocellular neurons, but not parvocellular neurons, show a strong peripheral (McIlwain) effect.

249.12

TEMPORAL VISUAL FILTERS IN THE HUMAN PERIPHERAL FIELD R.J. Snowden* and R.F. Hess* (SPONS: M. Husain) Kenneth Craik Laboratories, University of Cambridge, U.K.

It has long been suggested that the periphery is somehow specialized for moving stimuli, however, more recent theories claim that it is merely a coarser version of the foveal field. In this study we present evidence that the transient aspects of a stimulus are emphasized relative to the sustained aspects as one moves to the peripheral field. We used a novel masking technique to examine changes in temporal filtering properties with respect to eccentricity. **Probe stimuli** (Gabors in space, sinusoidal in time) were presented 4 dB above threshold. Superimposed **masking stimuli** (narrow band noise in space, sinusoidal in time) were then increased in contrast until the probe was no longer visible. Stimulus size was not scaled with eccentricity.

The number of temporal filter types isolated by this technique varied with eccentricity. Three (one low-pass & two band-pass with peaks of 8 & 16 Hz) were found foveally, two (low-pass & band-pass peak 8 Hz) at 10°, and only one (band-pass peak 8 Hz) above 30° eccentricity. Variations in spatial frequency did not reveal a low-pass channel above 30° eccentricity. This trend was also reflected in the modulation transfer function which was low-pass foveally and band-pass peripherally. This suggests that the sensitivity of the low-pass channel falls more rapidly with eccentricity than that of the band-pass (8 Hz peak) channel.

These results imply that the peripheral field is not merely a 'coarser' version of the fovea but has qualitative differences that emphasize transient information. The finding of a single channel at threshold for peripherally viewed stimuli can be used to explain why rapidly flickering stimuli viewed peripherally can be matched by foveal flicker of around 8 Hz, and why moving stimuli often appear slower when viewed peripherally.

249.13

SPATIAL FREQUENCY DISCRIMINATION FOR INPHASE AND COUNTER-PHASE RED-GREEN STIMULI: EFFECT OF APPARENT MOTION. R.L.P. Vimal* and R.Pandey* (spon: P.H.Hartline) Eye Research Institute Boston, MA

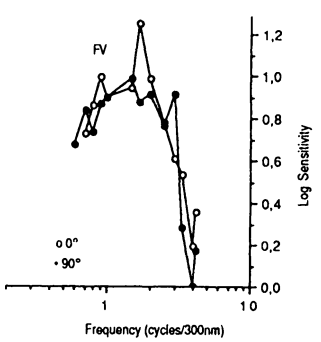
Based on the spatial (size of the stimuli) and/or spatial frequency (SF) (number of cycles/deg) criteria, the SF discrimination (SFD) thresholds to counter-phase stimuli were reported to be lower than the inphase stimuli at lower SF, but at higher SF the reverse was true[1] suggesting a possible interaction between form and color. We investigated whether this conclusion held when the criterion was apparent motion. For this purpose, high contrast spatially localized stimuli (sixth derivatives of spatial Gaussian function: $2^o \times 0.5^o$ on 6.4^o equiluminant inphase surround) were presented to two color normal observers at about 29 cd/m^2 using the Adage 3006 system. The relative luminances of red and green stimuli were determined by the minimum flicker isoluminant criterion. The method of constant stimuli with blocked two interval (each of 500 msec) forced choice procedure was used. The zero interstimulus interval (500 msec in previous study[1]) produced an apparent motion of contraction if second interval has high SF and apparent expansion if it has lower SF. The observer initiated a trial and reported the interval with lower SF using the contraction/ expansion criterion. The percent discrimination thresholds to inphase stimuli were found to be lower than that to counter-phase stimuli for all SFs. The results are consistent with the segregation of color and motion.[2] The *parvo-interblob* system may be sensitive to both achromatic and chromatic contrasts.[2] The achromatic channel might include both the *magno* system[2] as well as an achromatic component of the *parvo-interblob* systems. The stronger contribution of motion to inphase SFD may be due to the achromatic channel. The weaker contribution of motion to counter-phase SFD may be due to chromatic contrast component of *parvo-interblob* system. 1. Vimal R.L.P., Invest.Ophthalmol.Vis.Sci. (Suppl.) 29:448,1988. 2. Livingstone M.S. and Hubel D.H., J.Neurosci. 7(11):3416, 1987. (Supported by Eye Research Institute and NSERC grant to P.K. Kaiser)

249.14

RESPONSE OF HUMAN COLOR VISION TO SINUSOIDAL SPECTRAL DISTRIBUTIONS. V.Bonnardel* and F.Varela, Institute of Neuroscience, CNRS-U.Paris VI, Paris, France

We have constructed a device producing illuminants whose intensity varies sinusoidally over the visible spectrum to obtain the first measurements of the Modulation Sensitivity Function for human color vision. Frequency (13 values between 0.6 to 4.25 cycles/300 nm), contrast (through rotation of a polaroid to 0.25 deg precision), and phase (three phases, with 0 deg corresponding to maximum transmittance at 400 nm) were all under experimenter's control.

The threshold sensitivity when the baseline white became just detectably coloured were obtained. Each frequency and phase were also mapped in Munsell coordinates. The figure shows a typical result for one subject and two phases. These results can be compared with Fourier transforms of chromatic channels previously proposed.



(Supported by the Prince Trust Fund)

249.15

TWO ROD MECHANISMS IN DARK-ADAPTED VISION. P.E.Hallett*, C.S.Barnes* and M.I.Hofmann* (SPON:H.Kwan). Dept. of Physiology, University of Toronto, Toronto, ONT. M5S 1A8.

Experiments with continuous lighting have recognized three major regimes— scotopic, mesopic and photopic. In the present experiments, briefly flashed sine-wave gratings are presented to the dark-adapted peripheral retina. This procedure divides the scotopic regime into low and high luminance sections between which there is a fairly abrupt transition (at roughly -2.5 log scot td sec at 20° retinal eccentricity). Cone contributions are excluded by appropriate tests. Contrast sensitivity curves in the high rod regime are best fitted by Gaussian or Difference-of-Gaussian functions with a constant centre size ($\sigma_{centre} \cong 1.5°$). Simple Gaussians with a variable centre size ($\sigma \cong 1.5$ to $3°$) provide the best fit in the low rod regime, size increasing with decreasing flash energy. A comparison of brief flash results with long duration or flickering gratings suggests that vision in the low rod regime does not depend on stimulus duration, but may be impaired for brief flashes in the high rod regime.

249.16

LOSS OF SHORT-WAVE CONE SENSITIVITY AND POSSIBLE ACCELERATED AGING ASSOCIATED WITH CHRONIC EXPOSURE TO ULTRAVIOLET RADIATION J.S. Werner, V.G. Steele* and D.S. Pfoff*
Department of Psychology, University of Colorado, Boulder, CO 80309

The crystalline lens of the human eye absorbs most of the incident ultraviolet radiation (UVR), but when the lens is removed, this radiation can reach the photoreceptors. The consequences of UVR-exposure on cone receptor sensitivity were determined from psychophysical measurements in eight patients who had undergone bilateral cataract extraction and implantation of intraocular lenses. The lens implanted in one eye contained chromophores that absorb incident UVR, while the lens implanted in the other eye transmitted UVR. Two-color increment thresholds were measured to determine the spectral sensitivities of isolated cone mechanisms. Five years of differential exposure to ambient UVR was associated with a selective loss in sensitivity of the short-wave cone photoreceptors, although visual acuity and sensitivities of middle- and long-wave sensitive cones were not significantly different for the two eyes. These losses in short-wave cone sensitivity associated with UVR may be similar to losses in sensitivity that occur in normal aging. Using our sample of 76 normal subjects (ages 10-84 years) as a baseline, the results of this study show that five years of additional exposure to ambient UVR produces a loss in sensitivity that is equivalent to over 30 years of normal aging.

249.17

MECHANISMS UNDERLYING THE SPECTRAL TUNING OF X-CHROMOSOME LINKED HUMAN CONE PIGMENTS. J. Neitz*, M. Neitz* and G. H. Jacobs. Neuroscience Research Institute, University of California, Santa Barbara, CA 93106.

Nathans et al. (Science, 232:203, 1986) proposed that red-green color vision deficiencies arise from homologous recombination of the X-chromosome linked visual pigments genes that occur in individuals with normal color vision. They suggest that the fusion genes resulting from recombination encode pigments with the same spectrum as one or the other of the original normal pigments in dichromats and also encode pigments with spectra intermediate between the two original normal pigments in anomalous trichromats. The amino acid differences responsible for the spectral positioning of different pigments have not been identified. In the present experiments, an X-chromosome linked visual pigment gene was isolated from a red-green color blind male (a protanope) and its nucleotide sequence determined. The spectral properties of the pigment specified by this gene were determined by analyzing a corneally recorded gross potential, the electroretinogram. The spectrum of this photopigment (λ_{max} = 530 nm) is not different from that of a normal middle wavelength sensitive (MWS) pigment even though about half of the DNA coding sequences appear to be derived from a normal long wavelength sensitive (LWS) pigment gene. These results indicate which regions of the photopigment apoproteins govern the spectral tuning of the X-chromosome linked photopigments. A region in exon 4 appears to be responsible for the small spectral shift characteristic of the difference between X-linked pigments in anomalous trichromats, while a region in exon 5 is responsible for the larger spectral shift between MWS and LWS pigments.

HYPOTHALAMIC-PITUITARY-GONADAL REGULATION I

250.1

BINDING CHARACTERIZATION OF PROGESTERONE BOVINE SERUM ALBUMIN CONJUGATES (P-BSA) TO RAT BRAIN TISSUES. F.C.KE AND V.D.RAMIREZ. Department of Physiology and Biophysics, University of Illinois, Urbana, IL 61801

The membrane mechanism of progesterone (P) action on nerve cell remain unclear. Previously, we demonstrated specific binding of P-BSA to rat brain tissues. To examine the possibility that P-BSA interact with ion channel proteins, the binding of P-BSA to cerebellum and brain stem P_2 fractions was characterized by changing ionic condition of the incubation medium.

Depolarization of brain tissue by changing the Na^+/K^+ ratio increased the binding capacity but decreased the binding affinity. At either normal or depolarized conditions, the binding was diminished in presence of 2 mM EDTA. The presence of specific divalent cations (Mg^{++},Ca^{++},Sr^{++},Ba^{++}, Mn^{++}, but not Fe^{++},Co^{++},Ni^{++},Cu^{++},Zn^{++}) was essential for the binding of P-BSA. The binding was also regulated by guanine nucleotides (GTP and GDP).

The results indicate that the binding of P-BSA to rat brain tissue is sensitive to depolarization and requires specific divalent cations. This support the hypothesis that the membrane mechanism of P may be mediate by modulation of ion channel proteins on nerve cell membrane.

250.2

COLOCALIZATION OF PROGESTIN RECEPTOR- AND β-ENDORPHIN- OR ENKEPHALIN-IMMUNOREACTIVITY (IR) IN GUINEA PIG BRAIN. D.H. Olster and J.D. Blaustein. Psychology Department, Neuroscience and Behavior Program, University of Massachusetts, Amherst, MA 01003.

Many behavioral and physiological effects of steroid hormones have been hypothesized to be mediated by opiate peptides. We used double-label immunocytochemistry (ICC) to ascertain whether progestin receptor (PR) and β-endorphin (βEP) or enkephalin (ENK) were colocalized in the same neurons. Ovariectomized guinea pigs were treated with estradiol to induce high levels of PR-IR. Colchicine was injected intraventricularly 48 h prior to perfusion to improve visualization of βEP-IR and ENK-IR. Brain sections were processed for PR-, followed by βEP- or ENK-IR. The ENK antiserum (Incstar) was preferential for leucine-ENK, but cross reacted with methionine-ENK slightly. ENK-IR and PR-IR were both found in the preoptic area, dorsomedial (DMA) and ventrolateral areas of the hypothalamus and arcuate nucleus (ARC). However, it was only in the ARC that evidence of some colocalization was found. Many βEP-IR neurons were found in the ARC, with fewer in the DMA and ventromedial nucleus (VMN). Furthermore, substantial numbers of βEP-IR cells in the VMN (58%), DMA (69%) and ARC (30%) also contained PR-IR. These data provide neuroanatomical evidence that hypothalamic actions of progesterone may be mediated by opiate peptides. (Supported by NIH HD 23483, RCDA NS 00970 and NS 19327)

250.3

DOWN-REGULATION OF HYPOTHALAMIC PROGESTIN RECEPTOR-IMMUNOREACTIVITY (PR-IR) BY PROGESTERONE IN FEMALE GUINEA PIGS. J.D. Blaustein, Neuroscience & Behavior Program & Psychology Dept., Univ. Mass., Amherst, MA 01003

Progesterone injection in estradiol-primed ovariectomized guinea pigs results in down-regulation of hypothalamic progestin receptors (PRs) determined by in vitro binding assays. In order to determine if there are neuroanatomical areas in which progesterone preferentially down-regulates PRs, ovariectomized guinea pigs were injected with minimal behaviorally-effective doses of estradiol benzoate (10 µg 2 d before perfusion) and oil or progesterone (500 µg 4 h before perfusion), and PR-IR cells were observed immunocytochemically. Estradiol dramatically increased cell nuclear, and to a lesser extent cytoplasmic, immunostaining in defined regions of the preoptic area and hypothalamus. Subsequent progesterone injection resulted in significant decreases in PR-IR in all regions by 24 h after injection. The ventrolateral hypothalamus (VLH) was particularly sensitive showing a substantial decrease in PR-IR by 12 h after injection. This decrease is not due to occupation of receptor by hormone; while progesterone injection caused a nearly total absence of cytoplasmic immunostaining within 4 h, it did not decrease the intensity of cell nuclear staining.

The data extend earlier results of in vitro binding experiments. They point to the VLH as a site at which PRs may be particularly sensitive to down-regulation by progesterone. (Support: NIH NS 19327 and RCDA NS 00970)

250.4

ESTROGEN RECEPTOR-IMMUNOREACTIVITY IN THE FOREBRAIN OF THE FEMALE GUINEA PIG: DISTRIBUTION AND EFFECT OF STEROID HORMONE TREATMENT. L.L. DonCarlos, E. Monroy, K. Malik, and J.I. Morrell. Inst Animal Behav, Rutgers Univ, Newark, NJ 07102.

We have mapped the distribution of cells that contain estrogen receptors (ER) in the brain of the adult female guinea pig. Cellular ER content was detected using monoclonal antibody H222 (gift from Abbott Lab.) and the Vectastain-Elite method with nickel-intensified DAB. Deletion, titration and adsorption controls established the specificity of the staining. Dense collections of ER-immunoreactive (ER-IR) cells were found in medial preoptic, medial hypothalamic, and limbic nuclei (amygdala, bed n. stria terminalis, lat. septum). Numerous ER-IR cells were scattered throughout the remainder of the preoptic area, hypothalamus and limbic system, and also in the midbrain (central gray). Elsewhere, ER-IR cells were rare or absent. Treatment of ovx animals with Estrogen (E) or E plus progesterone decreased ER staining compared with oil. Our map of ER-IR cells in the guinea pig forebrain markedly extends previous maps based on E-binding. The presence of ER in many forebrain cell groups is a provocative reminder that E may modify, and thus coordinate, diverse behavioral and physiological processes. Supported by HD22983 and NS07997.

250.5

DIURNAL PATTERNS OF PROOPIOMELANOCORTIN (POMC) GENE EXPRESSION IN THE ARCUATE NUCLEUS OF PROESTROUS, OVARIECTOMIZED AND OVARIECTOMIZED, STEROID-TREATED RATS. P.M. Wise, K. Scarbrough, N.G. Weiland, G.H. Larson*. Dept. Physiology, U. Maryland, Baltimore, MD 21201

Opiate peptides are thought to modulate the pattern of LH release in female rats. Using *in situ* hybridization, we examined whether diurnal changes in POMC gene expression occur in proestrous, ovariectomized (OVX), or OVX steroid-treated rats and whether differences in the diurnal rhythm or average level of expression in these animal models may explain their different patterns of LH secretion. Four groups of young rats were used in this study: proestrus, OVX for 9 days, OVX for 7 days followed by 2 days of estradiol treatment (OVX-E_2), and OVX-E_2 plus progesterone treatment on day 9 at 1000h (OVX-E_2P). Rats (n=6-7/treatment/time) were killed at 2300, 0300, 1000, 1300, 1500, 1800 or 2300h. Brain sections (20um) were fixed in paraformaldehyde and hybridized at 45°C with a ^{35}S-labeled riboprobe prepared according to the methods of Chowen-Breed et al (Endocrinol 124:1697, 1989). After RNase treatment and stringent wash, slides were dipped in emulsion, exposed and developed. Experimental conditions were optimized for probe concentration and time of exposure to emulsion. POMC mRNA per cell was quantitated using a computerized image analysis system. On proestrus, POMC mRNA expression exhibits a diurnal rhythm: levels rise during the morning, plateau between 1000-1500h and decrease by 2300h. Assuming that there is a delay between a decrease in transcription rate and a measurable decrease in mRNA pool size, the observed decrease in POMC mRNA on proestrous evening may reflect decreased opiate tone that is hypothesized to occur at the time of the onset of the LH surge. OVX decreases the level of POMC mRNA per cell and no difference in the level of mRNA was observed between 1000 and 2300h in this group. The data demonstrate that steroidal milieu plays a role in the level and diurnal pattern of POMC expression in the arcuate nucleus and suggest that changes in these parameters may modulate the cyclic release of LH.

250.6

SEXUAL DIFFERENTIATION OF THE BRAIN IN MALE RATS IS ALTERED BY PRENATAL ACTH OR NICOTINE TREATMENT A.C.Segarra, M.Frankfurt, V.N.Luine and F.L.Strand. The Rockefeller Univ., New York, New York 10021.

Decreased sexual behavior was observed in male rats treated prenatally with nicotine or ACTH (Segarra & Strand, Brain Res.480:151,1989). We decided to investigate if this decrease was correlated with changes in catecholamines (CA) and/or serotonin(5HT) levels in the preoptic area (POA) of the brain. The POA was dissected from 6 day and 80 day old male rats treated prenatally with ACTH (0.1mg/day) or nicotine (1,0.5 mg/kg/day); CA and 5HT levels were measured by HPLC. ACTH-treated pups showed no difference in CA or 5HT levels whereas adults exhibited higher levels of 5HT in the POA. Prenatal nicotine treatment (1 mg/kg/day) decreased 5HT levels in the pups; adult POA levels were the same as controls. These studies provide further evidence of the remarkable plasticity of the brain during the process of sexual differentiation. Supported by the Council for Tobacco Research.

250.7

ESTROGEN INDUCED ALTERATION OF ADP-RIBOSYLATED PROTEIN EXPRESSION IN ANTERIOR PITUITARY BUT NOT IN STRIATUM OR UTERUS. B. Borgundvaag and S.R. George. Departments of Medicine and Pharmacology, University of Toronto, Toronto, Ontario, M5S 1A8 Canada.

Estrogen (E2) treatment reliably results in hyperplastic growth of anterior pituitary (AP) lactotrophs. We have previously shown attenuation of adenylate cyclase activity in AP after E2 treatment, with blunted responses to stimulation by VIP and inhibition by bromocriptine. The effect of E2 on the postreceptor mediators of function, guanine nucleotide binding proteins were studied.

Ovariectomized female rats were treated with E2 for 21 days. The studies were in accordance with the established guidelines for animal research. The guanine nucleotide binding proteins were studied using bacterial toxins to catalyse ADP-ribosylation. Cholera toxin mediated ADP-ribosylation (ADP-R) of several proteins, as determined by SDS-PAGE. The estrogen-treated pituitaries had an identical ADP-R Gs band (45K) as the untreated ovariectomized controls. There was a marked alteration in labelled protein expression in the 20-25K range. No effect of estrogen treatment on CT catalysed ADP-R in striatum or uterus were evident. Pertussis toxin mediated ADP-R in the same tissue was not affected by estrogen treatment. The potential significance of the novel 25K substrate for CT catalysed ADP-R in AP with respect to signal transduction, growth induction or oncocytic transformation remains to be clarified.

250.8

ESTROGEN RECEPTOR IMMUNOREACTIVITY IN HUMAN HYPOTHALAMUS. **E.G. Stopa, S. H. Hauser*, J.C. King, J.D. Blaustein, G. L. Greene*, and S. Reichlin. Depts. of Endocrinology, and Anat. and Cell Biology, Tufts U. Sch. of Med., Boston, MA, Ben May Institute, Univ. of Chicago, Chicago, IL, Neurosci. and Behavior Program, Univ. of MA, Amherst, MA, and Dept. of Pathology, SUNY Health Sci. Ctr, Syracuse, NY.**

The distribution of estrophilin-like immunoreactivity (ELI) in the human hypothalamus and adjacent regions was studied using a monoclonal antibody (D-75) raised against estrophilin derived from MCF-7 human breast cancer cells.

Hypothalami (n=5) were fixed by immersion in either 4% paraformaldehyde or Zamboni's fixative. The immunocytochemical reaction was performed on unmounted sections (30 - 60 um) using a modification of the PAP technique, after incubation in primary antiserum (11.5 ug/ml) for 72 hours.

Reaction product was found in restricted populations of neurons and astrocytes. ELI-positive neurons were medium-sized, multipolar, and diffusely distributed within the basal forebrain and preoptic area, infundibular region, central hypothalamus, basal ganglia, and amygdala. Immunoreactive astrocytes were noted within specific brain regions, including the lamina terminalis and subependymal layer. These data suggest that estrogens may influence the cellular functions of both neurons and supporting elements within the human brain. **(NIA: 1K11AG00295, DK16684)**

250.9

TEMPORAL EFFECTS OF E_2 AND P ON ARCUATE POMC MRNA LEVELS. S.L. Petersen. Dept. of Anatomy, Univ. of Missouri Sch. of Med., Columbia, MO 65212.

Opioid peptides have been implicated as mediators of both negative and positive feedback effects of estrogen (E_2) on LH release. Because opiate neurons in the arcuate nucleus contain E_2 receptors, this study examined changes in arcuate POMC mRNA in ovariectomized controls, in animals demonstrating both negative and positive feedback effects of E_2, and in those with progesterone-augmented LH surges. Representative 12 μm sections through the entire arcuate nucleus were hybridized *in situ* with a ^{35}S-labelled 48-base synthetic oligomer specific for POMC mRNA. E_2 decreased POMC mRNA levels at 0800 h (when E_2 exerts a negative feedback effect on LH release), but increased POMC mRNA significantly by 1200 h (just before the LH surge begins). At 1600 and 2000 h, POMC mRNA levels were similar to those seen at 0800 h. Progesterone increased POMC gene expression slightly, but not significantly, over that of animals treated with E_2 alone at 1200 and 1600 h. POMC mRNA levels were highest in OVX females at 0800 h, decreased by 1200 h and remained low at 1600 and 2000 h. These findings are consistent with the hypothesis that the negative feedback effect of E_2 may be opiate-independent. In addition, these results demonstrate that opiate gene expression appears to increase just prior to the onset of the LH surge. This increase may be necessary for the surge release of LH.

250.10

IN VITRO INHIBITION OF MUSCARINIC BINDING BY STEROID HORMONES. G.P. Dohanich, D.A. Cada* and B.E.F. Wee. Dept. of Psychology and Neuroscience Program, Tulane University, New Orleans, LA 70118.

The presence of micromolar concentrations of various steroid hormones inhibited binding of the muscarinic ligand [^{3}H]quinuclidinyl benzilate (QNB) in brain homogenates from ovariectomized rats. The order of potency was progesterone > corticosterone >> testosterone = androstenedione. Pregnenolone and dihydrotestosterone failed to inhibit binding. Progesterone inhibited [^{3}H]QNB binding in homogenates obtained from hypothalamus, brain stem, cortex, and forebrain. Under equilibrium conditions, progesterone inhibited [^{3}H]QNB binding at a K_I of approximately 5 uM. Scatchard analyses revealed that progesterone and corticosterone increased the apparent K_D of [^{3}H]QNB by 200-300% while reducing B_{MAX} by less than 15%, indicating a competitive inhibition of the steroids with [^{3}H]QNB for muscarinic binding sites. Although these steroids appear to be interacting with muscarinic binding sites throughout the brain, our procedures do not rule out the possibility that a direct ligand-steroid interaction could account for the observed inhibition. Data confirm and extend the original work of Klangkalya and Chan (Neuroendocrinology 47:294, 1988) that progesterone inhibits [^{3}H]QNB binding in vitro in rat brain homogenates.

Supported by USPHS grant HD-22235.

250.11

ESTRADIOL-CONCENTRATING FOREBRAIN AND MIDBRAIN NEURONS PROJECT DIRECTLY TO MEDULLA. K.P. Corodimas and J.I. Morrell. Inst Animal Behav, Rutgers Univ, Newark, NJ, 07102.

Estradiol (E2)-concentrating neurons afferent to medulla were investigated by combining a retrograde neuroanatomical tracing method with steroid hormone autoradiography. Injections of the fluorescent tracer Fluoro-Gold (FG) were made into the medulla of seven ovx/adx female rats. Seven days later, 3H-E2 (0.8ug/250g BW; SA=95.4 Ci/mMol) was given ip (Fahrbach et al, JCN, 247, '86, for protocol).

We examined the distribution of three neuron populations: medulla afferents, E2-concentrating neurons, and E2-concentrating neurons afferent to medulla. The largest proportion of E2-receptive neurons that projected to medulla were found in the paraventricular nucleus of the hypothalamus (PVN). Of the E2-concentrating neurons in PVN 69% projected to medulla. In central nucleus of the amygdala, 38%, and in lateral bed nucleus stria terminalis 13% of the E2-concentrating neurons were medulla-bound. The central gray was the only midbrain area which contained E2-target neurons that coursed to medulla (42%). Our findings suggest that E2-sensitive neurons may influence parasympathetic tone via direct projections.

Supported by HD22938 and Sigma Xi.

250.12

THE DISTRIBUTION OF NEURONS HAVING ESTROGEN RECEPTOR-IMMUNOREACTIVITY AND SUBSTANCE P INNERVATION OVERLAPS IN THE MIDBRAIN CENTRAL GRAY OF GUINEA PIGS. J.C. Turcotte* and J.D. Blaustein. (SPON:J. Desmond) Neuroscience and Behavior Program and Psychology Dept., University of Mass., Amherst, MA 01003

The midbrain central gray has been shown to play a role in hormonally induced female sexual behavior. Injections of Substance P (SP) into the central gray, an area known to contain estrogen receptors as well as SP fibers and terminals, facilitate lordosis in rats. In guinea pigs we found SP-immunoreactivity (SP-IR) and estrogen receptor-immunoreactivity (ER-IR) in the same areas of the central gray. ER-IR cell nuclei were distributed at all levels of the central gray, increasing in numbers along the rostrocaudal axis. Most ER-IR nuclei were found slightly ventrolateral to the cerebral aqueduct where SP-IR appears most dense. By using a multiple bridging technique to enhance ER-IR immunostaining, we have been able to visualize ER-IR in the cytoplasm of cell processes as well as in cell nuclei. This enabled us to observe SP-immunoreactive punctate structures, suggestive of terminal boutons, in close association with a small number of ER-IR cell processes and more frequently, with ER-IR cell bodies. This overlap of SP-IR innervation with ER-IR neurons suggests a neuroanatomical substrate for the facilitating effects of SP on lordosis.

(Supported by NS 19327 and RCDA NS 00970 from the NIH)

250.13

NORADRENERGIC INNERVATION OF HYPOTHALAMIC ESTROGEN RECEPTOR-IMMUNOREACTIVE CELL BODIES AND PROCESSES IN FEMALE GUINEA PIGS. M.J. Tetel and J.D. Blaustein. Neuroscience and Behavior Program and Psychology Department, University of Massachusetts, Amherst, MA 01003

Catecholamines regulate a variety of estradiol-influenced events in the hypothalamus of rats and guinea pigs. An immunocytochemical technique was used to study noradrenergic (NA) afferent input to estrogen receptor-immunoreactive (ER-IR) cells in the hypothalamus and preoptic area of ovariectomized guinea pigs. Brain sections were first immunostained for ER-IR by an immunoperoxidase technique using diaminobenzidine (DAB) as the chromagen, which stains ER-IR cell processes up to 250 um in length. Next, nickel-enhanced DAB was used for dopamine-B-hydroxylase-IR (DBH-IR). DBH-IR punctate structures were observed in close association with ER-IR cell bodies and processes throughout the hypothalamus. Furthermore, some ER-IR neurons in the dorsomedial hypothalamus appeared to have their surfaces completely enveloped by DBH-IR punctate structures, suggestive of terminal boutons. This NA interaction with ER-IR cells could provide a possible mechanism by which environmental stimuli could elicit neuronal changes in distinct subpopulations of estradiol-sensitive neurons.

(Supported by NS 19327 and RCDA NS 00970 from the NIH)

250.14

INVOLVEMENT OF THE CALCIUM MESSENGER SYSTEM IN THE STIMULATION OF LUTEINIZING HORMONE SECRETION BY NEUROPEPTIDE Y IN RATS. W.R. Crowley, G.V. Shah*, D. Kennedy*, M.E. Dockter*, and S.P. Kalra. Depts. of Pharmacology and Microbiology & Immunology, University of Tennessee, Memphis College of Medicine, Memphis, TN 38163 and Dept. of OB/GYN, University of Florida College of Medicine, Gainesville, FL 32610.

The stimulatory effect of neuropeptide Y (NPY) on luteinizing hormone (LH) secretion in female rats occurs via increased release of LH-releasing hormone (LHRH) from the median eminence and enhanced response of anterior pituitary cells to LHRH. Further studies investigated the involvement of the Ca^{++} messenger system in these effects. The stimulatory effect of NPY on *in vitro* LHRH release persisted in Ca^{++} free/EGTA-chelated medium, but was prevented by TMB-8, an agent that inhibits the mobilization of intracellular Ca^{++}. The facilitatory effect of NPY on LHRH-induced LH release from cultured anterior pituitary cells was mimicked by Bay K 8644, a dihydropyridine agonist at voltage-sensitive Ca^{++} channels, and the effects of either Bay 8644 or by NPY were blocked by the dihydropyridine Ca^{++} channel antagonist, nitrendipine. Dispersed pituitary cells were loaded with the Ca^{++} probe INDO-1 AM and scanned in a Flow Cytometer, at the rate of 1000 cells/sec, by a UV argon ion laser. LHRH alone increased intracellular Ca^{++} levels in a population of pituitary cells. After pretreatment with NPY, an increased number of cells responded to LHRH with increases in Ca^{++}. These results suggest that 1) the central action of NPY to stimulate LHRH release may occur via mobilization of intracellular Ca^{++}, and 2) the facilitatory effect of NPY on LHRH-induced LH release from the pituitary occurs through increased influx of extracellular Ca^{++}.

250.15

ENDOGENOUS INHIBIN SUPPRESSES BASAL FSH SECRETION WHILE E_2 SUPPRESSES PULSATILE FSH SECRETION IN THE FEMALE RAT. M.D. Culler* (SPON: E. Field), Repro. Neuroend. Sec., Lab. Mol. and Integrative Neurosci., NIEHS, NIH, Res. Tri. Pk, NC 27709.

In order to investigate the role of ovarian factors in regulating FSH secretion, cannulated female rats were either ovariectomized (OVX), with or without E_2 replacement, or sham-operated (SHAM) and injected with either anti-inhibin serum (AS) or normal sheep serum (NS) on the afternoon of diestrus II. Eighteen h later, blood samples were collected every 10 min for 4 h from the unanesthetized rats. The OVX, OVX-E_2 and SHAM-AS treatments all induced similar, significant increases in overall mean FSH and mean FSH trough levels as compared with the SHAM-NS controls. The parameters of pulsatile FSH secretion (pulse amplitude and frequency) were not altered by SHAM-AS or OVX-E_2 as compared with SHAM-NS rats. OVX, however, induced a significant increase in FSH pulse amplitude without affecting pulse frequency. Pituitary sensitivity to exogenous LHRH, in terms of FSH secretion, was the same in all groups. These results suggest that endogenous inhibin suppresses basal FSH secretion while E_2 suppresses pulsatile FSH secretion through a mechanism independent of pituitary sensitivity to LHRH.

250.16

OVARIAN STEROIDS INCREASE VERATRIDINE-INDUCED RELEASE OF AMINO ACID TRANSMITTERS IN PREOPTIC AREA SYNAPTOSOMES. A. Fleischmann*, M.H. Makman and A.M. Etgen. Albert Einstein College of Medicine, Bronx, NY 10461.

To evaluate the role of ovarian steroid hormones in the control of GABA and glutamate (GLU) neurotransmission, the release of GABA and GLU and the de novo synthesis of GABA from glutamic acid were studied in hypothalamic (HYP) and preoptic area (POA) synaptosomes from ovariectomized (OVX) and OVX, steroid-treated rats. Female Sprague-Dawley rats were injected with oil or 2 μg of estradiol benzoate (EB) 48 and 24 hr before sacrifice and with progesterone (P, 500 μg) or oil 4 hr before sacrifice. Synaptosomes isolated from the HYP and the POA were incubated with ^{3}H-glutamic acid for 30 min, then washed and reincubated in order to examine KCl- and veratridine-induced release of ^{3}H-GABA and GLU. Conversion of ^{3}H-glutamic acid to GABA in both HYP and POA synaptosomes was 7-9% in all groups. Neither basal nor KCl-induced release of GABA or GLU varied as a function of hormonal condition. In contrast, veratridine-induced release of GABA and GLU from POA synaptosomes of EB+P-treated animals was approximately twofold higher than that in OVX controls or OVX rats treated with EB or P alone. Steroid treatment had no significant effect on veratridine-induced release of GABA or GLU in HYP synaptosomes. Thus different mechanisms may underlie KCl- and veratridine-induced amino acid release, and these mechanisms may be affected differentially by steroid hormones.

250.17

MOST FOREBRAIN NEURONS PROJECTING TO THE ESTROGEN RECEPTOR-RICH VENTROLATERAL HYPOTHALAMUS DO NOT CONTAIN ESTROGEN RECEPTOR-IMMUNOREACTIVITY IN FEMALE GUINEA PIGS. Y.Delville and J.D.Blaustein. Neuroscience and Behavior Program and Psychology Department, University of Massachusetts, Amherst, MA 01003.

We have found that the stimulation of the rostral ventrolateral area (VLA) of the hypothalamus by estradiol is critical for the expression of sexual receptivity facilitated by progesterone. We looked at the forebrain afferent neurons to this area using a fluorogold retrograde tracing technique. Most noticeably, retrogradely-labelled neurons were found in areas known to contain many estrogen receptor-immunoreactive (ER-IR) neurons and also known to be involved in the control of female sexual behavior, such as the preoptic area (POA). We predicted that these retrogradely-labelled neurons in the POA would also contain estrogen receptors. Surprisingly, few if any retrogradely-labelled neurons also contained ER-IR. However, in many cases, retrogradely-labelled neurons were found adjacent to cells immunoreactive for estrogen receptors. Therefore neuronal connections between the POA and the VLA do not appear to be directly estrogen-responsive. (Supported by NS 19327 and RCDA NS 00970 from the NIH)

250.18

PERIPUBERTAL CHANGES IN ESTROGEN BINDING IN DISCRETE REGIONS OF THE FEMALE RAT HYPOTHALAMUS AND PREOPTIC AREA. T.J. Brown[1], F. Naftolin*[1], W. Jacobson*[2], and N.J. MacLusky[2] Dept. OB/GYN, Yale University School of Medicine[1], New Haven, CT 06510, and Division of Reproductive Science, Toronto General Hospital[2], Toronto, Ontario, M5G 2C4.

The events surrounding the onset of reproductive cyclicity in the female rat include a profound change in the sensitivity of the neural substrate to the feedback effects of estradiol on LH secretion. To test the hypothesis that this alteration in sensitivity could be associated with a change in intracellular receptors for estrogen, changes in mediobasal hypothalamus/preoptic area (MBH/POA) receptor content were assessed in 25-37d old female rats by *in vitro* binding assays and by quantitative analysis of *in vivo* neural uptake of 11β-methoxy-16α-[^{125}I]estradiol. Cytosol progestin receptors in the MBH and pituitary gland increased dramatically from d25 to d35. Occupation of nuclear estrogen receptors in the pituitary and MBH/POA of 34d old rats were increased 2-3 fold above that measured in 23 and 30d old rats. Nuclear estrogen receptor binding capacity in the arcuate nucleus increased by 48% from d25 to d32, and by 64% from d25 to d38. Similar but smaller changes in estrogen binding capacity were found in the ventromedial nucleus and medial POA. These results indicate that during the peripubertal period an increase in estrogen binding capacity occurs in specific regions of the rat brain known to play a vital role in the control of reproductive function. Supported by NIH grant HD13587.

250.19

BEHAVIORALLY-CORRELATED SINGLE UNIT DISCHARGE RECORDED FROM ENSEMBLES OF NEURONS IN THE PREFRONTAL CORTEX VARY ACROSS THE ESTROUS CYCLE. Sheryl S. Smith and Christina Bergqvist*, Dept. of Anatomy, Hahnemann Univ., Broad and Vine, Phila., PA 19102-1192.

Ongoing studies from this laboratory have shown that 17 β estradiol (E_2) augments excitatory responses of cerebellar Purkinje cells to the iontophoretically applied excitatory amino acids glutamate, quisqualate and NMDA. Using a behavioral paradigm, we have also shown that E_2 administration as well as estrous-associated hormonal changes increase cerebellar discharge during treadmill locomotion to a greater extent than during stationary periods, an effect compatible with the hypothesis that E_2 enhances evoked neuronal excitation, whether elicited pharmacologically or behaviorally.

In the present study, another CNS site was tested for possible locomotor-correlated discharge relative to spontaneous across the days of the estrous cycle. Neurons from the rat dorsomedial prefrontal cortex (PFC), an area shown to contain classic receptors for E_2 and which may be implicated in some of the psychoactive effects of this steroid, were recorded using chronically implanted arrays of 5-7 microwires (0.001" dia).Implanted animals were trained to locomote on a computer- controlled treadmill device (alternately on-off, at 10 s intervals) for a period of 1-2 hs daily during neuronal recording. PFC activity was found to be correlated either with locomotion (tread on) or non-movement (tread off) on almost an equal basis, when recorded from rats on diestrus 1 or 2 (D) during the light segment of the light:dark cycle. The tread off- correlated discharge was markedly augmented by three-fold on estrus (E) relative to values obtained on D, with little alteration in tread on correlated activity. In many cases, cells exhibiting tread on-correlated maximal activity were transformed to tread- off-correlated cells. Furthermore, increasing treadmill speed (from 4 to 11 cm/s) produced greater alterations in neuronal- correlated discharge when tested on D compared to E. When tested during the dark part of the cycle, tread-off correlated discharge was higher on E when compared with similar values during the light. Thus, the present results suggest that hormonal as well as light-dark fluctuations produce alterations in the response of PFC neurons to pre-movement conditions. *Supported by NS 25809 to SSS.*

250.20

POSTMENOPAUSAL HYPERTROPHY OF NEURONS EXPRESSING THE ESTROGEN RECEPTOR GENE IN THE HUMAN HYPOTHALAMUS. N.E. Rance*, N.T. McMullen§, D.L. Price and W.S. Young III†. Neuropathology Lab., The Johns Hopkins Univ. Sch. Med., Baltimore, MD 21205; §Dept. Physiology, Univ. MD Sch. Med., Baltimore, MD 21201; †Lab. Cell Biology, NIMH, Bethesda, MD 20892.

This study examines whether the loss of ovarian steroid secretion in postmenopausal (POSTM) women is associated with morphological alterations in the infundibular nucleus. Hypothalami from premenopausal (PREM) (n=3) and POSTM (n=3) women were serially sectioned and Nissl stained. Soma areas of >3500 infundibular neurons were digitized using a computer microscope. The mean cross-sectional area of neurons in POSTM women ($\bar{X}$ = 190 μm²) was 30% greater than that of PREM women ($\bar{X}$ = 147 μm²). To determine whether hypertrophied neurons were potential targets for estrogens, frozen sections from PREM (n=3) and POSTM (n=2) hypothalami were hybridized with a cDNA probe to estrogen receptor (ER) mRNA. Quantitative image analysis revealed that hypertrophied neurons in POSTM females contained ER mRNA. Finally, analysis of the infundibular nucleus from an ovariectomized 38-year old woman also revealed hypertrophied neurons containing ER mRNA. We propose that hypertrophy of infundibular neurons in POSTM women is not a function of chronological age *per se* but is secondary to loss of the inhibitory feedback of ovarian estrogen.

251.1

A MEDULLARY SITE MEDIATING INHIBITION OF SPINAL SEXUAL REFLEXES. L. Marson and K. E. McKenna Dept. of Physiology, Northwestern Medical School, Chicago IL 60611.

We have previously described a sexual response, the coitus reflex, elicited in anesthetized male and female rats. This reflex in under tonic descending inhibition. the goal of the present study was to identify the supraspinal site(s) mediating this inhibition.

Male rats were anesthetized with urethane and mounted in a stereotaxic frame. A urethral catheter was inserted for eliciting the coitus reflex, which was monitored by EMG recordings of the bulbospongiosus muscle. As previously reported, the coitus reflex cannot be elicited in this preparation in intact rats. Brainstem and spinal transection experiments identified the rostral medulla as the level of the neuraxis mediating the inhibition. Bilateral electrolytic lesions (1 mA, 15-60 sec) were made to identify the inhibitory site. Small (< 1 mm diameter) bilateral lesions of a site just rostral to the inferior olives and lateral to the pyramidal tract in the paragigantocellular recticular cell group were sufficient to release the coitus reflex. Bilateral injections of kainic acid (0.1M, 50-250nl) also released the inhibition of the coitus reflex, indicating that cell bodies in this region, and not axons of passage, mediate the inhibition. Injections of fluorogold (1 µl, 4%) into the lumbosacral cord retrogradely labelled numerous neurons in this area. These findings suggest that a population of bulbospinal neurons in the ventromedial rostral medulla mediate inhibition of spinal sexual reflexes.

251.2

EXTERNAL URETHRAL SPHINCTER EFFERENTS AND AFFERENTS IN THE SPINAL CORD OF THE MALE CAT. B. Mallory, M.N. Kruse, J.R. Roppolo and W.C. de Groat. Dept. of Pharmacol., Univ. of Pittsburgh, Pittsburgh, PA 15261.

Micturition involves the coordination of somatic and autonomic neuronal activity to the lower urinary tract (LUT). The main somatic innervation to the LUT is the afferent (AFF) and efferent (EFF) innervation of the external urethral sphincter (EUS). This study examined in the male cats the AFF and EFF projections of the EUS to the lumbosacral spinal cord using HRP tracing techniques. Under halothane anesthesia the EUS was exposed and injected bilaterally with either 1% WGA-HRP or 20% HRP. Three days later the animal was perfused with fixative and spinal cord removed. Labeled EFF neurons were seen at the base of the ventral horn in the S_1 and S_2 segments in an area known as Onuf's nucleus (ON). The majority of neurons were in the S_1 segment and at the most ventral part of ON (mean area 391 μm^2). HRP in the primary AFF pathway labeled neurons in the S_1 to Cx_1 dorsal root ganglia (DRG) with the majority of neurons in the S_2 DRG (mean area 849 μm^2). AFF fiber labeling could be seen along the lateral edge of the dorsal horn (DH) extending into lamina V and projecting toward the dorsal commissure (DC). AFF fiber labeling could also be seen at the medial edge of the DH projecting into lamina IV & V and into the DC. The close proximity of the EUS AFF and EFF with those of the sacral parasympathetic nucleus suggests the possible coordination of these systems during micturition.

251.3

THE EFFECTS OF MK-801 ON THE MICTURITION REFLEX IN THE CAT. J.R. Roppolo and W.C. de Groat. Dept. of Pharmacology, University of Pittsburgh, Sch. of Med., Pittsburgh, PA 15261.

A variety of evidence strongly suggests that several amino acid including L-glutamate and L-aspartate are important neurotransmitters at excitatory receptors in the vertebrate central nervous system. The present study was undertaken to determine if MK-801, a potent excitatory amino acid antagonist at NMDA receptors could modify the micturition reflex of the cat.

Cats anesthetized with α-chloralose 60 mg/kg were used in this study. Bladder pressure was recorded isovolumetrically via a urethral catheter connected to a pressure transducer.

Doses of MK-801 from 0.001 to 3 mg/kg given I.V. were examined in this study. Small doses 0.001 to 0.03 mg/kg had little or no effect on the amplitude or frequency of rhythmic bladder contractions. Doses of 0.1 to 0.3 mg/kg reduced the frequency and/or amplitude 10 to 30%. Doses of 1 to 3 mg/kg abolished all rhythmic bladder activity. Infusion of additional saline into the bladder could produce partial recovery of bladder activity. Complete recovery, however, was not seen.

This data suggests that excitatory amino acid neurotransmitter mechanisms maybe involved in the micturition reflex pathway.

251.4

BLADDER REFLEXES IN THE NEONATAL RAT. M.N. Kruse and W.C. de Groat. Depts. Pharmacology and Behavioral Neuroscience, Univ. of Pittsburgh, Pittsburgh, PA 15261.

Micturition in the adult rat is mediated via a supraspinal bladder-to-bladder reflex. The neonatal rat has a spinal perineal-to-bladder reflex that initiates micturition when the mother licks the perineal area. We report here on the development of the bladder-to-bladder reflex in the first few postnatal days. Urethane anesthetized, decerebrate and spinalized pups at 2-31 days of age were used. A needle was placed in the dome of the bladder through which bladder pressure was monitored and saline was infused. Awake pups of all age groups showed a perineal-to-bladder reflex. Urethane anesthesia depressed the micturition reflexes as many pups anesthetized with urethane did not exhibit a bladder-to-bladder or perineal-to-bladder reflex. Most pups of all age groups exhibited small amplitude, short duration bladder contractions following bladder distension which were probably intrinsic to the bladder smooth muscle as they occurred following ganglionic blockade with trimethaphan (25 mg/kg IM). Cystometrograms induced bladder-to-bladder micturition reflexes in decerebrate pups on day 6 and older, but not in spinal pups of the same age. A few 2-day-old decerebrate pups (3 of 13) showed the supraspinal bladder-to-bladder reflex. We conclude that rats develop a supraspinal bladder-to-bladder micturition reflex within the first few days after birth. During this period the perineal-to-bladder reflex is necessary to promote emptying of the bladder. If neonatal pups have a spinal bladder-to-bladder reflex, it is indistinguishable from the peripherally generated contractions.

251.5

CHOLECYSTOKININ HAS EXCITATORY EFFECTS ON TRANSMISSION IN VESICAL GANGLIA AND ON BLADDER CONTRACTILITY IN CATS. J.R. Keast, M. Kawatani and W.C. de Groat. Departments of Pharmacology and Behavioral Neuroscience, Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15261.

Cholecystokinin (CCK) is found in subpopulations of preganglionic autonomic neurons and primary afferent neurons. We have investigated the effects of exogenous CCK on transmission in vesical ganglia and bladder contractility in anesthetized cats in vivo. CCK 1-8 (sulphated form; 2-20 µg) was administered in 0.1-0.2 ml saline into the inferior mesenteric artery and caused a rapid, transient increase in firing on postganglionic nerves on the bladder surface (latency 2-5 s, duration 20-60 s). These effects were similar or greater in magnitude than those evoked by acetylcholine or methacholine (10-25 µg) and were abolished by hexamethonium (C_6, 1-2 mg). CCK in similar doses enhanced transmission in vesical ganglia, increasing the amplitude of pelvic nerve-evoked compound action potentials by 50-100% for up to 2-3 min. The excitatory effects of CCK were also seen on bladder activity. CCK (10 µg) caused a transient bladder contraction, which was reduced 60-80% by C_6 (1-2 mg) and the remaining effects were usually blocked by atropine (150-200 µg).

These results suggest that CCK causes the release of acetylcholine from nerve terminals in vesical ganglia and bladder smooth muscle, thereby enhancing transmission and contractility, respectively. CCK may play an important role in regulation of bladder function by facilitating excitatory pathways.

(JK is supported by an NH & MRC C.J. Martin Fellowship).

251.6

IMPROVED MICTURITION WITH DIRECT CURRENT STIMULATION OF THE SPINAL CORD IN THE SPINAL CAT. Khan, T., Dauzvardis, M., Walter, J., and Robinson, C.J., Rehab. R&D Center, Hines VA Hospital, Hines, IL 60141.

Changes in micturition behavior were noted as side effects in experiments that studied the effects of spinal cord stimulation on functional enhancement in six chronically spinalized female cats. A complete contusion injury was performed at the T9 level under anesthesia. These animals received daily post-operative care in accord with AAALC guidelines. Platinum disc electrodes were inserted 2 cm above and below the level of the lesion and connected to an 15 µA implantable DC source.

Three control animals were lesioned but did not receive stimulation. Two remained continent of urine during our 10-week study and required four or five Crede procedures (suprapubic pressure) for voiding. The third control animals was not distinguishable from experimental subjects.

Three stimulated animals were all incontinent by the third week and their bladders could be easily emptied with Crede applied once or twice.

High urethral resistance following spinal injury makes it difficult to empty the bladder. Improved voiding with spinal cord stimulation indicates inhibition of the urethral sphincter. This inhibitory effect may occur through activation of spinal inhibitory pathways or spinal cord regeneration.

Supported by funds from Veterans Administration, Rehabilitation R&D Service, Rehab. R&D Grant #B423R.

251.7

EFFECTS OF SUPRASACRAL SPINAL CORD STIMULATION ON BLADDER AND EXTERNAL URETHRAL SPHINCTER ACTIVITY IN THE DECEREBRATE CAT. B. Fedirchuk and S.J. Shefchyk. Depts. Med. and Physiol., Univ. of Manitoba, Winnipeg, Canada R3E 0W3.

The effects of electrical stimulation of the suprasacral spinal cord on urinary bladder pressure and external urethral sphincter (EUS) activity were examined in precollicular, postmammillary decerebrated male cats paralysed with Flaxedil. Electroneurographic recordings were made from the EUS and anal sphincter branches of the right pudendal nerve as well as from flexor and extensor hindlimb muscle nerves. Bladder pressure was monitored using a catheter inserted suprapubically into the bladder. Electrical stimulation (100-200 μA; 50 or 100 Hz; 0.2 ms) of the pontine micturition centre (PMC) elicited coordinated voiding characterized by synergistic bladder and EUS activity. Monopolar tungsten electrodes (70-110 kΩ) were placed within the superficial dorsolateral funiculus (DLF) at the T10 and T9 spinal segments. Stimulation (50-200 μA;100 Hz;0.2 ms square pulses) at these sites produced coordinated bladder pressure increase and decreased EUS activity during voiding. Subsequent transection of the dorsal columns rostral to the stimulating electrodes did not alter the response, nor did sacral dorsal root transections. Additional DLF lesions caused an apparent increase in tonic EUS activity and bladder pressure increases were no longer produced by stimulation of the spinal cord caudal to the DLF lesion, although the tonic EUS activity was inhibited by the stimulation. Voiding evoked from the PMC or the spinal cord rostral to the DLF lesion was not altered; distension-evoked reflex micturition was abolished. The results suggest that stimulation of the DLF at the thoracic spinal cord activates an ascending portion of a spino-bulbo-spinal loop which facilitates micturition. The apparent increase of activity of the EUS efferents following DLF lesion and the inhibition of EUS activity during DLF stimulation is consistent with reports by others which describe a descending pathway within the DLF which provides an inhibitory influence on a variety of spinal cord neurons. This pathway appears to be separate from the one responsible for the inhibition of the EUS activity during electrically evoked micturition. This research was supported by the Medical Research Council (MRC) of Canada. BF is supported by the Manitoba Health Research Council. SJS is an MRC Scholar.

251.8

COMPARISON OF SACRAL AND PUDENDAL NERVE STIMULATION FOR BLADDER INHIBITION IN THE SPINAL CAT

James S. Walter, Rebecca H. Sidarous*, John S. Wheeler*, Charles J. Robinson, Paul J. Zaszczurynski*, Robert D. Wurster, Rehab. R&D Center, Hines V.A. Hosp., Hines, IL 60141, and Dept. of Urology and Physiol., Loyola Medical Center, Maywood, IL 60153.

In order to develop neuroprosthetics for inhibiting the hyperreflexic bladder, electrical stimulation of pudendal and sacral nerves was compared in male unanesthetized cats (complete T1). For sacral stimulation, a quadripolar electrode (Pisces Quad, Medtronic), was inserted into the sacral canal on the midline. For pudendal nerve stimulation, wire electrodes (LifeTech) were inserted percutaneously adjacent to the bulbous urethra. Spontaneous bladder contractions were recorded before, during and after 10 min inhibitory procedures in animals 4 to 10 wk post-lesion.

Our first animal did not show inhibition. However, in our next two animals, bladder contractions of 40 to 60 mmHg were partially or entirely prevented; larger contractions were not inhibited. With sacral stimulation, effects of pulse duration and electrode location were inconclusive. Currents above threshold for pelvic floor stimulation were effective, from 1 to 2 ma, and 3.5 pps was better than 10 or 35 pps. Anesthesia of the pelvic floor prevented inhibition.

With pudendal stimulation, 1 to 4 ma, and 3.5 pps were most effective. Pudendal stimulation may be more selective for bladder inhibition than sacral stimulation. Supported by funds from Hines Veterans Administration Hospital, Rehabilitation R&D Center, and Rehab. R&D Grant B441 RA.

251.9

PHARMACOLOGICAL STUDIES OF VARIOUS AGENTS ON K^+ EVOKED RELEASE OF ^{3}H-NOREPINEPHRINE (T-NE) FROM RAT CORPORA CAVERNOSA. L. L. Truex and M. M. Foreman. Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285.

The sympathetic innervation of the corpora cavernosal (CC) smooth muscle is known to be important for control of penile erection. The focus of our studies is to establish a procedure to study the effects of pharmacologic agents on K^+ evoked T-NE release from CC tissue in vitro. CC tissue was removed from adult male Sprague Dawley rats. Transverse sections (0.5 mm) of CC tissue were made and preincubated for 30 minutes at 37° in buffer containing 119 mM NaCl, 4.6 mM KCl, 1.2 mM $KH_2PO_4 \cdot H_2O$, 25 mM $NaHCO_3$, 10 μM pargyline and 1 μM T-NE. Following the incubation period, tissue sections were superfused in a 0.2 ml chamber (3 per chamber) at a flow rate of 0.4 ml/min. Release of T-NE was induced by adjusting the K^+ concentration to 50 mM for 5 min. A double stimulus protocol was used and comparisons between treatments were made via the S2/S1 ratio. Release of T-NE was K^+ and Ca^{++} dependent and could be increased in the presence of 1 μM tomoxetine, a NE reuptake inhibitor. Release of T-NE was attenuated by both α_1 and α_2 agonists (methoxamine and clonidine) and increased by α antagonists (phentolamine, yohimbine, prazosin and phenoxybenzamine). Carbachol inhibited the release of T-NE. This procedure appears to be a useful model for studying pharmacological control of CC-NE release in vitro.

251.10

NEUROPEPTIDE Y INNERVATION OF PENILE ERECTILE TISSUE. Y. Carrillo*, E. Fernandez*, W. G. Dail and G. D. Walton*, Dept. of Anatomy, Univ. New Mexico Sch. of Med., Albuquerque, NM 87131.

Owing to the important role proposed for neuropeptide Y (NPY) in the regulation of blood vessels, the present study was designed to investigate the distribution of NPY immunoreactive fibers to the vascular tissue in the rat penis. In the corpora cavernosa penis (CCP), a dense plexus of NPY-IR fibers was present in relation to arteries, intrinsic cavernosal muscle, and veins, including the deep dorsal vein. The density and distribution of NPY-IR fibers was similar to that for noradrenergic nerves to this tissue. In the corpus spongiosum (CS), NPY-IR fibers were present on the scant cavernosal muscle in this tissue and at the base of the acini of the many periurethral glands. Noradrenergic fibers, stained by the glyoxylic acid method, were present over smooth muscle of the corpus spongiosum but were absent in the glandular acini. Immunohistochemistry of neurons, located by injection of fluorogold into the CCP or the CS, indicates that the majority of neurons in the sympathetic chain were NPY+ while less than 10% of the CCP and CS neurons in the pelvic plexus were NPY-IR. In summary, (1) NPY may be important in the regulation of blood flow in the penis, functioning on the venous as well as the arterial side, (2) the sympathetic chain is the most likely source of the NPY-IR fibers in the CCP and the CS, and (3) NPY may play a role in the secretory or absorptive functions of the periurethral glands.

251.11

THE EFFECT OF CHRONIC DECENTRALIZATION ON THE SYNAPTIC INPUT TO PENILE NEURONS. **N. M. Minorsky, G. D. Walton*, and W. G. Dail. Dept. of Anatomy, Univ. of New Mexico Sch. of Med. Albuquerque, NM 87131**

Synaptic connections within autonomic ganglia may become rearranged subsequent to deafferentation. To study this phenomenon further, the synaptic input to penile neurons in the major pelvic ganglion was examined following partial or total decentralization. Penile neurons were identified by injecting the retrograde dye, fluorogold into the corpora cavernosa penis. The synaptic plexus of nerve fibers surrounding penile neurons was immunolabeled with antibodies to met-enkephalin (m-Enk). The percentage of cells surrounded by a m-Enk plexus was averaged for each experimental group. Image analysis was used to obtain a measure of the degree of innervation around each penile neuron. Comparisons of synaptic profiles were made between animals which had received an acute or chronic nerve lesion of either pelvic or combined pelvic and hypogastric nerves. There was a marked increase in the number of neurons which receive innervation in chronically decentralized animals compared to the acute lesion paradigms. Also, the degree of m-Enk innervation increased for a small percentage of chronically decentralized neurons. Surprisingly, the reinnervation of penile neurons which were totally decentralized was comparable to that found for partial decentralization. Dependent upon the lesion paradigm, reinnervation in the pelvic plexus may involve the spared hypogastric nerve, intrinsic neurons or possibly contralateral preganglionic fibers.

251.12

SENSORY AND MOTOR BRANCHES OF PUDENDAL NERVE OF MALE RATS: ROLES IN COPULATION AND REFLEXIVE PENILE ERECTION. B.D. Sachs, Y.C. Liu*, D.B. McQuade*, J.X. Wang*, and G.M. Holmes. Univ. of Connecticut, Storrs, CT 06269-1020.

Male rats were tested for copulation and reflexive erections 5-7 days after bilateral section of the motor pudendal nerve (MPNx; n=10), which innervates the striated penile muscles, the sensory pudendal nerve (SPNx; n=7), which carries genital afference, or sham surgery (n=7). MPNx surgery was confirmed by EMG recording from the bulbocavernosus muscle; SPNx was verified by absence of response to probing the glans with forceps. Among SPNx males, 5/7 displayed intromissions. Only 5/10 MPNx males showed intromission patterns ($p<.05$), and a mean of 52% of these apparent intromissions missed the vagina. Of rats with SPNx or MPNx, only 1 male (SPNx) achieved ejaculation, and only he displayed reflexive erections, in contrast with 6/7 sham operated rats. Because reflexive vascular erections survive excision of the striated penile muscles, the absence of reflexive erection after MPNx was a surprise. This effect implies that the MPN carries some sensory or autonomic motor fibers essential for penile erection.

251.13

MOTOR CONTROL OF PENILE ERECTION: FUNCTIONAL HETEROGENEITY OF THE RAT BULBOCAVERNOSUS MUSCLE. G. M. Holmes, W. D. Chapple, and B. D. Sachs. Univ. of Connecticut, Storrs, CT 06269-1020, USA.

Erection of the glans penis results from vascular mechanisms augmented by constriction of the penile bulb by the bulbocavernosus (BC) muscle. We asked whether the recognized anatomical division of this muscle into a lateral (latBC) and a medial (medBC) portion was reflected by functional differences in their activity during erection. During copulation, the latBC had the characteristic EMG activity we have described previously (Soc. Neurosci. Abst. 14:41.5, 1988). Conversely, activity in the medBC, although synchronous in onset with that of the latBC, displayed uniform firing during the burst which did not vary significantly across changes in copulatory behavior and erection intensity. During reflexive erections, the medBC was periodically silent during latBC activity. The structure and dissociated EMG activity of the medBC and latBC suggest (a) that the BC muscle operates as a two-part pump in enhancing glans erection, and (b) that the two parts of the muscle may be regulated by separate motoneuron pools.

251.14

ULTRASTRUCTURAL CHANGES IN THE UTEROVAGINAL GANGLION OF THE PELVIC NERVE OF HENS DURING MOLT. S. L. Freedman and R. M. Kriebel. Depts. of Anatomy & Neurobiology, Univ. of Vermont, Burlington, VT 05405 and Philadelphia College of Osteopathic Medicine, Philadelphia, PA 19131.

The hen's ovary and reproductive tract undergo marked atrophy during molt. Extensive hormonal changes accompany the cessation of egg production, feather loss, and renewal. This study examined the fine structural modifications in the uterovaginal ganglion of the pelvic nerve in 3 hens concomitant with molt and reproductive inactivity. Prominent aggregations of ribosomes were present, but were not associated with cisternal membranes typical of RER. A marked alteration was the presence of crystalloid, ribbon-like inclusions often associated with dilated cisternal profiles. These structures may represent modified endoplasmic reticulum in storage form until the resumption of reproductive activity. Within synaptic profiles, which appeared to be degenerating, there was a noticeable decrease in the number of large granular vesicles. There was hypertrophy of the capillary endothelium. Plasma cells were present throughout the stroma, however, the absence of phagocytic activity suggests that tissue degeneration was not occurring. The question remains, if the neurons were quiescent or degenerating to be replaced when egg production was reestablished.

251.15

LATERAL HYPOTHALAMIC DAMAGE INDUCED BY KAINIC ACID OR ELECTROLYTIC CURRENT: EFFECTS ON GASTRIC EROSION FORMATION. B. Roland and C.V. Grijalva. BRI, Dept. of Psychology, UCLA, Los Angeles, CA 90024

Electrolytic lateral hypothalamic (LH) lesions in rats induce gastric mucosal erosions as well as other autonomic dysfunctions. In the present study, the effects of LH lesions were compared to infusions of Kainic Acid (KA) into the LH area on gastric mucosal injury. Rats were anesthetized with sodium pentobarbital and then given either bilateral LH lesions, N= 8 (1.2 ma anodal DC current/10 sec) or infused bilaterally with KA, N=9 (1.0µg/0.5µl/12 min) into the LH. Stomach were analyzed for gastric mucosal injury 24 hr postoperatively. Although gastric erosions were associated with both procedures, the incidence was significantly greater following electrolytic lesions (39.3mm^2 vs 5.0 mm^2; $p<0.05$). Furthermore, lesioned rats displayed more pronounced feeding deficits and overt autonomic dsyfunctions (salivation, lacrimation, diarrhea). Although these findings suggest that selective damage to LH neurons contribute to gastric lesion formation, the autonomic disruptions may be more closely linked to damage to axonal fibers of passage.

251.16

LATERAL HYPOTHALAMIC LESIONS ARE ASSOCIATED WITH STIMULATION OF GASTRIC CONTRACTILITY. C.V. Grijalva, T. Garrick*, and K. Jacobs*. VA Med.Ctr., CURE, BRI, Depts. of Psychology and Psychiatry, UCLA, Los Angeles, CA 90024

Changes in gastric contractility following lateral hypothalamic (LH) lesions were measured in urethane anesthetized rats. LH lesions were induced with anodal DC current (1.2ma/10sec) passed through stainless steel electrodes. Gastric contractility was recorded continously for 4 hr with acutely implanted strain gauge force transducers and analyzed by computer for cumulative force index. Rats with LH lesions consistently displayed stimulated gastric contractions in comparison to sham-operated controls (313.±96 vs 172±20 force units; $p<0.05$), and also had significantly more gastric mucosal erosions (5.7±2.4 vs 2.7±0.9 mm^2; $p<0.05$). Vagotomy, a procedure which blocks mucosal injury, also blocked LH lesion-induced gastric contractions as compared to sham vagotomy (25.6±2.1 vs 189.0±99 force units; $p<0.05$). These findings indicate that LH lesions are associated with stimulation of vagally mediated high amplitude gastric contractions which may be pathogenetically linked to erosion formation.

BEHAVIORAL PHARMACOLOGY: BENZODIAZEPINES AND STIMULANTS

252.1

A BEHAVIORAL TEST FOR ANXIETY IN RATS. J. C. Stout* and J. M. Weiss. (SPON: M. G. Caron). Depts. of Psychology and Psychiatry, Duke University, Durham, NC 27706.

A behavioral test for anxious behavior in rats has been developed by modifying Britton's open field test for anxiety (Britton & Britton, Pharmacol., Biochem, & Behav.:15:577, 1981). Sprague-Dawley rats are given access to water for 1 hour/day for 3 days. Rats are then tested in a novel, brightly-lit Plexiglas box (36 X 36 cm) containing a water spout at its center. Observations recorded for 10 min. include: latency to drink, seconds of drinking, number of drinking bouts, and approaches to the spout. Also recorded are grooming, rearing, inactivity, urination, and defecation.

Diazepam (.375-1.5 mg/kg) injected s.c. 30 min. before testing significantly increased measures of drinking, and decreased measures of inactivity, urination and defecation. This was not due to an effect of diazepam on thirst since the drug did not affect time spent drinking in the home cage. Behavior changes similar to those produced by diazepam resulted when rats were repeatedly exposed to the test condition, a procedure also designed to reduce anxious behaviors.

These experiments indicate that this test is useful for assessing anxious behavior in rats, and may thus be useful for studies of neurophysiological correlates of anxiety.

252.2

A COMPARISON OF ANXIOLYTIC AND NON-ANXIOLYTIC AGENTS IN THE SHOCK-PROBE/BURYING TEST. D. Treit. University of Alberta CANADA T6G 2E9.

The effects of IP pentobarbital (10.0-20.0 mg/kg), midazolam (1.0-2.0 mg/kg), chlorpromazine (2.5-5.0 mg/kg), and pentylenetetrazol (10.0-20.0 mg/kg) were compared in the shock-probe/burying test. Consistent with results found previously for chlordiazepoxide and buspirone, the anxiolytic agents midazolam and pentobarbital decreased rats' burying behavior toward a continuously electrified (2 mA) shock-probe, and increased the number of probe-shocks rats received. These concurrent, bidirectional, anxiolytic drug effects occurred at doses that did not affect rats' general activity. Although the non-anxiolytic agents pentylenetetrazol and chlorpromazine also suppressed burying behavior, neither of these drugs induced an increase in probe-shocks; in fact, pentylenetetrazol, which is believed to be anxiogenic, tended to reduce probe-shocks compared to vehicle controls. In addition, chlorpromazine, a standard neuroleptic, produced a significant suppression of general activity at 5 mg/kg. Thus, concurrent increases in probe-shocks and decreases in probe-burying seem to be characteristic effects of known anxiolytic agents, which distinguish them from non-anxiolytic agents.

252.3

MODULATION OF THE SEDATIVE EFFECTS OF DIAZEPAM BY THE VARIOUS BENZODIAZEPINE$_{BZ}$ RECEPTOR TYPES IN RATS. M.Massotti, C.De Luca* and L.Mele*. Lab. Farmacologia, Istituto Superiore di Sanità. 00161 - Roma, Italy.

In rats, diazepam (0.1-10 mg/kg iv) produces two distinct electrocorticographic(EEG) patterns: 1) synchronization and spindle bursts associated with behavioral sedation: 2) 20-30 Hz waves associated with signs of slight behavioral excitation (gnawing, eating,twitches of ears and vibrissae).

These effects are counteracted by administration of the selective antagonist of central benzodiazepine (BZ) receptor types Ro 15-1788 (0.01-1 mg/kg iv). A desynchronized record appears, associated with a state of arousal, lasting 10-20 min. In contrast, no significant effect is observed after the selective antagonist of peripheral BZ receptor type, PK 11195 (5 mg/kg iv).

In animals receiving diazepam (10 mg/kg) plus Ro 15-1788 (1 mg/kg), the subsequent administration of PK 11195, 1 min after Ro 15-1788, reduces the antagonizing effect of the imidazobenzodiazepine. A synchronized pattern rich in spindles is present in the EEG, associated with behavioral sedation.

These data suggest that peripheral BZ receptor type located in CNS can modulate the sedative effects of diazepam.

252.4

THE EFFECTS OF DIAZEPAM ON THE BEHAVIORAL TOPOGRAPHY OF RATS IN AN ELEVATED RUNWAY. S.L.M. Widgiz and C.H.M. Beck. Department of Psychology, University of Alberta, Edmonton, AB, Canada T6G 2E9.

The elevated plus-maze test has proven to be a suitable procedure for providing a behavioral correlate in rats for the clinical efficacy of the anxiolytic effects of drugs. Normal animals spend 80 to 90% of their time in the two maze arms with enclosing walls (walled arms) and only 10 to 20% of their time in the arms without walls (open arms). The generally accepted explanation of the effect is that the animals are fearful of the cliff-like edges of the open arms (Pellow, S. et al., J. Neurosci. Meth., 14:149, 1985). This hypothesis is supported by the demonstration that anxiolytic drugs increase the amount of time spent in the open arms (ibid.). An alternative interpretation is that normal animals have a thigmotaxic tendency to stay close to walls, whereas tranquilized animals do not (Treit, D. & Fundytus, M., Pharmacol. Biochem. Behav., 31:959, 1989). We tested this hypothesis in an elevated runway with no enclosing walls, but instead with a wall on the centre line of one end of the runway (walled end). The other end of the runway had no walls (open end). Rats treated with saline spent 80 to 90% of a 5-min session in the walled end. Animals receiving 2.0 mg/kg of diazepam, ip., 30-min before the test, doubled the time spent in the open end by the saline animals, a replication of the effect in the elevated plus-maze. The results favour the thigmotaxic hypothesis since the two ends of the runway differed in the length of wall but not in the length of elevated edge.

252.5

THE EFFECTS OF RO15-4513 ON ETHANOL DRUG DISCRIMINATION LEARNING. M. Kautz, J. Logan*, A. Romero*, M. Schwartz*, and A. Riley.
Psychopharmacology Laboratory, The American University, Washington, D.C. 20016.

The imidazole, RO15-4513, a benzodiazepine inverse agonist with high affinity for central benzodiazepine receptor binding, has been shown to block several behavioral effects of ethanol, including its reinforcing, anxiolytic and intoxicating properties. The present study further examined the interaction of RO15-4513 and ethanol by assessing the ability of RO15-4513 to antagonize the stimulus properties of ethanol within the conditioned taste aversion paradigm. Specifically, every fourth day rats were given ethanol (1 g/kg) prior to receiving a pairing of saccharin with LiCl. On intervening days, they were given distilled water prior to nonpoisoned access to saccharin. Following acquisition of the discrimination, RO15-4513 (3.0 and 5.0 mg/kg) was given alone and in combination with various probe doses of ethanol. RO15-4513 had no effect on the ethanol discrimination. The basis for the differences between these findings and others reporting antagonism remains unknown, although recent reports noting that antagonism may require an interaction of the intrinsic effects of RO15-4513 and ethanol suggest that antagonism is not a necessary outcome of the drug combination.

252.6

DISCRIMINATIVE STIMULUS PROPERTIES OF THE ANXIOLYTIC BETA-CARBOLINE ZK 112119. J.S. Andrews* and D.N. Stephens* (SPON: M. Reddington) Dept. Neuropsychopharmacology, Schering AG, POB 650311, D-1000 Berlin 65, FRG

The beta-carboline ZK 112119 has been identified as a partial agonist at the benzodiazepine (BZ) receptor. ZK 112119 has anticonvulsant and anticonflict properties in rats and is currently in Phase II trials as an anxiolytic. ZK 112119 substitutes for chlordiazepoxide (CDP) in rats trained to discriminate CDP from vehicle. However, as ZK 112119 also antagonises several BZ effects it may be that the interoceptive stimuli differ. The interoceptive effects of ZK 112119 and CDP were compared using a drug-discrimination procedure. Rats were trained to discriminate CDP (5mg/kg) or ZK 112119 (0.5 mg/kg) from vehicle in a standard 2-lever procedure. Following training, generalisation and antagonism testing was carried out using both BZ and non-BZ receptor ligands. Generalisation to the CDP cue by full, eg diazepam, or partial agonists eg ZK 95962 and CGS 9896 was similar to previous reports. However, these substances did not substitute for ZK 112119, although as with CDP the discriminative stimulus could be blocked by BZ antagonists such as RO 15-1788. These results suggest that the stimulus properties of beta-carboline partial agonists, though BZ receptor mediated, differ from those of full receptor agonists.

252.7

DISCRIMINATIVE STIMULUS EFFECTS OF ANXIOLYTICS IN THE HIPPOCAMPUS. S. Gleeson* & J.E. Barrett (SPON: H.C. Holloway). Dept. of Psychiat., Uniformed Services University of the Health Sciences, Bethesda, MD 20814.

Drug discrimination studies can provide information about the mechanisms of drug action. For example, both benzodiazepine (BZ) and serotonin (5-HT) compounds are anxiolytic, but generalization between these two drug classes does not occur. Because the limbic system has been implicated in the effects of anxiolytics and is rich in both GABA/BZ and 5-HT receptors, we employed either intramuscular (i.m.) injections or direct injections into the hippocampus in a drug discrimination procedure. Pigeons' pecks on one of two keys were reinforced on a fixed-ratio 30 schedule following (i.m.) injections of buspirone (right key) or saline (left key). In substitution tests, both buspirone and 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) resulted in generalization regardless of the route of administration, whereas hippocampal injections of midazolam produced primarily saline-key responding in all pigeons. Furthermore, i.m. injections of the putative 5-HT antagonist NAN-190 (1-(2-methoxyphenyl)-1-[4-(2-phthalimido)butyl] piperazine) attenuated the effects of hippocampal 8-OH-DPAT. The results suggest that receptors in the hippocampus are involved in mediating the discriminative stimulus and perhaps anti-conflict effects of 5-HT drugs, but not the effects of the benzodiazepines. Supported by DA-02873.

252.8

DISCRIMINATIVE STIMULUS CHARACTERISTICS OF A HIGH AND A LOW DOSE OF DIAZEPAM IN RATS. S.R.Franklin and A.H.Tang. CNS Research, The Upjohn Company, Kalamazoo, MI 49001

Two groups of rats were trained to discriminate either 1 mg/kg (LOW) or 10 mg/kg (HIGH) of diazepam (DZ) from IP injections of vehicle. The experimental paradigm was a 2-lever, FR-10 schedule for food reinforcement. For both dose groups, response rate at the training doses was equal to or greater than that in vehicle sessions.

Alprazolam, adinazolam, and pentobarbital completely generalized to both doses of DZ. Only pentobarbital reduced response rate at the dose of complete generalization. The benzodiazepine partial agonist, ZK 91296, generalized to DZ in the LOW group, but not in the HIGH group. Flumazenil (F) produced no drug-appropriate lever choice, and blocked the DZ cue effects from DZ or ZK 91296. Response rate was unchanged at the dose of cue-antagonism. The drug lever choice from pentobarbital in either group was not reversed by F, although F reversed the rate-reducing effect of pentobarbital. It is concluded that the DZ cue-effects in both HIGH and LOW groups are mediated by benzodiazepine receptors. It is not associated with sedation, as measured by reduction in response rate. There is a significant difference in the intensity of the drug cues, since the partial agonist generalized in the LOW dose, but not in the HIGH dose group.

252.9

EFFECTS OF NEONATAL TREATMENT WITH NORELEAGNINE ON ADULT MOTOR ACTIVITY AND CONVULSIONS INDUCED BY ELECTROSHOCK. P. McGuire, J. Tizzano, and D. Helton*. Pharmacol. and Toxicol., Ind. Univ. Med. Cen., Indianapolis, IN 46223, and Toxicol. Div., Lilly Res. Labs., Eli Lilly & Co., Greenfield, IN 46140.

Previously, it has been shown that neonatal treatment with noreleagnine (NOR) can result in increased juvenile activity in rats. This study examined potential persistent effects of such treatment on adult activity and electroshock-induced convulsions following NOR challenge. Twelve litters of CD rats were treated with NOR on postnatal days 13-20 (1 pup/sex/litter was dosed ip with 0, 2, 4 or 8 mg/kg/day NOR). There was no neonatal treatment related difference in adult baseline activity. When challenged with these doses as adults, dose-related decreases in activity were seen. Rats treated neonatally with 0 or 8 mg/kg were then challenged with 16 and 32 mg/kg NOR. Both doses decreased activity in a manner unrelated to neonatal treatment. Neonatal treatment did not alter the proportion of adult rats which showed tonic seizures produced by corneal electroshock. There also was no indication of a proconvulsant effect of NOR challenge with 32 mg/kg to adult rats treated neonatally with either 0 or 8 mg/kg. Neonatal treatment with NOR did not result in persistent changes in baseline motor activity, seizure susceptibility, or responsiveness in either end point after NOR challenge.

252.10

NEONATAL COCAINE TREATMENT DECREASED ACOUSTIC STARTLE RESPONSE LATENCY IN THE ADULT RAT. H. E. Hughes, L. A. Freed, L. A. Scribani*, T. H. Milhorat and D. L. Dow-Edwards. Laboratory of Cerebral Metabolism, Department of Neurosurgery, SUNY Health Science Center, Brooklyn, N.Y., 11203.

Our lab has demonstrated brain metabolic effects and changes in locomotor activity in adult female rats treated with cocaine during the first 20 days of postnatal life. The present study investigated the acoustic startle response (ASR) in adult rats treated with cocaine during this same period. Female littermates received 50 mg/kg/day cocaine HCl sc or vehicle during either 1-10 or 11-20 days of age. At age 90-100 days, animals from each group were tested on two consecutive days in an SR-Lab Startle Response System. Response amplitude was measured in voltage as the difference between the force of the animal at rest and at the height of the ballistic type movement that characterizes ASR. The latency to reach this maximum amplitude was also recorded. The results show that the latency to reach maximum ASR on the first trial was diminished on Day 2 of testing in the 11-20 day cocaine treated rats. There was no effect on response magnitude. These results are consistent with other studies that have dissociated ASR latency and magnitude (Horlington, M., *Physiol & Behav.*, 3: 839-844, 1968). Previous investigations using lesioning and stimulation have shown brain structures mediating ASR to include the v. cochlear nucleus, nuclei of the lateral lemniscus and v. nucleus reticularis pontis caudalis (Davis, M. et al., *J. Neurosci.*, 2: 791-805, 1982) We will report rates of brain glucose metabolism in ASR related structures of cocaine treated rats using the deoxyglucose method of Sokoloff. Supported by ADAMHA grant #DA04118.

252.11

BEHAVIORAL TOXICITY OF ORALLY ADMINISTERED COCAINE IS GREATER IN MALE, COMPARED WITH FEMALE S.-D. RATS. S.B.Sparber and M. Kubak* (SPON: G. Wilcox). Dept. of Pharmacology, Univ. of Minnesota, Minneapolis, MN 55455.

Prior to injection of pregnant rats with cocaine HCl, p.o., to determine the effects of this drug, if any, upon mature offspring exposed *in utero*, we compared the effects of 3 daily doses of 15 mg/kg or 45 mg/kg, spaced 3 hr & 40 min apart, upon food reinforced fixed ratio 15 operant behavior of rats maintained at 90% of their free-feeding weights (females: 274-324 gm; males: 459-599 gm, ca. 165 days old). Rats were given water (2 ml/kg) or cocaine 55 min after their 1st 15 min behavior session of the day and the effects of cocaine were measured 30 min and 2 hr & 30 min after treatment. Acute and subacute behavioral toxicity, including carryover effects from previous days' treatment, could thus be determined during subsequent daily sessions. Results indicated that the low dose of cocaine (15mg/kg x3/day) produced minimal behavioral suppression acutely or subacutely, 30min or 2 hr & 30 min after dosing of either sex (N=3/group). The high dose (45mg/kg x3/day) caused significant suppression of behavior 30 min, but not 2 hr & 30 min after the 1st dose, but only in the males. Carryover effects were evident in males given the high dose; behavior was suppressed prior to and after treatment. Females given the high dose started to show suppressed behavior 30 min after the 2nd daily dose after several days of treatment. We conclude that oral treatment of pregnant rats with such doses distributed over 8-10 hr should enable us to maintain exposure of the dam (and fetuses) without resorting to multiple i.p. or s.c. injections of pregnant rats, which lead to problems caused by local vasoconstriction, intrauterine injection and limited exposure to this short-acting drug of abuse. Supported in part by USPHS grant DA04979.

252.12

POST-CONDITIONING DRUG EFFECTS ON CONDITIONED PLACE PREFERENCE INDUCED BY THE COADMINISTRATION OF MAGNESIUM AND COCAINE. S.I. Lawley and K.M. Kantak. Lab. of Behav. Neurosci., Dept. of Psychol., Boston University, Boston, MA 02215.

It was hypothesized that $MgCl_2$ and cocaine would have interactive rewarding stimulus effects in a conditioned place preference (CPP) paradigm, using a submaximal dose of cocaine, based upon data which show a robust conditioning effect of 5 mg/kg cocaine and a moderate conditioning effect of 15 mg/kg $MgCl_2$. The interaction of 15 mg/kg $MgCl_2$ with 0.612 mg/kg cocaine was found to produce stronger conditioning effects than other submaximal dose combinations. In animals where this dose combination switched side preference, the acute administration of amphetamine (1.0 mg/kg) potentiated CPP relative to saline, haloperidol, or pentobarbital. Previously reported results show that this dose of amphetamine potentiates cocaine-induced CPP (using 5.0 mg/kg) and $MgCl_2$-induced CPP (using 15 mg/kg). In the present set of experiments, pentobarbital (10 mg/kg) attenuated cocaine + $MgCl_2$ CPP. It was previously shown that pentobarbital attenuates cocaine-induced CPP, whereas it maintains $MgCl_2$ induced CPP. Haloperidol (0.25 mg/kg) attenuated cocaine + $MgCl_2$ CPP as it does when each drug is used alone as the conditioning agent. It appears that the coadministration of MgCl2 with a submaximal dose of cocaine enhances the potency of cocaine and produces a profile of post-conditioning drug effects more similar to cocaine than MgCl2.

252.13

LONG TERM PREEXPOSURE TO MAGNESIUM INHIBITS THE REWARDING EFFECTS OF SELF ADMINISTERED COCAINE. K.M. Kantak, J.F. Bourg* and S.I. Lawley. Lab. of Behav. Neurosci., Dept. of Psychol., Boston University, Boston, MA 02215.

Previous research has shown that $MgCl_2$ robustly substitutes for self administered cocaine in rats in a dose dependent manner such that a steady intake pattern of $MgCl_2$ is maintained in the absence of cocaine. In contrast, in drug naive rats, $MgCl_2$ fails to be self administered which indicates that $MgCl_2$ has low abuse potential. These data are consistent with data obtained in a conditioned place preference procedure, and with a number of studies showing that $MgCl_2$ has stimulant - like behavioral effects. Additional research has shown that the disruptive effects of chronic cocaine on D1 receptor binding and on aggressive behavior could be prevented by concurrent administration of $MgCl_2$. Thus, besides serving as a non-addictive substitute for cocaine, $MgCl_2$ appears to have prophylactic effects against cocaine. This notion was further studied in the present experiments in drug naive rats who contingently responded for I.V. $MgCl_2$ for 4 weeks prior to access to I.V. cocaine. Normally, rats acquire a response for cocaine on the first day or two of access to cocaine. Preexposure to hypertonic saline results in a typical cocaine access pattern as well. In contrast, preexposure to $MgCl_2$ prevents the acquisition of a response for cocaine, even after two weeks of exposure to cocaine. The animals show a typical extinction curve of responding which indicates that cocaine is not rewarding under these conditions.

252.14

COCAINE AND OTHER LOCAL ANESTHETICS AS DOPAMINE AGONISTS. P.B. Silverman. Dept. of Psychiatry, U. Texas Health Science Ctr., Houston, TX 77030

Cocaine and some local anesthetics are self-administered and have similar stimulus properties. But unlike cocaine, other locals have little if any documented dopamine (DA) agonist activity. In order to determine if local anesthetics have previously undetected psychomotor stimulant-like effects, we tested locals in a unilaterally lesioned rodent preparation sensitive to both direct and indirect dopamine agonists. Under pentobarbital anesthesia, rats were lesioned in one substantia nigra by 6-hydroxydopamine infusion. They were subsequently tested for rotational behavior after apomorphine, cocaine and one or more of the locals.

Cocaine (10 mg/kg; 29 μmol/kg) induced ipsilateral rotation in the lesioned rats indicating indirect DA agonist activity. Chloroprocaine, etidocaine, lidocaine, mepivacaine, procaine and tetracaine induced no significant rotation. Dimethocaine at 50 and 100 μmol/kg, induced ipsilateral rotation at rates comparable to cocaine. Ritz et al. (Science 237:219, 1987) have reported a common DA uptake binding site for cocaine, related compounds and local anesthetics. Significantly, the local with highest affinity for the site is dimethocaine.

252.15

INTERACTIVE EFFECT OF COCAINE AND CAFFEINE AS ASSESSED BY CONDITIONED PLACE PREFERENCE. J. B. Bedingfield* and F.A. Holloway. (SPON: A.M. Revzin) University of Oklahoma Health Sci. Ctr., Oklahoma City, OK 73190-3000.

The conditioned place preference (CPP) paradigm is a behavioral assay where previously neutral environmental stimuli are classically conditioned to interoceptive drug-induced stimuli. CPP is an animal analogue to the "rewarding" effects of drugs in man. Eight groups of male Sprague-Dawley rats (n=9/group) were trained in the CPP paradigm using saline, cocaine (COC; 1.0, 3.2, or 5.6 mg/kg), or caffeine (CAF; .32 or 1.0 mg/kg) as conditioning stimuli. Saline failed to produce a significant level of CPP, but both COC and CAF produced dose-dependent increases in the amount of time subjects spent in the drug-paired environment. CPP was robust and statistically significant. When a marginally effective dose of COC (1 mg/kg) was combined with a marginally effective dose of CAF (1.0 mg/kg), a strong CPP resulted. When a non-effective dose of CAF (.32 mg/kg) was combined with a non-effective dose of COC (.32 mg/kg), a robust CPP again resulted. These results suggest that at certain doses, caffeine acts in a multiplicative fashion with cocaine. Motivational effects of these combinations may contribute to an abuse liability greater than each drug separately.

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252.16

CROSS-GENERALIZATION BETWEEN COCAINE AND "LEGAL" STIMULANTS. F.A. Holloway, D.V. Gauvin*, and K.M. Moore*. Department of Psychiatry and Behavioral Sciences, University of Oklahoma Health Sciences Ctr., Oklahoma City, OK 73190-3000.

To examine the abuse liability of "legal" over-the-counter stimulants, 4 groups of male Sprague-Dawley rats were trained in a two-choice drug discrimination task. Phenylpropanolamine (PPA; 17.8 mg/kg) or Ephedrine (EPH; 10 mg/kg), or Caffeine (CAF; 42 mg/kg), or a combination of these three stimulants (CEP; 15 mg/kg CAF, 3 mg/kg EPH, and 6 mg/kg PPA) and saline served as stimuli in separate groups (n=12/group). PPA and EPH groups displayed symmetrical cross-generalization between PPA and EPH. Each element of the CEP compound training stimulus produced at least partial generalization to that stimulus. Tests with cocaine resulted in complete generalization to the PPA, EPH, and CEP training stimuli. Cocaine produced only partial generalization to the CAF training stimulus. We have previously reported that a CEP compound test stimulus generalized to a cocaine training stimulus (Life Sciences, 44, 67-73). Such symmetrical cross-genereralization between cocaine and CEP suggest an abuse liability of these "legal" over-the-counter stimulants that is greater than would be predicted from existing self-administration data.

Supported by State of Oklahoma Department of Commerce (Contract #1686) and NIDA grant DA04444.

252.17

d-AMPETAMINE, MDMA, AND PCP EFFECTS ON AGGRESSIVE AND CONDITIONED BEHAVIOR: 5-HT AND DOPAMINE ANTAGONISTS. M. Haney*, K.A. Miczek (SPON: H.B. Barry III). Dept. Psychology, Tufts Univ., Medford, MA 02155.

Dopamine and 5-HT receptors maybe significant in several behavioral effects of amphetamine, MDMA and PCP. We developed an experimental preparation that permitted the concurrent assessment of behavioral performance under the control of a multiple schedule of reinforcement as well as aggressive behavior by a resident toward an intruder. First, the rate-increasing effects of amphetamine and, to a lesser extent, of MDMA and PCP were confirmed. Second, an aggression test was incorporated into the conditioning session. After ca. 22 min in the conditioned performance task, the mice confronted an intruder in their home cage for ca. 5 min, and then completed the remainder of the conditioning session. Aggressive behavior disrupted conditioned performance only transiently, and eventually enhanced responding in the second half of the conditioning session. Amphetamine, but not MDMA or PCP, increased aggressive behavior in a subset of individual subjects. The dose-effect curves for the suppression of aggressive behavior by amphetamine, MDMA and PCP paralleled those for FR and FI performance. SCH23390 blocked the enhancing but not the suppressive effects of amphetamine on FI performance and aggressive behavior. When mice were pretreated with 5-HT_2 receptor antagonists, MDMA and amphetamine decreased aggressive behavior and operant performance at lower doses than before. These observations indicate experience with aggressive behavior shares some rate-increasing effects with amphetamine. The D_1 antagonist effects suggest a role of this receptor subtype in the activation of aggressive and conditioned behavior by amphetamine.

252.18

NON-NEUROTOXIC RIGID ANALOGS OF 3,4-METHYLENEDIOXY-AMPHETAMINE (MDA). D. E. Nichols, W. K. Brewster* M. P. Johnson* R. Oberlender* and R. M. Riggs* Depts. of Pharmacol. and Toxicol., and Med. Chem. and Pharmacog., School of Pharmacy, Purdue Univ., W. Lafayette IN 47907.

MDMA and its α-ethyl analog (MBDB) have novel behavioral characteristics inconsistent with their classification as either hallucinogens or stimulants (Nichols et al., J. Med. Chem. 29:2009, 1986). Both MDMA and MBDB are selective neurotoxins for serotonin terminals (Johnson et al., Pharmacol. Biochem. Behav., in press).

Several methylenedioxy-substituted aminoindans and aminotetralins were examined in the rat drug-discrimination paradigm for MDMA or LSD like effects and were screened for serotonin neurotoxicity. Both 5,6-methylenedioxy-2-aminoindan (5,6-MDAI) and 6,7-methylenedioxy-2-aminotetralin (6,7-MDAT) but not 4,5-MDAI or 5,6-MDAT substituted for MDMA in drug-discrimination experiments. The ED_{50} values and 95% confidence limits were 0.64 (0.46-0.89), 0.59 (0.34-1.00), and 1.29 (0.75-2.21) mg/kg for MDMA, 5,6-MDAI and 6,7-MDAT, respectively. None of the compounds substituted for LSD. One week after a 40 mg/kg s.c. injection of 5,6-MDAI and 6,7-MDAT there was no change in any rat brain monoamine or metabolite level examined. There was also no change in B_{max} values for [^{3}H]-paroxetine binding, a measure of the number of serotonin uptake sites. In contrast, 40 mg/kg s.c. of MDMA significantly decreased 5-HT and 5-HIAA levels and decreased the B_{max} for [^{3}H]-paroxetine binding.

252.19

EFFECTS OF CAFFEINE ON FLASH EVOKED POTENTIALS OF HOODED RATS. B.E. Hetzler and A.C. Bauer*. Department of Psychology, Lawrence Univ., Appleton, WI 54912.

Caffeine is a widely consumed behavioral stimulant. However, its effects on evoked potentials have not been well characterized. In the present study, flash evoked potentials (FEPs) were recorded from the visual cortex of hooded rats 30 min following i.p. injections of distilled water, and of 25 and 100 mg/kg caffeine on separate days. Rats were tested at a standard (22°C) ambient temperature. The 25 mg/kg dose did not significantly alter the amplitude or latency of any FEP component. Amplitudes of components P2 and N3 were significantly reduced by the 100 mg/kg dose, and a reduction in the amplitude of component P3 approached significance. In contrast, the latencies of only the early components were significantly altered by the 100 mg/kg dose. Components P1, N1, and P2 were increased in latency by 1.42, 1.58, and 2.66 msec, respectively.

The increased latencies may be at least partially explained by caffeine-induced hypothermia. In a separate experiment, a 100 mg/kg dose produced hypothermia of about 0.5°C. The amplitude data suggest that manipulation of the brain adenosine system results in selective alterations of FEP components, since caffeine is an adenosine receptor antagonist. However, only the high dose of caffeine resulted in significant changes, so it is also possible that non-adenosinergic mechanisms are involved. (Supported by the Appleton Medical Center Foundation, Inc.)

252.20

UNILATERAL INTRASTRIATAL CAFFEINE PRODUCES A CONTRALATERAL BIAS IN THE TURNING BEHAVIOR OF RATS R J Beninger and S.A. Josselyn*. Dept. Psychol., Queen's University, Kingston, K7L 3N6, Canada

Caffeine stimulates locomotor activity but its site of action in the brain has not been established. Thus, the present study was undertaken to assess the possible effects of unilateral intrastriatal microinjections of caffeine on turning behavior in rats. 17 rats were implanted with chronic cannulae placed in the dorsoanterior striatum. They received 7 20-min tests, each separated by 72 h, in circular arenas (30 cm diam). The first and last sessions were preceded by no injection, the second and sixth by saline (1.0 µl) injections and the remaining three by caffeine (1.0, 10 or 20 µg in 1.0 µl), the order of doses being counterbalanced across rats. The total number of complete turns in each direction was recorded for each session. Results revealed no significant turning bias in rats receiving no injection or saline either before or after caffeine sessions. The low dose of caffeine produced no effect but the two higher doses produced a contralateral turning bias. Furthermore, the highest dose produced an increase in the total number of turns These results suggest that caffeine may produce its stimulant effects at least in part by an action in the striatum. (Funded by the Natural Sciences and Engineering Research Council of Canada.)

253.1

SIMULTANEOUS ASSOCIATIONS OF TASTES WITH NICOTINE. F.W. Flynn, D. Dracos and C. Ksir. Dept. of Psychology and Neuroscience Program, Univ. of Wyoming, Laramie, WY 82071.

Depending on the contingencies and doses, nicotine is reinforcing or an aversive stimulus to animals. Flynn, Webster and Ksir (1989) reported that, over days, rats develop a significant preference for a 1 µg/ml nicotine solution. In the current experiment, a simultaneous association procedure was used in an attempt to further demonstrate nicotine's reinforcing property in rats. On alternate days rats were given 1-bottle access to either a compound of a neutral taste (CS+) mixed with nicotine or a different taste stimulus presented alone (CS-) for 1 h/day. Rats having access to the compound solution with 1 µg/ml nicotine ingested 17.5 ± 0.7 µg of nicotine during each exposure and rats having access to the 5 µg/ml concentration ingested 61 ± 7 µg nicotine during each 1 h CS+ exposure, $p < .01$. Two-bottle preference tests between the CS+ and CS- were conducted every 14 days for 56 days. For each of the 4 tests, the preference ratios for tastes associated with 1 µg/ml nicotine (51 ± 3%) were greater than the preference ratios for tastes presented as a compound with 5 µg/ml nicotine (21 ± 3%), $p < .01$. In the present procedure, 1 µg/ml nicotine was found to have neither reinforcing nor aversive consequences whereas intake of 5 µg/ml nicotine was shown to have aversive consequences.

(Supported by NIH ROl NS24879 awarded to F.W.F.)

253.2

TOLERANCE TO ANXIETY-LIKE EFFECT OF NICOTINE (NIC) IN RATS. C. M. Harris and H. Lal, Depts. of Pharmacology, NYCOM, Old Westbury, NY 11568, and TCOM, Fort Worth, TX 76107.

NIC produces a stimulus similar to that of the anxiogenic drug pentylenetetrazol (PTZ) (Harris et al., Psychopharmacol., In Press). Eight rats trained to discriminate PTZ, 20 mg/kg, from saline in a 2-lever choice task were tested for tolerance to this effect during chronic NIC treatment. NIC base was infused at 0.477 ul/hr via Alzet osmotic pump. Rats first detected a PTZ-like direct effect of NIC (50% selected PTZ lever), then exhibited signs of toxicity, then recovered normal overt behavior and ceased to detect the PTZ-like effect of NIC. Convulsions abated by day 4; failure to eat and to complete the food-reinforced lever-choice task abated by day 6; selection of the PTZ-lever declined to 12.5% by day 10. After pump removal on day 12, a PTZ-like withdrawal effect emerged within 24 hours, as in a previous study (Harris et al., Psychopharmacol. 90:85-89, 1986). Within three days rats regained sensitivity to the PTZ-like effect of NIC (stimuli produced by withdrawal and by high doses of NIC were additive) but remained tolerant to the disruptive effect of NIC in the lever-choice task. Supported by AOA 82-11-045 and NIH-BRSG RR05879.

253.3

EFFECTS OF THE ACUTE AND CHRONIC ADMINISTRATION OF MK-801 ON BEHAVIOR AND BODY TEMPERATURE IN THE RAT. R.N. Pechnick, M. Hiramatsu*, R. George*, A.K. Cho and E. Di Stefano*. Department of Pharmacology, UCLA School of Medicine, Los Angeles, CA 90024-1735

Phencyclidine (PCP) binds to PCP and sigma receptors, but the differential roles of these two receptors in mediating the effects of PCP is not currently known. MK-801[(+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]-cyclohepten-5,10-imine maleate] has been found to have a high degree of selectivity for PCP over sigma receptors. The present study characterized the effects of the acute and chronic administration of MK-801 on behavior and body temperature in the rat. Like PCP, the acute administration of MK-801 produced ataxia, locomotion, sniffing and head weaving. MK-801 produced hyperthermia, in contrast to the hypothermia observed after the acute administration of PCP. There was tolerance to MK-801-induced ataxia after 6 days of daily administration; however, the hyperthermic response was enhanced. PCP produced less ataxia in chronically MK-801-treated rats, indicating the development of cross-tolerance. However, PCP, at a dose that did not affect body temperature in chronically saline-treated rats, caused hyperthermia in chronically MK-801-treated subjects, indicating the development of cross-sensitization to the effects on body temperature. The differences in response were not due to changes in biodisposition as the plasma levels of PCP were not significantly different. These results suggest that both MK-801 and PCP may produce ataxia by interacting with PCP receptors; however, PCP-induced hypothermia must be caused by interactions with receptors other than the PCP receptor. (Supported NIDA grants DA-02411, DA-04113 and DA-05448).

253.4

EFFECTS OF ACUTE ADMINISTRATION OF (+)-PENTAZOCINE ON BODY TEMPERATURE IN THE RAT. M. Bejanian[1], R.N. Pechnick[1], R. George[1], A. Thurkauf[2*], A.E. Jacobson[2*] and K.C. Rice[2]. Department of Pharmacology[1] and the Brain Research Institute, U.C.L.A. School of Medicine, Los Angeles, CA 90024-1735, and Lab. of Neuroscience[2], NIDDK, Bethesda, MD 20892.

(+)-Pentazocine has a greater selectivity for sigma versus phencyclidine receptors. The current study investigated the effect of acute administration of (+)-pentazocine on the body temperature of the rat. Baseline rectal temperatures of adult male Sprague-Dawley derived rats were recorded and the rats were injected subcutaneously with saline or (+)-pentazocine (20, 40 or 80 mg/kg). Rectal temperatures were recorded at 30 minute intervals for 180 minutes post-injection. Administration of 80 mg/kg (+)-pentazocine resulted in significant hypothermia when compared to saline-injected rats and rats injected with 20 and 40 mg/kg (+)-pentazocine. The hypothermic effect of 80 mg/kg (+)-pentazocine was evident for 120 minutes post-injection and was followed by a hyperthermic response. (+)-Pentazocine at doses of 20 and 40 mg/kg did not cause hypothermia, but showed a hyperthermic response in the rats at 120 minutes post-injection. In conclusion, (+)-pentazocine has a biphasic effect on body temperature such that at the dose of 80 mg/kg it causes an initial hypothermic response followed by hyperthermia. At the lower doses, only the hyperthermic response is evident. These results along with evidence from the studies of other selective ligands suggest that there may be different mechanisms underlying the hypothermic and hyperthermic effects of (+)-pentazocine. (Supported by NIDA grants DA-04113 and DA-05448).

253.5

PHENCYCLIDINE (PCP) EXPOSURE IN HUMAN ADDICTS DOES NOT ALTER REGIONAL SIGMA (σ) BINDING. A.D. Weissman, M.F. Casanova* and E.B. De Souza. Neurobiology Laboratory, NIDA Addiction Research Center, Baltimore, MD 21224; Clinical Brain Disorders Branch, NIMH, Washington, DC 20032.

PCP is an abused drug which is capable of precipating psychotic episodes in man. Some of the psychotomimetic properties of PCP are thought to be mediated by interactions with σ binding sites. Alterations in the σ site have also been associated with schizophrenia (Weissman et al., Soc. Neurosci. Abstr. 14, 104, 1988). The present study sought to examine whether chornic exposure to PCP in human addicts would alter σ binding in postmortem brain tissues. Saturation studies of [^{3}H]haloperidol binding in the presence of 50 nM spiperone were performed in the brains of addicts who had died by suicide and had high postmortem blood levels of PCP. Age and postmortem interval matched individuals who had committed suicide, but had no history or postmortem evidence of abusing drugs, served as controls. In our previous studies, both suicide and normal individuals showed similar σ binding. The present study showed that the affinity (K_D) and number of binding sites (B_{max}) was not different in the brain of PCP addicts in occipital, frontal and anterior cingulate cortex as well as in hippocampus and cerebellum. Potential changes in brain PCP binding sites are currently being examined in these tissues. The results suggest that the brain σ binding site remains unchanged in humans despite repeated challenges with a potent psychotomimetic drug.

253.6

THE SIGMA ANTAGONIST (±)BMY14802 DOES NOT REVERSE (+)N-ALLYLNORMETAZOCINE-INDUCED BEHAVIORAL CHANGES IN MEMBERS OF A NONHUMAN PRIMATE SOCIAL COLONY. **D.J. McGinness*, R.F. Schlemmer, Jr., N.L. Katz, J.M. Davis. Univ. of Illinois at Chicago & Illinois State Psychiatric Inst., Chicago, IL 60612.**

(+)N-Allylnormetazocine (NANM) is a drug that is commonly used as a sigma agonist in psychopharmacological studies. (+)NANM induces several significant behavioral changes in selected members of Stumptail macaque social colonies (McGinness et al., Neurosci. Abst. 14:525, 1988). The present study sought to determine if the preferential sigma antagonist and antipsychotic candidate (±)BMY14802 could reverse the behavioral changes induced in monkeys by (+)NANM. The subjects were 4 adult Stumptail macaques who formed a stable social colony. Following observation of baseline behavior, each monkey received treatment with (+)NANM (1 mg/kg, i.m.), then 3 escalating doses of (±)BMY14802 (3-10 mg/kg, i.m.) alone & in combination with (+)NANM. Only 2 monkeys received drug treatment/day & at least 72 hr separated each drug treatment. Two 1 hr observation sessions were conducted each day by a "blind" observer who rated behavior in the entire colony. The first observation session began 2 hr after administration of BMY14802. The second session began 30 min after the first concluded & was preceded by an injection of (+)NANM or saline 15 min before the next observation. In agreement with our previous study, (+)NANM decreased initiated social grooming, self grooming, checking (visual scanning) & locomotion, and increased resting with eyes open. None of the BMY14802 doses significantly antagonized the (+)NANM-induced behavioral changes. The results suggest that the behavioral changes induced by (+)NANM cannot only be attributed to mediation by sigma binding sites. One plausible explanation would be that behavioral effects of (+)NANM may be mediated by PCP receptors.

253.7

BOTH MORPHINE AND COCAINE INCREASE GLUCOSE METABOLISM IN THE OLFACTORY TUBERCLE IN FREELY-MOVING RATS.
D. Huston-Lyons, L.J. Porrino#, G.T. Bain*, and C. Kornetsky, Boston University School of Medicine, Boston, MA, #NINCDS, Bethesda, MD.

Although cocaine (COC) and morphine (MS) have markedly different spectra of actions on most behaviors, they are abused by humans, self-administered by animals, and facilitate brain-stimulation reward. However, previous experiments have reported that COC (ip) had little or no effect (London et al., 1986) and MS (sc) decreased or had no effect (Ito et al., 1983) on rates of local cerebral glucose utilization (LCGU) in structures of the meso-corticolimbic dopamine system, a system directly involved in motivation and drive. In order to compare the effects of COC and MS in the same laboratory and under the same conditions, the 2-[14-C]deoxyglucose method was used to determine rates of LCGU following the administration of COC 10 mg/kg (ip) or MS 4 mg/kg (sc) to freely-moving rats. COC increased LCGU in the olfactory tubercle (OT), medial prefrontal cortex, and substantia nigra reticulata. MS, however, caused a significant LCGU increase only in the OT, with either a decrease or no effect in other structures. Because of the significant role of the OT in the motivational system of the rat, these results support an hypothesis that drug-induced euphoria caused by COC or MS is mediated, at least in part, by activation of similar forebrain areas. (Supported in part by NIDA grant DA02326 and Research Scientist Award DA00099 to CK).

253.8

QUALITATIVE DIFFERENCES IN HEROIN AND COCAINE SELF-ADMINISTRATION BEHAVIOUR. D.C.S. Roberts and S.A.L. Bennett*, Department of Psychology, Carleton University, Ottawa, Ont., K1S-5B6.

Differences between heroin and cocaine motivated behaviour were investigated using progressive ratio (PR) schedules of reinforcement. On one schedule, the first response of the daily test session resulted in an IV injection. Thereafter, the response requirements increased through an escalating series (2,4,6,9,12,...). Breaking-points were defined as the last ratio completed prior to one hour of non-reward. On this schedule, animals self-administered cocaine in a regular fashion then stopped abruptly at relatively high "breaking-points". By contrast, rats responding for IV heroin self-administered in an irregular pattern, often pausing for long periods only to resume later. In general the breaking-points were substantially higher for cocaine (0.6mg/inj) than for heroin (25 ug/inj). On a different PR schedule, the requirements regulating reception of the session's first injection were adjusted. Higher breaking-points were obtained under heroin reinforcement than under cocaine. Animals often failed to respond for cocaine reinforcement without "priming," whereas rats would respond 60-80 times for their first injection of heroin without "priming". These data demonstrate that the intense motivation to receive heroin is diminished after the first few injections. The motivation to receive cocaine is cocaine-induced. (Supported by the M.R.C)

253.9

TOLERANCE TO MORPHINE-LIKE STIMULUS EFFECTS OF AGONIST AND AGONIST-ANTAGONIST MU OPIOIDS IN RATS. A.M. Young, G. Kapitsopoulos*, and E.S. Steigerwald*. Department of Psychology, Wayne State University, Detroit, MI 48202.

Experiments evaluated the ability of repeated treatment with morphine (MS) to confer tolerance to the MS-like discriminative stimulus effects of methadone (MET), nalbuphine (NBP), and buprenorphine (BUP). Male Sprague-Dawley rats were trained to discriminate saline and 3.2 mg/kg MS under FR15 schedules of food delivery. After establishment of stimulus control, cumulative doses of MET (0.1-3.2 mg/kg), BUP (0.00032-0.32 mg/kg), and NBP (0.032-5.6 mg/kg) were tested for generalization in separate groups of rats (N = 5 or 6). Then, training was halted and a dose of 10 mg/kg MS was administered b.i.d. for 14 to 17 days. Each drug was tested for generalization on the 7th and 14th day of MS treatment and at selected times after treatment ended. One or two weeks of MS treatment increased by 2- to 3-fold the doses of MET or BUP required for generalization. In contrast, repeated MS treatment produced an insurmountable increase in the dose of NBP required for generalization, so that doses of 32 to 56 mg/kg evoked only 40% MS-appropriate responding and suppressed response rates slightly. After MS treatment ended, the doses of MET or NBP required for generalization returned to initial values within 1 week; those of BUP, within 2 weeks. Such differential tolerance suggests that NBP has lower efficacy as a MS-like stimulus than do MET and BUP. (DA-03796.)

253.10

ACUTE DEPENDENCE AND WITHDRAWAL IN NEONATAL CHICKENS. M.E. Bronson* and S.B. Sparber. Dept. of Pharmacology, University of Minnesota, Minneapolis, MN 55455.

The importance of studying consequences of opioid exposure during development includes the need to study effects of withdrawal from opioids, which may be more detrimental than exposure to the opioid itself. Accordingly, we have previously used the chick embryo to study this phenomenon during earlier development and now report acute opioid dependence and withdrawal in neonates of this species. To characterize the signs of withdrawal, various doses of naloxone (Nx) were administered 1 hour after 8 mg methadone (M)/kg. Isobutylmethylxanthine (IBMX) was also given without prior opioid exposure to study quasi-opioid withdrawal. All drugs were given i.p. In chicks pretreated with M, mild to moderate withdrawal, after 0.8 mg Nx/kg, was manifest as distress vocalization and head shaking. More severe withdrawal (8-32 mg Nx/kg or 20 mg IBMX/kg) caused tremor, wing extension, splayed feet and , at the highest dose of Nx, loss of righting reflex and apparent convulsions. Some of these signs were observed in saline pretreated 1 day-old chicks injected with 48-64 mg Nx/kg, suggesting greater sensitivity to nonspecific actions of very high doses of Nx, or sensitization due to high titers of endorphins which may be caused by the hatching process, since most of these signs were not observed in older (ie., 3 day-old) chicks injected with 64 mg Nx/kg. These data demonstrate the utility of using the chick neonate to study further the consequences of dependence and/or withdrawal alone (IBMX) during early development in the absence of maternal influences. Supported by USPHS grant DA01880 and T32 DA 07097.

253.11

MORPHINE PHYSICAL DEPENDENCE DURING HYPOTHERMIA IN THE GROUND SQUIRREL (CITELLUS LATERALIS). M.L. Jourdan*, A.L. Beckman, and L.C.H. Wang*. Dept. of Zool., Univ. of Alberta, Edmonton, Alberta, Canada T6G 2E9 and Dept. of Psychol., California State Univ., Long Beach, CA 90840.

To test the possibility that hibernation induced blockade of morphine dependence (Beckman et al., Science 212:1527, 1981) may be due to the low body temperature (Tb) associated with deep hibernation, we examined the development of morphine dependence during deep hypothermia.

Morphine (M; 55 μg/hr), naloxone (Nx; 5 μg/hr) or artificial cerebrospinal fluid (aCSF; 1 μl/hr) were infused continuously via a chronic cannula in the lateral cerebral ventricle during 48 hr of induced hypothermia (Tb: 7°C). M-infused, aCSF-infused, and sham animals (no infusion) injected with Nx (1 mg/kg, s.c.) upon rewarming to normal Tb showed typical abstinence signs. Infusion of Nx during hypothermia significantly decreased the total amount of Nx-precipitated abstinence behavior below that of the other three groups.

The results suggest that the hibernation-block of morphine dependence is not due to low Tb alone. Further, the abstinence behavior of aCSF-infused and sham animals suggests that deep hypothermia is a stress that substantially increases the activity of brain opioid peptide systems.

253.12

INTERACTIONS BETWEEN SUCROSE FEEDING AND MORPHINE DEPENDENCE AND WITHDRAWAL. D.S. Roane*, G. Scheonbaum* and R.J. Martin (SPON: C. Phelix). Dept. of Foods and Nutrition, Univ. of Ga., Athens, Ga. 30602.

This study examines the effect of dietary sucrose on the development of tolerance to morphine analgesia and on the severity of subsequent naloxone-precipitated withdrawal.

In the first experiment, 15 male albino rats fed a 20% sucrose supplement ad libitum were given injections of morphine sulfate, (20 mg/kg) every 12 hours for one week. Tail flick latencies were measured every a.m. Tolerance to morphine analgesia developed similarly in sucrose-fed and control rats. Withdrawal was precipitated with naloxone HCl (2 mg/kg). Subsequent 18 h. weight loss - an index of withdrawal severity - was similar between sucrose-fed and control rats. During the development of tolerance sucrose consumption followed a bi-phasic pattern, first decreasing and subsequently increasing. Sucrose-fed animals were hyperphagic during withdrawal, consuming most of their dietary energy from sucrose.

In the second experiment 30 animals were assigned to the sucrose group and were treated as before. Immeditately prior to precipitated withdrawal sucrose access was denied to half this group. The severity of withdrawal, in terms of 18 h. weight-loss, was greater in the sucrose-denied group as compared to both the sucrose-maintained or control animals. The abrupt change in diet exacerbated a deleterious effect of precipitated morphine withdrawal.

253.13

ROLE OF SPINAL AND MEDULLARY α-ADRENERGIC RECEPTORS IN THE ANTI-MORPHINE WITHDRAWAL ACTION OF CLONIDINE. J.J. Buccafusco (SPON: F. Carl). Dept. Pharmacology and Toxicology, Medical College of Georgia and Veterans Administration Medical Center, Augusta, GA 30912.

Clonidine and related drugs have proved to be particulary useful in treating the autonomic symptoms narcotic withdrawal in addicts. The site of action of clonidine has been ascribed to the pontine locus coeruleus, however, recent studies in this and other laboratories have suggested that the spinal cord mediates many of the symptoms morphine withdrawal in the rat. We have also demonstrated that the magnitude of the increase in post-withdrawal mean arterial pressure (PWMAP) provides a direct index of the degree and intensity of the withdrawal response. Rats were made dependent upon morphine following a schedule of increasing dosage of morphine infused over a 5 day period through a permanent arterial catheter. They were also prepared either with chronic intrathecal (IT) or intracisternal (IC) catheters. Withdrawal was precipitated with 5mg/kg of naloxone injected through the i.a. line or by 60μg though the IT line. Naloxone produced a 30-45mmHg increase in PWMAP which lasted for 30-45min. Rats also exhibited several behavioral signis of withdrawal, including body shakes and escapes. Pretreatment with clonidine (0.5-10μg) IT produced a dose-dependent inhibition of the PWMAP elicited by i.a. naloxone. Clonidine administered IC did not affect precipitated withdrawal. When withdrawal was precipitated by IT naloxone, clonidine pretreatment did not alter the PWMAP. These results are consistent with a spinal site for the antiwithdrawal effect of clonidine, if the withdrawal response originates from higher centers. Sup: Vet. Admin.

253.14

STRAIN-SPECIFIC FACILITATION OF BRAIN STIMULATION REWARD BY Δ⁹-TETRAHYDROCANNABINOL IN LABORATORY RATS IS MIRRORED BY STRAIN-SPECIFIC FACILITATION OF PRESYNAPTIC DOPAMINE EFFLUX IN NUCLEUS ACCUMBENS. E.L. Gardner, J. Chen*, W. Paredes*, J. Li*, and D. Smith* (SPON: L. Goodman). Departments of Neuroscience and Psychiatry, Albert Einstein College of Medicine, New York, NY 10461.

We previously showed that Δ⁹-tetrahydrocannabinol (THC), the psychoactive constituent of marijuana, acutely facilitates brain-stimulation reward in the rat (Gardner et al. Psychopharmacology 96:142, 1988), and that this facilitation is rat strain-specific (Gardner et al. Collegium Internationale Neuro-Psychopharmacologicum 1988), implicating genetic variation in vulnerability to the brain reward facilitating effects (and thus, presumably, to euphorigenic effects and abuse potential) of marijuana. Due to the postulated role of CNS dopamine (DA) systems in mediating and/ or modulating the rewarding properties of drugs of abuse (Spyraki et al. Psychopharmacology 79:278 1983), we have now carried out in vivo microdialysis studies to see if the strain-specific brain-reward enhancing effects of THC are mirrored by strain-specific effects of THC on basal presynaptic DA efflux in the nucleus accumbens (Acc), a crucial anatomic convergence of brain-reward-relevant DA circuity. Lewis strain rats, which show robust brain-reward enhancement by THC, showed robust enhancement of basal DA efflux in Acc at 0.5 and 1.0 mg/kg THC. Sprague-Dawley rats, which do not show brain-reward enhancement by THC, show virtually no effect of THC on Acc basal DA within this dose range.

253.15

A PERTUSSIS TOXIN-SENSITIVE G PROTEIN IS INVOLVED IN THE MODULATION OF CYCLIC AMP BY CANNABINOID DRUGS IN THE RAT BRAIN. M. Bidaut-Russell* and A.C. Howlett. Dept of Pharmacology, St Louis Univ. Sch. of Med, St Louis, MO 63104.

The hypothesis that canabinoid drugs alter certain neuronal functions by affecting the adenylate cyclase/cyclic AMP system has been tested in the rat corpus striatum. Desacetyl-levonantradol (DALN) (10 μM), a synthetic cannabinoid analgetic (Pfizer), significantly decreased cyclic AMP accumulation in Forskolin-, VIP- or Dopaminergic D1 agonist (SKF 38393)-stimulated striatal slices by 30% to 50%. Neither spiperone nor naloxone reversed the inhibitory effects of DALN on cyclic AMP levels. This suggests that cannabinoid activity in the striatum is not taking place at the opioid or D2 dopaminergic receptors levels, but rather via a specific cannabinoid receptor. Unilateral injection of pertussis toxin (2 μg) in the striatum, hours before sacrifice of the rat, reduced the inhibitory effect of DALN by 70% compared with slices from the contralateral side. This suggests the involvement of a pertussis toxin-sensitive G protein in the mechanism of action of cannabinoid drugs in the brain.
(Supported by DA 03690).

253.16

Δ-9 TETRAHYDROCANNABINOL (Δ-9 THC) FACILITATION OF LATERAL HYPOTHALAMIC STIMULATION-INDUCED FEEDING. W. Trojniar* and R. A. Wise. Ctr. Stud. Behav. Neurobiol., Dept. Psychol., Concordia U., Montreal, Canada.

Δ-9 THC--the psychoactive agent in marijuana--has recently been shown to produce naloxone-reversible facilitation of self-stimulation (ICSS). Opiates facilitate both ICSS and feeding. We studied whether Δ-9 THC shares the feeding-facilitating effect of opiates. Six male Lewis rats which ate reliably in response to lateral hypothalamic stimulation were tested after i.p. injections of Δ-9 THC (0.4 mg/kg) alone or after naloxone ((1.0 and 2.0 mg/kg). Δ-9 THC reduced (by 20.5±4.3%) the stimulation frequency required to induce eating at a fixed latency. It also reduced the stimulation frequency necessary to induce maximal (asymptotic) performance. The effects of Δ-9 THC were blocked completely by 2.0 mg/kg and partially by 1.0 mg/kg of naloxone. These data suggest the involvement of endogenous opioid mechanisms in the appetite-enhancing effects of Δ-9 THC.

253.17

EFFECT OF PERINATAL DELTA-9-TETRAHYDROCANNABINOL (THC) ON RAT BRAIN MU OPIOID RECEPTORS. J.E. Margulies and R.P. Hammer, Jr. Dept. Anatomy & Reprod. Biol., Univ. Hawaii, Honolulu, HI 96822.

Delta-9-tetrahydrocannabinol (THC) has been shown to modulate mu opioid receptors *in vitro* in a noncompetitive manner (Vaysse et al., *J. Pharm. Exp. Ther.*, 241: 534, 1987). To evaluate the effect of THC on developing opioid receptors *in vivo*, we examined the binding properties of [^{3}H]DAGO to mu-opioid receptors following perinatal treatment with THC. Either 1.0 or 10.0 mg/kg of THC, or 5% propylene glycol and 0.5% Tween 80 vehicle was administered daily to gravid dams from gestation day 12 through postnatal day 9 (P9). Pups were sacrificed by decapitation on P10, 24 hours after the last THC treatment. Brains were removed, frozen and stored at -70°C during tissue collection. Saturation experiments using [^{3}H]DAGO were performed on cryosections taken through the caudatoputamen.

Scatchard analyses indicate that THC produced an increase in receptor affinity (K_D), but had no effect on receptor density (Bmax). The K_D of [^{3}H]DAGO binding in brain sections following vehicle treatment compared favorably to the K_D previously obtained for untreated adult sections (1.27 nM). Following perinatal THC treatment, K_D is reduced to 0.41 and 0.47 nM for 1.0 and 10.0 mg/kg THC-treated sections, respectively, compared to 1.29 nM for vehicle treatment. In addition, autoradiographic studies of similarly treated tissue sections suggest that THC has a selective effect on regional brain opioid receptor binding.

These results suggest that maternal THC administration has dramatic effects on the development of fetal brain opioid receptors, and they are consistent with the concept that the interaction of THC with opioid receptors is noncompetitive. (Supported by USPHS Awards DAO4081, NS01161 and RRO3061.)

253.18

ACUTE NEUROENDOCRINE RESPONSE TO DELTA-9-TETRAHYDROCANNABINOL (THC): THE INFLUENCE OF CHRONIC THC PRETREATMENT. L.L. Murphy*, R.W. Steger* and A. Bartke* (SPON: D. Smith). Department of Physiology, Southern Illinois University School of Medicine, Carbondale, IL 62901.

The influence of chronic THC pretreatment on the neuroendocrine response to acute THC administration was investigated. Adult male rats were treated for 7, 14 or 28 days with sesame oil vehicle or a low dose (0.5 mg/kg b.w., p.o.) of THC. Approximately 24 h after the last dose of chronic pretreatment, animals were given an acute dose of either vehicle or THC (0.5 mg/kg b.w., p.o.) and sacrificed 60 min later. Plasma LH levels in chronic vehicle controls were significantly reduced after acute THC administration ($p<0.05$). Chronic THC pretreatment did not alter the ability of acute THC to suppress LH levels. Acute THC treatment in vehicle pretreated animals significantly reduced norepinephrine turnover ($p<0.01$) within the median eminence (ME) but did not affect ME dopamine (DA) activity. Chronic THC pretreatment for 7 or 14 days prevented the reduction in NE activity by acute THC treatment. After 28 days of chronic treatment, however, NE and DA activities in the ME were significantly reduced by acute THC ($p<0.01$). Therefore, chronic THC pretreatment does influence the response of the ME catecholamine systems to acute THC but does not interfere with the ability of acute THC to inhibit LH secretion. This suggests that other neuromodulators, besides NE and DA, may be involved in the ability of THC to alter LH secretion. (Supported by DA 03875)

256

SYMPOSIUM. PROGRESS IN RESEARCH ON D1 DOPAMINE RECEPTORS. R.E. Chipkin, Schering-Plough Corp. (Chairperson); Marc Caron, Duke University; James Wamsley, (Western Inst. Neuropsychiatry); Francis White, Lafayette Clinic; and William Woolverton, Univ. of Chicago.

Dopamine receptors have been subtyped into two categories: D1 and D2. Although drugs affecting the D2 receptor have been known for decades, it has only been recently that specific agents for the D1 receptor have been available. These compounds have been of enormous value in elucidating the actions of D1 receptors. The purpose of this symposium is to discuss the progress in research in this area. Following opening remarks by the chairperson, Dr. Caron will present his work on the isolation and purification of the D1 receptor. These biochemical studies will orient the audience to the physiochemical nature of the receptor. Having this information as a background, Dr. Wamsley will discuss the localization of D1 receptors in the brain. These data are important since some of the functions of the D1 receptor can be linked with its heterogeneous distribution in the brain. To complement these in vitro studies, Dr. White will talk about his work on the electrophysiological effects of D1 agonists and antagonists. This work gives insight into the function of D1 receptors in vivo. Since the identification and function of the D1 receptor is based on the availability of selective ligands, Dr. Chipkin will discuss pharmacological work on new D1 receptor drugs. Finally, Dr. Woolverton will present his recent research on the behavioral actions associated with the D1 receptor. It is anticipated that this symposium will present a balanced, interdisciplinary view of the status of research on D1 receptors.

257

SYMPOSIUM. COMPUTING MOTION IN FLIES, MONKEYS AND MAN: LINKING PHYSIOLOGY WITH PSYCHOPHYSICS AND COMPUTATIONAL THEORY. C. Koch, Caltech (Chairperson); W. Reichardt*, Max-Planck Institute for Biological Cybernetics, Tübingen; T. Albright, Salk Institute; K. Nakayama, Smith-Kettlewell Institute; R. Andersen, MIT.

This symposium will highlight a number of convergent advances in understanding how optical flow fields and 3-D structure from motion is processed in the visual system of both invertebrates and vertebrates. W. Reichardt will discuss the correlation model of motion detection, first postulated in 1956 on the basis of behavioral experiments on insects and now thought to underlie human perception, and his recent extension of this model that can cope with the aperture problem. How 2-D motion and motion contrast information is processed in the middle temporal area (MT) in the primate's visual system is next discussed by T. Albright. C. Koch will give an account of a neural network model, based on the physiology of the magnocellular neuronal pathway from the retina to MT, which computes optical flow fields and mimics a number of psychophysical results. K. Nakayama will illustrate several motion phenomena and illusions and demonstrate how their study can serve to illuminate the physiological and computational constraints that underlie the computation of optical flow. Finally, R. Andersen will discuss his psychophysical (in man and monkey), electrophysiological (in area MT), and modeling work studying how 3-D surfaces can be inferred at both the single cell and the system level from optical flow fields.

LEARNING AND MEMORY: ANATOMY IV

258.1

INTRA-CEREBELLAR LIDOCAINE: DISSOCIATION OF LEARNING FROM PERFORMANCE. J.P. Welsh and J.A. Harvey. Univ. Iowa, Iowa City, IA 52242 & Med. Col. of Pa/EPPI, Phil., PA 19129.

This study examined whether rabbits could acquire CRs when performance was impaired by infusions of lidocaine (LID) into the n. interpositus (INT). There were 3 phases to the study. In Phase 1, rabbits were trained to make nictitating membrane CRs to a light CS that was paired with an air puff UCS. In Phase 2, rabbits received infusions of LID into INT during a conditioning session in which a tone CS was paired with the UCS. Interpolated light-CS trials were used to monitor the degree to which performance of CRs was being impaired by LID infusion. In Phase 3, two days later, animals received tone-CS alone trials to determine whether learning had occurred in phase 2 but was not observed due to a performance deficit resulting from inactivation of INT by LID. Infusion of LID into INT during Phase 2 eliminated CRs to the light CS and blocked any evidence of CR acquisition to the tone CS. However, in Phase 3, when no LID was infused and performance was not impaired, these animals demonstrated CR retention that was not different from that of controls that had received LID in adjacent sites and whose performance had not been impaired. Unpaired controls demonstrated less than 8% responding in Phase 3. These results confirm our previous suggestions that INT is essential for performance but not for acquisition or retention of learned responses. Supported by NIMH Grant MH16841.

258.2

EFFECT OF INFERIOR OLIVE LESIONS AND OLIVOCEREBELLAR TRACTOTOMY ON A CONDITIONED LIMB FLEXION RESPONSE IN THE CAT. T.J. Voneida, D. Jefford-Christie* & R. Bogdanski*, Dept. of Neurobiology, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272.

Cats trained to perform a right forelimb flexion to a tone conditioned stimulus underwent olivocerebellar tractotomy or left olivary lesions (radio frequency; quinolinic acid).

The most severe CR losses resulted from rostromedial lesions, as reported by McCormick et al, '85; Yeo et al, '86, in the rabbit nictitating membrane response (NMR). Furthermore, we observed a gradual postoperative decline in CR levels from high scores to zero or near zero level scores, similar to that observed during extinction, as reported also by McCormick et al ('85). Coincidental with CR decline was an increase in CR latency and decrease in amplitude, as noted also by Welsh & Harvey ('89) following anterior interpositus cerebellar lesions in NMR conditioned rabbits.

Prolonged postoperative training of our subjects resulted in recovery of limb flexion CR's, with concomitant decrease of response latency and increase of response amplitude. (Supported by NINCDS Grant NS26053).

258.3

PSYCHOPHYSICAL LAWS OF ELICITATION OF THE RABBIT'S NICTITATING MEMBRANE RESPONSE (NMR) TO ELECTRICAL STIMULATION OF BRAINSTEM AND CEREBELLAR NUCLEI. A. J. Nowak, B. Marshall-Goodell, E. S. Miller, and I. Gormezano, Dept. of Psychology, Univ. of Iowa, Iowa City, IA 52242.

Previous studies have reported NMR conditioning with electrical brain stimulation (EBS) of the spinal trigeminal nucleus (TRIG) and inferior olive (IO) as an UCS, whereas EBS of the interpositus (IP) and red (RN) nuclei have not. Since these studies have generally employed subject-defined EBS parameters, the behavioral laws relating EBS parameters at these sites to UCR elicitation and CR acquisition have yet to be determined. Accordingly, the present study sought to determine for the above sites, the psychophysical laws relating NM extension to EBS amplitude, frequency, pulse width, and train duration. The frequency of NMRs elicited by EBS of RN, TRIG, and IP was found to be a monotonic increasing function of the parameters of EBS with the highest values (250 uA, 500 Hz, 0.8 ms, 60 ms) producing levels of UCRs at RN (85%), TRIG (61%), and IP (40%) significantly above baseline. In contrast, stimulation of IO failed to yield any significant effects across parameters of EBS. The observed UCR frequencies suggest that EBS of IP, TRIG, or RN could serve as an increasingly effective UCS for NMR conditioning, whereas even at the highest levels of stimulation, IO may be an ineffective site. Ongoing research from our laboratory is directed at determining the validity of these expectations.

258.4

CLASSICAL CONDITIONING IN RABBITS WITH INTRACEREBELLAR ELECTRICAL STIMULATION AS THE US. P. G. Shinkman, R. A. Swain*, and R. F. Thompson. Dept. Psychology, Univ. Southern California, Los Angeles, CA 90089.

The purpose of this experiment was to replicate and extend observations reported by Brogden and Gantt about 50 years ago, in which classical conditioning was accomplished in dogs using a visual or auditory CS and electrical stimulation of the cerebellum as the US. Stainless steel electrodes for localized microstimulation were implanted in white matter underlying lobule HVI of cerebellar cortex in New Zealand White rabbits. Upon recovery animals were tested for unconditioned responses elicited by intracerebellar stimulation (ICS) and then given conditioning trials consisting of paired presentation of tone and ICS. Of 9 implanted animals, 8 showed reliable elicited behaviors to the ICS, including eyeblink, head turn or extension, or lip movement. Every animal showing such unconditioned responses developed clear, unequivocal conditioned responses to the tone during 2-8 daily sessions of 108 trials. For 7 animals training was extended to include extinction (tone alone) followed by reacquisition; each of these animals showed rapid extinction (1-2 sessions) and reacquisition (1-2 sessions) of the conditioned response.

Supported by NSF (BNS-8718300), ONR (N00014-88-K-0112), and a McKnight Foundation grant, all to RFT; and by USPHS (HD-03110) to the University of North Carolina.

258.5

EFFECTS OF DEEP CEREBELLAR LESIONS ON CLASSICAL CONDITIONING IN RABBITS USING INTRACEREBELLAR STIMULATION (ICS) AS THE US. R. A. Swain*, P. G. Shinkman, and R. F. Thompson. Dept. Psychology, Univ. Southern California, Los Angeles, CA 90089.

Paired presentations of tone and electrical stimulation of cerebellar white matter produce robust classical conditioning (Brogden & Gantt, 1937; Shinkman, Swain, & Thompson, 1989). The present experiment was designed as a first step in specifying the locus of synaptic plasticity underlying this phenomenon. New Zealand White rabbits were implanted with bipolar microstimulation electrodes in the white matter of HVI and with a lesioning electrode or cannula in the nucleus interpositus (IP). Following recovery, classical conditioning was established in paired tone-ICS trials. IP lesions were then made electrolytically or by kainic acid injection. These lesions produced deficits in responding. In particular, small electrolytic IP lesions selectively abolished conditioned eyeblink responses either permanently or temporarily. Unconditioned responses also disappeared, either immediately following the lesion or after a few days. These results provide additional support for the role of the IP as a critical site of plasticity underlying many classically conditioned behaviors.

Supported by NSF (BNS-8718300), ONR (N00014-88-K-0112), and a McKnight Foundation grant, all to RFT; and by USPHS (HD-03110) to the University of North Carolina.

258.6

THE EFFECTS OF COMBINED LESIONS OF CEREBELLAR CORTICAL AREAS ON ACQUISITION OF THE RABBIT CONDITIONED NICTITATING MEMBRANE RESPONSE. C. G. Logan, D. G. Lavond, and R. F. Thompson. Dept. of Psychology, University of Southern California, Los Angeles, CA 90089-1061

In order to test the hypothesis that multiple cerebellar cortical sites participate in a parallel fashion in classical conditioning of the rabbit nictitating membrane response, lesions of various cerebellar cortical areas were performed in combination, and the lesioned animals were then trained.

New Zealand white rabbits received either bilateral lesions of the flocculus and paraflocculus, bilateral lesions of the flocculus and paraflocculus and unilateral lesions of neocerebellar cortex, or bilateral lesions of flocculus and paraflocculus along with bilateral lesions of the hemispheric areas. The flocculus/paraflocculus group was trained to criterion on the left eye and then switched to right eye training. All rabbits in this group learned the conditioned response on both eyes, indicating that the flocculus and paraflocculus are not essential for classical conditioning of the eyeblink response.

All animals in the other 2 groups were given 10 days of tone-airpuff pairings on one eye, then the airpuff was switched for 10 days of training on the opposite eye. To date, animals in this group which did not have damage to the deep nuclei acquired the conditioned response, with the exception of one animal which did not learn on the eye ipsilateral to the hemispheric lesion. In all cases, damage to the deep nuclei was assessed by an observer blind to the animal's behavior.

Supported by the McKnight Foundation, ONR grant N00014-88-K-0112, and NSF grant BNS-8718300 to RFT.

258.7

INCREASED BRANCHING OF SPINY DENDRITES OF RABBIT CEREBELLAR PURKINJE NEURONS FOLLOWING ASSOCIATIVE EYEBLINK CONDITIONING B.J. Anderson, S.Lee, J.Thompson, J.Steinmetz, C.Logan, B. Knowlton, R.F.Thompson & W.T.Greenough Depts Psych & Cell & Struct Biol, & Neur &Behav Biol Prog, Univ IL, Champaign, 61820, and Dept Psych, Univ So Calif, Los Angeles, CA

Prior findings implicate cerebellum as essential for conditioned eyeblink responses in rabbits. When dorsolateral pontine n. (DLPN) stimulation serves as CS, cerebellar cortex lesions abolish CRs. Previous findings indicate increased Purkinje cell spiny branchlet dendrite, site of parallel fiber termination, after complex experience in rodents and monkeys. Acrobatic training also increases molecular layer synapse number in rats. We trained rabbits unilaterally, using DLPN stimulation as CS and corneal airpuff as US. Golgi-stained Purkinje neurons in lobule HVI were analyzed for the branching pattern of spiny branchlets. Altered pattern was indicated by greater numbers of 5th order branches and of 4th order bifurcating branches ($p<.05$). Mean total dendritic length for spiny branchlets was 5% greater in the conditioned hemisphere (ns). These results, taken with previous findings, suggest the formation of new parallel fiber to Purkinje cell connections as a consequence of conditioning.

--Supported by NIMH & NSF.

258.8

STRUCTURAL PLASTICITY IN CEREBELLAR CORTEX: LEARNING EFFECTS ON AFFERENT AND INTRINSIC FIBER SYSTEMS. W.T. Greenough, B.J. Anderson, K.R. Isaacs, J.E. Black, & A.A. Alcantara Depts Psych., Cell & Struct Biol, Neur & Behav Biol Prog, Beckman Inst. Univ Illinois, Champaign, 61820.

Quantitative Golgi studies indicate that weanling or old animals housed in complex environments have more spiny branchlet material per Purkinje neuron. Spiny branchlets receive parallel fiber (pf) input. One cannot directly assess other Purkinje afferents in light microscopy. Using electron microscopy, we found that visuomotor training increases cerebellar cortex thickness without significantly affecting synapse density, such that there are more synapses per Purkinje neuron (Black, et al SN Abs 13:1596; Anderson et al SN Abs 14:1239). We studied apex of paramedian lobule in 38 adult rats run for 30 days in 4 groups: an acrobatic condition (AC) given extensive motor-learning with relatively little exercise, two exercise conditions (FX, VX) that allowed little learning but given 10x the amount of AC exercise, and an isolated control group (IC). The AC group had about 30% more synapses than the others, all of which were roughly equivalent. As pf synapses are by far most numerous in the molecular layer (Eccles, Brain Res., 127:327), their numbers must have increased. Whether changes also occur in climbing and inhibitory fibers is now under study and will be discussed in the context of extant and plausible neural models of cerebellar cortical learning. NIMH-43830

258.9

LESIONS OF DEEP CEREBELLAR NUCLEI SELECTIVELY ABOLISH CONDITIONED BUT NOT "SPONTANEOUS" EYE BLINKS IN RATS. R. W. Skelton. Dept. of Psychology, Univ. of Victoria, B.C., Canada, V8W 2Y2.

The present study examined the topography of eyelid responses in rats given paired and unpaired presentations of tones and periorbital shocks, and evaluated the effects of deep cerebellar lesions on responses under both conditions.

In the paired condition, eyelid responses to tones contained two distinct components. The "CR" component consisted of multiple bursts of EMG with variable amplitude and frequency. It was clearly distinguishable on 40-60% of trials and resembled the well-known conditioned eyelid response (CRs) of rabbits. The "spontaneous blink" component consisted of a single burst of EMG that was invariably large and brief. It was present on 10-20% of trials and resembled eye blinks emitted by the rats "spontaneously" before conditioning, during the intertrial interval, and shortly after the unconditioned response to the eye shock. In the unpaired condition, "spontaneous blink" responses occurred on 10-20% of tone trials, but "CR" responses were rare.

After bilateral lesions of the deep cerebellar nuclei eyelid responses under paired and unpaired conditions were equivalent and were almost entirely "spontaneous blink" responses.

Supported by grant #URF0034961 from NSERC of Canada.

258.10

LESIONS OF THE CEREBELLAR VERMIS SEVERELY IMPAIR ACQUISITION OF PAVLOVIAN CONDITIONED BRADYCARDIC RESPONSES IN THE RABBIT. W. F. Supple, L. Archer* and B.S. Kapp. Department of Psychology, Univ. Vermont, Burlington, VT 05405.

Recent lesion data in the rat (Supple & Leaton 1986) and electrophysiological data in the rabbit (Supple & Kapp, 1988) has indicated an important contribution of the midline cerebellar cortex to the acquisition of fear-conditioned heart-rate(HR) responses. This experiment examined the effects of restricted vermis lesions on the acquisition of classically conditioned bradycardia in rabbit.

Electrolytic or aspiration lesions of the anterior vermis blocked the development of conditioned bradycardic responses to a pure tone CS paired with a 2.0 mA eyelid shock UCS. Importantl the lesions did not affect baseline HR, the unconditioned HR orienting response or HR responses to the UCS, thus indicating that these lesions selectively disrupted conditioned but not unconditioned HR responses.

These data implicate the anterior lobules of the vermis as being importantly involved in this rapidly acquired CR in the rabbit; a species in which the lateral cerebellum has been shown to be essential for acquisition of the classically conditioned eyeblink CR.

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258.11

THE CEREBELLUM AND LONG-TERM HABITUATION OF ACOUSTIC STARTLE IN RATS: LESIONS OF FASTIGIAL (MEDIAL) AND DENTATE (LATERAL) NUCLEI. R. N. Leaton and W. F. Supple, Jr. Department of Psychology, Dartmouth College, Hanover, NH 03755.

In a series of experiments we have shown that the vermis of the cerebellum is essential for long-term habituation (LTH) of the acoustic startle response in rats. Lesions of the vermis of the cerebellum prevent long-term habituation but leave short-term habituation unaffected (Leaton & Supple, Science, 1986). The effects are at least partially response specific (Supple & Leaton, SNS, 1986) and are anatomically specific within the cerebellum: vermal lesions blocked LTH while lesions of the cerebellar hemispheres were without effect on LTH (Leaton & Supple, SNS, 1986).

The present experiment was an effort to expand the anatomical substrate of the LTH circuitry. We compared rats with lesions of the fastigial nuclei (a projection target for the cerebellar vermis) with rats with lesions of the dentate nuclei (a projection target for the cerebellar hemispheres). Startle response was measured to 118-dB, 100-msec white noise bursts on a 60-sec interstimulus interval (ISI) in 8 daily 6-trial sessions. Retention of LTH was assessed 6 days later. Then responsiveness to a systematic variation of ISI was tested over intervals of 5,10,30,60, and 120 sec. Three groups of rats were tested following (1) bilateral electrolytic lesions of the fastigial nuclei (n=12), (2) bilateral electrolytic lesions of the dentate nuclei (n=11), and (3) sham surgery (n=9).

Fastigial lesions blocked LTH suggesting that these projection nuclei are part of the LTH pathway from the cerebellar vermis. Dentate lesions were without affect on LTH but in the ISI series appeared to reduce sensitization relative to controls. These data are consistent with and extend our previous findings that the medial cerebellum is part of an essential circuitry for LTH of the acoustic startle response in rats.

GABA AND BENZODIAZEPINE RECEPTORS I

259.1

STRUCTURAL BASIS OF TYPE I AND TYPE II $GABA_A$/BENZODIAZEPINE RECEPTORS. D.P. Pritchett, H. Lüddens* and P.H. Seeburg*. ZMBH, University of Heidelberg, INF 282, 6900 Heidelberg, F.R.G.

$GABA_A$ receptors expressed upon transfection of mammalian cells with cDNA expression vectors and assembled from one of three different α subunit variants (α_1 or α_2 or α_3) in combination with a β_1 and a γ_2 subunit displayed the pharmacological properties of the previously described type I and type II subtypes of the $GABA_A$/benzodiazepine receptor. Each of the three expressed receptors contained high-affinity binding sites for benzodiazepines. However, the triazolopyridazine CL 218872 showed a 10-fold higher affinity for receptor containing an α_1 rather than an α_2 or α_3 subunit. A similar selectivity for α_1 subunit containing receptors was seen using the benzodiazepine 2-oxo-quazepam and the β-carboline β-CCM. Four other benzodiazepines (clonazepam diazepam Ro 15-1788) or β-carbolines (DMCM) with no type I or type II selectivity displayed indistinguishable binding characteristics for the three recombinantly expressed receptors. The affinity of all compounds tested, except 2-oxo-quazepam, was indistinguishable from their affinity for brain membranes. The selectivity of CL 218872 and 2-oxo-quazepam compounds was reduced at 37°C, as observed with BZ I receptors from cerebellar membranes. No such temperature-induced decrease in affinity for benzodiazepines was observed for receptors carrying α_2 or α_3 subunits, again consistent with results using brain membranes. These results strongly suggest that α subunit heterogeneity constitutes the structural basis for type I and type II receptors.

259.2

4'-CHLORODIAZEPAM DECREASES FUNCTION OF Cl^- CHANNELS COUPLED TO NATIVE AND TRANSIENTLY EXPRESSED $GABA_A$ RECEPTOR SUBUNITS IN A MANNER INSENSITIVE TO FLUMAZENIL. G.Puia, M.R.Santi, S.Vicini*, D.B.Pritchett[1]*, P.Seeburg[1]*, & E.Costa*. F.G.I.N. Georgetown Univ.,Wash.,D.C.20007. [1]ZMBH, Univ. Heidelberg, FRG.

4'-chlorodiazepam (RO 5-4864), the peripheral benzodiazepine receptor agonist, also acts as a negative modulator of central $GABA_A$ receptors. We employed whole-cell recordings of GABA-induced Cl^- currents in rat cortical neurons in primary culture. RO 5-4864 decreased these currents with a potency 10 fold lower than that of DMCM. Flumazenil antagonized DMCM but was without effect on 4'-chlorodiazepam reduction of Cl^- currents. Utilizing the transient expression of the different subunits constituting the $GABA_A$ receptor in a mammalian cell line(1), it has been shown that transfection of a third subunit (γ), along with the previously identified α and β subunits, is necessary to observe the benzodiazepine increase of GABA-activated Cl^- currents (2). The transiently expressed combination α_1 and β_1 subunits produced Cl^- channels downregulated by picrotoxin but not affected by benzodiazepine receptor ligands (1). We did not observe any decrease of Cl^- currents induced by GABA in these transfected cells either by RO 5-4864 or by DMCM. Conversely, when the GABA receptor Cl^- channels were reconstituted together with the γ_2, subunits both compounds were effective with similar potency and efficacy, as on the native receptors. Our results indicate that there is no similarity between the picrotoxin and the 4'-chlorodiazepam sites of action, as previously thought. 1) D.Pritchett et al. Science 242:1306 (1988). 2) D.Pritchett et al. Nature 338:582 (1989).

259.3

THE TRIAKONTATETRANEUROPEPTIDE (TTN) A BRAIN PROCESSING PRODUCT OF DIAZEPAM BINDING INHIBITOR (DBI): A PUTATIVE ALLOSTERIC MODULATOR OF $GABA_{A3}$ RECEPTOR. E. Slobodyansky, H. Alho, P. Bovolin, A. Guidotti and E. Costa. FGIN Georgetown Univ., Washington, D.C.

Several lines of investigation suggest that 4'Cl diazepam (Ro-5 4864) binds to neuronal membranes and functions as a $GABA_{A3}$ receptor modulator. ^{3}H-4'Cl diazepam binds to synaptosomes (K_D 53 nM) and mitochondria (K_D 7nM) of brain. In synaptosomes ^{3}H-4'Cl diazepam can be displaced with picrotoxinin, but not protoporphyrin and in mitochondria with protoporphyrin IX but not picrotoxinin. mRNA levels (monitored by Northern blot analyses) for the peripheral BZ receptor are much lower in the brain than in kidney, implying that 4'Cl diazepam binds in brain to some other receptor in addition to the mitochondrial one. The functional role of TTN in brain is supported by following data 1) Immunocytochemical investigations done with antibodies directed against TTN reveal its coexistence with GABA in cortical, reticulothalamic and cerebellar neurons. 2) Its proconflict effect is blocked by PK-11195 (antagonist of 4'Cl diazepam), but not by flumazenil. 3) TTN displaces 4'Cl diazepam from synaptosomes with K_i 5uM, but does not displace flumazenil. Similarly to 4'Cl diazepam TTN potentiates the effect of picrotoxinin on ^{3}H-flunitrazepam binding to brain homogenate. The effect of TTN on 4'Cl diazepam binding in cultured cells expressing $GABA_A$ receptors with different subunit composition is currently being studied.

259.4

DIFFERENCES IN THE PHARMACOLOGICAL PROFILES OF BENZODIAZEPINE RECOGNITION SITE LIGANDS REFLECT INTERACTIONS WITH HETEROGENEOUS POPULATION OF $GABA_A$ RECEPTORS. J.L. Schlichting, M. Massotti, M.D. Antonacci* and A. Guidotti, FGIN, Georgetown University, Washington, D.C. 20007.

Heterogeneity of $GABA_A$ receptor is supported by binding and molecular studies (Memo et al., Neurosci. Abst. 1989). $GABA_{A1}$ is abundant in cerebellum, cortex, olfactory bulb but absent in spinal cord; $GABA_{A2}$ is most abundant in spinal cord, but also present in striatum, hippocampus and thalamus; $GABA_{A3}$ is abundant in olfactory bulb. In rats, the pharmacological profiles of acutely adminstered diazepam (DZP), alprazolam (AZP), clonazepam (CZP), or zolpidem (ZPD) suggest an action at selective populations of $GABA_A$ receptor subtypes. CZP and ZPD elicit anticonvulsant, anticonflict, and sedative effects but have weak myorelaxant action suggesting that $GABA_{A1}$ receptors are responsible for sedative and anticonvulsant activity, $GABA_{A2}$ receptors for myorelaxant action, whereas the pharmacology of $GABA_{A3}$ receptors is less well understood. When DZP or CZP are administered to rats for 14 days, tolerance to the effects of DZP results but not to the effects of CZP. The induction of tolerance to effects of DZP can be correlated with changes in the dynamic state of endogenous DBI (diazepam binding inhibitor) in specific brain regions. On-going studies on tolerance characteristic of AZP and ZPD suggest that AZP, like DZP, can elicit changes in the dynamic state of DBI.

259.5

EXPRESSION OF A cDNA ENCODING A PROTEIN COMPRISING PERIPHERAL-TYPE BENZODIAZEPINE RECOGNITION SITES.
A.G. Mukhin*, M.R. Santi*, D.R. Grayson*, and K.E. Krueger. (SPON: W. Norman). Fidia-Georgetown Institute for the Neurosciences, Georgetown University Medical School, Washington, D.C. 20007.

PKBS (PK binding site) is a protein of approximately 17 kDa which was identified earlier as being intimately associated with peripheral-type benzodiazepine recognition sites (PBR). A full-length rat cDNA probe for PKBS was inserted into an eucaryotic expression vector, pSV-globin, placing it under transcriptional control of the β-globin promoter and SV40 enhancer. The transformed human embryonic kidney cell line, 293, was transfected with this construct and transient expression of PBR was studied. Cells transfected with the pSV-PKBS vector exhibited about a 1 pmol/mg of protein increase in specific binding for [^{3}H]PK11195 and for [^{3}H]4-chlorodiazepam when compared to cells subjected to a mock transfection or transfected with the original pSV-globin vector. The dissociation constants of the PBR expressed was 5-8 nM and of higher affinity than the human form of PBR endogenous to 293 cells (K_D = 19-55 nM). The rank order of potency at inhibiting [^{3}H]4-chlorodiazepam binding to the expressed PBR was PK11195 > 4-chlorodiazepam > diazepam > clonazepam. These studies demonstrate that expression of PKBS is required and apparently sufficient for the manifestation of PBR.

259.6

BENZODIAZEPINE SENSITIVITY AND STRUCTURE OF GABA- A RECEPTOR SUBTYPES.
M. Memo, M. Massotti, A. Guidotti, D.R. Grayson* and E. Costa. FGIN, Georgetown Univ., Washington, D.C. 20007.

Recent transient expression of various cDNAs coding for GABA-A receptor subunits suggests that the structural composition of the reconstituted receptor determines its senistivity to various benzodiazepines (BZs). The experiments were performed with poly A+ mRNA and neuronal membranes prepared from cerebellum and spinal cord. The Bmax for tritiated diazepam, clonazepam, zolpidem, flumazenil and CCM (a beta carboline) binding is approximately the same in cerebellum. Spinal cord membranes fail to bind clonazepam, zolpidem and CCM, but bind tritiated diazepam and flumazenil with Kd similar to the cerebellum and a Bmax which was 5 fold lower. The steady state mRNA content of the alpha-1-subunit of GABA-A receptor was measured by northern blot analysis of RNA isloated from either tissue. The alpha-1- subunit cDNA clone gave two strong hybridization signals in cerebellum but neither poly A+ species was detected in multiple RNA preparations from spinal cord. These results indicate that the binding profile of Bz to GABA-A receptor populations present in spinal cord might be correlated with the different subunit composition of GABA-A receptors.

259.7

RESOLUTION AND IDENTIFICATION OF THE PUTATIVE SUBUNITS OF THE PERIPHERAL BENZODIAZEPINE RECEPTOR (pBr) OF RAT KIDNEY MITOCHONDRIA.
M.W.McEnery, A.M.Snowman, and S.H.Snyder Dept. Neuroscience, Johns Hopkins Sch. of Med.,Baltimore, MD 21205.

Benzodiazepines have been shown to bind with nanomolar affinity to two distinct classes of sites. The central site has been shown to be components of the GABA-A receptor associated with Cl^- channels. The peripheral site (pBr) is pharmacologically distinct from the central site and is found in kidney and adrenals. The pBr has been localized to the mitochondrial fraction of these tissues, specifically to those complexes associated with the outer membrane. We have previously reported a procedure for the purification of the pBr from rat kidney mitochondria. The intact complex is composed of two proteins with apparent molecular weights of 30-35kDa and 18kDa. This preparation has been characterized with regard to its ability to bind those drugs which are diagnostic for the pBr (the benzodiazepine Ro 5-4864 and the isoquinoline carboxamides PK11195 and PK14105) in either a reversible or covalent manner. In all respects, the soluble and purified pBr is identical to the native, membrane-bound receptor. We have recently resolved the 30-35kDa protein from the 18kDa protein and herein report our attempts to examine the binding characteristics of the components. The function of the pBr will be discussed as well as our conclusions as to the identity of the 30-35kDa component.

259.8

GABA RECEPTOR HETEROGENEITY: NEW INSIGHTS FROM RECEPTOR RADIOAUTOGRAPHY AND IN SITU HYBRIDIZATION HISTOCHEMISTRY.
J.G. Richards, J.M. Séquier*, P.Malherbe* T. Giller* and H. Möhler*. Pharma Research CNS, F. Hoffmann-La Roche AG., CH-4002 Basel, Switzerland.

Allosteric sites on the $GABA_A$ receptor complex, a pentameric (?) heterooligomer in pre-, post- as well as extra-synaptic neuronal membranes in the CNS, are targets for a variety of psychoactive drugs including benzodiazepines and barbiturates. Recent evidence from receptor mapping and cloning studies suggest a structural and possibly functional heterogeneity of the receptor in rat CNS. The distribution of rat α_1 and β_1 mRNA in rat brain has been described (Séquier, J.M. et al., Proc. Natl. Acad. Sci. USA, 85: 7815, 1988).

Recently, a variety of receptor subunits and subtypes thereof have been cloned (5 α's, 1 β, 1 γ); their regional expression in the CNS, mapped by in situ hybridization histochemistry (using ^{35}S- or ^{3}H-labelled cRNA and synthetic oligonucleotide probes), differed profoundly. α_1 was the most ubiquitously and highly expressed subtype but was not detected in A9 DA neurones, for example. $\alpha_{2,3,5}$ and β_1 were restricted to the olfactory bulb, caudate putamen (α_2), hippocampal formation and cerebellum. α_4 and γ_2 (highly homologous to human γ_2) were much more widely expressed but again differed from the α_1 pattern of distribution, e.g. absence of signal in pallidum, most thalamic regions and substantia nigra (except α_4 which was present in the zona compacta). None of the clones to date have been detected in spinal cord. Moreover, the interaction of GABA with a variety of benzodiazepine receptor ligands (GABA-shift), determined for 13 rat brain regions by quantitative receptor radioautography and image analysis, revealed markedly different profiles.

The functional implications of these findings will be discussed.

259.9

ISOLATION OF MULTIPLE PHARMACOLOGICALLY DISTINCT GABA AND BENZODIAZEPINE BINDING SUBUNITS OF THE GABA-A RECEPTOR.
M. BUREAU* and R.W. OLSEN (SPON: D.J. Jenden). Department of Pharmacology, UCLA School of Medicine, Los Angeles, CA 90024.

At least two different α subunits (photoaffinity labeled by [^{3}H]flunitrazepam [FLU]) and two different β subunits (photolabeled by [^{3}H]muscimol) were detected on SDS gels of GABA-A/benzodiazepine (BZ) receptor from rat cortex purified by BZ affinity column. The two bands labeled by each ligand showed differential sensitivity to inhibition by nonradioactive analogs. [^{3}H]Muscimol photolabeled two bands at 56 kDa (β_{56}) and 58 kDa (β_{58}) to equal extent, despite a greater amount of β_{56} than β_{58}, indicating a higher affinity for β_{58}. β_{58} also showed a higher affinity for nonradioactive THIP, (IC_{50} = 0.2 μM versus 3 μM for β_{56}), GABA, bicuculline, SR-95531, and taurine. [^{3}H]FLU photolabeled two bands at 51 kDa (α_{51}) and 53 kDa (α_{53}) with a higher affinity of α_{51} for FLU, and the BZ1-specific ligands CL 218872 (IC_{50} = 50 nM versus >1 μM for α_{53}), quazepam, and beta-carbolines. Barbiturates showed differential modulation of GABA and BZ binding to the two bands. Thus, multiple subunit subtypes on SDS gels show different binding affinities for drugs in the native oligomeric state. These can be correlated with brain regional variation in ligand binding, and appear to represent different gene products.

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260.1

GLUTAMATERGIC REGULATION OF GABAERGIC NEURONAL ACTIVITY
R. Bernasconi*, P. Martin*, A.F. Steulet*, T. Leonhardt*, M. Schmutz*, P. Baumann*, H. Bittiger* and M. Williams. (SPON: S. Bischoff). Pharmaceuticals Div. CIBA-GEIGY Ltd. CH-4002 Basel.

The nature of the GLU receptors which mediate the excitatory synaptic modulation of GABAergic neurons in the striatum is still unknown. Therefore, we examined the effects of competitive NMDA antagonists (CPP, CGS 19 755, CGP 37 849, CGP 39 551) on GABA turnover in comparison with those of non-competitive antagonists (PCP, MK-801, dextromethorphan). The rate of GABA accumulation after irreversible inhibition of GABA-T by gabaculine, an index of GABA turnover, was measured in 4 brain structures of the mouse. Anticonvulsant doses of all competitive NMDA inhibitors did not alter steady-state levels of GABA, but dose-dependently decreased its turnover rate. MK-801, PCP and dextromethorphan had no effect on GABA turnover. In the absence of any direct effect of the competitive NMDA antagonists on GABA-A, GABA-B, chloride channel, central benzodiazepine binding sites and on GABA uptake (at conc. > 100 uM) in vitro and on GABA levels in vivo, these results confirm that GABAergic pathways are subject to regulation by GLUergic transmission. This effect seems to be mediated, at least in part, via NMDA receptors located on GABA neurons. Furthermore, NMDA receptors which modulate GABA synthesis are not blocked by PCP-like channel ligands.

260.2

PROTEIN KINASE C TRANSLOCATION IN PRIMARY CULTURES OF NEURONS: REGULATION BY EXCITATORY AND INHIBITORY NEUROTRANSMITTERS.
F.M. Vaccarino AND J.F. Tallman, ABRAHAM RIBICOFF RES. FACILITIES, DEPT. PSYCHIATRY, YALE UNIV. SCHOOL OF MEDICINE, NEW HAVEN, CT 06508.

In primary cultures of cerebellar granule cells, Protein Kinase C (PKC) translocates from the soluble to the membrane compartment (with subsequent activation) after stimulation of intact neurons with agonists, such as glutamate, NMDA and kainate, which promote the influx of calcium through receptors-gated transmembrane channels (Vaccarino et al, Proc.Natl.Acad.Sci, 84:8707,1987). No significant PKC translocation was elicited after treatment of cell monolayers with agonists primarily affecting PI turnover, such as carbachol, quisqualate or noradrenaline. In primary cultures of cortico-striatal neurons, PKC translocation, assessed by the binding of radiolabelled β phorbol-12,13-dibutyrate (PDBu) to intact cell monolayers, was stimulated by the excitatory amino acid glutamate in the absence of Mg with the same efficacy and potency as in cerebellar granule cells and this stimulation was blocked by the specific NMDA allosteric antagonist MK-801. Differently than in cerebellar granule cells, PKC translocation could also be stimulated by ligands for the quisqualate-type excitatory amino acid receptor and by the muscarinic cholinergic agonist carbachol, which cause an increase in PI turnover in these cultures. Moreover, bicuculline, which antagonizes $GABA_A$ receptor-activated chloride channels, greatly enhanced the efficacy and the potency of glutamate (in Mg-free medium) in inducing PKC translocation. Bicuculline's potentiation was blocked by MK-801 as well as by tetrodotoxin, suggesting that it involved a depolarization-induced enhancement of NMDA receptor action. These data support a tonic antagonism of glutamate-mediated PKC translocation by GABA, which may explain the blockade of bicuculline-induced seizure activity by NMDA receptor antagonists.

260.3

EFFECTS OF EXCITATORY AMINO ACID (EAA) ANTAGONISTS ON SYNAPTIC CURRENT IN PHRENIC MOTONEURONS.
G. Liu and J. L. Feldman, Systems Neurobiology Lab., Dept. of Kinesiology, UCLA, Los Angeles, CA 90024-1586.

In spite of the presumed importance of EAA receptors in synaptic transmission, little is known about their role in mediation of synaptic drive during ***natural behaviors***. Our previous results suggest the involvement of EAAs in the bulbospinal transmission of inspiratory drive to phrenic motoneurons (e.g., Soc. Neurosci. Abs. 14:938, 1988). The present study used single electrode voltage-clamp techniques to explore the effects of different EAA receptor antagonists on the endogenous rhythmic respiratory synaptic drive current to phrenic motoneurons *in vitro*. When the membrane potential of a phrenic motoneuron was clamped at end-expiratory potentials (-65 to -75 mV), the peak amplitude of synaptic current during inspiration (duration 200-500 msec) was ~1 nA. After bath application of 10 μM 6-cyano-7-nitroquinozaline-2,3-dione (CNQX), a non-NMDA antagonist, the amplitude and duration of the inspiratory drive current was reduced. This concentration of CNQX did not produce any change in resting membrane potential, action potential characteristics, input resistance, cell excitability, or membrane frequency/current relationship. These results suggest that CNQX specifically blocked inspiratory-modulated synaptic transmission without changing intrinsic (passive or active) membrane properties. Bath application of 100 μM 2-amino-5-phosphonovaleric acid (AP5) induced only a small reduction of synaptic drive current, and did not affect membrane properties. Combined with our previous work, these data indicate the involvement of EAA receptors, especially of the non-NMDA type, in synaptic transmission of bulbospinal inspiratory drive to phrenic motoneuron. Supported by NIH Grant NS 24742.

260.4

WHEAT GERM AGGLUTININ INHIBITS QUISQUALATE DESENSITIZATION IN CULTURED POSTNATAL RAT HIPPOCAMPAL NEURONS.
L.L. Thio, D.B. Clifford, & C.F. Zorumski, Depts. of Psychiatry & Neurology, Washington U. Sch. Med., St. Louis, MO 63110

Quisqualate evokes a rapidly desensitizing inward current in postnatal rat hippocampal neurons. We have studied this current using the whole cell mode of the patch clamp technique. We found that the amplitude of the desensitizing current produced by 100μM quisqualate is irreversibly increased by 240% after applying 25μg/ml of the lectin wheat germ agglutinin (WGA) for 30s. WGA potentiates the desensitizing current in a dose dependent manner with an ED_{50} of 3μg/ml, and decreases the rate of desensitization markedly. The lectin does not appear to unmask a new set of quisqualate receptor-channel complexes since the ED_{50} for the quisqualate current in WGA treated neurons is comparable to that for the desensitizing current in naive neurons. In addition, the current-voltage relationship for the current in WGA treated neurons is linear with a reversal potential of 0mV as it is untreated neurons. WGA also augments the rapidly desensitizing current evoked by α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid and glutamate, but it has no effect on N-methyl-D-aspartate, kainate, and γ-aminobutyric acid (GABA) currents. In particular, it has no effect on NMDA and GABA desensitization. Thus, WGA may be useful in determining both the physiological role and molecular mechanism of quisqualate desensitization.

260.5

QUISQUALATE ACTIVATES A DELAYED AP5-SENSITIVE INCREASE IN $[K^+]_o$ AND DECREASE IN $[Ca^{2+}]_o$ IN THE GRANULAR LAYER OF THE TURTLE CEREBELLUM.
M.E. Rice and C. Nicholson, Dept. Physiol. & Biophys., New York University Medical Center, New York, NY 10016.

Iontophoresis of excitatory amino acid agonists changes $[K^+]_o$ and $[Ca^{2+}]_o$ in the granular layer (GrL) of the isolated turtle cerebellum (Rice & Nicholson, Soc. Neurosci. Abstr. 13:764, 1987). Quisqualate, in contrast to glutamate, aspartate, NMDA and kainate, had little effect on $[K^+]_o$ and $[Ca^{2+}]_o$ during iontophoresis, but increased $[K^+]_o$ and decreased $[Ca^{2+}]_o$ 4-40 s after application ended. We have investigated this secondary response using ion-selective microelectrodes for K^+ and Ca^{2+} located 50-60 mm from the double-barreled iontophoresis pipette.

Quisqualate-activated secondary ion shifts in the GrL were enhanced in zero Mg^{2+} and low Ca^{2+} solutions and inhibited by 10 mM Mg^{2+} or 20 μM AP5. Iontophoresis of quisqualate in the molecular layer (MoL) produced ion changes only during iontophoresis but secondary $[K^+]_o$ and extracellular potential changes were recorded in the MoL following quisqualate iontophoresis in the GrL; the latter MoL events were simultaneous with those in the GrL. Co-iontophoresis of NMDA or kainate (at currents insufficient to elicit ion changes) with quisqualate in the GrL produced only direct ion changes, with no secondary response. Co-iontophoresis of GABA with quisqualate enhanced the secondary effect slightly.

The lack of a primary effect and the characteristics of the secondary response to quisqualate application suggest that quisqualate receptors are located on inhibitory interneurons in the GrL. The secondary response may represent the relief of tonic inhibition of granule cells by inhibitory receptor desensitization. We cannot exclude, however, a modulatory action of quisqualate at kainate or NMDA receptors. Funded by USPHS Grants NS-13742 and NS-07745.

260.6

LIPOXYGENASE MEDIATES THE FORMATION OF CYCLIC GMP INDUCED BY EXCITATORY AMINO ACIDS IN CEREBELLAR GRANULE CELLS.
B. Wroblewska*, J.T. Wroblewski, P.W. Ramwell* and E. Costa. (SPON: A. Berkovich). Dept. of Physiology and Fidia-Georgetown Institute for the Neurosciences, Georgetown Univ. Sch. of Med., Washington, D.C. 20007.

In primary cultures of cerebellar granule cells, the activation of N-methyl-D-aspartate and kainate-sensitive excitatory amino acid (EAA) receptors leads to increased entry of extracellular Ca^{2+} and to Ca^{2+}-dependent transient accumulation of cGMP. Our results indicate that the EAA-induced accumulation of cGPM in granule cells depends on the activity of phospholipase A_2, since the enzyme inhibitors quinacrine, p-bromophenacyl bromide or 7,7-dimethyleicosadienoic acid reduced cGMP formation induced by glutamate, aspartate or kainate, but failed to affect the stimulation of guanylate cyclase by sodium nitroprusside. Further experiments on the role of arachidonic acid metabolites in receptorial signal transduction showed that indomethacin, a cyclooxygenase inhibitor, and BW755C, a monoxygenase inhibitor, failed to affect the EAA-induced cGMP accumulation. In contrast, nordihydroguaiaretic acid, an inhibitor of lipoxygenases, abolished the EAA receptor-mediated cGMP accumulation. These resuls indicate that increased accumulation of cGMP, induced by EAA receptors, is mediated by the consecutive action of phospholipase A_2 and one of the lipoxygenases. The transient character of the cGMP response may suggest the regulatory involvement of short-lived lipoxygenase products - hydroperoxyeicosatetraenoic acids.

260.7

DISTRIBUTION OF QUINOLINIC ACID PHOSPHORIBOSYLTRANSFERASE IN THE HUMAN HIPPOCAMPAL FORMATION. F. Du, [1]W.O. Whetsell, Jr., E. Okuno, C. Köhler and R. Schwarcz. Maryland Psych. Research Center, Baltimore, MD 21228 and [1]Dept. Pathology, Vanderbilt Univ., Nashville, TN 37232.

For better understanding of the metabolism of the endogenous excitotoxin quinolinic acid, the distribution of its catabolic enzyme, quinolinic acid phosphoribosyltransferase (QPRT), was studied in the human hippocampal formation with immunohistochemical techniques. In 7 normal human brains obtained at autopsy, QPRT-immunoreactivity (QPRT-i) was found in glial cells and neurons. Glial cells exhibiting QPRT-i were observed in all hippocampal subfields. The polymorphic layer of the dentate gyrus contained the highest density of QPRT-i glial cells. Numerous QPRT-i glial cells also occurred along both sides of the fused hippocampal fissure and in the alveus of Ammon's horn, whereas only a few were observed in the granule cell layer and the stratum pyramidale. Neurons containing QPRT-i were found mainly in the subiculum and in strata oriens and pyramidale of CA1. In addition, moderate numbers of QPRT-i glial cells and neurons were observed in layers II-IV of parahippocampal cortex. The preferential localization of QPRT-i in selected glial cells and neurons suggests that these cellular elements might play specific roles in the regulation of extracellular concentrations of quinolinic acid in the regions examined. Supported by USPHS grants NS 16102 and NS 20509.

260.8

ELECTROPHYSIOLOGICAL EVIDENCE FOR AN EXCITATORY AMINO ACID PATHWAY FROM MEDIAL PREFRONTAL CORTEX TO LATERAL DORSAL TEGMENTAL NUCLEUS AND ROSTRAL LOCUS COERULEUS. D. Highfield and S.J. Grant, Dept. Psychology and Program in Neuroscience, Univ. Delaware, Newark, DE. 19716

We previously reported that electrical stimulation of the Medial Prefrontal Cortex (MPFC) in the rat reliably activated single units in the Lateral Dorsal Tegmental nucleus (LDT), rostral Locus Coeruleus (rLC), and adjacent brainstem areas. The present experiments test the hypothesis that this activation is mediated by an Excitatory Amino Acid (EAA) receptor.

Extracellular single unit recordings from cells driven orthodromically from the MPFC were obtained from chloral hydrate anesthetized rats. All recording sites were histologically verified. In 12/12 units tested, Kynurenic acid (0.1-0.4 µmoles, icv.) produced a dose dependent blockade of the orthodromic response with complete suppression at the highest dose. The NMDA antagonist AP7 (0.04-0.16 µmoles, icv.) did not block the orthodromic response (4/4). Vehicle injections had no effect (2/2).

The results thus far support the hypothesis of an EAA pathway from the MPFC to the LDT-rLC region, but suggest that the receptor is probably not of the NMDA subtype. Work is in progress to determine whether the receptor is the Kainate or Quisqualate subtype.

Supported by a Biomedical Research Support Grant to SJG.

260.9

DISTRIBUTION OF GLUTAMATE-LIKE IMMUNOREACTIVITY IN RAT BRAIN. L. Thai*, J.S. Hong, S. Merchenthaler*, W.E. Stumpf, and M. Sar*. Neurobiology program, Department of Cell Biology and Anatomy, University of North Carolina, Chapel Hill, NC 27599; and Laboratories of Molecular and Integrative Neuroscience, National Institute of Environmental Health Sciences, P.O. Box 12233, RTP, NC 27709.

The distribution of Glutamate-like immunoreactivity in forebrain and mid-brain was studied by Avidin-Biotin peroxidase technique using anti-serum to Glutamate (Hepler et al, J. Histochem. Cytochem 36;13-22 1988.) Male rats were perfused with 4% paraformaldehyde solution and 50µm vibratome brain sections were immunostained. Immunostaining with anti-serum to Glutamate (1:7000) was observed in several areas of the brain with varying intensities. No immunostaining was detected with immunoserum pre-absorbed with Glutamate. In the forebrain, specific immunostaining of perikarya was found in the cortex, amygdala, hippocampus, thalamus, preoptic-septal region, anterior hypothalamic areas, and central hypothalamus. In the mid-brain, perikarya staining was detected in the central gray, substantia nigra, and interpeduncular nucleus. The results show a wide distribution of Glutamate containing cells and suggest an excitatory role of these neurons in brain functions (supported in part by NIH grant NS17479.)

260.10

COLOCALIZATION OF ASPARTATE- AND GLUTAMATE-LIKE IMMUNOREACTIVITY IN RAT VESTIBULAR NUCLEI. C.S. Toomim and P. Petrusz*. Dept. of Cell Biology & Anatomy, University of North Carolina, Chapel Hill, NC 27599-7090.

Neuronal perikarya in the four main vestibular nuclei (spinal, medial, lateral, superior) stain with antisera specific for aspartate (anti-Asp) and glutamate (anti-Glu) (Toomim et al., Soc. Neurosci. Abst. 12:419, 1986 & 13:1562, 1987). The present experiments were undertaken to determine if staining with the two antisera was colocalized in the same cells. Consecutive 4 um paraffin sections were stained with either anti-Asp or anti-Glu, counterstained with toluidine blue and compared for position and appearance of stained cells. All distinguishable neurons within the vestibular nuclei stained strongly with anti-Asp and less strongly with anti-Glu. Also, strong staining was present in the fibers of the ascending medial longitudinal fasciculus, the path projecting from the vestibular nuclei to the oculomotor nucleus. These data are consistent with the presence of high concentrations of both Asp and Glu in the neurons of the vestibular nuclei. Alternatively, the results may be explained by the presence in these neurons of a single unknown antigen recognized by both antisera.

260.11

PROLINE AS AN EXCITATORY AMINO ACID IN THE HIPPOCAMPAL FORMATION: UPTAKE AUTORADIOGRAPHY AND RECEPTOR PHARMACOLOGY. J.V. Nadler, D. Martin, S.D. Bray* and D.A. Evenson*. Depts. Pharmacology and Neurobiology, Duke Univ. Med. Ctr., Durham, NC 27710.

Proline is an excitatory and excitotoxic amino acid in the rat hippocampal formation. We have used slice preparations to determine the sites of proline uptake and the mechanism of proline-induced neuronal excitation.

An autoradiographic method was developed to localize Na^+-dependent L-[^{3}H]proline uptake sites. The laminar pattern of uptake sites closely resembled that of D-[^{3}H]aspartate uptake sites. Lesion studies indicated that the perforant path and Schaffer collateral-commissural projections take up proline. The laminar pattern of uptake was largely obliterated by co-incubation with non-radioactive L-proline, but not by co-incubation with L-glutamate or threo-3-hydroxyaspartate. These results suggest that excitatory pathways in the hippocampal formation express transport carriers for proline as well as for glutamate/aspartate.

Pharmacological studies performed on a grease-gap preparation of area CA1 indicated that L-proline depolarizes CA1 pyramidal cells at concentrations of 0.5 mM or higher. D-Proline was inactive. Depolarizing responses to L-proline were largely blocked by D-AP5, Mg^{2+} and Zn^{2+}, but were insensitive to concentrations of Mn^{2+} that abolished synaptic transmission. CNQX was a relatively weak antagonist. TTX reduced L-proline-evoked depolarizations (30-40%) much more than NMDA-evoked depolarizations (10-15%). These results suggest that L-proline depolarizes CA1 pyramidal cells largely by activating postsynaptic NMDA receptors, but may also directly or indirectly activate postsynaptic TTX-sensitive Na^+ channels. (Supported by NIH grant NS 16064.)

260.12

HOMOCYSTEIC ACID AS TRANSMITTER CANDIDATE IN RAT CEREBELLAR CLIMBING FIBERS. M. Cuénod, E. Audinat*, K.Q. Do, P. Grandes*, T. Knöpfel, P. Streit, F. Vollenweider* and B.H. Gähwiler. Brain Research Institute, University of Zürich, CH-8029 Zürich, Switzerland

Homocysteic acid (HCA), known as a strong excitant, has recently been proposed as transmitter candidate, with mixed agonist properties and a preference for NMDA receptors (Do et al., 1986, J. Neurochem. 46:779, J. Neurosci. 6:2226). Upon depolarization of cerebellar slices by 50 mM K^+, the Ca^{++}-dependent HCA efflux was increased by 48% in hemispheres and by 125% in vermis. Ten days after 3-acetyl-pyridine injections, which leads to degeneration of olivo-cerebellar climbing fibers (cf), the stimulated HCA release was abolished, indicating its cf origin.

Highly specific polyclonal antibodies were obtained with glutaraldehyde-linked HCA-albumin conjugates. Immunohistochemistry was performed on semithin sections of perfusion-fixed rat cerebellar tissue. HCA-like immunoreactivity was conspicuous in terminal-like dots and beaded fibrous profiles mostly in close association with Purkinje cell (PC) perikarya and dendrites. Moreover, the immunoreactivity was totally abolished in cf deprived cerebella. These observations suggest that HCA is localised in cf.

In organotypic rat cerebellar slice cultures, PC were voltage-clamped at a potential of -55 to -65 mV. L-HCA (500 uM), NMDA (50 uM to 1 mM), and the non-NMDA receptor antagonist CNQX (10 uM) were superfused in presence of 1 uM TTX. Inward currents were induced by HCA in PC, as well as by aspartate and glutamate, whereas NMDA had no direct effect on PC. The agonist currents were fully antagonized by CNQX. Thus, in mature PC where no-classical NMDA receptor has been reported, HCA activates a non-NMDA receptor.

Taken together, these results support the proposal that HCA is a transmitter in rat cerebellar cf, acting on non-NMDA receptors of PC.

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261.1

SYNERGISTIC ACTIVATION OF PROENKEPHALIN GENE EXPRESSION BY CAMP, PHORBOL ESTERS, AND DEPOLARIZATION. S.E. Hyman, M.J.Comb, T.V. Nguyen. Molecular Neurobiology Lab, Massachusetts General Hospital East, 149, 13th St. Charlestown, MA 02129

The integration of multiple intracellular signals is an important mechanism regulating neuronal responses. Here we present evidence that multiple second messenger pathways interact to regulate proenkephalin gene expression. cAMP acts synergistically with phorbol esters, calcium entry, and membrane depolarization to produce high levels of transcription from the human proenkephalin enhancer-promoter. The magnitude of this synergy suggests that pairing of signal transduction pathways is an important mechanism regulating gene expression. Although cAMP is required as one of the partners for maximal stimulation of proenkephalin transcription, the observed synergy is not simply a result of increased activation of the cAMP pathway: the level of gene expression produced by paired inputs is significantly greater than the maximum achievable by cAMP alone. Moreover, prolonged exposure to cAMP agonists results in desensitization of the transcriptional response to cAMP, but even when the cAMP pathway is desensitized, phorbol esters remain active. Surprisingly the synergistic responses to cAMP, phorbol esters, calcium entry, and membrane depolarization all map within the proenkephalin enhancer, suggesting that this short region of DNA confers responsiveness to multiple second messengers.

261.2

MULTIPLE RNA CAP SITES ARE INVOLVED IN THE TISSUE-SPECIFIC EXPRESSION OF THE RAT PREPROENKEPHALIN GENE. G. Weisinger*, J.D. DeCristofaro & E.F. La Gamma (SPON: L. Fochtmann) Depts of Peds and Neurobio, SUNY, Stony Brook, NY

Cholinergic agonists can elevate rat adrenal preproenkephalin (preproenk) steady state mRNA by >100 fold and 3 fold in the brain striatum (see DeCristofaro et. al. Neurosci. Abstr., 1989). One molecular process that could result in these tissue-specific observations, at the transcriptional level, is the regulation of RNA initiation at the gene's cap site(s). To address this, we treated groups of rats with: nicotine (5mg/kg sc ql2h), oxotremorine (1mg/kg sc ql2h), both agents, saline vehicle, or no drug for 4d. Twelve hours after the last injection total RNA was extracted and hybridized to a 133 bp end labelled PvuII-SacI preproenk fragment overlapping the putative RNA cap site(s). S1 nuclease analysis (40ug RNA) revealed multiple cap sites from brain striatum derived from unmanipulated animals and 5-10 fold increased initiation (preserving a similar start site ratio) from all the treated groups striatal RNA. In contrast, 40ug of unmanipulated rat adrenal RNA showed essentially no preproenk initiation but large increases in RNA initiation in the cholinergic agonist treated groups. Changes in the pattern of adrenal preproenk cap site usage observed in these experiments will be verified by primer extension analysis. These studies demonstrate that preproenk RNA expression involves multiple RNA initiation sites, tissue-specificity, and is induced by cholinergic agonists. Supported by NSF #BNS8719872.

261.3

PREPROENKEPHALIN DNA BINDING PROTEINS ARE TISSUE-SPECIFIC. E.F. La Gamma, G. Weisinger* and J.D. DeCristofaro. Depts of Peds & Neurobio, SUNY at Stony Brook, NY 11794-8111.

Transcript initiation and levels of rat preproenkephalin mRNA differ by as much as 10- to 100-fold between the adrenal medulla and brain striatum in baseline and induced states, respectively (DeCristofaro & Weisinger, Neurosci '89). This indicates tissue-specific expression exists in this species. Regulation appears to reside at the level of membrane-linked signal-transduction or at "cis"-acting elements/DNA binding proteins. To address this, we are exploiting gel shift assays to initially characterize nuclear proteins from adrenal medulla, brain striatum, liver, AtT-20 cells, HeLa cells, and other lines. These tissues show unique as well as shared patterns of protein binding to fragments of the rat preproenkephalin gene from -360 to -249, from -248 to -89, and from -88 to +51 (relative to the putative Cap site). Strongest banding is localized to the region -360 to -88; is competed away by unlabeled homologous DNA; but not by non-specific DNA (dA/dT). Competition studies using fragments of the double stranded DNA tumor virus SV40 (38-fold excess) indicate that two striatal-specific bands can be competed away by the promoter region containing the 21 bp repeats but not by the 72 bp enhancer. This suggests an SP1-like DNA binding protein may exist in native rat tissues. Whether differences in banding represent site-specific binding or function is being evaluated by footprinting and transfection studies. Supported by NSF grant #BNS8719872.

261.4

STRUCTURAL LINKAGE OF RAT VASOPRESSIN AND OXYTOCIN GENES. T.G. Sherman and S.J. Watson. Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109-0720.

The rat vasopressin/neurophysin-I and oxytocin/neurophysin-II precursors are encoded by a pair of genes whose close homology strongly suggested that these genes arose via a duplication event from an ancestral gene during evolution. As part of ongoing studies into the neuroendocrine regulation of vasopressin and oxytocin mRNA expression, vasopressin and oxytocin genes were isolated from a rat lambda gt10 library. Closer examination of the 3' non-transcribed regions of two lambda inserts, one from a vasopressin positive and one from an oxytocin positive, revealed a short segment of DNA that cross-hybridized between the two clones. The possibility existed, therefore, that the vasopressin and oxytocin genes existed side-by-side in the rat genome, analogous to what had been shown in a human small cell lung cancer cell line, H378. To investigate this further in rat, a cosmid library was prepared in pWE15 using MboI-partially digested liver DNA with an average insert size of 40-45 Kbases. Screening of duplicate lifts of 100,000 recombinants with either vasopressin gene exon C or oxytocin gene exon C resulted in 4 positives, three of which were double positives. The fourth was positive for only vasopressin. Southern mapping of these double positives revealed that the EcoRI, PstI, and NcoI positive vasopressin or oxytocin hybridizing fragments were identical in size to those on the previously isolated individual lambda clones. The 3' localization of the shared fragments in the lambda clones suggests that the vasopressin and oxytocin genes reside on opposite DNA strands, with their 3' ends facing each other at a distance of 10-15 Kbases. This orientation has been closely associated with recent gene duplication events. Studies are continuing in the mapping of the inter-genic domain, as well as elucidating the apparent divergence of duplicated tissue-specific and transcriptional regulatory regions.

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261.5

RAT CORTICOTROPIN RELEASING HORMONE GENE: ANALYSIS OF TRANSCRIPTIONAL CONTROL ELEMENTS. A.F. Seasholtz* and R.C. Thompson. Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109-0720.

The rat corticotropin releasing hormone (CRH) gene has been isolated and characterized (Thompson, R.C., Seasholtz, A.F., and Herbert, E., Mol. Endo., 1:363, 1987). The CRH gene is expressed in many regions of the brain as well as several peripheral tissues including rat testis which expresses a significantly larger CRH transcript. CRH mRNA structural analyses have mapped the brain transcription initiation sites and suggest that the testicular CRH mRNA represents an alternatively spliced mRNA arising from a more distant 5' promoter element. In addition to the CRH mRNA structural analyses, transcriptional control elements in the 5' flanking sequence of the rat CRH gene are being identified and localized using cultured cells that have been transfected with a chimeric gene (CRHCAT) containing the rat CRH 5' flanking DNA linked to the bacterial chloramphenicol acetyltransferase gene. A cAMP responsive element has been localized to the region between 238 and 180 bp 5' to the major brain CRH mRNA cap site (Seasholtz, A.F., Thompson, R.C., and Douglass, J.O., Mol. Endo., 2:1311, 1988). The endogenous rat CRH gene has been shown to be negatively regulated by glucocorticoids in the hypothalamus, and we have been able to demonstrate regulation of rat CRHCAT expression by glucocorticoids in several transfected cell lines. The exact location of the glucocorticoid responsive element is being determined using in vivo competition studies and deletion analysis in both transiently and stably transfected cell lines.

261.6

CLONING AND CHARACTERIZATION OF cDNAS AND GENES ENCODING RAT AND HUMAN NEUROMEDIN B, A MAMMALIAN BOMBESIN-LIKE PEPTIDE. I.M. Krane*, W.W. Chin* and E.R. Spindel (SPON: P.C.K. Leung). Div. of Genetics, Dept. of Medicine, Brigham & Women's Hospital; Harvard Med. School; Boston, MA 02115 and Div. Neurosci., Oregon Regional Primate Research Ctr.; Beaverton, OR 97006.

The diverse biological effects of amphibian bombesin and its mammalian homolog, gastrin-releasing peptide (GRP), are well documented. Less is known about the amphibian bombesin-like peptide ranatensin and its homolog, neuromedin B (NMB). NMB has been isolated from porcine spinal cord as a decapeptide (NMB-10) and as a 32 amino-acid peptide (NMB-32). GRP and NMB have a similar but distinct distribution throughout the brain and GI tract. Our laboratory has reported previously the isolation of the human NMB cDNA and we now report the isolation and characterization cDNAs encoding rat NMB as well as the genes encoding both the human and rat forms. Using a full-length human NMB cRNA probe, human genomic clones containing the three exons of the NMB gene were isolated from a genomic library. The intron/exon organization of the human NMB gene is similar to that previously reported for the human GRP gene in that there are two introns, one of which interrupts a codon coding for a Gly residue in the bioactive portion of the peptide. In order to characterize tissue specific NMB gene expression more fully, the human NMB cDNA was used to isolate both the rat NMB gene and its corresponding cDNA. Sequence analysis of hybridizing cDNA clones revealed a 670 bp cDNA encoding an 83 amino-acid preprohormone. In the precursor, a signal peptide is followed by NMB-32 and a short C-terminal extension peptide. The amino-acid conservation among the rat, porcine and human NMB-32 peptides is quite remarkable; the only differences occur in the amino-extended form of the peptide where the rat and human differ by three amino acids and the rat and porcine sequences differ by only two. RNA blot analysis shows hybridizing RNAs of approximately 900 bases, with highest mRNA concentrations found in the pituitary.

261.7

NEUROENDOCRINE EXPRESSION OF A SOMATOSTATIN-SV40 T ANTIGEN FUSION GENE IN TRANSGENIC MICE. R. Ventimiglia, V. Fairchild*, R. H. Goodman*, and M. J. Low*. Division of Molecular Medicine, New England Medical Center, Tufts U. School of Med., Boston, MA 02111

Somatostatin is a peptide hormone produced in neurons in discrete regions throughout the central nervous system as well as in non-neural tissues including the endocrine pancreas and gut. To identify the elements within the somatostatin gene that direct tissue-specific expression, we have examined the expression of a somatostatin fusion gene in transgenic mice. Fertilized mouse eggs were injected with a 4.0 kilobase fragment of the rat somatostatin gene fused to a 2.6 kb fragment containing a mutant (non-transforming) SV40 T antigen. Five mice carrying the integrated hybrid gene were identified by dot blot analysis. T antigen (TAg) expression was detected immunohistochemically in four of the five lines of mice. A consistent pattern of expression was observed in each of the four lines. TAg immunoreactivity was restricted to cell nuclei, and was detected in layers II-VI of the cerebral cortex, the stratum oriens, pyramidal cells, and hilus of the dentate gyrus of the hippocampus, and in the caudate nucleus, reticular nucleus of the thalamus, and zona incerta. In the retina, TAg immunoreactivity was detected in presumptive amacrine cells in both the inner nuclear layer and ganglion cell layer. TAg immunoreactivity was also detected in peripheral cells of the pancreatic islets, characteristic of somatostatinergic D cells. We conclude that elements within 4.0 kb of the 5' flanking region of somatostatin are sufficient to direct both neural and endocrine expression of the gene.

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261.8

IDENTIFICATION OF GENETIC ELEMENTS IMPORTANT FOR CELL-SPECIFIC EXPRESSION OF THE HUMAN VIP GENE. J. S. Fink Lab. of Molecular Neurobiology, Dept. of Neurology, Massacusetts General Hospital, Boston, MA 02129.

The cell-specific expression of many genes has been determined to result from the action of one or more genetic elements that act in *cis* to control transcription. To begin to understand the molecular mechanisms which control the cell-specific and developmental regulation of neural genes, genetic elements in the human VIP gene that are important for directing its neural-specific expression were identified. VIP is a 28 amino acid peptide that is present in neurons of the central and peripheral nervous systems, sparsely in cells of the adrenal medulla and ectopically in some tumors of neural crest origin. Fusion genes containing portions of the 5'-flanking region of the VIP gene linked to a reporter gene chloramphenicol acetyltransferase (CAT) were transfected into several VIP-producing or VIP-lacking cells lines. CAT activity in the cell lysates assayed 48-72 hours after transfection provided an index of transcriptional activity under the control of portions of the 5'-flanking sequences. Both positively and negatively-acting genetic sequences that direct cell-specific expression of the VIP gene were identified. VIP-CAT fusion genes containing 94 bp of 5'-flanking sequence and 146 bp of exon 1 (VIP94CAT) were 7 to100-fold more active in the VIP-expressing human neuroblastoma cell lines SH-SY5Y and NB-Fl than in cells that do not synthesize VIP (PC12, HeLa, C6 glioma, COS-7). A 31 bp fragment within this postively-acting region was capable of transferring cell-specific expression to a heterologous viral promotor. In cells that do not synthesize VIP the activity of VIP94CAT was much less than a fusion gene containing 2000 bp of 5'-flanking sequence. In the VIP-expressing neuroblastoma cell lines, the activity of VIP-CAT fusion genes containing 94 or 2000 bp of 5'-flanking sequence were comparable. These studies demonstrate that (1) a positively-acting cell-specific genetic element is contained within 94 bases of the 5'-flanking region of the human VIP gene, and (2) a negatively-acting genetic element(s) or "silencer" acts to repress VIP gene transcription in non-neuronal cell types.

261.9

ESTROGEN REGULATION OF RAT GALANIN TRANSCRIPTION IS MEDIATED BY SEQUENCES IN THE 5'-FLANKING REGION OF THE GALANIN GENE. L.M. Kaplan, D. Abraczinskas*, M. Davidson*, W.W. Chin*. Gastrointestinal Unit, Massachusetts General Hospital and Division of Genetics, Brigham & Women's Hospital, Boston, MA 02114.

Galanin is a neuroenteric peptide which regulates the secretion of several pituitary and pancreatic hormones. We have previously shown that galanin gene expression in the rat anterior pituitary (AP) varies over a several thousand-fold range depending on the levels of circulating estrogens (PNAS **85**:7408, 1988). We investigated the mechanisms by which estrogen regulates galanin gene expression. Nuclear run-on studies were performed using rat pituitaries isolated after treatment *in vivo* with 17β-estradiol benzoate (100 μg/kg s.c.) or carrier. These studies revealed that estrogen increases AP galanin gene transcription up to 70-fold with a maximal effect 6 hr after treatment. In order to examine transcriptional regulation further, we isolated and characterized the gene encoding rat galanin. This gene contains 6 exons and extends approximately 6 kb. Plasmids containing the native galanin promoter and up to 1.8 kb of 5'-flanking sequence inserted upstream of the firefly luciferase gene were transfected into AP-derived GH_3 cells that were subsequently incubated for 48 hr in medium ± 17β-estradiol (10^{-9} M). Expression of the chimeric plasmids was determined by luciferase activity in GH_3 cell extracts. Estrogen stimulated the expression of plasmids containing the full 1.8 kb fragment approximately 30-fold. Mutants deleted in various portions of the galanin 5'-flanking region abolished the response to estrogen in this system. We have used these mutants to identify sequences which confer the observed estrogen-induced stimulation of galanin gene transcription.

261.10

MOLECULAR CLONING OF THE TRYPTOPHAN HYDROXYLASE (TPH) GENE IN *DROSOPHILA*. W. Neckameyer* and K. White * (spon: T. Tully). Dept. of Biology, Brandeis University, Waltham MA 02254

A full-length cDNA encoding rabbit TPH (Grenett *et al*, PNAS **84**:5530, 1987) was used to isolate *Drosophila* cDNA clones on the basis of hybridization at reduced stringency. Sequence analysis of an essentially full-length *Drosophila* cDNA showed considerable homology to rabbit TPH at both the DNA and deduced protein levels, but greater homology to rat phenylalanine hydroxylase (PAH). However, Southern analysis of *Drosophila* genomic DNA with rabbit TPH and and several of the *Drosophila* cDNA isolates indicated a single gene that was recognized by all probes used. The *Drosophila* cDNA clone hybridized to a single site on larval polytene chromosomes at region 66A. Northern analysis showed a single transcript of 1.75 kb which was enriched in adult heads, but which was also present in adult bodies and 0-6 hour embryos. This data raises the possibility that *Drosophila* has only two hydroxylase-family genes: tyrosine hydroxylase (TH, Neckameyer and Quine, Neuron **2**:1167, 1989) and a gene encoding both TPH and PAH functions.

261.11

MAPPING OF A FUNCTIONAL GLUCOCORTICOID RESPONSE ELEMENT IN THE RAT PHENYLETHANOLAMINE N-METHYLTRANSFERASE PROMOTER. M.E. Ross, M.J. Evinger, T.H. Joh, D.J. Reis, and H.M. Goodman Depts of Mol. Biol., Mass. Gen. Hosp., Boston, MA 02114 and Neurol. and Neurosci., Cornell Univ. Med. Coll., New York, NY 10021. The catecholaminergic phenylethanolamine N-methyltransferase (PNMT) gene was cloned in rat and a consensus sequence for a glucocorticoid response element (GRE) was found at -513 bp, 5' to the transcriptional start site (Ross et. al., Soc. Neurosci. 1988, 14:1250). In order to define the function of this element, fusion genes containing the PNMT promoter and a chloramphenicol acetyltransferase (CAT) reporter were constructed. However, they did not express in any of 7 continuous cell lines transfected, none of which endogenously produce PNMT. A system for transfecting bovine adrenal chromaffin cells in primary culture was therefore devised. Using these chromaffin cells, both basal and regulated CAT expression of the PNMT fusion constructs was detected. In the presence of dexamethasone (dex) (20 μM) pPNMT3000 and pPNMT900, containing the putative GRE and 3000 bp or 863 bp of PNMT promoter sequence, respectively, displayed a 4 to 10 fold induction in CAT activity. Both pPNMT300 and pPNMT100, which lack the GRE and contain 273 bp or 99 bp of PNMT promoter sequence, respectively, were unaffected by dex. Addition of the PNMT region spanning -490 bp to -863 bp conferred full dex responsiveness to a thymidine kinase promoter. Deletion of the putative GRE by oligonucleotide directed mutagenesis abolished the response to dex. These data identify the sequence at -513 bp in the rat PNMT gene as a functional GRE.

261.12

GAD SEQUENCES MAP TO TWO MOUSE CHROMOSOMES. M. H. Brilliant[1], G. Szabo[2*], Z. Katarova[2*], C. A. Kozak[3*], T. M. Glaser[4*], R. J. Greenspan[5] and D. Housman[4*]. [1]Fox Chase Cancer Center, Philadelphia, PA; [2]Hungarian Academy of Sciences, Hungary; [3]NIH, Bethesda, MD, [4]MIT, Cambridge, MA; [5]Roche Institute, Nutley, NJ.

The chromosomal locations of mouse DNA sequences homologous to a feline cDNA clone encoding glutamic acid decarboxylase (GAD; Kaufman et al., Science 232:1138, 1986) were determined. Although cats and humans are thought to have only one gene for GAD (Erlander et al., Soc. Neurosci. Abstr. 13:857, 1987), GAD cDNA sequences hybridize to two distinct loci in the mouse, on Chromosomes (Chrs) 2 and 10. The mapping of sequences homologous to GAD cDNA utilized Southern hybridization analysis of DNA from mouse-hamster hybrid cells, an inter-specific backcross and recombinant inbred strains. Mouse genomic sequences homologous to GAD cDNA were isolated and used to determine that GAD is encoded by a locus on mouse Chr 2 (Gad-1) and that an apparent pseudogene locus is on Chr 10 (Gad-1ps). The Gad-1 locus maps near several neurological mutations and is part of a homology of synteny between mouse Chr 2 and the long arm of human chr 2.

262.1

QUANTITATIVE AND QUALITATIVE CHARACTERIZATION OF MULTIPLE APP RNA FORMS IN NORMAL AND ALZHEIMER'S BRAINS BY S_1 PROTECTION ANALYSIS. J. Steven Jacobsen*, Robert J. Donnelly*, Arthur J. Blume, Bernard Beer and Michael P. Vitek. Molecular Neurobiology, CNS Research, Lederle Laboratories, American Cyanamid Company, Pearl River, NY 10965.

Three Amyloid Peptide Precursor RNAs (*i.e.*, APP 695, 751 & 770) have been identified in Alzheimer's diesease (AD) and normal brain. To determine if the amount of one or more of these APP RNAs changes in AD, we used an anti-sense [^{32}P]-riboprobe derived from the protease inhibitor region of APP 770 (*i.e.* the *Dde*1-*Xho*1 fragment) in an S_1 nuclease protection assay [Donnelly, R.J. *et al. in* Alzheimer's Disease and Related Disorders, Alan R. Liss, Inc., NY, 1989]. All brain regions examined and some peripheral tissues contain 695, 751 and 770 APP RNAs. The rank order in frontal cortex of normal and AD brains is 695>751>770. In temporal cortex from AD brains, there are individuals with the frontal rank order and others which rank 751>695>770. Data from additional brain regions (*e.g.*, parietal and occipital cortex) are complex. Using microtubulin-associated protein (MAP2) and cyclophilin internal controls, our data imply a change in the absolute amounts of APP RNA forms between normal and AD brains. S_1 analyses also reveal protected probe fragments which are not predicted by the known APP cDNA sequences indicating the existence of additional as yet uncharacterized APP RNAs. In addition, S_1 analysis has been used to determine the effects of several hormones on the quality and quantity of APP gene expression.

262.2

SYNAPTIC LOCALIZATION OF THE A4 PROTEIN PRECURSOR OF THE AMYLOID OF ALZHEIMER'S DISEASE. J. Beer*, A. Weidemann*, P. Fischer*, D. Bunke*, C. Masters* and K. Beyreuther* (SPON: S. Scheff). Center for Molecular Biology of the University of Heidelberg, 6900 Heidelberg, FRG.

Alzheimer's disease is a progressive neurodegenerative disorder which is characterized by the deposition of amyloid A4 protein in the central nervous system. The A4 protein is part of a precursor protein (PreA4). Three different mRNAs arise from the PAD-gene (Precursor of Amyloid in Alzheimer's disease and Down's Syndrome) by alternative splicing and encode primary translation products of 695, 751 and 770 amino acids.

We have raised polyclonal and monoclonal antibodies against the brain specific $PreA4_{695}$ and the synthetic cytoplasmic domain common to all three primary translation products. Using standard immunolabelling techniques we demonstrated the neuronal and synaptic localization of PreA4 proteins in cryosections of snap frozen tissues from rat brain. This immunolocalization supports the hypothesis of a role for PreA4 proteins in cell-cell interactions. Furthermore these proteins may contribute to synaptic function and may be degraded to amyloid at synaptic sites. These results forward the hypothesis of the neuronal origin of A4 amyloid deposition.

262.3

BETA AMYLOID PROTEIN PRECURSOR (BAPP) DEPOSITION AND CYTOSKELETAL DISRUPTION MAY BOTH BE PREREQUISITES FOR ALZHEIMER'S DISEASE (AD) AC McKee, LI Binder, KS Kosik and NW Kowall Depts Neurol & Neuropathol, Harvard Med Sch, Boston MA 02114; Dept Cell Biol Anat, UAB, Birmingham AL 35294.

We found isolated deposits of BAPP (antiserum courtesy of DJ Selkoe) in temporal isocortex of aged adults and of a Down's child. Staining with MAP2, MAP 1b and tau was normal. MAP 1b stains dentate granule cells and forms a trilaminar pattern in the molecular layer. Pyramids in CA3>CA2>CA4 were intensely MAP 1b ir, while CA1, pre-, and subiculum were not. Pro- & para-subicular pyramids were strongly ir as were entorhinal star cells and layer III-VI neurons. In AD, BAPP forms variably sized globular deposits in the dentate molecular layer, CA fields and subiculum.Superficial pro- and para-subiculum contain clouds of punctate BAPP. Entorhinal layers III-VI, subpial and layers II-VI temporal isocortex are positive.MAP 1b staining in AD shows enhanced ir of CA1 pyramids and loss of the laminar pattern in dentate molecular layer.Tau abnormalities do not parallel BAPP deposition. Tau dystrophic neurites are numerous where BAPP deposition is absent, BAPP deposits outnumber tau neurites of senile plaques, and the confluent areas of punctate BAPP staining are not tau ir.These findings show that, in AD, BAPP deposition and tau ir are not co-extensive, and that BAPP deposition may precede tau neurites and neurofibrillary tangles and MAP2 proliferative abnormalities.Furthermore, MAP 1b, which is under strong developmental regulation, is reexpressed in CA1 pyramids in AD suggesting that neuritic proliferation is associated with the re-emergence of fetal characteristics.BAPP deposition and cytoskeletal disruption may both be necessary for the development of AD.

262.4

SPATIAL RELATIONSHIP OF AMYLOID PLAQUES AND CAPILLARIES IN ALZHEIMER DISEASE. M. Kawai*, S.I. Harik, R.N. Kalaria and G. Perry. Inst. Pathology and Dept. Neurol., Case Western Reserve Univ., Cleveland, OH 44106.

The theory that amyloid plaques (AP) of Alzheimer disease (AD) are formed by abnormal leakage from brain capillaries finds much of its support in reports that AP contain capillaries. Since these studies did not perform statistical analysis, we quantitatively analyzed the spatial relationship between capillaries and APs in AD. Vibratome sections (60μm) of AD hippocampus were immunostained with a MAb to β-protein (gift of G. Glenner) (APAPP) and counterstained with a rabbit serum to collagen IV (PAP). Classification of the APs showed that while 60% of AP are associated with capillaries only 8% are penetrated by a vessel, remainder being adjacent. To test whether (1) the interior of the AP or (2) the borderzone (10μm rim outside the AP) has statistically higher density of capillaries than (3) the rest of the cortical gray matter, similarly double-stained 6μ sections from 3 cases of AD were photographed and the capillary densities in the 3 areas calculated by using a BioQuant. The capillary densities of (1), (2) and (3) in one of the 3 cases were 36.5/mm^2, 250.0/mm^2 and 127.6/mm^2 respectively. x^2-test demonstrated that (1) and (3), (2) and (3) are significantly different at p=0.01. The combined area of (1) and (2) had a density of 137.9/mm^2, not significantly different from the rest of the gray matter (3). Essentially identical results were obtained for the other 2 cases. It is concluded that the frequently observed association of AP to capillaries can be statistically explained by chance contact as well as by exclusion of capillaries from the plaque interior. Supported by Fulbright and Fogarty Fellowships (MK) and NIH K04-AG00415 and AG007775.

262.5

WITHDRAWN

262.6

ARYLESTERASE AND PARAOXONASE LEVELS IN SUSPECTED ALZHEIMER PATIENTS AND AGE-MATCHED CONTROLS. D.E. Moss, D. Wong*, A. Enriquez*, S.F. Sands, and R.H. Whitworth*. Alzheimer's Disease Research Project, Univ. Tex. El Paso, El Paso, TX 79968-0553.

Reduced levels of serum arylesterase (ARYL) and paraoxonase (PXN), proteases implicated in degrading amyloid A, are found in reactive systemic amyloidosis [Maury et al., J. Lab. Clin. Med. 104:761, 1984]. Beta amyloid in Alzheimer's disease may also come from extracellular sources [e.g., Glenner and Wong, Biochem. Biophys. Res. Commun. 122:1131, 1984]. Serum ARYL and PXN were studied in 10 patients suspected of SDAT and 10 age-matched controls. There was no difference between SDAT and normals in ARYL. Surprisingly, however, PXN was significantly higher in SDAT; almost double control values. In brain bank tissue, 6 samples from autopsy-confirmed Alzheimer cases or 6 normals showed no difference in ARYL. However, the one available case of Creutzfeld-Jakob disease had ARYL levels about twice all other brains. There was insufficient PXN activity in brain for reliable measurement. Increased expression of serum PXN in patients with neurological disorders may mark an enzyme response related to amyloid metabolism. Further research will be required to extend this limited study and determine if this change is unique to Alzheimer's disease.

Supported in part by NIMH (RR08012) and MBRS Program.

262.7

PROTEOLYTIC PROCESSING OF β-PROTEIN PRECURSOR-RELATED SYNTHETIC PEPTIDES. C.R. Abraham and H. Potter. Dept. Neurobiology, Harvard Med. Sch., Boston, MA 02115.

We have shown that the serine protease inhibitor α_1-antichymotrypsin (ACT) is found in the amyloid deposits of the β-protein type in Alzheimer's disease (AD), Down's syndrome, normal aging of humans and monkeys and hereditary cerebral hemorrhage with amyloidosis of Dutch origin. It is believed today that an aberrant proteolytic processing of the β-protein precursor or inadequate clearing of the β-protein is the cause of the β-protein accumulation. The identification of brain-specific proteases involved in this process is therefore crucial. Since ACT is known to inhibit serine proteases such as chymotrypsin and cathepsin G, we tested whether these proteases can cleave synthetic peptides made around the extracellular N-terminal cleavage site of the β-protein. Using thin-layer chromatography (TLC) and HPLC, we showed that these two proteases are able to cleave between methionine and aspartic acid, aspartic acid being the N-terminus. Chymotrypsin and cathepsin G, though, are not found in the brain. In order to detect brain serine proteases that are able to cleave at the met-asp bond, we added a histidine at the N-terminus of the peptide and iodinated it so we could follow the cleavage products after incubation with brain extracts. Cathepsin G cleaved the labeled peptide, as did the soluble fractions of brain homogenates from both control and AD brain, as seen on TLC. Specific cross-linking of the iodinated peptide to brain fractions and separation on SDS gels revealed bands of approximately 20-35 kD, which are in the process of being characterized. Labeling of brain homogenates with ^{3}H-DFP also reveals bands of similar molecular weights, suggesting that these proteins may be serine proteases.

262.8

ALZHEIMER AMYLOID PRECURSOR PROCESSING: RAMIFICATIONS TOWARD DISEASE ETIOLOGY. B.D. Greenberg, D.E. Lowery*, P.A. Gonzalez-DeWhitt* and R.A. Altman*. Molecular Biology Research, The Upjohn Company, Kalamazoo, MI 49001.

The role of amyloid precursor processing in the etiology of Alzheimer's Disease is still unclear. We and our collaborators, as well as several other groups have shown that protein sequences within the amyloid precursor, but outside the plaque core protein domain are associated with amyloid deposits in Alzheimer brain. These observations, however, do not distinguish two likely scenarios. The amyloidogenic β-protein might be created by *in situ* processing of the precursor, or full length precursor may adventitiously associate with preexisting amyloid deposits following release by degenerating neurites. This is an important question since the two pathways significantly differ, impacting potential therapies based on etiological models of the disease. We are proceeding with our efforts to resolve such questions, employing recombinant DNA technology toward generating precursor protein and related antisera. These approaches and the application of these reagents will be the topic of this presentation.

262.9

PCR AMPLIFICATION OF REVERSE TRANSCRIBED RNA REVEALS A FOURTH ALTERNATIVELY SPLICED βAPP mRNA. T. E. Golde, S. Estus, L. H. Younkin, and S. G. Younkin. Institute of Pathology, Division of Neuropathology, and Department of Pharmacology, Case Western Reserve University School of Medicine, Cleveland, OH 44106

The ß amyloid protein precursor (βAPP) gene has been shown to produce at least three mRNAs (βAPP_{695}, βAPP_{751}, and βAPP_{770}) through alternative splicing of two adjacent exons that encode 56 and 19 amino acid inserts. We have used polymerase chain reaction (PCR) amplification of reverse transcribed mRNA to unambiguously detect the various βAPP mRNAs and to quantitate their relative levels. Using oligonucleotide primers that flank the site of alternative splicing, we specifically amplify 87, 255, and 312 bp fragments derived respectively from the βAPP_{695}, βAPP_{751}, and βAPP_{770} mRNAs. In RNA isolated from U87-MG, a human glioblastoma cell line, and from a variety of human brain regions, an additional band at 144 bp is also specifically amplified. Sequencing of this band reveals that it is derived from a previously undocumented βAPP_{714} mRNA containing only the insert that encodes the 19 amino acid domain. As expected, relative quantitation of the various βAPP mRNAs reveals expression of βAPP_{695} > βAPP_{751} >> βAPP_{714} and βAPP_{770} in all regions of the brain examined, whereas levels of βAPP_{751} and βAPP_{770} > βAPP_{695} in the liver. Significantly, we also observed high levels of expression of all forms of the message in white matter RNA. This indicates that non-neuronal cells in the brain produce appreciable amounts of βAPP mRNA. To assess expression of the βAPP gene in Alzheimer's disease, we are currently using the polymerase chain reaction to analyze RNA from a variety of brain regions in a number of Alzheimer's disease cases and in well matched controls.

262.10

PHYSIOLOGICAL FUNCTION OF AMYLOID β-PROTEIN PRECURSOR M. Sundsmo*, J.-M. Roch, T. Oltersdorf*, D. Schenk*, and T. Saitoh. University of California, San Diego, Dept. of Neurosciences, M-024, La Jolla, CA 92093, U.S.A. and Athena Neurosciences, Inc., 800 F Gateway Blvd., South San Francisco, CA 94080, U.S.A.

Amyloid β-protein precursor (ABPP) has a nearly ubiquitous tissue expression and is conserved through varying species from Drosophila to man, suggesting a potential major role in cell biology. This work elucidates a possible function for ABPP. A fragment of amyloid β-protein precursor cDNA was conjugated in the antisense orientation to an eukaryotic promoter and transfected into fibroblasts. The fibroblasts harboring this construct (pNCA, neuritic core antisense) produced 75% less ABPP mRNA (by Northern blot analysis) and 60% less ABPP (by Western blot analysis using an antibody directed against a synthetic peptide from the N-terminal region of ABPP). These transfected cells grew poorly in the presence of 10% serum as compared to nontransfected control cells . Normal growth was restored when medium conditioned by parent cells (final concentration of 30%) or partially purified ABPP (final concentration of 10 ng/ml) was provided. The capacity of the conditioned medium (CM) to restore cell growth was entirely abolished by passage through an anti-ABPP affinity column; the activity was recovered in the bound fraction. These results are consistent with the hypothesis that ABPP is released from cells into the medium and has an autocrine function, suggesting its basic role in growth regulation.

262.11

INDUCTION OF THE AMYLOID PRECURSOR PROTEIN GENE PROMOTER IN CULTURED CELLS. R. J. Donnelly*, A. J. Friedhoff, B. B. Beer#, A. J. Blume#, and M. P. Vitek#. Millhauser Laboratories, Department of Psychiatry, NYUMC, 550 First Avenue, NY, NY 10016 and #Molecular Neurobiology, CNS Research Dept., Lederle Laboratories, Pearl River, NY.

Using sequences from the APP 770 cDNA (Donnelly et al., Neurobiol. Aging 9(1988)333), we have isolated the promoter region of the single copy gene for Amyloid Precursor Protein (APP). DNA sequencing has identified several elements homologous to either constitutive or inducible genes. We have constructed a reporter gene system in which fragments of the promoter were fused to the human growth hormone (hGH) gene. We have found that cells transfected with different promoter fragment constructs produce varied levels of hGH suggesting that constitutive elements within the promoter are functional. Inducibility of the promoter fragments containing different groups of elements was tested by treating the transfected cells with human recombinant interleukin-1 (IL-1). This treatment resulted in a two fold stimulation of hGH production. Promoter constructs containing nucleotides -485 to +101 responded to the induction but constructs containing nucleotides -305 to +101 did not. Therefore the IL-1 responsive sequences map between nucleotides -485 and -305, a region which may also be responsive to other trophic factors. We have now produced stably transfected cells containing promoter fragment constructs to enable characterization of the nature of IL-1 induction and the effect of other trophic factors on this promoter.

262.12

MITOGENIC ACTIVITY OF MEDIUM CONDITIONED BY TRANSFECTED CELLS EXPRESSING A NEUROTOXIC FRAGMENT OF THE ALZHEIMER AMYLOID PRECURSOR. S. Fidel*, L.R. Dawes, R.L. Neve. (SPON: K. Walton) The Children's Hospital, Boston, MA 02115

Neuritic plaques in Alzheimer disease often contain core deposits containing beta-amyloid (a peptide derived from the C-terminus of the amyloid precursor protein [APP]) in association with dystrophic neurites and proliferating glial cells. Recent work has shown that peptides derived from the C-terminal region of APP have effects on neuronal outgrowth and survival.

Conditioned medium (CM) from transfected NIH 3T3 cells expressing the C-terminal 105 amino acids (AB1) of APP has previously been shown to be toxic to differentiated PC12 cells, as well as to cultured hippocampal neurons. We are now examining the mechanism of the neurotoxic activity of this AB1 CM. One possibility is that the AB1 CM is mitogenic, which could have deleterious effects on nonproliferating terminally differentiated neurons. Significant mitogenic activity, as measured by DNA synthesis in quiescent BALB/c 3T3 cells, was present in AB1 CM compared with control CM from nontransfected cells. Genetic and biochemical studies are currently underway to further characterize the mitogenic activity of the AB1 CM. We thank Paul Fanning for technical assistance, and Dan Raben for helpful discussions.

262.13

THE PROTEASE INHIBITOR CONTAINING FORM OF β-AMYLOID PRECURSOR PROTEIN PROMOTES NEURITE OUTGROWTH L.D.Altstiel[1,2], N.K. Robakis[1], (SPON: P. Knott) [1]Department of Psychiatary and Fishberg Neurobiology Center, Mount Sinai School of Medicine, New York, N.Y. 10029. [2]Department of Psychiatry, Bronx VA Medical Center, Bronx, NY.

The major component of the neuritic plaque and cerebrovascular amyloid of Alzheimer disease is a 4.2 KDa protein termed β-protein or A_4 peptide. The sequence of β-protein is part of at least three distinct precursors βAPP_{695}, βAPP_{770}, βAPP_{751}. βAPP_{770} and βAPP_{751} contain a 57 amino acid insert with high sequence homology to the Kunitz-type serine protease inhibitors (KPI) which is not present in βAPP_{695}. Synthetic peptides with amino acid sequence derived from the sequence predicted by βAPP cDNA were used as antigens to prepare antisera against specific regions of βAPP. Immunofluoresence and immunohistochemical microscopy indicated that the KPI containing βAPP is located on the cell bodies, axons, and growth cones of cultured neuronal cells (PC12 and Sy5y cells). Antisera specific to the KPI insert inhibited nerve growth factor (NGF) induced neurite outgrowth in both PC12 and Sy5y cells while antisera specific to βAPP regions common to all three precursors had no effect on neurite outgrowth. NGF-treated PC12 cells shed large amounts of the KPI containing βAPP into the culture medium. In addition, NGF treated PC12 cell culture medium contained high levels of protease inhibitory activity. Antibodies specific to the KPI-insert present in βAPP blocked the protease inhibitory activity present in the conditioned medium. These results indicate that 1) KPI-containing βAPP promotes neurite outgrowth and 2) that this effect may be mediated via the protease inhibitory activity of the KPI.

PROCESS OUTGROWTH, GROWTH CONES AND GUIDANCE MECHANISMS IV

263.1

MOLECULAR CLONING OF UNC-116, A GENE INVOLVED IN AXONAL GUIDANCE IN C. ELEGANS. Jorge R. Mancillas and Danielle Thierry-Mieg, Dep. of Anatomy and Cell Biology, UCLA and CNRS, Paris, France.

Unc-116 is a mutant of C. elegans which was isolated during a screen for mutants with abnormalities in backward locomotion. Unc-116 displays anatomical defects which include mispositioning of a number of longitudinal nerve processes normally occupying a lateral position.

We cloned unc-116 by a combination of transposon tagging and use of the C. elegans physical map: 1) a polymorphic 1.67 kb Hind III fragment was identified by probing genomic Southern blots with a set of overlapping cosmid clones in the mab-5/unc-86 contig of the physical map; 2) probing with TC5 (one of the C. elegans transposons), revealed a band that consistently segregates with the unc-116 phenotype.

A 2.8 kb transcript that hybridizes to the Hind III fragment and to the cosmid clones was identified by Northern blot analysis of wild type mRNA. In unc-116 mRNA, this band appears as a 6.4 kb transcript, suggesting that the TC5 sequence is transcribed in the mutant. We have isolated 3 cDNAs and placed them and the transcript in a detailed restriction map of the region, allowing us to select a complete set of genomic fragments (putative coding region) for sequence analysis. Injection of clones from that region into unc-116 oocytes rescues the mutant phenotype. We are now sequencing the 3 cDNAs and genomic fragments as a first step in the molecular characterization of unc-116.

263.2

PATHFINDING OF SENSORY NEURONS IS DISRUPTED IN THE MOTH HOMEOTIC MUTATION *OCTOPOD*. R. Booker* (SPON: C. Miles). Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853.

In the moth *Manduca sexta* the projections of larval mechanosensory (MS) neurons form a somatotopic map within the CNS. The rules governing the formation of this map are believed to be both segment and tissue specific. Thoracic and abdominal MS neurons show distinct projection patterns. In addition, MS neurons located on the thoracic legs arborize in a specialized region of the ganglia known as the leg neuropile. The moth homeotic mutatant *Octopod* was used to test these rules. This mutation results in the appearance of ectopic thoracic legs on the first abdominal segment (A1). Cobalt backfills were used to examine the projections MS neurons from the affected segment into the CNS. In mutant animals, only MS neurons on the ventral surface of A1 were transformed. Based on their projection patterns, the segmental identity of 80% and 20% of MS neurons in the anterior and posterior half of the ventral surface of A1, respectively were transformed; their projection pattern was thoracic like. The segmental identity of the remaining ventral A1 MS neurons appeared to be abdominal. In addition, all of the MS neurons located on the ectopic leg appeared to be determined as leg tissue, independent of their segmental identity. All ectopic leg MS neurons had anamolous branching in the lateral margin of A1 neuropile. Those MS neurons on the anterior half of the ectopic leg whose segmental identity was wild-type (i.e. abdominal), sent a branch anteriorly which arborized along the midline of the 3rd thoracic ganglion. In addition to their normal midline projection these abdominal like anterior MS neurons on the ectopic legs of A1 had an anamolous branch which aborized in the region of the T3 leg neuropile. Thus, while their segmental identity was abdominal they remained leg MS neurons. These results suggest that the determination of *Manduca's* MS neurons occurs in at least two independent steps involving both positional and tissue specific information.

263.3

SELECTIVE AGGREGATION OF DISSOCIATED AFFERENT GRASSHOPPER NEURONS. Maureen L. Condic. Neurobiology Group, University of California, Berkeley, CA 94720.

The growth cones of embryonic grasshopper neurons orient towards and selectively contact the somata of other neurons as they migrate *in vivo*. Recent work has shown that this selective affinity is due, at least in part, to adhesive interactions between neurons. We have previously reported that many of the neuronal interactions observed *in vivo* are replicated by dissociated neurons *in vitro*. In particular, dissociated neurons adhere to other neuronal cells when rotated in suspension culture. Neuronal and non-neuronal aggregation were further quantified by determining the time course and selectivity of cell aggregation. Limbs and antennae were dissected from embryos at 40-43% of embryonic development, incubated in 0.4% elastase for 1.5 hrs, rinsed in calcium-magnesium free saline for 1.5 hrs, then triturated until a single cell suspension of 1-5 x 10^6 cells/ml (in 100-200 ul) was obtained. Neurons were labeled during the cell dissociation by including 0.8 mg/ml rabbit anti-horseradish peroxidase (HRP) serum antibodies during the elastase digestion and 0.08 mg/ml FITC-conjugated goat anti-rabbit IgG during the Ca-Mg free rinse. Aliquots were taken at 20 minute intervals, and the number of single cells remaining in suspension was determined by cell counts. The number of single cells rapidly declined to approximately 20% of the initial value within the first 80 minutes of rotation. The rate of this decline during the first 20 minutes was higher when calcium was included in the aggregation media. Aggregates were optically sectioned using confocal microscopy to determine the distribution of neuronal cells. After 80 minutes of rotation, 22% of the neurons were found in large, purely neuronal aggregates of 15-75 cells. This selective aggregation represents over a ten fold purification relative to the initial density of neurons in the single cell suspension. These results indicate that afferent embryonic neurons strongly and selectively adhere to other neurons. This adhesion is relatively resistant to the dissociation conditions, including elastase digestion, low calcium concentration and the presence of high concentrations of antibodies directed against neuronal cell surface components.

263.4

DEVELOPMENTAL EXPRESSION OF A GLIAL ANTIGEN IN GRASSHOPPER EMBRYOS E. M. Carpenter and M. J. Bastiani, Dept. of Biology, University of Utah, Salt Lake City, UT 84112

During development, glial cells may provide pathway cues or favorable substrates for axonal growth. Identification and characterization of glial cells associated with developing axonal pathways may reveal information dictating how initial nerve pathways are established. We have examined an antigen, recognized by the 7F7 monoclonal antibody, which is expressed on subsets of glial cells during embryonic development in the grasshopper embryo. Antigen expression in the CNS begins at 27% of development. Labeling appears in three regions, one associated with the developing anterior commissure and two associated with the longitudinal connectives. Observations on wholemount embryos using Nomarski optics show that the antigen is expressed on cell processes. Labeling is also apparent on live wholemount embryos, indicating that epitope is expressed on a surface molecule. As embryonic development proceeds, antigen expression diminishes and finally disappears from the CNS by 55% of development. However, the antigen is present in sections through adult ganglia suggesting that a second wave of expression must occur at a later stage of development. In adult ganglia, the 7F7 antibody labels small cell bodies in a ring around the neuropil, corresponding to the neuropilar glia of Hoyle (JCN 246: 85-103, 1986). These observations suggest that the 7F7 antibody recognizes an antigen present on a subset of early glial cells which are positioned appropriately to play a role in the formation of nerve pathways. Immunoprecipitation using the 7F7 antibody reveals that the antibody recognizes a single molecule having a molecular weight of 60kD. Supported by the NIH and the McKnight Foundation.

263.5

SEGREGATION OF PRESYNAPTIC INPUTS ON AN IDENTIFIED TARGET NEURON IN VITRO VISUALIZED OVER TIME. M. J. Bank and S. Schacher. Ctr. for Neurobiol. & Behav., Columbia CPS & NYS Psych. Inst., New York, NY 10032.

Sensory cells of Aplysia form synaptic connections with the motor cell L7 in culture, and appear to segregate their processes to different areas on the target (Glanzman et al., Soc. Neurosci. Abstr. 14:840, 1988). To investigate how this mature pattern is generated, two sensory cells were cocultured with L7 to maximize the neuritic interactions between each other and L7. The pattern of growth of each sensory cell was visualized by video fluorescense microscopy at two times (on day 2 and day 4 or 5 after plating) following injection of each cell with a different fluorescent dye (n=8 cultures). The strength of the synapses increased from 12.0 ± 1.8 mV on day 2 to 17.5 ± 2.8 mV on day 4-5. Segregation of the sensory cell processes on L7 in mature cultures is established in two ways. In some cases (n=3), areas of neurite overlap observed on day 2 appear to be occupied by only one sensory cell on day 4-5. In other cases (n=2), sensory cells neurites are segregated on day 2, and this segregation persists on day 4-5. In contrast, segregation of neurites is absent even in mature cultures if the sensory cell bodies are in direct contact with one another (n=3); conditions which facilitate the formation of electrical coupling between the sensory cells. The importance of interactions between the presynaptic cells and their target cell L7 in establishing this segregated patttern of neuritic outgrowth is currently being investigated.

263.6

NICOTINE INDUCES RETRACTION OF NEURITES IN CULTURED RAT RETINAL GANGLION CELLS. T.P.O. Cheng* and Stuart A. Lipton (SPON: A. Ganser). Dept. of Neurology, Children's Hospital & Harvard Medical School, Boston, MA 02115.

Previous evidence indicated that nicotinic antagonists enhanced neurite outgrowth by rat retinal ganglion cells (Lipton et al., *Science* 239:1293, 1988). We have now used real-time, computer-enhanced video microscopy to show that application of nicotine to identified retinal ganglion cells induces neurite retraction. Rat retinal ganglion cells were labeled, dissociated, and plated in MEM containing 10% fetal calf serum and 5% rat serum at 37° C, or frozen in DMSO for later use. To protect cultures against photodamage during video imaging, mineral oil and antioxidants were used. Identified retinal ganglion cells were microperfused with different concentrations of nicotine (10-100 μM) in the presence or absence of antagonists. Neurites retracted consistently with 40 μM nicotine in solutions containing 1.25 and 2.5 mM Ca, but they did not retract in 0.2 mM Ca, suggesting that Ca is involved in their retraction. Also, application of solutions containing 5.8 or 50 mM K did not produce retraction, indicating that membrane depolarization is not the cause of neurite retraction. Typically, retraction began 30 sec after nicotine exposure, was complete within 2-5 min, and could be blocked by 10 μM *d*-tubocurarine, mecamylamine or hexamethonium. Most (80%) of the retinal ganglion cells reacted in this fashion to nicotine. In addition, the degree of labeling of the growing neurite tips with rhodamine-phalloidin, an indicator of assembled F actin, was greatly diminished in the presence of ≥50 μM nicotine. Collectively, these findings suggest that binding to nicotinic cholinergic receptors leads to an influx of Ca and disassembly of actin fibers, resulting in neurite retraction.

263.7

GROWTH OF RAT SYMPATHETIC NERVE FIBERS LOCALLY DEPRIVED OF CALCIUM IN VITRO. R. B. Campenot. Dept. of Anatomy and Cell Biology, Faculty of Medicine, Univ. of Alberta, Edmonton, Alta., Canada T6G 2H7.

Experiments were conducted employing three-compartmented cultures of newborn rat sympathetic neurons to determine the effects of extracellular calcium deprivation on neurite growth. Nerve growth factor (NGF) was supplied in left, center, and right compartments, and neurons plated in center compartments extended neurites on a collagen substratum, across silicone grease barriers, into left and right side compartments.

When the center compartments were calcium-deprived (0 added calcium, 0.5 mM EGTA), neurite elongation ceased in left and right side compartments after 3 days, and the neurites subsequently degenerated. In cultures in which center compartments and left side compartments were supplied with normal calcium (1 mM), but right side compartments were given no added calcium and 0.5-5 mM EGTA, neurites readily grew into and elongated 4 mm within the right side compartments. The calcium-deprived neurites were very dense although they elongated at about 70% of the rate of neurites in calcium-supplied, left compartments.

Thus, the entry of extracellular calcium into the neuron locally at or near the growth cone does not appear to be required for sustained neurite growth in this experimental system. Neither does calcium-dependent adhesion to the collagen substratum appear to be required. Since previous work (Campenot, R. B., *Proc. Natl. Acad. Sci. USA*, 74:4516, 1977) has shown that NGF must be supplied in side compartments for rat sympathetic neurites to grow into them, the growth of neurites into NGF-containing, calcium-deprived compartments shows that the local requirement of NGF for neurite growth is not mediated by the entry of extracellular calcium.

263.8

NON-UNIFORM DISTRIBUTION OF FREE CALCIUM WITHIN A NEURONAL GROWTH CONE. V. Rehder*, P.B. Guthrie, P. Dou, S.B. Kater. Program in Neuronal Growth and Development and Dept. Anatomy & Neurobiology, Colorado State University, Ft. Collins, CO 80523

We have recently used fura-2 to demonstrate that cell bodies of different neurons of the snail *Helisoma* and of the rat hippocampus have distinct intracellular free calcium concentrations ($[Ca]_i$) (Guthrie et al. 1988, Soc. Neurosci. Abstr. 14:582; Kater et al. 1988, Soc. Neurosci. Abstr. 14:582). Upon perturbation by a variety of stimuli, these cells regulate their calcium concentrations back to their previous values; i.e. they display a high degree of calcium homeostasis.

We demonstrate here that there is a dramatic heterogeneity in the calcium distribution in the motile growth cones of *Helisoma*. The $[Ca]_i$ in the lamellipodial veil appears quite low. (This region is quite thin and therefore potentially subject to error). The $[Ca]_i$ in the leading edge of the organelle containing region is markedly higher and sharply decreases towards the growth cone/neurite junction.

Exposing growth cones to cobalt or low extracellular calcium concentrations leads to a transient decrease in $[Ca]_i$ but the original calcium distribution is rapidly restored, demonstrating calcium homeostasis of the neuronal growth cone as well.

We have begun studying the formation of the gradient during the generation of a new growth cone. New growth cones are generated by experimentally severing neurites. An immediate influx into the neurite's cut end, leading to a homogeneous local calcium rise, is detected. As the growth cone develops, the calcium concentration becomes non-uniform, establishing the characteristic gradient. This new calcium map is essentially indistinguishable from the maps displayed by control growth cones.

Our work is now directed towards understanding the generation and functional role of this non-uniform calcium distribution in growth cones.

263.9

SUBCELLULAR DISTRIBUTION OF MAP1.2 AND ß-TUBULIN DURING NGF-TRIGGERED NEURITE OUTGROWTH. J.M. Aletta, D.J. Asai* and L.A. Greene Columbia U. NY, NY 10032; Purdue U. W.Lafayette, IN 47907.

The phosphorylation of several cytoskeletal proteins in PC12 cells is enhanced concomitantly with NGF-triggered neurite outgrowth. Among these are the microtubule-associated protein1.2 (MAP1.2) and ß-tubulin (ßT). The functions of these molecules are not understood, but their distributions in different subcellular compartments impose certain constraints on their possible actions. We have therefore studied their localizations in PC12 cells, by western blotting and 32P-metabolic labeling, before and after NGF-exposure. Separation of neurites from cell bodies (Meth.Enzymol. 147 207) enabled us to measure the enrichment in neurites of the phospho-forms of these proteins relative to total radiolabeled phosphoprotein; MAP1.2=5-fold (n=3), ßT=2.1-fold (n=4). >90% of 32P-labeled MAP1.2 is removed from non-treated or NGF-treated cells with 0.1% Triton in a microtubule-stabilizing buffer. Although similar results were obtained from isolated cell bodies, only ~33% of 32P-labeled MAP1.2 was removed by the same treatment of neurites. In contrast, phospho-ßT is detectable only in long-term NGF-treated cells and is most abundant in an extract containing Ca-, cold-sensitive microtubules. Immunoblotting with a polyclonal MAP1.2 Ab or a mAb to ßT indicates that the proteins partition differently than their radiolabeled forms. Based upon blotting with mAb 7-1.1, which recognizes a phosphoepitope of MAP1.2, ~30% of the total antigen in whole cells is Triton-extractable. Further experiments should explain whether these data reflect different rates of phosphate turnover or protein exchange between compartments. We conclude that phospho-MAP1.2 exists in a dynamic equilibrium among different subcellular loci and that the associations of phospho-MAP1.2 and -ßT with the cytoskeleton are relatively enhanced in neurites.

263.10

GROWTH FACTOR-REGULATED CHANGES IN METALLOPROTEASE RELEASE FROM PC12 CELLS. J.C.Noszek* R.B. Nelson, and R. Siman. (SPON: B. Wolfson). Neuroscience Group, The DuPont Company, Wilmington, DE. 19880-0400.

Protease release by neurons has been hypothesized to serve as a mechanism for tissue penetration by developing neurites, and as a means by which neurites alter the extracellular matrix to allow for growth. Using substrate-containing polyacrylamide gels, we studied how the release of proteases by PC12 cells was altered by addition of growth factors already having defined effects on neurite outgrowth. PC12 cells released three metalloproteases, designated MP-92, MP-70, and MP-50, the former two of which we have previously described in rat brain. Dibutyryl cAMP or bFGF, which produce transient formation of short neurites, each caused a selective increase in MP-70 activity. Conversely, NGF, an inducer of stable neurite outgrowth, selectively reduced activities of MP-92 and MP-70. Combining NGF with dibutyryl cAMP or bFGF, which causes synergistic induction of neurite outgrowth, also decreased MP-92 and MP-70 and caused a massive increase in MP-50. The decreases in MP-92 and MP-70 only occurred after several days in growth factor, whereas the increase in MP-50 was essentially immediate. NGF, which includes a putative serine protease as part of its structure, did not directly alter the metalloprotease profile. Phorbol esters and EGF elicited no neurite outgrowth and had no effect on metalloprotease release. These results suggest that specific shifts in extracellular metalloprotease activities may be an important factor underlying the extent and stability of neurite outgrowth in PC12 cells.

263.11

THY-1 DIMERIZATION, THY-1-DERIVED PEPTIDES, AND NEURITE OUTGROWTH. N.K. Mahanthappa and P.H. Patterson, Div. of Biol., 216-76, Caltech, Pasadena, CA 91125.

We previously demonstrated that anti-Thy-1 antibodies can enhance neurite outgrowth from rat sympathetic neurons, adrenal chromaffin cells, and PC12 cells, and that Thy-1-deficient mutant PC12s spontaneously sprout neurites. Thus we have suggested that Thy-1 can stabilize neuronal membranes and inhibit sprouting, and that this inhibition is reduced by mutation or antibody binding. Affinity purification of Thy-1 has now revealed a 50 kD protein that co-purifies with the 25 kD Thy-1 protein. By 2-D peptide mapping it appears that the larger protein is a Thy-1 dimer, yet it is resitant to boiling in SDS and disulfide reduction. Superimposition of the Thy-1 sequence onto the IgV tertiary structure (a presumed homolog) enabled us to select candidate sequences that could be involved in homophillic binding and dimerization. Synthetic peptides corresponding to these sequences are being tested for effects on neurite outgrowth *in vitro*, and at least one of these peptides promotes outgrowth from sympathetic neurons. This peptide could disinhibit sprouting by perturbing either homophillic Thy-1 binding and dimerization, or the binding of Thy-1 to other surface or matrix ligands.

263.12

CHARACTERIZATION OF AN ENDOGENOUS RECEPTOR FOR THY-1 AND ITS ROLE IN NEURITE OUTGROWTH. E. B. Dreyer, N. K. Mahanthappa, P. H. Patterson, R.L. Neve, C. J. Barnstable, L. A. Levin,* & Stuart A. Lipton. Dept.of Neuro., Harvard Med. Sch., Boston, MA; Yale Med. Sch., New Haven, CT; Cal. Inst. Tech, Pasadena, CA.

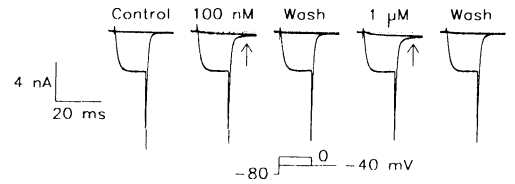
Specific Thy-1 monoclonal antibodies have been shown to promote the outgrowth of postnatal rodent retinal ganglion cells in culture [Leifer et al., *Science* 224:303, 1984; Lipton et al., *Soc. Neurosci. Abstr.*, 1988]. Using two anti-idiotypic antibodies as probes, we have therefore sought to identify a natural analog for this antibody effect -- a Thy-1 receptor. A Thy-1 receptor has now been tentatively identified on rodent astrocytes. Competitive binding to astrocytes between purified Thy-1 and the anti-idiotypes has been demonstrated. In Western blots and isoelectric focusing gels of whole brain and astrocyte preparations, a Thy-1 receptor binding site has been identified. By employing these anti-idiotypes as probes of a brain cDNA library in λ-gt11, tentative identification has been made of a clone for this receptor. The anti-idiotypes have been shown to affect the outgrowth of retinal ganglion cell neurites over astrocyte monolayers. These data strongly suggest that a receptor for the neuronal glycoprotein Thy-1 exists on the surface of astrocytes and that this receptor plays a role in modulating neurite outgrowth.

CALCIUM CHANNELS III

264.1

BAY K 8644 IS HIGHLY POTENT, BUT HAS SMALL MAXIMAL EFFECTS, ON CALCIUM CURRENTS OF ISOLATED FROG SYMPATHETIC NEURONS. Stephen W. Jones and Leila S. Jacobs*. Dept. Physiology & Biophysics, Case Western Reserve University, Cleveland, OH 44106.

Dihydropyridine calcium channel agonists and antagonists are relatively inactive against calcium currents of neurons, and most studies have used high (1 - 10 μM) concentrations in order to obtain clear effects. We also find that the agonist Bay K 8644 has little effect on the peak calcium current, in contrast to its actions on muscle. However, currents at negative membrane potentials can be greatly increased, and tail currents measured at -40 mV 5-6 ms after repolarization from -10 or 0 mV were enhanced several fold by Bay K 8644 with half maximal effect at ~50 nM. This suggests that Bay K 8644 acts on neuronal L currents as it does on calcium currents of muscle, but that the major calcium current of these cells is a dihydropyridine-resistant N current.



264.2

Ca^{++}DEPENDANT INACTIVATION OF N-TYPE Ca^{++}CURRENTS IN THE MELANOTROPH **.P.J.Williams, B.A.MacVicar, & Q.J.Pittman. Neuroscience Research Group, U of Calgary , Calgary , Canada.** Ca^{++} currents were analyzed by S.E.V.C in melanotrophs in intact pituitary maintained in vitro. Following pharmacological blockade of Na^{+} & K^{+} currents 3 protocols were tested . 1) with cells held at -90mV a small inactivating current was evoked by 200msec pulses to test potentials above -40mV. A larger, more slowly inactivating current was activated above -10mV and was maximal around 0mV. When the cell was held at -30mV a pulse to 0mV elicited 2 seconds after the shift to -30mV contained an initial transient component followed by a more sustained current. The transient current in subsequent test pulses declined until , after 12 sec, only a sustained , non-inactivating current remained. This suggests that an inactivating portion of the current was abolished when the cell was held at -30mV. 2) In a second protocol test pulses from -90mV to 0mV were elicited 150msec after a pre-pulse of 200msec to potentials between -70mV and +70mV. The peak current was reduced significantly following a pre-pulse to +10mV and the reduced current was non-inactivating. The maximal inhibition of Ca^{++} currents (70%) occurred at a pre-pulse potential between +10 and +30mV. As the pre-pulse potential was increased above +30mV the inhibition of peak current was reduced until, with a pre-pulse to +70mV , only a 20% reduction was seen. The prepulse potentials that maximally inhibited peak current are in the same range as those which evoke maximal Ca^{++} current which suggests that Ca^{++} entry rather than voltage alone may be responsible for the inactivation of peak Ca^{++} current. 3) The magnitude of the transient current evoked by a pulse from -90mV to 0mV was the same in 2mM Ca^{++} (47.5±17 pA , mean±S.D. ,n=3) and in 2mM Ca^{++} plus 2mM Ba^{++} (52.5±13 pA , n=4) but was greatly increased in 4mM Ca^{++} (125±22 pA , n=4). Although transient current did not increase on addition of Ba^{++} sustained current was increased from 116±15 pA in 2mm Ca^{++} to 160±20pA in 2mM Ca^{++} plus 2mM Ba^{++}. The size of the transient current is therefore dependant on the concentration of Ca^{++} in the medium and Ba^{++} does not substitute for Ca^{++} in this regard. We conclude this N-type current is Ca^{++} rather than voltage inactivated.

264.3

CALCIUM-DEPENDENT INACTIVATION OF A PERSISTENT CALCIUM CURRENT OF RAT HIPPOCAMPAL NEURONES. A. Nistri[1] and E. Cherubini. INSERM U29, 123 Bd Port-Royal, 75014 Paris, France and [1]Pharmacology Dept., St. Bartholomew's Hospital Med. Coll., London EC1M 6BQ., England.

Hippocampal neurones possess a persistent Ca^{2+} current presumably due to voltage-dependent opening of L-type Ca^{2+} channels. The present experiments were initiated to examine the mechanisms responsible for the inactivation of this current and used single electrode voltage clamp of CA1 neurones of the rat hippocampal slice at room temperature. K^{+} currents were depressed by Cs^{+} and TEA while fast Na^{+} currents were blocked by TTX. At -40 mV holding potential 1 s long depolarizing pulses elicited an inward Ca^{2+} current (threshold about -25 mV) comprising an early transient and a sustained component which usually reversed between 0 and +30 mV. Depolarizing pulses applied at frequencies higher than 0.03-0.05 Hz attenuated the slow current. Double pulse inactivation protocols showed 55% (maximal) inactivation of the persistent current at +10 mV with half-maximal reduction at -10 mV. While in control conditions no inward tails were found, such tails were seen with intracellular BAPTA or extracellular Ca^{2+} replacement by Ba^{2+} which also increased the slow current size. With intracellular BAPTA the inactivation of inward tail currents was only 30% at +10 mV. Intracellular free Ca^{2+} seems thus to contribute to the macroscopic process of inactivation of a persistent Ca^{2+} current.

264.4

Facilitation of Calcium Channel Current in Bovine Chromaffin Cells is Due to The Recruitment of a Different Class of Calcium Channels. A.P. Fox*, M. Dahmer, R.L. Perlman, C.R. Artalejo* (SPON: P. Hoffman), Dept. of Pharmacol. and Physiol. Sci., Univ. of Chicago, 947 E. 58th St. Chicago, IL 60637

Facilitation of calcium currents by a prior depolarization pulse has been previously described in chromaffin cells (Fenwick et al., J. Physiol. 331,1982; Hoshi et al., Proc. Natl. Acad. Sci. 81,1984). Our data suggest that the facilitation seen in chromaffin cells represents the recruitment of a new class of Ca channels rather than a novel property of a single type of Ca channel. Whole-cell patch clamp recordings showed that facilitation currents were sensitive to dihydropyridine antagonists while control currents were not. Inactivation of the control Ca^{2+} currents showed no voltage-dependent inactivation, while the facilitation Ca^{2+} currents did. Tail currents measured at -30 mV were different before and after the induction of facilitation. Recordings in 90 mM extracellular Ba^{2+} showed that control and facilitation Ca^{2+} currents could activate at different potentials; the threshold of activation for facilitation was approximately 20 mV more depolarized than control. Single-channel recordings showed three different types of unitary events 14 pS, 18 pS and 27 pS. We believe that the large-conductance 27 pS channel mediates facilitation. As the Ca^{2+} influx through voltage-dependent calcium channels regulates the secretory response in these cells, it will be very interesting to uncover the various roles that the different Ca^{2+} channels play in the physiology of release.

264.5

Is Facilitation of Calcium Channel Current in Bovine Chromaffin Cells Due to Long-lived Openings of a 27 pS Ca^{2+} Channel? C.R. Artalejo*, M.K. Dahmer, R.L. Perlman, A.P. Fox* (SPON: A. Heller), Dept. of Pharmacol. and Physiol. Sci., Univ. of Chicago, Chicago, IL. 60637

Experiments were performed to explore the process of Ca^{2+} channel facilitation at the single-channel level (Hoshi & Smith, J. Neurosci. 7, 571, (1987)). Three different types of unitary currents (14, 18 and 27 pS) were found to exist. Neither the 14 pS channel nor the 18 pS channel were sensitive to dihydropyridines (DHP). The 27 pS channel, in some respects similar to L-type Ca^{2+} channels previously described, was sensitive to both DHP agonists and antagonists, consistent with our whole-cell facilitation results. Under control conditions, the 27 pS channels were relatively silent. The addition of the DHP agonist BAY K 8644 increased the activity dramatically by promoting very long-lived openings (mode 2), usually uncovering the presence of multiple channels under the pipette. Under drug free conditions, long-lasting openings, similar to those seen in the presence of Bay K 8644, could be elicited by protocols shown to induce facilitation (rapid trains of depolarizations or by changing the cells to a depolarized HP). Fig. 1 shows single-channel currents recorded while holding the patch potential at -10 mV; the first trace was recorded at time=0 sec, the second trace was obtained at time=25 sec, while the last trace was acquired at time=75 sec. At first there was little activity of the 27 pS channel; 15 seconds after changing to HP=-10 mV, long-lasting openings were observed. After another 50 seconds, the activity subsided. This behavior correlated very well with whole-cell facilitation. Alternate long-lived open states of Ca^{2+} channels may play an important role in governing Ca^{2+}-influx into cells.

TIME = 0 SEC. TIME = 25 SEC. TIME = 75 SEC.



264.6

ω-AGATOXINS: A FAMILY OF NEURONAL CA^{++} CHANNEL ANTAGONISTS FROM FUNNEL WEB SPIDER (*AGELENOPSIS APERTA*) VENOM. M. E. Adams[1], V. P. Bindokas*[1], A.C. Dolphin*[3], J. S. Imperial*[2], B.M. Olivera[2], R.H. Scott*[3], and V. J. Venema*[1]. [1]Div. Toxicol. & Physiol., Dept. of Entomology, Univ. of California, Riverside, CA 92521, [2]Dept. Biology, Univ. of Utah, Salt Lake City, UT 84112 and [3]Dept. of Pharmacology, St. Georges Hospital Medical School, London, SW17 0RE, UK.

Five polypeptide antagonists (ω-agatoxins) of insect neuromuscular transmission were purified from *Agelenopsis aperta* spider venom. Physiological and biochemical data indicate that the ω-agatoxins comprise a family of presynaptic voltage-sensitive Ca^{++} channel antagonists. Although all ω-agatoxins block insect neurotransmission, biochemical criteria distinguish them as two subclasses. Type I toxins (ω-Aga-IA, IB IC) are 7-8 kDa, have closely related N-terminal amino acid sequences, and exhibit high tryptophan-like UV absorbance. Type II toxins (ω-Aga-IIA and IIB) are 10-11 kDa, show minor sequence homology to Type I toxins, and exhibit low tryptophan-like absorbance. Type II, but not type I toxins inhibit specific binding of ^{125}I-labeled ω-conotoxin GVIA to chick synaptosomal membranes. In accordance with this binding data, both Type II toxins and ω-conotoxin block ^{45}Ca flux in chick synaptosomes, while Type I toxins are either inactive (IA, IB) or less potent (IC) under identical conditions (see Pocock, J.M. *et al*, this meeting).

Type I and II ω-agatoxins may define distinct Ca^{++} channel subtypes in both insects and vertebrates. ω-Aga-IA (10 nM) blocks high threshold Ba^{++} and Ca^{++} currents in rat dorsal root ganglion neurons. It also blocks transmission in insect and frog skeletal muscle (Bindokas and Adams, *J. Neurobiol.* in press). Our binding data suggest that these actions involve a unique binding site unrelated to that defined by ω-conotoxin. However, Type II toxin inhibition of ω-conotoxin binding suggests that they may block Ca^{++} channels in a similar manner. Thus, the ω-agatoxins provide two novel sub-classes of polypeptide calcium channel antagonists active in diverse animal phyla.

Supported by NIH grant NS24472 and USDA grant 86-CRCR-1-2097.

264.7

EFFECTS OF ω-AGATOXINS ON VOLTAGE-DEPENDENT CA^{++} FLUX IN CHICK BRAIN SYNAPTOSOMES. J. M. Pocock*, V. J. Venema* and M. E. Adams (SPON: R. Farley) Division of Toxicology and Physiology, Department of Entomology, University of California, Riverside, CA 92521.

ω-agatoxins purified from *Agelenopsis aperta* spider venom are presynaptic calcium channel antagonists which block insect neuromuscular transmission (Adams et al.; Bindokas and Adams, this meeting). These toxins also appear to interact with vertebrate calcium channels. For example, Type II (ω-Aga-IIA, IIB), but not Type I (ω-Aga-IA, IB) ω-agatoxins inhibit the specific binding of ^{125}I-labeled ω-conotoxin GVIA, a known neuronal calcium channel antagonist, to chick brain synaptosomal membranes. We compared the effects of ω-agatoxins with those of ω-CgTx on voltage-activated Ca^{++} flux in intact synaptosomes.

Synaptosomes prepared from whole brains of 2-day old chicks were pre-incubated for 5-25 minutes in toxin followed by a 5 sec depolarization in 100 mM K^+ saline. Type II ω-agatoxins and ω-conotoxin directly inhibit voltage-dependent Ca^{++} influx into synaptosomes at 10-100 nM concentrations within 15 minutes. ω-Aga-IA and IB do not block Ca^{++} influx at concentrations up to 500 nM. ω-Aga-IC blocks Ca^{++} influx, but is less potent than the Type II toxins.

We conclude that the Type II ω-agatoxins constitute a subclass of calcium channel antagonists interacting with the ω-conotoxin binding site to block Ca^{++} flux through neuronal calcium channels.

Supported by NIH grant NS4472 and USDA grant 86-CRCR-1-2097.

264.8

FURTHER STUDIES ON THE P TYPE CALCIUM CHANNEL IN LIPID BILAYERS AND ON THE NATURE OF FTX CALCIUM CHANNEL BLOCKER. B. Cherksey,* J.W. Lin, M. Sugimori and R. Llinás (SPON: D. Hillman) Dept. Physiol. & Biophysics., New York Univ. Med. Ctr., New York, NY 10016.

Funnel web spider venom contains a potent and selective neuronal calcium channel blocker, FTX, which was purified from the crude venom of A. aperta by deproteination followed by size-exclusion and ion-exchange chromatography. The final product exhibited a single, sharp absorption band at 220 nm. The purified FTX was subjected to structural analysis using NMR and FT-IR spectroscopy and mass spectrometry. The results obtained show that this toxin is not a peptide nor does is resemble the known polyamine channel blockers but rather possesses a unique and unexpected aliphatic structure. A purified FTX-Sepharose affinity gel was used to isolate protein from guinea pig cerebellar homogenate which was then studied functionally in the lipid bilayer (Cherksey, et al. Neurosci Abstract, 1988). The reconstituted protein exhibited voltage-dependent single channel activity with the divalent ion selectivity Br>Sr>Ca. In 80 mM solutions, the conductance for Ba++ was 10-12 pS, for Sr++ 8-10 pS, and for Ca++ 6-8 pS. The conductance was pH sensitive with no channel openings present at pH 6.8 and below. In the absence of divalent cations, a monovalent ion conductance could be determined. The permeabilities, determined from the reversal potentials, were: Na>K>Cs>TEA. The channel was not blocked by either dihydropyridines or conotoxin thus ruling out its identity as an L-type channel. Macroscopic currents derived from ensemble averages of channel activity during pulses indicated that the channel does not inactivate as would be expected for either a T- or N-type channels. However, the voltage dependence, lack of inactivation and specific block by FTX suggest its identification as a P-type channel (Llinas, et. al., 1989, PNAS, 68: 1689).NINCDS13742, NEI08002 and FIDIA Research Foundation.

264.9

A FUNNEL-WEB SPIDER TOXIN (FTX) FRACTION BLOCKS CALCIUM CURRENTS INDUCED BY RAT BRAIN mRNA IN XENOPUS OOCYTES. J.W. Lin, B. Rudy, B. Cherksey, M. Sugimori and R. Llinás. Dept. Physiol. & Biophys., New York Univ. Med. Ctr., NY 10016.

A fraction of funnel-web spider toxin has been shown to block Ca conductances in Purkinje cells and the squid giant synapse (Llinás et al., 1989, PNAS, 89: 1689). To further characterize this toxin, we examine its effect on the Ca currents (I_{Ca}) expressed by rat brain mRNA in Xenopus oocytes. Similar to the P channel reported for Purkinje cells and the squid giant synapse, this I_{Ca} differs from conventional Ca channel types in that it is not blocked by organic Ca channel blockers (dihydropyridine) or conotoxin (Leonard et al., 1987, J. Neurosci, 7:875). The fact that this current is the only Ca channel type expressed by the whole brain mRNA suggests its abundance in CNS. After mRNA injection, a Ca activated Cl current ($I_{Cl(Ca)}$) can be activated upon depolarization. The tail of this Cl current has a threshold of -40mV and peaks between 0 to +10mV when $[Ca]_o$ is 1.8 mM. FTX blocks this current irreversibly while the amplitudes of Na and K currents are unmodified. The blockade affects Ca channel directly since $I_{Cl(Ca)}$ can still be activated by intracellular Ca injection in the presence of FTX. Furthermore, a small intrinsic $I_{Cl(Ca)}$ of uninjected oocytes is not affected by the toxin. In 40 mM Ba, 1 mM TTX and Cl free solution, FTX eliminates an inward current, presumably carried by Ba. This FTX sensitive I_{Ba} has similar properties as that reported by Leonard et al. (1987), namely it does not inactivate over a duration of 400 msec, has a threshold of -20mV and reaches the maximal amplitude at +20mV. The diffference in the voltage sensitivities between I_{Ba} and the $I_{Cl(Ca)}$ is probably due to the increased extracellular divalent cation concentration, since a similar shift to the right is observed for $I_{Cl(Ca)}$ when $[Ca]_o$ is raised to 15 mM. In addition, Cd blocks the same I_{Ba} as does FTX.

Support: NS13742, GM26976, NE108002 and FIDIA Research Foundation.

264.10

A FAMILY OF PUTATIVE VOLTAGE-DEPENDENT CALCIUM CHANNELS FROM RAT BRAIN. T.P. Snutch[1], J.P. Leonard[2], M.M. Gilbert*[1], H.A. Lester[3] and N. Davidson[3]. [1]Biotechnology Laboratory, U. of British Columbia, Vancouver, Canada V6T 1W5, [2]Dept. of Biology, U. of Illinois at Chicago, Chicago IL 60680 and [3]Dept. of Biology, California Institute of Technology, Pasadena CA 91125.

Calcium entry through voltage-dependent calcium channels (VDCCs) contributes both to the membrane potential of neurons and also to increase the intracellular concentration of this important second-messenger. Electrophysiological and pharmacological analyses have identified a number of distinct types of VDCC in mammalian neurons. In an attempt to determine the molecular relationships between the various described VDCCs we are utilizing a molecular genetic approach to isolate rat brain cDNAs which may encode neuronal VDCCs.

Northern blot analysis of 26 isolated cDNAs shows that they can be grouped into four distinct classes. Class A cDNAs hybridize with approx. equal intensity to messages of 8.0 and 8.7 kilobases (kb). Class B cDNAs hybridize to a major message 9.5 kb in size. Class C cDNAs hybridize to two messages of approx. 8.8 and 12 kb, while Class D cDNAs hybridize mainly to a 9.3 kb mRNA. DNA sequencing shows that the four classes of cDNA are related to the brain Na channel and rabbit skeletal muscle dihydropyridine (DHP) receptor/Ca channel. Antisense experiments in Xenopus oocytes injected with rat brain mRNA indicate that these cDNAs are not voltage-dependent Na channels. The class C cDNAs show a very high degree of amino acid similarity to the muscle DHP receptor/Ca channel (>90%); this suggests that this group may represent the brain DHP receptor. (Supported by MRC of Canada and NIH grants GM-10991 and GM-29836).

264.11

LOW THRESHOLD, TRANSIENT CALCIUM CURRENT IS PRESENT IN YOUNG, BUT NOT ADULT, ISOLATED RAT HIPPOCAMPAL PYRAMIDAL CELLS. Scott M. Thompson and Robert K. S. Wong, Dept. of Neurology, Columbia University, NY, NY 10032

Several types of Ca++ currents may be distinguished on the basis of their threshold, rate of inactivation, and pharmacology. Ca++ currents in acutely isolated adult guinea-pig CA1 cells are predominantly high-threshold and sustained (Kay and Wong, 1987). We examined the development of Ca++ currents in pyramidal cells obtained from rats between 4 days and 5 weeks after birth. Ca++ currents were recorded with whole-cell voltage-clamp using 10 mM Ba++. Na+ and K+ currents were blocked with pharmacological antagonists and substitution of impermeant ions. In cells of all ages, depolarizing steps from -50 mV activated inward currents at a mean threshold of -33 mV. The current was non-inactivating near threshold, and slowly inactivating at more positive potentials. In cells > 4 weeks of age, the threshold was little affected by hyperpolarizing prepulses. In contrast, in all cells < 2 weeks of age, hyperpolarizing prepulses (3 sec, > -85 mV) revealed a component of Ca++ current with a lower threshold (mean = -58 mV) and rapid decay rate (time constant = 38 msec at -40 mV). The low threshold, transient current in young cells was selectively reduced by ethosuximide (350 uM) and amiloride (200 uM), as are Ca++ currents with similar kinetics in other cell types. No significant differences in Ca++ currents were observed between CA1 and CA3 cells at any age. These results suggest that low threshold, rapidly inactivating Ca++ channels are expressed in the soma and proximal dendrites of young pyramidal cells and are lost or redistributed to distal dendrites during development. Supported by NIH grants F32 NS08306 (SMT) and NS24682 (RKSW).

264.12

THREE-DIMENSIONAL RECONSTRUCTION OF VOLTAGE-DEPENDENT CALCIUM CHANNEL DISTRIBUTION IN HIPPOCAMPAL SLICES BY CONFOCAL MICROSCOPY. KJ. Angelides', B.D. Mensh*', and O.T. Jones*. (SPON: H. Epstein). 'Division of Neuroscience and Dept. of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, TX 77030.

Influx of calcium through voltage-dependent calcium channels (VDCCs) may be critical in the induction of long-term potentiation (LTP) at the mossy fiber synapse in the hippocampus (Gray, R. & Johnston, D. *Nature* 327:620, 1987). To examine the distribution of VDCCs on CA1/3 neurons in the hippocampus we have utilized biologically active fluorescent analogs of ω-conotoxin. Previous studies on cultured CA1 hippocampal neurons show that VDCCs are segregated in clusters at synapses and on dendrites. The goal of the present work was to extend our studies in primary culture to hippocampal slices using confocal microscopy in order to reveal the three-dimensional organization of VDCCs on CA1/3 neurons with the aim of exploring VDCC dynamics during LTP.

Adult rat hippocampal slices (300 μm) were incubated at 25°C in solutions of artificial CSF containing fluorescent ω-conotoxin. In some experiments the sections were incubated with DiI, a voltage-dependent dye, to outline cells. Images at low magnification showed staining in the pyramidal cell body layer. At high magnification, single neurons were selected to include cell bodies and basal and apical dendritic arbors, and a through-focus series was performed at ~1.0 μm sectional resolution. Non-uniform staining of cell bodies in CA1 and CA3 regions, as well as strings of clustered staining particularly visible in the region of apical dendrites was observed. Further optical sectioning on processes revealed hotspots which appeared as thin, short branches projecting from the thicker processes. While synapses have not been identified, these areas of punctate staining are candidate dendritic spines. Reconstruction of VDCC distribution in three-dimensions will be presented. Supported by NS24606, NS01218 and MSTP.

GENETIC MODELS OF NERVOUS DISORDERS III

265.1

THE deg-1 GENE OF *CAENORHABDITIS ELEGANS* MAY ENCODE A CELL MEMBRANE RECEPTOR E. J. Wolinsky and M. Chalfie, Dept. Biol. Sci., Columbia University, New York, NY 10027

The u38 mutation of the deg-1 gene induces late-onset vacuolar degeneration of the PVC interneurons. This phenotype is dominant and temperature sensitive. Our goal is to determine the normal function of the wild type deg-1 gene, and how the u38 mutant gene product causes selective neurodegeneration.

We have cloned both wild type and mutant deg-1 DNA. Our physical map of the gene is defined by four DNA rearrangements which inactivate the dominant u38 mutation (2 deletions, 2 transposon insertions), spanning about 25 Kb. Germ line transformation experiments using a fragment of u38 DNA have resulted in transformed lines expressing the degeneration phenotype.

We have sequenced wild type deg-1 cDNA clones to deduce a partial amino acid sequence of 293 residues. The most striking features of the predicted deg-1 protein are a 27 amino acid hydrophobic sequence, two possible glycosylation sites, and a cystine rich region. The possible glycosylation sites and cystine residues are probably located extracellularly. The cystine rich sequence can be aligned with the EGF-like repeats of thrombomodulin, a cell surface receptor of the same class as the EGF receptor. We hypothesize that deg-1 encodes an integral membrane protein which may function as a receptor.

265.2

STUDIES OF CNS LUPUS IN TRANSGENIC AUTOIMMUNE MRL-lpr MICE EXPRESSING A SINGLE T CELL RECEPTOR. J.D. Mountz*, J.M. Mountz. Univ of Alabama, Birmingham, Alabama 35294, and Univ of Michigan Medical Center, Ann Arbor, Michigan 48109.

Lupus cerebritis has been postulated to be due to antineuronal tissue autoreactive T cells. In support of this, autoreactive T cell clones from patients with lupus cerebritis have been isolated. The MRL-lpr/lpr mouse develops many symptoms of Systemic Lupus Erythematosus (SLE), including arthritis, glomerulonephritis, arteritis, Sjögren's Syndrome, and infiltrates the (CNS) with T cells. To further clarify the role of CNS cell infiltrates in the development of CNS lupus, a "non-lupus" C57 BL/6 transgenic mouse expressing a single T cell receptor (α-A heterodimer) against the male HY antigen in association with class Ib restrictive element was backcrossed to MRL-lpr/lpr C57 BL/6-lpr/lpr mice to produce transgenic mice homozygous for the lpr gene. These mice demonstrated a decrease in T cell mediated autoimmunity as opposed to the MRL-lpr/lpr strain.

CNS lupus cerebritis and cerebral blood flow (CBF) alterations in these mice strains were studied utilizing CBF radionuclide tracer and autoradiographic techniques. MRL-lpr/lpr mice were found to have an increase in CBF. In contradistinction, neither the MRL- +/+ or the MRL-lpr/lpr with the transgenic T cell receptor showed abnormal CBF. In addition, these mice showed decreased T cell activity in comparison to the autoimmune strain of mice. The presence of T cell dependent CNS lupus suggest anti-T cell antibodies and perhaps T cell immunization may be of therapeutic value. These results suggest improved methods of diagnosis and therapeutic monitoring of CNS lupus in humans may be possible using single photon emission computed tomography (SPECT) imaging of ^{99m}Tc-Hexamethylpropyleneamine oxime.

265.3

IN SITU LOCALIZATION OF ANTI-SENSE MYELIN BASIC PROTEIN TRANSCRIPTS IN MYELIN DEFICIENT MICE Robert T. Fremeau,Jr.[1] and Brian Popko*[2], 1. Glaxo Research Laboratories, Research Triangle Park, NC, 27709 and Department of Neurobiology, Duke University; 2. Biological Sciences Research Center CB#7250, U. of North Carolina, Chapel Hill, NC 27599

Mice homozygous for the myelin deficient (mld) mutation have an unusual phenotype in which the gene encoding the myelin basic protein (MBP) is expressed at low levels and on an abnormal developmental schedule. Mld mice are characterized by severe central nervous system (CNS) hypomyelination, hindquarter tremors, tonic seizures, and premature death. The mld mutation is the result of a DNA rearrangement within the myelin basic protein gene. The mld MBP locus consists of two tandem MBP genes with the upstream gene containing an inversion of its 3' region (Popko, B. et al. Neuron 1:221,1988). In this study we have used RNA probes from the inverted region of the upstream mld MBP gene to identify anti-sense MBP transcripts in the CNS of mld animals by in situ hybridization. These RNA molecules apparently initiate off the upstream mld MBP promoter and elongate through the inverted region of the upstream gene.

265.4

HUMAN DYSTONIA GENE FOUND ON CHROMOSOME 9q32-q34 X.O. Breakefield, L. Ozelius*, P. L. Kramer*, C.B. Moskowitz*, D.J. Kwiatkowski*, M.F. Brin*, S.B. Bressman*, D.E. Schuback*, D. DeLeon*, Y.-P.P. Hsu*, C. Craft, G. Hu*, J. Haines*, R.E. Burke, T. Nygaard*, J. Raese, J.F. Gusella*, S. Fahn* E.K. Shriver Ctr, Waltham, MA, 02254 Neurology & Hematology Depts., Mass. General Hosp., Neuroscience Program & Genetics Dept., Harvard Med. Sch. Boston, MA; Dept. Neurology, Columbia CPS, New York, NY; VA Med. Ctr, Dallas, TX.

Torsion dystonia is a movement disorder characterized by sustained muscle contractions and/or abnormal posture. The etiology of this disease is unknown, but symptoms are thought to arise from abnormalities in the basal ganglia. About one-third of cases are familial with most being inherited in an autosomal dominant manner with reduced penetrance. Genetic linkage analysis using DNA polymorphisms has been used to locate the gene for torsion dystonia to the q32-q34 region of chromosome 9 in a large, non-Jewish kindred with early onset. Multi-point linkage analysis using six DNA marker probes in this region gives a lod score of >+4 in this family. Two-point linkage analysis is being used to evaluate the role in this disease of two candidate genes encoding gelsolin and dopamine beta-hydroxylase, which lie in this region. Studies are also underway to evaluate whether other ethnically and/or clinically distinct forms of hereditary dystonia are caused by gene(s) in this same chromosomal region.

265.5

NGF EFFECTS ON PC12 CELLS TRANSFECTED WITH THE B-AMYLOID GENE R.E. Majocha, S.B. Zain, W.G. Chou, B. Tate-Ostroff, M. Ventosa-Michelman and C.A. Marotta Mass. Gen. Hosp., Harvard Med. Sch., Boston, MA; U. Rochester Sch. Med.; McLean Hosp., Belmont, MA.

PC12 cells were permanently transfected with DNA of the A4 to C terminal region of the amyloid precursor protein (APP; Marotta et al., PNAS 86, 33, 1989). Transfectants that received in frame DNA (IF) as well as out of frame DNA (OF) and untransfected cells (UF) were grown on polylysine-treated plates for two days at 10,000 cells/well. Morphological and immunological differences were apparent between UF and IF cells (Tate-Ostroff et al., these abstracts). Cells treated with NGF at 100ng/ml for 48 hrs exhibited increased neurites/cell and neurite length in both UF and OF cells but not IF cells. In addition, IF cells did not cease dividing. After two weeks in NGF at concentrations up to 200ng/ml, IF cells showed no apparent morphological response. Efforts are under way to determine if these effects are due solely to overaccumulation of the A4 region. Supported by AG02126, CA 11198, CA 36432, AHAF and the Sandoz Found.

265.6

MORPHOLOGICAL AND IMMUNOLOGICAL STUDIES OF PC12 CELLS TRANSFECTED WITH THE B-AMYLOID REGION OF THE AMYLOID PRECURSOR PROTEIN B. Tate-Ostroff, E.C. Walcott, P. Paskevich, S. Zain, W.G. Chou, C. Labonne, R.E. Majocha and C.A. Marotta McLean Hosp, Belmont, MA; Mass Gen Hosp, Harvard Med Sch, Boston, MA; U. Rochester Sch Med, Rochester, NY

PC12 cells were permanently transfected with cDNA of the A4 to C terminal region of the amyloid precursor protein (APP; Marotta et al., PNAS 86,33:1989). Cells transfected with in-frame (IF) and out-of-frame vectors were examined by light and electron microscopy (EM) and compared to untransfected cells. IF cells were morphologically different from controls. IF cells immunoprocessed with antibodies to B-amyloid (A4) and C terminal regions of the APP were darkly stained compared to controls. At the EM level, the A4 staining of IF cells was heaviest in the membrane. These studies indicate that over production of the B-amyloid to C terminal region of the APP can affect the morphology of cells. In addition, insertion of A4 into the membrane requires only the A4 to C terminal region of the APP. Supported by AG02126, AHAF, the Sandoz Found. CA 11198, CA 36432.

265.7

ULTRASTRUCTURAL PATHOLOGY OF MIDBRAIN DOPAMINE NEURONS IN THE WEAVER MUTANT MOUSE AND PROGRESSION OF NEURON LOSS WITH AGING. B. Ghetti and L.C. Triarhou. Dept. of Pathology & Med. Neurobiology Pgm, Indiana Univ. Sch. of Med., Indianapolis, IN 46202

Homozygous weaver mutant mice (wv/wv) on the B6CBA-A^{w-J}/A hybrid strain may live up to 24-30 months. In these mice, loss of midbrain dopamine (DA) neurons is part of the neuropathologic phenotype at P20 and P90. The specific aims of this study were (1) to analyze the fine structure of degenerating neurons in substantia nigra (SN) and ventral tegmental area (VTA) and (2) to investigate whether loss of DA neurons progresses with old age. Electron microscopic examination of 4 wv/wv and 4 +/+ mice at P20-P45 showed osmiophilic cytoplasm, disrupted organelles and a pyknotic nucleus in neurons of the SN and VTA of wv/wv; cell debris was surrounded by astrocytic processes. Immunocytochemical study of wv/wv at P355-P756 (n=7) and +/+ (n=4) at P538-P894 with tyrosine hydroxylase (TH) antibodies revealed the presence of some surviving TH cells in the SN of wv/wv at P355, most of which are lost by P756. Furthermore, we re-examined 3 wv/wv and 3 +/+ mice at P90 from previously studied material to compare cell losses at maturity and in old age. In rostral VTA, loss of TH neurons appears to be severer on P756 than on P90. A quantitative analysis of midbrain DA neurons will establish whether the progression of losses beyond 90 days of age in wv/wv is part of the wv phenotype or represents an age-related phenomenon superimposed on the wv gene-induced condition.

(Supported by USPHS-RO1-NS14426)

265.8

MONOAMINE UPTAKE KINETICS IN BRAIN REGIONS FROM GENETICALLY RELATED WISTAR-KYOTO RATS WITH SPONTANEOUS HYPERTENSION AND/OR HYPERACTIVITY. E.D. Hendley and W.G. Ohlsson*. Dept. Physiology & Biophysics, Univ. of Vermont, Burlington, VT 05405.

Four inbred strains derived from the Wistar-Kyoto (WKY) rat were used to study the neurochemical correlates of genetic hypertension and behavioral hyperactivity. Spontaneously hypertensive rats (SHR strain) when compared with WKY controls are known to exhibit both hypertension and hyperactivity. Two additional, new, inbred Wistar-Kyoto strains, WK-HT and WK-HA, were developed in this laboratory from an original SHR X WKY cross in order to separate the two traits using recombinant inbreeding procedures. WK-HTs are hypertensive and not hyperactive, and conversely, WK-HAs are normotensive but hyperactive in behavior. Together, these four related strains allowed us to search for neurochemical correlates of both of these genetic traits. Previous studies in this laboratory revealed few significant correlations with altered levels of monoamines and metabolites in brain regions from the four strains. However, when functional changes in monoaminergic transmission were studied, using high affinity uptake of norepinephrine, dopamine and serotonin, significant correlations were observed in selected brain regions. Supported by NSF grant R11-860679.

265.9

THE SPONTANEOUSLY HYPERTENSIVE RAT (SHR) AS AN ANIMAL MODEL OF ATTENTION-DEFICIT HYPERACTIVITY DISORDER: IMPROVED TIME DISCRIMINATION AND NO "PARADOXICAL EFFECT". T. Sagvolden* and B. Wultz* (SPON: J.A. Bevan). Inst. of Neurophysiology, Univ. of Oslo, N-0162 Oslo 1, Norway.

SHR may be used as an animal model of attention-deficit hyperactivity disorder (ADHD), a disorder frequently treated with psychomotor stimulating drugs. Several studies have addressed the hyperactivity of SHR, but none has investigated attention processes in these animals. The present study investigated attention processes of SHR and Wistar-Kyoto control rats. SHR were hyperactive. In particular, SHR had elevated rates of responses with short inter-response times. At the same time, SHR timed their responses more effectively than WKY. It is concluded that the behavior of SHR may have two components: One consists of responses with short inter-response times and is primarily expressing the hyperactivity of these animals. The other consists of the timing of the response according to the schedule of reinforcement and is reflecting attention. Methylphenidate did not change the animals' timing of their responses. Neither were there any "paradoxical" effects. Response rates started to decrease following high doses in SHR, but in WKY already following medium doses, indicating that both groups are affected similarly by the drug, but SHR are less sensitive to the drug.

266.1

FAILURE TO DETECT INCREASES IN BRAIN DOPAMINE TURNOVER IN CORN OIL AND SUCROSE SHAM FEEDING RATS. S.C. Weatherford, J. Gibbs and G.P. Smith Dept. Neurobiology and Obesity Research, Hoffmann-La Roche, Nutley, NJ 07110 and Dept. Psychiatry, New York Hospital-Cornell Medical Center, White Plains, NY 10605.

Sham feeding (SF) rats prefer corn oil (CO) >> 10% sucrose (suc) >> 6% suc. The preferences are evidence for the rank order of reward value of these solutions. The relative potency of dopamine (DA) antagonists for decreasing sham intake is 6% suc >> 10% suc >> CO. To test that the relative antagonist potencies are due to differential release of DA, dependent on the reward value of the sham-fed solution, DA turnover was measured using HPLC-electrochemical detection in forebrain-DA terminal fields microdissected from rats that had sham fed CO, 6% and 10% suc immediately before decapitation. The results did not support our hypothesis: no increase in DA turnover was observed after SF of any solution.

266.2

DIFFERENTIAL D1/D2 ANTAGONISM OF SUCROSE SHAM FEEDING. L.H. Schneider, C.A. Watson*, J.D. Davis, J. Gibbs & G.P. Smith. E.W. Bourne Lab, NY Hosp-Cornell Med Ctr, White Plains, NY 10605 & U. Illinois, Chicago, IL 60680

Selective antagonists for D1 (SCH 23390) and D2 (RAC; raclopride) receptors inhibit sucrose sham feeding (Schneider et al, 1986, 1987, 1988). To determine if equivalent reductions in the volume sham fed in 30 min after ID_{50} doses (ip) of the D1 and D2 antagonists are achieved through the same effects on the pattern of licking, the microstructure of licking was analyzed in 8 male, 4.75h food-deprived rats sham feeding 40% sucrose. The only significant differential effect on the microstructure of D1 vs D2 blockade ($p<.005$) was that SCH 23390 shortened the interval from the first to the last lick [min, mean $\pm$SE; Veh=25.7 $\pm$ 1.0, SCH=17.6 $\pm$ 2.4, $p< .02$)], but RAC did not [Veh=25.5 $\pm$ 0.9, RAC=26.0 $\pm$ 1.4]. Neither SCH 23390 nor RAC changed licking rate [6.0 $\pm$ 0.1/sec] or efficiency [163.1 $\pm$ 9.7 licks/ml], providing further support for our hypothesis that antagonists of D1 or D2 receptors reduce sham intake by attenuating the rewarding potency of sucrose without impairing the motor mechanisms of licking. The differential effect on lick duration suggests that under these conditions, antagonism of dopamine at D1 but not D2 receptors decreased the positive reinforcing effect of sham fed sucrose below the threshold necessary to sustain licking.

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266.3

MAPPING THE NEURAL CIRCUITRY OF A FEEDING RESPONSE: REGIONAL BRAIN CHANGES IN ^{14}C-2DG UPTAKE INDUCED BY INTRAHYPOTHALAMIC NOREPINEPHRINE INJECTIONS. J.N. Nobrega and D.V. Coscina, Clarke Institute of Psychiatry, Toronto, Ont., M5T 1R8, Canada.

Norepinephrine (NE) injections into the paraventricular hypothalamus (PVN) are well known to induce feeding in satiated rats. There is, however, a paucity of information on the neural pathways involved in this response. The PVN has extensive afferent and efferent connections and is involved in neuroendocrine and autonomic functions, which may be related to its role in feeding. Thus one area of interest in defining the functional anatomy of PVN feeding responses relates to the extent to which such feeding circuits may overlap autonomic/endocrine pathways. In this study the quantitative ^{14}C-2-deoxyglucose (^{14}C-2DG) technique was used to investigate regional brain changes induced by PVN NE injections. Male Wistar rats, bearing unilateral PVN cannulae and previously screened for NE feeding, were given 125 μCi/ kg ^{14}C-2DG i.v. immediately following a PVN injection of either 40 nmol NE or vehicle, then killed 45 min later. ^{14}C-2DG uptake was examined in 97 brain structures using computerized densitometry. PVN NE injections resulted in small but statistically significant changes in a discrete number of areas. Forebrain areas included the somatosensory parietal cortex (+15%), the CA3 hippocampal field (-8%), and the reticular thalamic nucleus (+14%). Midbrain areas included the anterior pretectal (+8%) and central gray areas (-11%). At the hindbrain level the lateral reticular nucleus showed the most pronounced changes in the brain (-24%), followed by the nucleus of the solitary tract (-16%) and the laterodorsal tegmental nucleus (+16%). No changes were seen in the median eminence or in other hypothalamic areas. This pattern of results is compatible with recent proposals for the anatomy of a PVN-hindbrain feeding pathway. In addition, however, it suggests that some forebrain structures may also be involved in this response.

266.4

5-HT$_{1A}$ AND 5-HT$_{1B}$ RECEPTOR BINDING SITES IN DISCRETE HYPOTHALAMIC NUCLEI: RELATION TO FEEDING. S.F.Leibowitz and M.Jhanwar-Unival. The Rockefeller Univ, New York, N. Y. 10021

In this study, we examined biochemically, in albino rats, hypothalamic serotonin (5-HT) receptors that may control feeding behavior. Eight hypothalamic nuclei (paraventricular (PVN), ventromedial (VMH), suprachiasmatic (SCN), dorsomedial (DMN), supraoptic (SON), arcuate-median eminence (ARC-ME), perifornical lateral (PLH) and preoptic (POM)) were micropunched and assayed. Using the radioligand binding technique, we estimated the binding of the 5-HT$_{1A}$ receptor antagonist [^{3}H]8-OH-DPAT (2 nM) to 5-HT$_{1A}$ receptor sites (in presence or absence of 10 µM 5-HT). For the 5-HT$_{1B}$ receptors, [^{3}H]5-HT (2 nM) was incubated with or without 10 µM 5-HT and in presence of 8-OH-DPAT (100 nM) and mesulergine (100 nM).

The results revealed a considerably higher concentration of 5-HT$_{1B}$ (average = 150 fmol/mg P) than 5-HT$_{1A}$ (20-40 fmol/mg P) binding sites throughout the hypothalamic nuclei. The 5-HT$_{1B}$ sites were densest in the VMH and PVN (>200 fmol/mg P), moderate in the SCN, SON and POM, and lowest in the DMN, PLH and ARC-ME (<100 fmol/mg P). This agrees with cannula-mapping results, showing 5-HT's inhibitory effect on feeding (a 5-HT$_{1B}$ response) to be strongest in VMH, PVN and SCN, and weaker or absent in POM, DMN, and PLH. The 5-HT$_{1A}$ binding sites were found to be uniformly low throughout the hypothalamic nuclei. Moreover, in 48-hr food deprived rats, 5-HT$_{1B}$, but not 5-HT$_{1A}$, binding sites were significantly increased in the medial, but not lateral, hypothalamus.

266.5

PERIPHERALLY ADMINISTERED 5-CARBOXAMIDOTRYPTAMINE (5-CT) INHIBITS FEEDING IN RATS. K. J. Simansky and K. Eberle-Wang. Dept. Pharmacology, Med. Coll. of Pennsylvania, Philadelphia, PA 19129.

Peripheral administration of serotonin (5-HT) reduces feeding in rats. Pharmacological evidence suggests that 5-HT$_2$ receptors mediate this anorexia. We assessed the actions of the selective 5-HT$_1$ agonist, 5-CT, in male albino rats maintained with 6 h access to a milk diet during the light period. 5-CT (.015-.24 umol/kg, i.p.) produced a dose-related decrease of milk consumption during the 30-min test period (controls, 15.9 $\pm$ 1.3 ml). 5-CT (estimated ID_{50} = .04 umol/kg) was 100-fold more potent than 5-HT. Pretreatment with 5 umol/kg of the ergots methysergide or LY53857 but not with ketanserin or xylamidine antagonized the anorectic effect of 5-CT (.03 umol/kg). This dose of xylamidine maximally inhibited 5-HT-induced anorexia. In rats with gastric cannulas, .03 umol/kg 5-CT reduced "real" feeding with closed cannulas but not sham-feeding with open cannulas. Although 5-CT decreased real feeding, .03 umol/kg of this indole did not act as an unconditional stimulus for a conditioned taste aversion using a two-bottle test. This dose of 5-CT also failed to reduce locomotion, rearing or investigation of novel objects in an open field. Based upon these data, we are pursuing the hypothesis that 5-CT potently and selectively inhibits feeding by stimulating a peripheral, xylamidine-insensitive, 5-HT receptor. Supported by NIMH Grant 41987.

266.6

PARTITIONING OF LABELED AMINO ACIDS INJECTED INTO THE PREPYRIFORM CORTEX OF RATS FED AMINO ACID IMBALANCED DIETS. J.L.Beverly*, B.J.Hrupka*, D.W.Gietzen, P.M.B.Leung and Q.R.Rogers, Dept. Physiol. Sci. and Food Intake Lab, Univ. Calif., Davis, CA, 95616.

Injection of the dietary limiting amino acid (DLAA) into the anterior prepyriform cortex (PPC) ameliorates the depression in intake of amino acid imbalanced diets (IMB). This response, specific for the DLAA, becomes apparent approx. 6 hr after injection possibly involving protein synthesis. Incorporation of injected amino acids into protein in the PPC was evaluated in rats fed a low protein basal diet and fitted with chronic bilateral 24 gauge cannulae 3 mm above the PPC. Immediately prior to the onset of the dark cycle, and the feeding of thr IMB, rats received bilateral injections, 0.5 µl/5 min via 32 gauge injection needles, into the PPC of aCSF containing [^{3}H]-leu (100 pmole/5µCi/µl) +/- [^{14}C]-thr (4 nmole/µCi/µl). Rats were sacrificed at 15, 30, 60, 90, 120, 180 and 360 min post-injection and the PPC dissected from consecutive 0.5 mm brain slices. After homogenization in 1 ml Krebs-Ringer buffer, 0.1 ml 10% TCA was added and the homogenates separated into supernatant and protein fractions by centrifugation. Supernatants were decanted and 1 ml of 0.3 M KOH added to each protein pellet. [^{3}H] and [^{14}C] were analyzed in 0.5 ml aliquots of supernatant and protein. Incorporation of [^{3}H] and [^{14}C] into the protein fraction plateaued 60-90 min post-injection. 50% of injected [^{3}H] and 30% of [^{14}C] were recovered in the protein fraction within 1.25 mm of the injection site. Supernatants contained 8% and 3% of labels, respectively. In rats receiving DLAA [^{3}H] in protein was $\geq$150% of the level measured in rats not receiving the DLAA ($p=.02$). [^{3}H] and [^{14}C] in the hypothalamus and cingulate cortex were at or near background. The results of this study suggest that following injection of the dietary limiting amino acid into the brains of animals fed IMB there is an increase in protein synthesis in the immediate area of injection. Supported by NIH AM-07355, DK-13252; USDA CRCR1-2418; CNRU DK35747-04.

266.7

BLOOD GLUCOSE DYNAMICS, MEAL INITIATION AND METABOLIC RESPONSES IN FREE-FEEDING RATS HABITUATED TO A POLYCOSE DIET OPTION. L.A.Campfield, D.W.Driscoll* and F.J. Smith Department of Neurobiology and Obesity Res. Hoffmann-La Roche Inc., Nutley, NJ 07110

The goal of this study was to determine if transient declines in blood glucose (TDBG) precede meals of highly preferred polycose diet option in the presence of chow and to define the metabolic responses to these meals. Rats with cardiac cannulas were habituated to a CHO option (CO) of 32% polycose gel or powder in addition to chow. Meal pattern and blood glucose were continuously monitored at the light/dark transition in free-feeding rats. TDBG, within the limits previously reported, were observed prior to each polycose meal recorded in the late light and early dark (n=13). Each meal initiated after a normal latency was either CO or CO plus chow and the rise in blood glucose was larger than that observed following a chow meal. The metabolic response to oral polycose and glucose (1 ml of 50% solution) were compared; blood glucose responses were similar, while the plasma insulin response was increased following polycose. These studies suggest that TDBG reliably predict meal initiation in rats habituated to a preferred diet option and that differential metabolic responses occur following these meals. The experiments provide additional support for the role of blood glucose dynamics in the control of feeding.

266.8

TRANSPORT OF INSULIN FROM PLASMA TO CSF IS NOT ALTERED BY CAFETERIA DIET OBESITY IN THE RAT. D.F. Lattemann*, P. Israel*, M. Schwartz*, P. Green, A. Sipols, F. Mukaida*, D. Porte, Jr.*, and S.C. Woods. University of Washington, 98195, and VA Medical Center, 98108, Seattle WA.

We have hypothesized that steady-state plasma insulin (I) provides an adiposity signal at the CNS. I transport from plasma to CSF is impaired in obese Zucker rats. In this study we tested whether I transport is altered in cafeteria diet obesity. Osborne-Mendel rats were fed either chow (C;n=20) or chow and snacks (S;n=20) to achieve a 23% weight difference (359±7 vs 292±6 gm;Lee Index=.319±.002 vs .302±.002). Rats were infused SQ 24 hr with I, or with vehicle with an overnight fast. Basal plasma I was elevated in S vs C rats (113±10 vs 45±7 uU/ml;n=5 each) but CSF I was 10% of plasma I in both (10.0 vs 4.6±1.5 uU/ml). Plasma I (395±117 vs 369±58 uU/ml) and CSF I (5.7±1.2 vs 7.8±2.9 uU/ml) were comparable for C (n=7) vs S (n=10) rats with I infusion. Thus at both basal and elevated plasma I there is no defect of I transport into CSF in the S rats. This suggests that rats with mild non-genetic obesity receive an appropriate I adiposity signal at the CNS.

266.9

CIRCADIAN CHANGES IN I.V. NUTRIENT SATIETY. E.K. Walls*, A.E. Willing* and H.S. Koopmans, Dept.Med.Physiol., Univ. of Calgary, Calgary, Alberta, Canada T2N 4N1.

Most previous studies have delivered i.v. nutrients over 24 hours to study their effect on daily food intake. In this study, nutrients were delivered to dark fed rats during different portions of the 12:12 hr light/dark cycle to determine whether feeding-generated gut signals act in conjunction with i.v. nutrients to suppress food intake. During 4 day test periods, 6 rats were infused with 35.5 - 37.0 kcal of a solution containing 25% glucose and 4.25% amino acids (Travasol). Saline baseline intakes were 63 - 75 kcal/day. During 8 and 12 hr infusions in the dark phase, food intakes were reduced by 40.5 ± 1.1 and 30.7 ± 1.8 kcal/day (or 110 and 87% of infused calories) respectively ($p < .01$). These rats gained 1.1 ± 0.2 and 1.1 ± 0.3 g/day. Following 8 or 12 hr infusions in the light phase, food intakes were reduced significantly less than in the dark, 21.3 ± 1.1 and 19.6 ± 0.7 kcal/day (or 57 and 55% of infused calories, $p < .01$) and greater weight gains were observed 3.0 ± 0.3 and 2.1 ± 0.3 g/day ($p < .01$). With 24 hour infusions, as in previous studies, food intake was reduced by 24.5 ± 2.1 kcal/day (or 68% of infused calories, $p < .01$) and rats gained 2.4 ± 0.3 g/day. This study shows that gut signals generated by concurrent feeding act with i.v. glucose and amino acids to produce a more compensatory reduction of voluntary intake. In the light phase when gut signals are absent and fat is being burned, i.v. nutrients produce a much smaller reduction in subsequent food intake.

266.10

SUPPRESSION OF FEEDING BY INTRAINTESTINAL MALTOSE IS MEDIATED BY PHLORIZIN-SENSITIVE MECHANISM. R.C. Ritter and E. Simon*. Department of VCAPP, Washington State University, Pullman, WA 99164-6520.

Intraintestinal infusion of maltose suppresses food intake. This effect is mediated by vagal sensory neurons that are destroyed by the neurotoxin capsaicin. To further examine the mechanism by which maltose suppresses food intake, we made intraintestinal infusions of monosaccharides or maltose in sham feeding rats. We also made coinfusions of these substances with phlorizin, a blocker of sodium-coupled intestinal glucose transport. Glucose, which is absorbed by a sodium-coupled carrier, suppressed food intake in a dose dependent manner. 3-o-methylglucose, a glucose analogue that is absorbed but does not move by the sodium-coupled glucose carrier, did not suppress sham ingestion. Suppression of ingestion by either glucose or maltose was attenuated by phlorizin. Our data suggest that suppression of food intake by maltose is mediated by glucose liberated from maltose digestion. Furthermore, the data indicate that for suppression of feeding to occur, glucose must interact with the sodium-coupled carrier or a similar receptor site. (Supported by NIH RO1 NS20561).

266.11

HEPATIC PORTAL INFUSION OF GLUCAGON ANTIBODY INCREASES SPONTANEOUS MEAL SIZE IN RATS. J. Le Sauter and N. Geary, Psychology Department, Columbia University, NY, NY 10027.

Pancreatic glucagon (PG) administration reduces the size of test meals elicited by food deprivation, by presentation of palatable food, and, as we recently reported (Le Sauter et al., Proc. EPA 60: 51, 1989), spontaneous meals in free feeding rats. All these data suggest exogenous PG may signal postprandial satiety. Endogenous PG has also been implicated in satiety because intraperitoneal injection of PG antibodies increases the size of post-deprivation meals (Langhans et al., Science 218: 894, 1982). We now report that endogenous PG also appears necessary for the normal control of spontaneous meals. Nine rats with chronic intraportal cannulas were infused twice each with either rabbit PG antibodies (Radioassay Sys., Carson, CA) or non-immunized serum according to separate crossover tests. A dose sufficient to neutralize 3 ng PG in vitro was remotely infused at .034 ml/min during the first 2 min of the first spontaneous meal in the last quarter of the dark phase. Antibody infusion increased meal size in 16 of 18 tests. The mean increase was 57%, from 2.8 ± 0.3 to 3.8 ± 0.4 g, $t(8)= 2.36$, $p<.05$. Meal duration increased 71%, from 11.2 ± 1.3 to 16.2 ± 2.6 min, but this was not statistically reliable. These data suggest pancreatic glucagon operates physiologically to control spontaneous meals.

266.12

PREFERENCES BETWEEN CONCURRENTLY PRESENTED FLAVORS REINFORCED BY DELAYED NUTRITIONAL DIFFERENCES. B.J. Baker* and D.A. Booth, School of Psychology, University of Birmingham, B15 2TT, U.K.

The site and nature of a nutritional signal to the brain can be characterised by the effects on ingestive behavior of administering particular nutrients selectively to parts of the gut, liver or brain. Flavor preference reinforcement effects (conditioned choices) are more specific than (food-intake suppression) satiety effects. Rats were conditioned by differences between two concurrently presented high- and low-carbohydrate-containing diets and protein-containing and non-nutritive diets so that they preferred the flavor of the richer or nutritive diet. Such learning occurred with a 10-minute delay between the presentation of both dietary stimuli and the intubation of the nutrient mixture proportional to the flavor intakes. Thus, rats are able to perceive the difference between the nutritional aftereffects of two distinctive diets presented concurrently and to learn to respond differentially to associated flavors.

Therefore, reinforcement that has been suggested to arise from gastric chemoreception may be attributable to delayed postgastric effects.

267.1

EFFECTS OF MAITOTOXIN (MTX) ON cGMP GENERATION IN PC12 CELLS. Fabian Gusovsky, Andrew Schulick*, Takeshi Yasumoto* and John W. Daly, LBC, NIDDK, NIH Bethesda, MD 20892

The marine toxin MTX is a calcium channel activator and it stimulates phosphoinositide breakdown in most cells. In PC12 cells MTX has no effect alone on cAMP or cGMP formation, but as was the case for phorbol esters that activate protein kinase C, MTX potentiates forskolin-stimulated cAMP accumulation, probably through formation of diacylglycerol. Atrial natriuretic factor (ANF) induces a dose dependent formation of cGMP in PC12 cells through the stimulation of a particulate membrane bound guanylate cyclase. ANF-mediated cGMP formation is not affected by varying $[Ca^{2+}]_o$. MTX inhibits ANF-induced cGMP formation. The inhibitory effect is calcium-dependent and is eliminated in the absence of $[Ca^{2+}]_o$. But the inhibition by MTX is not reduced by the calcium channel blocker nifedipine. The calcium ionophore ionomycin does not affect ANF-mediated cGMP formation. Phorbol-12-myristate-13-acetate (PMA), but not 4α-phorbol, also inhibit ANF-mediated cGMP accumulation. Sodium nitroprusside (NPS) induces large increases in cGMP through the stimulation of a soluble guanylate cyclase in PC12 cells. Neither MTX nor PMA inhibit NPS-induced cGMP accumulation. The results indicate that in PC12 cells, protein kinase C (PKC) activation leads to inhibition of ANF-mediated cGMP accumulation. Such PKC activation can be attained either directly with PMA, which binds to and activates PKC, or with MTX, through calcium-dependent phosphoinositide breakdown and subsequent diacylglycerol formation. Since NPS-mediated stimulation was not affected with MTX or PMA, it is possible that the particulate, but not the soluble guanylate cyclase, is phosphorylated by PKC resulting in an inhibition of activity.

267.2

TETRAHYDROPAPAVEROLINE INHIBITS IN VITRO BRAIN PROTEIN KINASE C ACTIVITY. R.Del Vesco*, S.Govoni*~, F.Battaini~, C.Lopez* and M.Trabucchi*~ (SPON. V.Olgiati).Ins. Pharmacol. Sci. Univ. of Milan; ~Pharmacobiol. Dept. Univ of Bari;~Chair of Toxicol., 2nd Univ. of Rome , Italy.

It has been demostrated that isoquinolinsulfonamides, in particular H-7, inhibit in vitro brain PKC activity interacting, in a competitive manner, with the ATP site on the catalytic domain of the enzyme. Moreover H-7 doesn't modify 3H-Phorbol dibutyrate (3H-PDBu) binding further suggesting that this compound interacts specifically with the catalytic domain of the enzyme. We have investigated the in vitro effect of tetrahydropapaveroline (THP), which has a chemical structure related to H-7, on 3H-PDBu binding and on partially purified PKC activity from rat brain. THP doesn't modify 3H-PDBu binding up to a concentration of 300 μM. On the other hand THP inhibits in vitro, in a concentration dependent manner, from 10 to 300 μM, the enzyme activity both in the soluble and the particulate fraction. The IC50 is 50 μM, a value similar to that of H-7 (basal activity: 1.5±0.11 nmoles 32P incorporated/min./mg protein).

The present data demostrate that THP, a tetrahydroisoquinoline derivative, inhibits PKC activity with the same potency and, probably, the same mechanism of H-7. Moreover THP has been isolated from mammalian brain and urine of human subjects suggesting the possibility that endogenously formed THP could act as a PKC regulator.

267.3

DUAL MECHANISMS OF INHIBITION BY DOPAMINE OF BASAL AND TRH STIMULATED INOSITOL PHOSPHATE PRODUCTION IN ANTERIOR PITUITARY CELLS. A. Enjalbert, G. Guillon*, B. Mouillac*, V. Audinot*, R. Rasolonjanahary*, C. Kordon and J. Bockaert*. U. 159 INSERM (Paris) and CCIPE (Montpellier), France.

In primary culture of anterior pituitary cells dopamine inhibited basal and thyrotropin releasing hormone inositol monophosphate, inositol diphosphate and inositol triphosphate production. This inhibition by dopamine was a biphasic phenomenon. One of the component of the inhibition was very rapid and already present after 10 seconds. The other was slower, starting after 1 min, and was mimicked by nimodipine, a dihydropyridine calcium channel antagonist. The effects of dopamine and nimodipine were not additive on both basal and TRH stimulated inositol phosphates production. Furthermore, the dopamine inhibition in the presence of TRH was much higher (51 %) than the inhibition induced by nimodipine (20 %).

In conclusion dopamine appears to inhibit inositol phosphates production by two distinct mechanisms. The slow inhibition is likely due to an inhibition of voltage dependent calcium channel. This is further substantiated by the fact that ionomycine (10 μM) was able to reverse this slow component. In contrast the rapid inhibition induced by dopamine was not reversed by ionomycine. This fast component of the dopamine inhibition could represent a direct coupling, though a pertussis toxin sensitive G protein of D2 dopamine receptors with phospholipase C.

267.4

REGULATION OF INOSITOL 1,4,5-TRISPHOSPHATE (IP_3)-INDUCED Ca^{2+} RELEASE FROM CANINE CEREBELLUM MEMBRANE PREPARATIONS. P. Volpe, B.H. Alderson-Lang* and M. Tzinas*. Department of Physiology and Biophysics, UTMB, Galveston, TX 77550.

Intracellular Ca^{2+} stores capable of accumulating and releasing Ca^{2+} have been shown in CNS neurons. The Ca^{2+} store sensitive to IP_3 seems to be particularly enriched in membrane preparations obtained from the cerebellum. Regulation of IP_3-induced Ca^{2+} release is not well understood yet. To this end, we have studied the effects of cAMP, cAMP-dependent protein kinase (PKA) and $[Mg^{2+}]$ on IP_3-induced Ca^{2+} release from canine cerebellum membrane preparations actively preloaded with Ca^{2+}. The catalytic subunit of PKA increases the extent of IP_3-induced Ca^{2+} release, shifts the dose-dependence curve for IP_3-induced Ca^{2+} release to the right, and does not affect Ca^{2+} uptake rate. cAMP appears to have no effect on both Ca^{2+} uptake and Ca^{2+} release. Reduction of free $[Mg^{2+}]$ causes no change of Ca^{2+} uptake rate, provided that [MgATP] is kept well above K_m for Ca^{2+} transport. Furthermore, low $[Mg^{2+}]$ increases the extent of IP_3-induced Ca^{2+} release, and shifts the dose-dependence curve for IP_3-induced Ca^{2+} release to the left. These results suggest that: a) PKA-dependent phosphorylation and high $[Mg^{2+}]$ may decrease the affinity of the receptor for IP_3; b) no appreciable, endogenous membrane-bound PKA is detected; c) dephosphorylation and high $[Mg^{2+}]$ favor the transition to a closed state of the IP_3-sensitive Ca^{2+} channel. Supported by NIH grant GM40068.

267.5

EFFECTS OF SINGLE AND REPEATED DOSES OF LITHIUM ON BRAIN REGIONAL INOSITOL, INOSITOL-1-PHOSPHATE AND CALCIUM CONCENTRATIONS. K.M. Savolainen and M.-R. Hirvonen*. National Public Health Institute, Department of Environmental Hygiene and Toxicology, P.O.Box 95, SF-70701 Kuopio, Finland.

The effects of an i.p. single (10 mEq/kg) dose of LiCl during 24 h, and of repeated doses (2 mEq/kg daily) at one and two weeks on brain regional inositol, inositol-1-phosphate (InsIP), intermediates of brain phosphoinositide (PI) cycle, and calcium (Ca^{2+}) concentrations were studied in male Han/Wistar rats. Inositol and InsIP were measured by GLC, and confirmed with a mass selective detector. Ca^{2+} and Li^+ were measured by atomic absorption sepectrophotometer. There was a slight gradual, but significant increase in cerebral InsIP after a single dose of LiCl during 24 h, and a much more pronounced decrease in brain inositol. Brain Ca^{2+} and Li^+ increased during the same time period. During repeated dosing of LiCl there was an increase in brain InsIP levels at one week which were decreased at two weeks; brain nositol and Ca^{2+} were unchanged, but there was a constant decrease in brain Li^+. These results suggest that a high singl. dose of LiCl disturbs brain PI cycle, not only by inhibiting InsIP monophosphatase but other enzymes of the cycle as well. Elevated brain Ca^{2+} levels support this interpretation. Accumulation of Li^+ in the brain may also partly explain these results. During repeated administration of LiCl, InsIP levels increased at one week but decreased again at two weeks indicating an adaptation of PI metabolism to the effects of LiCl. Alternatively, decreased brain Li^+ may contribute to his effect.

267.6

PHOSPHOINOSITIDE (PI) SECOND MESSENGER MARKERS IN RAT CEREBELLAR PURKINJE CELLS. C.A Ross, A. Verma, T. Milner, M.W. MacCumber, S. Suppatapone, J. Meldolesi, and S.H. Snyder. Dept. of Neuroscience, Johns Hopkins University, School of Medicine, 725 N. Wolfe Street, Baltimore, MD 21205.

Cerebellar Purkinje cells are enriched in elements of the PI cycle, such as protein kinase C, G_o protein, and IP_3 receptors (Worley et al. Ann. Neurol. 21:217-229, 1987). By EM immunocytochemistry, we find Purkinje cell IP_3 receptors in smooth and rough endoplasmic reticulum (ER) and the nuclear membrane, sites where IP_3 could influence synaptic transmission, protein synthesis, and nuclear events. IP_3 releaseable $^{45}Ca^{++}$ uptake into ER in brain sections is highly concentrated in Purkinje cells. In situ hybridization studies show that mRNA for an intracellular calcium pump is also most highly concentrated in Purkinje cells. Another mRNA wich is most enriched in Purkinje cells is that of one particular isozyme of phospholipase C (Bennett et al. Nature 334:268, 1988). The high concentration of PI elements in Purkinje cells is explained by the finding that the synapse from parallel fibers to Purkinje cells, quantitatively the most prominent in the brain, involves a specific glutamate receptor which activates the PI cycle (Blackstone et al. PNAS, in press). These studies suggest that cerebellar Purkinje cells are an excellent model system for studying brain PI system function.

267.7

AUTORADIOGRAPHIC VISUALIZATION OF INOSITOL-1,4,5-TRIPHOSPHATE (IP_3) SENSITIVE CALCIUM POOLS IN RAT BRAIN. A. Verma, C.A. Ross, S. Supattapone, D. Verma* and S.H. Snyder. Johns Hopkins Univ. Sch. of Med., Dept. of Neurosci., Baltimore, MD 21205 and *Univ. of MD Med. Sch., Baltimore, MD 21201.

Fresh frozen 10 μm rat brain sections demonstrated ATP-Mg^{2+} dependent uptake of $^{45}Ca^{++}$ which was markedly enhanced by oxalate. This uptake involved a non-mitochondrial pool, being insensitive to ruthenium red or azide, but inhibited by vanadate and abolished by A23187. Autoradiographic distribution of accumulated $^{45}Ca^{++}$ oxalate precipitate overlapped the distribution of IP_3 binding sites in serial brain sections. Highest levels of both $^{45}Ca^{++}$ uptake and IP_3 binding were in cerebellar Purkinje cells.

Accumulation of $^{45}Ca^{++}$ oxalate precipitate was diminished 30-40% by 10μM IP_3, an effect blocked by heparin. A similar effect was seen with I(2,4,5)P_3 but not I(1,3,4)P_3 or other inositol phosphates. The effect of IP_3 on $^{45}Ca^{++}$ accumulation was limited to brain regions with IP_3 binding. Thus, CNS IP_3 binding sites appear to represent functional receptors which modulate a non-mitochondrial, oxalate permeable calcium pool.

267.8

FUNCTIONAL RECONSTITUTION OF PURIFIED INOSITOL 1,4,5-TRISPHOSPHATE RECEPTOR FROM RAT CEREBELLUM. C.D. Ferris, R.L. Huganir, S. Supattapone and S.H. Snyder. Dept. of Neuroscience, The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 20205.

IP_3 receptor was purified as described (Supattapone, S., et al., JBC 263:1530-1534, 1988). Purified receptor protein was mixed with sonicated, solubilized liposomes. After detergent removal reconstituted vesicles were used for both [^{3}H]IP_3 binding and [$^{45}Ca^{++}$] flux assays. The affinity and inositol phosphate specificity of binding in the vesicles is nearly identical to that of the native membranes and the purified receptor. [^{3}H]IP_3 binding in the vesicles has a K_d of approximately 80 nM at pH 7.4, is largely insensitive to other inositol phosphates, and is completely inhibited by heparin (100ug/ml). [$^{45}Ca^{++}$] flux was assayed by mixing reconstituted vesicles with [$^{45}Ca^{++}$] in the presence or absence of Ins (1,4,5) P_3 or other inositol phosphates followed by cation exchange chromatography. In the presence of 10 uM IP_3 maximum [$^{45}Ca^{++}$] flux occurs within 10 sec. Dose response analysis for Ins (1,4,5) P_3 shows an EC_{50} of 100 nM. All other inositol phosphates are inactive at micromolar doses. Thus, a single protein receptor contains both the IP_3 binding site and the calcium release mechanism.

267.9

ANGIOTENSIN II MOBILIZES CYTOSOLIC CALCIUM IN NG108-15 CELLS. E.A. Tallant*, M.C. Khosla and C.M. Ferrario. Research Institute, Cleveland Clinic Foundation, Cleveland, OH 44195.

Angiotensin II (Ang II) participates in the central regulation of cardiovascular function and has been identified, along with its receptor, in regions of the CNS which participate in cardiovascular regulation. To define the signal transduction mechanisms of Ang II in neuronal cells, we have used a transformed cell line of neural origin, the neuroblastoma x glioma hybrid NG108-15, which contains a specific, high affinity binding site for Ang II. Incubation of intact NG108-15 cells with ^{125}I-Ang II revealed a single population of binding sites with a K_D of 1.09±0.06 nM and a Bmax of 7.35±0.94 fmol/mg protein. Ang peptides competed with Ang II for binding, with an order of potency of Ang II>Ang-(2-8)>Ang I>Ang-(1-7)>Ang-(2-7)>>Ang-(1-6). Incubation of NG108-15 cells with 1 mM dibutyryl cAMP for 6 days, which produces morphological differentiation of the cells, resulted in a 50-fold increase in the number of Ang II binding sites (362.7±98 fmol/mg protein) and a smaller decrease in their affinity (5.38±1.6 nM), suggesting that a cAMP-dependent mechanism may be involved in neuronal Ang II receptor regulation. Nanomolar concentrations of Ang II elevated the level of cytosolic Ca^{2+}, measured in NG108-15 cells loaded with the fluorescent Ca^{2+} indicator dye Fura 2, and the response was specifically blocked by the Ang II antagonist Sar^1Ileu^8-Ang II or Sar^1Thr^8-Ang II. Because Ca^{2+} mobilization in response to Ang II was not blocked by chelation of extracellular Ca^{2+} with EGTA or by verapamil, the peptide mobilizes Ca^{2+} from intracellular stores. The data suggest that Ang II in neuronal cells is coupled to hydrolysis of inositol lipids and the subsequent mobilization of cytosolic Ca^{2+}. (Supported in part by HL-6835 and the Storer Foundation).

267.10

G-PROTEIN CROSSTALK IN RECEPTOR COUPLING TO PHOSPHOLIPASE C. E.M. Landau, T.M. Moriarty, S.C. Sealfon*, D.J. Carty*, G. Omri*, J.L. Roberts and R. Iyengar* Depts. of Psychiatry, Pharmacology and Fishberg Research Center, Mount Sinai School of Medicine, New York, N.Y. and Dept. of Psychiatry, Bronx V.A. Medical Center, Bronx, N.Y.

Heterotrimeric G-proteins can be categorized into molecularly divergent groups by their differential sensitivity to pertussis toxin (PTX). Receptors specifically use either PTX sensitive or insensitive G-proteins to activate effectors. To determine if this specificity is absolute we tested the possibility that receptors which utilizes PTX insensitive pathways in native tissues might use PTX sensitive G-proteins in a foreign environment. *Xenopus* oocytes injected with rat liver RNA expressed liver V_1-type vasopressin receptors which are known to couple to a PTX insensitive G-protein in liver. Oocytes injected with rat brain RNA expressed brain CCK-A receptors which are also thought to use a PTX insensitive G-protein. We studied coupling to phospholipase C by monitoring a receptor-evoked IP_3 dependent Cl^- current. The coupling of the liver V_1 receptor in the oocyte was inhibited by PTX, whereas the brain CCK-A receptor coupling was not sensitive to PTX. Both responses were regulated by purified G-protein βγ subunits. The results indicate that the V_1 receptor can utilize two different G-proteins as signal transducers whereas the brain CCK-A receptor maintains specificity for a PTX insensitive pathway, and further suggest the hitherto unrecognized possibility of the existence of closely related G-proteins that are differentiated only by their PTX sensitivity.

267.11

ENDOTHELIN AND SARAFOTOXIN STIMULATE PI TURNOVER IN CLONAL HSDM1C1 CELLS. N.A.Sharif* and R.L.Whiting (SPON:R.P.Rosenkranz), Inst. of Pharmacology, Syntex Research, Palo Alto, CA 94303 (USA).

The peptides, endothelin (ET) and sarafotoxin (ST), are 21-amino acid-containing peptides that share 67% structural homology but which have been isolated from porcine cells and snake venom respectively. We have studied the mechanism of action of these peptides in clonal murine fibrosarcoma cells (HSDM1C1) using phosphoinositide (PI) hydrolysis as an index of receptor activation.

In [^{3}H]inositol-labelled HSDM1C1 cells, ET and ST produced a dose-related (0.1 nM - 10 μM) accumulation of [^{3}H]inositol phosphates. Human/porcine ET (HP-ET) and ST were more potent than rat ET (R-ET) at stimulating PI turnover in these cells. The ED_{50} values were (nM; $\bar{X}$ ± SEM; n = 3-6) : HP-ET = 87.4 ± 16.8; ST = 61.4 ± 5.3 and R-ET = 224 ± 22. However, all three peptides were full agonists and yielded a 4.5-5.6-fold increase in PI turnover at 10 μM. Although the actions of these peptides were reduced in Ca^{2+}-free-EGTA-containing buffer, the addition of the Ca^{2+}-channel blockers, nifedipine and w-conotoxin (0.1nM-1μM), failed to alter ET-induced PI hydrolysis. In contrast, numerous other neuroactive agents failed to induce PI hydrolysis in this cell-line.

In conclusion, these data suggest that the clonal cell-line, HSDM1C1, contains specific receptors for ET and ST which are linked to a PI turnover mechanism.

267.12

ENDOTHELIN-INDUCED PHOSPHOINOSITIDE TURNOVER IN CEREBELLAR GRANULE CELLS. W.-W. Lin[*1,2], C.Y. Lee[2] and D.-M. Chuang[1] (Spon: V. Gilad). [1] Lab. of Preclinical Pharmacol., National Institute of Mental Health, Washington, DC 20032 and [2] Dept. of Pharmacol. College of Medicine, National Taiwan Univ. Taipei, Taiwan, ROC.

The effect of endothelin (ET) on phosphoinositide (PI) hydrolysis was studied in primary culture of cerebellar granule cells. ET induced approximately 10-fold increase in ^{3}H-inositol monophosphate accumulation. The EC_{50} and saturation concentrations of ET were 1.3 and 10 nM, respectively. The PI response elicited by ET was dependent on the presence of extracellular calcium but was only slightly affected by 1 mM of Co^{++} or Mn^{++} and 10 μM of nimodipine or nisoldipine. Sodium depletion resulted in a marked increase in basal PI turnover and a corresponding decrease in the fold-stimulation elicited by ET; however, the net increase of PI turnover was not attenuated. ET-induced PI breakdown was partially inhibited by a phorbol ester and was unaffected by pertussis toxin. The ET-induced PI turnover appeared to be additive to that induced by carbachol, histamine, NE and serotonin. Prestimulation of cells with ET for 30 sec or longer resulted in virtually complete loss of the ability of ET to stimulate PI hydrolysis, without affecting subsequent responsiveness induced by carbachol, histamine, NE, serotonin and maitotoxin. However, the response induced by sarafotoxin was desensitized by ET prestimulation.

268.1

RESPONSES FROM PRIMARY SOMATOSENSORY CORTEX IN M. MULATTA DURING ACTIVE DISCRIMINATION OF TEXTURED SURFACES. R. Sinclair & H. Burton. Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

Two trained monkeys (M. Mulatta) stroked their fingertips over pairs of horizontal gratings (Sinclair & Burton, Somato Res 5,1989, 283) with constant ridge (250um) and varying groove (500-2900um) width (roughness), and identified the smoother (smaller groove), while recordings were made from somatosensory cortex. All findings are preliminary. From ~140 single units in SI areas 1 & 3b, 3 response types were found: 1. Response proportional to surface roughness; vigorous response to roughest and none to smoothest surface. Instantaneous firing rates appear linearly related to roughness. This graded response is similar to that of peripheral receptors. 2. Graded response, but smaller response difference to same roughness range as #1. 3. Response not correlated with roughness. All 3 response types were seen in areas 1 & 3b. No significant differences found between 1 & 3b in cells with rapidly adapting (RA) responses to punctate stimuli. Slowly adapting (SA) cells in 3b showed similar response patterns. Some SA responses correlated both with applied force and roughness. A few 3b cells (SA) showed inverse responses to roughness (greater activity on smoother surfaces). Contrast effects were noted where response rate was greater if smoother surface was stroked first. No difference was found in SI responses preceding correct or incorrect discrimination. Discrimination of horizontal gratings differing by groove width could be served by activity proportional to groove size in a large population of SI cells. Supported by NIH NS09809.

268.2

PARALLEL PROCESSING OF MOVING BAR PATTERNS IN PRIMATE S-I CORTEX. S. Warren, H.A. Hamalainen, C.I. Palmer and E.P. Gardner. Dept. of Physiology and Biophysics, NYU School of Medicine, NY, NY 10016.

Horizontal bar patterns swept across the tactile array of an OPTACON stimulator have been used to map receptive fields of cutaneous afferents and SI cortical neurons. Cortical single unit recordings in alert macaques reveal a remarkable central transformation of peripheral sensory information, involving (a) amplification, prolongation and merger of sensory signals; (b) temporal frequency filtering; and (c) frequency dependent receptive field plasticity. Cortical responses are stronger, longer in duration and less tightly phase locked to OPTACON pulses than receptor activity. Four types of spike trains are observed. ***Pulse neurons*** resemble sensory afferents discharging highly synchronized, short duration bursts of spikes which are tightly time-locked to OPTACON pulses; they are found in area 3b and anterior area 1, and respond at shortest latencies (15-18 ms). ***Burst neurons*** also show tight response coupling to stimulus pulses, but display longer lasting discharges; spikes evoked by successive stimuli merge, forming a continuous discharge at high pulse frequencies. Burst neurons have similar cytoarchitectural distributions in SI, but show longer latency peak responses (18-25 ms). Firing patterns of ***modulated neurons*** are more loosely coupled to OPTACON pulses, while those of ***non-modulated neurons*** are completely uncoupled from the pulse train, showing only a generalized rise in excitability. The pulsatile characteristic of the stimulus is smoothed into a signal of continuous motion by modulated and non-modulated neurons. They form 60% of the activated cortical population, and are generally located posteriorly. Burst neurons show the greatest increase in spikes/pulse, and non-modulated neurons the least.

Unlike peripheral afferents whose firing rates mimic stimulus frequency, *average firing rates* of cortical neurons are relatively *uniform* at all frequencies. Temporal frequency of stimulation appears to be coded primarily by the spike trains of pulse and burst neurons. Frequency coding is degraded by the other classes; at high frequencies they fire continuous discharges in which individual pulse responses are merged. Non-modulated neurons require closely spaced temporal inputs, as they fire at higher rates to 100 Hz than to 25 Hz stimuli. We suggest that differences in conduction velocities of individual afferents spread out central inputs, allowing separate pulse responses of receptors to be merged into a continous cortical discharge. This temporal amplification may provide the neural basis for the sensation of apparent motion across the skin. *(Supported by USPHS Research Grant NS11862.)*

268.3

DIFFERENCES IN SENSORIMOTOR INTEGRATION IN CORTICAL AREAS 3b AND 1. OF THE MONKEY. R. J. Nelson and V. D. Douglas* . Dept. of Anatomy and Neurobiology, College of Medicine, University of Tennessee, Memphis, 875 Monroe Avenue, Memphis, TN 38163.

Areas 3b and 1 of monkey somatosensory cortex receive similar afferent input and are topographically mirror-images of each other. However, their functional differences in sensorimotor integration are not completely understood. To determine the differences in the activity of area 3b and 1 neurons during sensory triggered motor behaviors, Rhesus monkeys were taught to make identical wrist flexion and extension movements in response to either vibration delivered to the palm by the apparatus' control handle or visual cues consisting of changes in the display lamps that also signaled the current wrist position. These cues were presented randomly in blocks of ten trials. Animals were required to make the same movements in response to either sensory stimulus. From single-unit activity records of 35/44 and 90/115 area 3b and 1 neurons the onset times and magnitudes of activity changes related to the onset of sensory cues and premovement activity were measured. In general, the onsets and magnitudes of non-stimulus related premovement activity were significantly different for vibratory as compared to visually cued trials for area 1 but not for area 3b neurons ($p<0.01$ and $p<0.05$; t-test). Premovement activity differences of area 1 neurons were related to vibratory stimulus frequency, receptive field type and the direction of movement. Area 3b premovement activity differences were not. For 40 area 1 cue related-neurons, increases in vibratory responsiveness were correlated with proportional decreases in the magnitude of premovement activity when movements were made against a load and in the direction of the stimulated body hand. Seventeen area 3b neurons did not show this trend.

These findings and previously reported differences in sensory and premovement related activity suggest that while areas 3b and 1 are similar in many respects, neurons in these areas respond differently to peripheral and centrally generated modulatory inputs. This study met NIH animal utilization guidelines.

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268.4

DISTINCTIVE PATTERNS OF PROJECTIONS TO STRIATUM FROM PHYSIOLOGICALLY-MAPPED SOMATOSENSORY REPRESENTATIONS IN PRIMATE CORTEX. A. W. Flaherty*, A. M. Graybiel, M. Sur, P. Garraghty (SPON: M. Nastuk). Dept. of Brain and Cognitive Sciences, M.I.T., Cambridge, MA 02139.

To help understand the way the striatum represents bodily sensation, we have investigated how electrophysiologically-defined areas 3a, 3b, and 1 of the primate primary somatosensory cortex (SI) project to the striatum. With multiunit recording, we mapped hand or mouth receptive fields (RFs) in 8 squirrel monkeys, and injected small volumes of distinguishable anterograde tracers (HRP-WGA and 35S-methionine) into mapped regions. **(1) Pattern of SI projections to the striatum.** Projections appeared as a constant pattern of patches and bands of label, exclusively in the extrastriosomal matrix of the putamen. Some abutted striosomes identified by enkephalin-like immunostaining. **(2) Different representations in the same functional areas.** When we injected two different parts of the hand representation within a single cortical area (e.g. the thumb and fifth fingers in area 3b), we saw partially overlapping and interdigitating projection patterns. In contrast, corticostriatal projections from the hand and mouth representations within a single cortical area projected to distinctly different, non-overlapping areas of the putamen. **(3) Matched representations in different functional areas.** We injected homologous representations of a single body part within different functional areas--e.g. representations of the index finger in areas 1 (primarily cutaneous RFs) and 3a (primarily deep, muscle spindle RFs). Such injections produced overlapping zones of projection in the putamen, but in the rostral putamen the regions of densest labeling did not always overlap. These anatomical findings suggest that there is a functional mosaic of somatosensory fields in the primate striatum. Supported by Javits Award 2 R01 255290101 and The Seaver Institute.

268.5

RETROGRADE LABELING OF STRIATONIGRAL AND STRIATOPALLIDAL NEURONS IN THE RHESUS MONKEY. L.D. Selemon, and P.S. Goldman-Rakic, Sect. of Neuroanat., Yale Univ. Sch. Med., New Haven, CT 06510.

The topography of striatonigral and striatopallidal neurons and their relative apportionment in the caudate and putamen was examined in five rhesus monkeys. In Cases 1-3, following simultaneous retrograde transport of the fluorescent dyes, diamidino yellow (DY) and fast blue (FB), from the rostral globus pallidus (GP) and rostral substantia nigra (SN), respectively, labeled neurons were densely distributed within corresponding topographic zones of the precommissural neostriatum. Both striatofugal populations were observed in the caudate, as well as in the putamen. Despite intermingling of striatonigral and striatopallidal cells in two of the cases, very few double-labeled neurons were observed. In Case 4, in which DY was injected into the caudal GP, striatopallidal neurons were found in the posterior putamen and dorsolateral caudate. Although labeling was largely dense and homogeneous in the fluorescent cases, clusters of DY and FB-labeled neurons were observed. More prominent clustering was observed in the posterior putamen in Case 5 following an injection of WGA-HRP into the caudolateral SN.

Retrograde labeling in the present study indicated that striatofugal neurons are not segregated on a gross morphological level but may be organized as a mosaic of GP- and SN- projecting clusters. The observation that each population is widely distributed in both the caudate and putamen, suggests that strict parallelism is not maintained in the cortical-basal ganglia loop. (MH38546, MH00298, AG05310).

268.6

TRANSNEURONAL TRANSPORT OF HSV1 FROM THE PRIMARY MOTOR CORTEX OF PRIMATES. M.C. ZEMANICK,* P.L. STRICK and R.D.DIX*. VA Med. Ctr. and Depts. of Neurosurg. & Physiol., SUNY-HSC @ Syracuse, Syracuse NY, 13210 and Depts. of Ophthal. and of Microbiol. and Immunol., School of Medicine, Univ. of Miami, Miami, FL, 33101.

We injected Herpes Simplex Virus Type 1 (HSV1, strain H129, titre: 10^{12} PFU/ml) at 4-12 sites (0.05ul/site) in the 'arm' area of the primary motor cortex in 2 monkeys (Cebus apella). After a 3 or 4 day survival time, immunohistochemical procedures were used to demonstrate virus transport. We observed intense labeling of neurons in regions which receive input from the injection site (e.g., portions of the putamen, thalamic reticular nucleus, red nucleus, and pontine nuclei). Brain regions which project to the injection site (e.g., portions of the nucleus basalis) or are reciprocally connected with the injection site (e.g., portions of the ventrolateral and intralaminar thalamus, and frontal and parietal cortex) also contained intensely labeled neurons. In addition, we observed several striking instances of anterograde transneuronal transport to 'third-order' neurons. For example, clusters of labeled granule cells were located at multiple sites in the cerebellar cortex, and labeled neurons were seen in the external segment of the globus pallidus. Our results suggest that anterograde transneuronal transport of HSV1 can be used to examine connections between primate cerebral cortex and the cerebellum and basal ganglia. Support: VA Res. Serv. and USPHS 24328, 8705, 2957.

268.7

INTRACELLULAR STAINING WITH BIOCYTIN REVEALS THE COMPLETE TELENCEPHALIC ARBORIZATIONS OF RAT PYRAMIDAL TRACT NEURONS. R. L. Cowan and C. J. Wilson, Department of Anatomy and Neurobiology, College of Medicine, University of Tennessee, Memphis TN 38163.

Incomplete labeling of distant axonal processes limits the usefulness of intracellular staining techniques. The most desirable marker would completely label distant axon collaterals after intrasomatic injection. Horikawa and Armstrong have recently introduced biocytin, whose characteristics suggest it might be a less toxic and more mobile intracellular label than HRP. We tested this possibility by injecting antidromically identified pyramidal tract neurons with biocytin *in vivo*. After incubation in avidin-biotin-HRP solution and reaction with diaminobenzidine, all the injected neurons were recovered, and no degenerating neurons were seen with survival times up to 4 hours. Intracortical collaterals of these neurons could be traced from their origin to fine distant terminal branches. The main axons of the neurons could be traced through the internal capsule and were darkly labeled at least as far as the cerebral peduncle at the level of the pons. Many neurons had intrastriatal collaterals arising in the internal capsule and dividing into smaller varicose branches. These could be traced to their terminal arborizations in widely separated regions of the neostriatum. Some neurons with intrastriatal collaterals also had small diameter varicose collaterals in the basal forebrain. These collaterals were also stained completely, including their finest terminal arborizations.

Biocytin injection is a substantial improvement over previous intracellular staining techniques, causing less damage to the injected neurons, and more effectively staining distant axonal arborizations.

268.8

ELECTROPHYSIOLOGICAL PROPERTIES OF IDENTIFIED CORTICOSPINAL TRACT NEURONS STUDIED IN VITRO. Guo-Fang Tseng, David A. Prince, Dept. of Neurology and Neurological Sciences, Stanford Univ. School of Medicine, Stanford, CA 94305.

Intracellular recordings were obtained from 37 double labelled neurons in rat motor cortical slices. Cells had been retrogradely filled in vivo with Rhodamine beads (spinal cord injections), and intracellularly labelled in vitro with biocytin. Three types of neurons were distinguished based on patterns of spike discharge elicited by intracellular current pulses. 1) Regular spiking cells (RS; N = 19) fired at a relatively constant frequency after a brief period of spike adaptation. Distinct fast (f) and medium (m) AHPs followed each spike but slow (s) AHPs were not readily evoked. Subpial stimulation evoked EPSPs and early and/or late IPSPs in these neurons. 2) Adapting neurons (n = 10) showed significant spike frequency accommodation and generated no clear f AHP. Subthreshold current pulses evoked an early graded active depolarization in these neurons. Orthodromic stimuli elicited EPSPs and large amplitude, early and late IPSPs more readily than in RS cells. 3) A third class of neurons (n = 8) generated prominent depolarizing spike afterpotentials which often evoked spike doublets or triplets during current injection. Preliminary examination showed no gross differences in the general morphology of these three types of pyramidal cells. Thus, even a seemingly uniform population of corticospinal tract neurons possesses subsets that have strikingly different intrinsic properties, as well as differences in the character of their synaptic inputs. We speculate that this physiological diversity may underlie different functional properties in vivo. Supported by NIH grants NS06477 and NS12151 from the NINDS.

268.9

INTRINSIC OSCILLATORY PROPERTIES OF LAYER 5 NEOCORTICAL NEURONS. L.R. Silva, Y. Chagnac-Amitai and B.W. Connors. Section of Neurobiology, Box G, Brown University, Providence, RI 02912.

We have observed that a majority of pyramidal neurons in layer 5b have the intrinsic tendency to fire rhythmically at about 5 to 15 Hz. Neurons were recorded intracellularly from slices of rat SmI neocortex maintained *in vitro*. Periods of oscillatory firing lasting as long as 20 sec were elicited by brief stimuli (EPSPs or positive or negative current pulses of a few msec) when the membrane potential was held a few mV negative to spike threshold. Oscillations were comprised of either repetitive bursts (each burst = 2 to 5 spikes firing at 150-300 Hz) or single spikes. Repetitively bursting neurons had minimal rates of 4-9 Hz, while single-spike neurons had minimal rates of 6-15 Hz. When stimulated with long (~1 sec) depolarizing pulses at resting potential, neurons displayed all-or-none responses at threshold; i.e. either they did not spike at all, or they generated long, rhythmic trains. As current was increased, mean oscillatory bursting frequency rose from about 5 to a maximum of 15 Hz; oscillatory single-spiking increased from about 9 to >35 Hz. During strong, sustained stimuli, oscillatory bursting cells often shifted abruptly from bursting to single-spiking, with an accompanying 2-fold increase in frequency. The firing rates of oscillatory neurons did not adapt. Oscillatory firing was unaffected by doses of kynurenic acid that blocked EPSPs; thus, oscillations probably arise from intrinsic membrane properties rather than local synaptic circuitry. Injections of biocytin revealed that oscillatory neurons were large pyramidal cells of layer 5b. Intrinsically oscillating neurons of layer 5b may contribute to the generation of certain neocortical EEG rhythms and seizures.

Supported by NS01271, NS25983 and MHO9641.

268.10

INTRINSIC 40 HZ OSCILLATORY PROPERTIES OF LAYER IV NEURONS IN GUINEA PIG CEREBRAL CORTEX *IN VITRO* R. Llinas and A.A. Grace,* (SPON: S. Ferris) Dept. of Physiology and Biophysics, N.Y.U. School of Medicine, New York, NY 10016 and Depts. Behav. Neurosci & Psychiat., Univ. Pittsburgh, Pittsburgh, PA 15260.

Oscillations at 40 Hz have been recorded in mammalian EEG during high vigilance states (Bouyer et al., Neurosci. 22:863, 1987). Single unit and field potentials recorded from cat visual cortex show that visual stimuli elicit 40 Hz oscillations in these cells (Gray et al., Nature, 338:34, 1989). The origin of this 40Hz rhythm is presently unknown. *In vitro* intracellular recordings were obtained from brain slices of guinea pig frontal cortex, following standard techniques. Smooth or sparsely spinous multipolar neurons (SMNs) of layer 4 (identified after HRP or Lucifer yellow filling) demonstrated sustained subthreshold oscillatory activity at 38-45 Hz spontaneously or upon direct depolarization. In some cells the oscillatory event was all-or-none and was superimposed on a plateau potential which could outlast the depolarizing current pulse. Depolarization sufficient to bring the cell to the firing level elicited well-defined spike activity at the frequency of the oscillations. Similar results were observed in 20 SMNs. The ionic conductances underlying the oscillations were a voltage-dependent sodium conductance which could be blocked by bath application of TTX (1-2 $_{\mu}$M) and a delayed rectifier. Thus, in sharp contrast to other CNS neurons, cortical SMNs require only sodium and potassium conductance increases to generate their oscillations. These results suggest that the well-defined 40 Hz rhythm in the cerebral cortex is driven by the intrinsic properties of neurons located in the same cerebral cortical layer that receives the specific thalamo-cortical afferents. The results imply that the thalamic input to the cortex serves as a trigger for the inherent rhythmic activation of specific cortical columns.

268.11

SPONTANEOUS AND EVOKED CORTICAL ACTIVITY IN THE ISOLATED ADULT GUINEA PIG BRAIN MAINTAINED *IN VITRO* Marco de Curtis* and Rodolfo R. Llinas (SPON: R. Llinás) Dept. Physiology and Biophysics, New York University Medical Center, 550 First Ave, New York, N.Y. 10016.

Cortical activity has been recorded extracellularly from the *in vitro* isolated, arterially perfused brain of guinea pig. The preparation combines the advantages of the *in vitro* slice preparations with the preservation of intact neuronal circuits. Substantial improvements in the methodology has been developed, which have allowed studies on the isolated brainstem cerebellar (Llinas and Muhlathaler 1988, J.Physiol. 404, 215-240) and whole brain preparations (Llinas, Muhlethaler and Walton 1989 J. Physiol. in press).

Field potentials evoked by electrical stimulation of the ipsilateral optic tract were recorded in the primary cortical visual areas both from the surface and at different cortical dephts. Evoked activity from the lateral geniculate nucleus and from the superior colliculus were recorded and correlated with the simultaneously recorded cortical potentials. Most of the recordings were obtained at 23-26^{0} C. The waveform observed in these structures together with the pattern of temporal invasion of the optic tract-driven activity closely resemble the results reported in the mammalian brain in vivo. In preliminary experiments the perfusion fluid temperature was slowly raised to 36^{0} C, at which temperature spontaneous electrocortical activity could be recorded. Spindle sequences with a duration of 2-4 sec and an inner rate of 4-6 Hz were observed by adding barbiturates to the perfusate.

These results confirm the viability of the *in vitro* whole brain preparation maintained under complete isolation and offer a new tool for the study of the mammalian brain rhythmicity.

269.1

INJECTION OF NPY INTO THE RAT HYPOTHALAMIC PVN SUPPRESSES GASTRIC ACID SECRETION THROUGH POSSIBLE AUTONOMIC NERVOUS INTERACTIONS IN THE STOMACH. G.A. Humphreys*, J.S. Davison and W.L. Veale. Dept. of Medical Physiology, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

We have shown previously that injection of Neuropeptide Y (NPY) into the hypothalamic paraventricular nucleus (PVN) of anesthetized rats inhibits basal and pentagastrin stimulated gastric acid output. This antisecretory effect of NPY was abolished by truncal vagotomy, coeliac ganglionectomy, atropine, phenoxybenzamine, yohimbine, and by bethanecol stimulation. The effect was present with electrical stimulation of the distal cut ends of the cervical vagus nerves and under α_1 receptor blockade using prazosin. These results indicate that the antisecretory action of NPY in the PVN is dependent on the integrity of the subdiaphragmatic sympathetic and parasympathetic nerves and on the function of α_2 adrenergic and peripheral muscarinic acetylcholine receptors. The effect was absent during exogenous administration of muscarinic cholinergic agonist which indicates that the inhibitory effect of central NPY may be acting through inhibition of peripheral acetylcholine release. Together, these results indicate that the antisecretory effect of NPY in the PVN may be mediated by sympathetic nerves which may suppress gastric acid output by inhibiting vagal release of acetylcholine via activation of α_2 receptors.

269.2

VAGAL INHIBITORY EFFECTS ON THE RAT STOMACH: INVOLVEMENT OF DOPAMINERGIC NEURONES. E.K.Tayo*, P.Trye* and R.G.Williams* (SPON: Brain Research Association). Dept. of Phys. & Pharmacol., Univ. Southampton, Southampton SO9 3TU, U.K.

Dopaminergic vagal abdominal efferents have been described and dopamine has been shown to have inhibitory effects on gut motility. However, a physiological role for dopamine in the control of the gut in the rat has not been established.

Male Wistar rats (fasted overnight but allowed free access to water) were anaesthetised with urethane (125mg/100gm body weight I.P.). A balloon catheter was inserted into the stomach for measurement of intragastric pressure, the jugular vein was catheterised for administration of drugs, the carotid artery for recording arterial blood pressure and the peripheral cut end of left cervical vagus was placed over silver stimulating electrodes. Supramaximal stimulation (20 volt. 20Hz) produced an increase in intragastric pressure which was followed by a decrease below the baseline. Both responses were inhibited by hexamethonium (hex., 10-20mg/kg). In the presence of hex. carbachol (0.0125mg/kg) produced a sustained increase in intragastric pressure which was inhibited by vagal stimulation. This effect was partially but not completely antagonised by domperidone (5-10mg/kg). The results support a role for dopamine as an inhibitory transmitter in the rat stomach and support morphological evidence for the existence of dopaminergic vagal abdominal efferents.

269.3

RAPHE OBSCURUS STIMULATION INCREASES GASTRIC PRESSURE AND MOTILITY IN THE RAT. P.J. Hornby, D.H. Kuhn* and R.A. Gillis*. Dept. of Pharmacology, Georgetown University, Washington, D.C. 20007.

Recently, we have demonstrated that chemical stimulation of the caudal medullary raphe nuclei in the cat results in increases in gastric motility, acid and pepsin secretion. These effects of raphe stimulation on gastric function are vagally-mediated, and are likely due to activation of neurons in the raphe subnuclei which project to the dorsal motor nucleus of the vagus (DMV) where vagal neurons controlling gut function are located. The present study was designed to evaluate the role of specific medullary raphe subnuclei, the raphe obscurus (Ro) and raphe pallidus (Rp) in control of gastric motility and pressure in rodents, a species where extensive projections from the raphe subnuclei to the dorsal vagal complex are known to exist. This was accomplished by microinjection of 20nl L-glutamate (0.5M) into the Ro and Rp in urethane-chloralose anesthetized rats while monitoring pyloric motility (g.tens.), stomach pressure (ccH_20) and blood pressure (mmHg). Stimulation of Ro (N=9), from the level 0.5-1.5mm rostral to obex, resulted in significant increases in pyloric motility (2.6±0.7 peak g.tens. $P<0.05$) and stomach pressure (0.8±0.3 cmH_20, $P<0.05$). However, stimulation of Rp (N=5) did not result in statistically significant increases in pyloric motility (O.8±0.4 peak g.tens.) or stomach pressure (0.1±0.1 cmH_20). Blood pressure increases (5-25mmHg,N=9) and decreases (10-15 mmHg, N=5) were not specific to one or other subnucleus. These data suggest that activation of neurons in an anatomically defined region of the medullary raphe, i.e. Ro but not Rp, results in excitation of gastric smooth muscle. Supported by NIH grant AM29975.

269.4

RAPHE OBSCURUS STIMULATION PRODUCES INCREASES IN GASTRIC SECRETION AND MOTILITY. R.L. White*, C.D. Rossiter*, P.J. Hornby, D.K. Kasbekar*, J.W. Harmon*,W.P. Norman, R.A. Gillis* (SPON: K.L. Dretchen). Depts. Pharm., Surgery, Physiol., Georgetown Univ. Med. Sch., Washington, D.C. 20007

Previous data from our laboratory indicate that the raphe obscurus provides major afferent innervation to the portion of the dorsal motor nucleus of the vagus (DMV) that innervates the stomach. The purpose of the present study was to elucidate the role of the raphe in control of gastric function. The studies were performed in chloralose anesthetized cats while monitoring gastric pH, pyloric motility, and pepsin production. After sealing the pylorus and attaching a pyloric strain gauge a gastrotomy was created through which two catheters were inserted into the stomach. The stomach was continuously irrigated with saline and the pH constantly monitored. Acid output was determined by titration to pH 7.0. Chemical excitation of neurons in the raphe increased gastric motility(MMI +3.1±1.3, $p<0.05$) and acid secretion [baseline was 6±2 microequivalents (μeq)/15 min increasing to 11±4 μeq/15 min (n=8), 34±12 μeq/15 min($p<0.05$), and 39±19 μeq/15 min ($p<0.05$) during the first, second and third 15 minute periods after microinjection, respectively]. Pepsin output increased from a baseline of 287±67 pepsin units (p.u.) to 507±126 p.u. 15 min. post injection (n=4), 541±118 p.u. 30 min. after injection ($p<0.05$), 608±92 p.u. 45 min. after injection ($p<0.05$), and 700±156 p.u. 60 minutes post injection ($p<0.05$). Atropine methylbromide (0.5 mg/kg I.V.) abolished the increases in gastric acid and motility. These data indicate that activation of raphe neurons results in cholinergically mediated increases in gastric function. These are the first findings to our knowledge that implicate raphe nuclei in gastric function.

269.5

GASTRIC VAGAL AND GREATER SPLANCHNIC INPUT TO THE TEGMENTAL NUCLEI IN THE CAT. C.S. Yuan* and W.D. Barber. Dept. of Anatomy, Coll. of Med., Univ. of Arizona, Tucson, AZ 85724.

Our previous studies demonstrated gastric vagal and greater splanchnic (GS) evoked unitary responses in nucleus tractus solitarius (NTS). Immunohistochemical studies localized neuropeptides common to both NTS and the dorsal and ventral tegmental nuclei (TN) suggesting a neuronal link between the caudal brainstem and pons. In the present study unitary responses were recorded in the dorsal and ventral TN in anesthetized cats in response to electrical stimulation of proximal gastric vagal and/or left GS fibers. The mean latency of 181 gastric vagally-evoked orthodromic responses, bilaterally distributed in the TN, was 352.2 msec, while the latency of the splanchnic-evoked response was 63.2 msec. Convergence of input from gastric vagal and GS fibers upon single cells was observed in 151 units (83 percent). Stimulation of the GS fibers resulted in a short latency excitation followed by an inhibition of the gastric vagally-evoked response. Projections from NTS and the parabrachial nucleus upon cells in the TN were also identified electrophysiologically by direct microstimulation of the two former structures. The significant number of gastric vagal and splanchnic evoked unitary responses recorded in the dorsal and ventral TN suggested that they may serve an important role in the neuronal link for processing visceral information between NTS and forebrain structures. (Supported by USPHS Grants DK 35434 and DK 36289)

269.6

MESENTERIC NERVES MEDIATE PLASMA VASOPRESSIN (AVP) RESPONSE TO SPLANCHNIC OSMORECEPTOR STIMULATION IN CONSCIOUS RATS. S.C. Kwon* and A.J. Baertschi. Dept. of Physiology, Univ. of Virginia, Charlottesville, VA 22908

Hypertonic (598 mOsm/kg) or isotonic (290 mOsm/kg) saline solution was infused over 4 min via stomach tube at a rate of 0.57 ml/min in conscious rats with indwelling arterial catheters. The average baseline of plasma AVP was 4.7 ± 0.4 pg/ml. Plasma AVP in time controls (isotonic gastric infusion) was unchanged. Mean changes of plasma AVP over 30 mins following gastric infusions of hypertonic saline solutions were 5.9 ± 0.8 pg/ml ($p < 0.01$) in intact rats. The response was significantly ($p < 0.001$) attenuated by 59.4 % in rats with lesion of side branches of splanchnic nerves innervating the mesentery of the upper small intestine and the portal vein area, but not affected by subdiaphragmatic vagotomy. Right splanchnic nerve lesion decreased the plasma AVP response by 32.2 % between 11 and 21 min ($p < 0.025$). Left splanchnic nerve lesion also caused a decrease in the plasma AVP response by 35.5 % between 11 and 16 min ($p < 0.05$). Plasma osmolality over 30 mins in each group was not significantly different from baseline, indicating that central osmoreceptors could not have been involved. The results show that 1) splanchnic receptors are located in the mesentery of the upper small intestine and possibly the portal vein area ; and 2) splanchnic osmoreceptors project to the spinal cord via right and left major splanchnic nerves (supported by NSF BNS-8819877).

269.7

THE RELATIVE CONTRIBUTION OF ALPHA- AND BETA-ADRENOCEPTORS TO THE METABOLIC RESPONSE OBSERVED DURING A PROSTAGLANDIN E_1 HYPERTHERMIA IN THE RAT. D.M.Fyda, K.E.Cooper, and W.L. Veale, Department of Medical Physiology, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

Although the thermal effects of pyrogens have been extensively examined, few studies have assessed the effector systems responsible. Thus, these experiments investigated the contribution of nonshivering thermogenesis (NST) and its effector system to a prostaglandin E_1 (PGE_1) hyperthermia. Thirty-two male rats had guide cannulae directed to a lateral cerebral ventricle (LCV) and were fitted with indwelling carotid and jugular catheters and radio-transmitters for the remote monitoring of core temperature (Tc). During the LCV injection of PGE_1 (30 ng/µl), Tc rose by 0.9°C, metabolic rate more than doubled and brown adipose tissue temperature (Tbat) increased by 1.1°C. When the rats were pretreated with the β-blocker propranolol (1 mg/kg), PGE_1 evoked only a 0.2°C increase in Tc and both metabolic and Tbat responses were reduced by 80%. Following the α_1-antagonist prazosin (50 µg/kg), PGE_1 elevated Tc by 0.4°C, metabolic increase was reduced by 26%, whereas the rise in Tbat was unaffected. The results suggest that brown adipose tissue-mediated NST is, at least in part, responsible for a PGE_1 hyperthermia and that 80% of the response is due to β- and 20% to α_1-adrenoceptors. Although the relative contribution of shivering, NST and vasoconstriction is unclear, it appears that about 25% of the hyperthermia may result from an α-mediated vasoconstriction.

269.8

DENDRITIC MORPHOLOGY OF FUNCTIONALLY IDENTIFIED NEURONS IN THE DORSAL MOTOR NUCLEUS OF THE VAGUS. E.A. Fox and T.L. Powley, Lab. of Regulatory Psychobiology, Purdue U., W. Lafayette, IN 47907.

The dorsal motor nucleus of the vagus (dmnX) is composed of five primary, topographically separate, longitudinal columns of neurons; each is associated with a different subdiaphragmatic vagal branch (ie. one of the two celiac, two gastric, or unpaired hepatic branches), and each mediates a different but overlapping set of functions. To investigate the relation of morphology to function of dmnX neurons, randomly selected cells (n=250/column) from each functionally identified dmnX column were injected with Lucifer yellow (Neurosci. Abstr. 14(1988) 315) in 100µm coronal, sagittal or horizontal brain slices.

The location, extent and complexity of branching of dendritic fields was different for celiac vs. gastric vs. hepatic cells. Within all columns mid-rostrocaudal neurons differed from rostral and caudal ones in terms of dendritic field extent and complexity of branching, soma profile area, percentage of neurons with extra-dmnX dendrites, and dendritic orientation (dendrites ramified most extensively in the horizontal plane, with those of the most rostral and caudal cells oriented longitudinally and those of middle ones oriented radially). Additionally, classification of neurons demonstrated that all cell types were found in each column, but in different proportions.

The different location and extent of dendritic fields associated with each column provide the basis for differential interaction with inputs and local circuits. Thus, the differential distribution of cell types within and across columns may provide the basis for the different but overlapping subsets of functions mediated by each column (NIH grant DK 27627).

269.9

THE FIBER COMPOSITION OF THE RAT ABDOMINAL VAGUS. J.C. Prechtl and T.L. Powley. Laboratory of Regulatory Psychobiology, Purdue University, W. Lafayette, IN, 47907.

The subdiaphragmatic vagal trunks and all of the primary abdominal branches of six rats were analyzed with complete cross-sectional electron microscopic (EM) montages (X10,000). All fibers were counted, and more than 10% of them were morphometrically evaluated with an image analyzer. Nerve whole mounts were used to analyze the branching patterns and intraneural organization.

The mean diameters (perimeter-derived) of unmyelinated axons in each of the branches were similar (0.75-0.83 µm). But the shapes of the size distributions as summarized by their coefficients of skewness, revealed significant differences between the bilateral gastric branches and the two celiac branches; the hepatic branch size distribution differed from all others. Most of the myelinated fibers (85%) in the vagal branches were less than 2.6 µm in diameter, and had myelin sheath widths between 0.1 and 0.5 µm. The gastric branches, however, consistently contained a few larger myelinated fibers with sheath widths as great as 0.85 µm. Whole mounts also revealed fiber bundles within the vagal branches which were not of supradiaphragmatic origin; these adventitial bundles could be traced along the perineurium between the adjacent vagal branches. The sum of the fibers in the combined branches (26,930) was 21% more than the number of fibers counted in the parent trunks (22,272); this excess probably reflects the adventitial fiber content.

The results demonstrate a cytological diversity between and within the individual vagal branches. The fiber counts, when considered with observations on the number of efferents and adventitial fibers in the nerve, indicate that the abdominal vagi contain considerably fewer nodosal afferents and more autonomic efferents than have been traditionally inferred. (DK27627)

269.10

INNERVATION OF URINARY BLADDER IN BB DIABETIC RATS: FUNCTIONAL AND STRUCTURAL ALTERATIONS ARE PREVENTED BY GANGLIOSIDES ADMINISTRATION. M. Paro*, M.G. Fiori, M. Prosdocimi*, and A.A.F. Sima*(1) (SPON: V. Magri). FIDIA Research Laboratories, Abano Terme 35031, Italy and (1) Faculty of Medicine, University of Manitoba, Winnipeg, Canada R3EOW3.

A previous study in the spontaneously diabetic BB-rat has demonstrated functional and structural autonomic polyneuropathy affecting bladder function (Paro et al., Diabetes, in press). BB-Wistar rats (7-11 mo) underwent cystometrographic (CMG) analysis and morphological evaluation of pelvic and hypogastric nerves after gangliosides (GA) administration. BB rats, in comparison with their respective controls, had significantly greater bladder capacity and weight, symptoms of polyuria and polydipsia. In particular the rate of bladder contractions (RC) (index of vesico-vesical excitatory reflex) was significantly decreased in diabetic animals (48% normal). These functional findings were correlated with morphometric changes in the autonomic nerves (hypogastric and pelvic) constituting the neural reflex arc. Treatment with GA (10 mg/kg i.p. die) for 1 month was able to restore RC (91% normal) and the morphometric changes due to diabetic autonomic neuropathy in BB-rats.

269.11

METABOLIC ALTERATIONS IN BRAINS FROM RATS WITH STREPTOZOTOCIN-INDUCED DIABETES MELLITUS. T.L. Krukoff and K.P. Patel*. Dept. of Anatomy & Cell Biology, Univ. of Alberta, Edmonton, Canada, T6G 2H7, and Dept. of Physiol. & Pharmacol., Univ. of S. Dakota, Vermillion, USA, 57069.

Effects of streptozotocin (STZ)-induced diabetes mellitus on metabolic activity of discrete regions of the brain were studied with hexokinase (HK) histochemistry. Two weeks after 1 injection of STZ (65 mg/kg i.p.), blood glucose and osmolarity levels were elevated; blood sodium was depressed. These changes were reversed in diabetic rats treated with insulin (2 U/day, s.c.). In the brain after 2 weeks, significant increases in HK were seen in the magnocellular paraventricular n. (mPVH, 12.1%), medial division of the n. tractus solitarius (mNTS, 15.5%), and commissural division of the NTS (cNTS, 10.9%). (An increase just below significance was seen in the supraoptic n. (SON, 11.5%)). In insulin-treated rats, increases in HK activity were reversed in cNTS (and SON) and only partly reversed in mPVH and mNTS. No changes in HK activity were found in subfornical organ, medial preoptic area, parvocellular PVH, locus coeruleus, or dorsal motor n. of the vagus of diabetic rats. These results demonstrate that the brain is not exempt from changes associated with diabetes. Metabolic alterations in mPVH and NTS of diabetic rats are likely related to changes in vasopressin production and blood volume, respectively.

Supported by the MRC of Canada and the NIH.

269.12

CARDIOVASCULAR RESPONSES TO ADMINISTRATION OF BICUCULLINE INTO THE PARAVENTRICULAR NUCLEUS (PVN) OF CONSCIOUS RATS. D. S. Martin*, T. Segura* and J. R. Haywood. Department of Pharmacology, The University of Texas Health Science Center, San Antonio, Texas 78284-7764.

This study was undertaken to determine whether GABA exerts a tonic sympathoinhibitory action at the PVN of the hypothalamus. Rats were instrumented with guide cannulae directed at the PVN and with femoral venous and arterial catheters. Artificial CSF (aCSF) or bicuculline methiodide (BMI; 2 mM) was infused bilaterally (100 nl) to achieve a steady state response. Blood samples (1 ml) were taken during the baseline period and during the response to BMI for the measurement of plasma catecholamines. The BMI infusion was repeated after treatment with either a β_1 adrenergic antagonist, metoprolol tartrate (2 mg/kg/i.v.), or a ganglion blocking agent, chlorisondamine chloride (12.5 mg/kg/s.c.).

Infusion of BMI into the PVN caused blood pressure (BP) to increase by 20 ± 2 mm Hg from a resting level of 118 mm Hg, while heart rate (HR) rose by 108 ± 10 b.p.m. from a control level of 372 b.p.m.. Plasma norepinephrine (NE) and epinephrine (EPI) increased by 623 ± 100 and 1212 ± 250 pg/ml, respectively. aCSF did not alter BP (-1 ± 1 mm Hg), HR (-1 ± 6 b.p.m.) or plasma NE (11 ± 21 pg/ml) and EPI (-89 ± 53 pg/ml). After β_1 adrenergic blockade, the BP response to BMI was unchanged (27 ± 7 mm Hg), but the tachycardia was reduced significantly (17 ± 8 b.p.m.). Ganglionic blockade abolished both the BP (-1 ± 2 mm Hg) and HR (14 ± 23 b.p.m.) responses. These results suggest that an endogenous GABA system exerts a tonic depressor effect at the level of the PVN. Blockade of this GABA-ergic tone results in activation of the sympathoadrenal axis. (Supported by HL36080 and HL32977. DSM supported by a Fellowship from MRC Canada.)

270.1

OPIATE-INDUCED MUSCLE RIGIDITY IS MEDIATED BY MU, AND NOT DELTA OR KAPPA RECEPTORS, IN THE RAT. M. B. Weinger and J. B. Bronson*, Dept. of Anesthesiology, Univ. Calif. San Diego and V.A.M.C., San Diego, CA. 92161.

Opiate-induced muscle rigidity is a significant clinical problem. We examined the role of receptor subtypes in opiate rigidity by comparing the effects of selective mu (D-Ala^2,N-Me-Phe^4-Gly^5-ol-enkephalin [DAGO]), delta (D-Ala^2,D-Leu^5-enkephalin [DADL]), and kappa agonists [U50488h] on alfentanil-induced rigidity in spontaneously ventilating rats. Forty-five anesthetized male Wistar rats received a 10 µl "blinded" intracerebroventricular (ICV) injection of either saline, DAGO (2.5, 5.0, or 10.0µg), or DADL (17.75 or 35.5µg). U50488h (2.5, 5.0, or 10.0 mg/kg) was administered intraperitoneally to an additional 37 animals. Muscle rigidity was assessed using electromyographic (EMG) activity from the left gastrocnemius muscle. After a 15 min baseline, all rats were injected with either alfentanil (ALF, 0.5mg/kg s.c.) or saline, and data were then collected for 60 min. Data from the 3 experiments were analyzed individually and for each group the mean (±SEM) of the area under the EMG voltage-versus-time curve (AUC) was calculated. The addition of ICV DAGO increased the magnitude of ALF rigidity in a dose-dependent fashion ($P<0.05$ by ANOVA). In contrast, pretreatment with U50488h or DADL had no significant effect. Neither DADL (37.5µg) or U50488h (5mg/kg) in the absence of ALF produced significant rigidity (AUC 44% and 53% of ALF controls, respectively; $P<0.05$). This data supports the hypothesis that mu but not delta or kappa receptors play the major role in mediating opiate rigidity. Since mu receptors are critical for analgesia, opiates with high mu affinity will be more likely to produce rigidity. Thus, development of more potent mu agonists may not further the quest for opiate anesthetics with fewer clinical side-effects.

270.2

USE OF COMPUTER SIMULATION TO PREDICT THE MAGNITUDE AND DURATION OF ANALGESIA PRODUCED BY EXOGENOUS OPIOIDS. M. J. C. Holland. Baruch College CUNY, New York, NY 10010 and New York Univ. Med. Ctr., New York, NY 10016.

A computer program for the simulation of binding of exogenous opioid agonist (EOA) to specific opioid binding sites in brain has been constructed. Program assumes that plasma EOA concentration (following i.v. administration) can be expressed as the sum of three exponential terms, EOA molecules pass the blood brain barrier in both directions by passive diffusion, and EOA molecules in brain extracellular fluid bind reversibly to specific opioid binding sites on exterior face of cell membranes. For each simulated experiment user supplies: (1) initial plasma EOA concentration, (2) total concentration of opioid binding sites in brain region of interest, (3) pharmacokinetic constants, and (4) EOA-receptor interaction rate constants. Program calculates the concentration of EOA in brain extracellular fluid and amount bound to specific opioid binding sites as functions of time following injection. Levels of analgesia predicted by two models of drug action: Occupation Theory (as modified by Stephenson, R.P. 1956. Br. J. Pharmacol. 11:379.) and Rate Theory (Paton, W.D.M. 1961. Proc. R. Soc. B. 54:21.) are compared. Simulated experiments have been performed for three EOAs (morphine, etorphine and buprenorphine) for which good pharmacokinetic and receptor binding data are available.

270.3

HIGH AFFINITY SIGMA RECEPTORS COUPLED TO GTP BINDING-PROTEIN(S). Y. ITZHAK* (SPON: D. Wilson). Dept. of Biochemistry & Mol. Biology, REPSCEND Labs., University of Miami School of Medicine, Miami, FL 33101.

Sigma receptors have been postulated to be involved in the psychotomimetic effects of certain opiate-benzomorphans (pentazocine, cyclazocine and (+)SKF 10047). This receptor is selectively labeled with (+)-3-(3-hydroxyphenyl)-N-(1-propyl)piperidine[(+)-3-PPP]. The effect of the stable GTP analog, GppNHp, on the binding kinetics of (+)[^{3}H]-3-PPP and on the affinities of sigma receptor agonists and postulated antagonists was examined. The association rate of (+)-3-PPP (1nM; 25°C) binding in rat brain membranes is inhibited by~6 fold in the presence of GppNHp (0.1mM). The dissociation kinetics (25°C) of (+)[^{3}H]-3-PPP in control brain preparations display biexponential decay, suggesting the occurrence of multiple steps in the formation of the ligand receptor complex. However, inclusion of GppNHp during the association eliminates the slow-dissociation binding component. Hill coefficients for cyclazocine, pentazocine and (+)SKF 10047 but not haloperidol and chlorpromazine, for competing for (+)-3-PPP binding are less than 1 (0.5-0.6). In the presence of GppNHp the affinity of the agonist, but not the "antagonist", is reduced and Hill values are close to 1. These results suggest that the sigma receptor exists in a high affinity state that is coupled to GTP-sensitive binding protein(s).

270.4

PERTUSSIS TOXIN DIFFERENTIALLY AFFECTS MORPHINE ANALGESIA AND SUPERSENSITIVITY. V. Sierra*, S.C.J. Lee*, K. Lutfy* and B.C. Yoburn. Coll. of Pharmacy, St. John's Univ., Queens, NY 11439.

Pertussis toxin (PT) interferes with inhibition of adenylate cyclase (AC) by ADP-ribosylation of the guanine nucleotide regulatory protein (G_i). Opioid agonists will inhibit AC via G_i; and correspondingly, PT will reduce morphine analgesia. This study examines if morphine analgesia and opioid antagonist-induced supersensitivity are mediated by a common PT-sensitive mechanism. Mice were injected intracerebroventricularly and intrathecally (ICV+IT) with saline or 0.5ug PT. Morphine analgesia (4mg/kg,sc) was blocked at 6 days but not at 1 day following PT. These results indicate that morphine analgesia is sensitive to PT treatment in a time-dependent manner. To determine if PT altered opioid antagonist-induced supersensitivity, mice were injected ICV+IT with saline or PT, and 24hr later implanted sc with a placebo or 15mg naltrexone (NTX) pellet. Pellets were removed in 8 days, and 1 day later, mice were injected with morphine (1-15mg/kg,sc). PT inhibited the analgesic effects of morphine, while NTX treatment increased the analgesic potency of morphine in both control and PT-treated groups. These results suggest that morphine antinociception is mediated through the G_i protein but that some other mechanism is involved in opioid antagonist-induced supersensitivity. (supported by NIDA DA 04185).

270.5

THE EFFECT OF CHRONIC MORPHINE ON SPINAL OPIOID ANALGESIA AND CONVULSIONS. B.C. Yoburn, K. Lutfy*, V. Sierra*, J. Candido*, and F. Tortella!. (SPON: J. WURPEL). Coll. of Pharmacy, St. John's Univ., Queens, NY 11439; !Walter Reed Army Inst. of Research, Wash., DC 20307-5100.

Opioid receptors in the spinal cord have been shown to mediate the analgesic effects of opioid agonists. In the course of examining tolerance to spinal opioids, we found that supra-analgesic doses of spinal morphine produced prominent hindlimb convulsions in the mouse. These studies examined the opioid nature of these effects. Administration of morphine into the spinal intrathecal (IT) space produced dose-dependent analgesia (tailflick) in the mouse (ED_{50}=0.6µg/mouse). At higher doses IT morphine induced seizures of the hindlimbs (ED_{50}=8.7 µg/mouse). Mice treated chronically with morphine (75mg pellet, sc) for 72hr and tested with the pellet in place were tolerant to the analgesic effects of IT morphine (ED_{50}=4.8µg/mouse), but not to the proconvulsant action (ED_{50}=6.3µg/mouse). Chronic morphine treatment produced physical dependence as determined by naloxone-precipitated withdrawal jumping. Spinal morphine analgesia was dose-dependently attenuated by naloxone (5 & 10mg/kg, sc), whereas IT morphine-induced seizures were not. These results indicate that spinal opioid receptors mediate analgesia, but not seizures following IT morphine treatment in the mouse. (supported by NIDA DA 04185)

270.6

F-8-F-Amide AND A-18-F-AMIDE, MAMMALIAN FaRPs, ACT LIKE OPIOID AGONISTS, NOT ANTAGONISTS IN MOUSE COLON. R.B. Raffa and H.I. Jacoby.* Dept. of Biol. Res., Janssen Res. Fndtn., Spring House, PA 19477.

Morphine and the two endogenous mammalian FMRFamide (Phe-Met-Arg-Phe-NH_2)-related peptides known as morphine-modulating neuropeptides, F-8-Famide (Phe-Leu-Phe-Gln-Pro-Gln-Arg-Phe-NH_2) and A-18-Famide (Ala-Gly-Glu-Gly-Leu-Ser-Ser-Pro-Phe-Trp-Ser-Leu-Ala-Ala-Pro-Gln-Arg-Phe-NH_2), were administered intracerebroventricularly (i.c.v.) to mice and the effect of each on colonic bead expulsion time was measured. Each of the three compounds delayed expulsion of a 3 mm glass bead placed in the distal colon. A-18-Famide was more potent than F-8-Famide [ED_{50} = 2.3 µg (1.2 nmole) and 13.9 µg (13.0 nmole), respectively]. A-18-Famide: (i) did not block morphine-induced delay of bead expulsion time, and (ii) was blocked by simultaneous administration (i.c.v.) of 1.0 µg of the competitive opiate antagonist naloxone. These data demonstrate apparent opioid modulatory or agonist-like, rather than antagonist-like, properties of A-18-Famide and F-8-Famide.

270.7

ROLE OF METABOLISM AND RECEPTOR-SUBTYPE INVOLVEMENT IN β-ENDORPHIN (β-END) ANESTHESIA. J.L. Lewis & F.S. LaBella, Lovelace Inhal. Tox. Res. Inst., Albq.,NM and Dept. of Pharmacol., U of Manitoba, Wpg., Man. Canada.

We have reported that ICV infusions of β-END in rats result in naloxone reversible general anesthesia which is mediated within or periventricularly to the inferior third (I3V) or fourth (4V) ventricles. Mean latency to onset of anesthesia from both areas is nearly one hour. We now assess the role of metabolism of β-END in the delayed onset and the type(s) of opioid receptor(s) that mediate the response. Presumed inhibition of β-END degradation by PMSF did not modify latency to onset nor duration of anesthesia. ICV administration of β-END fragments 1-27, 1-17, and 30-31 did not produce anesthesia. Neither the δ ligand DPDPE nor the μ ligand DAGO alone produced anesthesia, but the less specific ligand metkephamid produced anesthesia in 50% of the animals tested with a latency to onset of only 25 min. DAGO + DPDPE, each at one-half the dose used individually, effected anesthesia in all animals tested at either ventricular region. Compared to β-END, latency to onset with the combined peptides was significantly shortened (by nearly 50%), but duration was not affected. These results indicate that (a) metabolic fragments of β-END do not mediate the anesthetic response, (b) the entire β-END molecule is required for anesthesia, (c) both μ and δ receptors must be activated in concert, and (d) the latency to onset reflects recruitment of a yet unidentified physiological system. (Work supported by MRC of Canada)

270.8

ABNORMAL ACTIVITY OF BRAINSTEM NOCICEPTIVE MODULATORY NEURONS IN OPIATE TOLERANCE.
J.B. Bederson*, H.L. Fields and N.M. Barbaro. Depts. of Neurosurgery, Neurology, and Physiology, University of California, San Francisco, CA, 94143

There is evidence that spinal nociceptive reflexes are modulated by reciprocally active inhibitory ("off-cell") and facilitatory ("on-cell") medullary neurons. Opiate analgesia is associated with off-cell activation and on-cell suppression. Tolerance to opiate analgesia could be due in part to increased on-cell activity (and thus increased nociception for a given concentration of opioid).

In rats lightly anesthetized with methohexital, recordings of on-on, off-off, or on-off cell pairs were made. Activity relationships in opiate-naive rats were compared to those in rats with morphine pellets implanted five days earlier.

In opiate-naive rats, the activity of on-on, or off-off pairs was positively correlated (mean Kendall rank correlations, r, =.68 and .60, respectively, $p < .05$, Wilcoxon signed rank test). On-off cell pairs demonstrated strict reciprocity and an inverse activity relationship ($r = -.53$, $p < .05$). In opiate-tolerant rats, although the positive correlation between like pairs was retained, on- and off-cells consistently showed simultaneous activity, i.e. loss of the usual reciprocal discharge pattern, with absence of the normal inverse activity correlation ($r = -.07$, p=ns). On-cells were active despite the continued presence of a morphine pellet.

Thus, opiate tolerance may be explained in part by an increased tendency of the on-cell to fire while opiate-induced off-cell activity continues. It is the increased on-cell firing that leads to decreased antinociception at a maintained concentration of opiate.

(Supported by PHS grant DA01949 and Bristol- Myers Foundation.)

270.9

ANXIOGENIC EFFECTS OF TWO SELECTIVE SIGMA LIGANDS IN THE RAT. N.L. Lai*[1], W.D. Bowen[2], R.R.Matsumoto, A. Thurkauf[3], K.C. Rice[3], and J.M. Walker[1], (SPON: R.M. Church) [1]Department of Psychology and [2]Division of Biology and Medicine, Brown University, Providence RI, 02912 and Laboratory of Neuroscience, Section on Drug Design and Synthesis, NIDDK, Bethesda, MD 20892.

1,3 di-o-tolylguanidine (DTG) and (+)-pentazocine appear to be selective ligands for the sigma receptor. Previous work suggested that sigma ligands affect movement and posture, but the effects of sigma ligands on more complex behavior remain obscure. A role in emotional behavior is suggested by the presence of sigma receptors in limbic areas and reports of anxiety in humans following injections of (+)-pentazocine. To test whether sigma ligands have effects on fear in rats we used a modified version of the Vogel test, a conflict test that predicts anxiolytic potency in humans. Thirsty rats licked a tube for water and were administered a mild electric shock after every 21st lick. As in previous work, chlordiazepoxide reduced the lick suppression produced by this regimen, while a beta-carboline caused a greater suppression. The sigma ligands, DTG and (+)-pentazocine had effects similar to the anxiogenic drug in that they too significantly suppressed licking under both shocked and unshocked conditions. These findings suggest that sigma ligands have fear-inducing effects.

270.10

(+)-OPIATE INHIBITION OF OXOTREMORINE ANALGESIA. J. Michael Walker[1], Saundra L. Patrick[1], Andrew Thurkauf[3], Kenner C. Rice[3] and Wayne D. Bowen[2]. [1]Department of Psychology and [2]Division of Biology and Medicine, Brown University, Providence RI, 02912 and Laboratory of Neuroscience, Section on Drug Design and Synthesis, NIDDK, Bethesda, MD 20892, USA.

Previous work showed that (+)- but not (-)-pentazocine reverses acetylcholine-induced analgesia in mice. Considering the inhibition of carbachol-stimulated phosphoinositol turnover by sigma ligands, it seemed plausible that this effect was mediated by sigma receptors. However, our data suggest that this effect of (+)-pentazocine is not mediated by sigma receptors. (+)-Pentazocine antagonized the analgesic effects of oxotremorine with 50% reversal occurring around 10mg/kg sc. However, this effect was not produced by other sigma ligands including 1,3 di-o-tolylguanidine (DTG), (+)-SKF 10,047, or dextrallorphan. Furthermore, a steep parallel dose curve of nearly equal potency was produced by (+)-nordihydrocodeinone, a compound with negligible (K_i>10,000 nM) affinity for sigma receptors. Yet, (-)-nordihydrocodeinone failed to produce the effect. Several other sigma-inactive (-)-opiates also lacked activity. These findings suggest that some (+)-opiates can produce effects outside the Sigma or PCP system.

270.11

EFFECTS OF SELECTIVE OPIOID AGONISTS ON HIPPOCAMPAL CA3 RESPONSES EVOKED IN OPIOIDERGIC AND NON-OPIOIDERGIC AFFERENTS J. L. Martinez Jr. & B. E. Derrick, Dept. of Psychology, University of California, Berkeley, 94720

Induction of LTP of mossy fiber-CA3 responses is blocked by naloxone and may involve opioid mechanisms at presynaptic sites (Soc. Neurosci. Abstr., 13:767, 1988; Neuron, 1:96, 1988). We therefore compared the effects of selective opioid agonists on CA3 responses evoked in two projections to the CA3 region in vivo: the putatively opioidergic mossy fibers (MF), and the non-opioidergic commissural projection (COMM).

DAGO, a mu-selective agonist (1-100 nmols), produced large transient increases in both MF- and COMM-evoked CA3 responses. Dynorphin A (1-13) amide (10 nmols), a kappa-selective agonist, produced a transient depression of only the MF-evoked CA3 response. The delta-selective agonist D-Pen^2-[D-Pen^5]-enkephalin (DPDPE, 1-10 nmols) produced increases in both the MF- and COMM- evoked field EPSP. The increases in MF-evoked field EPSP were significantly greater than COMM-evoked field EPSPs. The effects observed with DAGO are consistent with an attenuation of GABAergic inhibition. The different effects produced by kappa- and delta-selective agonists on MF and COMM responses suggests that these peptides are not acting exclusively through disinhibition, but through other mechanisms, perhaps at presynaptic sites.

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270.12

NALOXONE INCREASES DELTA ACTIVITY IN THE EEG OF OPIOID NAIVE RATS DURING PERIODS OF HEIGHTENED AROUSAL. K.Grasing* and H.Szeto* (SPON: D.Hinman), Department of Pharmacology, Cornell University Medical College, New York, NY 10021.

Exogenous opioids such as morphine cause a dose dependent increase in high voltage, slow wave EEG activity with stuporous behavior which is followed by a period of increased motor activity with an alert EEG pattern. In previous studies, naloxone has shown no effect on the EEG of opioid-naive animals. Rats were prepared with chronic jugular catheters and electrocortical electrodes, and EEG was analyzed on line by fast fourier transformation. A single 1 mg/kg intravenous infusion of naloxone given at night, when rats are normally active, is followed by an increase in delta power over the 1 to 3 Hz range, a decline in beta 2 power over the 15 to 30 Hz range, and diminished motor activity. These effects did not occur when naloxone was administered during daytime, inactive periods. Dose response analysis revealed that a maximal response was achieved with .3 mg/kg of naloxone.

Endogenous opioid activity is elevated during periods of heightened arousal, and this may explain why naloxone EEG effects are only apparent at night when rats are more active. Activity of endogenous opioids may be associated with different effects on arousal than pharmacologic doses of exogenous opioids.

271.1

STRUCTURE DETERMINATION AND CELLULAR LOCALIZATION OF A NOVEL MYOMODULIN RELATED OCTOPEPTIDE IN *APLYSIA*. E.S.Vilim, E.C.Cropper, A. Alevizos, R.Tenenbaum, I.Kupfermann and K.R.Weiss. Cntr. Neurobiol.& Behav., P & S. Columbia Univ., and NYS Psych. Inst., NYC, NY 10032.

The ARC muscle has provided a model for the study of the behavioral role of neuromodulation and cotransmission. Our previous studies have demonstrated that contractions of this muscle are potentiated by several neuropeptides that are present in the cholinergic motoneurons B15 and B16 . When applied exogenously these peptides interact to modify the properties of muscle contractions. To gain insight into the physiological role of modulation we have undertaken to define the complement of modulatory peptides in the ARC., and have been purifying and sequencing the peptides (e.g. myomodulin and buccalin) that are present in the ARC. motoneurons. We have now purified a new bioactive peptide, and localized it to B16. The structure of the peptide is; Gly-Ser-Tyr-Arg-Met-Met-Arg-Leu-amide. This peptide (MMb) shares a significant homology with another peptide, myomodulin (MM) which is also present in B16. Radiolabeling experiments suggest that B16 synthesizes approximately ten times more MM than the related MMb. Similar synthesis patterns were obtained in cell L10, which also synthesizes both peptides. The homology of peptide structures and the similarity of the rate of their synthesis in L10 and B16, suggests that these peptides may be encoded by the same gene and that MM may be represented by a higher number of copies than MMb. While both peptides potentiate contractions at low concentrations (threshold below $5x10^{-9}$M), their actions diverge at higher concentrations. MM powerfully depresses contractions at concentrations higher than 10^{-7}M, while MMb acts as a depressant only at concentrations higher than 10^{-5}M. The possible physiological significance off these differences is being investigated.

271.2

THE BUCCALIN NEUROPEPTIDE FAMILY IN *APLYSIA*: PURIFICATION OF BUCCALIN C AND SEQUENCE OF ADDITIONAL PEPTIDES PREDICTED BY A cDNA CLONE.
M.W. Miller*, E.C. Cropper, K. Eisinger*, F. Vilim, R. Tenenbaum*, S. Beushausen*, J. Brosius, I. Kupfermann, and K.R. Weiss . (SPON: D. I. Schuster) Center for Neurobiol. and Behav., Columbia Univ., Coll. P & S, and NYS Psychiat. Inst., New York, NY 10032; and Fishberg Ctr. for Neurobiol., Mount Sinai Med. Ctr., New York, NY 10029.

The neuropeptide buccalin, originally purified and sequenced from the accessory radula closer (ARC) muscle of *Aplysia*, has been shown to be present in the two cholinergic motor neurons (B15 & B16) innervating this muscle, where it has been proposed to act as a modulatory cotransmitter (Cropper et al.,1988). The identification of a related peptide, buccalin B (parabuccalin of Weiss et al., 1988), with similar actions, i.e. depression of ARC contractions (and EJPs) produced by motor neuron stimulation, suggested that these peptides may be members of a larger peptide family. Utilizing the methods originally developed for the purification of buccalin A and buccalin B, i.e. HPLC fractionation of an ARC extract and bioassay of fractions for modulatory actions on ARC contractions produced by motor neuron stimulation, we have purified and sequenced a third related peptide, buccalin C, with the following sequence: Gly-Phe-Asp-His-Tyr-Gly-Phe-Thr-Gly-Gly-Ile-amide. A 20-base oligonucleotide derived from this sequence was used to isolate a clone from an *Aplysia* buccal ganglion cDNA library (provided by R. Scheller). The partial cDNA sequence that has been obtained predicts an amino acid sequence which could comprise part of a propeptide containing at least nine distinct, but related neuropeptides. Successive peptides are separated by two or three basic amino acids, possible proteolytic cleavage sites. A glycine residue, which could serve as a donor for amidation, is present on the carboxy terminus of each peptide. Sequences encoding the identified peptides buccalin B and buccalin C are present on this clone.

271.3

RELEASE OF PEPTIDE COTRANSMITTERS UNDER PHYSIOLOGICAL CONDITIONS. E.C. Cropper,[1] D. Price,[2] R. Tenenbaum,[1] I. Kupfermann,[1] and K.R. Weiss.[1] Ctr. Neurobiol. & Behav., Columbia Univ. P&S, & NYS Psych. Inst. NY,NY 10032[1]; C V Whitney Lab., St. Augustine, FL 32086[2].

The cholinergic motor neuron B15, which innervates the ARC muscle of *Aplysia*, contains the neuropeptides, SCP_A and SCP_B. When exogenously applied these peptides increase muscle cAMP levels, and modulate parameters of muscle contraction; they increase contraction size and relaxation rate.

To determine whether B15 releases the SCPs under physiological conditions, electrodes were implanted in ARC muscles of intact animals, and *in vivo* firing patterns of neuron B15 were determined by recording synaptic currents from ARCs of feeding animals. During ingestion of strips of food B15 fired in bursts for about 3.5 sec (duty cycle 50%) at about 7.5-10 Hz. This firing pattern was simulated on one side of buccal ganglion-buccal mass preparations and comparisons were made for control and stimulated levels of SCP_A and SCP_B. Stimulation produced a decrease in SCP levels, indicating that peptide release occurred. In a second group of animals the relaxation rates of contractions produced by physiological patterns of stimulation of B15 were compared to those of contractions produced by a nonphysiological pattern of stimulation with long interburst intervals. Relaxation rates under physiological conditions progressively increased; those under nonphysiological conditions remained unchanged.

These results indicate that the SCPs are released when neuron B15 fires at physiological frequencies, and suggest that the SCPs are released in sufficient quantities to act physiologically as cotransmitters.

271.4

SORTING OF THE BAG CELL PEPTIDES AND EGG-LAYING HORMONE: LOCALIZATION OF THE SORTING EVENT. W.S. Sossin and R.H. Scheller. Department of Biological Sciences, Stanford University, Stanford, CA 94035-5020.

We have recently demonstrated, using immunohistochemical techniques, that two regions of the egg-laying hormone (ELH) prohormone are localized to different classes of dense core vesicles (DCVs) (Fisher et al., Cell, 54: 813-822, 1988). By taking advantage of the asymmetric distribution of amino acids in the two regions of the prohormone, and the technique of electron microscopic autoradiography, we have independently demonstrated that the different regions of the prohormone are sorted into distinct DCVs. Furthermore, we have identified the time course and locale of the sorting event. Sorting occurs soon after the cleavage of the prohormone in immature vesicles. The sorting compartment is part of the Trans-Golgi network as defined by staining with acid-phosphatase. A model for the mechanism of sorting based on these findings will be presented.

271.5

DIFFERENTIAL PEPTIDE SECRETION FROM THE BAG CELLS OF *APLYSIA*. G. Whitney* and S. Arch. Biological Laboratories, Reed College, Portland, OR 97202

We have employed a combination of electrophoretic and HPLC procedures to investigate the secretory output of the bag cells. Our principal interest has been to use K^+-evoked depolarization to evaluate previous indications that bag-cell derived peptides are not secreted in fixed stoichiometries. When bag cell organs (BCO) are isolated from the abdominal ganglion, incubated in ^{3}H-leu for 18hr, and exposed to high-K^+ medium, 8 peaks of radiolabeled material are reliably resolved by HPLC. To evaluate secretion stoichiometry, we have concentrated on the 3 most abundantly labeled of these (p4, p10, and p12). Under conditions of 2 sets of 3 successive 15min superfusions with high-K^+ medium separated by 60min of normal saline incubation, the radiolabel content ratio between p4 and p10 remains effectively constant. However, the ratio between p12 and either p4 or p10 changes systematically in a manner indicating increasing relative amounts of p12 secretion with increasing depolarization exposure. The IEF- and SDS-PAGE profiles obtained from p10 and p12 are consistent with their identification as the peptides AP and ELH respectively. Moreover, neither of these species is detected in secretion medium collected from cells labeled with ^{3}H-phe. Analyses of p4 indicate that it is labeled with ^{3}H-phe, has an acidic pI and a molecular weight of *ca.* 20kD. Although speculative at present, the labeling and physical characteristics of p4 are consistent with those expected of the N-terminal intermediate derived from the initial cleavage of the proELH molecule at positions 181-184. Extraction of neurites and terminals separated from cell bodies demonstrates the presence and secretory depletion of p4 along with AP and ELH. The secretory pattern indicates that p4 and AP are cosecreted while ELH secretion is kinetically distinct.

Supported by NIH-NS 11149.

271.6

NEUTRAL METALLOENDOPEPTIDASE (NEP)-LIKE ENZYME CLEAVES ALPHA-BCP IN CNS OF *APLYSIA*. G.A. Phares*¶, B.S. Rothman¶, A. Arvand*¶, M. Talebian*¶, D.B. Borson*#, J.A. Nadel*#, J. Calaycay*§, J.E. Shively*§, M.P. Nusbaum¶, S.T. Lockhart*¶, L.D. England¶*, and K.A. Briggs-Crawford¶*. ¶ Dept. of Biology, San Francisco State University, San Francisco, CA 94132; # Cardiovascular Res. Inst., U.C.S.F., San Francisco, CA 94143; § Beckman Research Institute, City of Hope Med. Ctr., Duarte, CA 91010.

We are studying inactivation of the peptide neurotransmitter alpha-bag cell peptide (α-BCP[1-9], single letter code: **APRLRFYSL**) in the CNS of *Aplysia*. We mimic release of α-BCP without the concomitant release of the other bag cell peptides by arterially perfusing synthetic α-BCP into an isolated abdominal, pedal-pleural or cerebral ganglion. The perfusate is analyzed by reverse phase-HPLC. When perfused at 1-50 μM, 5% to 15% of α-BCP[1-9] is recovered as α-BCP[6-9], and somewhat lower amounts recovered as α-BCP[1-5], and [7-9]. These products were identified by amino acid composition, sequence, and FAB-mass spectroscopic analyses. (α-BCP[6-9] was incorrectly identified as α-BCP[1-8] in a previous abstract (Rothman et al., *Neurosci. Abstr.* 13: 39, 1987)). Thiorphan, phosphoramidon or carboxymethyl-Phe-Leu (10 μM each) each significantly inhibit generation of these products, whereas captopril (10 μM) is ineffective. The same set of products, plus α-BCP[1-6], is generated by incubating α-BCP with recombinant human NEP. Our results suggest that α-BCP[1-9] is cleaved in the CNS of *Aplysia* by an enzyme resembling NEP (E.C. 3.4.24.11). NEP exists in mammalian brain, where it cleaves enkephalin and possibly other neuropeptides at bonds N-terminal to hydrophobic residues. In *Aplysia* the NEP-like enzyme is likely to inactivate α-BCP and other peptides after their release by the bag cells. *Supported by NIH grants RO1-NS-24046 (BSR) & K04-NS-01177 (BSR).*

271.7

AMINOPEPTIDASES IN BLOOD AUGMENT DEGRADATION OF ALPHA-BAG CELL PEPTIDE IN *APLYSIA*. C. R. Squire*¶, B. S. Rothman¶, J. Calaycay*§, J. E. Shively*§, and E. H. MacNeale*¶. (SPON: J.S. Williston¶). ¶ Dept. of Biology, San Francisco State University, San Francisco, CA 94132; § Beckman Research Institute, City of Hope Med. Ctr., Duarte, CA 91010.

We are investigating the role that enzymes in blood play in the inactivation of the neurotransmitter alpha-bag cell peptide (α-BCP[1-9], single letter code: APRLRFYSL) by incubating synthetic α-BCP (1 and 10 μM) in plasma. Enzyme activity is halted by TCA-precipitation and samples are analyzed by reverse phase HPLC. α-BCP[1-9] is slowly degraded in blood ($T_{1/2}$=36 min), whereas α-BCP[3-9], a neuroactive product of a membrane-bound ganglionic peptidase (Rothman et al., *Neurosci. Abstr.* 13: 39, 1987), is degraded rapidly ($T_{1/2}$=3 min). Bestatin (65 μM) considerably extends the life of α-BCP[3-9] ($T_{1/2}$=32 min). α-BCP[3-9] yields four cleavage products in blood: α-BCP[4-9], [5-9], [6-9], and [7-9]. These were identified by amino acid composition, sequence, and FAB-mass spectroscopic analyses. When each cleavage product alone is incubated in blood, [4-9] yields [5-9], [6-9] and [7-9]; [5-9] yields [6-9] and [7-9]; [6-9] yields [7-9]. Each of these cleavages is blocked by bestatin. These findings are consistent with the hypotheses that: 1) α-BCP[3-9] is sequentially cleaved in blood by one or more aminopeptidases, and 2) α-BCP[1-9] resists aminopeptidase cleavage due to the Pro residue at position #2. Because blood perfuses *Aplysia* ganglia *in vivo*, and there is no functional blood-brain barrier, the aminopeptidases in blood most likely act in concert with membrane-bound ganglionic peptidases to degrade α-BCP[1-9] into inactive fragments, thereby limiting the range of its actions. This experimental system may be a useful model of the actions of peptidases in the cerebrospinal fluid of vertebrates.

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271.8

RELEASE OF PEPTIDE COTRANSMITTERS (SCPs) FROM A CHOLINERGIC MOTOR NEURON IN *APLYSIA*. P.E. Lloyd and M. D. Whim* Dept. Pharm. Physiol. Sci. & Comm. on Neurobiol. Univ. Chicago, Chicago, IL 60637.

Cholinergic motor neuron B15 innervates buccal muscle I5 (also termed ARC) and contains and synthesizes the SCPs (A & B)(Cropper et al.,PNAS 84:3486, '87). SCPs synthesized in the buccal ganglia are transported to terminals in I5 (Lloyd, J.Neurosci.8: 3507,'88). However buccal nerves contain only ~1% of the SCPs found in muscle so, over periods <4 h , release cannot be replaced by axonal transport and should produce depletion of SCPs from muscle. SCP levels were found to be identical for bilateral muscles from the same animal when measured with the sensitive accurate snail heart bioassay. Thus, release can be measured by comparing SCP levels of I5 muscles after B15 stimulation with those of contralateral control muscles. Stimulation of B16, a cholinergic I5 motor neuron which does not contain the SCPs, at 50 Hz for 1s every 9s, or sustained hyperpolarization of B15, for 1 h produced no depletion (stim/control ratio =1.04±0.04 & 1.05±0.03 SEM, N=4 for each) . The first experiments used stimulation of B15 at an overall rate of 5 Hz for 1 h with varied spike grouping. Tonic stimulation at 5 Hz, or 25 Hz for 2s every 8s produced no sigificant depletion (1.04±0.04 & 0.99±0.07, N=4 for each) while 50 Hz for 1s every 9s produced a depletion of 27±3% (N=4). This corresponds to release of ~10 fmol SCPs/min in 10 mg muscles. Increasing the burst duration (25 Hz for 4s every 6s for 1 h) also depleted SCP levels by 26±6% (N=4). Thus significant release of SCPs from B15 occurs only during highly facilitated release conditions such as high frequency firing or lower frequency firing for longer durations. Some physiological consequences of this release are addressed in the accompanying abstract (Whim & Lloyd,'89). Supported by NS 23569.

271.9

RELEASE OF PEPTIDE COTRANSMITTERS (SCPs) FROM AN IDENTIFIED MOTOR NEURON ELEVATES cAMP IN ITS TARGET MUSCLE IN *APLYSIA*. M.D. Whim* and P.E. Lloyd. (SPON: J. Goldberg). Dept. Pharm. Physiol. Sci. & Comm. on Neurobiol. Univ. Chicago, Chicago, IL 60637.

Cholinergic motor neuron B15 synthesizes the SCPs and innervates muscle I5. SCPs potently elevate cAMP in the I5 muscle (Lloyd et al., PNAS 81: 2934, '84). We tested the ability of B15 stimulation to increase cAMP in an I5 muscle compared to the unstimulated contralateral muscle. After 10 min stimulations, muscles were frozen and cAMP levels determined. Stimulation of B15 at 50 Hz for 2s at 3s intervals produced very large increases of cAMP (stim/control ratio = 330.8±63.0; SEM, N=4). Using the same paradigm, stimulation of a second cholinergic motor neuron (B16) which innervates I5 but does not contain the SCPs resulted in very little elevation (1.44 ±0.66; N=4). Several B15 stimulation paradigms at an overall rate of 5 Hz were tested. Tonic 5 Hz or 25 Hz for 2s every 8s produced very little elevation (respectively, 1.39±0.47; 1.44±0.46; N=4), however 50 Hz for 1s every 9s resulted in a 5.34 ±2.11 (N=4) increase in cAMP. Qualitatively, these results parallel very closely the direct measurements of SCP release using an unrelated procedure (see accompanying abstract). Frequency, length of stimulation and interburst intervals were systematically varied. B15-induced elevation of cAMP levels was very sensitive to these parameters. For example, stimulation of B15 for 4s at 3s intervals now produced an increase in cAMP at a much reduced frequency (15Hz; 16.48±5.0; N=4). We conclude that the elevation of muscle cAMP levels are produced by the release of the SCPs from B15 terminals in the muscle. Supported by NS 23569.

271.10

MOLECULAR STUDIES ON LUQ-SPECIFIC NEUROPEPTIDE GENES AND ON NEUROPEPTIDE DEGRADATION IN APLYSIA CALIFORNICA.
L. DesGroseillers*, L. Wickham* and X. Yang* (SPON; A. Ferron) Department of Biochemistry, University of Montreal, Montreal, Canada H3C 3J7

My laboratory is interested in the characterization of molecules involved in neural cell communication. We used molecular genetic approaches to isolate these molecules and study their regulation and functions. LUQ cells seem to play important roles in the control of the kidney. In order to study neurotransmitters involved in kidney functions, we used differential screening to isolate LUQ-specific neuropeptide genes. The LUQ-1 cDNA clone is 3.7kb long. Dot blot and in situ hybridization experiments have revealed that the gene is expressed in only 1 of the 5 LUQ cells. Southern blot experiments also show that the gene is present in 1 copy per haploid genome.

To understand the homeostasis of the peptidergic nervous system, we also want to study the mode of peptide degradation. To do this we have cloned a neutral endopeptidase-like enzyme (NEP-like) whose function is believed to terminate the action of some neuropeptides. This clone shows 82% homology with the mammalian NEP sequences around the active site. Characterization of this enzyme in the simple Aplysia nervous system will help to understand the fine tuning of neural cell communication.

271.11

INSULIN RELATED SUBSTANCES IN THE CENTRAL NERVOUS SYSTEM OF PULMONATES AND *APLYSIA CALIFORNICA*. J. van Minnen and A.B. Smit. (SPON: European Neuroscience Association) Dept. of Biology, Vrije Universiteit, Amsterdam, The Netherlands

In the snail *Lymnaea stagnalis* the presence of an insulin-related substance was demonstrated by means of molecular biological techniques. Using *in situ* hybridization it was shown that transcription of this molluscan insulin-related peptide (MIP) occurs in 2 clusters of giant neuroendocrine cells that are located in each cerebral ganglion. There exists endocrinological evidence that these neurons are involved in the regulation of processes related to body growth, such as growth of the soft body parts and shell, glycogen metabolism and ornithine decarboxylase activity. Screening of the cDNA of the CNS and *L. stagnalis* genomic library revealed the presence of at least five closely related MIP genes, all encoding prohormones that share the characteristics of vertebrate proinsulin, *i.e.* they are composed of an B- and A-chain and a connecting C-peptide and have amino acids that are essential for the tertiary structure of proinsulin (*viz.* cysteins) at identical positions as vertebrate insulins. Remarkably, the C-peptide of the various MIPs showed a high degree of homology at the amino acid level, which may suggest an important function for this peptide (in vertebrates no function is known for the C-peptide). By means of immunocytochemistry, using antibodies that were raised against the C-peptide of MIP, the CNS of other pulmonates and the opisthobranch *A. californica* were investigated. In basommatophoran snails immunoreactive neurons were found at identical locations as in *L. stagnalis*; in the stylommatophorans *Helix aspersa* and *Limax maximus* in each cerebral ganglion one cluster of immunoreactive neurons was found. Previous experiments have shown that those neurons in *H. aspersa* produce a growth hormone. In *A. californica* all central ganglia contained immunoreactive neurons. The highest number was observed in the abdominal ganglion (approximately 50 with diameters varying from 30-150 μm). In each pedal ganglion a cluster of about 40 neurons (diam. 20-40 μm) was found.

271.12

DOPAMINE-DEPENDENT MODULATION OF IONIC CONDUCTANCES AND PROTEIN PHOSPHORYLATIONS IN HELIX ASPERSA NEURONS.
S. Tiwari¶ Y-K. Kim*and M.L. Woodruff. Depart. of Chem. and Biochem., Southern Ill. Univ., Carbondale, Ill 62901.

Dopamine (DA) causes an inhibition of neurons in the circumesophageal ganglion of the land snail Helix aspersa. The neurons hyperpolarize and their spontaneous action potentials cease with DA. In addition, the shape of evoked action potentials is modified--action potential height and duration are decreased. Voltage-clamp experiments reveal that Ca^{2+} conductance, Ca^{2+}-dependent K^+ conductance and a transient K^+ conductance are decreased by DA. The Ca^{2+} current may be mediated by two different channel types, a transient and a sustained Ca^{2+} channel current. Both are inhibited by DA.

Forskolin, a potent activator of adenylate cyclase, effects the physiology, but does not mimic the DA effect. Oleoyl acetyl glycerol (OAG), an activator of protein kinase C, partially blocks the DA-dependent decrease in Ca^{2+} current. The possible role of protein phosphorylation in mediating the effects of DA is being examined by in vivo ^{32}P labeling experiments. Intact ganglia and F-lobe cells in primary cell culture both show DA-dependent phosphorylations and dephosphorylations; however the pattern of labeling is different in the two cases. Cells in primary cell culture eliminate glial cells and this could be partially responsible for the different patterns. We are presently trying to relate specific phosphorylations and dephosphorylations to decreases in ionic conductances.

272.1

NO-949: ANXIOLYTIC GABA-UPTAKE INHIBITOR. Erik B. Nielsen. CNS Pharmacology Lab., NOVO Industri A/S, CNS Division, DK-2880 Bagsvaerd, Denmark.

NO-949 (N-((4-phenyl-4-N-methyl pyrrol-2-yl)buten-3-yl)guvacine hydrochloride) is a potent, selective GABA-uptake inhibitor (IC_{50} for synaptosomal GABA-uptake ~185 nM) which was characterized in various anxiolytic models. NO-949 was active in a modified Vogel conflict test in rats (MED 1 mg/kg i.p.) and in a conditioned emotional response (CER) test with a MED of 0.3 mg/kg i.p. Further, NO-949 was also active in a "light-dark" test in mice with a MED of 1 mg/kg (i.p.). Finally, since GABA-uptake inhibitors fail to substitute for diazepam in drug discrimination situations, it indicates that anxiolytic effects of GABA-uptake inhibition may be obtained in the absence of benzodiazepine-like subjective effects.

272.2

BIOCHEMICAL CHARACTERIZATION OF CI-966: A CENTRALLY ACTIVE GABA UPTAKE INHIBITOR. L.J. Brahce,* R.D. Schwarz, D.K. Boyd, L.L. Coughenour,* T.A. Pugsley, and C.R. Clark (SPON: L. Copeland). Parke-Davis Pharm. Res. Div., Warner-Lambert Co., Ann Arbor, MI.

Recent studies have shown that enhancing synaptic GABA-ergic activity in rodents results in a variety of anti-convulsant behaviors. 1-[2-[bis(trifluoromethyl)-phenyl]methoxy]ethyl]-1,2,5,6-tetrahydro-3-pyridine-carboxylic acid, HCl (9CI-966) is a novel GABA uptake inhibitor which has anticonvulsant properties. CI-966 was found to selectively block [^{3}H]-GABA uptake into rat hippocampal slices (IC_{50}=0.32uM) while having no effects on $^+$DA, NE, 5-HT, or D-aspartate uptake. Inhibition of Na^+ flux was observed at 10uM. There was no detectable affinity for most receptors examined, however, some binding was observed at DA_2 and sigma receptors as well as site 2 of voltage-sensitive Na^+ channels. CI-966 did not affect the rate of DA and 5-HT synthesis in rat brain, but did appear to slightly increase (+28%) the rate of NE synthesis in cortex (but not brain stem) following the IP administration of 30mg/kg. These results, together with those of Taylor, et al. (this meeting), suggest that the mechanism by which CI-966 blocks convulsant activity is inhibition of GABA uptake. In initial clinical trials, CI-966 produced adverse effects.

272.3

ANTICONVULSANT AND BEHAVIORAL EFFECTS OF CI-966 AND PD 126141, POTENT GABA-UPTAKE INHIBITORS, IN ANIMALS. C.P. Taylor and M.G. Vartanian*. Parke-Davis Pharmaceut. Res. Div., Warner-Lambert Co., Ann Arbor, MI 48105.

CI-966 selectively inhibits GABA uptake in vitro (IC_{50} = 322 nM) and is an anticonvulsant in rodents, but was associated with adverse events in initial clinical trials.

CI-966 potently blocks clonic threshold seizures from pentylenetetrazole (PTZ, ED_{50} = 0.4 mg/kg PO mice) but prevents maximal electroshock extensor seizures only at much higher doses (ED_{50} ≈ 60 mg/kg PO mice). CI-966 (ED_{50} = 2.6 mg/kg PO) raises afterdischarge threshold in rat hippocampus, a model for partial seizures. PD 126141 (GABA uptake IC_{50} = 200 nM) blocks PTZ seizures in mice and causes ataxia with potency similar to CI-966 while its enantiomer is inactive for either effect at 100 mg/kg PO.

CI-966 reduces spontaneous locomotor activity (30 mg/kg PO mice) and causes ataxia (64 mg/kg PO mice, 27 mg/kg PO rats, approx. 5 mg/kg PO dogs, approx. 3 mg/kg IM rhesus monkeys). At ataxic doses, reactions to sensory stimuli are reduced with tremors or myoclonus of extremities; patellar reflexes are enhanced with splayed hindlimbs. Behavioral changes reverse within 4-10 hr. CI-966 has only slight cardiovascular effects.

PD 126141 blocks GABA uptake in vitro and is active in vivo with much greater potency than its enantiomer. We conclude that both anticonvulsant and behavioral actions are mediated by stereospecific blockade of GABA uptake.

272.4

ELECTROPHYSIOLOGICAL ACTIONS OF A POTENT GABA-UPTAKE INHIBITOR. D.M. Rock, C.P. Taylor, U. Ebert* and K. Krnjevic. Parke-Davis Pharmaceutical Res. Div., Warner-Lambert Co., Ann Arbor, MI 48105 and Physiol. and Anaesth. Res. Depts., McGill Univ., Montreal.

CI-966 is a GABA uptake inhibitor (IC_{50} = 322 nM) that has anticonvulsant activity in animals but was associated with adverse events in initial clinical trials. We tested potential mechanisms of CI-966 in vivo and in vitro using electrophysiological techniques.

In urethane-anesthetized rats, CI-966 (1-10 mg/kg IP) tended to reduce the amplitude of population spikes, and increased inhibition of paired orthodromic (commissural stimulus) population spikes in hippocampal area CA1. Recurrent inhibition (alvear stimulus) was also increased. CI-966 (10 mg/kg IP) extended the duration of orthodromically-induced inhibition by as much as 30-fold and also enhanced and prolonged the depressant action of iontophoretically-applied GABA on population spikes.

In cultured mouse spinal cord neurons, CI-966 (10 μM) did not change intracellular responses to iontophoretically-applied GABA or glutamate. However, CI-966 blocked sustained rapid firing (IC_{50} ≈ 10 μM), presumably by acting on voltage-sensitive sodium channels.

These results with CI-966 are consistent with a functional decrease in the uptake of GABA in vivo, and an additional effect on neuronal sodium channels at relatively high concentrations.

272.5

ACTIONS OF A GABA-UPTAKE INHIBITOR, CI-966, AND MUSCIMOL ON NEUROTRANSMITTER OVERFLOW IN THE RAT STRIATUM USING IN VIVO MICRODIALYSIS. L.W. Cooke, L. Ball*, C.P. Taylor, and M.D. Davis* (SPON: M.J. Callahan) Parke-Davis Pharm. Res. Div., Warner-Lambert Co., Ann Arbor, MI 48105.

CI-966 is a selective GABA reuptake inhibitor (IC50 = 320nM) as established in a number of biochemical and pharmacological tests that also exhibits anticonvulsant properties in rodents. We report here changes in amino-acid and catecholamine overflow from the rat striatum after systemic administration of CI-966 and muscimol.

Adult male Sprague-Dawley rats were anesthetized with urethane, mounted in a stereotaxic frame and implanted with a Carnegie Medicin microdialysis probe (4mm) into the left striatum. Artificial CSF was infused through the probe (2ul/min) and samples were collected at 20 min intervals for analysis by HPLC with electrochemical detection. After a suitable baseline was established drugs were injected IP.

In preliminary studies, CI-966 (5,10,25 mg/kg) caused a dose-dependent increase in dopamine overflow of up to 105% with little change in DOPAC or HVA. At 10 mg/kg, GABA and glycine levels were also elevated in the dialysates by 123% and 168% respectively. The GABAa agonist, muscimol (4mg/kg), increased dopamine overflow by 98%. These results demonstrate that CI-966 is a modulator of GABA neurotransmission in vivo and that this action may also influence brain dopamine neuronal function. In initial clinical trials with CI-966, adverse side-effects were observed.

272.6

EFFECTS OF THE GABA UPTAKE INHIBITOR, CI-966, ON THE FIRING ACTIVITY OF MIDBRAIN DOPAMINE NEURONS. K.A. Serpa, C.L. Christoffersen and L.T. Meltzer. Department of Pharmacology, Parke-Davis Pharmaceutical Research Division, Warner-Lambert Co., Ann Arbor, MI 48105.

There is a complex interaction between dopamine (DA) and GABA. Depending on the intensity, electrical stimulation of the mainly GABAergic striatal-nigral pathway can either excite or inhibit DA neurons in the substantia nigra (SN) (Grace and Bunney, Brain Res. 333:271,1985). Additionally, systemic administration of the GABA-A agonist, muscimol, paradoxically increases the activity of DA neurons (Grace and Bunney, Eur. J. Pharmacol. 59: 211, 1979). We have begun to evaluate the effects of CI-966, a potent GABA uptake inhibitor in vitro (IC-50 = 322 nM), on the firing activity of SN DA neurons extracellularly recorded in chloral hydrate anesthetized male S-D rats.

CI-966, 10 & 20 mg/kg IP, increased, decreased or did not change DA neuron firing. In contrast, muscimol, 5 mg/kg IP, consistently increased firing activity. The different effects due to GABA uptake blockade and direct GABA-A agonist action may be due to low tonic GABA release in the anesthetized rat. Studies are now examining the effect of CI-966 on the response of DA neurons to stimulation of the striatal-nigral pathway.

Adverse effects occurred during the initial clinical trial of CI-966.

272.7

EFFECT OF GABA-BENZODIAZEPINE RECEPTOR LIGANDS ON SEIZURE THRESHOLD IN DIAZEPAM-SENSITIVE AND -RESISTANT MICE. E.J. Gallaher* and S.E. Gionet* (SPON: JK Belknap). VA Medical Center and Depts. of Pharmacology and Medical Psychology, Oregon Health Sci. Univ. Portland, OR 97201.

Diazepam (DZ)-sensitive (DS) and DZ-resistant (DR) mouse lines were developed by selective breeding based on rotarod impairment (Gallaher et al., Psychopharmacol., 93:25, 1987). DS and DR mice do not differ in pentylenetetrazol (PTZ) seizure threshold or DZ protection against PTZ, suggesting different mechanisms for rotarod and seizure activity. Here we study other GABA-BZ receptor ligands.

Bicuculline (competitive GABA antagonist), DMCM (BZ inverse agonist), and picrotoxin (chloride channel blocker) were infused into the tail vein until seizures were observed; DS and DR lines did not exhibit differences in seizure threshold. Since they do not produce seizures themselves, the partial inverse agonists FG-7142 (0-550 mg/kg) and beta-CCE (0-300 mg/kg) were injected ip, followed by PTZ infusion. Dose-dependent decreases in PTZ threshold indicated pro-convulsant activity; again no line differences were observed. Ro 15-1788, a BZ receptor antagonist, was injected ip (0-100 mg/kg) followed by PTZ infusion; this drug did not affect the PTZ threshold in DS or DR mice.

These data further suggest that the mechanism underlying seizure activity differs from that involved in the rotarod behavior used for selective breeding. (Supported in part by VA Research Service and PHS RO1 NS23927.)

272.8

PRENATAL EXPOSURE TO DIAZEPAM ACCENTUATES AGE-RELATED DIFFERENCES IN SEIZURE SENSITIVITY TO BICUCULLINE.
Daniel Bitran, Rajesh Miranda, Renee Primus, and Carol K. Kellogg.
University of Rochester, Department of Psychology, Rochester, NY 14627

Prenatal diazepam (DZ) exposure has been shown to alter neurochemical and functional indices of early interaction at the GABA/BDZ receptor complex long after the drug has disappeared from the brain and periphery (see Kellogg, *Prog Brain Res* 73: 207, 1988). Since GABAergic mechanisms have been implicated in convulsive disorders, we examined seizure susceptibility in juvenile (21 day old) and adult (75 day old) male offspring of female rats treated with DZ (1 or 2.5 mg/kg, SC) over gestational days 13 to 20.

Seizure was induced by IV administration of bicuculline (BIC, 50 μg/ml) at an average rate of 200 μl or 2 ml per minute, in juvenile and adult males, respectively. EEG recordings were used to discern the appearance of epileptogenic activity, continuous high-amplitude rapid spiking, and post-ictal depression. Animals were anesthetized, paralyzed, and ventilated during seizure induction.

Onset of seizure activity was found to vary as a function of age and prenatal drug exposure. Control animals were less sensitive to the convulsive effects of BIC at 21 days of age, relative to 75 day-old control animals. Prenatal treatment with DZ enhanced this age-related difference in seizure threshold: Juvenile drug-exposed animals showed a 30-40 percent increase in the convulsant dose of BIC (333 ± 10 μg/kg), relative to age-matched controls (250 ± 9 μg/kg); in adult males seizure threshold was lower in DZ- exposed males (130 ± 10 μg/kg) relative to age-matched controls (200 ± 4 μg/kg). Thus, prenatal treatment with DZ decreased seizure susceptibility to BIC in juvenile males but increased the sensitivity to BIC-induced seizure in adult animals. In contrast, neither age nor prenatal drug treatment affected the duration of high intensity rapid spiking activity and post-ictal depression. In order to facilitate interpretation of these functional changes, studies examining GABA/BDZ receptor-mediated chloride influx and receptor binding in prenatally DZ-exposed offspring are underway. Supported by grant MH31850.

272.9

GLUTAMATERIC PROCESSES INVOLVED IN KINDLING INDUCED BY THE BENZODIAZEPINE RECEPTOR INVERSE AGONIST; FG 7142.
D.N. Stephens*, L. Turski, J.D. Turner* and H.H. Schneider*. (SPON: W. Kehr) Dept. Neuropsychopharmacology, Schering AG, P.O.B. 65 03 11, 1000 Berlin 65, FRG

FG 7142, a beta-carboline partial inverse agonist at central benzodiazepine receptors induces kindling in rats and mice on repeated administration. Kindling depends on FG 7142's action at benzodiazepine receptor, but no changes in the biochemical pharmacology of the benzodiazepine/GABA receptor complex have been found which could explain the increased sensitivity. Now we report that in mice both the expression of FG 7142 (40 mg/kg, i.p.) kindled seizures and the development of kindling were antagonised by 2-aminophosphonoheptanoic acid (2-APH; 0.025 μmol, i.c.v.) and MK 801 (0.5 mg/kg, i.p.), respectively competitive and non-competitive antagonists at glutamate receptors of the NMDA subtype. The quisqualate antagonist, CNQX (0.025 μmol i.c.v.) and kainate antagonist, AMPA (0.025 μmol i.c.v.) did not antagonise the expression of FG 7142 kindled seizures. Preliminary autoradiographic studies suggest an increase in ^{3}H-glutamate binding in hippocampus and cortex of FG 7142 kindled rats (15 mg/kg, i.p.). These results suggest that FG 7142-induced kindling, in common with electrical kindling, may be associated with changes in glutamatergic activity.

272.10

IN VITRO AND IN VIVO ACTIONS OF 3α-OH-5α-REDUCED PREGNANE STEROIDS. D. Belelli*, M. Bolger* and K.W. Gee, Univ. of So. Calif, School of Pharmacy, Los Angeles, CA 90033

Progesterone (P) and some of its metabolites modulate the $GABA_A$/Benzodiazepine receptor linked Cl^- ionophore (GBRC) by specific interactions with a putative steroid recognition site. The most potent P metabolite, 5α-pregnan-3α-ol-20-one (3α-OH-DHP), has been reported to inhibit completely the binding of [^{35}S]t-butylbicyclophosphorothionate ([^{35}S]TBPS) to a site on or near the Cl^- ionophore whereas 5α-pregnan-3α,20α-diol (5α-pregnanediol) was found to have limited efficacy as an inhibitor. This allosteric modulation of [^{35}S]TBPS binding led us to evaluate the possibility that the action of the two pregnanes are mediated through a common site associated with the chloride ionophore. In this study 3α-OH-DHP and 5α-pregnanediol behave like full and partial agonist respectively, in the modulation of GABA-stimulated ^{36}Cl uptake. Moreover, the high potency of 3α-OH-DHP in both binding and functional assays is reflected in in vivo studies. 3α-OH-DHP has potent anticonvulsant actions in standardized anticonvulsant screens with maximum potency against (+)bicuculline (ED_{50} = 4.1 mg/kg, i.p.) induced seizures and no activity against maximum electroshock and strychnine induced convulsions. These studies suggest that therapeutically useful anticonvulsant steroids can be identified. (Supported by NIH Grant NS 24645)

272.11

DIFFERENTIAL BEHAVIORAL EFFECTS OF RO 15-4513 AND FG 7142 IN CHRONIC ETHANOL-TREATED RATS. A. K. Mehta* and M. K. Ticku. Dept. of Pharmacology, Univ. of Texas Hlth. Sci. Ctr., San Antonio, TX 78284-7764.

Pentylenetetrazol (PTZ)-induced convulsions were studied in control, chronic ethanol-maintained, and ethanol-withdrawal rats. Ethanol-maintained rats required higher doses of PTZ to produce convulsions, compared to control and ethanol-withdrawal rats. Ro 15-4513 (2 mg/kg, i.p.) and FG 7142 (20 mg/kg, i.p.) produced proconvulsant effect in control and ethanol-withdrawal rats as they potentiated the effect of subconvulsive dose of PTZ. A higher dose of Ro 15-4513 (4 mg/kg, i.p.), but not FG 7142 (up to 80 mg/kg, i.p.) also produced proconvulsant effect in ethanol-maintained rats. Furthermore, Ro 15-4513 (5, 10 mg/kg, i.p.), but not FG 7142 (up to 80 mg/kg, i.p.) produced clonic-tonic seizures of short duration in ethanol-withdrawal rats. These effects of Ro 15-4513 and FG-7142 were reversed by diazepam, as well as by Ro 15-1788, thereby, indicating the involvement of central benzodiazepine (BZ) receptors in the action of Ro 15-4513 and FG 7142. These observations suggest that chronic ethanol treatment selectively alters the receptor sensitivity to Ro 15-4513, a partially negative ligand for BZ sites.

272.12

PARALLELES BETWEEN THE BEHAVIORAL EFFECTS OF DMCM & CONDITIONAL FEAR. M. S. Fanselow, F. J. Helmstetter, & D. J. Calcagnetti* Dept. of Psychology, UCLA, LA, CA 90024 & Dept. of Psychology, Dartmouth College, Hanover, NH 03755.

The benzodiazepine inverse agonist methyl 6,7-dimethoxy-4-ethyl-ß-carboline-3-carboxylate (DMCM) has been reported to act as an anxiogenic. Therefore, we examined its behavioral effects to determine if it would produce behaviors similar to those produced by Pavlovian conditional fear stimuli. In situations associated with electric shock rats exhibit a species typical freezing response, increased defecation, and analgesia. DMCM produced similar responses in nonshocked rats. That is, it produced freezing (in a small chamber and an open field), increased defecation, and analgesia (formalin and hot plate tests). DMCM also enhanced freezing to a shock associated context. Opioid antagonists reverse conditional fear-induced analgesia but naltrexone produced only a weak attenuation of DMCM analgesia. Conditional fear stimuli potentiate the acoustic startle reflex. However, DMCM attenuated acoustic startle. Thus there are some similarities between conditional fear-induced behavior and the behavioral effects of DMCM but there are also some important differences. While benzodiazepine antagonists (Ro 15-1788 & ZK 93-426) reversed DMCM's effects, they had no effect on fear conditioning itself. It seems unlikely that an endogenous inverse agonist is a critical mediator of fear.

272.13

ANXIOLYTIC EFECT RESULTING FROM CHRONIC EXPOSURE TO FLUMAZENIL (Ro 15-1788). M. Urbancic* and T.J. Marczynski. Dept. of Pharmacology, Univ. of Illinois, College of Med., Chicago, IL. 60612.

The Vogel drinking-punishment test was used in order to evaluate the response to conflict situation in adult rats chronically treated with the benzodiazepine (BDZ) antagonist, flumazenil (4mg/kg/day for 3 weeks in drinking water). Tests were repeated on day 3, 6 and 10 following drug withdrawal, each time in 44 hr water- deprived rats. One min. of unpunished drinking was followed by 5 min. of punished drinking (0.35 mA shock after each 20 licks). In the first session, there was no difference in unpunished drinking between control and flumazenil-treated rats. However, in control animals shock experience caused a precipitous decrease in unpunished drinking during next two trials, while the shock experience did not affect the unpunished drinking in the flumazenil-exposed rats. In addition, the rate of punished drinking was significantly higher in the flumazenil-exposed rats on day 3 and 6 after drug withdrawal, as compared to controls. Nociceptive treshold, measured by the tail-flick test and tail-shock vocalization test, was not altered during the same time period. Hence, our data show a strong anxiolytic effect resulting from chronic exposure to a "neutral" BDZ antagonist, flumazenil, the nature of which warrants further investigation. Supported by USAF grant 87-0364.

272.14

INTRASEPTAL ADMINISTRATION OF GABA & BENZODIAZEPINE AGONISTS & ANTAGONISTS: ALTERATIONS IN HIPPOCAMPAL CHOLINE UPTAKE AND COGNITIVE BEHAVIOR. R.W. Stackman, D.F. Emerich, L.A. Taylor and T.J.Walsh. Rutgers University, Department of Psychology, New Brunswick, NJ 08903.

The present studies examined the neurochemical and behavioral effects of pharmacologically manipulating the GABA or benzodiazepine (BDZ) receptor in the septum. A second study examined the effects of intraseptal chlordiaxepoxide (CDP) upon working memory processes.

Sprague-Dawley rats implanted with intraseptal cannulae were injected with either vehicle, or an agonist: muscimol (0.75, 3.0nM), baclofen (3.0, 6.0nM), CDP (1.5, 3.0nM), or an antagonist: bicuculline (0.5, 1.0nM), RO15-1788 (5.0, 10.0nM), or ß-CCM (1.0, 2.0nM), of the GABA or BDZ receptor. One hour later, rats were sacrificed and hippocampal high affinity choline uptake (HAChU) evaluated. Dose-related decreases in HAChU were produced by each of the agonists: muscimol (15-30%), baclofen (15-26%), CDP (30-49%). Dose-related increases in HAChU were produced by only the high dose of RO15-1788 (30%) and ß-CCM (31%).

In a second experiment, Sprague-Dawley rats were trained to perform a working memory version of the eight arm radial maze (RAM) task in which a one-hour delay was imposed between the fourth and fifth arm choices. Following acquisition of the task, animals were implanted with intraseptal guide cannulae, allowed one week to recover, and returned to the RAM for further testing. Intraseptal injection of 10 µg, but not 5 µg CDP or vehicle, immediately after the pre-delay RAM session, significantly impaired performance on the working memory task, as did muscimol or baclofen in a previous report (Chrobak et al., in press). These studies provide additional evidence that GABA/BDZ mechanisms in the medial septum modulate (1) cholinergic neurons (2) their afferents to the hippocampus and (3) the behavioral processes served by these neurons. Supported by BRSG Grant 07058 to TJW.

272.15

EFFECTS OF Ro 5-4864 AND CHLORDANE ON LIMBIC EVOKED POTENTIALS IN THE FREELY BEHAVING FEMALE RAT. K.-S. DAI*, Z. A. HASAN* and D. E. Woolley. Dept. of Animal Physiol., Univ. of Calif., Davis, CA 95616.

The atypical benzodiazepine Ro 5-4864 (15 mg/kg i.p. in DMSO) and the insecticide cis-chlordane (25 mg/kg p.o. in oil) produced similar effects on limbic evoked potentials, though the effects of Ro 5-4864 appeared earlier and the effects of chlordane were more marked and lasted longer. The doses used produced minimal seizure activity of the myoclonic type. Both produced the following signs of enhanced limbic excitability: 1. an increase in appearance and amplitude of afterpotentials in the response produced in the prepyriform cortex (PPC) by stimulation of the lateral olfactory tract; 2. a nearly 2-fold increase in amplitude of the PPC-evoked response recorded in the dentate gyrus (DG); 3. paired pulse potentiation of the PPC-evoked DG response; and 4. a several-fold increase in the population spike interrupting the slow wave recorded in the DG in response to stimulation of the dorsal perforant path (DPP). Chlordane produced a transient decrease in recurrent inhibition during paired pulse stimulation of the DPP, in keeping with its reported effect at the PTX/TBPS site on the GABA-activated chloride channel, whereas we have not yet observed this effect with Ro 5-4864.

272.16

BENZODIAZEPINE RECEPTOR-MEDIATED CONTROL OF BICUCULLINE-INDUCED CHANGES IN CEREBRAL ENERGY METABOLISM. R. Miranda, D. Bitran, T. Ceckler,* and C.K. Kellogg. Dept. of Psychology, Univ. of Rochester, Rochester, NY 14627.

Changes in rat brain cellular energy metabolism following IV injection of the GABA antagonist bicuculline (BIC) were studied using 31P-NMR spectroscopy. Rats were anesthetized, cannulated, paralyzed and ventillated. Spectra were averaged over 10 min. bins using a GE 2.0 Tesla CSI system. Bic (125-1000 µg/kg) induced dose-related alterations in inorganic phosphate, cellular pH and in EEG activity. A nonconvulsive dose of BIC (170 µg/kg) induced changes in energy metabolism not accompanied by EEG patterns characteristic of a seizure, indicating that changes in brain cellular metabolism may precede full seizure expression. Diazepam (DZ, 1.0 mg/kg) given alone had no effect on metabolism, but it prevented the metabolic changes induced by 170 µg/kg BIC. Concurrent administration of the benzodiazepine (BDZ) antagonist Ro 15-1788 (10 mg/kg) attenuated this effect of DZ. The results indicate that changes in cellular energy metabolism may precede seizures rather than be merely a consequence of seizure and that the BDZ receptor may mediate influences on energy metabolism during states of neuronal activation but not during basal states. Supported by Grant MH31850.

272.17

PK 11195 AND Ro 15-1788 ALTER THE TOXICITY OF Ro 5-4864 AND LINDANE. H. L. Drummer and D. E. Woolley. Dept. of Animal Physiol., Univ. of Calif., Davis, CA 95616.

We have shown that the effects in vivo of Ro 5-4864, lindane, and picrotoxin are similar and include hypothermia, convulsions, reduced food intake and long-term enhancement of the limbic evoked potential elicited in the dentate gyrus of the hippocampal formation by single and paired-pulse stimulation of the prepyriform cortex in the rat. PK 11195 and Ro 15-1788, putative antagonists for peripheral and central benzodiazepine sites, respectively, have been reported to antagonize some of the in-vivo effects of Ro 5-4864. Further, PK 11195 is reported to antagonize some of the effects of the type II pyrethroids which, like lindane, are convulsant insecticides. Surprisingly, in the current study, a dose of PK 11195 (30 mg/kg), which by itself had no effect, exacerbated the hypothermic and anorexic effects of Ro 5-4864 (10 mg/kg) and exacerbated the hypothermic but not the anorexic effects of lindane (10 mg/kg). A dose of Ro 15-1788 (20 mg/kg), which alone had no effect, unexpectedly exacerbated the hypothermic effects of lindane (10 mg/kg) but did not alter the anorexic effects. All drugs were administered i.p. in DMSO (0.5 ml/kg) to adult female rats. The complex interactions in vivo of these drugs suggest that caution should be used with the clinical use in humans of PK 11195, Ro 15-1788 and lindane.

273.1

ANATOMICAL LOCALIZATION OF GUANINE NUCLEOTIDE-SENSITIVE AND GUANINE NUCLEOTIDE-INSENSITIVE VIP RECEPTORS IN RAT BRAIN.
J.M. Hill, D. Lambie* and A. Harris* NSB NIMH, Bethesda, MD 20892 and Peptide Design, 12321 Middlebrook Rd, Germantown, MD 20874.

A heterogeneity of VIP receptors in mammalian tissues has been demonstrated by pharmacological studies, electrophoretic patterns of labeled VIP crosslinked to cell membranes and in studies of adenylate cyclase activity. In intestine, two binding sites can be differentiated by their sensitivity to GTP regulation and the stimulation of adenylate cyclase.[1] In the present study the GTP inhibition of VIP receptor binding at the GTP sensitive site was used to differentiate GTP-sensitive from GTP-insensitive VIP receptors in rat brain.

In vitro autoradiography with ^{125}I-VIP was performed on rat brain slices with and without GTP. The presence of GTP (10^{-5}M) abolished ^{125}I-VIP binding at several VIP binding sites including those in the caudate putamen, many cortical regions, locus coeruleus, and central grey. ^{125}I-VIP binding was reduced to varying degrees in many regions including the hippocampus, cerebellum, and entorhinal cortex. In a third set of brain areas, including the olfactory bulb, thalamus, pituitary, superior olive, and superior colliculus superficial grey, ^{125}I-VIP binding appeared to be unaffected by the addition of GTP.

These results suggest that VIP binding sites not linked to G protein/adenylate cyclase are distributed unevenly throughout the brain and that in some regions, e.g., the olfactory bulb and thalamus, they represent the primary subtype of receptor.

[1] Calvo *et. al.*(1989) Biochem. 28, 1667.

273.2

INFLUENCE OF HISTONES ON AN ANATOMICALLY DI STINCT SUBSET OF VIP RECEPTORS IN RAT BRAIN SUGGEST AN INTERACTION BETWEEEN VIP AND HISTONE. J. B. O'Neill, A. Harris,* P.Laing,* and J.M. Hill. NSB, NIMH Bethesda Md 20892 and Peptide Design 12321 Middlebrook Rd. Germantown, Md 20874

Affinity labeling of VIP receptors by crosslinking ^{125}I-VIP to Sub T1 cells (human T cells) and brain membranes produces a band at ~16 kD, the molecular weight range of histones, which is displaceable with VIP and peptide T. Nuclear VIP receptors have been reported (Omary *et al* Science, 238:1578, 1987) A histone-like protein has been found on activated T cell surfaces (Stricker *et al*, IV International AIDS Conference, 1988). This study examines the possibility that VIP might bind to histones or homologous proteins.

^{125}I-VIP was bound to Sub T1 cells and rat brain membranes, displaced with cold VIP and peptide T, crosslinked with DSS and subjected to SDS-PAGE. SDS-PAGEs were blotted and probed with anti-histone H2b. The H2b immunoreactive band occurred at ~16kD and in Sub T1 cells this band was reduced with VIP and peptide T. *In vitro* autoradiography was performed on rat brain sections using ^{125}I-VIP pretreated with histone or myelin basic protein. Pretreatment of ^{125}I-VIP with histone changed the binding pattern of VIP by reducing binding in the thalamus, olfactory bulb, and other VIP binding sites which are insensitive to GTP but did not reduce binding in the GTP-sensitive sites (Hill *et al.* Neuroscience Abstacts 1989.

This data is consistent with the hypothesis that VIP interacts with a histone.

273.3

VASOACTIVE INTESTINAL PEPTIDE RECEPTORS IN DISPERSED ACINI FROM RAT SUBMANDIBULAR GLAND MEDIATE CYCLIC AMP PRODUCTION AND MUCIN RELEASE. J.T. Turner and J.M. Camden, Department of Pharmacology, University of Missouri-Columbia School of Medicine, Columbia, MO 65212.

Vasoactive intestinal peptide (VIP) has been identified as an important regulator of submandibular salivary gland function, consistent with its co-localization with acetylcholine in parasympathetic neurons innervating this gland. Enzymatically dispersed acini from rat submandibular gland are a useful system in which to study gland regulation at the cellular level. In this study, we examined three aspects of VIP interactions with acini: inhibition of binding of ^{125}I-VIP, stimulation of cyclic AMP production and enhancement of mucin release. VIP and peptide histidineisoleucineamide (PHI) inhibited ^{125}I-VIP binding to intact acini with IC_{50} values of 16 ± 3 nM and 46 ± 17 nM, respectively. This rank order of potency agrees with that observed previously in assays using rat submandibular gland membranes and is similar to values obtained in assays measuring increases in cyclic AMP production in which the ED_{50} values for VIP and PHI were 3.1 ± 1.8 nM and 29 ± 13 nM, respectively. Although VIP stimulation of cyclic AMP production was only about 10% of that seen in response to isoproterenol, mucin release levels induced by the two agents were more similar. The ED_{50} for VIP-stimulated mucin release was 0.12 ± 0.05 nM, thus suggesting a "spareness" in the VIP receptor-coupled signal transduction pathway at a point between cyclic AMP production and mucin release. Supported by NIH Grant DE07389.

273.4

LOCALIZATION OF ^{3}H-ARGININE-VASOPRESSIN BINDING SITES IN THE CNS OF THE GOLDEN HAMSTER. P. Szot, C. F. Ferris and D. M. Dorsa. GRECC, Seattle VAMC, WA 98108 and Dept. of Physiology, Univ of MA Medical Ctr, Worcester, MA 01605.

Microinjection of arginine^{8}-vasopressin (AVP) into specific brain regions of the golden hamster elicits dramatic changes in behavior. This study was undertaken to localize AVP binding sites in the CNS of the golden hamster. Autoradiography with ^{3}H-AVP at a concentration of 2.5 nM was performed on coronal sections from 3 animals as previously described (J Neurosci 4:1764, 1984). Non-specific binding was defined in the presence of 1μm unlabeled AVP. ^{3}H-AVP binding sites were concentrated in the dorsal, ventral and lateral septum, bed nucleus of the stria terminals (BNST), central amygdala and hippocampus in the golden hamster. This pattern of labeling is very similar to that observed in the adult rat. In contrast, the anterior cingulate cortex and the endopiriform nucleus were also intensely labeled by ^{3}H-AVP in the hamster CNS. ^{3}H-AVP binding was further characterized using septum-BNST membranes. Saturation analysis performed in 3 groups of 12 animals resulted in a B_{max} of 12.4 ± 0.82 fmol/mg protein and a K_D of 2.5 ± 0.36nM. These values are similar to those obtained from crude septal membranes prepared from adult rat. Comparisons of the pharmacologic specificity of hamster and rat brain ^{3}H-AVP binding sites are in progress.

273.5

GUANINE NUCLEOTIDES AND PERTUSSIS TOXIN ALTER AGONIST BINDING TO RAT SEPTAL V_1-VASOPRESSIN RECEPTORS. M. W. Swank* and D. M. Dorsa. GRECC, VA Med. Ctr., and Depts. of Physiol. and Psychol. Univ. of WA, Seattle, WA 98108.

To determine whether the septal V_1-vasopressin (VP) receptor is coupled to phospholipase C by a GTP-binding protein, we investigated the effects of GTP analogues and pertussis toxin (PTX) on binding of ^{3}H-VP to synaptosomal membranes prepared from rat septum. Nucleotides were present in the reaction mix at a concentration of 1μM. Saturation isotherms revealed that none of the nucleotides significantly altered the B_{max}. All GTP analogs increased the Kd significantly, but ATP had no effect, indicating the specificity of the effect. Nonhydrolyzable analogs Gpp(NH)p ($p<0.01$) and GTPγS ($p<0.001$) had the greatest effect on receptor affinity.

	CONTROL	GTP	GDP	GPPNHP	ATP	GTPγS
B_{max} (fm/mg)	12.28	9.78	12.88	11.06	10.03	12.78
Kd(nM)	0.81	1.29	1.29	1.70	0.75	3.41

Synaptosomal membranes were incubated with 10μg PTX per mg membrane protein and binding of 2nM ^{3}H-VP was measured. PTX reduced specific binding by over 50% ($p<0.05$) suggesting that the septal VP receptor is associated with a G_o/G_i-type G protein.

273.6

THE RAT BRAIN CONTAINS EXCLUSIVELY VASOPRESSIN BINDING SITES OF THE V_1 SUBTYPE. E.M. v.d. Beek*, F.W. van Leeuwen*, J.J. van Heerikhuize* and Y.P. Wan**. (SPON: ENA) *Neth. Inst. for Brain Res., Amsterdam, The Netherlands, **Dupont-NEN, Boston, MA.

In the rat brain vasopressin (VP) binding sites have been revealed using radiolabeled VP and VP antagonists. Many studies pointed to the existence of V_1 type binding sites. In addition, central effects of the V_2 agonist dDAVP and effects of VP on cyclic AMP accumulation were shown. The present study examined directly the presence of V_2 binding sites in the rat brain, pituitary, kidney and liver. For that purpose the localizations of the tritiated V_2 antagonist desGly$(NH_2)_5$[D-Ile^2,Ile^4]VP and the V_1 antagonist d$(CH_2)_5$Tyr(Me)VP were compared (Van Leeuwen et al., Neurosci. Lett., 80: 121, 1987). The V_2 antagonist used is the most selective V_2 antagonist known to date (Manning et al., J. Med. Chem., 30: 2245, 1987). Whereas in the brain the V_1 antagonist appeared to bind to many areas, there was a total lack of V_2 antagonist binding. In the pituitary neither compound showed any labeling. In the kidney, cortical regions were labeled by the V_1 antagonist. The V_2 antagonist showed a dense labeling of the medulla; the cortical part was less intensely labeled. In the liver only V_1 binding was present. The present results support electrophysiological and pharmacological data indicating the exclusive existence of V_1 binding sites in the rat brain. Therefore central effects thought to be mediated via V_2 binding sites most probably reflect indirect effects.

273.7

"VASOTOCIN" RECEPTORS ARE INTRINSIC TO THE SONG CONTROL SYSTEM IN THE CANARY BRAIN. T.A.M. Voorhuis*, J.P.M. Elands*, E.R. de Kloet and D. de Wied* (SPON: Tj.B. van Wimersma Greidanus). Rudolf Magnus Institute, University of Utrecht, Vondellaan 6, 3521 GD Utrecht, The Netherlands. Vasotocin (VT) immunoreactive fibers were visualized in extra-hypothalamic areas in the brain of the canary. A strong VT innervation was observed in regions thought to be involved in mediation of limbic and autonomic function (septum), in sensory function (optic tectum), and in vocal control (nucleus robustus archistriatalis (RA)). The VT immunostaining in septum and dorsal diencephalon is increased following testosterone administration. In vitro autoradiography revealed a discrete neuroanatomical pattern of binding sites labelled with 3H-vasopressin. A low density was observed in the RA region, which increased however two fold after testosterone treatment. Using an ^{125}I-oxytocin antagonist (OTA) a high density of binding sites (Kd:0.05nM) was found exclusively in the RA region. VT has to these sites a high affinity (Ki:0.43nM), while mesotocin (Ki:7.95nM), oxytocin (Ki:2.91nM) and vasopressin (Ki:5.13nM) have a lower affinity. Accordingly the RA seems to contain two classes of binding sites, one of which is testosterone sensitive.

273.8

EFFECT OF ENDOTHELIN ON Ca^{2+} INFLUX, INTRACELLULAR FREE Ca^{2+} LEVELS AND LIGAND BINDING TO N AND L TYPE Ca^{2+} CHANNELS IN RAT BRAIN. Paul M. Lundy*, Robert Frew* and Murray G. Hamilton* (SPON: A.L. Miller). Defence Research Establishment Suffield, Ralston, Alberta, Canada, T0J 2N0.

Endothelin is a potent agonist on various peripheral smooth muscle preparations. It appears to cause vasoconstriction via stimulation of Ca^{2+} influx by activation of specific endothelin receptors and also by promotion of Ca^{2+} influx through the peripheral L type voltage sensitive Ca^{2+} channel (VSCC). The possibility that endothelin has similar effects on Ca^{2+} movements in central neurons was investigated as a result of the discovery of specific endothelin receptors in mammalian brain (Jones et al., Neurosci. Lett. 97:276, 1989). In the present study, endothelin by itself failed to induce Ca^{2+} influx nor did it potentiate Ca^{2+} influx in partially depolarized (15 mM K^+) synaptosomes. Endothelin failed to alter intracellular free Ca^{2+} concentrations nor did it induce acetylcholine release from brain slices. Endothelin also did not displace specifically bound ^{3}H nitrendipine or ^{125}I-conotoxin from L or N type VSCC's in synaptic membranes respectively. The results suggest that endothelin does not alter Ca^{2+} influx either by a direct effect on its own receptors or through L or N VSCC's in neural tissue.

273.9

GALANIN RECEPTORS IN BASAL NUCLEUS OF MEYNERT IN THE POSTMORTEM HUMAN BRAIN - CHANGES IN ALZHEIMER'S DISEASE. Ch.Köhler[1] and V.Chan-Palay[2]. [1]Astra Research Centre AB, Dept. of Neuropharmacology, S-Södertalje and [2]Neurology Clinic, University Hospital, CH-Zürich.

Recent studies in the postmortem human brain have shown that the nucleus basalis of Meynert (nbM) is densely innervated by galanin (GAL)-immunoreactive fibers arising from local GAL interneurons as well as possibly from cells situated outside this region. In brains from patients with senile dementia of the Alzheimer type (SDAT) taken from the Zürich dementia study a hyperinnervation by the GAL-fibers is observed. We have now studied the distribution of ^{125}I-GAL binding sites in the nbM of postmortem human brains using quantitative autoradiographic techniques with subtraction. The nbM is rich in specific binding sites for ^{125}I-GAL. The binding sites are distributed in distinct patches of high density on a background of low densities. Correlation with sections stained for AchE showed that the high density of ^{125}I-GAL binding sites did not correlate with areas of highest AchE staining suggesting that ^{125}I-GAL binding sites are not necessarily always in register with cholinergic cells giving rise to cortical projections. There is considerable interindividual variation in the findings from each patient. Comparisons between normal and SDAT cases showed no significant reductions in ^{125}I-GAL binding in the nbM in SDAT, despite the drastic loss of cholinergic neurons.

273.10

CHARACTERIZATION OF GP120 BINDING IN CEM CELLS AND HIPPOCAMPUS. M.R. Kozlowski, E. Hall, A. Watson. Department of Screening and Biochemical Research, Bristol-Myers Company, Wallingford, CT 06492-7660 and Oncogen, Seattle, WA 98121.

Entry of HIV into cells involves, in most cases, the binding of the viral protein, gp120, to the CD4 cell surface antigen. Since HIV infection is frequently associated with neurological abnormalities, neurons may also contain CD4-like binding sites. This report first examines gp120 binding to a CD4 antigen-bearing cell line, CEM, and then searches for similar binding sites in a brain region, the hippocampus.

Binding of radio-iodinated virion gp120 to intact CEM cells or hippocampal homogenates from the cow or the rat was measured at 37C in either PBS with BSA and glucose (PBG); PBG with bacitracin, aprotinin, and leupeptin; or HEPES (50mM) with the same protease inhibitors. Specific binding, defined as that inhibited by the CD4a antibodies OKT4a or G17-2, amounted to 85% of total.

The binding of gp120 to CEM cells was unusual. Thus, only 50% of the binding dissociated even after 4h. Furthermore, Scatchard plots of the saturation data were non-linear, with prominent convexities. We were unable to demonstrate specific gp120 binding to either rat or cow hippocampus. These data suggest that gp120 binding to CEM cells is not a simple competitive process.

273.11

CHARACTERIZATION OF [1-SARCOSINE, 8-THREONINE] AII IN RAT BRAIN. V.I. Cook and J.W. Harding. Dept. of VCAPP, Washington State University, Pullman, WA 99164-6520.

The specific AII antagonist [1-Sarcosine, 8-Threonine] AII (Sarthran) is known to significantly lower pressor activity in both hypertensive and normotensive rats when given i.c.v. Sarthran also exhibits less inherent agonist activity than [1-Sarcosine, 8-Isoleucine] AII and [1-Sarcosine, 8-Alanine] AII. Preliminary reports implicate Sarthran as a valuable compound in hypertensive research, with possible clinical applications.

Radioligand binding assays were carried out utilizing the HTSA portion from the brains of male Sprague-Dawley rats. Using ^{125}I-Sarthran, receptor binding assays included association and dissociation kinetics, saturation isotherms, and a series of competition curves with several angiotensin associated ligands. Using HPLC, metabolism data was also gathered, comparing Sarthran, AII, and AIII. Equilibrium dissociation constants were determined by both kinetic (K_D = .15 nM) and equilibrium (K_D = .3 nM) methods. Analysis of data from saturation isotherms indicated a Bmax of approximately 30 fmol/mg protein competition curves indicated the following rank order of affinity for the various angiotensin analogs: Sar^1-AII = Sar^1,Ile-AII > Sar^1, Thr^8-AII > AIII ≥ AII > AI > AII(3-8) > AII(4-8) >> AII(1-7). ^{125}I-Sar^1, Thr^8-AII was shown to be very stable in the binding as revealed by HPLC analysis.

273.12

MOLECULAR CLONING OF THE HUMAN BRAIN β-DENDROTOXIN ACCEPTOR. J.D. Hirsch, L. Malkowitz*, B. Beer, A.J. Blume and M.R. Ziai*. Molecular Neurobiology Group, Dept. CNS Research, Medical Research Division, American Cyanamid Co., Pearl River, NY 10965.

The snake venom-derived toxin, β–dendrotoxin (β-DTX), blocks the voltage-dependent slow or delayed-rectifier-type K^+ channel (Kdr), and is used to study its function. The Kdr is responsible for repolarization of neurons following action potentials and represents an intriguing target for drug discovery. We attempted to clone the β-DTX acceptor as a first step toward cloning the entire Kdr. With a fetal human brain-derived cDNA library in bacteriophage λgt11 as the starting point, we screened this library using β-DTX as the probe. After FPLC purification from crude snake venom, the β-DTX was biotinylated. This permitted us to detect positive (i.e. toxin-binding) plaques following incubation with avidin conjugated to alkaline phosphatase. Out of one million plaques screened, ten putative clones were identified. A detailed characterization of these clones may elucidate the molecular profile of K^+ channels.

273.13

ISOLATION AND PARTIAL CHARACTERIZATION OF AN ELH-BINDING PROTEIN FROM THE OVOTESTIS OF APLYSIA CALIFORNICA. J.V.A. Choate, T.E. Kruger* and J.E. Blankenship. Marine Biomed. Inst., Univ. Tex. Med. Br., Galveston, TX 77550.

Egg-laying in Aplysia californica has served as a model for studying the control of a behavior by a peptidergic neuroendocrine system. The neuroendocrine bag cells in this animal release a family of peptides, including the amidated 36-amino acid egg-laying hormone (ELH). ELH diffuses into the hemolymph and interstitial spaces to reach its target tissues, which include the ovotestis and certain identified neurons in the abdominal and buccal ganglia. While ELH has been well characterized, very little is known about its receptor. In an attempt to isolate and characterize the ELH receptor, we have covalently linked synthetic ELH to a solid matrix (Reacti-Gel 6X, Pierce Chemical Co.) and used this to form a ligand-affinity column. Ovotestes were homogenized using a Potter-Elvehjm glass-teflon homogenizer and then solubilized by sonication. The solubilized membranes were loaded onto the affinity column and eluted with 0.1M glycine (pH 2). The column eluate was analyzed by SDS-PAGE and by IEF. We have purified a 50 kDa protein which can be reduced with β-mercaptoethanol to subunits of 25 kDa. The intact protein has a pI of 4.9, while the subunits, which appear to be highly similar, have a pI of 4.3-4.4. Supported by NINCDS NS 23169 (JEB), 07185 (TEK), 11255, and NSF BBS8711368.

273.14

EXPRESSION OF THE NEUROTENSIN RECEPTOR IN *XENOPUS* OOCYTES WITH RNA TRANSCRIBED FROM λ AND pCDM8 LIBRARIES. B.F. O'Hara*, J.M. DiGiorgianni*, S. Shimada, L. Kushner, C.E. Spivak, J. Lerma, R.S. Zukin, M.V.L. Bennett, and G.R. Uhl. NIDA/ARC, Baltimore, MD, 21224, Depts. of Neurology & Neuroscience, Johns Hopkins University Sch. of Med., Baltimore, MD, 21205, and Dept. of Neuroscience, Albert Einstein Col. of Med., Bronx, NY, 10461.

Following injection of brain mRNA, *Xenopus* oocytes express several neurotransmitter receptors. In this study, mRNA from rat cerebral cortex or ventral midbrain was injected into the oocyte cytoplasm. Application of neurotensin produced an inward current with a concomitant increase in membrane conductance, while control eggs gave no response.

cDNA libraries derived from these mRNAs were constucted in λ-ZAP and in the eukaryotic expression vector pCDM8. Both vectors contain a T7 promoter for *in vitro* transcription. However, pCDM8 also contains a CMV promoter and an SV40 origin for eukaryotic replication, and can be used in conjunction with ligand-autoradiographic receptor screening (Rattray, *et al.* this meeting). In addition, receptor binding properties conferred by pooled or individual clones can be readily tested in appropriate cell lines. Further, with its strong eukaryotic promoter, injection into the oocyte nucleus may allow direct expression of cDNAs. Plasmid libraries also offer greater ease for virtually all manipulations and can be size fractionated even after construction.

In vitro transcripts from both pCDM8 and λ libraries produced positive signals in oocytes following neurotensin application. Under voltage-clamp conditions (-60mV) responses were up to 26nA in size, and displayed the same characteristics seen after injection of brain mRNA. Fractionation of these libraries should allow isolation of cDNAs coding for a functional neurotensin receptor.

273.15

IDENTIFICATION OF SIZE CLASSES OF mRNA ENCODING KAINATE AND NEUROTENSIN RECEPTORS. S.G. Fan*, L. Kushner*, J. Lerma*, B.F. O'Hara*, G.R. Uhl, M.V.L. Bennett and R.S. Zukin. (SPON: S. Salinas) Dept. of Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461, and NIDA/ARC, Baltimore, MD 21224.

Binding, electrophysiological, and pharmacological studies have defined receptors responsive to the neuropeptide neurotensin and the excitatory amino acid analog kainic acid. However, little is known about the molecular properties of these receptors or of the mRNAs encoding them.

In *Xenopus* oocytes injected with rat brain mRNA neurotensin evoked delayed oscillatory inward currents, ascribable to coupling of activated receptors to the inositol triphosphate system, followed by opening of Ca^{2+}-dependent chloride channels. Kainate evoked smoothly rising, non-desensitizing inward currents. Fractionation of rat brain poly(A$^+$)RNA over a 5-25% linear sucrose gradient produced mRNA samples of 0.3-9.5 kb. Individual fractions were injected into *Xenopus* oocytes. After 6 days, responses to application of γ-aminobutyric acid (GABA), serotonin, neurotensin and kainate were assessed by voltage clamp analysis. mRNA encoding serotonin-1c and $GABA_A$ receptors were in the size range of 5-7 and 3-7 kb, respectively, as expected from reported data. mRNA encoding the kainate receptor was found in fractions of 6.0-8.5 kb and was enriched approximately 10-fold. mRNA encoding the neurotensin receptor (3.1-4.8 kb) was enriched several-fold in one fraction. These partially purified mRNAs provide enriched source material for the cloning of kainate and neurotensin receptors. [Supported by NIH grants NS20752 and NS07512, and Fogarty Fellowship TW 04040].

273.16

AUTORADIOGRAPHIC DISTRIBUTION OF CALCITONIN GENE-RELATED PEPTIDE (CGRP) BINDING SITES IN THE MONKEY SPINAL CORD. K. YASHPAL*, T. DENNIS and R. QUIRION (Spon R. MALMO). Douglas Hospital Res. Ctr. and Dept of Psychiatry, Verdun, Quebec, Canada H4H 1R3.

Immunohistochemical studies show the presence of CGRP in dorsal root ganglion cells as well as in axons of the dorsal root. Nerve terminals containing CGRP are localized predominantly in laminae I, II and V. Physiological studies provide evidence for its actions at the spinal level. Therefore, to provide further information on the possible involvement of CGRP in spinal transmission, the present study examines the distribution of [^{125}I]-hCGRP binding sites in the monkey spinal cord. Male green monkeys (cercopithecus aethiops) were sacrificed by an overdose of nembutal. Spinal cords were removed, sectioned, snap frozen and stored at -80^{0}C. Each segment was cut in 20 micron sections and thaw mounted on gelatin coated slides. The autoradiographic procedure, as described by Sexton *et al* (Neurosci 19, 1235 1986), was followed. Binding of 0.1nM [^{125}I]-hCGRP was observed throughout the length of the spinal cord and was relatively constant. However, high densities were seen in layers I and II and in the inner borders of layers III to V, around lamina X and in the lateral horn region. Moderate densities were present in the ventral horn, thus showing differences in distribution as compared to the rat spinal cord where high densities of these sites were localized in the medial borders of the dorsal horn and around the central canal only.

273.17

HUMAN CALCITONIN GENE-RELATED PEPTIDE (hCGRP) ANALOGUES DIFFERENTIATE RECEPTOR SUB-TYPES. T. Dennis[1], A. Fournier[2], S. St Pierre*[2] and R. Quirion[1]. [1]Douglas Hospital Res. Ctr., Dept of Psychiatry, McGill University, Verdun, QC, H4H 1R3. [2]INRS-Santé, Pointe Claire, QC, Canada.

The use of a variety of hCGRP analogues and fragments to study the structural requirements of the CGRP receptor showed that the radioligand binding characteristics in brain and spleen membrane preparations are similar. Furthermore, the *in vitro* biological activities of these compounds revealed differential agonist and antagonist potencies in a number of preparations suggesting the existence of CGRP receptor subtypes. Binding of the ligand to the CGRP receptor is independent of the N-terminal 2,7 disulfide bridge as indicated by the nM affinity of the analogues [Cys(ACM)^{2,7}]-hCGRP and cyclo^{2,7}[Asp^2,Lys^7]-hCGRP and the C-terminal fragments $hCGRP_{8-37}$ and $hCGRP_{12-37}$. Indeed, 6 to 8 amino acid residue cyclo^{2,7}-N-terminal fragments showed a total lack of affinity. The linear analogue [Cys(ACM)^{2,7}]-hCGRP retained high potency in the inhibition of the twitch response of the rat vas deferens but displayed no agonistic activity in the right and left guinea pig atrial preparations. Moreover, competitive antagonistic properties were displayed by $hCGRP_{8-37}$ and $hCGRP_{12-37}$ in atrial but much less so in vas deferens preparations. We propose that the receptor present in atria, which is highly sensitive to the antagonistic properties of C-terminal fragments be classed as the $CGRP_1$ subtype while $CGRP_2$ refers to the [Cys(ACM)^{2,7}]-hCGRP-sensitive sub-type resistant to the antagonist properties of $hCGRP_{8-37}$ and $hCGRP_{12-37}$.

274.1

CLONING, mRNA DISTRIBUTION, FUNCTIONAL EXPRESSION OF THE RAT SEROTONIN 1A RECEPTOR GENE. P. Albert*, Q.Y. Zhou*, H. Van Tol*, J. Bunzow*, O. Civelli. (SPON: E. Lewis)
Vollum Institute, Oregon Health Sciences University, Portland, OR 97201.

We have screened a rat genomic library under low stringency hybridization conditions with the hamster β_2-adrenergic receptor gene to isolate new members of the G protein-coupled receptor gene family. We show that one of this gene codes for a functional 5-HT_{1a} receptor on the basis of five criteria: (1) it possesses an intronless open reading frame encoding a protein with seven putative transmembrane domains and 89% amino acid identity with the human 5-HT_{1a} receptor; (2) its corresponding mRNA's are present in rat brain as three RNA species with the distribution expected for the 5-HT_{1a} receptor; (3) when transfected into fibroblast cells it expresses a ligand binding site with the pharmacology of the 5-HT_{1a} subtype; (4) it encodes a binding site which interacts with G protein; (5) it encodes a functional receptor which mediates agonist-induced inhibition of basl and stimulated cAMP accumulation in transfected GH4 C1 pituitary cells.

274.2

CLONING OF MOUSE GENOMIC 5-HT_2 RECEPTOR

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Based on ligand binding specificities, 5-hydroxytryptamine (5-HT) receptors have been classified into at least six subtypes: 5-HT_{1A}, 1B, 1C, 1D, 2 and 3. The cDNAs encoding human 5-HT_{1A}, rat 5-HT_{1C} and 5-HT_2 receptors have been cloned. The latter two receptors share 78% of sequence similarity in their transmembrane domains. However, Northern and Southern blot analyses show that the 5-HT_2 receptors from human and rat have high sequence homology, whereas the 5-HT_{1C} receptor appears to have low sequence similarity between these two species.

The restriction patterns of Southern blotting of mouse genomic DNA using the 5-HT_2 receptor cDNA as a probe suggest that the 5-HT_2 gene contains intron(s) and probably occurs as a single copy in the mouse genome.

Using the 5-HT_2 receptor cDNA probe, a Balb/c mouse genomic library constructed in the pWE 15A cosmid vector was screened. Two positive clones were obtained with insert sizes of approximately 35Kb. Comparing the restriction patterns of cloned DNAs to that of the genomic DNA indicates that partial genomic 5-HT_2 clone was obtained. The structural organization and DNA sequences of these genomic clones will be discussed. (Supported by MH39085, MH00796, MH37020 and Welin professorship)

274.3

EXPRESSION OF FUNCTIONAL ADULT HUMAN SEROTINERGIC RECEPTORS IN XENOPUS OOCYTES.

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Problems inherent in obtaining tisssue complicate analyses of human neurotransmitter receptor function.These problems can be circumvented by the use of *in ovo* expression of mRNA extracted from adult human brain tissue at post-mortem.

RNA was extracted from frontal cortex of adult human brain. Poly(A)$^+$mRNA was purified by oligo(dT) chromatography and the integrity of extracted RNA was examined by formaldehyde agarose gel electrophoresis. *In vitro* translation yielded ^{35}S-methionine labelled proteins up to a molecular weight of 200KD as determined by PAGE.

Samples of mRNA (50nl of 1mg/ml in H_2O) were injected into Xenopus oocytes which had been denuded of their outer layer of follicular cells by collagenase treatment and mechanical stripping. Two electrode voltage-clamp measurements (holding potential -80 mV) of oocyte responses to bath applied 5HT revealed a dose dependent inward current. Control oocytes (uninjected or sham injected with H_2O) were unresponsive to 5HT, suggesting that 5HT receptors in mRNA injected oocytes were exogenous. Repeated application of equal doses of 5HT produced an apparent desensitisation of response in some oocytes. The ramping of the command voltage (-100mV to 0mV /1s, constant dV/dT) during the stable phase of the drug response showed an increased conductance in response to 5HT with a reversal potential of approximately -20mV. These results suggest that the *in ovo* expression of human brain mRNA is a viable approach to the investigation of human neurotransmitter receptor systems.

274.4

Decreased density of platelet [^{3}H] imipramine, but not [^{3}H] paroxetine binding sites in major depression. B.E.Suranyi-Cadotte, L. Iny, P. Desjardins, R. Yassa and S. Welner.
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A reduction in the number of binding sites for imipramine on platelets has been proposed to serve as an index of altered 5-HT uptake and a potential biochemical correlate of depression. Recent findings, however, suggest that ^{3}H imipramine binding sites are not consistently associated with 5-HT uptake, whereas [^{3}H] paroxetine, a selective 5-HT uptake inhibitor labels the 5-HT transporter. To further characterize the relationship between [^{3}H] imipramine and [^{3}H] paroxetine binding we investigated these binding sites in parallel on platelets of 11 depressed patients (RDC-major depression) and 10 age-matched healthy volunteers, who were drug-free prior to [^{3}H] imipramine (Briley et al, Science 209;303;1980) and [^{3}H] paroxetine (Mellerup et al, Eur. J. Pharmacol,96;303;1983) binding assays. Consistent with previous findings, the density (Bmax) but not the affinity (Kd) of [^{3}H] imipramine binding sites was significantly lower in depressed patients than in controls. In contrast [^{3}H] paroxetine binding variables did not differ between the two groups. There were no correlations between the two binding site variables. These results provide further evidence that [^{3}H] imipramine and [^{3}H] paroxetine bind to distinct sites and that reduced platelet [^{3}H] imipramine binding may serve as an index of depression.

274.5

RESPONSIVENESS OF SEROTONIN-STIMULATED PHOSPHOINOSITIDE HYDROLYSIS IS INCREASED IN PLATELETS FROM UNMEDICATED DEPRESSED PATIENTS. M.Mikuni, I.Kusumi*, A.Kagaya*, H.Yamamoto*, Y.Kuroda*, T.Nishikawa* and K.Takahashi*.
Div. of Mental Disorder Res., Natl. Institute of Neurosci. NCNP, Kodaira, Tokyo, 187, Japan.

Studies of 5HT receptor function should provide important information about the role which receptor modulation or dysfunction may play in alterations of serotonergic function in affective disorders. The presence of 5HT-2 receptors on the human platelets as a readily accessable peripheral model for the certain function of 5HT neurons, may permit direct study of function of 5HT-2 receptors in living persons. Recently, it has been suggested that 5HT-stimulated phosphoinositide(PI) hydrolysis in rat cerebral cortex, rat aorta and human platelets is mediated by the 5HT-2 receptors. In this preliminary study, we have assessed 5HT-stimulated PI hydrolysis in platelets from a normal control group and depressed subjects. In a group of unmedicated patients with major depression matched for age with normal control group, we found a significant increase in 5HT(100uM)-induced accumulation of inositol-monophosphate, one metabolic product of PI hydrolysis (150$\pm$7% of basal for depressed patients, 132$\pm$3% for controls). This result may reinforce the hypothesis that serotonergic receptor abnormality play a role in affective disorder.

274.6

ANTI-IMIPRAMINE ANTIBODIES RECOGNIZE POTENT ENDOGENOUS SUBSTANCES WHICH INHIBIT SEROTONIN UPTAKE AND IMIPRAMINE BINDING. S.W. Tang, A. Strijewski*, S. Cheung*, D.M. Helmeste and J. Chudzik*. Psychopharmacology Unit, Clarke Institute of Psychiatry, Toronto, Ontario, Canada M5T 1R8.

Anti-Imipramine antibodies raised in rabbits were tested against a variety of antidepressant compounds, uptake inhibitors, antihistamines, neuroleptics and other psychotropic drugs and hormones. Compounds which show high affinity for the antibodies all possess a similar partial structure. Serotonin uptake inhibitors were generally not recognized. Brain, plasma and urinary extracts further purified by multiple chromatographic procedures all contained substances which showed high affinity for the antibody preparation. Some, but not all of these substances also demonstrated potent action when tested for inhibition of serotonin reuptake and ^{3}H-Imipramine binding. The data suggest the possible existence of endogenous compounds which modulate serotonin reuptake. Such compounds may show partial molecular structure similar to that identified by the antibodies.

274.7

LECTIN CHROMATOGRAPHY FOR PURIFICATION AND CHARACTERIZATION OF SOLUBILIZED PLATELET ^{3}H-IMIPRAMINE BINDING SITES. D.M. Helmeste, J. Chudzik* and S.W. Tang (SPON: P. Brawley) Clarke Institute of Psychiatry, Toronto, Ontario, Canada, M5T 1R8.

The glycoprotein nature of cell surface receptors offers purification strategies based on the affinity of lectins for different carbohydrate moieties. Accordingly, a variety of lectins have been tested for their ability to bind and retain the platelet serotonin transport complex, as labelled with the antidepressant ^{3}H-Imipramine. Human platelets were solubilized with CHAPS and passed through lectin-sepharose columns of differing sugar specificities. ^{3}H-Imipramine or RO-11-2465 binding was used to monitor receptor specifically eluted with sugar (0.5 M). Of the lectins tested, wheat germ (Triticum vulgaris), Lens Culinaris and Ulex Europaeus I appear to be useful for affinity purification of solubilized ^{3}H-IMI binding sites. Peanut (Arachis hypogaea) and Dolichus biflorus Agglutinins had no affinity for this receptor, whereas Concanavalin A showed strong non-specific adsorption of the applied protein. None of these lectins interfered with the binding of ^{3}H-Imipramine. The results suggest that the carbohydrate moieties are extrinsic to the ligand-binding domain and may be useful for purification of solubilized ^{3}H-Imipramine binding sites.
(Supported by Ontario Mental Health Foundation Grant OMHF # 1019-88-90).

274.8

NEW EVIDENCE FOR MULTIPLE ANTIDEPRESSANT BINDING DOMAINS IN SEROTONIN TRANSPORTER. J. Chudzik*, D.M. Helmeste and S.W. Tang. Psychopharmacology Unit, Clarke Institute of Psychiatry, Toronto, Canada M5T 1R8.

The interaction of imipramine and paroxetine with the serotonin transport protein provides a useful model for the study of mechanisms of actions of antidepressant drugs. Previous data imply both a single and a two-site binding model for ^{3}H-imipramine and ^{3}H-paroxetine. We have systematically investigated the effects of temperature, various protein and lipid modifying agents, as well as proteolytic, lipolytic and glycolytic enzymatic digestions, on the binding parameters of these two ligands in intact and solubilized platelet membrane preparations. The results clearly suggest that the binding of ^{3}H-paroxetine and ^{3}H-imipramine to the membrane preparations could be differentially manipulated. The presence of multiple binding domains for antidepressant compounds in the serotonin transport protein is proposed. (Supported by Ontario Mental Health Foundation Grant OMHF #1019-88-90).

274.9

COMMON EFFECTS OF 5HT REUPTAKE INHIBITORS AND 8-OHDPAT: REVERSAL OF 5HT-1B EFFECTS ON 5HT RELEASE AND SYNTHESIS. M.P. Galloway, E.A.Novak*, B.N.Mathews*, Lafayette Clinic, CCN, Psychiatry, Wayne State Univ Sch Med Detroit MI

We have previously reported that 5HT synthesis in cortical brain slices is inhibited by 5HT-1B, but not 5HT-1A agonists, suggesting that synthesis modulating 5HT autoreceptors are present on 5HT nerve terminals. Although 5HT-1A agonists have no effect on 5HT synthesis in vitro, compounds such as 8-OHDPAT actually block the inhibition produced by 5HT-1B agonists suggesting a possible interaction between 1A and 1B receptor subtypes. We have extended these studies to examine the relationship among 5HT-1 agonists, 5HT release and synthesis (5HTP accumulation after NSD-1015) in cortical slices. TFMPP, a 5HT-1B agonist, induces a 3-5 fold Ca^{++} independent increase in both basal and K^+ stimulated 5HT release. As with 5HT synthesis, the 5HT releasing effect of TFMPP is blocked by 8-OHDPAT, which is without effect by itself. Removal of tryptophan from the media does not block the effect of TFMPP on 5HT synthesis or release, nor does it alter the antagonism afforded by 8-OHDPAT on release. Fenfluramine, a 5HT releaser, promotes 5HT release and inhibits 5HT synthesis, both effects reversed by 8-OHDPAT. 5HT reuptake inhibitors sertraline, imipramine and fluoxetine also blocked the effects of TFMPP on both 5HT release and synthesis. These studies provide functional evidence for an interaction of 5HT-1A and 1B ligands with the 5HT reuptake or transporter site. Support: MH-41227 DA-04120 State of Michigan, DMH.

274.10

SEROTONIN (5HT) UPTAKE AND [^{125}I]-CYANOPINDOLOL ([^{125}I]-CYP) BINDING TO $5HT_{1B}$ RECEPTORS IN SPINAL CORD SYNAPTOSOMAL SUBFRACTIONS. I. Matsumoto*, M.R. Combs*, D.J. Jones. Dept. of Anesthesiology, UTHSC, San Antonio, TX 78284-7838

Based on anatomical studies, the percentage of 5HT terminals compared to the overall population of neuronal terminals in spinal cord is small. In order to utilize a cellular fraction enriched in 5HT terminals, the present studies compared presynaptic 5HT uptake and $5HT_{1B}$ receptor binding in subfractions of spinal cord synaptosomes isolated via Percoll gradients of 23, 15, 10 and 3% (Robinson and Lovenberg, Neurochem Int 9:1986).

Electron microscopic analysis indicated enrichment of synaptosomes in the 10-15% interphase (Fraction 3) and 15-23% interphase (Fraction 4). The uptake of [^{3}H]-5HT (fmols/mg protein) in Fraction 3 was 19.25 $\pm$ 1.63, in Fraction 4, 37.50 $\pm$ 1.78 and 6.48 + 0.22 in the crude synaptosomal preparation (P_2). The receptor site labelled by [^{125}I]-CYP in the presence of isoproterenol has been proposed as the autoreceptor which regulates 5HT release (Engel et al, NS Arch Pharmacol 332:1986). Using crude synaptosomal preparations, specific binding of [^{125}I]-CYP in spinal cord averaged around 30%. A comparison of saturation isotherms in crude vs purified subfractions of synaptosomes demonstrated 50-60% specific binding in Fraction 3 with 30-40% specific binding in Fraction 4. Bmax's (fmol/mg protein) in Fractions 3 and 4 and the crude synaptosomal preparation were 113 $\pm$ 7, 79 $\pm$ 11 and 34 + 6, respectively. The Kd's were similar (0.16 $\pm$ 0.22 nM). Displacement of [^{125}I]-CYP binding by 5HT and RU 24969 was monophasic in both the crude preparation and Fraction 3. However, displacement occurred over a range of concentrations from 10^{-8} - 10^{-4} in the crude preparation vs 10^{-8} - 10^{-6} in the purified synaptosomal preparation. Ki's for displacement were the same. It is concluded that purified subfractions can provide a more suitable tissue preparation for studying the neurochemistry of spinal cord 5HT terminals. Supported by NINCDS 14564 and VA Grant

274.11

DECREASED ^{125}I-AMIK BINDING TO 5HT2 SITES IN RAT BRAIN FOLLOWING CHRONIC ADINAZOLAM TREATMENT.

F.Lafaille, S. Welner, P. Desjardins*, R.Quirion, B. Suranyi-Cadotte. Douglas Hospital Research Center, Verdun, Canada H4H 1R3

Prolonged antidepressant treatment down-regulates $5HT_2$ binding sites, an effect possibly related to the therapeutic efficacy of these agents in depression. We postulated that adinazolam, a triazolobenzodiazepine with both anxiolytic and antidepressant efficacy may exert part of its action via $5HT_2$ binding sites. We have tested this hypothesis by chronic administration to rats of either adinazolam (4 mg/kg), desipramine (DMI), a classical antidepressant (10 mg/kg) and diazepam , an anxiolytic benzodiazepine (5 mg/kg). Animals were injected i.p. with drugs or vehicle for 21 days. Binding parameters (Kd and Bmax) of 7-amino-8-[^{125}I]-ketanserin ([^{125}I]-AMIK) using homogenates of frontal cortex 24 hours after the last injection were determined according to a modified method of. W. Wouters et al., (Biochem. Pharmacol. 35: 3199-3202). Chronic DMI treatment elicited a significant, 25% reduction in the density (Bmax) of [^{125}I]-AMIK binding sites without altering Kd values. Adinazolam, like DMI produced a 27% reduction in Bmax values, whereas diazepam an anxiolytic agent devoid of antidepressant activity produced an increase by 21% in the number of [^{125}I]-AMIK binding sites following chronic treatment. These results confirm previous reports that chronic antidepressant treatment can decrease the density of $5HT_2$ binding sites and suggest that this mechanism may also be operative in the antidepressant activity of adinazolam.

274.12

MODULATION OF 5-HT1A BINDING SITE AFFINITY BY MAO INHIBITION. R. Mongeau, S. Welner, R. Quirion, C. de Montigny, Y. Chaput and B.E. Suranyi-Cadotte. Douglas Hospital Research Center and Dept. Psychiatry, McGill Univ., Montreal, Canada H4H 1R3.

The purpose of the study was to investigate the effect of MAO inhibition on [3H]-8-OH-DPAT binding which specifically label 5-HT1A receptors. [3H]-8-OH-DPAT binding was performed as previously described by Peroutka et al. (J. Neurochem., 47, 1986). Specific binding was defined using 10 uM 5-HT and represented 80-90 % of the total binding. Radioligand concentrations ranging from 0.3-10 nM identified a single population of sites, however,a tendency toward a second site was observed. When the range was expanded to 100 nM ,this tendency was confirmed with a ten-fold difference between the affinity of the two sites; this represented statistically a better fit than the one site model. Biphasic scatchards have already been reported in cerebral cortex (Hall et al.,J. Neurochem.,44,1985); we now report similar results in hippocampus and striatum.

Monophasic curves with loss of the high affinity component were observed following chronic (21 day) and acute MAO A inhibition by clorgyline (1 mg/kg),as well as by the addition of this compound to the preincubation membrane preparation. A blockade of the high affinity component by endogenous 5-HT cannot, however,explain this effect,since inhibition of [3H]-8-OH-DPAT by nM concentations of 5-HT did not affect the curvilinearity of the scatchards. The mechanism and the clinical relevance of this modulation is under further investigation.

274.13

EFFECT OF CHRONIC ANTIDEPRESSANT TREATMENTS ON SEROTONIN$_{1A}$ (5HT$_{1A}$) RECEPTOR DENSITY AND RESPONSIVENESS. J. Hensler & A. Frazer, Dept. Psychiatry, Univ. of Pennsylvania & VA Med. Ctr., Philadelphia, Pennsylvania 19104.

Chronic treatment of rats with monoamine oxidase inhibitors (MAOIs) or 5HT uptake inhibitors produces subsensitivity of responses elicited by somatodendritic autoreceptors in the dorsal raphe nucleus and a diminished serotonin behavioral syndrome or hypothermia in response to 5HT$_{1A}$ receptor agonists. In agreement with this, we have found that DPAT (1mg/kg, sc) produced a decrease in body temperature of 1.3±0.6°C (n=7) in rats injected chronically with saline but only 0.5±0.4°C in rats treated for 21 days with the MAOI tranylcypromine (5mg/kg, qd; ip, n=7). Chronic treatment of rats with either trazodone or DMI did not alter the hypothermic response to DPAT. Experiments were done to determine whether subsensitive responsiveness is mediated by changes in the binding of ^{3}H-DPAT to 5HT$_{1A}$ receptors as measured by quantitative autoradiography. Chronic administration (14-21 days) of 5HT uptake inhibitors (citalopram or sertraline) or MAOIs (phenelzine or clorgyline) did not alter the binding of ^{3}H-DPAT (1nM) to 5HT$_{1A}$ receptors in the dorsal or median raphe nuclei or regions of the frontal cortex, hippocampus or hypothalamus. From these data, it may be inferred that decreased sensitivity of 5HT$_{1A}$-mediated responses may not result from decreased density of 5HT$_{1A}$ receptors but probably occurs through some other mechanism. **(Supported by Vet. Admin. & USPH grants MH14654 & GM34781.)**

274.14

AGE-RELATED ALTERATIONS IN 5-HT$_1$-LIKE AND ADENOSINE A$_1$ RECEPTOR SYSTEMS IN THE CNS OF FISCHER 344 RATS.

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Different membrane components of signal transduction may be affected by age. To test whether receptors, their coupling to G-proteins or their effectors are altered by age, membrane receptor systems (receptor/G-protein/effector) in Fischer 344 rat CNS were examined by three assays: functional (inhibition of forskolin-stimulated adenylyl cyclase, FSAC; "response"), occupancy (quantitative receptor autoradiography) and modulation of occupancy by guanyl nucleotides. Three Gi-linked membrane receptors, the adenosine A$_1$ (AD A$_1$), the 5-HT1$_A$, and the 5-HT$_1$-like, ("non-5-HT1$_A$"), were studied in 3 and 23 month-old animals, using their respective agonists, (R)-phenylisopropyladenosine (PIA), 8-hydroxy-2-(di-n-propylamino)-tetralin (DPAT) and 5-HT. Similar to reported observations in Sprague-Dawley rats, the receptor systems are differentially distributed across the Fischer rat CNS. In membrane preparations, the response to 5-HT (5-HT$_1$-like receptor) and PIA were region dependent, and the former showed age-related, region dependent decreases. Occupancy of AD A$_1$ and 5-HT$_{1A}$ receptors and their attenuation by 5'-guanylimidodiphosphate (Gpp(NH)p) (100 uM) and NaCl (1 mM) appeared to be receptor, region, and age dependent. These two receptor systems may serve as a model to study age-related alterations in receptor systems that may play a role in the pathophysiology of CNS aging. (Supported by USPH GM 34852 and HL 42585).

274.15

DEVELOPMENTAL CHANGES IN THE 5-HT$_3$ RECEPTOR OF NG108-15 CELLS. M.X. Shao, J.L. Yakel, and M.B. Jackson. Department of Biology, UCLA, Los Angeles CA 90024-1606.

NG108-15 cells, a rat glioma-mouse neuroblastoma somatic cell hybrid, undergo morphological differentiation when PGE$_1$ and theophylline are added to the culture medium. This cell line is also a useful system for the study of the 5-HT$_3$ receptor, which is found in both central and peripheral mammalian nervous systems. We have used whole-cell patch-clamp techniques to compare the responses mediated by the 5-HT$_3$ receptor in differentiated and undifferentiated NG108-15 cells, and have found that a number of changes in receptor function accompany morphological differentiation. These changes include a reduction in the rate of desensitization, an increase in the voltage dependence of desensitization, and an increase in the mean response amplitude. There may also be changes in the curvature of the current-voltage curve and in the susceptibility of the receptor to modulation of desensitization. The desensitization of the 5-HT$_3$ receptor in primary cultured neurons is also voltage dependent. Thus, the increased voltage dependence of desensitization is consistent with the morphological change in that it represents the acquisition of a neuronal characteristic. The changes we observed in the 5-HT$_3$ receptor indicate that it has different functional forms which are expressed in a manner that corresponds with different stages of development. This may also reflect the existence of different subtypes of the 5-HT$_3$ receptor.

NICOTINIC RECEPTORS

275.1

CRYSTALS OBTAINED FROM PREPARATIONS OF NICOTINIC ACETYLCHOLINE RECEPTOR FROM TORPEDO ELECTRIC TISSUE. Hucho, F.*, and Hertling-Jaweed, S.* (SPON. W.Huttner). Institut für Biochemie, Freie Universität Berlin, Thielallee 63, looo Berlin 33.

Crystals were obtained from preparations of nicotinic acetylcholine receptors in detergent solutions. The receptors were prepared by a novel procedure avoiding affinity columns. This procedure allows large scale purification of receptor protein in mg quantities within two days. The conditions are chosen to preserve the receptor's activity and to obtain a homogeneous population of undenatured receptor molecules. Crystals appeared reproducibly and abundantly within three days. They had the shape of rods of about 25 /um length with a hexagonal cross-section of about 8 /um. They are too small yet for X-ray diffraction. Their abundance but not their phenotype is affected by the crystallization conditions, especially by the type of detergent used for solubilization.

275.2

CHARACTERIZATION OF THE NICOTINIC ACETYLCHOLINE RECEPTOR OF *MANDUCA SEXTA*. M.L. Perez and D.J. Prescott*, Biology Dept., Bryn Mawr College, Bryn Mawr, PA 19010.

The CNS of *Manduca sexta* is responsive to bath applied cholinergic ligands but is less sensitive to nicotine than is *Periplaneta americana* CNS (C. Morris, J. Exp. Zool. 229: 361-374, 1984). We are characterizing the receptor in *Manduca* cerebral ganglia to determine if it is a variant type as has been suggested (C. Morris, Ibid.). The Triton X-100 solubilized receptor forms complexes with monoiodinated α-bungarotoxin. The DEAE filter disk assay is used to follow complex formation (Schmidt, J. and Raftery, M.A., Anal. Biochem. 52: 349-354, 1973). Nicotinic ligands such as methyllycaconitine, dihydro β erythroidine, curare and nicotine are effective competitors of α-toxin binding. Also, strychnine and bicuculline are effective competing ligands illustrating binding correlates to the molecular homology seen within the ligand-gated channel superfamily. The competition binding curves for dihydro β erythroidine, methyllycaconitine and especially nicotine exhibit unusually shallow slopes over a concentration range of several orders of magnitude. The shallow slopes may indicate a heterogeneity of affinities within the two ligand binding areas of the receptor and explain the insensitivity to nicotine.

The preliminary results of structural studies show a monomer which has a sedimentation coefficient which corresponds to a M_r of 300,000. The curare eluate of the toxin-affinity column shows four polypeptides on non-denaturing gel electrophoresis and two major polypeptides on denaturing gel electrophoresis. These results indicate the receptor is composed of hetero-oligomers.

275.3

BIOCHEMICAL CHARACTERIZATION OF A NEURONAL NICOTINIC ACETYLCHOLINE RECEPTOR FROM DROSOPHILA. P.Wu*, X.Mai*, J.Wu*, X.Chang*, L.C.Yang*, H.Meones*, S.Bobin* and T.Schmidt-Glenewinkel*. Department of Biological Sciences, Institute for Biomolecular Structure and Function, Hunter College of CUNY, New York, NY 10021

The central nervous system of Drosophila contains an α-bungarotoxin binding site with the properties expected of a nicotinic acetylcholine receptor. The receptor was purified 5500-fold by affinity chromatography. Sodium dodecyl sulfate polyacrylamide gel electrophoresis revealed four subunits with apparent molecular weights of 42000, 57000, 65000, and 79000. Polypeptides of identical size were also identified by in situ photoaffinity labeling of the membrane fraction. The receptor has a $s_{20,w}$ of about 9.5 and a Stoke's radius of 7.4nm. The frictional coefficient was calculated to be 1.7 indicating a highly asymmetrical protein complex. From sedimentation analysis in H_2O and D_2O a molecular weight of 270000 was determined. The individual polypeptides were blotted on a nitrocellulose membrane, digested with trypsin and subjected to internal amino acid sequence analysis. Screening of cDNA library from heads of Drosophila with mixed oligonucleotide probes derived from the amino acid sequence allowed identification of several cDNA clones.

275.4

ACETYLCHOLINE RECEPTOR TRANSCRIPTS ARE COMPARTMENTALIZED IN NUCLEI OF MYOTUBES. S.Bursztajn[1], S.Berman[2], and W.Gilbert[3]. [1]Baylor College of Medicine, Houston, Tx. 77030; [2]University of Chicago, Chicago, Il. 60637; [3]Harvard University, Cambridge, Ma. 02138

We have previously demonstrated that not all nuclei of the multinucleated skeletal myotube are equally active in expressing the acetylcholine receptor (AChR) α subunit message. In order to further understand AChR transcript processing within individual nuclei we have used in situ hybridization, while simultaneously staining nuclei with bisbenzamide to localize the position of intron and exon RNA sequences in intact cells with respect to the nuclei. We employed probes for chicken AChR exon #2 and intron #1 (Fontaine, B and Changeux, J. Cell Biol. 108: 1025-1037, 1989). For comparison we used actin intron and exon sequences as well as a sequence for U1 small nuclear RNA. We made ^{35}S labelled single stranded probes and hybridized these in situ to cultured chick embryonic muscle cells.

The cells hybridized with the AChR α subunit intron probe showed a striking perinuclear localization characterized by grains confined to the periphery of the nuclei, frequently forming a ring. Both the actin and AChR exon showed a uniform cytoplasmic distribution whereas the actin intron and U1 probes showed a homogeneous grain distribution within nuclei. Quantitative analysis showed variations in the level of activity among nuclei of cells hybridized with the AChR intron and actin intron but no significant differences were observed when cells were hybridized with U1 probes. Our data indicates that the AChR α subunit intron is compartmentalized in the myotube nuclei.

275.5

TE671 CELLS EXPRESS AN ABUNDANCE OF UNASSEMBLED α SUBUNITS OF THE HUMAN MUSCLE ACETYLCHOLINE RECEPTOR WHICH HAVE PROPERTIES OF AN ASSEMBLY INTERMEDIATE. William G. Conroy* and Jon Lindstrom. The Salk Institute, La Jolla, CA 92037

The muscle-type nicotinic acetylcholine receptor (AChR) is composed of four homologous subunits which assemble into an $\alpha_2\beta\gamma\delta$ pentamer which forms an ACh-gated ion channel. The α subunit, before assembly, goes through a maturation process in which it acquires the conformation for binding α-bungarotoxin (α-Bgt) and antibodies to the main immunogenic region (MIR). Whether the maturation process involves covalent modification and/or conformational changes of the α subunit is unknown.

We have found that TE671 cells express an abundance of unassembled α subunits of the human AChR which have properties of an assembly intermediate. Sucrose gradient density sedimentation analysis of Triton X-100 extracts of TE671 cells indicates the presence of a 5S species which binds α-Bgt and MIR-specific monoclonal antibodies (mAbs). The conformation of this α subunit was compared and contrasted to the α subunits of native TE671 AChRs by analyzing the binding of α-Bgt, mAbs, and the small ligands d-tubocurarine and carbamylcholine. The unassembled α subunit has lower affinity for α-Bgt (Kd = 0.6 nM) than the native AChR (Kd = 0.1 nM) and has negligible affinity for d-tubocurarine or carbamylcholine ($K_i > 10^{-2}$ M). These binding properties are similar to the assembly intermediate identified in BC3H-1 cells and rat myotubes. Thus, the TE671 cell line may be a useful model for studying the conformational changes which take place during the maturation of the α subunit of AChRs.

275.6

A SYNTHETIC PEPTIDE (RESIDUES 172-227) OF THE α-SUBUNIT OF THE NICOTINIC ACETYLCHOLINE RECEPTOR (AChR) CONTAINS BINDING SITES FOR COMPETITIVE ANTAGONISTS AND NONCOMPETITIVE INHIBITORS (NCIs). D. L. Donnelly-Roberts*, M. Gastka* and T. L. Lentz. Dept. of Cell Biology, Yale University School of Medicine, New Haven, CT 06510.

A 56 residue peptide (172-227) (α56mer) from the α-subunit of the AChR was synthesized because it contains a region (172-204) previously shown to bind α-bungarotoxin (α-Btx) and it contains the first 18 residues of the first transmembrane spanning region (M1). α-Btx bound to the α56mer in a solid phase assay. Binding was markedly enhanced by .02% sodium dodecyl sulfate (SDS) and was competed by d-tubocurarine. Equilibrium saturation binding experiments in the presence of SDS revealed an apparent K_D of 3.5 nM for α-Btx. The NCI, [^{3}H]phencyclidine (PCP) was photolabeled to the α56mer. PCP binding was saturable and inhibited by unlabeled PCP, chlorpromazine and tetracaine. Saturation binding experiments revealed two saturable sites, high and low affinity, for [^{3}H]PCP. The binding of [^{3}H]PCP to the receptor peptide was also enhanced by .02% SDS. These studies demonstrate that this 56 residue region contains sites for both the antagonist, α-Btx, and an NCI, [^{3}H]PCP, supporting the suggestion (Dipaola et al., 1986 Neurosci. Abstr. 12:961) that these sites are tightly coupled and part of one functional domain. Supported by NIH NS21896 and the Osserman/McClure Fellowship of the Myasthenia Gravis Foundation.

275.7

NICOTINIC ACETYLCHOLINE RECEPTORS AND N-GLYCOSYLATION REQUIREMENTS. V.M. Gehle*, R. Miledi, and K. Sumikawa. Lab. of Cellular and Molecular Neurobiology, Dept. of Psychobiology, Univ. of Calif., Irvine, CA 92717.

The role of N-glycosylation in the function, assembly, and membrane insertion of nicotinic acetylcholine receptors (nAChR) was studied using the Xenopus oocyte as an expression system. Initially, oocytes were injected with normal subunit mRNAs and incubated with tunicamycin. These oocytes showed reduced responses to acetylcholine (ACh). Biochemical data indicates this may have been at least partially due to inefficient insertion of the receptors into the plasma membrane, but was not attributable to a lack of assembly of the subunits.

To further study this problem, we also expressed mutant nAChRs by injection of various combinations of mutant (lacking N-glycosylation consensus sequences) and normal subunit mRNAs into oocytes. Data from cells injected with normal β, γ, δ, and mutant α subunit mRNAs indicate that mutant α subunits can assemble with normal β, γ, and δ subunits, but the resultant receptors are not inserted into the plasma membrane. Oocytes injected with normal α, β, γ, and mutant δ subunit mRNAs show responses to ACh which are smaller than those of control oocytes. Since assembly occurs with the mutant δ subunits, the reduced response may also be due to inefficient insertion. These results support the conclusions derived from the earlier experiments with tunicamycin. (Supported by NIMH Training Grant MH14599, NIH grants NS25928 and NS 23284, and the Muscular Dystrophy Association)

275.8

THE HYDROPHOBIC PHOTOREAGENT 3-(TRIFLUOROMETHYL)-3-(m-[^{125}I]IODOPHENYL)DIAZIRINE ([^{125}I]TID) AFFINITY LABELS A UNIQUE SITE ON THE TORPEDO NICOTINIC ACETYLCHOLINE RECEPTOR (AChR). B.H. White* and J.B. Cohen. Dept. of Anatomy and Neurobiology, Washington Univ. Sch. of Med., St. Louis, MO 63110.

Studies of [^{125}I]TID photolabeling of AChR in Torpedo postsynaptic membranes indicate that this compound specifically labels the AChR at a site (or sites) distinct from, but probably coupled to, the agonist and noncompetitive antagonist binding sites (White, B.H. and Cohen J.B., Biochemistry, 27:8741, 1988). To further characterize the binding of [^{125}I]TID we have studied the effects of nonradioactive TID on [^{125}I]TID photolabeling of the AChR and on the binding of tritiated cholinergic agonists and noncompetitive antagonists to Torpedo membranes. We find that nonradioactive TID inhibits [^{125}I]TID photolabeling with an IC_{50} of 10 μM and that specific photoincorporation saturates at 0.6 moles of TID per mole of AChR. Since [^{125}I]TID specifically labels all AChR subunits, this suggests a single TID site per AChR lying in close proximity to all subunits, probably within the central pore. We further find that TID causes a (maximal) five-fold increase in the K_D of [^{3}H]histrionicotoxin providing direct evidence for allosteric coupling of the noncompetitive antagonist and TID binding sites. A smaller inhibitory effect of TID is noted on the binding of agonists. We conclude that the AChR contains a central hydrophobic binding site for [^{125}I]TID that is distinct from, but linked to, the agonist and noncompetitive antagonist binding sites.

275.9

IDENTIFICATION OF NICOTINIC RECEPTORS ON CULTURED CORTICAL NEURONS USING ANTI-IDIOTYPIC ANTIBODIES. P.M. Lippiello, K.G. Fernandes*. Research and Development Dept., R.J. Reynolds Tobacco Co., Winston-Salem, NC 27102 and J.J. Langone*, R.J. Bjercke*. Baylor College of Medicine, Houston, TX 77030.

Based on the binding of L-[3H] nicotine to cell membrane preparations,we previously reported the presence of putative high-affinity nicotinic receptors on cultured neurons derived from fetal rat brain. (Lippiello and Fernandes, J. Pharmacol. Exp. Ther. 246:409, 1988). In the present studies we have identified these sites on intact cells by indirect immunofluorescence, using two anti-idiotypic monoclonal antibodies (422F11, 422G11) that represent the internal image of nicotine. Primary monolayer cultures of cortical neurons were fixed with 4% paraformaldehyde, followed either by incubation with L-[3H] nicotine (to assess receptor binding properties) or by successive incubations with anti-idiotypic antibodies and rhodamine-conjugated anti-mouse IgG (to determine the locations of cell-surface receptors). The affinity, kinetic binding properties and pharmacological specificity of cell-associated receptors were the same as those previously determined for cell membrane preparations and adult brain tissue. Approximately 10-20 % of the cells exhibited specific staining with both anti-idiotypes. Neurons with bipolar and pyramidal morphology exhibited the most intense fluorescence, primarily at the distal portion of cellular processes. No staining of glial cells was observed. The specificity of both anti-idiotypes was indicated by their ability to completely block the binding of L-[3H] nicotine to the receptors. Binding was not blocked by the myeloma protein MOPC. The snake toxins, alpha- and kappa-bungarotoxin, did not block the binding of either anti-idiotype to cells. The results suggest that anti-idiotypic antibodies to nicotine may provide appropriate structural, and possibly functional probes that are specific for high-affinity nicotinic receptors in mammalian brain.

275.10

CO-LOCALIZATION OF PHOSPHOTYROSINE WITH THE NICOTINIC ACETYLCHOLINE RECEPTOR AT THE RAT NEUROMUSCULAR JUNCTION Z.Qu* and R.L.Huganir. HHMI, Dept. of Neuroscience, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205

The nicotinic acetylcholine receptor (nAChR) is a ligand gated ion channel which has served as an excellent model system for the study of the structure, function and regulation of neurotransmitter receptors and ion channels. The nAChR is phosphorylated by cAMP-dependent protein kinase, protein kinase C and by a protein tyrosine kinase on a total of seven different phosphorylation sites. We have previously demonstrated that the nAChR isolated from Torpedo californica electroplax is highly phosphorylated on tyrosine residues. In contrast, the nAChR isolated from rat primary muscle cell cultures has a very low level of tyrosine phosphorylation. To examine whether tyrosine phosphorylation of the nAChR in muscle requires some signal from the nerve that is not present in the primary muscle cell cultures, we have used immunocytochemical techniques with affinity purified antibodies against phosphotyrosine to examine tyrosine phosphorylation in intact skeletal muscle. Immunofluorescent labelling of frozen sections of rat diaphragm with anti-phosphotyrosine antibodies shows specific intense labelling of the neuromuscular junction which directly co-localizes with the nAChR at the postsynaptic membrane. These results suggest that the nAChR is highly phosphorylated on tyrosine residues at the neuromuscular junction. The effect of denervation and development on tyrosine phosphorylation is currently being investigated.

275.11

SITE-SPECIFIC MUTAGENESIS OF THE PHOSPHORYLATION SITES OF THE NICOTINIC ACETYLCHOLINE RECEPTOR. P.Hoffman*, K.L.Choi*, P.Kienker*, G.Yellen and R.L.Huganir. HHMI, Dept. of Neuroscience, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205

The nicotinic acetylcholine receptor (nAChR) is a ligand gated ion channel which has served as an excellent model system for the study of the structure, function and regulation of neurotransmitter receptors and ion channels. The nAChR is phosphorylated by cAMP-dependent kinase, protein kinase C and a protein tyrosine kinase on seven different phosphorylation sites. The phosphorylation of the nAChR by all three of these protein kinases has been reported to modulate the rapid phase of receptor desensitization. We have now removed the known phosphorylation sites by site specific mutagenesis and have expressed these mutants (0phos receptor) in Xenopus oocytes. Wild type nAChR expressed in Xenopus oocytes is phosphorylated on the gamma and delta subunits. Peptide maps of the phosphorylated gamma and delta subunits are consistent with these subunits being phosphorylated at the known cAMP-dependent protein kinase sites. Expression of the 0phos receptor subunits in Xenopus oocytes produces a functional receptor, which is not phosphorylated, with single channel properties similar to the wild type channel. The 0phos receptor still displays slow desensitization in response to prolonged exposure to acetylcholine. These results suggest that although phosphorylation of the nicotinic receptor may regulate the rate of desensitization, it is not an absolute requirement for desensitization.

275.12

MEMBERS OF THE NEURONAL NICOTINIC ACETYLCHOLINE RECEPTOR FAMILY DISPLAY DISTINCT PHARMACOLOGICAL AND TOXICOLOGICAL PROPERTIES. C. W. Luetje* and J. W. Patrick. Division of Neuroscience, Baylor College of Medicine, Houston, TX 77030.

A family of genes has been identified which encode subunits of nicotinic acetylcholine receptors (nAChR) expressed in the brain. The alpha2, alpha3 and alpha4 subunits can each form functional nAChR when expressed in *Xenopus* oocytes in combination with the beta2 subunit (Boulter et al., 1987, P.N.A.S. 84: 7763; Wada et al., 1988, Science 240: 330). We find that these neuronal nAChR each possess distinct pharmacological and toxicological properties. Neuronal subunit combinations, as well as subunits encoding the muscle nAChR, were expressed in *Xenopus* oocytes and studied under two electrode voltage clamp. The nicotinic antagonist dihydrobetaerythroidine (DHBE) more effectively blocks the alpha4-beta2 receptor than the alpha2-beta2 receptor (16 fold difference in IC50), the alpha3-beta2 receptor (27 fold difference) and the muscle nAChR (at least a 200 fold difference). Neosurugatoxin is 100 fold more effective in blocking neuronal nAChR responses than muscle nAChR responses. Neuronal bungarotoxin blocks the alpha3-beta2 receptor 100 fold more effectively than the alpha4-beta2 receptor, while the alpha2-beta2 is insensitive to this toxin. These results demonstrate that homologous but distinct members of the neuronal nAChR alpha subunit gene family encode subunits with distinct functional properties.

275.13

EXPRESSION OF NEURAL NICOTINIC RECEPTOR SUBUNIT GENES IN GOLDFISH RETINA AND TECTUM. K.A. Cauley*, B.W. Agranoff and D. Goldman. Mental Health Research Institute and the Dept. of Biological Chemistry, University of Michigan, Ann Arbor, MI 48109.

Nicotinic Acetylcholine receptors (nAchRs) are expressed in the vertebrate retina. The numbers of different types of receptors, their cellular distribution, and their function within the retina, is not known. We have begun addressing these issues by characterizing cDNA clones encoding neural nAchR subunits isolated from a goldfish retinal cDNA library. One of these clones, referred to as goldfish non-alpha 2 (GFnα-2), was found to encode a putative structural subunit of the nAchR. The GFnα-2 gene is expressed predominantly by the ganglion cells of the retina (Cauley et. al., J. Cell Biol. 108:637, 1989). Here we report the identification of a second novel nAchR structural subunit sequence, GFnα-3, and a partial sequence of a ligand binding subunit, GFα-3, believed to be the goldfish homologue of the rat α-3 clone (Boulter et. al., Nature 319:368, 1986). In situ hybridization reveals that, like GFnα-2, the GFnα-3 and GFα-3 genes are expressed by the ganglion cells. However, GFnα-3 is expressed at a significantly higher level by cells of the inner-nuclear layer. In the tectum, which recieves synaptic input from the retinal ganglion cells, we find low expression of the GFα-3 and GFnα-2 genes. GFnα-3 is expressed throughout the tectum with highest levels of expression in the deepest layers. These data indicate that multiple types of neural nAchRs are expressed in retina and tectum.

275.14

IN SITU HYBRIDIZATION IDENTIFIES MEMBERS OF THE NICOTINIC RECEPTOR GENE FAMILY EXPRESSED IN DEVELOPING AND ADULT RAT RETINA F. Hoover* and D. Goldman (SPON: S. Easter). Department of Biological Chemistry and Mental Health Research Institute, University of Michigan, Ann Arbor MI, 48109.

Expression of neural nicotinic acetylcholine receptor (nAChR) subunit genes in the rat retina has been determined using c-RNA *in situ* hybridization techniques. Three members of the nAChR family have been investigated; two ligand binding subunit genes, α-3 and α-4 (Boutler *et al.*, Nature, 319:368, 1986 and Goldman *et al.*, Cell, 48:965, 1987) and a structural subunit gene, β-2 (Deneris *et al.*, Neuron, 1:45, 1988).

In the adult rat retina, these genes are expressed heterogeneously in the ganglion cell layer (GCL) and inner nuclear layer (INL). In the GCL, which consists of both ganglion and amacrine cells, approximately half of the cells contain detectable levels of α-3 RNA, while the majority of the cells contain α-4 and β-2 RNA. In the INL, which consists of predominantly amacrine cells, high levels of α-4 and β-2 RNA but low levels of α-3 RNA are found. *In situ* hybridization was used to investigate when nAChR genes are expressed during retinal development. At the earliest experimental time point studied (embryonic day 18), α-3, α-4 and β-2 genes are expressed by neuroblasts and post-mitotic cells, with the highest level of RNA found in cells closer to the inner wall of the retina. Many of these cells are likely to represent ganglion or amacrine cells. At postnatal day 1 (PN1), when a distinct GCL has been formed, α-3, α-4 and β-2 RNA levels are highest in this layer. From PN4-PN12, when ganglion cell death is occurring, nAChR gene expression gradually becomes adult-like.

These data indicate that multiple nicotinic receptor systems are used in the mammalian retina.

275.15

THE DEVELOPMENT OF ACH RESPONSES ON CHICK CILIARY GANGLION NEURONS IS NOT DEPENDENT ON THE TARGET.
K.L. Engisch* and G.D. Fischbach. Dept. of Anat. and Neurobiol., Wash. Univ. Sch. Med., St. Louis, MO 63110.

ACh responses on chick ciliary ganglion (CG) neurons increase in two discrete steps during development. A 2-fold increase in ACh responses occurs between embryonic day 8 (E8) and E9; a 3-fold increase between E14 and E18. Both occur after the establishment of 100% ganglionic transmission.

We investigated whether the development of ACh responses on embryonic CG neurons is dependent upon interaction with their target by removing the primordial eye on E2. Cells were dissociated from "eye-less" ganglia and studied 3-6 hours after plating. Neurons were clamped to -50 mV in the whole-cell configuration of the patch-clamp technique and ACh (250 μM) was applied by pressure-ejection. Peak ACh-induced currents in neurons that develop without target structures are reduced by 1.5-fold as early as E7, prior to the period of cell death. Responses of target-deprived neurons increase 2.7-fold between E7 and E10, and 3.5-fold between E12 and E18. The 1.2-1.5-fold reduction throughout development following target removal is not specific to ACh responses: GABA currents, voltage-activated G_{K+} and cell size are all reduced by a similar proportion in target-deprived neurons.

There is no profound effect of target removal on the ACh responses of embryonic neurons. This is in contrast to the 8-fold reduction in ACh sensitivity following axotomy of adult chick ciliary ganglion neurons.

275.16

TOXINS OF *CONUS* MARINE SNAILS BIND THE NICOTINIC ACETYLCHOLINE RECEPTOR AT SITES NOT IDENTICAL TO THOSE OF TRADITIONAL ANTAGONISTS.
R. A. Myers* (SPON: B. Olivera) Dept. of Biology, University of Utah, Salt Lake City, UT 84112.

The fish-hunting cone snails use venom to subdue their more agile prey. Paralysis is achieved with "conotoxins" against a multitude of neuronal and muscle receptors, including both voltage-activated Ca^{++} and Na^{+} channels, and the nicotinic acetylcholine receptor (nAChR) (Olivera et al. (1985) *Science* 230, 1338-1343). The α-conotoxins are a class of small (13-19 amino acids), basic, and homologous peptide antagonists of the nAChR which are lethal upon intraperitoneal injection in mice. α-Conotoxins compete for all α-bungarotoxin (BTX) and d-tubocurarine binding sites of the nAChR. However, there is evidence that the α-conotoxin's binding sites are not identical with those of other cholinergic antagonists. For example, α-conotoxin does not compete with BTX for binding to the 32 amino acid BTX-binding fragment of the α subunit (Wilson et al., (1985) *Proc. Natl. Acad. Sci. USA* 82, 8790-8794). Furthermore, crosslinking of α-conotoxin to the nAChR with bifunctional reagents of decreasing (12-, 8-, and 4-atom) tether lengths labels the β and γ subunits with increasing selectively. Photoaffinity labeling tags these subunits exclusively. The α subunit, putative site of cholinergic ligand binding, is not significantly labeled. Photolabeled segments have been preliminarily localized to segments β138-213 and γ121-183. These results suggest that the α-conotoxin binding site is distinct from those of other cholinergic ligands, and that with their smaller size, α-conotoxins are potentially sensitive probes of nAChR fine structure.

275.17

INTERACTIONS OF ANATOXIN-a ANALOGUES WITH NEURONAL ACETYLCHOLINE RECEPTORS.
S Wonnacott[1], S Jackman[1]*, VB Cockcroft[1]* RS Aronstam[2], H Rapoport[3]*, KL Swanson[4] & EX Albuquerque[4], [1]Dept Biochem, Univ Bath, BATH, BA27AY, U.K; [2]Dept Pharm Toxicol, Med Coll GA, Augusta, GA 30912; [3]Dept Chem, Univ CA, Berkeley CA 94720; [4]Dept Pharm Exp Ther, Univ MD Sch Med, Baltimore, MD 21201.

(+)Anatoxin-a (AnTx), a potent, stereospecific nicotinic agonist, has a semi-rigid structure that makes it attractive for structure-activity studies. Synthetic analogues of AnTx have been compared in ligand binding assays for cholinergic receptors in rat brain. The rank order of potency was the same at both [^{3}H]nicotine and [^{125}I]αBgTx binding sites: **AnTx > dihydro-AnTx = AnTx-methylester > AnTx-dimethylamide = dimethyl-AnTx = AnTx-al = AnTx-ol = AnTx-ol-O-methyloxime = αhydroxy-AnTx > nor-AnTx-ol = N-methyl-AnTx acid methyl ester.** All compounds were two orders of magnitude more potent at [^{3}H]nicotine sites than at [^{125}I]αBgTx sites. Interestingly, the potency at brain αBgTx sites correlated with binding to muscle nAChR.

With respect to functional groups involved in agonist recognition (the secondary amine and the conjugated carbonyl), N-methylation coupled with removal of >C=O results in total loss of binding, shown by (S)- and (R)N-methyl-AnTx-ol, whereas either change alone does not eliminate binding. These studies are being used to define acetylcholine binding sites in the CNS.

Supported by MRC Grant G8722675N and NATO Award 0243/87.

275.18

THYMOPOIETIN COMPETES WITH α-BUNGAROTOXIN FOR ITS BINDING SITE IN BRAIN SECTIONS: AN AUTORADIOGRAPHIC STUDY.
R. Afar*, P.B.S. Clarke*, T. Audhya*, G. Goldstein* and M. Quik. Dept. Pharmacol., McGill U., Montreal, Canada & Immunobiol. Res. Inst., Annandale, NJ.

Studies in neuronal tissues now suggest that the α-bungarotoxin (α-BGT) receptor is distinct from the neuronal nicotinic acetylcholine receptor, at least in some species. Recent studies in our laboratory have shown that thymopoietin (Tpo), a thymic peptide, can block α-BGT binding to rat brain membranes, possibly implicating a role for this polypeptide at this receptor. As an extension of this work, the interaction of Tpo with the α-BGT receptor was investigated in unfixed 20 μm thick rat brain sections. A dose-dependent inhibition of ^{125}I-α-BGT binding was observed with Tpo (IC_{50}=6 nM); this was similar to results obtained with non-radioactive α-BGT. The inhibitory effect of Tpo was not mimicked by splenin, a peptide differing in only 1 amino acid in its active site from Tpo. In contrast to its effects at the α-BGT receptor, Tpo ($\leq$1 μM) did not affect high affinity ^{3}H-nicotine binding to brain sections. Autoradiographic analysis of a number of brain areas provided no evidence for regional variation in the inhibitory effect of Tpo on ^{125}I-α-BGT binding. Thus Tpo can interact with the α-BGT binding protein in brain.

275.19

AGMATINE ACTS AS AN ANTAGONIST OF NICOTINIC RECEPTORS.
R.H. Loring and Y. Xie* Dept. of Pharmacology, Northeastern University, Boston, MA 02115

^{3}H-Agmatine is a cation at a physiological pH and has been used by others in ion flux methods to demonstrate nicotinic receptor function in neurons (e.g. Yoshikami, D. Science, 212:929, 1981). However, at high concentrations, we find that agmatine blocks nicotinic receptor function in both the chick retina and the rat superior cervical ganglion. In intact chick retina, 1 mM agmatine decreases depolarizations measured from the optic nerve that are induced by the nicotinic agonist, dimethylphenylpiperazinium (DMPP, 100 μM) by approximately 70%. In contrast, 5 mM agmatine has little or no effect on depolarizations in the retina induced by the glutamate analogs kainic acid or quisqualate (100 μM each), suggesting that agmatine selectively affects the nicotinic receptor. DMPP dose-response curves are reduced in a manner consistent with a non-competitive effect of agmatine. Furthermore, agmatine at 1 mM does not prevent binding of ^{125}I-neuronal bungarotoxin, a snake venom neurotoxin that competitively binds to functional nicotinic receptors in chick retinal homogenates (Loring et al., J. Neurosci., in press).

Agmatine influx has been used to demonstrate activation of nicotinic receptors in the rat superior cervical ganglion (SCG) at concentrations of 1-3 μM ^{3}H-agmatine (Quik, M., Brain Res., 325:79, 1985). However, 10 mM agmatine substantially blocks both DMPP-induced depolarizations of the rat SCG (80%) and synaptic transmission through the ganglia. These data raise the possibility that although agmatine is a cation that will traverse the channel of neuronal nicotinic receptors, agmatine also acts as a weak blocker of the open channel in a manner similar to hexamethonium or high concentrations of acetylcholine (e.g. Sine, S.M. and Steinbach, J.H., Biophys. J., 46:277, 1984). Supported by NIH grant NS22472.

275.20

A SERUM FACTOR MODULATES AGONIST ACTIVITY AT FUNCTIONAL NICOTINIC ACETYLCHOLINE RECEPTORS EXPRESSED BY THE PC12, SH-SY5Y AND TE671 CLONAL LINES.
Linda Lucero* and Ronald J. Lukas (SPON. T.J. Tarby). Division of Neurobiology, Barrow Neurological Institute, Phoenix AZ 85013.

Studies were initiated toward the identification of neuroactive substances that might act as cofactors in the regulation of nicotinic acetylcholine receptor (nAChR) function, which was assessed in clonal cell lines using a 86-Rb efflux assay. In the presence of as little as 1% horse serum, dose-response profiles for suberyldicholine (SbdCh) are shifted to higher concentrations by about 1, 2, and 3 log units, respectively, for activation of nAChR expressed by the human neuroblastoma lines SH-SY5Y and IMR-32 and the TE671 human medulloblastoma. Conversely, SbdCh (at concentrations as high as 10 mM) does not activate nAChR function on the PC12 rat pheochromocytoma cell line except in the presence of 1% horse serum. No effects of fetal calf serum are observed on SbdCh functional activity, nor are there observed effects of horse serum on activity of other nicotinic agents including a bis-choline compound (succinyldicholine) structurally related to SbdCh. The horse serum factor appears to have a molecular mass greater than 8 kDa and is selectively depleted upon passage over the anion exchanger, DEAE-Sepharose. Yet to be demonstrated is whether the horse serum factor acts on SbdCh or nAChR and whether it or SbdCh recognizes distinct ligand binding subsites on nAChR isotypes.

275.21

IN VITRO AND IN VIVO CORTICOSTERONE MODULATES NICOTINIC CHOLINERGIC RECEPTOR BINDING. J.R. Pauly, E.A. Ullman*, M.J. Marks* and A.C. Collins. Institute For Behavioral Genetics, Univ. of Colorado, Boulder, CO 80309.

Previous studies from our laboratory have shown that corticosterone (CCS) modulates nicotine sensitivity in the mouse. Adrenalectomy increases the sensitivity of animals to nicotine while chronic administration of CCS induces nicotine sub-sensitivity. These experiments were performed to determine if CCS has direct actions on nicotinic cholinergic receptor binding. For in vivo experiments, animals were adrenalectomized and supplemented with various concentrations of CCS in pelleted form. Following one week of chronic CCS therapy, nicotinic sites labeled by alpha-[^{125}I]-bungarotoxin (BTX) were decreased by 30-50%, depending on brain region; however, the binding of [^{3}H]-nicotine was unaltered. In vitro experiments were also performed to test for CCS-induced changes in the kinetics of NIC and BTX binding. Inclusion of CCS in binding assays (100 uM - 1mM) caused partial inhibition of single concentration binding (10-40%) for both ligands and also induced a concentration-dependent shift to the right of the K_I for nicotine inhibition of BTX binding. These data suggest that acute or chronic administration of CCS has profound effects on the binding of nicotinic cholinergic ligands to their receptors. Supported by DA-05131, DA-00116 and a grant from R.J. Reynolds Tobacco Company.

275.22

NICOTINE-EVOKED CALCIUM CHANGES IN SINGLE FETAL RAT CORTICAL NEURONS MEASURED WITH THE FLUORESCENT PROBE FURA-2. E.N. Fluhler*, P.M. Lippiello, K.G. Fernandes, R. J. Reynolds Tobacco Co., Winston-Salem, NC 27102 (SPON: J. Robinson)

The presence of nicotinic acetylcholine receptors (nAChRs) in the mammalian CNS is now widely accepted. Others have shown nicotine-evoked release of neurotransmitters from various CNS synaptosome preparations, indicating that these receptors may be of functional importance (1,2). We are using fluorescence microscopic and image analysis techniques to investigate the functional properties of putative nAChRs which we previously identified on cultured fetal rat cortical neurons (3). Administration of nicotine (100-1000 nM) to cultures loaded with the fluorescent calcium probe fura-2 resulted in a transient increase in intracellular calcium in approximately 5-15% of the neurons measured. The magnitude of the response was enhanced under partially depolarizing conditions, in some cases causing the cells to "fire" with a concomitantly large change in intracellular calcium. The ability of classical nicotinic antagonists to attenuate changes in calcium was consistent with the involvement of nicotine receptor activation in the response. Our results suggest that nicotine receptor-mediated events can lead to an increase in intracellular calcium, which has been proposed to play a role in neurotransmitter release.

1. Rapier, C., Lunt, G.G., Wonnacott, S. (1988) J. Neurochem. Vol. 50, No. 4, pp.1123-1130
2. Rowell, P.P., Winkler, D.L. (1984) J. Neurochem. Vol. 43, No. 6, pp.1593-1598
3. Lippeillo, P.M., Fernandes, K.G. (1988) J. Pharmacol. Exp. Ther. Vol. 246, No. 1, pp.409-416

275.23

LONG TERM NICOTINE TREATMENT IN RATS WITH BILATERAL NUCLEUS BASALIS LESIONS. N. N. Sjak-Shie*, J. Burks*, W. J. Millard and E. M. Meyer. Dept. of Pharmacology, Univ. of Florida, College of Medicine, Gainesville, FL 32610.

Bilateral nucleus basalis magnocellularis (nbm) lesions were shown previously to elevate cortical neuropeptide Y (NPY) levels and cause cell loss of cerebral cortical neurons by 10 months post-lesioning. In the present study, we examined the potential role of nicotinic transmission in these transsynaptic effects. We also investigated whether the increase in NPY levels may be due to increased transcription of the NPY gene. Male Sprague Dawley rats received bilateral nbm-infusions of 5 ug ibotenic acid. Starting 6 months post-lesioning, groups of sham-operated or lesioned rats received daily injections of saline or nicotine (0.2 mg/kg) until their sacrifice 2 months later. By 6 months post-lesioning, partial recovery of passive avoidance behavior was observed, and nicotine had no additional ameliorative effect. Behavioral recovery was complete by 8 months post-lesioning. High affinity [3H]acetylcholine-binding to nicotine receptors increased only 20% at 8 months post-lesioning, down from a 40% increase at 5 months. Nicotine-treatment in the lesioned animals increased receptor levels by an additional 30% by 8 months, suggesting that nicotine may partially block the loss of cells possessing nicotinic receptors. Brain sections from these animals are being analyzed for Nissl-staining cell bodies to corroborate this hypothesis. Our preliminary results indicate that 2 months post-lesioning, mRNA encoding pre-proNPY was reduced 24% using a NPY cDNA probe (from Dr. Janet Allen), while total NPY levels were unaffected. Levels of pre-proNPY mRNA are currently being measured from the animals in the aforementioned chronic nicotine study.

275.24

TOLERANCE TO NICOTINE IN CEREBELLAR CORTEX NEURONS. R. de la Garza. Univ. of Colo., Health Sci. Ctr., Dept. of Pharmacol. C236, 4200 E. 9th St., Denver, CO 80262 and Denver VAMC.

Recent studies from our laboratory have shown the heterogenous electrophysiological actions of nicotine in the rat cerebellum (de la Garza et al., J. Pharmacol. Exp. Ther., 240: 689, 1987). More recent studies have shown the pharmacological specificity of these actions of nicotine on cerebellar neurons (de la Garza et al., Psychopharmacology, in press). However, an important characteristic of the actions of nicotine in the peripheral nervous system is the rapid desensitization to its agonist actions and the present study attempted to determine the development of tolerance to the excitatory actions of nicotine applied from glass micropipettes.

Local application of nicotine produces excitations of cerebellar cortex interneurons. This study found that the mode of local application determines the extent of desensitization or acute tolerance. Repeated pressure- ejection of nicotine leads to a reduction in the response to nicotine if applications are spaced by less than 30 sec. Repeated iontophoretic applications of nicotine lead to reductions in the response to nicotine even if applications are spaced 90 sec apart.

Acute tolerance to nicotine may occur in cells of the cerebellar cortex if the mode of drug application maximizes the exposure of the tissue to nicotine and local concentrations of nicotine are higher after iontophoresis than after pressure-ejection. These findings are further evidence of the pharmacological nature of these cerebellar responses. (USPHS grant DA 07043 and VAMC).

275.25

INTRACELLULAR RECORDING OF NICOTINIC RESPONSES IN LATERAL SPIRIFORM NEURONS OF THE CHICK. E.M. Sorenson and V.A. Chiappinelli. Dept. of Pharmacology, St. Louis Univ. Sch. of Med., St. Louis, MO 63104.

The lateral spiriform nucleus (SPL) in the chicken mesencephalon is innervated by cholinergic fibers as demonstrated by choline acetyltransferase immunohistochemistry (Sorenson, E.M., et al., J. Comp. Neurol. 281:641, 1989). Autoradiography reveals a high density of ^{3}H-nicotine sites in the SPL, but little or no ^{125}I-alpha-bungarotoxin (ABgT) or ^{125}I-kappa-bungarotoxin sites (in preparation). The SPL is also recognized by monoclonal antibody mAb35 (Swanson, L.W., et al., J. Neurosci. 7:3334, 1987). The immuno-purified mAb35 binding site has a high affinity for ^{3}H-nicotine but does not bind ABgT. These results suggest that nicotinic transmission occurs in the SPL and that the functional receptor has a high affinity for nicotine. We now report that SPL neurons in chick brain slices respond to nicotine and carbachol in the presence of 1 μM atropine. Intracellular recording from SPL neurons display a depolarization and a decrease in input resistance following exposure to nicotinic agonists. Evoked action potentials are often seen during the depolarization. The nicotinic responses are reversibly blocked by 60 μM tubocurarine and 100 μM dihydro-β-erythroidine. ABgT (1 μM) does not antagonize these responses. We conclude that the high affinity ^{3}H-nicotine binding sites found in SPL neurons are functional receptors. (Supported by NS17574 to V.A.C.).

275.26

IRREVERSIBLE ORGANOPHOSPHATE EFFECTS ON NICOTINIC ACETYLCHOLINE RECEPTORS/ION CHANNEL COMPLEX. D.E. Menking*, R.G. Thompson and J.J. Valdes. Biotechnology Div., Chem. Res. Devel. & Engr. Ctr., Aberdeen Proving Ground, MD 21010-5423.

Organophosphate (OP) toxicity is primarily due to its inhibition of acetylcholinesterase. In sublethal doses, OPs induce symptoms which cannot be solely attributed to cholinesterase inhibition, with preliminary data indicating a direct interaction of OPs with postsynaptic nicotinic acetylcholine receptors (nAChR). Membrane fragments from Torpedo electric organ were used to determine OP interactions with the nAChR-coupled Na+ channel using 3H-phencyclidine (3H-PCP) as a probe. Highly toxic OPs (O-ethyl S-(diisopropylaminoethyl) methylphosphonothiolate, ethyl-N,N-dimethylphosphoramidocyanidate, isopropyl methylphosphonofluoridate and pinacolyl methylphosphonofluoridate) were found to potentiate binding of 3H-PCP to the nonactivated acetylcholine ion channel and to inhibit carbachol-activated 3H-PCP binding. To determine the irreversibility of the OP effects, membrane preparations were preincubated with OPs and washed several times with Tris-HCl buffer before assessing binding with 3H-PCP. Results show that OPs irreversibly potentiate 3H-PCP binding. These results are consistent with the hypothesis that OPs bind to, and irreversibly phosphorylate, an allosteric site on the ion channel associated with the nAChR.

276.1

ANTIBODIES AGAINST RAT LIVER CONNEXIN32 RECOGNIZE SUBSURFACE CISTERNS IN MOTONEURONS: IMMUNOHISTOCHEMICAL EVIDENCE FOR SIMILARITIES BETWEEN GAP JUNCTIONAL AND SUBSURFACE CISTERNAL PROTEINS. T. Yamamoto[1], E.L. Hertzberg[2] and J.I. Nagy[1] [1]Dept. of Physiol.,[2]Univ. of Manitoba, Winnipeg, Man., Canada R3E 0W3 and [2]Dept. of Neurosci., Albert Einstein College of Medicine, Bronx, N.Y., U.S.A. 10461

A polyclonal antibody against the 27kD rat liver gap junctional protein (connexin32) and a monoclonal antibody against a synthetic peptide corresponding to the carboxy-terminus of this protein gave discrete immunohistochemical labelling patterns in rat liver and in spinal cord and brain stem motoneurons. In sections of liver, staining with both antibodies was localized to puncta by double immunofluorescence and to gap junctions in this tissue by immuno-EM. In the facial motor nucleus, the polyclonal antibody labelled widely distributed fine puncta, the Golgi apparatus within somas of some motoneurons and large puncta on these somas and their initial dendrites. By immuno-EM the punctate labelling was localized to glial gap junctions and axon terminals in apposition to motoneurons at sites of subsurface cisterns. These subsurface cisterns were labelled by the monoclonal antibody. These results suggest that there may be some homology between gap junction proteins and the protein constituents of subsurface cisterns.

276.2

IMMUNOHISTOCHEMICAL LOCALIZATION OF THE 27KD AND 43KD GAP JUNCTION PROTEINS IN THE RAT CENTRAL NERVOUS SYSTEM. J.I. Nagy[1], T. Yamamoto[1], P.A. Carr[1] and E.L. Hertzberg[2] [1]Dept. of Physiol.,[2]Univ. of Manitoba, Winnipeg, Man., Canada R3E 0W3 and [2]Dept. of Neurosci., Albert Einstein College of Medicine, Bronx, N.Y., U.S.A. 10461

Intercellular communication in peripheral tissues is mediated by gap junctions composed of various junctional proteins referred to as connexin21, 32 and 43. Polyclonal antibodies against connexin32 as well as monoclonal and polyclonal antibodies against oligopeptides corresponding to different regions of each protein were used to determine immunohistochemically the localization of gap junctions, junctional proteins and what may be polypeptides related structurally or functionally to junctional proteins in the rat CNS. Antibodies against presumed cytoplasmic domains of connexin43 gave dense, heterogeneously distributed punctate staining throughout the CNS which in some structures was associated with glial gap junctions as seen by immuno-EM. Polyclonal anti-connexin32 gave immunoreactive neuronal and glial gap junctions as well as immunoreactive synaptic vesicles and dendritic and axonal cytoplasmic membranes. Antibodies against presumed extracellularly located regions of connexin32 and 43 gave intracellular immunoreactivity in neurons. These results indicate a differential and heterogeneous distribution of various connexin and possibly connexin-like proteins in the rat CNS.

276.3

SPECTROSCOPIC CHARACTERIZATION OF THE INTRINSIC FLUORESCENCE OF SYNAPSIN I. F. Benfenati, P. Neyroz°, M. Bähler+, F. Masotti*, L.F. Agnati* and P. Greengard+ Institue of Human Physiology, University of Modena, Italy, °Institute of Biological Chemistry, University of Parma, Italy and +Laboratory of Molecular and Cellular Neuroscience, The Rockefeller University.

Synapsin I is a major neuron-specific phosphoprotein which binds to synaptic vesicles and F-actin in a phosphorylation-dependent fashion. The existence of conformational transitions of synapsin I in response to microenvironmental changes were investigated by analyzing the steady-state and time-resolved intrinsic fluorescence of the protein. The steady state emission spectrum of native synapsin I was centered at 330 nm and underwent a clear-cut red shift upon denaturation, as expected for tryptophan residues mostly segregated from the external aqueous environment. The intrinsic fluorescence of synapsin I was resolved in its time-dependent components and their distribution as a function of wavelength was defined. Three major decay components with lifetimes of about 0.2, 3, and 7 nsec were resolved. Possible changes in synapsin I conformation upon site-specific phosphorylation were investigated by comparing the decay associated spectra obtained from the dephospho-form and the various in vitro phosphorylated forms. Upon phosphorylation of the tail sites, the spectra associated with the medium and long lifetimes were shifted to the red region of the spectrum, whereas the spectrum associated with the shortest lifetime was shifted to the blue region, in the absence of significant changes of the lifetimes. Similar changes were observed for the fully phosphorylated form of synapsin I. Supported by a NATO grant (0039/89) for collaborative research.

276.4

RETINAL RIBBON SYNAPSES LACK SYNAPSIN IMMUNOREACTIVITY J.W. Mandell*[1], E. Townes-Anderson[1], R. Cameron[2], P. Greengard[3], and P. De Camilli[2]. Cornell U. Med. Coll.[1], New York, 10021, Yale Univ. Sch. Med.[2], New Haven, 06510 and Rockefeller U.[3], New York, 10021

The vertebrate retina contains two ultrastructurally distinct types of vesicle-containing synapses. Conventional synapses are made predominantly by amacrine cells in the inner plexiform layer (IPL), while ribbon synapses are made by bipolar cells in the IPL and by photoreceptors in the outer plexiform layer (OPL). We have investigated, using immunocytochemical techniques, whether ribbon and conventional synapses differ in their content of three characterized synaptic vesicle proteins. Immunofluorescence labeling of adult rat retinas with antisera to P38 (synaptophysin) or with a monoclonal antibody to the synaptic vesicle protein SV2 revealed identical labeling patterns: intense labeling of large terminals, presumably rod cell spherules, in the OPL and bright labeling throughout the IPL. At the vitreal border of the IPL, large bulbous structures, possibly bipolar cell terminals, were intensely labeled. In contrast, antisera to synapsin I labeled the OPL extremely weakly. Labeling in the IPL was very bright though the bulbous structures were not labeled. To positively identify the cell types possessing the synapsin-negative, P38/SV2-positive terminals, enzymatically isolated retinal neurons were examined. The synaptic endings of intact rod photoreceptors and bipolar cells contained immunoreactive P38 and SV2 but not synapsin . In addition, protein A-gold labeling of ultrathin frozen sections demonstrated the localization of P38 to synaptic vesicles of photoreceptors terminals. Synapsin has been proposed to mediate the clustering and exocytosis of synaptic vesicles at the active zone. Its absence from rod and bipolar cells suggests that the mechanism of transmitter release is different in conventional and ribbon synapses . Supported by: NIH grant EY06135 (ETA), Life & Health Ins. Med. Res. Fund Scholarship (JWM) and Muscular Dystrophy Association grant (PDC)

276.5

LOCALIZATION OF SYNAPSIN I IN ADULT RAT BRAIN. L.J. DeGennaro, P.J.Apostolides*, D.Pulaski-Salo*, and J.E. Hamos. Dept. of Neurology, Univ. of Mass. Med. Ctr., Worcester, MA 01655.

To gain insight into the relationship between the neuronal phosphoprotein Synapsin I and synaptic function, we used anti-Synapsin I antibody to map the distribution of the protein in the brains of 6 adult rats. Synapsin I immunoreactivity was restricted to the neuropil and essentially no staining was seen in fiber tracts. There was marked regional heterogeneity in staining with a generally increasing caudorostral gradient along the neuraxis. Immunoreactivity was highest in the pyriform and cingulate cortices, the CA1 and CA3 fields of the hippocampus, the septal nuclei, ventral pallidum, globus pallidus, substantia nigra, and the median eminence of the hypothalamus. More modest staining was found in layers I, II, III, and V of cortex, the acellular glomerular and external plexiform layers of the olfactory bulb, the molecular layers of the cerebellum and dentate gyrus, the caudate-putamen, thalamus, amygdala, central gray, and spinal cord. Specific staining differences were also noted within various nuclei of the thalamus and amygdala. This heterogeneous staining pattern raises the question of whether the distribution of Synapsin I solely reflects variation in synaptic size and/or density, or whether it represents a variation of the protein's concentration within individual synapses that may be dependent on synaptic function and/or activity.

276.6

SYNAPSIN I EXPRESSION DURING POSTNATAL DEVELOPMENT OF THE RAT HIPPOCAMPUS. P.J. Apostolides*, D. Pulaski-Salo*, L.J. DeGennaro, and J.E. Hamos. Dept. of Neurology, Univ. of Mass. Medical Center, Worcester, MA 01655.

Synapsin I is a neuronal phosphoprotein whose phosphorylation has been implicated in the regulation of neurotransmitter release. To gain insight into the molecular mechanisms of synaptogenesis in the rat hippocampus, we employed immunocytochemical techniques using antibodies directed against purified Synapsin I to study the expression of this protein during a range of postnatal time points. Synapsin I immunoreactivity was present in the newborn rat (P0) despite the paucity of mature synapses within the hippocampus at this time and was present in both fiber bundles as well as select synapses. During early postnatal days, immunoreactivity revealed diffuse staining within the molecular layer of the dentate gyrus and a distinctively increasing mediolateral gradient of immunostaining within the mossy fiber terminals of the dentate granule cells. By the second postnatal month, Synapsin I immunoreactivity assumed its adult-like characteristics and was limited to the neuropil. Our results suggest that the temporal and spatial expression of Synapsin I within the developing rat hippocampus reflects the process of synaptogenesis. These findings are consistent with previous studies of Synapsin I expression in other developing regions of the rat brain, and provides further support for its developmental regulation.

276.7

DISTINCT PLATELET-ACTIVATING FACTOR (PAF) BINDING SITES IN SYNAPTIC MEMBRANES AND MICROSOMES FROM RAT CEREBRAL CORTEX. NG Bazan[1], VL Marcheselli[1]*, P Braquet[2]*, and JM Cluzel[1]*. LSU Eye Center, New Orleans, LA[1], Institut Henri Beaufour, Le Plessis Robinson, Paris, France[2].

PAF binding sites were studied in enriched subcellular fractions from rat brain cortex. The Kd in synaptic membranes is 1.2 nM and the Bmax is 0.99 pmole/mg protein. The high-affinity binding site in microsomes has a Kd of 22.5 pM and a Bmax of 82.5 fmole/mg protein; the low-affinity binding site Kd is 25.0 nM and the Bmax is 60.0 pmole/mg protein. The order of potency of antagonists on synaptic binding sites is BN-50726 (IC_{50}=1.33·10^{-7}M), BN-50727 (IC_{50}=1.46·10^{-7}M), and BN-52021 (IC_{50}=1.64·10^{-7}M). Even if BN-50727 has identical potency in both membrane preparations, the other BN compounds are more potent in the synaptic membranes than in microsomes. BN-52021 appears to be a non-competitive inhibitor for both membrane preparations. A comparison of other PAF antagonists shows that CV-3988 is the most potent in microsomes, with an IC_{50} of 1.57·10^{-9}M. In synaptic membranes it has an IC_{50} of 2.54·10^{-7}M. Because of its different subcellular location and its different kinetics and response to antagonists three binding sites of PAF appear to be present in cerebral cortex. Supported by NS 23003.

276.8

DISTRIBUTION OF VESICLE ASSOCIATED MEMBRANE PROTEIN mRNA IN RAT CENTRAL NERVOUS SYSTEM. T.S. Gray, L.A. Elferink, W.S. Trimble, R.H. Scheller and M.C. Wilson. Loyola Univ., Maywood, IL 60153, Stanford Univ., Stanford, CA 94305, Res. Inst. Scripps Clinic, La Jolla, CA 92037.

Recently, cDNA clones of mRNAs encoding two synaptic terminal vesicle associated proteins (VAMP) of rat brain have been isolated that are 84% and 75% homologous to a VAMP-1 protein isolated in Torpedo ray (Elferink et al., J.B.C., 1989). The rat VAMP-1 & -2 predicted protein sequences consist of 118 and 116 amino acids and are encoded by distinct genes. Here we report the distribution of VAMP-1 & -2 mRNA within rat brain via in situ hybridization of ^{35}S RNA probes directed against the distinct 3' untranslated sequence of mRNA. In spinal cord VAMP-1 mRNA levels were highest in ventral horn cells while VAMP-2 was higher in dorsal horn. In brainstem VAMP-1 mRNA was localized to the lateral reticular formation, the 12th, 7th, 5th, 4th and 3rd cranial nerve motor nuclei. VAMP-2 mRNA levels were highest in inferior olivary, the vagal motor, dorsal raphe', parabrachial and parabigeminal nuclei. mRNA levels for both VAMPs was high within the pontine gray. In forebrain VAMP-1 mRNA was localized to the thalamic reticular, entopeduncular and the lateral mammillary nuclei. VAMP-2 hybridization was generally higher within the forebrain and was localized within the hypothalamus, amygdala and cortex. High levels of mRNA to both VAMPs was observed within the anterior thalamic nucleus. The present findings indicate some functional correlations for the differential VAMP mRNA expression; e.g., VAMP-1 is associated with somatomotor neurons while VAMP-2 is expressed in autonomic motor neurons. Supported by NS20041 (TSG) and NS23038 (MCW).

276.9

A SUBPOPULATION OF SYNAPTIC VESICLES FROM RAT BRAIN CONTAINS TUBULIN. E. Floor, Dept of Anatomy, Univ. of Wisconsin Medical School, Madison, WI 53706.

Many studies have reported tubulin in partially purified synaptic vesicles from brain. At the final step in preparation of >90% pure synaptic vesicles from rat brain, tubulin co-purifies with synaptophysin, a synaptic vesicle protein. Thus isolated brain synaptic vesicles do appear to contain tubulin. About 30% of total protein in >90% pure synaptic vesicle preparations can be immunoprecipitated by polyacrylamide beads (Immunobeads, BioRad) coated with KMX-1, a monoclonal antibody to β-tubulin (Birkett et al. (1985) *FEBS Lett.* **187**, 211-218). In contrast, ~90% of total protein is immunoprecipitated specifically by monoclonal antibodies to synaptic vesicle proteins SV2, synaptophysin, or p65 (Floor and Feist (1988) *J. Neurochem.* **52**, In press). Antibody KMX-1 immunoprecipitates all of the tubulin in these experiments as shown by Western immunoblot analysis with polyclonal tubulin antibody of proteins remaining in the supernatant after immunoprecipitation. Tubulin, then, is present on a subpopulation of rat brain synaptic vesicles. One of the major proteins in these synaptic vesicles observed by SDS-PAGE with Coomassie staining, protein R2, can be identified as tubulin by Western immunoblotting. Protein R2 is a tightly-bound, extrinsic membrane protein. It remains bound at high or low ionic strength but dissociates in 50mM NaOH. The strong association of vesicular tubulin with synaptic vesicles suggests that its presence there is not artifactual. Because tubulin is an abundant protein in brain, its presence on a subpopulation of synaptic vesicles also argues that tubulin does not bind nonspecifically to these organelles. The function of tubulin in synaptic vesicles is not known, although a role in mediating interactions with the cytoskeleton seems likely. (Supported by NIH Grant NS 24890.)

276.10

WITHDRAWN

276.11

DOES MUTAGENESIS OF ASP-135 IN CA^{2+}/CALMODULIN-DEPENDENT PROTEIN KINASE II (CK-II) ALTER SUBSTRATE BINDING? S. A. Westgate*, P.T. Kelly and M.N. Waxham. Depts. of Neurology, and Neurobiology and Anatomy, Univ. of Texas Medical School, Houston, TX 77225.

Through comparison of conserved sequences in protein kinase catalytic domains it was determined that serine/threonine specific kinases share a conserved stretch of amino acids not found in tyrosine specific kinases (Hanks, S.K. et al., Science, 241:42, 1988). This consenses sequence Asp-Leu-Lys-Pro-Glu-Asn (DLKPEN) is located at residues 135-140 in the amino terminal half of the 50 kDa subunit of CK-II and was proposed to be involved in protein substrate binding. Using site directed mutagenesis we have substituted Asn for Asp-135 in the DLKPEN region of the 50 kDa subunit and expressed the protein in bacteria. The expression in bacteria of the 50 kDa subunit of CK-II using the expression vector PK233-2 was previously reported by this lab. The Asn-135 substituted enzyme was purified and compared with non-mutated bacterially expressed CK-II using phosphorylation assays as an indicator of activity. The Asn-135 substituted enzyme showed no autophosphorylation or phosphorylation of any substrates in the presence or absence of calcium and calmodulin, whereas the original Asp-135 enzyme phosphorylated itself and synapsin only with calcium and calmodulin present. In order to determine whether the absence of phosphorylation could be due to alterations in ATP binding, photoaffinity labeling experiments were done using alpha-^{32}P-8-azido ATP. After labeling, both the Asp-135 and Asn-135 enzymes were analyzed by SDS-PAGE and the autoradiographs showed the 50 kDa proteins to be labeled suggesting both were able to bind the azido-ATP analogue. These results suggest that the Asn-135 mutant binds ATP but is unable to undergo the phosphoryl transfer reaction presumably due to an alteration in its ability to bind protein substrates.

276.12

A MONOCLONAL ANTIBODY SPECIFIC FOR TAP-1. M. Iwata*, A. Davis* and S.S. Carlson. Dept. of Physiology and Biophysics, University of Washington, Seattle, WA 98195.

TAP-1 is a large (10^6 dalton) chondroitin sulfate proteoglycan (CSPG), hypothesized to act as a glue at the elasmobranch electric organ synapse (Carlson and Wight, 1987, *JCB*, 105:3075-3086). This proteoglycan was originally identified because it shares a carbohydrate epitope (SV4) with a smaller synaptic vesicle proteoglycan (Carlson, et al., 1986, *JCB*, 103,509-520).

We have produced a monoclonal antibody which is specific for TAP-1 and does not react with the synaptic vesicle proteoglycan. The antigenic site (T1) identified by this monoclonal antibody is destroyed by protease digestion. That is, when TAP-1 is subjected to overnight digestion with pronase, it can no longer inhibit the immunoprecipitation of ^{125}I-TAP-1 with the anti-T1 mAb. This is in contrast to the SV4 epitope which is pronase resistant in this test. Thus, the anti-T1 mAb may be directed against the polypeptide core of the TAP-1 CSPG.

By immunocytochemistry at the light level, the T1 antigenic site has a broad tissue distribution in electrically excitable tissue of the elasmobranch electric fish. It is present on both the innervated and non-innervated face of the electrocyte, and on the surface of muscle cells. It is present in the electromotor nucleus which innervates the electric organ, other regions of the fish brain, and spinal cord. We also find the antigen present in the mammalian amphibian retina.

The SV4 antigenic site behaves like a glycosaminoglycan. It can be released from TAP-1 by alkaline borohydride cleavage. When chromatographed on Sepharose 6B, the released antigenicity elutes as a broad peak with an apparent molecular weight range of 23,000-76,000. The SV4 antigenicity requires approximately 0.5 M NaCl at pH 4.9 to be eluted from DEAE nitocellulose. It is resistant to all glycosaminoglycan lyases except keratinase by which it is partially digested.

276.13

VAT-1: AN ABUNDANT MEMBRANE PROTEIN FROM TORPEDO CHOLINERGIC SYNAPTIC VESICLES. M. Linial*, K. Miller* and R.H. Scheller. Department of Biological Sciences, Stanford University, Stanford, CA 94035-5020.

Expression screening of a lambda gt-11 library constructed from electromotor nucleus of Torpedo was used to isolate cDNA clones encoding a synaptic vesicle membrane protein, VAT-1. The RNA is specifically expressed in the electric lobe and to a much lower extent in the brain. The predicted protein has a molecular weight of 41,572 daltons and contains several hydrophobic regions. The best homology found is to calcium translocase of rabbit muscle and several other ATPases. An antibody raised against a fusion protein recognizes an abundant 42 kD protein which copurifies with synaptic vesicles. The integrity of VAT-1 to vesicles was confirmed by direct amino-acid sequencing and by tryptic digestion of vesicles. Hydrodynamic studies suggest VAT-1 is part of a high-molecular weight complex of the vesicle.

276.14

NEURONAL EXCITABILITY IN LONG-SLEEP AND SHORT-SLEEP MICE: II. POTASSIUM-STIMULATED CALCIUM-DEPENDENT RELEASE OF GABA FROM FRONTAL CORTEX SYNAPTOSOMES. B. C. Jones*, C. C. Duncan and V. G. Erwin. Alcohol Research Center, University of Colorado, Boulder, CO 80309.

Long-sleep (LS) and short-sleep (SS) mice, are known for their differential initial sensitivity to the hypnotic effect of ethanol (EtOH); the LS line being the more sensitive both in sleep time and in blood ethanol content upon awakening from a hypnotic dose of EtOH. We recently completed an experiment in which we examined calcium-dependent, potassium-stimulated release of GABA from synaptosomes prepared from the frontal cortex of LS and SS brains. Enriched synaptosomal fractions were prepared from frontal cortex tissue and incubated in Krebs-Ringers-HEPES (KRH) buffer containing ^{3}H-GABA. Using a superfusion technique, (Tapia, R. and Sitges, M., *Brain Res.*, 250:291, 1982) synaptosomal samples (0.3-0.5 mg protein), samples were washed in KRH buffer containing 4.7mM potassium and 2.5mM calcium. Following the wash, KRH containing 4.7, 9.4, 18.8, 37.6 or 47mM potassium was added to the superfusion chamber and 0.5 ml samples were collected for liquid scintillation counting of released GABA. Findings included calcium dependency and differential thresholds for GABA release in LS and SS synaptosomes. While there was no difference between LS and SS in GABA release at 37 or 47 mM potassium, 9.4 and 18.8 mM potassium produced greater GABA release (percent of baseline) in LS, compared to SS synaptosomes. EtOH (22 and 88 mM) added to all release buffers produced a dose related tendency for reduction in GABA release with no differential sensitivity seen between the two lines of mice. Supported by USPHS Grants no. AA03527, AA00079 and AA07330.

276.15

Na^+-Ca^{2+} EXCHANGE ACTIVITY IN SYNAPTIC PLASMA MEMBRANES FROM POSTMORTEM HUMAN BRAIN. **G. Hoel*, and M.L. Michaelis. Dept. Pharmacology/Toxicology and Center for Biomedical Research, University of Kansas, Lawrence, KS 66046**

Procedures have been developed for the preparation of resealed plasma membrane vesicles from postmortem human brain. The vesicles were enriched in synaptic junctions and exhibited the capacity to transport Ca^{2+} in the presence of a Na^+ gradient. The kinetic characteristics of the Na^+-Ca^{2+} exchange process were determined in synaptic plasma membrane vesicles isolated from human brain hippocampus and cortex. The K_{act} for Ca^{2+} was calculated with computer assisted curve fitting to be 33.7 uM for the hippocampal tissue and 17.6 uM for the cortical tissue. The maximal rate of Ca^{2+} uptake (V_{max}) was determined to be 3.6 nmol/mg protein/15 sec and 3.34 nmol/mg protein/15 sec for hippocampal and cortical tissue respectively. Dependence of Ca^{2+} transport on the magnitude of the Na^+ gradient was found to be fairly complex as might be expected from the stoichiometry reported in other tissues. The effect of varying the pH of the incubation medium was examined and an optimum observed at pH ~9. The results of these studies demonstrate that Na^+-dependent Ca^{2+} transport activity can be preserved in membranes isolated from postmortem human brain. Such preparations could potentially be used to determine the influence of various pathological conditions on this transport system. **[Supported by grants AA 04732 from NIAAA, DAAL-03-88-K0017 from ARO, and AG 04762 from NIA].**

276.16

SYNAPTIC VESICLES AND CALCIUM HOMEOSTASIS AT THE SYNAPSE. M.Mata and D.J.Fink. University of Michigan and VA Medical Center, Ann Arbor, MI, 48105

Using the oxalate-pyroantimonate technique to demonstrate the ultrastructural localization of calcium *in vivo*, we have previously found that calcium appears to be sequestered in synaptic vesicles of the neuromuscular junction (Histochem. 87:339, 1987). Similar localizations of calcium in synaptic vesicles have been reported by others in CNS synapses (J.Anat. 129:869, 1979).

We have now used EM enzyme cytochemistry (modified from Ando, Acta Histochem.Cytochem. 14:705, 1981) to localize the calcium pump (Ca-ATPase) at synapses in the central nervous system and the neuromuscular junction. Vibratome sections from rat cerebral cortex, spinal cord, and muscle were incubated in medium containing 3 mM ATP, 10 mM $CaCl_2$, 4 mM lead citrate and 8 mM levamisole, rinsed with cacodylate buffer, post-fixed with osmium, and embedded in Epon-Araldite. Ca-ATPase activity, visualized as the electron dense lead phosphate deposits, was demonstrated in the plasma membrane and was prominent intracellularly along all synaptic vesicle membranes.

We propose that calcium entry accompanying depolarization at the synapse may be buffered in part by pumping of calcium into synaptic vesicles by Ca-ATPase. This calcium pool would then be released from the presynaptic terminal when vesicle release is triggered by depolarization. Such a mechanism may constitute a very efficient method for dealing with calcium entry that accompanies stimulation at the synapse.

Supported by grants from the JDF, the NINDS, and the VA.

276.17

EFFECT OF CHRONIC ETHANOL ADMINISTRATION ON THE INTERMEDIATE STEPS OF (Na,K)-ATPase ACTIVITY. P.M. Wixom and A.Y. Sun. Sinclair Research Farm and Dept. of Biochem. Univ. of Missouri, Columbia, MO 65203

(Na,K)-ATPase activity depends greatly on the structural integrity of the membrane. Previous studies have indicated that ethanol, which partitions into membranes freely, hinders the conformational changes of the (Na,K)-ATPase in the reaction sequence. Using (γ-^{32}P)ATP and autoradiography, we have been able to separate the steps involved in the conformational changes of the enzyme by manipulating the Na^+ and K^+ in the assay medium. Previous studies have found that in vitro ethanol did not block the phosphorylation process, but did inhibit the protein dephosphorylation step.

In this study, synaptic plasma membranes were isolated from cerebral hemispheres of C57/Bl mice who had been on a liquid diet containing 5% ethanol for 5 weeks. In the ethanol tolerant membranes, the phosphorylated intermediate was increased, possibly reflecting an increase in the amount of enzyme. This provides a structural basis for the increase in specific enzyme activity of (Na,K)-ATPase after chronic ethanol administration observed in our laboratory as well as others. Also in ethanol tolerant membranes the dephosphorylation process in the presence of K^+ was increased, reflecting a faster turnover rate. Since (Na,K)-ATPase is responsible for the regulation of membrane repolarization by the transport of the Na^+ and K^+ across plasma membranes, the increase in enzyme activity after chronic ethanol ingestion may be related to the adaptational process leading to ethanol tolerance. (Supported in part by NIAAA grant # AA02054.)

277.1

PREFERENTIAL RELEASE OF EPINEPHRINE FOLLOWING MUSCARINIC STIMULATION OF BOVINE ADRENAL MEDULLA CELLS: EFFECT OF LITHIUM. R.H. Lenox, A. Hussein* and O. Zinder*, Neurosc. Res. Unit, Dept. of Psychiatry, UVM Med. Coll., Burlington, VT; Dept. of Clin. Biochem., Rambam Med. Ctr., Haifa, ISR.

Acetylcholine (ACH) stimulation of adrenal medullary chromaffin cells results in the release of both epinephrine (EPI) and norepinephrine (NEPI). Nicotinic (NIC) stimulation produces a depolarization dependent influx of calcium, triggering release; while muscarinic (MUSC) receptors are coupled to phosphoinositide metabolism and intracellular mobilization of calcium. We have been examining the pattern of catecholamine (CAT) release following MUSC vs NIC activation and the effects of lithium on this process.

Medullary chromaffin cells were isolated from fresh bovine adrenal glands and stabilized in incubation medium. Basal secretion was 75% EPI reflecting the CAT content of the cells. Following stimulation with either ACH or NIC, the pattern of CAT released was 62% EPI and 38% NEPI; a significant shift to NEPI release. Mecamylamine partially blocked ACH mediated release and significantly increased the EPI/ NEPI ratio. Release of CAT following MUSC activation was diminished relative to ACH with an EPI/NEPI ratio similar to basal conditions; and was blocked by atropine. Pre-exposure of cells to lithium markedly reduced MUSC mediated release of CAT and decreased the EPI/NEPI ratio. The MUSC receptor appears to mediate preferential release of EPI which predominates during basal secretion. Lithium appears to preferentially block MUSC mediated release.

277.2

MODULATION OF CATECHOLAMINE SECRETION FROM ADRENAL CHROMAFFIN CELLS BY ADENOSINE AND ADENINE NUCLEOTIDES. M. Diversè-Pierluissi, W.N. Kopell, K.T. Kim and E.W. Westhead. Program in Molecular and Cellular Biology, University of Massachusetts, Amherst, MA 01003.

We have previously reported that adenosine and adenine nucleotides can inhibit or enhance nicotinic or K^+ triggered release of catecholamine from chromaffin cells (Chern et al, J. Neurochem,1987,1988). Enhancement by ATP is instantaneous but inhibition develops over about 3 min.. Cholera toxin prevents the former effect, pertussis the latter. ATP elevates IP_3 causing release of Ca^{2+} from internal stores but this effect appears unrelated to enhancement or inhibition since neither effect is mimicked by UTP which also causes IP_3 production and Ca^{2+} release. ADP-ribosylation of membrane proteins by cholera and pertussis toxins identifies at least two proteins whose labeling is altered by the presence of ATP or ADP during toxin treatment. Studies with antibodies to specific G-proteins are in progress to identify the nucleotide-sensitive proteins. Phenylisopropyl-adenosine (PIA) can inhibit secretion by >90%,an effect apparently not related to its effect on cAMP. Preincubation of chromaffin cells with ^{32}P phosphate, followed by stimulation, shows that many proteins are phosphorylated or dephosphorylated in response to stimulation. A subset of these shows marked sensitivity to preincubation with PIA under conditions in which PIA inhibits secretion.
Supported by NIH Grant 26606

277.3

ARACHIDONIC ACID METABOLITES AS MEDIATORS OF THE LONG TERM EFFECTS OF ANGIOTENSIN ON CHROMAFFIN CELL SECRETION. A.M. Poisner*, M.K. Stachowiak, H.K. Jiang*, P. Hudson* and J.S. Hong (SPON: E.J. Walaszek). Dept. of Pharmacology, University of Kansas Medical Center, Kansas City, KS 66103 and NIEHS, Research Triangle Park, NC 27709.

We recently reported that 24 hr treatment of chromaffin cells with Sar-1-angiotensin II (ANG) (0.2-20 nM) causes the secretion of catecholamines (CAT) and met-enkephalin (MET), while simultaneously increasing the levels of mRNA for tyrosine hydroxylase, PNMT and proenkephalin (FASEB J. 2:A1799, 1988). We now report that some of the effects of ANG appear to be mediated by arachidonic acid (AA) and/or its metabolites. AA and PGE2 caused a marked increase in CAT and MET secretion. The effects of ANG and AA were greatly reduced by 1-10 uM indomethacin, whereas the stimulant effect of veratridine was unaffected. Similar results have been found when examining mRNA induction. The results suggest that some of the long term effects of ANG on chromaffin cell function are mediated by cyclooxygenase metabolites. Other experiments implicate protein kinase C and lipoxygenase products. Interaction between AA metabolites and protein kiase C seems likely.

277.4

MUSCARINIC BINDING AND EFFECT ON CATECHOLAMINE RELEASE IN OVINE ADRENAL MEDULLA. C.Y. Cheung. Dept. Repro. Med., Univ. Calif., San Diego, CA 92093

Catecholamine(CA) release from the adrenal medulla(AM) is mediated through nicotinic receptors. In species such as the rat, muscarinic agonists can also stimulate CA release. The present study investigated the presence and function of muscarinic receptors in the AM of fetal and adult sheep. Muscarinic binding was determined by incubating aliquots of purified AM membrane fraction with [^{3}H]QNB at 5 to 1000 pM in the presence or absence of 2.5 uM atropine (to define nonspecific binding). Specific binding of [^{3}H]QNB to adult ovine AM membrane was saturable and of high affinity, where K_D=20±2pM and Bmax=90.6±3.9fmol/mg. Scatchard analysis revealed a second binding site with K_D=19±8nM and Bmax= 3.3±1.6pmol/mg. In AM of fetuses at 130 days gestation (term=147 days), [^{3}H]QNB binding was observed with K_D and Bmax of 24±4pM and 91.2±11.9fmol/mg, resp. for the first, and 23±11nM and 2.1±0.8pmol/mg, resp. for the second site. These values were not different from those in the adult. Muscarine at 5 to 50 uM had no effect on CA release from AM cells in vitro. Thus, muscarinic binding sites can be identified in the AM of the adult and fetal sheep. However, it appears that these receptors may not be playing a role in regulating CA release.

277.5

CHARACTERIZATION OF GLUCOCORTICOID RECEPTORS IN CULTURES OF BOVINE ADRENAL MEDULLARY CELLS K. Betito, M.J. Meaney, D.H. Aitken*, J. Diorio* and P. Boksa Douglas Hosp. Res. Ctr., Depts. of Pharmacol. & Psychiatry, McGill Univ., Verdun, Quebec, H4H 1R3

The adrenal cortex and medulla are closely interrelated, such that levels of catecholamines (CAs) and other neurohormones secreted by the chromaffin cells of the adrenal medulla are influenced by the glucocorticoids (GCs) from the adrenal cortex. In the present study, we have measured GC receptors in cultured bovine adrenal medullary cells isolated using a Percoll gradient. We observed specific binding of [^{3}H]-dexamethasone (DEX) (Kd=3.5±0.7 nM; Bmax=40.2±4.0 fmol/mg protein) and of the pure GC receptor agonist, [^{3}H]-RU 28362 (Kd=1.5±0.2 nM; Bmax=33.3±3.4 fmole/mg protein). The GC receptor is a typical type II GC receptor, as observed by competition studies, and has relative binding affinities of RU 28362 > cortisol > aldosterone. In the presence of cortisol, translocation of the GC-receptor complex from a soluble to a chromatin-bound compartment was dose-dependent, and the soluble receptor levels were replenished within 30 minutes following removal of steroid. Nuclear uptake of GCs was observed by measuring the amount of [^{3}H]-DEX in the nuclear fraction. Incubation of these cultured cells with 100 nM cortisol produced increased PNMT activity. Treatment with insulin or reserpine produced no change in GC receptor Bmax, while treatment with 8-bromo-cAMP, an analogue of cAMP, decreased soluble GC receptors. In chromaffin and non-chromaffin cell cultures further purified by differential plating, preliminary data indicate that GC receptor numbers did not correlate with CA content. This suggests the presence of GC receptors in both chromaffin and non-chromaffin cells. Supported by FRSQ & MRC of Canada.

277.6

ADRENAL CHROMAFFIN CELLS EXPRESS IMIDAZOLE RECEPTORS WHICH BIND CLONIDINE AND AN ENDOGENOUS CLONIDINE-DISPLACING SUBSTANCE. P. Ernsberger, M.J. Evinger, M.P. Meeley, G. Feinland* & D.J. Reis. Div. Neurobiology, Cornell Univ. Med. Coll., NY, NY 10021.

In the medulla oblongata, clonidine binds not only to α_2-adrenergic receptors but also to non-adrenergic sites specific for imidazoles (Ernsberger et al. *Eur J Pharmacol* 134:1 '87). Since clonidine induces mRNA for PNMT in bovine adrenal chromaffin cells (BACC) by a non-adrenergic mechanism (Evinger et al. *this meeting*) we sought to determine whether BACC express imidazole receptors which bind clonidine. BACC were isolated, homogenized, and fractionated into P1, P2, and P3 by differential centrifugation. Membranes (P2) were incubated (40 min, 25°C) with the clonidine analog ^{3}H-*p*-aminoclonidine (^{3}H-PAC) prior to vacuum filtration. Nonspecific binding was defined by piperoxan or phentolamine (0.1 mM). Specific, saturable, high-affinity ^{3}H-PAC sites were abundant in BACC P2 membranes (B_{max}=-345±111 fmol/mg protein; K_d=0.66±0.24 nM). Most of the ^{3}H-PAC sites in BACC were present in P2 (72±12%); lesser amounts were recoved in P1 (19±3%) and P3 (9±2%). Adrenergic agents with imidazole rings, e.g. phentolamine, piperoxan, PAC and idazoxan, completely and potently inhibited ^{3}H-PAC binding (K_i<300 nM), whereas non-imidazoles such as epinephrine, phenylephrine and SKF-86466 were inactive (K_i>1 mM). Imidazoles lacking adrenergic potency exhibited high affinity for ^{3}H-PAC sites on BACC (cimetidine: K_i=3.3±0.9 µM; IAA: K_i=5.2±1.3 µM). Brain extracts containing clonidine-displacing substance (CDS), the putative natural ligand at imidazole receptors, potently inhibited ^{3}H-PAC binding (K_i=0.21±0.04 Units). We conclude that clonidine binds to imidazole but not to α_2-adrenergic receptors in BACC. Imidazole receptors may mediate the actions of clonidine on BACC, and CDS may be the endogenous ligand.

277.7

EFFECT OF MPTP ON THE MORPHOLOGY OF THE PRIMATE ADRENAL MEDULLA. S.W. Carmichael, L.E. DeLanney, D.A. DiMonte*, L.S. Forno, I. Irwin*, and J.W. Langston, Dept. Anatomy, Mayo Clinic/Foundation, Rochester, MN 55905 and California Parkinson's Foundation, San Jose, CA 95128.

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) was administered systemically to 15 squirrel monkeys. The doses varied from 1-3 s.c. injections of 2.5 mg/kg. Animals were sacrificed 1 to 41 days after the last injection. Two monkeys served as naive controls. Following sacrifice, the adrenal glands were removed and the adrenal medulla was prepared for transmission electron microscopy. There was little cytological evidence of toxic stress in chromaffin cells from animals treated 24 hours earlier. By three days post-injection, some necrotic and some functioning chromaffin cells were evident. The majority of cells from animals treated 5 to 10 days previously were necrotic, indicating the effects of stress. Evidence of stress was also seen in chromaffin cells from two animals who received repeated doses of MPTP. Some chromaffin cells from one animal sacrificed after 41 days were necrotic and others were depleted, indicating that the toxic effects of MPTP on the adrenal medulla can be long-lasting. The present study demonstrates that systemically-administered MPTP has a toxic effect on the adrenal medulla. This effect is manifested a few days after administration, but can be long lasting.

277.8

DETECTION OF TRANSMITTER SPECIFIC CNS NEURONS REGULATING THE ADRENAL GLAND WITH THE RETROGRADE TRANSNEURONAL VIRAL LABELING METHOD. A.D. Loewy, A.M. Strack, W.B. Sawyer, K.B. Platt*, and Dept. of Anatomy & Neurobiology, Washington Univ. School of Medicine, St. Louis, MO 63110, and *Dept. of Veterinary Microbiology, College of Veterinary Medicine, Iowa State Univ. Ames, IA.

The retrograde transneuronal viral cell body labeling technique was used to detect the CNS cell groups that innervate the sympathoadrenal preganglionic neurons. A suspension of pseudorabies virus (PRV) was injected into the adrenal gland of rats with a glass micropipette. After two days, the rats received intracerebroventricular injections of colchicine (100 μg/10 μl saline). Two days later, the rats were perfused with 4% paraformaldehyde. Viral infections were detected using a pig antibody against PRV. Neurotransmitter enzymes and neuropeptides were detected using antibodies simultaneously with PRV antibodies.

Five key areas of the brain were labeled: parvocellular paraventricular hypothalamic nucleus, A5 noradrenergic cell group, rostral ventrolateral medulla, ventromedial medulla, and the caudal raphe nucleus. Putative neurotransmitters are found to co-localize with PRV infected cells. In the brain stem: substance P, serotonin, neuropeptide Y and PNMT immunoreactive cells co-localize with PRV; also smaller numbers of somatostatin and enkephalin cells co-localize. In the A5 cell group, most of the PRV infected cells co-localize with tyrosine hydroxylase and some co-localize with somatostatin. In the parvocellular paraventricular hypothalamic nucleus, several transmitters co-localize with a small percent of the PRV infected cells, including substance P, tyrosine hydroxylase, enkephalin, oxytocin, neurotensin, somatostatin and vasopressin. These data indicate that the sympathoadrenal preganglionic neurons are innervated by a wide spectrum of chemically coded CNS neurons.

277.9

CENTRAL AND PERIPHERAL REGULATION OF SYMPATHOADRENAL ACTIVITY DURING EXERCISE. AJW Scheurink* and AB Steffens* (SPON: O Hathaway). Dept of Psychology, Univ of Washington, Seattle WA, 98195 USA, and Dept of Animal Physiology, Univ of Groningen, 9750 AA Haren, The Netherlands.

The role of the sympathetic nervous system in the regulation of energy substrate release was investigated in exercising rats. Exercise consisted of strenous swimming against a counter current for 15 min. Before, during, and after exercise blood samples were taken through permanent heart cannulas for determination of plasma glucose, FFA, insulin, E, NE, and corticosterone concentrations.

Infusion of adrenoceptor antagonists into distinctive areas of the hypothalamus (VMH, LHA, or PVN) interfered with the normal hormonal and metabolic responses to exercise. Glucose, FFA, corticosterone, and catecholamine levels changed independently of each other after hypothalamic adrenoceptor blockade and exercise. Experiments dealing with intravenous administration of selective adrenoceptor agonists and antagonists in intact and adrenodemedullated exercising rats demonstrated that the two branches of the sympathoadrenal system are functionally and metabolically dissociated. The experiments also showed that presynaptic adrenergic regulatory mechanisms at the peripheral sympathetic nerve endings influence the activation of the sympathetic nervous system during exercise.

The results suggest that the organization of an organ specific activation of sympathetic output during exercise may take place at different levels of the CNS. The hypothalamus can be considered as the major of these levels. Further, the activity of the sympathetic nervous system may be affected at the level of extrahypothalamic areas in the limbic system, in various nuclei of the brain stem, in the intermediolateral column of the spinal cord, in the sympathetic ganglia and the adrenal medulla, and at the level of the peripheral nerve endings of the sympathetic nervous system. (Supported by the Netherlands Organization of Scientific Research).

DEVELOPMENTAL DISORDERS: GENETIC AND CHEMICAL MODELS

278.1

ENHANCED PRODUCTION OF SUPEROXIDE BY MICROGLIA FROM TRISOMY 16 MICE. Carol A. Colton, Jibin Yao*, Daniel Gilbert and Mary Lou Oster-Granite. Department of Physiology and Biophysics, Georgetown University Medical School, Developmental Genetics Laboratory, Johns Hopkins University School of Medicine and the Lab of Biophysics, NINDS, NIH.

Disruption of normal oxygen radical metabolism in the CNS may contribute to the neuropathological changes associated with Down Syndrome (trisomy 21) and its mouse counterpart, the trisomy 16 (Ts16) mouse. Our lab has shown that one potent source of oxy-radicals is the CNS-specific macrophage, the microglial cell. We prepared primary glial cultures from the cerebral cortices of Ts16 and normal littermate mice taken at day 15 of gestation. Microglia were isolated from confluent cultures after 14 days in vitro and assayed for superoxide anion production using a cytochrome C reduction assay. Stimulation by either opsonized zymosan (OPZ) or phorbol myristate acetate (PMA), produced significantly higher levels (2.8 to 20 fold) of superoxide per mg protein in Ts16 microglial cultures. Resting, i.e., unstimulated secretion, was not significantly different from littermate controls. Astyrocyte enriched cultures, stimulated by OPZ, exhibited low levels of superoxide production which ranged from 1.4 to 2 fold higher than astrocytes from normal littermate cultures. Rat microglial enriched cultures were exposed for 24 hours to medium from the Ts16 glial cultures. Superoxide production in the Ts 16-media treated rat microglia was significantly higher compared to those treated with littermate conditioned media. Supported in part by HD 19920.

278.2

OLIGOSACCHARIDE ACCUMULATION IN BRAIN AND SPINAL CORD IN CAPRINE β-MANNOSIDOSIS. P.J. Boyer, M.Z. Jones, E.J.S. Rathke*, N.K. Truscott* and K.L. Lovell. Dept. Pathol., Mich. State Univ., East Lansing, MI 48824.

Goats affected with β-mannosidosis have deficient activity of the lysosomal enzyme β-mannosidase along with tissue accumulation of a trisaccharide (TS, Manβ1-4GlcNAcβ1-4GlcNAc), a disaccharide (DS, Manβ1-4GlcNAc), and small quantities of complex oligosaccharides. A caudal-to-rostral pattern of increasingly severe central nervous system myelin deficiency is a major pathological feature of caprine β-mannosidosis. In this study, TS and DS content in cerebral hemisphere (CH) gray matter and white matter and in spinal cord from three affected and two control neonatal goats was measured to determine the extent of association between oligosaccharide storage and myelin deficiency. In affected goats, content of TS and DS (expressed as ug hexose per mg protein) was 40% higher in spinal cord (moderate myelin deficiency) than in CH white matter (severe myelin deficiency) or gray matter. The ratio of TS:DS content was about 6:1 in all three regions. Thus, in affected animals, greater accumulation of TS and DS is not associated with more severe myelin deficiency. The similarity of TS and DS content in gray and white matter was an unanticipated result, considering the higher concentration of lysosomal storage vacuoles in gray matter cells. Supported by NS 20254 (KLL) and NS 16886 (MZJ).

278.3

SPATIAL LEARNING DEFICITS IN TWO YEAR OLD MICE HETEROZYGOUS FOR THE *TWITCHER* (C57Bl/6j/*twi*) MUTATION. Ch. E. Olmstead UCLA Sch. of Med., Mental Retardation Res. Center Group at Lanterman Developmental Center, Pomona, CA 91769.

The *twitcher* mouse is an enzymatically authentic model for recessively transmitted globoid cell (Krabbe) leukodystrophy. We have previously described subtle neurological (Behav. Brain Res. 25(1987) 143-153) and learning (Soc. Neurosci. Abstr. 13, 1987) differences between developing heterozygotes (*twi/+*) and their normal (*+/+*) littermates. Aging *twi/+* show decreased nerve conduction velocities compared to *+/+* (Soc. Neurosci. Abstr. 14, 1988), suggesting that this mutant is also a model for heterozygote risk during lifespan development.

We report here for the first time a longitudinal investigation of spatial learning in the Morris water maze in 32 mice of both sexes (*twi/+*, N = 19; *+/+*, N = 13) studied during the first month of life and after two years of age. Genotype was determined by assay of galactosylceramidase from short segments of tail in the first week of life. As we originally reported there were substantial differences between *twi/+* and *+/+* as juveniles and these differences, as measured by both water and radial maze indices, disappeared in early adulthood. Those behavioral deficits were again seen with increasing frequency in aged animals where *twi/+* showed significantly longer latencies in finding the platform, swam slower and showed more error trials. The features most associated with adolescence, floating, escape, etc, were seen on a significantly higher number of trials in the *twi/+* than in the *+/+*.

278.4

METHYLMERCURY-INDUCED MOVEMENT AND POSTURAL DISORDERS IN THE DEVELOPING RAT: INHIBITED DEVELOPMENT OF INTRACORTICAL CAPILLARY NETWORKS. J.R. O'Kusky. Dept. of Pathology, Division of Medical Microbiology, University of British Columbia, Vancouver, B.C., Canada V5Z 1M9.

The neurotoxicity of methylmercuric chloride (MeHg), administered to neonatal rats (5 mg/kg/day) beginning on postnatal day 5, produces movement and postural disorders during the fourth postnatal week. Stereological analyses of the length density of intracortical capillaries (L_V, total capillary length per unit volume) were performed in individual laminae of the visual cortex (area 17) at the onset of neurological impairment and at three subclinical stages of toxicity. When MeHg-treated rats were compared to weight-matched controls, the L_V of capillaries was significantly less in lamina IV and in the adjacent regions of laminae III and V at the onset of neurological impairment (30-40%) and at two subclinical stages of toxicity (20-35%). The laminar distribution of this decrease in the L_V of capillaries correlates with the distribution of degenerating stellate neurons in laminae III, IV and V of the visual cortex (O'Kusky, J., Exp. Neurol., 89:32, 1985). Since the inhibited development of intracortical capillaries precedes the onset of neuronal pathology in this animal model, it would appear that the degeneration of neurons in the visual cortex is secondary to these vascular abnormalities. Supported by the United Cerebral Palsy Research and Educational Foundation, Inc.

278.5

ADULT HYPERACTIVITY RESULTING FROM POSTNATAL ADMINISTRATION OF DELTA, BUT NOT MU OR KAPPA, OPIOID AGONIST. J. W. Spain, Dept. Biomedical Sciences, Univ. Illinois College Medicine, Rockford, IL 61107.

Chronic perinatal morphine or methadone treatments have been reported to induce hyperactivity in young adult rats. However, the opioid site responsible for this persistent developmental effect has not been identified. Rat pups were treated (s.c.) daily from postnatal days 1 to 16 with either DAGO (0.5 mg/kg), DPDPE (1 mg/kg), U-69593 (1 mg/kg), or vehicle (0.1 ml/15 g). On the last day of treatment, there was a tendency for mean body weight of all treatment groups to be lower control but only U-69593 treated rats were significantly lower. When rats were between 40 and 55 days old, hourly activity of a single rat was measured over a 24 hr period using a commercial optical activity monitor. Rats treated with the delta agonist, DPDPE, were significantly hyperactive ($p>.05$; one-way ANOVA with Tukey post-hoc test) with a 37% increase over controls during the dark phase of the circadian cycle (6 pm-6 am). Non-significant increases of 19% and 9% were seen in rats treated with DAGO and U-69593, respectively. Activity during the light phase was similar for all groups. When the data was further compared for each sex, only in the DAGO treatment group were sex differences detected (6% vs. 33% increase for males vs. females, respectively). Our results implicate the delta opioid site in hyperactivity resulting from perinatal opioid exposure.

278.6

THE EFFECT OF PERINATAL HYPOXIA-ISCHEMIA IN A UNILATERAL RODENT MODEL ON STRIATAL TYROSINE HYDROXYLASE(TH)-POSITIVE FIBER INNERVATION. J Kent,* A Karanas,* RE Burke.(SPON: Y STERN). Dept. Neurology, Columbia Univ., NYC, NY, 10032.
Little is known of the effect of perinatal asphyxial injury on the neurochemical anatomy of the brain. The striatum often shows the predominant pathology in dyskinetic cerebral palsy. Johnston (1983) had previously shown in a unilateral rodent model that striatal dopaminergic biochemical indices are relatively preserved. We studied whether there is morphologic evidence for sparing of these systems. Rat pups underwent left carotid ligation and exposure to 8% oxygen at 7 days of age, and were sacrificed at 3-4 weeks. Cryostat cut sections were stained for TH (ETI). Because striatal TH-positive fibers were too numerous to count, we used optical density as an ordinal measure, at the population level, of degree of TH-positive fiber innervation, to compare striata on the same section. This approach had been validated in 6-OHDA lesioned animals in relation to rotational behavior. We found that pups with mild striatal asphyxial injury (no volume loss) showed no change in extent of TH-innervation. Pups with moderate injury (decreased striatal volume) (N=7) showed an increased density of staining on the asphyxiated side (Wilcoxon $p<.001$, for N=83 sections in 4 striatal planes). Thus, there is clear morphologic evidence of sparing of striatal dopaminergic systems, with an increase in their innervation density, in the asphyxia-injured striatum. NINDS #KO7NS00746, DMRF, PDF.

278.7

DENDRITIC EFFECTS OF EXPERIMENTAL INFANTILE HYDROCEPHALUS AND VP SHUNTS. J.P.McAllister, M.H.Park*, S.D.Katz* and R.M.Kriebel. Depts. of Anatomy, Temple U. Sch. of Med. & PCOM, Philadelphia, PA 19140.

Since dendritic damage may underlie the neurological deficits which persist following surgical treatment of infantile hydrocephalus, we have examined a feline model suitable for placement of ventriculoperitoneal (VP) shunts. Hydrocephalus was induced in neonatal kittens by intracisternal injection of kaolin. Hydrocephalic animals were sacrificed 15-25 days later and compared to age-matched controls. VP shunting was performed 10-18 days post-kaolin on those animals that were confirmed to be hydrocephalic. Tissue from cortical areas 22 and 17 was processed for Golgi-impregnation and 370 pyramidal cell dendrites were analyzed light microscopically. Apical and basal dendrites from hydrocephalic brains consistently displayed decreased numbers of spines and thinner shafts. Some dendrites were nearly devoid of spines and numerous constrictions gave the shafts a beaded appearance. In contrast, shaft thickness and spine density of dendrites from shunted brains increased steadily over the 30 day post-shunt period to reach levels similar to controls. However, more dendrites from area 17, which was most affected initially, retained their retarded appearance. These results suggest that most, but not all, of the dendritic alterations caused by hydrocephalus can be corrected with VP shunts.

278.8

NEURAL TUBE DEFECTS INDUCED BY AMNIOTIC INJECTION OF CON A IN MOUSE EMBRYOS. S. Nakahara*, J. Fiacco*, D.G. McLone, M.G. Hvizd*, R.G. Higbee, and P.A. Knepper. Division of Neurosurgery, Children's Memorial Medical Center, Northwestern University, Chicago, IL 60614.

Carbohydrates may serve as determinants of differentiating cells in cell-to-cell interaction and maturation. To test the possibility that an exogenous lectin (Concanavalia ensiformis, Con A) may bind to mannose/glucose containing glycoconjugates of neuroepithelium during the process of neurulation and induce neural tube defects (NTD), a novel method of administration of Con A was tested in mice embryos. The amniotic cavity of individual embryos of time-bred C57BL/6J mice was injected with 100 ng of Con A (in a volume of 2 µl) on gestation day 8.2, 8.4 (prior to anterior neuropore closure, which normally closes at gestation day 8.5), and 9.0, 9.2 (prior to posterior neuropore closure, which normally closes at gestation day 9.5). Control embryos received 2 µl of saline, a sham injection, or no treatment. Con A administered prior to anterior neuropore closure resulted in exencephaly without spinal NTD, whereas Con A administered on gestation day 9.0 resulted in thoracic and/or lumbar NTD without exencephaly. Thus, these results indicate that the effects of an exogenous lectin were dependent on the time of its administration, and Con A administration resulted in target-specific NTD.
(Supported in part by Kiwanis International.)

278.9

FITC-CON A BINDING IN NEURAL TUBE DEFECTS INDUCED BY HYPOTHERMIA DURING NEURULATION IN CHICK EMBRYOS.
S. Robinson*, D.G. McLone, R.C. Dauser*, R.G. Higbee, W. Goossens*, and P.A. Knepper. Division of Neurosurgery, Children's Memorial Medical Center, Chicago, IL 60614.

Neurulation involves cell-to-cell recognition and adhesion mediated in part by cell surface and extracellular glycoconjugates (GC). The timing of the expression and post-translational modification of GC may be influenced by environmental factors such as temperature. To test this hypothesis, chick embryos were incubated at 38°C for 30 hr (start of neurulation) and then at 35°C for 18 hr (during neurulation). Stage 11 embryos were examined by SEM and by serial sections of FITC-Con A-incubated 1-µm araldite sections using low-intensity light video microscopy. The majority of embryos subjected to hypothermia had a large (400-600 µm) zone, i.e., neural tube defect (NTD), characterized by closely apposed neural folds that did not adhere or fuse, whereas the control embryos had a small (50 µm) zone of neural fold apposition prior to closure. In abnormal embryos, the dorsal basal lamina of the neural fold was FITC-Con A positive within a few microns of the zone of closure, whereas in control embryos, it was FITC-Con A positive 50 µm rostral to the zone of closure. These results are consistent with the hypothesis that NTD may relate to an alteration in the intrinsic developmental timetable of the neuroepithelium and the expression of GC.
(Supported in part by Kiwanis International.)

278.10

ALTERED PATTERN OF SHOCK-MOTIVATED ESCAPE IN RATS WITH PRENATALLY INDUCED MICRENCEPHALY.
H.K. Chang*, B.K. Chung*, S.K. Park* and M.H. Lee (SPON: A. Rabe). Yeungnam University, Korea; NY State Institute for Basic Research in Developmental Disabilities, Staten Islandy, NY 10314.

The rat with methylazoxymethanol (MAM) induced forebrain hypoplasia is a model of congenital brain defects. With its forebrain reduced by 60% and neurotransmitter imbalance, it displays, in addition to maze learning deficits, behavior indicative of emotional/motivational changes. It is hyperactive, shows enhanced performance in a shuttle box, and altered responsivity to different motivational conditions. In order to further explore its altered emotionality, we examined the effect of inescapable shocks on escape behavior. Male Sprague-Dawley rats (3 mo) received either 80 inescapable shocks (1.0 mA for 6s) or simple confinement in a chamber, followed in 24h by 30 shock-escape trials during which escape was allowed 6s after the onset of shock. In the no-preshock condition, the MAM rats displayed a significantly longer escape latency than the controls. Following inescapable shocks, however, no group difference was revealed; thus, compared to the no-preshock condition, the inescapable shock significantly increased the latency of the control rats, while it had no effect on the MAM rats. It indicates the MAM rats' performance is severely curtailed by a moderate level of stress, possibly reflecting an imbalance in relative concentrations of monoamines and acetylcholine.

278.11

MASKING OF ATTENTIONAL DYSFUNCTION BY NUTRIENT RESTRICTION.
M. Bunsey,* B.J. Strupp, J. Stasior,* D.A. Levitsky* and S. Ginsberg.* Dept. of Psychology and Div. of Nutritional Sci., Cornell Univ., Ithaca, NY 14853.

The present study was designed to test the hypothesis that attentional dysfunction (AD) may be obscured when nutrient restriction is used to motivate learning; prenatal ethanol (PE) exposure was used to induce AD. On days 5-20 of gestation, dams were maintained on a liquid diet containing 0 or 35% ethanol-derived calories. At 42 days of age, the female offspring (n=260) were tested. Half of the subjects were given a simple discrimination task (the Non-Distraction Task), while the other half was tested on a Distraction Task with both predictive and non-predictive cues present. For both tasks, half of the rats were fed ad libitum and half were maintained on a food restriction (FR) schedule. The pattern of results indicated increased distractability in the PE rats. While FR, as predicted, reduced distractability, its effect on learning rate was comparable for the PE and control groups. However, evidence for the hypothesized masking of AD by FR was obtained in further analyses of the types of errors committed by the animals. One particular type of error -- failing to pick the same object after a correct choice on the previous trial -- was evident in the PE rats only in the ad libitum feeding condition; it was masked by FR.

278.12

POLYLYSINE-DTPA-Gd ENHANCED MR IMAGES OF C6 GLIOMA AT 1.5 AND 9.4 TESLA.
S. Kornguth, M. Anderson, P. Turski, H.I. Robins, J. Cohen, J. Sorenson, A. Rappe, J. Markley. University of Wisconsin, Madison WI 53705.

A novel agent, polylysine-DTPA-Gd (PL-DTPA-Gd), was employed to define the margin between C6 glioblastoma and normal brain by magnetic resonance imaging (MRI) at field strengths of 1.5 and of 9.4 Tesla. Sixteen Wistar Furth rats (300 gms) were injected with $2X10^6$ C6 cells and four rats were injected with control vehicle. Seven days later the rats were injected intra-aortically with 0.5 ml of PL-DTPA-Gd (either 100, 10, 1 or 0 ug PL) and on the tenth day they were imaged by MRI. The PL (DP88) was modified with 1 Gd per 15 lysine residues. Images obtained at 1.5 Tesla were 3 mm slices while those obtained at 9.4 Tesla were 125 or 500 um slices. Without the PL-DTPA-Gd contrast agent, the tumors were barely detectable at either field strength. The tumors in animals that received PL-DTPA-Gd (1 ug or greater) were readily identified at 1.5 and 9.4 Tesla. The central tumor region had a reduced signal intensity (SI) with a high SI at the margin between tumor and normal brain. The size, location and histology of the tumors were examined in frozen brain sections that were stained with thionine. The high SI margin of the tumor was heavily vascularized and the 9.4 T instrument permitted detailed images of the margin structure. This study is the first to demonstrate the utility of the PL-DTPA-Gd imaging agent for definition of intracranial tumors and the utility of the 9.4 Tesla instrument as a microscopic analyzer of brain and gliomas.

NEUROTOXICITY II

279.1

NEUROTOXIC EFFECTS OF 3,4-METHYLENEDIOXYAMPHETAMINE (MDA) ON DOPAMINE (DA) AND SEROTONIN (5-HT) NEURONS IN REAGGREGATE TISSUE CULTURE.
L. A. Won, P. J. Kontur, P. C. Hoffmann and A. Heller. Dept. of Pharm. and Phys. Sciences, Univ. of Chicago, Chicago, IL 60637.

Methylenedioxyamphetamine is a synthetic amphetamine analog with abuse potential. MDA is toxic to 5-HT-containing, but not DA-containing, neurons in adult animals (Ricaurte et al., Science, 1985). (+)-MDA possesses amphetamine-like properties, while (-)-MDA has properties resembling hallucinogens. Neurotoxicity of these isomers was examined in three-dimensional reaggregate tissue cultures formed from dissociated cells of fetal mesencephalic tegmentum and corpus striatum. Reaggregates were exposed to (+)-MDA or (-)-MDA in concentrations ranging from 10^{-7}M to 10^{-4}M between 15 and 22 days in culture. Reaggregates were then analyzed for endogenous DA and 5-HT levels using HPLC, and DA and 5-HT cells were examined. DA and 5-HT cells were visualized using tyrosine hydroxylase (TH)- and 5-HT-immunocytochemistry, respectively. 10^{-4}M and 10^{-5}M (+)-MDA and (-)-MDA significantly decreased endogenous DA levels, with the (+) isomer being more potent. Both isomers of MDA at 10^{-4}M were equally effective in reducing endogenous 5-HT levels. At 10^{-4}M (+)-MDA and (-)-MDA, both DA and 5-HT cells showed evidence of damage including vacuolization and fragmentation. These results indicate that (+)-MDA and (-)-MDA affect both DA and 5-HT neurons in reaggregate cultures. In contrast to studies in adult animals, MDA decreased DA levels and at high concentrations produced evidence of cellular damage in the reaggregates suggesting that developing DA neurons may be more susceptible to this agent.
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279.2

PRENATAL EXPOSURE TO METHYLENEDIOXYMETHAMPHETAMINE (MDMA)IN THE RAT: BEHAVIORAL AND NEUROCHEMICAL EFFECTS.
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The developmental toxicity of MDMA was assessed by measuring behavioral and neurochemical endpoints in offsprings after prenatal MDMA treatment. Pregnant CD rats were gavaged with 0, 2.5 or 10 mg/kg/day MDMA during gestation on alternate days 6-18. Pregnancy weight gain, gestational length, litter size, birth weight, physical appearance at birth, progeny growth and several behaviors were not altered by prenatal treatment. Olfactory discrimination on PND 9-11 and Figure 8 maze activity on PND 22-23 were enhanced in both male and female MDMA-treated progeny while negative geotaxis at PND 7-10 was delayed. Whole brain serotonergic uptake sites on PND 9 and 21 and 5-HT and 5-HIAA concentrations on PND 27 in frontal cortex (FC), hippocampus (HC) and caudate nucleus (CN) were not altered by treatment. Maternal 5-HT concentrations on PND-27, however, were significantly decreased in FC, HC and CN at 10 mg/kg. These data suggest that prenatal exposure to MDMA produces selective behavioral alterations without affecting 5-HT neurochemistry in the developing rat brain. Persistent neurochemical alterations, however, were observed in maternal brain.

279.3

COMPARED EFFECTS OF CHRONIC INFUSION OF GABA INTO THE NUCLEUS BASALIS AND FRONTAL CORTEX IN RATS.

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GABAergic neurons exert an inhibitory control on the cholinergic neurons of the nucleus basalis magnocellularis (NBM) which project onto the fronto-parietal cortex. Behavioral and morphological effects of a chronic infusion (by osmotic minipumps) of GABA into the NBM were compared to those of similar infusions into the frontal cortex. Rats were infused with GABA (at either 10, 50 or 100 µg/µl/h) in one hemisphere and simultaneously with saline in the contralateral hemisphere for 4 days ; the catheters were then exchanged and infusion pursued for 4 more days. Both cortical and NBM GABA treatments induced an ipsilateral turning bias (followed by a rebound effect after treatment with high doses) . Only cortical GABA infusion at the highest dosage induced a locomotor deficit in beam walking, which recovered after treatment. The highest doses of GABA, whether infused in the cortex or the NBM, induced a dose-dependent lesion (with extensive gliosis in NBM) only on the side first infused with GABA. These data corroborate previous results which also showed preventive effects of chronic intracerebral saline treatment. They may be of interest for interpreting some aspects of neurodegenerative diseases such as Alzheimer's disease.

279.4

CHANGED SETTING OF NORADRENALINE NEUROTRANSMISSION IN ADULT RAT BRAIN DUE TO NEONATAL AGONIST AND ANTAGONIST TREATMENT

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Noradrenaline(NA) is functionally active early in brain development. Interference with NA neurotransmission during maturation therefore brings about advanced or delayed signals. Unlike reversible effects seen in adults such pharmacological interferences in neonates may reveal life-span changes in the NA system.

Male rat pups were injected twice daily with the α-agonist clonidine(0.1mg/kg), α-antagonist yohimbine(2.5mg/kg) or the β-antagonist propranolol(15mg/lg) either between birth and day 10 or between day 10 and 20. Brain monoamines and metabolites were determined in nine areas by means of HPLC/ED 2h after the last injection (direct effects) and at 2 or 3 months of age (long-term consequences).

Direct effects confirmed the functional activity of the NA system. Neonatal clonidine decreased, whereas propranolol and yohimbine increased MHPG. Early clonidine or yohimbine treatments gave no longlasting effects on NA or MHPG values. However, for clonidine αMpT-induced NA depletion was significantly retarded in some areas. Day 1-10, but not 11-20 propranolol injections revealed higher MHPG/NA ratios in the adult brain. The latter strongly indicate that antagonistic interference of the NA system during maturation can alter regional NA brain activity in adult stages.

279.5

PRENATAL HALOPERIDOL (H) EXPOSURE REDUCES OFFSPRING BRAIN WEIGHT.

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The effect of prenatal H exposure on offspring brain weight was assessed in Sprague-Dawley derived rats. In two independent experiments, pregnant dams were given daily sc injections of vehicle, 2.5 or 5.0 mg/kg H, over gestational days (GD) 6 through 20. In both experiments, GD 20 maternal weight was reduced by 13% in the high-dose H group. Offspring body weight was reduced in experiment 1 (around 10%) but not experiment 2. In both experiments, whole brain weight at postnatal day (PND) 30 was significantly depressed (5 to 10%) in both male and female high-dose offspring. This reduced brain weight was seen at birth and on PNDs 15, 30, and 60. Dry weight of PND 30 H brains was also significantly reduced by a similar percentage. Gross dissection of PND 30 brains revealed that weight loss was restricted to certain brain regions. Caudate and brainstem were most affected. Weights of thalamus, hypothalamus and posterior cortical remnants were not reduced by prenatal H. It is concluded that dopamine may play a trophic role in early brain development, since prenatal receptor blockade seems to reduce brain weight even when body weight is not altered.

279.6

IN VIVO ELECTROCHEMICAL AND ELECTROPHYSIOLOGICAL STUDIES OF THE CAUDATE NUCLEUS OF NEONATAL 6-OHDA-LESIONED RATS.

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Neonatal destruction of dopamine (DA)-containing neurons via intracerebroventricular or intracisternal (i.c.) treatment with 6-hydroxydopamine (6-OHDA) has been shown to produce rats that exhibit hyperactivity. The permanent decrease in DA levels is also associated with an increase in serotonin (5-HT). However, the neuronal mechanisms underlying the hyperactive behavior remain unclear. In the present study, high-speed electrochemical measurements of potassium-evoked monoamine overflow and extracellular single-unit electrophysiological recording methods were used to characterize the caudate nucleus of rats that had received neonatal i.c. injections of 6-OHDA (75 micrograms). Potassium-evoked electrochemical signals were diminished in amplitude as compared to control caudate nucleus, and the electrochemical responses were characteristic of extracellular 5-HT overflow rather than DA. Local applications of 5-HT, DA or phencyclidine into the caudate nucleus elicited excitations of caudate cells rather than the normal inhibitory effects seen in control animals. These data suggest that the function of remaining monoamine striatal circuits is dramatically changed as a consequence of the neonatal 6-OHDA exposure. Supported by USPHS grants AG06434, NS09199 and the VA.

279.7

INHIBITION OF RETROGRADE AXONAL TRANSPORT BY 3,4-DIMETHYL-2,5-HEXANEDIONE.

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Previous studies from this laboratory have shown that single injections of high doses of 2,5-hexanedione (2,5-HD) produce reversible decreases in the rate of retrograde axonal transport (RT). We have examined the question whether 3,4-dimethyl-2,5-hexanedione (DMHD; a gift from Dr. L. Sayre), is capable of inhibiting RT. Rats were dosed daily with DMHD (0.25 mmol/kg I.P.) for nineteen days. DMHD-treated rats lost considerable body weight and developed severe hind limb weakness. Twenty-four hours after the last injection, gastrocnemius muscles of control and DMHD-treated rats were injected unilaterally with ^{125}I-labeled tetanus toxin. At 3 and 5 hours after tetanus-toxin injection, five rats, from each group, were sacrificed, ipsilateral and contralateral sciatic nerves, including tibial branches innervating the gastrocnemius muscle, were removed, cut into 3-mm segments and radioactivity in each segment determined with a gamma counter. The leading edge of retrogradely-transported ^{125}I was determined by plotting percent total radioactivity in each nerve segment against the distance transported. The apparent rate of RT in the control group was 6.8 ± 0.4 mm per hour. Animals treated with DMHD showed a reduced rate of RT (3.7 ± 0.9 mm per hour). These data show that multiple injections of DMHD inhibit RT velocity by approximately 45%. Dose-dependent inhibition of RT with DMHD is currently under study. Supported by NS 19611.

279.8

SUBTLE BEHAVIOURAL CHANGES PRODUCED IN RATS BY IN UTERO EXPOSURE TO DIAZEPAM

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The purpose of this experiment was to evaluate in rats whether the prenatal treatment with diazepam (0.1 or 1.0 mg/kg) could affect the ultrasonic vocalization which seems to be a sensitive indicator of emotional and motivational changes (Cagiano, R. et al., Life Sci., 38:1417, 1986 and Cagiano R. et al., Eur. J. Pharm.,157:45, 1988). In particular, the following parameters were evaluated: a) ultrasonic vocalization elicited by the removal of the rat pups from their nest; b) ultrasonic vocalization of male rats during sexual behaviour.

Primiparous pregnant Sprague Dawley dams were administered a single daily s.c. injection of diazepam or vehicle, over gestation days 14-20. The results show that the administration of diazepam during gestation produced in rat pups removed from their nest marked changes in the lenght of ultrasonic calls. Finally, adult male rats (120 days of age) prenatally exposed to diazepam showed a notable impairment in copulatory activity as well as a significant decrease in the duration of ultrasonic 22 kHz post-ejaculatory calls emitted during sexual behaviour.

These results further confirm that prenatal treatment with diazepam produces short and long-term behavioural changes at dose levels below those associated with overt signs of neurotoxicity.

279.9

NUCLEUS BASALIS LESIONING IN NEONATE RATS: A SELECTIVE CORTICAL CHOLINERGIC HYPOFUNCTION AND BEHAVIORAL IMPAIRMENT DURING ADULTHOOD. G.J. Sengstock[1]*, K.B. Johnson[1]*, E.M. Meyer[2], A.J. Dunn[3] and G.W. Arendash[1]. (SPON: C. Edwards) Dept. of Biology, U. of South Florida, Tampa, FL 33620[1], Dept. of Pharm., U. of Florida, Gainesville, FL 32610[2] and Dept. of Pharm., Louisiana State U., Baton Rouge, LA 70803[3].

Ibotenic acid was infused bilaterally into the nucleus basalis magnocellularis (nBM) of 2 day-old neonate rats to eliminate immature cholinergic neurons within the nBM before they develop functional termination points in the neocortex. Behavioral testing was initiated 2 months post-lesion and all animals were sacrificed at 8 or 12 months of age. Histological analysis of the subcortex from these lesioned animals revealed a gliosis essentially restricted to the globus pallidus (containing the nBM). Neurochemically, cortical choline acetyltransferase (CAT) activity was reduced significantly by 25% at 8 months and by 18% at 12 months after lesioning. Cortical concentrations of biogenic amines and their metabolites were unaffected, indicating the selectivity of these lesions. Behaviorally, lesioned animals had a marked deficit in passive avoidance retention and 2-way active avoidance acquisition. They also demonstrated significant deficits in two spatial tasks: Lashley III maze and 17-arm Radial maze. These data indicate that neonate nBM lesions may provide a useful animal model for elucidating changes in the plasticity of the developing brain as a result of cortical cholinergic anervation.

279.10

6-HYDROXYDOPAMINE TOXICITY IN MESENCEPHALIC CULTURES IS POTENTIATED BY THIOLS. H. Kalir* and C. Mytilineou. Dept. of Neurology, Mt. Sinai Sch. of Med. New York, N.Y. 10029.

Mesencephalic neuronal cultures from 14 day old rat embryos were treated with 6-hydroxydopamine (6OHDA; 0.1mM), 6OHDA + N-acetylcysteine (NAC; 1mM) or 6OHDA + cysteamine (CAM; 1mM) for 1 hour. Toxicity was assessed by measurement of [^{3}H]dopamine uptake 24 hours later. A reduction in uptake was noted in all 3 cases, but adding NAC or CAM to 6OHDA resulted in a potentiation of the toxic effect (1.75 and 2.94 fold increase in toxicity respectively, compared to 6OHDA alone). The potentiating effect of NAC was not due to protection from 6OHDA auto-oxidation in the medium, since HPLC measurements showed no change in the extra- or intracellular levels of 6OHDA when NAC was added. Extracellular auto-oxidation of NAC or CAM, which could result in formation of reactive oxygen species, was not a factor in the potentiation of 6OHDA neurotoxicity, since addition of catalase and superoxide dismutase to the medium had no protective effect. The mechanism of potentiation of 6OHDA toxicity by thiols is currently being investigated. Supported by NIH grants NS-11631 and NS-23107.

279.11

COMPARISON OF THE EFFECTS OF INTRADENTATE VERSUS INTRAVENTRICULAR INJECTIONS OF COLCHICINE: NEUROBIOLOGICAL AND BEHAVIORAL CORRELATES. D.F. Emerich, R.W. Stackman and T.J. Walsh. Rutgers University, Department of Psychology, New Brunswick, NJ 08903.

Intradentate injection of colchicine (COL) produces an extensive loss of dentate granule cells, a 25% loss of cholinergic neurons within the medial septum and persistent memory impairments (Emerich and Walsh, Physiol. Behav., 45, 93-101, 1989). In the following studies we compared the behavioral and neurobiological effects induced by COL injected into either the hippocampus (HPC) or lateral ventricles (ICV)

Sprague-Dawley rats were trained on a standard radial arm maze (RAM) task. Following training, COL was injected bilaterally into the HPC or ICV (3.5 or 7.0 ug/.5 ul/site). Animals receiving intradentate injections of COL, regardless of dose, were markedly impaired on the RAM task and showed no evidence of recovery. Depending on the dose, ICV COL produced either a transient impairment which recovered within 15 trials (3.5 ug) or a long lasting impairment (7.0 ug).

Quantitative histological analysis (9 weeks following COL) revealed that intradentate COL produced a significant decrease in the thickness of both blades of the dentate gyrus and a 15-18% loss of cholinergic neurons within the medial septum. ICV COL had no effects on hippocampal morphology but did produce a 40% loss of septal cholinergic neurons (7.0 ug).

An analysis of the time course of cholinotoxicity (7.0 ug) revealed that both intradentate and ICV COL produced significant decreases in hippocampal ChAT activity and HAChU at 1 and 3 but not 9 weeks following surgery. No alterations were observed in either ChAT or HAChU in the striatum, frontal cortex or olfactory bulbs.

These data suggest that, in these model systems, COL exerts a pronounced and selective effect on the septohippocampal cholinergic system which is not dependent upon the loss of granule cells but appears to result from a direct cholinotoxic effect.

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279.12

NEUROTOXICITY OF INTRAVENTRICULARLY INJECTED RIBOSOME INACTIVATING PROTEINS. R.G. Wiley and K. Leone*, VAMC, Nashville, TN 37212.

Immunotoxins consisting of monoclonal antibodies coupled to ribosome inactivating proteins (RIPs) are potentially useful for making experimental lesions and in the treatment of CNS cancer. We sought to compare the neurotoxicity of 3 free RIPs that might be used to construct such toxins to 3 toxic lectins. Test agents were pressure injected into the left lateral ventricle of anesthetized, adult, male Sprague-Dawley rats using stereotactic technique. Animals were observed and weighed daily for up to 2 weeks. The toxic lectin, volkensin, was most potent with a minimal lethal dose (MLD) <2.7 ng/rat. The MLD for ricin was <50 ng/rat and for viscumin <100 ng/rat. By comparison, the RIPs that had no specific binding activity were much less potent. The MLD for ricin A chain was >6.5 ug/rat and >4.2 ug/rat for deglycosylated ricin A chain. The MLD for saporin was <50 ug/rat. Only 1 of 42 rats died in <42 hrs. The neurologic deficits seen at doses near the MLD often suggested cerebellar dysfunction, but at higher doses inanition appeared rapidly followed by death. The results will form a basis for comparison with specific immunotoxins. (This work supported by Veterans Administration.)

279.13

EFFECTS OF CHRONIC DELTA-9-THC ON HIPPOCAMPAL ULTRASTRUCTURE AND ADRENAL HYPERTROPHY. L.B. Cadwallader*, S.L. Vinsant* and P.W. Landfield (SPON: M. Levitt). Dept. Physiol. & Pharmacol., Bowman Gray Sch. Med., Wake Forest Univ. Winston-Salem, NC 27103

In a previous study (Landfield, et al, Brain Res, 1988) we found that long-term treatment with 8 mg/kg delta-9-tetrahydrocannabinol (THC) induced aging-like neuronal loss and glial reactivity in the hippocampus of young rats. In addition, the chronic THC treatment increased adrenal-pituitary hormonal release during restraint stress. The similarity between THC and glucocorticoid molecular structures led to the hypothesis that THC may mimic the hippocampal aging-like neurotoxic effect of corticosteroids. Alternatively, THC might interfere with negative feedback, causing increased release of endogenous corticosteroids.

In the previous study, however, no clear effects on synaptic density were seen, possibly due to sprouting or remodeling. It was also unclear whether adrenal steroids were elevated continually in THC-treated rats. In the present study, we attempted to increase the effect with a higher dose of THC (10 mg/kg, s.c., 5 x weekly) given for 9 months to young, male rats. Hippocampal structure was quantified in 50 electron micrographs and 5 semithin sections from each of 11 vehicle controls and 14 THC rat brains that met all criteria for good fixation. Stereological methods were used, and all micrographs were coded and scored blind.

The results replicated our previous work on the loss of CA1 neurons and increased astrocyte reactivity. However, only a borderline effect on one category of synapses was seen. Nevertheless, ultrastructural effects were found in the neuronal somata, such that cell volume occupied by the Golgi apparatus was increased. Endoplasmic reticulum also exhibited an increase. These data seem consistent with greater genomic activation, perhaps due to increased steroid-like stimulation.

Adrenal gland weights were significantly elevated in THC-treated animals, indicating that THC interactions with corticosteriod systems are persistent and, therefore, viable mechanisms for the neurotoxic effects of THC (supported by DA 03637, ADAMHA).

279.14

EFFECTS OF IMINODIPROPRIONITRILE ON AUDITORY FUNCTION IN THE RAT. K.M.Crofton, R.Janssen, L.D.Williams* and R.C.Hamrick*. Neurotoxicology Division, US EPA, RTP, NC 27711 and NSI Technology Services, RTP, NC 27709.

3,3-Iminodiproprionitrile (IDPN) is a neurotoxicant that induces a hyperkinetic syndrome in rats consisting of spontaneous head movements, abnormal circling, backwards locomotion, and sensory disruption. The present study was designed to compare IDPN-induced auditory dysfunction using behavioral and electrophysiological techniques. Adult male Long Evans hooded rats (n=9-12/group) were exposed to saline, 100, 150, or 200 mg/kg IDPN for 3 consecutive days. Auditory thresholds were determined for 5 and 40 kHz tones using acoustic reflex modification of the auditory startle response (ARM). Motor function was assessed using figure-eight maze activity and hindlimb gripstrength. Behavioral endpoints were measured 24hrs, 1, 3, and 9 weeks post-dosing. Brainstem auditory evoked response (BAER) thresholds were determined for 5 and 40 kHz filtered clicks at 11 weeks post-dosing. ARM thresholds for 5 kHz tones were elevated 25-35 dB in the high dosage group starting 3 weeks post-dosing, and 40 kHz thresholds were increased 50-55 dB within 48 hrs in the 200 mg/kg group and 15-25 dB in the 150 mg/kg group. BAER thresholds for 5 and 40 kHz clicks were elevated 50-55 dB in the 200 mg/kg group only. Motor function tests indicated a 400% increase in locomotor activity in the 200 mg/kg group that persisted for up to 9 weeks, whereas gripstrength was unaltered at any dosage. These data demonstrate dosage- and time-dependent alterations in auditory and motor function following IDPN exposure.

280.1

DEVELOPMENT OF MAJOR HISTOCOMPATIBILITY COMPLEX ANTIGEN EXPRESSION IN RATS FOLLOWING EYE REMOVAL AT VARIOUS POSTNATAL AGES K. T. Yee*, A. M. Smetanka*, R. D. Lund, K. Rao. Department of Neurobiology, Anatomy and Cell Science, Univ. of Pittsburgh, School of Medicine, Pittsburgh, PA 15261

Cells in the mature nervous system, unlike other tissues in the body, do not normally express major histocompatibility complex (MHC) antigens. However, expression is seen in certain disease states such as multiple sclerosis and Alzheimer's disease. We have found that MHC antigen expression can be induced in mature animals after eye removal--class I MHC antigens are expressed in degenerating optic terminal regions while class II MHC antigens are expressed mainly in degenerating pathways. A retinal graft implanted into the brain of a mature animal is subject to a higher rate of spontaneous rejection than one implanted into the brain of an early postnatal animal. This study examines the relationship between MHC antigen expression, myelination and transplant rejection.

The time course of MHC antigen expression was studied in Sprague-Dawley rats after unilateral eye removal on postnatal day (PND) 0, 5, 7.5, 10, 12.5, 15, 17.5. Normal and eye lesioned animals were perfused, fixed, and sections were stained with OX-6 for class II MHC, OX-18 (class I MHC), OX-42 (microglia), W3/25 (reactive microglia), and GFA (astrocytes). As in adults, normal postnatal rats do not express class II MHC antigens. In contrast, however, large areas of the normal postnatal rat brain contain cells which express class I antigen. After eye removal on PND 0, an increase in class I expression is seen in the superficial layers of the superior colliculus and optic tract by PND 5. In contrast, the first evidence of class II expression is seen in the optic tract after eye removal on PND 12, and in the superior colliculus after enucleation on PND 15; this is at the time when the optic pathway becomes myelinated.

These results support the hypothesis that class II expression is associated with myelin degeneration. The absence of lesion induced class II expression may explain why eye removal in early postnatal animals does not cause rejection of xenografts placed in neonatal brains, while eye removal at later times does result in graft rejection.

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280.2

DEGENERATING UNMYELINATED AXONS INDUCE CLASS I BUT NOT CLASS II MHC ANTIGEN EXPRESSION. A. M. Smetanka*, K.T. Yee*, R.D. Lund, and K. Rao. Department of Neurobiology, Anatomy, and Cell Science, Univ. of Pittsburgh, School of Medicine, Pittsburgh, PA 15261

Major histocompatibility (MHC) antigen (Ag) induced expression in the adult rat following eye lesion is known to follow two distinct patterns of expression in the superior colliculus of the brain (Rao et al. Brain Res., in press). Class I Ags are present in degenerating tracts and terminal regions, while class II Ags are predominantly associated with degenerating fiber tracts. To test the hypothesis that class II Ag expression is uniquely associated with degenerating myelinated pathways, we have examined expression patterns after lesions of an unmyelinated pathway. Small lesions confined to the molecular layer of the cerebellum were made, using a 26 gauge needle, in adult Sprague-Dawley rats. Animals were perfused with zinc-aldehyde after 1-9 days survival. Alternate sections were then stained with OX-6 (for class II Ags), OX-18 (for class I Ags), OX-42 (for microglia), and GFAP (for astrocytes).

Class I and II Ag staining was always found around the lesion and positively stained large cells with few processes, presumed to be invasive macrophages. These presumptive macrophages were seen extending variable distances into the brain from the lesion site. Class I Ag staining appeared in cells in the molecular layer, being heaviest 2-3 days after the lesion. The cells had the morphology of microglia. No class II Ag staining was seen in these cells throughout the survival period studied. When lesions extended into the granule cell layer, class II Ag expression on these microglia-like cells was still not seen in the molecular layer; however, large numbers of these labelled cells were present in the white matter, extending into the deep nuclei of the cerebellum.

These findings support the hypothesis that there is a special relationship between degenerating unmyelinated axons and the induction of class II Ag expression.

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280.3

SCHWANN CELL PROLIFERATION IS UNDER NEGATIVE AUTOCRINE CONTROL

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Unlike most other cell types, purified populations of rat sciatic nerve Schwann cells (SCs) divide very slowly (10-20 day cycle) under standard serum-containing culture conditions. However SCs are capable of robust proliferation if provided with mitogens such as cholera toxin or neurons in coculture. Here we have examined whether such cultured SCs release substance(s) that inhibit their proliferation in the absence or presence of added mitogens. SC proliferation was monitored in subconfluent microcultures by direct cell counting and [3H]thymidine incorporation. When serial dilutions of medium conditioned by confluent SC cultures were added to mitogen stimulated microcultures, a concentration-dependent and reversible inhibition of cell proliferation was observed. The inhibition of proliferation by SC conditioned medium occurred when test SCs were under the influence of mitogens believed to have different mechanisms of action, suggesting that negative regulation involves a common process in the proliferative pathway. A similar inhibitory activity was found in medium conditioned by rat RN22 Schwannoma cells, but not in media conditioned by rat astroglial or mouse 3T3 fibroblastic cells. The above results indicate that SC proliferation is under negative autocrine control.

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280.4

BASAL LAMINA DEPOSITION ON SCHWANN CELLS CULTURED WITH FIBROBLASTS IN THE ABSENCE OF NEURONS. M.B. Bunge and P.M. Wood*. Dept. Anat. & Neurobiol., Washington U, St. Louis, MO.

In well differentiated neuron-Schwann cell (SC) cultures, the abaxonal surface of SCs is covered with basal lamina (BL). Additional culture studies suggest that substantial axonal contact (as during the process of SC ensheathment of an axon) is critical for SCs to fully assemble this BL (Clark and Bunge, '89). We as well as other investigators have repeatedly observed that, although SCs grown without neurons in differentiation supporting medium deposit BL constituents, typical BL is not seen (e.g., Baron-Van Evercooren et al., '86). When neurons and SCs are co-cultured but kept separate, the same result is obtained, even though mRNA for a BL component, laminin, is increased in SCs (Dean et al., '89). In this study cultures consisting of SCs and fibroblasts (Fbs) were studied electron microscopically; to our surprise, we observed that a BL-like structure enclosed singly situated SCs or encircled the external border of clustered SCs. These neuron-free cultures had been prepared as follows. Purified SCs were obtained from cultures of embryonic rat dorsal root ganglia first treated with fluorodeoxyuridine to eliminate Fbs and then maintained in defined medium to allow proliferation of BL-free SCs. The ganglia were excised and the remaining SCs were resuspended and plated ; 1 to 3 d later Fbs obtained from outgrowth regions of periosteum explants were added. Assembled cultures were grown in medium containing serum and embryo extract for 6-8 wk. The BL (lamina densa) was as thick or thicker than that observed in neuron-SC cultures; the lamina lucida was sometimes obscured. This is the first observation of substantial BL assembly on SCs (initially devoid of BL) cultured without neurons. Because SCs (without neurons) given exogenous BL components (Matrigel; PMW, unpublished) do not assemble BL to as great a degree, we suggest that the Fbs are not simply providing additional BL components for assembly of the SC. (NIH NS09923).

280.5

WITHDRAWN

280.6

A NEW GLIAL CELL SPECIFIC IMMUNOPROBE SHOWS PARTIAL TISSUE SITE SPECIFICITY. L. Zhang* AND N. G. F. Cooper. Dept. of Anatomy and Neurobiology, Univ. of Tenn., Memphis, TN 38163

The regional distribution of glia in the developing cerebral cortex, hippocampus, basal ganglia and cerebellum of the rat was studied with the immunoprobe, GLIAS, a glial cell specific antiserum. The serum was prepared by immunizing Balb/c mice with a subcellular fraction of cultured glial cells isolated from fetal rat striatum. Aldehyde perfused brains of anesthetized rats were used to obtain vibratome sections. Glia cells were recognized with a light microscope by the presence of dark brown horseradish peroxidase product after immunocytochemical staining. Radial glia were strongly stained at prenatal stages and were substituted by the less extensive and more astro-like immature glia at early postnatal stages in all areas. In cerebellar cortex, strongly stained radial glial processes of Bergmann cells were evident in early postnatal and adult animals. Mature astrocytes were predominantly present in the basal ganglia, cerebellar white matter, hippocampus, and corpus callosum in the adult brains. GLIAS labeled fewer glia cells than did anti-GFAP in the CNS of adult animals which suggests that a distinctive subpopulation of glia exists in the CNS of mature animals. Thus GLIAS may provide a sensitive method for the study of the organization and the histogenic role of glia during development. Finally, when glial antigens derived from fetal rat striatum were used to immunize mice, the resultant antibodies recognized a subpopulation of glia present in adult rat striatum, cerebellum, hippocampus and corpus callosum, but not cerebrum, thalamus, and brainstem. Supported by NIH:NEI EY02708 (NGFC) and a Neuroscience Center of Excellence predoctoral fellowship (LZ).

280.7

NEUROPEPTIDE GENE EXPRESSION IN CULTURED ATROCYTES: BRAIN REGION AND GENE SPECIFICITY.
J.P.Schwartz, H.Shinoda, A.M.Marini* and C.Cosi
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Astrocytes exhibit many neuronal characteristics, such as neurotransmitter receptors, ion channels and neurotransmitter uptake systems. We now present evidence that cultured astrocytes express certain neuropeptide genes, with a specificity shown for both the gene expressed and the brain region from which the cells were prepared. Somatostatin (SS) mRNA and peptide are present only in cerebellar astrocytes whereas proenkephalin (PE) mRNA and enkephalin peptides are present in astrocytes of cortex, cerebellum and striatum. Cholecystokinin is not expressed in any of the cells. Exposure of either cortical or striatal astrocytes to forskolin, which elevates cyclic AMP, increasesd both PE mRNA and enkephalins. The content of neuropeptide mRNA decreases with age of the donor animal: cells prepared from E20 and postnatal day 3 rat cerebellum contain the highest levels of SS and PE mRNA, the levels are reduced by PND8 and undetectable in adult. We propose that peptides synthesized in astrocytes may play a role in CNS development.

280.8

EPHEMERAL PATTERN OF CELL PROLIFERATION IN THE DEVELOPING OPOSSUM SUPERIOR COLLICULUS. L.A. Cavalcante*, P. C. Barradas* and A.M. Vieira* (SPON: C. Timo-Iaria), Instituto de Biofísica Carlos Chagas Filho, UFRJ, Rio de Janeiro, RJ, 21944, Brasil.

The appearance of GFAP$^+$ astrocytes in the opossum superior colliculus (SC) follows an outside/in gradient (Barradas et al., Glia, in press, 1989). To verify whether this gradient of glial differentiation is linked to patterns of non-neuronal cell proliferation, we have mapped the distribution of cells able to take up tritiated thymidine (3H-T) in pouch youngs of ages from 18-58 days. In selected stages, sections were also reacted for non-specific esterase or nucleoside diphosphatase. 3H-T$^+$ cell nuclei were few and dispersed in most stages but were numerous and arranged in 3 tiers in 30 day SC. No evidence was found for the involvement of macrophage/microglial precursors in this pattern of proliferation. The results show no link between the patterns of cell proliferation and sequence of GFAP expression. In addition they suggest that either macroglial percursors in the collicular parenchima reenter the mitotic cycle or that there is short-term immigration of macroglial precursors in the SC. (Supported by CNPq, FINEP and CEPG/UFRJ).

280.9

PRIMARY ENCEPHALOCELE CULTURES CAN YIELD PURE POPULATIONS OF NORMAL HUMAN ASTROCYTES.
R.L. Alterman*, J.T. Goodrich, R.M. Morrison and J.R. Moskal. Department of Neurological Surgery, Albert Einstein College of Medicine/Montefiore Medical Center, Bronx, NY 10461

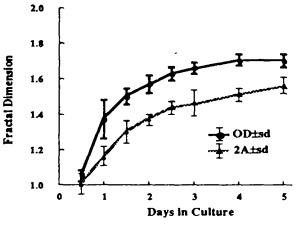
In order to develop a reliable source of human astrocytes for *in vitro* studies, we established a primary explant culture of a human encephalocele. A sample of encephalocele-derived white matter was minced and the pieces placed in a 25 cm^2 T-flask containing RPMI-1640 supplemented with 10% fetal bovine serum and antibiotics. A confluent monolayer of cells was obtained after two weeks at which time the cells were passaged by mild trypsinization (0.025%). Two cell-types with morphologies corresponding to fibrous and protoplasmic astrocytes were identified. All of the cells expressed glial fibrillary acid protein as determined by indirect immunofluorescence but none expressed somatostatin, galactocerebroside or neuron-specific enolase. Cytogenetic analysis revealed a normal 46XY karyotype. Cell growth was anchorage dependent. By passage 13 the cells exhibited phase III degeneration. The cells demonstrated a mitogenic response to epidermal growth factor (10ng/ml) and to basic fibroblast growth factor (10ng/ml). These results demonstrate that tissues derived from human encephaloceles can be used to generate pure populations of normal astrocytes.

280.10

FRACTAL ANALYSIS OF THE MORPHOLOGY OF DIFFERENTIATING GLIA. G.D. Lange[3], T.N. Behar*[1], T.G. Smith, Jr.[1], W.B. Marks[2] and W.H. Sheriff, Jr.*[3]. [1]LNP, [2]LNLC, [3]SIC, NINDS, National Institutes of Health, Bethesda, MD, 20892, USA.

Bi-potential progenitor cells (O-2A) derived from 7 day old rat optic nerve were used to generate synchronized, differentiating cultures of oligodendrocytes (OD) or type 2 astrocytes (2A). Populations enriched for each phenotype were produced by modifying the growth medium. Cultured cells were fixed at 12 hour intervals and identified immunocytochemically (GC$^+$ for OD; A2B5$^+$-GFA$^+$ for 2A). Video images of representative cells from each time were digitized and the borders were extracted. Fractal dimensions (FD) were calculated with a border dilation method.

FD correlated well with perceived morphological complexity. FD monotonically increased in both populations. It increased most rapidly and reached a plateau in OD. The plateau was not the result of a cessation of growth but its onset does coincide with the end of the period when a maturing OD can still be transformed to a 2A.



We believe that FD could replace biochemical markers as an indicator of the stage of differentiation of cultured cells and therefore provide a continuous "timeline" along which to study functional development.

280.11

UNIQUE MORPHOLOGY OF OLFACTORY BULB RADIAL GLIA: ROLE IN GLOMERULAR DEVELOPMENT? M.S. Bailey, M.R. Poston*, M.T. Shipley. Dept. of Anatomy and Cell Biology, Univ. of Cincinnati, Cincinnati, Ohio 45267.

Radial glia are considered an immature type of astrocyte which functions as a scaffold for the migration of neurons during development. In the rat olfactory bulb (OB), neurons migrate in an inside-outside manner similar to that in cerebral cortex; migration continues into postnatal development. We have examined the development of astrocytes in the postnatal rat immunohistochemically, using a polyclonal anti-GFAP antibody. Our observations suggest that radial glia in the OB may function in the formation of glomeruli, the multineuronal substrate of first order olfactory synaptic integration.

At the day of birth (P1) abundant radial glia course from deep layers of the bulb to the superficial glomerular layer (GL). Upon reaching the GL, many of these glia branch profusely, forming a plexus of glial processes. This plexus is in contrast to the continuous parallel course of cortical radial glia to the pial surface. In addition, mature stellate astrocytes can be found in GL at P1. In the GL at P5, P9, and P18, there is a decrease in the number of radial glia and an increase in the number of mature astrocytes prior to the increased numbers of mature astrocytes in the deeper layers of the OB. The adult pattern of astrocyte distribution is present by P36.

The differentiation of radial glia in embryonic development is suggested by the abundance of specialized branched radial glia and the presence of mature astrocytes in the GL of the OB at P1. Glial processes may interact with elongating mitral cell dendrites and/or primary olfactory neuron axons to form the glomeruli. Embryonic studies are now in progress. (Supported by NS23348.)

280.12

CATALASE-CONTAINING PEROXISOMES IN CULTURED GLIAL CELLS OF RAT AND CHICK. M. Beard, M. Saito and V. Sapirstein (SPON:G.Stone). The Nathan Kline Institute for Neuropsychiatric Research, Orangeburg NY 10962.

Peroxisomes, previously identified by others in neonatal and adult rat brain and adult chick spinal cord by their content of catalase, are implicated in the formation of myelin by virtue of their content of enzymes catalyzing the early steps in plasmalogen synthesis and their numerical increase in glia at the onset of myelination in cerebellum.

In order to investigate the more global relationships between brain and peroxisome function, primary glial cultures were established from perinatal rat and pre-hatching chick brains. Peroxisomal presence was monitored microscopically using the 3,3'-diaminobenzidine technique demonstrating catalase, the peroxisomal "marker" enzyme. We established that catalase-reactive peroxisomes are expressed in glial cells in culture. Peroxisomes were most prominent in oligodendrocytes grown in the presence of astrocytes; astrocytes from these cultures had few peroxisomes. Peroxisomes became more easily found in astrocytes when cultures contained fetal calf serum. Thus, it appears that culture conditions affect peroxisome expression, and that neural peroxisomes are responsive to environmental cues as are peroxisomes in non-neural systems.

280.13

CELLULAR INTERACTIONS BETWEEN BRAIN ENDOTHELIAL AND NEURONAL CELLS IN VITRO. J.H.Tao-Cheng, L.Chang*, O.Okuda* & M.W.Brightman.Lab Neurobiol.,NINDS,NIH,Bethesda,MD 20892

Bovine brain endothelial cells (BE) were co-cultured with primary cultures of cerebral tissues from 17-20 day fetal rats (1°brain cultures), or with PC12 cells, which were primed with nerve growth factor or infected with the K-Ras virus to express neuronal features, to see whether the different milieu of the co-cultures has any effect on the differentiation of the various cell types.

BE greatly enhanced the survival and growth of neurons from the 1° brain cultures. There were many more (>10x) neurons in the co-cultures with BE than in the solo 1° brain cultures. These neurons were positive for MAP2 and neuron specific enolase. Their neurites had many microtubules and some synaptic vesicles and occasionally formed mature synapses. BE also enhanced PC12 cells' neurites to bear more small, clear vesicles in the co-cultures than in the solo PC12 cultures. In return, BE were induced by the 1° brain cultures and PC12 cells to have (1) greatly distended rough endoplasmic reticulum (0.5 μm in lumen diameter) filled with fuzzy material, (2) abundant and tightly packed actin-like filaments and (3) an excessive extracellular matrix up to 1.5 μm in thickness. These BE cellular responses appeared to be induced by the neuronal elements in the co-cultures because enriched astroglial cultures without neurons did not induce such responses from BE. Thus, BE and neuronal cells reciprocally influence each other's differentiation and growth in vitro.

CONTROL OF POSTURE AND MOVEMENT VII

281.1

MODULATION OF THE H-REFLEX DURING STATIONARY CYCLING IN NORMAL HUMANS AND PATIENTS WITH SPINAL SPASTICITY G.I. BOORMAN *, W. J. BECKER, R.G. LEE. Dept. of Clinical Neurosciences, University of Calgary, Calgary, Canada.

It has been shown that the H-reflex is modulated during the step cycle in normal humans during walking (Capaday, C. and Stein, R., J. Neurosci. 6:1308, 1986). To further investigate modulation of spinal stretch reflexes during patterned voluntary activity we recorded H-reflexes while subjects were pedalling a stationary bicycle.

Normal subjects and patients with spasticity due to incomplete spinal cord injury were studied. Using a modified exercise bicycle, electrical stimuli were delivered to the posterior tibial nerve at defined points in the pedal cycle. EMG was recorded from gastroc-soleus and tibialis anterior muscles using surface electrodes. The M-wave amplitude was used to ensure that stimulus strength was constant at each pedal position. Background EMG was measured during a 30 ms interval prior to nerve stimulation.

In normal subjects the H-reflex varied in magnitude during the pedal cycle. The H-reflex was increased during the downstroke as compared to the upstroke. Patients with spasticity also showed H-reflex magnitude changes during the pedal cycle. The patterned changes in H-reflex amplitude were greater than could be accounted for by variations in prestimulus background EMG.Normal subjects are able to modulate spinal stretch reflexes. Some modulation also occurs in patients with spasticity.

281.2

LIMB TRAJECTORY DISORDERS IN HEREDITARY CEREBELLAR ATAXIA. Thomas Zeffiro. Human Motor Control Section, Medical Neurology Branch, NINDS, National Institutes of Health, Bethesda, MD 20892.

Damage to the cerebellum in man commonly results in an incoordination of goal-directed limb movement referred to as cerebellar ataxia. This study examines the kinematics of ataxia under varying conditions of visual guidance.

Hand trajectories were recorded during visually-elicited planar movements in 8 patients with hereditary cerebellar ataxia and 20 age-matched, healthy controls. Each subject, while sitting in total darkness, tracked a visual stimulus on the surface of a 20" x 20" digitizing tablet. In order to explore the effects of vision on trajectory properties, the appearance of the target was manipulated as follows: In one condition the target vanished as the movement began (T_{off}); and in the other the target remained visible throughout the trial (T_{on}).

Trajectories generated by patients with clinically mild ataxia exhibited primarily spatial abnormalities. Although errors in the initial direction of movement were seen in both visual guidance conditions, dysmetria, as measured by errors in final position, was greater in the T_{off} condition. Accuracy improved with visual guidance due to directional changes occurring during the decelerative phase of the movement. In contrast to the observed spatial abnormalities, temporal trajectory properties, including the scaling of peak velocity with movement size, were normal.

These results are consistent with the notion that specification of movement direction is impaired in cerebellar dysfunction, resulting in errors in final position. Visual information may be used to correct the trajectory as the hand nears the target.

281.3

POSTURAL INSTABILITY IN HUNTINGTON'S DISEASE (HD) J-R. Tian*, S.J. Herdman, D.S. Zee, The Johns Hopkins University, Baltimore, Maryland, 21205.

We measured A-P sway using moving platform posturography in 20 patients with HD and 20 age-matched normal controls. We determined the relative contributions of sensory cues to postural stability by eliminating proprioception and/or vision. Because of chorea, results were normalized to sway under normal vision and proprioception. With a criterion of abnormality of > 2 SD from the mean, 10 of 20 patients could not use vestibular cues alone to maintain postural stability. Thus, they relied more upon vision and proprioception to maintain balance.

In response to translations of the support surface, patients showed normal response strength but increased latencies (by 30-50 ms). In response to rotations patients had greater response amplitude than controls though, with repetition, response amplitude decreased normally.

HD patients have a consistent pattern of abnormality on posturography that may be clinically useful. Since labyrinthine function is thought to be normal in HD, our patients may have had difficulty in central processing of vestibular information.

281.4

SHORT LATENCY POSTURAL RESPONSES TO HEAD/NECK PERTURBATIONS. C.Shupert,F.B.Horak,V.Dietz* and G.Horstmann*.R.S.Dow Neuro. Sci.Inst.,Good Samaritan Hosp.,Portland,OR,97209&Dept.Clin. Neurol./Neurophysiol.,Univ.Freiburg,D-78 Freiburg,FRG.

To explore the relative contribution of sensory information from the head and neck and from the feet and legs to automatic postural responses during stance, we compared responses to A/P perturbations induced at the head (using a backpack-mounted apparatus) or at the feet (using a hydraulically-driven platform). Ten healthy adults and two patients with profound bilateral vestibular loss were tested with eyes closed. Head perturbations caused large, early head accelerations, but negligible center of mass movements, while platform perturbations produced small early head head accelerations and large center of mass movements. Both types of perturbations resulted in activation of the same ankle, knee, and hip muscles, but with different timing relationships. Ankle muscle response latencies were shorter for head perturbations (70 ms vs. 97 ms), but muscle burst amplitudes were 3 times larger for platform perturbations. Responses to head perturbations were nearly absent in the patient who lost vestibular function as an adult, but similar to normals in the patient who lost function as an infant. These results suggest that the contribution of different senses to coordinated postural responses depends on the nature of the body's displacement.

Supported by NIH grants NS01094, AG06457, and NS19222 and the Deutsche Forschungsgemeinschaft SFB 325.

281.5

RESPONSE CHARACTERISTICS OF SPASTIC MUSCULATURE TO PASSIVE CYCLING MOTION. J.J. Fuller*, R.M. Herman, R.A. Yapp*, A.H. Seif-Naraghi*, S.C. D'Luzansky* (SPON: M.J. Perlow). Samaritan Rehabilitation Institute, Phoenix, AZ 85006.

Traditionally, the magnitude and sensitivity of stretch reflexes in patients with spinal spasticity have been measured during and following passive movement of a single joint. A motor-driven stationary bicycle has been developed that produces a passive, rhythmic, cyclical movement of the lower limbs. Electromyographic (EMG) activity, kinetics and kinematics related to velocity sensitivity, interlimb relationships, and phase modulation of spinal reflexes in patients with spinal lessions were recorded. The following observations were made during passive cycling: a) cycling rates beyond a threshold velocity produced intralimb dependent, sustained, rhythmic muscle activity of both flexor and extensor muscles which increased in magnitude with cycling velocity, b) spasms, often induced during the first cycle and during continuous cycling at higher rates, could "reset" or enhance rhythmic motor activity, c) phase modulation of electocutaneously induced flexor reflexes was not observed, and d) changes in hip angle may alter spinal reflex sensitivity. These results support the notion of velocity sensitive stretch reflexes and describe other features of spinal reflex behavior among patients with spinal spasticity.

281.6

TIME COURSE OF CHANGES IN TRICEPS SURAE ACTIVATION DURING GAIT IN SPASTIC CEREBRAL PALSY AFTER WEARING AN ORTHOSIS. F. Dumas, F. Malouin and C.L. Richards. Neurobiology Lab. and Dept. of Physiotherapy, Fac. Medicine, Laval University, Quebec, Canada, G1K 7P4.

We studied the time course of changes in muscle activations of a child with spastic hemiparesis (31 months old at first evaluation) after wearing an ankle foot orthosis (AFO) that maintained the ankle in about 5° of dorsiflexion. Following baseline measures of the gait movements (electrogoniometer) and electromyographic activity of the triceps surae (TS) and tibialis anterior (TA) muscles, the child wore the orthosis (4 hrs/day, 5 days/wk) during daily activities. Post-tests were made monthly during a 6 month consecutive AFO-wearing period, then after the AFO was removed for 1.5 months, and again after a second 2.5 month AFO-wearing period. Changes in TS activation began to emerge after 3 months of AFO-wearing. At the end of the 10 month evaluation period, the spastic TS activation profile was remodelled and showed a prominent late stance burst which resembled that found in 3 year old normal children but an abnormal early stance phase peak persisted. This early peak activation was accentuated by a higher cadence in the early months but became less sensitive to cadence with time of AFO-wearing. In contrast, only minor changes were observed in the TA. One can question whether the emergence of a near-normal TS activation pattern is due primarily to the effects of AFO-wearing (prolonged TS stretching) which helps to unmask a basic activation pattern, to the gradual maturation of the activation pattern or to their interaction. This work was supported by a grant from the Fondation du Conseil des Clubs de Service, Québec.

281.7

MUSCLE PATTERNS DURING WALKING AND PEDALLING IN SUBJECTS WITH HEMIPLEGIA. C.A. Giuliani. Division of Physical Therapy, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7135.

The purpose of this study was to compare the EMG patterns during walking and pedalling in a group of subjects with hemiplegia.

Ten subjects with hemiplegia secondary to stroke walked and pedalled on a stationary bicycle at two speeds. EMG was recorded from the vastus lateralis (VL), biceps femoris (BF), tibialis anterior (TA), and triceps surae (LG/SO). Myopotentials were sampled off-line, full-wave rectified, and analyzed with computer software (ANAPAC). The onset latency of BF, TA, and LG/SO was referenced to the onset of the VL and expressed as a percent of cycle time.

During pedalling and walking, the TA and LG/SO were reciprocally active, and the SO, BF, and VL were active as extensor synergists. During both activities, TA onset latency occurred between 46 and 85% and the LG/SO and BF were within 10% of the cycle. There was less cocontraction between flexor and extensor muscles, and more consistent muscle patterns with fewer abnormal cycles during pedalling than during walking. Average I-EMG values for the LG and BF decreased during pedalling while the VL value increased significantly ($p < .05$).

These results suggest that the temporal pattern of muscle activity of walking and pedalling are similar. A more consistent pattern with fewer periods of cocontraction during pedalling than walking provide a rationale for using pedalling as a method of exercise for improving muscle patterns in patients with movement dysfunction.

Supported in part by a grant from the Foundation for Physical Therapy.

281.8

FORCE CONTROL IN SCHIZOPHRENICS WITH DYSKINESIA. P. B. Vrtunski, L. D. Alphs* and H. Y. Meltzer. VA Med. Center and CWRU Sch. of Med., Cleveland OH 44141.

Recent studies of force control of neurologically impaired (Mai et al, Brain, 111:973, 1988) and schizophrenic patients (Biol Psychiat, 25:529, 1989), suggest the usefulness of this paradigm for evaluating movement dysfunction in patients with tardive dyskinesia (TD). 33 subjects participated in this study, 12 with mild to moderate TD, and 21 nondyskinetic controls. Force control (FC) was measured by means of five force transducers fitted into a bite-piece (for oro-mandibular FC), both armrests of the test chair (for index finger FC) and two footrests (for pedal FC), respectively. The task consisted of matching the target force with a generated force. A score was developed from the mean error of matching performance. Results indicate significant impairment of the FC function in TD subjects: with high target forces, impairment was greater with the dominant hand than with the nondominant, whereas with low target forces, impairment was greater in the nondominant hand. Pedal FC (with higher target forces) showed a greater impairment than index finger FC. Oro-mandibular FC was the least valuable for discriminating the two groups of patients. Our results on force and laterality differentiation of patients with TD suggest a more complex mechanism for dyskinetic impairment than has previously been believed.

281.9

RELATIONSHIP BETWEEN RIGIDITY AND BRADYKINESIA DURING WEARING-OFF IN PARKINSON'S DISEASE. M.P. Caligiuri. Motor Function Laboratory, VA Medical Center, San Diego, CA 92161

The traditional view that rigidity underlies bradykinesia in Parkinson's disease (PD) contrasts with evidence showing that many rigid PD patients are not bradykinetic. In the present study, quantitative measures of rigidity (muscle stiffness) and bradykinesia (movement velocity) were used to test the hypothesis that rigidity and bradykinesia do not follow the same pattern of deterioration during the wearing-off phase of the medication cycle.Idiopathic PD patients were studied for four hours immediately following their morning dose of Sinemet. Upper extremity rigidity and bradykinesia were evaluated hourly. Preliminary findings indicated that the wearing-off patterns of rigidity and bradykinesia were independent of one another. An increase in stiffness associated with end-of-dose deterioration was not phasically related to slowing of movement. These results support the hypothesis that muscular rigidity may not underlie the slowness of movement and underscore the value of serial quantitative measures in revealing differential effects of Sinemet on parkinsonism. (Research supported by Veterans Administration)

281.10

DELAYED VISUOMOTOR CONTROL: OSCILLATIONS IN NORMAL AND PARKINSON'S PATIENTS. A. Beuter, C. Labrie, Milton*, J. L. Glass* Univ. of Québec at Montréal, C.P. 8888, Succ A H3C 3P8 and McGill Univ., Montréal.

Patients with Parkinson's disease (PD) have deficits in planning and executing voluntary movements. We compared the effect of delayed visual feedback on a simple motor task for 12 subjects: 4 Stage II PD, 4 age-matched and 4 young adults. This task required the subject to maintain a constant finger position relative to a stationary baseline displayed on an oscilloscope. The finger positions (not directly seen by the subject) were recorded for 60 sec. using a LVDT and digitized at 150 Hz. In the absence of delayed visual feedback, power spectral analysis revealed a baseline oscillation in finger position with a peak at 5.5-7 Hz for 4/4 PD patients and 9-11 Hz for 4/8 normal subjects. With the addition of delayed visual feedback, a complex higher-amplitude, low-frequency oscillation was superimposed on the baseline oscillation. The average peak-to-peak time interval (INT) of this low frequency oscillation increased linearly as the visual delay was increased from 600-1400 msec. No significant difference was found in the change INT as a function of added delay between PD and normal subjects. These observations indicate that adjustments to alterations in the information guiding movement are preserved in Parkinson's disease.

281.11

MOTOR CONTROL IN SPINAL MUSCULAR ATROPHY. A. Beric, J. Bojakowski*[1], M.R. Dimitrijevic, I. Hausmanowa-Petrusewicz*[1] and A. Michalik*[1], Baylor College of Medicine, Houston, TX 77030, and [1]Warsaw Academy of Medicine, Poland.

We compared voluntary and automatic motor activity in humans with intact neuromuscular systems and in persons with degenerative disorders of the common final path. We studied a group of 18 subjects with spinal muscular atrophy (SMA) in comparison to an group of 10 healthy subjects. Surface EMG electrodes were placed over paraspinal, abdominal, tensor fasciae latae, quadriceps, adductors, hamstrings, tibialis anterior and triceps surae muscles. Ink jet recordings were carried out during quiet standing, forward and backward leaning, standing on one leg, and various maneuvers while sitting or lying supine. When responses of SMA and healthy subjects were compared, the patterns of activity induced by skillful voluntary efforts were not obviously different between the two groups. In a comparison of postural activity during standing, the SMA subjects demonstrated a noticeable increase in EMG amplitude as well as a spread of activity to muscles not active in the healthy subjects. There was more coactivation of antagonistic pairs, which was not restricted to the muscles with most clinically evident weakness. There was a more pronounced loss of selectivity and loss of local response in the more severely disabled individuals. This decrement in performance is similar to that observed in intact subjects at the limits of endurance.

281.12

CHANGES IN MUSCLE ACTIVATION DURING THE MAINTENANCE OF A POSTURE AT THE HUMAN ELBOW JOINT. T.S. Buchanan, J.P.A. Dewald, G.P. Rovai*, and W.Z. Rymer. Sensory Motor Performance Program, Rehabilitation Institute of Chicago, Chicago, IL 60611.

We examined the activation of muscles that act in shoulder and elbow flexion/extension and wrist supination/pronation during various static postures. Intramuscular and surface EMGs for up to ten muscles and force at the wrist in 3 dimensions (flexion/extension, varus/valgus, and supination/pronation) were recorded while subjects produced steady-state forces at the wrist. Subjects held one of 3 forearm pro-supination angles and two shoulder flexion/extension angles. Data was recorded from the normal side of spastic hemiparetic subjects (see abstract by Dewald et al.).

It was observed that some elbow-joint muscles that undergo no change in length with shoulder angle changes (ie. do not cross the shoulder joint) are nonetheless varied significantly with postures at different shoulder horizontal abduction angles. For example, when the shoulder was held in 25° horizontal abduction, the brachialis EMGs increased compared to those collected when the shoulder was at 0° (extension). Inverse relationships were observed in the pronator teres which, similarly, is far removed from the shoulder.

It is surprising to see muscles being modulated with changes in the joint angle of a secondary joint at which they have no obvious mechanical action. Changes in joint angles induce changes in lengths for muscles which cross the joint. Such is the case in this situation for shoulder muscles and two-joint muscles such as the biceps brachii which shortens with increased abduction. With muscle shortening, length/tension relations would predict that biceps tension would drop, barring changes in activation. The increased activation of the brachialis could be a result of compensation for force/EMG changes in such two-joint muscles or for other changes in the roles of these muscles associated with their actions at the secondary joint.

These observations point to a complex control strategy that alters a muscle's activation level with the load conditions at joints where the muscle may have no mechanical action. Such coupling is perhaps important for compensating for torques produced by multi-joint muscles that are necessary for torque production. This underscores the importance of taking into account the load state at nearby joints when building models of muscle coordination.

This work is supported by NIH grant NS-19331.

281.13

CONTROL OF MUSCLE ACTIVATION DURING THE MAINTENANCE OF ARM POSTURES IN THE SPASTIC HEMIPARETIC SUBJECT. J.P.A. Dewald, T.S. Buchanan, G.P. Rovai*, and W.Z. Rymer. Sensory Motor Performance Program, Rehabilitation Institute of Chicago, Chicago, IL 60611.

In an effort to study the effects of different perturbations on muscle coactivation patterns in hemiparetic stroke we compared electromyographic activation levels in both upper extremities of such subjects for different positions. EMG activities in up to ten muscles that act in shoulder and elbow flexion/extension and wrist supination/pronation were recorded with surface and intramuscular electrodes during very controlled static postures. Forces were applied at the wrist and were measured in three dimensions (flexion/extension, varus/valgus, and supination/pronation) while the arm position was fixed at 90° elbow flexion, neutral pro-supination position, and one of two different shoulder horizontal abduction angles angles (0° and 25°).

The EMGs were examined as a function of the direction of the force at the wrist in the plane orthogonal to the forearm. For most muscles, the EMGs revealed a broadening of muscle activation as a function of torque direction in the spastic limb compared to the normal limb. This is a result of an increase in co-contraction of muscle activity. However in moderate to severely involved subjects this increase in range of activation was observed to change significantly at different shoulder horizontal abduction angles. As the shoulder horizontal abduction angle increased, EMGs became more focused (ie. had a narrower range) in the spastic limb. Compared to 0°, at 25° shoulder horizontal abduction spatial EMG characteristics of elbow muscles appeared more 'normal'. The fact that the 25° horizontal abduction angle places the arm in a position which is close to the typical hemiplegic arm posture is an interesting observation. It appears that in this arm posture the moderate to severely affected subjects are able to produce more normal muscle coactivation combinations during the static tasks of our experimental paradigm.

The loss of corticospinal descending fibers in the spastic hemiparetic subject due to cortical or subcortical lesions thus favors not only a particular arm position through the intact vestibulo- and reticulospinal systems, but also appears to affect muscle coactivational behavior in the involved upper extremity.

This work is supported by NIH grant NS-19331.

281.14

Effect of Head Orientation on Human Postural Stability Following Unilateral Vestibular Ablation (UVA). C.R.Fox and G.D.Paige. Washington Univ, St Louis, MO 63110.

Vestibular input to postural control is important but remains poorly understood. Appropriate postural responses to vestibular input during sway requires that orientation of the head on the body is known. Postural stability might deteriorate when vestibular input and information about head on body are not properly coupled. Perhaps this condition arises in subjects with vestibulopathy. Postural sway was assessed in 9 normals, and in 9 vestibulopathic patients before and 1 wk, 1 mo and 4 mo after UVA. Postural stability with eyes closed was quantified as scaled pk-pk sway during 20 s trials in which the support surface was modulated proportionally with sway. Subjects were tested with the head upright and facing forward, 45 deg R and 45 deg L. In normals, sway was uninfluenced by head orientation. Sway was greater in patients than in normals both before and after UVA. Further, UVA subjects showed head orientation-dependent asymmetries. Before UVA, 22% of subjects showed abnormally large R-L asymmetry, but split as to whether sway was less with the normal (contra) or abnormal (ipsi) ear facing forward. 1 wk after UVA, 60% showed asymmetry, with 2/3 of them displaying better stability with the contra ear forward. The proportion of subjects with asymmetry declined to 44% after 1 mo, and the contra-ipsi difference was eliminated by 4 mo. It is concluded that head orientation, in conjunction with vestibular input, is important in postural control.
(Supported by NIH grants AG06442 and EY07057.)

281.15

EMG ANALYSIS OF CO-CONTRACTION IN ACUTE HEMIPARESIS: EMG-INITIATED ELECTRICAL STIMULATION VS CONVENTIONAL THERAPY. S.S.Fitts,M.C.Hammond* and G.H.Kraft*. Department of Rehabilitation Medicine, University of Washington, Seattle, WA 98195.

Electromyographic (EMG) analysis of isolated agonist recruitment and co-contraction was used to assess poststroke motor recovery in patients treated with EMG-initiated electrical stimulation (EMG-Stim) and/or conventional therapy (CONV). Patients were recruited within four (X=2.9) wks of their first unilateral, non-hemorrhagic cerebrovascular accident (CVA). Five received EMG-Stim, plus conventional therapy; five received only conventional therapy. Intramuscular EMG was recorded simultaneously from flexor carpi radialis (FCR) and extensor carpi radialis longus (ECR) during isometric wrist flexion and extension at an average of 8.4 wks post-CVA. The number of events exceeding 250 recorded from agonist muscles did not differ between the two groups. However, fewer antagonist FCR events were recorded from EMG-Stim than from CONV patients during wrist extension (X's=28.4 vs 61.5; $p<.10$); and more antagonist ECR events were recorded from EMG-Stim than from CONV patients during wrist flexion (X's=130.9 vs 11.6; $p<.025$). Compared to CONV, EMG-Stim patients had lower co-contraction ratios (antagonist events divided by the total of agonist plus antagonist events) during extension (X's=.06 vs .14; $p<.10$) and higher co-contraction ratios during flexion (X's=.18 vs .06; $p<.05$). The difference between groups was maintained at least 3 months for wrist extension (X's=.06 vs.13; $p<.10$); but not for flexion (X's=.034 vs .037). We conclude that EMG-Stim patients achieved a more functional balance of opposing flexor and extensor muscles, although post-CVA recovery of isolated agonist recruitment was not affected.
NIDRR grant #133CH70074 supported this research; Biometer International and Medtronics, Inc. provided equipment.

281.16

EFFECTS OF SOMATOSENSORY THRESHOLD TRAINING ON MANUAL MOTOR PERFORMANCE IN NORMALS AND PATIENTS WITH HEMIPARESIS. M. Pause*, E. Kern*, M. Haller*, V. Hömberg* (SPON: K.H. Mauritz). Dept of Rehabilitation, Univ. of Cologne and Dept. of Neurology, Univ. of Düsseldorf, FRG.

Data of motor performance in non-paretic patients with isolated marked sensory deficits suggested that afferent feedback is crucial for the precision of fine motor acts (Rothwell et al., Brain 105, 515-542,1982). This leads to the hypothesis that systematic (re-)training of somatosensory functions might induce an improvement of fine motor functions as well. In our study therefore we investigated the relative impact of somatosensory training on manual motor performance in normals and hemiparetic patients.

The non-dominant hand of 10 healthy subjects was examined with a standardized battery of 14 different motor tests comprising automated-proximal-visually guided (APV) movements and non-automated- distal-somatosensory guided (NADS) movements. Under occlusion of vision these normals were trained by verbal feedback over ten days for tactile discrimination of threshold stimulus pairs in 10 different paradigms (*Proprioceptive:* position sense, load estimation, distance estimation, form discrimination; *cutaneous* discrimination of vibration, roughness, dynamic and successive two-point stimuli, movement direction and pressure) at their non-dominant hand, whereby active movements were minimized. After the training the motor test battery was repeated. The same protocol was used in the affected hand of 8 patients suffering from focal cerebral lesions causing mild unilateral motor disturbances with or without additional somatosensory deficits.

Motor performance either for APV- or NADS- movements of *normal subjects* was not altered by the somatosensory training despite a mean improvement of somatosensory discrimination thresholds of 31%. In the *patients* the mean initial sensory discrimination thresholds were elevated by 77% compared to normals. After training this value was reduced to 48%. The mean of the initial motor test of the patients was 41.8% of the average value of the normal subjects. After sensory training the APV-movement-score showed an amelioration by 10.3%, the NADS-movement-score by 21.5%. From these preliminary investigations it can be concluded that somatosensory threshold training is beneficial for reeducation of non-automated fine manual movements in patients with upper motor neuron lesions.

281.17

EFFECT OF HYPOXIA ON REACHING IN NORMALS AND IN PATIENTS ON LONG-TERM OXYGEN. M.C.Verrier,K.R.Chapman*,W.G.Tatton,A.S.Rebuck* and C.Diep*. Restorative Motor Control Lab. Departments of Rehab. Medicine, Medicine and Physiology, Univ. of Toronto, Toronto, CANADA. M5T 1W5.

Hypoxia has been shown to have complex effects on lever press accuracy and response time in highly trained primates(Nicholson,A.N. and Wright,C.M.,Exp. Neurol. 47:535- 543,1975). In an ABAB design, reaction time (RT), movement time (MT), 3-D kinematics and oxygen saturation (SaO_2) were used to determine if hypoxia alters routine reaching in a simple RT task in young normals (n=10) and chronic obstructive pulmonary disease patients (COPDs) on chronic O_2(n=8). Individual $\bar{x}$ RTs (35 trials) in 4 of 15 and $\bar{x}$ MTs in 6 of 13 cases decreased significantly ($p < 0.05$) in COPDs when O_2 was removed, with a change in $\bar{x}$ SaO_2 from 93 to 88%. Mild hypoxia improved reaching performance in the COPD group by a $\bar{x}$ of 63 msec ($\bar{x}$RT=25msec;$\bar{x}$MT= 38msec). The results in normals were more complex. Mean SaO_2 levels were decreased from 97 to 80% using a rebreathe technique in 6 subjects. Four subjects were unable to tolerate hypoxia. In contrast to COPDs individual $\bar{x}$ RTs in normals were marginally but significantly increased in 4 of 11 and decreased in 1 of 11 cases while $\bar{x}$ MTs decreased in 5 of 11 and increased in 1 of 11 cases. However, the $\bar{x}$ group increase was only 8msec (RT) and 2msec (MT) demonstrating marked variability. These results in normals could be accounted for by the difference in individual ability to tolerate marked hypoxia which causes temporary alterations in both processing (RT) and execution (MT) functions of neural structures essential for reaching. In contrast, mild hypoxia in COPDs improves performance which could indicate their adaptive ability to tolerate mild hypoxia. Supported by NIH.

SPINAL CORD AND BRAINSTEM: CORD ANATOMY

282.1

THE ORIGINS OF DESCENDING PATHWAYS TO THE SPINAL CORD IN THE BRAZILIAN OPOSSUM, MONODELPHIS DOMESTICA. M-C Holst, R. Ho and G.F. Martin. Department of Anatomy, The Ohio State University, Columbus, OH 43210.

The North American opossum, Didelphis virginiana, is a good model for developmental studies of descending spinal pathways because of its immaturity at birth and protracted postnatal development. The smaller, gray short-tailed Brazilian opossum, Monodelphis domestica, can be bred more easily however, and offers other advantages as a laboratory animal. Since little is known about the organization of descending pathways in adult Monodelphis, we have begun studies to determine their cells of origin. Cervical, lumbar and sacral cord injections of WGA-HRP or True Blue (TB) labeled supraspinal areas comparable to those labeled by similar injections in Didelphis, plus some additional ones. For example, cervical injections labeled neurons in the amygdala and basal forebrain. As in Didelphis, the neocortex was labeled only after cervical injections and the distribution of labeled neurons in the red nucleus indicates that rubrospinal somatotopy is not as distinct as in placental mammals. The origin of monoaminergic projections to the cervical cord was studied using the retrograde transport of TB combined with immunofluorescence for serotonin or tyrosine hydroxylase. The results are similar to those obtained in Didelphis, but differences were noted. This is the first comprehensive report on descending pathways to the spinal cord in a marsupial other than Didelphis. Supported by the Bremer Foundation and NS25075.

282.2

ORIGIN OF THE CERVICAL PROJECTIONS TO THE LUMBOSACRAL ENLARGEMENT (LS) OF THE SPINAL CORD IN THE OPOSSUM, MONODELPHIS DOMESTICA, T. Cabana and J.P. Parent*, Département de Sciences biologiques, Université de Montréal, C.P. 6128, Succ. "A", Montréal, Canada H3C 3J7.

The origin of the cervical projections to LS was investigated with the retrograde tracing of WGA-HRP and TMB technique in adult opossums as a basis for interpreting developmental studies on interlimb coordination. Injections of 1-5µl of 1.5-2%WGA-HRP were placed in LS, involving, in some animals, some or all areas of the gray matter. The 8 cervical & 1st thoracic levels were examined. Labeled cells were found in all the segments studied but in relatively greater number in the lower cervical and first thoracic segments. Lamina VII, then lamina VIII, contained the greatest number of labeled neurons. Labeling was also present in laminae VI and V, especially their lateral portion, and ventrally and ventrolaterally to the central canal. In all these areas, the labeled cell bodies were often large (mean diameter of 30 to 50µm). A few small sized, labeled neurons were observed in the lateral part of lamina I. No attempts were made at establishing the laterality of the projections by performing hemisections rostral to the injections, but the little spread of the injectate to the contralateral side in some cases indicated that the labeling is bilateral, except in lamina I where it is ipsilateral. These data generally conform with those known of other mammals.

282.3

DIFFERENCES IN SEXUALLY DIMORPHIC SPINAL MOTOR NUCLEI AS REVEALED BY TRANSNEURONAL TRANSPORT OF WGA. K.R. Peshori, J.T. Erichsen and W.F. Collins, III, Dept. of Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook, NY 11794

We have previously demonstrated retrograde transneuronal transport of wheat germ agglutinin [WGA] between motoneurons [MNs] in the dorsal medial nuclei [DMN or SNB] of the rat spinal cord (Rose et al., 1987). The present study was undertaken to examine the distribution of transneuronally labeled MNs and interneurons [INs] following injection of WGA into different penile muscles. In adult male rats (Sprague-Dawley), WGA (1%, 0.5 ul) was injected into either the left lateral bulbospongiosus [LBS] or ischiocavernosus [IC] muscles. Prior to injection of WGA, the left and right pudendal sensory nerves, the contralateral pudendal motor nerve, and the nerves to either (1) the ipsilateral IC and urethral sphincter muscles (LBS injections) or (2) the bulbospongiosus and anal sphincter muscles (IC injections) were cut. Following 1 to 7 days survival, rats were sacrificed and the spinal cords processed immunohistochemically for visualization of WGA. With LBS injection, direct retrograde labeling of ipsilateral DMN MNs (1-7 days) and bilateral transneuronal labeling of DMN MNs (2-7 days) and INs (3-7 days) were observed. Transneuronal labeling increased with longer survival times. Labeled INs were located bilaterally in segments L_5 and L_6 lateral to the DMN and extended dorsally to the dorsal grey commissure. In contrast, IC injection resulted in direct retrograde labeling of the medial portion of the ipsilateral dorsal lateral nucleus [DLN] (1-7 days) with little or no transneuronal labeling of other MNs or INs (1-7 days). These results suggest differences between the DMN and the DLN with respect to (i) afferentation, (ii) MN interactions, and/or (iii) baseline activity. Supported by EY04587 and a grant from the Center for Biotechnology SUNY Stony Brook (JTE), NS24206 (WFC) and NS14899 & NS16996 to L.M. Mendell.

282.4

ALTERED LEVELS OF CALCITONIN GENE-RELATED PEPTIDE (CGRP)-LIKE IMMUNOREACTIVITY OF CAT AND RAT MOTONEURONS AFTER DIFFERENT TYPE OF LESIONS. U. Arvidsson[1*], S. Cullheim[1*], B. Ulfhake[1*], H Johnson[1*], F. Piehl[1*], T. Hökfelt[2], M. Risling[1*] and L. Terenius[3]. (Spon. T. Carlstedt). Departments of [1]Anatomy and [2]Histology and Neurobiology, Karolinska Institutet, Stockholm (Sweden) and [3]Department of Pharmacology, Uppsala University, Uppsala (Sweden).

The indirect immunofluorescence technique has been used to study CGRP-like immunoreactivity (LI) in motoneuron cell bodies in the lower lumbar segments after sciatic nerve transection (cat and rat) and in chronic thoracic spinal cord transection (SCT) alone (cat) or in combination with unilateral rhizotomy of all dorsal roots below the SCT (cat).

In the lower lumbar motor nucleus of normal cats and rats almost all large neurons (of alpha motoneuron size) express CGRP-LI. However, dorsally located neurons in the nucleus, innervating distal muscles groups, are less intensely labeled than ventrally located neurons innervating proximal muscles groups. Seven days after sciatic nerve transection in the cat, the CGRP-LI in motoneurons with a location corresponding to the sources of the sciatic nerve was significantly stronger on the axotomized side. In rats, the effects of sciatic nerve transection was studied at various post-operative intervals (1-21 days). These experiments revealed a stronger CGRP-LI expression in motoneurons on the operated side after 2-5 days, while no clear side difference could be detected after 11 or 21 days.

After SCT in the cat (44 days post-operatively) there was a decrease in CGRP-immunoreactive labeling of the neurons in the motor nucleus compared to normal. When SCT was combined with unilateral rhizotomy, the CGRP-LI in the motor nucleus displayed an apparantly normal pattern on both sides. Thus, removal of the primary afferent input on one side of the cord seems to diminish the effects of spinal cord transection on both sides.

The present results indicate that surgical interventions such as axotomy or manipulation of afferent inputs to motoneurons induce changes in the level of CGRP-LI in motoneurons.

282.5

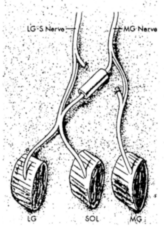
DISTRIBUTION OF CATECHOLAMINERGIC AND MU-OPIOID RECEPTORS IN THE CAT SPINAL CORD. C.D. Barnes, S.J. Fung, R.C. Speth, L. Churchill, and H. Zhuo*. (SPON: J. Chan) Dept. of VCAPP, Washington State University, Pullman, WA 99164-6520

In the cat, it has been reported that the locus coeruleus complex, including the Kölliker Fuse nucleus, supplies the vast majority of catecholaminergic fibers to the spinal cord. Additionally, it has been shown that a significant percentage of these descending catecholaminergic neurons contain met-enkephalin. The present study was done to determine if there is a differential distribution of α_1, α_2 and β adrenergic and mu-opioid receptors, the presumed targets of these fibers, in the spinal cord. 60-65 pM of ^{125}I-HEAT ± 1 μM prazosin, 7nM of ^{3}H-idazoxan ± 10 μM phentolamine, 60-65 pM of ^{125}I-(-) cyanopindolol ± 1 μM l-propranolol and 0.2 nM [^{125}I] Tyr-D-Ala-Gly$_5$-me Phe-Gly(ol) ± 5 μM naloxone, were used to autoradiographically label these receptors, respectively.

In the ventral horn all four receptor types are present in Rexed's lamina IX, but mu-opioid and β are also present in lamina VIII. There is a concentration of α_2 receptors near the nucleus intermedio-medialis, and both β and mu-opioid receptors appear in area VI near Clark's column. Both α_1 and α_2 receptors appear in the nucleus intermedio-lateralis, and all four receptors were represented in differing degrees in the dorsal horn areas I, II and III. A dorsal rizotomy eliminated the mu-opioid receptors of the dorsal horn but not those of the ventral horn. A relatively heavy concentration of β receptors was also found in fibers of both the dorsal column and the dorsal lateral funiculus (Supported by grant NS 24388).

282.6

LONG-TERM DIRECT INNERVATION OF SOLEUS MUSCLE BY MEDIAL GASTROCNEMIUS NERVE IN THE CAT. JB Munson & RC Foehring. U of FL Med Ctr, Gainesville, & U of TN Med Ctr, Memphis.



The proximal portion of ~1/3 of the MG nerve was coapted with soleus nerve distally; ~2/3 of MG nerve remained innervating the MG muscle. 18 mo later, MN and/or muscle-unit properties were determined for MG MNs innervating MG, soleus or no muscle. Properties and percents of motor units in the MG muscle were normal: thus a normal population of MG motor axons had been directed to soleus. 74% of cross-innervated soleus (Xsol) units were type-S (by contractile measures), despite cross-connection by ~75% fast MNs. 35% of MG MNs cross-connected to soleus nerve failed to innervate muscle (cf ~10% when MG nerve was coapted with MG or LG-S nerve): MG MNs innervating soleus muscle thus may be disadvantaged. Electrical properties of axotomized MG and soleus MNs, and of Xsol MG MNs, differed from normals; Xsol MN properties approached those of axotomized MG MNs. It appears that fast MG MNs which innervated large (strong) soleus muscle units failed to convert those muscle fibers to fast types, and were thereby prevented from recovering their normal electrical properties. Conversion and recovery did occur for fast MG MNs which innervated small (weak) soleus muscle units, and for cross-reinnervated slow MG MNs. Supported by NS-15913.

282.7

CHARACTERIZATION OF GABA-LIKE IMMUNOREACTIVE MOTONEURONS IN THE VENTRAL HORN OF THE CHICK SPINAL CORD. **E. PHILIPPE* and C. DELAGRAVE*** (SPON: L. Larochelle), Dept. of Anatomy, Laval University and Center of Neurobiology, 2075 Vitré, Quebec - CANADA.

In order to determine the role of peripheral and central tissues on the phenotypes expressed by chick lumbo-sacral spinal neurons, characterization of motoneurons was performed according to a combination of anatomical, immunocytochemical and ultrastructural criteria.

Motoneurons were characterized by the means of retrograde transport of fluorescent latex spheres from the Sartorius and the Femoro-tibialis muscles of one hindlimb.

Immunocytochemistry was performed with polyclonal antibodies raised against gamma aminobutyric acid (GABA) according to the peroxydase anti-peroxydase procedure (PAP).

Among the whole population of neurons located in the lumbo-sacral spinal cord, a few large neuronal cell bodies were both retrogradely labeled with the fluorescent latex spheres and immunostained with anti-serum raised against GABA. These immunostained motoneurons, mainly located in the lamina 7 and 9, according to the laminar distribution of the chick spinal cord (Martin, 1979) were large, ovoid and mainly characterized by a well developed rough endoplasmic reticulum and numerous straight Golgi apparatus.

These preliminary results raised the questions of the role of amino acid in the motor innervation of skeletal muscles in the chick.(Supported by grants of C.R.M. and F.R.S.Q.)

282.8

ORGANIZATION OF MOTOR NUCLEI SUPPLYING INTERVERTEBRAL MUSCLES OF THE CAT REVEALED BY MULTIPLE LABELLING. D.C. Gordon* and F.J.R. Richmond, Dept. of Physiology, Queen's University, Kingston, Ontario, Canada, K7L 3N6.

In previous electromyographic studies we have shown that deep neck muscles have highly modulated patterns of recruitment during head movement. Spinalis dorsi, semispinalis cervicis and rectus capitis posterior (RC) are primarily active during elevation of the head whereas obliquus capitis caudalis (OC) is more strongly recruited during head turns. In this study we have used a multiple labelling method to examine the topographical organization of motoneurons supplying different intervertebral muscles. Intervertebral muscle nerves were isolated, cut and exposed separately to one of three different retrograde tracers, Fluorogold, Fast Blue and horseradish peroxidase. This method permitted us to compare directly the location of different motoneuron groupings in a single cat. Motoneurons supplying dorsal intervertebral muscles were arranged in overlapping cell columns from rostral C1 to mid C5. The segmental location of motor nuclei corresponded to the rostrocaudal location of the muscles along the vertebral column. Cells serving the extensor RC interdigitated with those supplying the turning muscle OC. Results suggested that the topographical organization of the cell groupings may not be a major factor influencing the specialized recruitment of deep neck muscles. Supported by the MRC of Canada.

282.9

LUMBAR INPUT TO RAT SACROCAUDAL SPINAL CORD. M.L. Sparkes*, C.R. Murray* and L.A. Ritz. Depts. of Neurosurgery and Neuroscience, Univ. of Florida, Gainesville, Fl. 32610.

The sacrocaudal spinal cord, that portion of the neuraxis which innervates the tail, has been the subject of anatomical, physiological and behavioral analyses in our laboratory. The present study investigates propriospinal input to rat sacrocaudal spinal cord. Rats were injected at spinal level S3 with 0.5-1.0 ul of 2% fluoro-gold. After 3-10 days the rats were sacrificed and the lumbar, sacral and caudal segments processed. Injection sites involved, bilaterally, portions of dorsal horn, medial intermediate zone and ventral horn, but not the ventral white matter. To date, data have been analyzed for the L4-S1 segments. Most labelled neurons were localized to the intermediate zone, adjacent to the central canal and to the medial portion of the ventral horn. Cells were also found at the base of the dorsal horn. About 10-20 neurons were labelled per 50 micrometer section. The present data support the idea that there are propriospinal connections between lumbar and sacrocaudal spinal cord, which could contribute to coordination of hindlimb-tail interactions. Research supported by NS23683.

282.10

MORPHOLOGY AND SEGMENTAL DISTRIBUTION OF INHIBITORY INTERNEURONS IN WHOLE-MOUNTS OF GOLDFISH SPINAL CORD. J.R. Fetcho. Dept. of Physiology, SUNY at Buffalo, NY 14214.

The morphological variability and segmental distribution of spinal inhibitory interneurons were studied with a newly developed whole-mount technique. Crossed inhibitory interneurons in the spinal network for escapes were physiologically identified based on an electrotonic input from the Mauthner cell and then filled by intracellular pressure injection of HRP. All of the filled cells were morphologically similar to one another and to the small number crossing interneurons studied previously in serial sections. The cell body gave rise to a single axonal process that crossed the cord and then bifurcated into a relatively short, thin rostral branch and a thicker, longer caudal one. Branches arose from these processes and terminated near M-axon collaterals. The axons of interneurons extended longitudinally over a distance of from one to two body segments. However, the location of cells with respect to segmental boundaries (as defined by ventral roots) was variable; axons always straddled segments even when the axons were only one segment long. These observations indicate that the interneurons form a morphologically homogeneous class that distribute inhibition to motoneurons (and other interneurons) in a local, but not strictly segmental manner. Supported by NS 26539.

282.11

RETROGRADE LABELING OF SPINAL NEURONS FOLLOWING INJECTIONS OF FLUORESCENT MICROSPHERES AND HRP INTO DIFFERENT REGIONS OF THE CAT'S LUMBAR CORD. J.E. Hoover* and R.G. Durkovic. (SPON: S. Nord). Dept. of Physiol., SUNY-Health Science Center, Syracuse, NY 13210.

In order to retrogradely label interneurons projecting to motor nuclei, fluorescent latex microspheres were pressure injected into motoneuron pools of anesthetized cats. Single small injections of red and green beads (+HRP in one) were made at the L-7 level (one fluorochrome on each side) into either deep peroneal or PBST motoneuron pools. The micropipette tip was positioned by recording the antidromic field potential elicited by stimulation of the motor nerves. Animals remained anesthetized 60 hr before sacrifice.

Retrogradely labeled neurons were observed on both sides of the spinal cord from L-1 to S-2. Most cells labeled ipsilateral to the injection site were located in laminae VI and VII. However, labeled neurons were also found in laminae V, VIII, IX and X. Cells labeled contralateral to the injection site were restricted to laminae VII, VIII, and X, the majority being located in VIII. A few double labeled cells (red and green) were observed in lamina X. Even though diffusion of HRP occurred far outside the area of bead injection, HRP labeled cells were located in qualitatively the same spinal cord regions as those labeled with fluorescent microspheres. Supported by NSF grant BNS 8808495.

282.12

VARIATION IN ULTRASTRUCTURAL CHARACTERISTICS OF BOUTONS OF INDIVIDUAL MG Ia FIBERS CONTACTING MOTONEURONS.

J.P. Pierce, H.R. Koerber and L.M. Mendell. Dept. of Neurobiology and Behavior, SUNY, Stony Brook, NY 11794

Individual Ia fibers project to large numbers of α-motoneurons (MNs) from dif ferent subgroups (innervating FF, FR and S motor units), and the physiological properties of each connection correlates with the type of MN contacted. While single fiber EPSPs in high rheobase MNs (associated with FF motor units) display facilitation in response to high frequency stimulation, single fiber EPSPs in low rheobase MNs (associated with S motor units) display depression (Collins et. al., J. Neurophysiol., 52, 1984). Studies in vertebrate cortex (Bower and Haberly, PNAS, 83 1986) and at the lobster NMJ (Meiss and Govind, J. Neurobiol., 11, 1980) have shown that these types of response characteristics are associated with presynaptic ultrastructural differences. If a similar correlation exists at the Ia-MN synapse, then there should be as much variation in the given feature among the boutons of a single fiber as within the whole population. In the present study medial gastrocnemius Ia afferents were identified physiologically in barbiturate anesthetized cats, injected with HRP, and processed for light and electron microscopic analysis. Light level reconstructions were made to define the location of individual boutons and neighboring boutons were serially thin sectioned. MN contacts were identified by the size of the postsynaptic profiles. Ultrastructural features measured included cross sectional area, shape, vesicle density (VD), fractional mitochondrial area (FMA), fractional apposed perimeter (FAP) and active zone length (AZL). Within the boutons of an individual fiber, VD and shape were the most variable features (VD varied up to 3-fold). FMA and FAP were relatively constant. All characteristics were consistent through virtually all of the sections for a given bouton. These findings suggest a possible correlation between ultrastructure and physiological properties in this system. Sup. by NSO8239 (JPP), NS23725 (HRK) and NS16996 (LMM).

282.13

MORPHOMETRIC ANALYSIS OF DEVELOPING CAT PHRENIC MOTONEURONS LABELED BY INTRACELLULAR INJECTION OF HRP. He,F.*, J.S.Jodkowski*, R.D.Guthrie* and W.E.Cameron (SPON: J.Fernstrom), Depts. Neurobiology and Pediatrics, Univ. of Pittsburgh and Magee-Womens Hospital, Pittsburgh, PA 15213

A detailed description of the morphologic changes of phrenic motoneurons during postnatal development is prerequisite to our interpretation of the intrinsic electrical properties of these cells measured in physiological experiments. Phrenic motoneurons were impaled, identified by antidromic activation of thoracic phrenic nerve and filled with HRP. Spinal cord sections were reacted with DAB and neurons reconstructed at X100. Data derived for motoneurons of 1 and 2 month old kittens are compared to earlier data from the adult (Cameron et al, JCN 231:91,1985).

	1 month	2 month	Adult
Comb. stem dendrite diam. (um)	32.6	55.4	58.3
# terminal branches/dendrite	3.5	5.8	7.0
Length to dendrite termination (um)	569	754	1236
Total surf area of cell (um^2)	72,712	162,629	351,073
Somal/dendritic surf area (%)	5.4	3.7	2.6

The diameter of primary was positively correlated with combined dendritic length, dendritic surface area and volume for the motoneurons at one month. This relationship was not as strong in the 2 month old and adult animals; the diameter of the primary was only a fair predictor while the diameter of the second order dendrite provided a much stronger correlation. Supported by grants from the NIH (HD 22703) and American Lung Association.

282.14

ROLE OF INTRINSIC PROPERTIES IN DETERMINING SPONTANEOUS ACTIVITY OF CAT PHRENIC MOTONEURONS DURING POSTNATAL DEVELOPMENT. Cameron,W.E.,Jodkowski,J.S.*,He F.* and Guthrie,R.D.* Depts. of Neurobiology and Pediatrics, Univ. of Pittsburgh and Magee-Womens Hospital, Pittsburgh, PA 15213

The activity patterns and intrinsic membrane properties of phrenic motoneurons (PMs) were studied at 4 different postnatal ages (2,4,8,12 weeks). The cells were impaled then identified by antidromic activation of the thoracic phrenic nerve. All cells analyzed had membrane potentials less than -60mV and positive overshoots. There were no differences in the mean amplitude of the action potential/overshoot or the membrane potential among the age groups studied. PMs were classified as early (E) or late (L) recruited relative to the onset of phrenic nerve activity or quiescent (Q). Significant differences ($p<.05$) were found between the axonal conduction velocities of E and L cells in all groups except 2 wks and between L and Q for 8 and 12 wks. Mean discharge frequency was found to decrease with age from 31.6Hz (4wks) to 25.4Hz (8wks) to 16.5Hz (12wks) without any significant change in the duration of the afterhyperpolarization (AHP; 62.2, 59.9, 66.2ms, respectively). Differences were found between the mean duration of the AHP of E and L cells at all ages except 2wks while no differences were found for this same parameter between L and Q cells at any age. In all experiments in which 4 or more cells were fully characterized, the E cells could be segregated from L cells based on input resistance and rheobase current and the active cells (E and L) could be separated from Q cells based on axonal conduction velocity. The strongest predictor of recruitment order in the developing phrenic nucleus was input resistance. Supported by NIH grants HD22703 and HL34875.

282.15

A Probabilistic Description of Motoneuron Dendrites. W. B. Marks and R. E. Burke, Lab. of Neural Control, NINDS, NIH, Bethesda, MD 20892

We used morphological data from 43 dendrites belonging to four type-identified cat motoneurons (one type S, two FR, and one FF; Cullheim et al., *J. Comp. Neurol.* 255:68, 1987) in order to determine whether relatively simple probabilistic rules can be used to describe features of dendritic morphology. When branches ended in branch points (**parent branches; PAR**), the mean branch length was a monotonically decreasing function of the initial branch diameter, $\mathbf{d_0}$. In contrast, mean branch length of **terminating branches (TRM)** was a monotonically increasing function of $\mathbf{d_0}$. The probabilities of branching or terminating ($\mathbf{P_{br}}$ and $\mathbf{P_{trm}}$) were determined from these means for relatively short segments (Δlength = 25 μm). Surprisingly, $\mathbf{P_{br}}$ and $\mathbf{P_{trm}}$ were not correlated with order of branching or distance from the soma, except as these relate to local segment diameter, $\mathbf{d_i}$. The length distributions of **PAR** ($\mathbf{d_0}$ between 0.75 and 13.5 μm) and **TRM** ($\mathbf{d_0}$ between 0.25 and 2.75 μm) depended not only on $\mathbf{P_{br}}$ and $\mathbf{P_{trm}}$ but also on the rate of branch diameter taper ($\mathbf{R_{tpr}}$) during probabilistic growth. The best fits to date between the modeled and observed branch length distributions for all $\mathbf{d_0}$ have been obtained when $\mathbf{R_{tpr}}$ monotonically decreases with $\mathbf{d_i}$. In mature cat alpha-motoneurons, the lengths of dendritic branches can be described as relatively simple probabilistic functions of local dendritic diameter.

282.16

QUANTITATIVE MORPHOLOGY OF GAMMA MOTONEURONS IN THE CAT SPINAL CORD. A. K. Moschovakis, W. B. Marks and R. E. Burke. Lab. of Neural Control, NINDS, NIH, Bethesda, MD 20892.

We have examined the quantitative morphology of the dendritic trees of three triceps surae gamma-motoneurons after intracellular injection with horseradish peroxidase. In keeping with earlier studies, these cells had fewer and thinner dendrites, much less branching, and a greater proportion of unbranched dendrites, in comparison with alpha-motoneurons. However, the distances of dendritic terminations from the soma were comparable. Because of the more restricted branching, gamma-motoneuron dendrites occupied their perisomatic dendritic territory much more sparsely than alpha-motoneurons and the occupied territories were more restricted in three-dimensional space than found for alpha-motoneurons, particularly in the rostro-caudal direction. The distributions of individual dendritic branch lengths as a function of initial diameter showed the same basic pattern found in alpha-motoneuron dendrites (Marks and Burke, this meeting), suggesting that gamma-motoneuron dendrites can be described according to probabilistic rules that are fundamentally similar to those that apply to alpha-motoneurons.

282.17

MORPHOLOGY OF PRIMATE TRICEPS SURAE (TS) MOTONEURONS AND THEIR PRIMARY AFFERENT CONNECTIONS: INITIAL STUDIES. C.L. Lee, J.S. Carp, and J.R. Wolpaw, Wadsworth Laboratories, New York State Dept Health, Albany, NY, 12201.

Detailed morphologic data are not available for primate spinal motoneurons and their Ia-afferent connections. In preparation for seeking the anatomic basis of the spinal cord memory traces produced by operant conditioning of the H-reflex (J Neurophysiol 61:563-572, 1989; Wolpaw et al, this vol), we are labelling TS motoneurons and primary afferents in Macaca nemestrina with HRP.

Animals are deeply anesthetized throughout study. HRP is injected into identified TS motoneurons. HRP crystals are crushed onto appropriate dorsal rootlets 12-18 h before animals are sacrificed by overdose and perfused. Motoneurons are reconstructed by camera lucida.

Soma diameters of injected motoneurons range from 40 um to 61 um. Motoneurons have 7-13 primary dendrites. Dendritic trees are ellipsoid in shape. The largest extends at least 2100 um rostrocaudally, 680 um dorso-ventrally, and 400 um mediolaterally. Putative Ia synaptic boutons on these motoneurons range from 1.6 x 1.8 um to 3.0 x 7.2 um, and are located on somata as well as on proximal and distal dendrites.

Morphologic data from naive animals will be compared to data from conditioned animals to assess the spinal cord changes caused by H-reflex conditioning.

(Supported by NIH NS22189 & Paralyzed Veterans of America)

282.18

PHYSIOLOGIC PROPERTIES OF PRIMATE LUMBAR MOTONEURONS: INITIAL STUDIES. J.S. Carp, C.L. Lee, and J.R. Wolpaw. Wadsworth Laboratories, NYS Dept Health, Albany, NY 12201.

Operant conditioning causes side-to-side asymmetry in the H-reflex, the electrical analog of the monosynaptic spinal stretch reflex (J Neurophysiol 57:443-459, 1987). Conditioning changes the spinal cord itself, since reflex asymmetry is still present in the anesthetized animal after transection removes supraspinal influence (J Neurophysiol 61:563-572, 1989; Wolpaw et al, this vol). The most likely sites of change are the elements of the H-reflex pathway--the Ia afferent and the motoneuron.

In preparation for studying the spinal cord alterations produced by H-reflex conditioning, we are recording intracellularly from triceps surae (TS) motoneurons of anesthetized naive (i.e., unconditioned) Macaca nemestrina. In 48 TS motoneurons from the first five animals, axonal conduction velocities were comparable to human values and slower than those of the cat. Mean values and ranges for input resistance, resting potential, action potential amplitude and threshold, rheobase, afterhyperpolarization amplitude and duration, and composite EPSP amplitude were comparable to those previously described for cat and baboon.

Data from naive and conditioned animals will be compared to elucidate the sites and mechanisms of the spinal cord changes produced by H-reflex conditioning.

(Supported by NIH NS22189 & Paralyzed Veterans of America)

282.19

COMPUTER GENERATION OF ISOPOTENTIAL CONTOUR MAPS. G.R. Detillieux[1]*, L.M. Jordan[2], and D.J. Kriellaars[2]*. [1]INFO WEST Inc., Box 130, St. Boniface, Winnipeg, Canada, R2H 3B4; and [2]Dept. Physiol., Univ. Manitoba, Winnipeg, Canada, R3E 0W3.

The various steps and algorithms involved in generating isopotential contour maps on a computer are presented. This covers the data collection techniques, and the major components to the contour mapping program: 1) a two-dimensional spline interpolation algorithm used to calculate a smooth surface over a matrix of measurements of limited resolution, 2) the contour scanning and contour following algorithms which locate and trace out the contour lines on the calculated surface, and 3) the coordinate transformations used to position the generated map over a traced diagram.

Contour maps have various uses in science, such as isopotential mapping of voltage fields, isobar mapping of pressure fields, isotherm mapping of temperature, radiography, etc. A contour map can also be sued for mapping source-sink distributions. Any two-dimensional matrix of measurements can be viewed as a contour map. Examples of isopotential, source-sink and isobar contour maps are presented.

Supported by the Medical Research Council of Canada and the Health Sciences Centre Reearch Foundation, Winnipeg, Canada.

MENTAL ILLNESS: SCHIZOPHRENIA

283.1

LAMINA SPECIFIC CLONIDINE BINDING IN HUMAN CORTEX Grant N. Ko, M.D.; ; V. Haroutunian Ph.D.; T.B. Horvath M.D.; S. Lim, M.D.; E Scala; K.L. Davis, .MD. Dep. of Psychiatry Mt. Sinai School of Medicine/Bronx VAMC, New York, NY

Norepinephrine (NE) dysfunction has been implicated in the pathophysiology of schizophrenia. For instance, elevated CSF NE as well as increased NE in limbic brain regions post mortem have been reported. The purpose of the present study was to compare α_2 adrenergic receptors in the frontal cortex of postmortem brains from patients with schizophrenia and control subjects. Unfixed frontal cortex material from Brodmann's Area 9 was obtained, cut and mounted on brass microtome chucks. Coronal cryostat sections of 10μ thickness were thaw mounted on subbed microscope slides and allowed to air dry at room temperature before being stored. (^{3}H) para-aminoclonidine (PAC) was used to label α_2 adrenergic binding sites in the mounted tissue sections, prior to exposing tritium sensitive film for 6-8 weeks. Cresyl Violet was used to stain cells in alternate serial sections. At 1.0 nM PAC, lamina I binding=610 nC/g. wet weight of tissue; lamina III=930 nC/g.; lamina VI=390nC/g. (ANOVA $F=13, p=0.0001$). Schizophrenic binding and controls did not differ, suggesting that these cortical receptors are not involved in the pathogenesis of schizophrenia.

This work was supported by the Veteran Administration.

283.2

THE COMPARATIVE BEHAVIORAL EFFECTS OF THE D1 AGONIST SKF38393 AND THE D2 AGONIST QUINPIROLE IN SELECTED MEMBERS OF PRIMATE SOCIAL COLONIES. **R.F. Schlemmer, Jr., D.J. McGinness* & J.M. Davis. University of Illinois at Chicago and Illinois State Psychiatric Institute, Chicago, IL 60612.**

This study sought to evaluate the role of D1 & D2 subtypes of dopamine (DA) receptors in DA agonist-induced behavior in monkeys using acute doses of the D1 agonist SKF38393 (SKF) & the D2 agonist quinpirole (QUIN). Two stable adult Stumptail macaque social colonies each composed of 1 male & 4 females who were continually housed in a large group cage served as subjects. Following determination of baseline behavior, each of the 10 monkeys received 5 acute doses of SKF (0.3-10 mg/kg) & QUIN (1-10 mg/kg) i.m. in random order. Only 1 monkey received drug treatment/day & at least 6 days elapsed between each drug treatment. A 1 hr observation session was conducted daily by a "blind" observer beginning 15 min after drug or saline administration. QUIN induced behavioral changes which were qualitatively similar to those induced by amphetamine & apomorphine including increased scratching, submissive gestures, checking, stereotypy & distancing from other monkeys, & decreased initiated social grooming, self grooming, locomotion & feeding. SKF failed to induce similar dramatic changes, but decreased social & self grooming, locomotion, & increased huddling. These results suggest that the behavioral changes induced in primates by dopamine agonists such as apomorphine & amphetamine are predominantly mediated by D2 receptors which is in agreement with similar studies in most other species. Importantly, behaviors modelling human psychotic behavior such as increases in scratching, submissiveness & social withdrawal appear to by mediated by D2 receptors since SKF did not significantly affect these behaviors. (Supported by the Campus Research Board, University of Illinois at Chicago.)

283.3

NEUROLEPTICS HAVE TWO MODAL EFFECT ON SKIN CONDUCTANCE RESPONDING IN SCHIZOPHRENIC PATIENTS. S.Alfisi*, M.Mintz and M.Sigal* (SPON: H.Frenk). Psychobiol. Unit, Tel-Aviv Univ. Ramat-Aviv, 69967 and Pardesia Mental Health Hosp., Israel.

To test whether neuroleptic treatment affects the skin conductance response (SCR) to loud tones, chronic schizophrenic patients with and without drug-induced abnormal involuntary movements were tested before and after drug withdrawal. Patients demonstrated a high rate of SCR on both sessions. The daily neuroleptic dose correlated negatively with SCR, suggesting that the immediate effect of neuroleptics is to inhibit the autonomic nervous system (ANS) reactivity. Only patients with tardive dyskinesia (TD) showed habituation of SCR while on treatment and facilitation of reactivity after short drugs withdrawal, indicating that TD pathophysiology is associated with development of brain susceptibility to the attenuating effect of neuroleptics.

These findings confirm that alterations of ANS reactivity, considered by some as a potential markers of schizophrenia, might in fact reflect an iatrogenic side effect due to both acute and chronic effects of neuroleptic treatment.

283.4

AMPHETAMINE PSYCHOSIS AS A MODEL OF SCHIZOPHRENIA IN THE RAT: NEUROTRANSMITTER RELEASE FROM BRAIN SLICES. Pirjo Kontro, S.M. Lillrank*, T. Seppälä* and S.S. Oja. Tampere Brain Research Center, Department of Biomedical Sciences, University of Tampere and National Public Health Institute, Helsinki, Finland.

In addition to dysfunction of dopaminergic neurons also hypoactivity of glutamatergic tracts has been proposed as a primary cause for schizophrenia. As an animal model for schizophrenia, adult rats were exposed to amphetamine sulphate for 7 days using implanted osmotic minipumps (Alzet) (24 mg/kg/day). The rats exhibited loss of appetite and hyperkinesia. The potassium-stimulated (50 mM) and resting release of preloaded labeled dopamine, GABA and D-aspartate (a nonmetabolized analogue of glutamate) was characterized in superfused striatal and frontal cortical slices. The levels of amphetamine in the brain and blood serum were analyzed by gas chromatography. The potassium-stimulated release of dopamine from striatal and cortical slices was attenuated in the amphetamine-treated rats. The release of D-aspartate was slightly increased in the striatum. Potassium-evoked GABA release was potentiated in the frontal cortex more than in the striatum. When brain slices were exposed to amphetamine in vitro the resting and potassium-stimulated release of dopamine was considerably enhanced but not the release of D-aspartate. Nor did amphetamine stimulate the release of D-aspartate in the treated rats.

283.5

THE PRESENCE OF γ-ENDORPHIN IN EBSTEIN BARR VIRUS (EBV)-TRANSFORMED LYMPHOCYTES DERIVED FROM HEALTHY DONORS AND SCHIZOPHRENIC PATIENTS. M. Diamant*, C.J. Heijnen*, V.M. Wiegant*, D. de Wied* (SPON: M.F. Von Meyenfeldt). Rudolf Magnus Institute, Medical Faculty, State-univ. of Utrecht, 3521 GD Utrecht, The Netherlands.

Processing of the pro-opiomelanocortin (POMC)-derived peptide β-endorphin (βE) leads to the generation of γ-endorphin (γE) and its non-opioid fragments des-TYR^1-γE (DTγE) and des-ENK-γE. These γ-type endorphins have been demonstrated to possess neuroleptic-like activity in behavioural tests in rats. The observed beneficial effects of DTγE-treatment in schizophrenics and the deviant concentrations of γE found in post mortem hypothalamic tissue obtained from schizophrenic patients point towards a possible role for γE in the pathogenesis of schizophrenia. Recent demonstration of POMC-peptide production by cells of the immune system has provided a tool for studying endorphin production and metabolism in lymphocytes derived from schizophrenic patients versus controls. Peripheral blood lymphocytes were immortalized by in vitro transformation with EBV and cultured to yield up to 1400.10^6 cells. βE- and γE-immunoreactivity was demonstrated in EBV-cell homogenates (350 - 450pg and 3 - 6pg/10^7 cells respectively). Currently, γE-isolation from EBV-cell homogenates by specific affinity chromatography is performed. Immunoreactivity versus bioactivity of the POMC-peptides will be discussed.

283.6

NEW ANTIBODIES TO STUDY SCHIZOPHRENIA

W.G. Honer*, C.A. Kaufmann, J.E.Kleinman, M.F. Casanova*, P.Davies* (SPON: C.S. Raine), Dept. of Pathology, Albert Einstein College of Medicine, Bronx, NY 10461

With an approach similar to that used to develop the antibody Alz-50 for the investigation of Alzheimer's disease (Science 1986; 232:648) we have developed new antibodies for the study of schizophrenia. Five limbic regions of schizophrenia brain were used for immunization. Twelve antibodies were selected from 7601 hybrids screened. Enzyme-linked immunoadsorbent assay comparison of homogenates from four cases of schizophrenia and four controls revealed differences in reactivity from two- to eightfold. Three antibodies were more reactive with specific regions in the schizophrenia brains, four were more reactive with regions in the control brains, the relative differences in reactivity for the final five antibodies depended on the region tested. Preliminary studies using antibody EP10 were of particular interest. Immunocytochemistry revealed staining essentially confined to gray matter. In the cerebellum, the molecular layer was stained diffusely, the Purkinje cell layer was essentially devoid of reactivity, and the granule cell layer exhibited focal areas of staining. Other antibodies appear to be reactive with neuronal cell processes, white matter elements and astrocytes.

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283.7

FIBROBLASTS: NEUROPATHOLOGICAL MODEL FOR SCHIZOPHRENIA. H. Laev, R. Reddy*, S. Mukherjee* and S. Mahadik. NYS Psychiatric Inst., and Dept. of Psychiatry, College of P. & S. Columbia Univ., New York.

Fibroblast cultures are useful as cellular models for studying some of the molecular mechanisms underlying the pathophysiology of CNS diseases, and for determining the genetic linkage of certain inheritable diseases. In schizophrenia, neuropathological changes have been detected in CNS. These neuropathologies may be associated with one or more genetic abnormalities.

Fibroblast cultures have been established from skin biopsies of 10 schizophrenic patients and 12 age-matched normal subjects. Fibroblast cultures from these patients showed differences on the following growth parameters. Initial growth: fibroblasts from schizophrenic patients took considerably longer to establish that those from controls. Established cultures were obtained from most normals at <1 month as compared to 2-4 months for schizophrenic patients. Rate of growth: the doubling time for fibroblasts from schizophrenics was markedly longer than that of normals. Morphological differences: fibroblasts from normals showed typical uniform, long, spindle-like appearance and unidirectional orientation; fibroblasts from schizophrenics exhibited random sizes (shorter and flatter) and orientation. These differences probably reflect 1) common cellular pathophysiological changes associated with the disease process, or 2) result from the treatment of the disease.

283.8

DECREASED ANTICONVULSANT RESPONSE TO ELECTROCONVULSIVE SEIZURES IN SCHIZOPHRENIC AND SCHIZOAFFECTIVE PATIENTS: A PHYSIOLOGICAL FEATURE THAT CORRELATES WITH SCHIZOPHRENIFORM SYMPTOMS. A.L. Politoff* and H.S. Lee. LIJMC, New Hyde Park, NY 11042.

Normal experimental animals and depressive illness patients are known to respond to electroconvulsive treatment (ECT) with an anticonvulsant response, which consists of shortened seizure duration (SD) and raised seizure threshold (electrical energy required for eliciting a seizure). The anticonvulsant responses of 11 schizophrenics, 7 schizoaffective disorder patients and 38 depressed illness patients were analyzed retrospectively. At the onset of ECT, SD and stimulus energy were similar in all diagnostic groups; with time, there was simultaneous decrease in SD and increase in stimulus energy in depressed illness patients, independently of medication. In contrast, SD and stimulus energy remained unchanged troughout ECT duration in schizophrenic and schizoaffective disorder patients, with or without medication. Thus, the central nervous system of schizophrenic and schizoaffective disorder patients seems to be deficient in the adaptive mechanism(s) necessary for developing an anticonvulsant response.

283.9

GLIAL PROTEINS IN SCHIZOPHRENIA. C.N. Karson, W.S.T. Griffin, M. Cassanova* & J.E. Kleinman, Depts. of Psychiatry/Behavioral Sci. Pediatrics & Anatomy, UAMS & J. L. McClellan VAMC, Little Rock AR., 72205 & Lab. of Clinical Brain Studies, NIMH, Wash. D.C., 20857.

We are examining gliosis in schizophrenia in postmortem brain tissue from regions of cortex, cerebellum, rostral brain stem and diencephalon using Western blot to determine relative levels of glial proteins (glial fibrillary acidic protein (GFAP) and interleukin-1 (IL-1)). Initial negative results from frontal lobe (below) suggest this approach is practical and may ultimately answer whether gliosis (neurodegeneration) occurs in this disorder.

GLIAL PROTEINS IN FRONTAL LOBE

DIAGNOSIS	N	AGE	GFAP*	TOTAL IL-1
CONTROLS	5	39±16	0.11±0.04	0.52±0.05
SCHIZ	7	34±10	0.10±0.04	0.52±0.07

* area integrated densitometric values

283.10

THE MORPHOMETRY OF THE CORPUS CALLOSUM IN SCHIZOPHRENIA. M.F. Casanova*, M. Zito*, E.F. Torrey*, T. Goldberg*, L. Bigelow*, R. Sanders*, D.R. Weinberger and J.E. Kleinman. (SPON: P. Oliver) CBDB, NIMH Neurosciences Center at St. Elizabeths, Washington, D.C. 20032.

It has been suggested that faulty interhemispheric communication underlies some of the symptoms observed in schizophrenic (SC) patients. Since the corpus callosum (CC) is the major conduit for corticocortical fibers, this structure has been the focus of several morphometric studies in SC patients. Unfortunately, it has been difficult to replicate many of the reported findings and the role of the CC in SC still remains controversial. In an attempt to improve the reliability of morphometric measurements of the CC we used a computer image analysis system to quantitatively examine the shape, area, thickness and length of the CC in MRI scans in 12 pairs of monozygotic twins discordant for SC. The study failed to replicate previous findings, but partially supported the notion of disease related shape differences in the middle segment of the CC. In a follow up study the mean curvature of the middle segment of the CC in the same MRI scans was measured to define the basis of this structural distortion. The results indicated that the CC of SC patients have an increased upward convexity. In order to determine whether the structural distortion was the result of a primary histological lesion involving the CC, axonal counts in postmortem tissue were performed in the CC of 11 SC and 13 age matched controls. Independent t-tests (SC vs controls) were non-significant at the 0.01 level for the anterior, middle and posterior sites. Since hydrocephalus is associated with an upward bowing of the CC, it seems possible that the conformational disturbance observed in our SC patients is secondary to ventriculomegaly and not the result of an intrinsic CC lesion.

283.11

AN ANIMAL MODEL OF SCHIZOPHRENIA: EFFECTS OF HIPPOCAMPAL CELLULAR DAMAGE. K.S. Seybold*, J.L. Stotler*, P.W. Parsons*, and R.L. Port. Depts of Psychology, Grove City College, Grove City, PA 16127, and Slippery Rock University, Slippery Rock, PA, 16057.

Recent evidence suggests that neuropathy within the hippocampal formation may provide an anatomical substrate for schizophrenia (Conrad & Scheibel, Schizo. Bull. 13, 1987). In the present experiment, slight pyramidal cell loss was induced in rats via intraventricular injection (1 ul) of kainic acid (0.5 and 1.5 nM). Animals were tested in shuttlebox avoidance for sixty trials over two days. The results showed a facilitation of acquisition in damaged animals that is consistent with reports of accelerated simple conditioning in schizophrenics and dissimilar to the deleterious effects which often accompany disruption of hippocampal activity. Performance of the low-dosage group was most similar to the control group early in training and resembled the high-dosage group later in training. These effects are suggestive of an interaction between the compromised hippocampus and the induction of long-term potentiation over training.

283.12

100ms AUDITORY RESPONSES IN PATIENTS WITH SCHIZOPHRENIA AND NORMAL CONTROLS USING MAGNETOENCEPHALOGRAPHY. A.Reeve, D.F.Rose, S.Sato, D.R.Weinberger. NIMH and NINDS, 9000 Rockville Pike, Bethesda, MD. 20892.

Anatomical studies have shown decreased temporal lobe volume and loss of cortical cells of temporal lobe in patients with chronic schizophrenia. We studied a physiologic measure of temporal lobe function in chronic schizophrenics on neuroleptic medication and controls. Three patients (2 male, 1 female, ages 30-40) and four controls (2 male, 2 female, ages 24-50) were administered tone bursts (1000Hz, 20ms plateau, 2ms rise/fall, 65dB SL, 0.25/s, 100 epochs, from Nicolet CA-1000) to each ear unilaterally. All subjects were alert; state of arousal was monitored with EEG. The 100ms magnetic field response (N100m) was recorded contralateral to the stimulated ear with a 7-channel magnetometer in a shielded room (Biomagnetic Technologies,inc.). Simultaneous EEG (10-20 system to balanced non-cephalic reference) recording of N100 permitted verification of the presence of N100m and its reproducibility over time. Locations of equivalent current dipoles varied without a consistent pattern between or within groups. Using MRI, the N100m localized to the temporal lobe in right and left hemispheres in all subjects.

These results suggest that previous reports of interhemispheric differences in dipole locations may reflect differences in anatomical substrate, rather than in processing of auditory information in either hemisphere.

283.13

SCHIZOPHRENIA: LINKAGE ANALYSIS WITH CHROMOSOME 5 MARKERS. K.L. Newell[1]*, E.I. Ginns[2]*, J. Schreiber[2]*, R. Long[2]*, J.R. Kelsoe[2], T. Manschreck[3]*, C.N. Pato[2]*, D. Pickar[2]*, S.M. Paul[2]*. [1]Howard Hughes Medical Institute-NIH; [2]Clinical Neuroscience Branch, National Institute of Mental Health, Bethesda, MD 20892; [3]Massachusetts General Hospital, Boston, MA.

Schizophrenia is a psychiatric disorder affecting approximately 1% of the human population. Family and adoption studies of this disorder have suggested that both environmental and genetic factors may be involved in its etiology. The initial report of a partial trisomy of the 5q11.2-13.3 region in association with schizophrenia in an Asian family (Bassett, A.S. et al., Lancet 1:799-801, 1988) prompted further study using DNA probes at this site resulting in the discovery of linkage between a disease locus and this region in seven Icelandic and British families (Sherrington, R. et al., Nature 336:165-67, 1988). We have utilized three chromosome 5 probes from the region 5q11-13 (D5S76, D5S39 and D5S78) to analyze genomic DNA for co-segregation of schizophrenia and these marker loci in a large North American pedigree exhibiting a high incidence of chronic psychosis and affective disorder. Preliminary results do not support linkage between schizophrenia and the 5q11-13 probes used in this investigation. These data further suggest the presence of genetic heterogeneity in schizophrenia.

283.14

PREFRONTAL CORTICAL BLOOD FLOW IN MONOZYGOTIC TWINS CONCORDANT AND DISCORDANT FOR SCHIZOPHRENIA. K.F. Berman, E. Fuller Torrey*, D.G. Daniel* and D.R. Weinberger. CBDB, NIMH Neurosciences Center at St. Elizabeths, Washington, D.C. 20032.

Several converging lines of evidence suggest abnormal prefrontal cortical function in schizophrenia (SCH). However, nothing is known about the etiology of such a deficit in this illness. To explore one of the proposed major etiologies, that of genetic determination, we used the Xe133 inhalation technique to measure regional cortical blood flow (rCBF) in 10 pairs of monozygotic twins discordant for SCH as well as in 8 pairs of monozygotic twins concordant for SCH. Each subject completed 3 rCBF measurements during 3 different cognitive conditions: first during the resting state and then during 2 tasks: an automated version of the Wisconsin Card Sort (WCS), which is specifically linked to prefrontal cortex; and a simple numbers matching task which controlled for non-prefrontal aspects of the procedure. Within each discordant twin pair the twin with SCH had lower relative prefrontal flow during the WCS (for well twins mean(±SEM) = 111±2, for ill twins mean = 104±2, matched pairs T = 5.6, p = .0004). The mean relative prefrontal flow during the WCS for the twins concordant for SCH (105±2) did not differ from that of the SCH discordant twins. Within 6 of the 8 concordant pairs, the twin who had more lifetime neuroleptic treatment had the higher relative prefrontal flow, suggesting that neuroleptic treatment cannot explain prefrontal dysfunction in SCH. These data reconfirm a pathophysiological defect in prefrontal function in patients in SCH, even when compared to genetically identical individuals without schizophrenia. While these results do not lend strong support for genetic control of "hypofrontality," they suggest that neuroleptic treatment does not account for prefrontal dysfunction in SCH.

284.1

REDUCTION OF PARVALBUMIN-IMMUNOREACTIVITY IN THE SUBCORTICALLY DENERVATED HIPPOCAMPUS. L.S. CHEN, T.F. FREUD, G. BUZSAKI, K.D. BAIMBRIDGE AND F.H. GAGE. Dept. of Neuroscience, UC San Diego, CA92093.

Removal of subcortical and commissural inputs to hippocampus (HP) by fimbria-fornix (FF) lesions permanently produces large amplitude EEG spikes and decreases threshold to stimulus-induced seizure (Neuroscience 28:527-538,1989). In this study, we examined parvalbumin (PV, a calcium binding protein specific to GABA neurons) immunoreactivity to test the hypothesis that hyperexcitability of the subcortically denervated HP results from a decrease in GABA-mediated inhibition.

Two months after FF lesions, female Sprague-Dawley rats (n=7) were perfused and coronal sections were taken from dorsal HP for PV immunohistochemistry. Age-matched, non-lesioned rats (n=7) were served as control. The cell density (number of cells/area) of PV-immunoreactive cell (PV-IR) was the parameter used for comparison.

In normal rats, a gradient of the cell density of PV-IR was observed in the dorsal HP. The cell density in the anterior 1/3 was significantly greater than that of the posterior 2/3 in the stratum granulosum (DG), hilus (H) and CA3c regions by 75%, 75% and 50% respectively ($P<0.01$). In rats with FF lesions, a reduction in the cell density was demonstrated in the DG (20%, $P<0.05$), H (40%, $P<0.01$) and CA3c (50%, $P<0.01$) in the anterior region. While in the posterior region, the reduction was found only in the H (30%, $P<0.05$).

We propose that a decrease of calcium buffering capacity of interneurons consequent to the reduction of PV expression may be causally related to the hyperexcitability of the denervated hippocampus.

284.2

THE SUBCORTICALLY DEAFFERENTED HIPPOCAMPUS: EPILEPTIC PATTERNS AND PLASTIC PROPERTIES. Z. Horvath*, S. Berger*, M. Hsu*, F. H. Gage and G. Buzsaki. (SPON: R. Livingston) Dept. of Neurosciences, UCSD, La Jolla, CA 92093

Spontaneous and evoked electrical activity was studied in the subcortically denervated (fimbria-fornix; FF) hippocampus in rats. Within one week after FF surgery short-duration, large amplidude (up to 12 mV) interictals spikes (IIS) emerged and persisted for up to 10 mo. Simultaneous microelectrode recordings along the longitudinal axis of the hippocampus revealed that IIS invaded the entire hippocampus simultaneously (<5 ms) or slowly (<0.5 m/s) by recruiting new sets of neurons successively. IIS occurred independently in the two hemispheres, but occasionally IIS could spread to the contralateral hippocampus via the retro-hippocampal commissural system. IIS occurred more frequently during immobility and slow wave sleep than during movement. Very large IIS were frequently followed by startle reactions, indicating that limbic activity could invade motor structures. In contrast to intact rats large amplitude, multiple population spikes were recorded in the dentate gyrus and CA1 region in response to stimulation of the perforant path (PP). IIS-triggered evoked responses were several times greater than responses elicited between the IIS. High frequency stimulation of PP increased the incidence of IIS for several hours and the morphology of the IIS became similar to the stimulus-evoked responses. These finding indicate that a) the deafferented hippocampus is hyperexcitable, b) the excitability of the hippocampal network is increased substantially during the IIS and c) tetanic stimulation of PP produces long-term changes of the spontaneous excitability of the hippocampal circuitry.

284.3

LOCAL CIRCUIT INTERACTIONS IN THE CA1 REGION OF HYPER-EXCITABLE KAINATE-LESIONED RAT HIPPOCAMPUS. S. Nakajima*, J.E. Franck and P.A. Schwartzkroin. Dept. of Neurological Surgery, Univ. of Washington, Seattle, WA 98195.

Intraventricular (icv) injection of kainic acid (KA), with loss of hippocampal CA3 pyramidal cells (PCs), produces a model of temporal lobe epilepsy. Hyperexcitability in remaining CA1 PCs is associated with a loss of IPSPs and possible recurrent excitation. Despite the functional loss of inhibition, the purported inhibitory interneurons remain viable. The present study evaluates local circuit interactions in this hyperexcitable CA1 in order to determine the cause of IPSP failure and identify excitatory collateralization.

Paired intracellular recordings were obtained *in vitro* from physiologically identified interneurons and PCs 2-4 weeks following bilateral icv KA (0.6 μg/1 μl saline/side). Recorded slices had an abnormal population burst response to stimulation of stratum radiatum. No synaptic interactions were detected in simultaneous recordings from 33 pairs of PCs. Five of six PC/basket cell pairs (interneurons at the pyramidale/oriens border) and two PC/OA interneuron pairs (interneurons at the oriens/alveus border) also showed no interactions. In one pair, PC discharge evoked EPSPs in a basket cell; the PC was unaffected by interneuron discharge.

These data are consistent with reports that there are few, if any, excitatory synaptic connections among CA1 PCs. The development of such connections does not appear to underlie KA-induced CA1 hyperexcitability. In normal hippocampus, previous data indicate that 30% of CA1 PCs show inhibitory PSPs to basket cell activation. Our failure to detect any such connections in KA-lesioned hyperexcitable tissue suggests a functional disconnection at the interneuron to PC synapse.

Supported by NIH, NINDS grants NS 15317, NS 20482, and NS 25155, and a grant from the Shimamura Fund.

284.4

THE SEIZURE-ASSOCIATED LOSS OF INHIBITION IN THE RAT HIPPOCAMPUS; INHIBITORY CELLS SURVIVE AND ARE FUNCTIONAL IN VIVO. R.S. Sloviter, Neurol. Res. Ctr., Helen Hayes Hosp., W. Haverstraw, NY 10993

We hypothesized that the seizure-associated loss of inhibition in the hippocampus was not due to inhibitory cell loss or dysfunction but, rather, to the loss of cells that excite basket cells (Sloviter, R.S., Science, 235:73-76, 1987). This "dormant basket cell" hypothesis implies that inhibition might be restored if the basket cells could be activated. Weeks after perforant path stimulation (PPS) that produced dentate and CA3 damage, CA1 pyramidal cells responded abnormally to ipsilateral PPS (0.1-3Hz) with large amplitude multiple spikes. Twin pulse inhibition (40ms) was decreased in CA1 despite no apparent CA1 damage. Contralateral PPS restored CA1 pyramidal cell inhibition immediately and completely (n=20). We suggest that inhibition in CA1 is decreased as a result of CA3 damage that decreases feedforward activation of ipsilateral CA1 basket cells. Contralateral stimulation activates the same basket cells via the commissural projection from CA3 to CA1. Thus, the hypothesis that the loss of inhibition **in vivo** is due to the loss of excitatory input to surviving basket cells, rather than to basket cell loss or malfunction, is confirmed.

284.5

DO CHANGES IN SYNAPTIC EXCITATION OR INHIBITION UNDERLIE GENERATION OF LONG-LASTING, BURSTING-INDUCED LATE EPSPs IN PIRIFORM CORTEX? W.H. Hoffman and L.B. Haberly (SPON: J. Hind) Neurosci. Training Prog. and Dept. of Anatomy, Univ. of Wisconsin, Madison WI 53706.

Stimulation of afferent or association fibers in piriform cortex slices in Mg free bathing medium evokes burst responses which lead, upon return to normal Mg concentrations, to the appearance of long-latency, all-or-none EPSPs that persist for the duration of experiments (Hoffman & Haberly, J. Neurosci. 9:206). Evidence has also been presented that these late EPSPs are initiated in layer III of piriform cortex and propagate synaptically to pyramidal cells in layer II (Hoffman & Haberly, Soc. Neurosci. Abs. 14:278). The present experiments were designed to test the hypothesis that late EPSPs in deep cells result from reverberating positive feedback following a disruption in the balance between excitatory and inhibitory processes as postulated for the hippocampus (Traub et al, J. Neurophysiol. 58:739). Possible sources of such a disruption include potentiation of EPSPs mediated by intrinsic association fibers or decreases in the early Cl mediated or late K mediated IPSPs.

Measurements of rise times of monosynaptic EPSPs evoked by stimulation of association fibers revealed an enhancement in only 1 of 6 layer III cells following induction of late EPSPs, but this increase disappeared within 50 min despite the continued presence of late EPSPs. Blockage of the K mediated IPSP by phaclofen (a specific $GABA_B$ receptor antagonist) failed to induce late EPSPs (n=6), suggesting that any reduction in the late IPSP plays a minor role. To determine if reduction in Cl IPSPs underlies late all-or-none EPSP generation, bicuculline was applied over a range of concentrations (1.25-10 μM) (n=9). Substantial reduction in Cl IPSP amplitudes did not directly lead to the appearance of late EPSPs. Late EPSPs only appeared after onset of bursting evoked by the accentuated early EPSP. Moreover, comparison of Cl mediated IPSPs before and after induction of late EPSP by 0 Mg revealed an average increase in amplitude of 16% in layer II cells (n=5) and 32% in layer III cells (n=4). These results suggest that changes in known synaptic processes are not responsible for the generation of late EPSPs. It is postulated that enhancement of slowly developing synaptic or endogenous currents underlies development of the observed late EPSPs. Such slow excitatory activity could occur while the Cl IPSP is refractory following activation by the early EPSP and lead to all-or-none EPSPs via positive feedback. Supported by grant NS 19865 to LBH.

284.6

MULTIPLE POPULATION SPIKES IN THE DENTATE GYRUS TO HILAR STIMULATION AFTER BLOCKADE OF $GABA_A$ RECEPTORS. J. CRONIN[1] and F. E. DUDEK[2]. [1]Psychology Dept., Tulane Univ., New Orleans, LA 70118 [2]Mental Retardation Research Center, UCLA Med. Sch., Los Angeles, CA 90024.

The hippocampal mossy fibers of the kainate-treated rat sprout collaterals that project to the granule cell dendrites. Hilar stimulation, which normally evokes a single population spike, could trigger multiple spikes in slices from kainate-treated rats; this suggested that the collaterals had formed a functional pathway (Tauck & Nadler, J. Neurosci. 5:1016, 1985). To evaluate hilar stimulation as a test for new recurrent excitatory circuits, we examined this response in the normal dentate gyrus and under conditions of increased excitability.

After bath application of picrotoxin (50-100 μM, 5 of 5 slices) or bicuculline (100 μM, 5 of 9 slices), hilar stimulation evoked 2 to 8 population spikes. Intracellular recordings revealed stimulus-evoked EPSPs. Raising extracellular $[K^+]$ from 3 mM to 8 mM, or use of Mg-free medium, caused 2 to 4 additional spikes per stimulus. In one slice that did not respond to bicuculline, raising extracellular $[K^+]$ led to multiple spikes from hilar stimulation.

We conclude that hilar stimulation can evoke EPSPs and multiple population spikes from granule cells. Since the response recorded in the normal dentate gyrus from hilar stimulation can have an orthodromic component, multiple population spikes do not necessarily indicate the formation of a new recurrent excitatory pathway.
Supported by NS16683.

284.7

THE EFFECTS OF OSMOLALITY ON SYNCHRONOUS BURSTING IN THE ABSENCE OF CHEMICAL SYNAPTIC TRANSMISSION IN HIPPOCAMPAL SLICES. J.G. Tasker and F.E. Dudek, Mental Retardation Research Center, UCLA School of Medicine, Los Angeles, CA 90024.

Numerous studies using low-[Ca^{2+}] solutions have indicated that non-synaptic mechanisms can synchronize electrical activity in the hippocampus. We examined the effects of altered extracellular osmolality on CA1 population responses after blocking chemical synaptic transmission in slices of rat hippocampus. Synaptic responses to single and repetitive electrical stimuli were completely blocked in solutions in which Ca^{2+} was replaced with EGTA (1-2 mM) and kynurenate (3 mM). Bursts of population spikes and/or negative shifts were induced in CA1 when [K^+] was raised to 5 mM. When negative shifts occurred without population spikes, reduction of the extracellular osmolality by adding water (5-20%) or lowering NaCl (10-20 mM) caused bursts of population spikes. When bursts occurred in solutions of normal or lowered osmolality, addition of mannitol or sucrose (+5-40 mOsm/kg), which are membrane impermeant, dramatically reduced the bursts. Addition of glycerol (+5-40 mOsm/kg), which is membrane permeant, had little or no effect. The effects of mannitol and sucrose could be reversed by diluting the medium (i.e., decreasing osmolality). All effects of changing osmolality were at least partially reversible. Thus cellular swelling in dilute media, and the resultant reduction of the extracellular space, enhance neuronal synchrony, even in the absence of chemical synaptic transmission. Similarly, cell shrinkage from increased extracellular osmolality reduces synchrony. These data strongly support the hypothesis that electrical field effects and/or changes in extracellular [K^+] play an important role in the synchronization of hippocampal neurons. Supported by AFOSR 87-0361.

284.8

FUNCTIONAL RE-ORGANIZATION OF THE PERIRHINAL-PYRIFORM BURST RESPONSE BY PROLONGED EXPOSURE TO O-Mg++:SUPPORT FOR AN INVITRO MODEL OF KINDLING. J.R.Plant* & D.C. McIntyre. Dept. of Psychology, Carleton Univ., Ottawa, Canada K1S 5B6.

It has been previously demonstrated that the Perirhinal (PRc) & Pyriform (Pc) cortices are capable of O-Mg++ induced discharges INVITRO, & exhibit a functional relation ship determined by the history of the donor rat. In slices from unstimulated controls, onset & development of O-Mg++ induced discharges originated exclusively from the PRc & led those in the Pc. In contrast, discharges in slices from amygdala kindled rats originated in the Pc & led those in the PRc, suggesting a kindling-induced re-organization of the relationship between the PRc & Pc, & a long-term increase in Pc excitability. In the present study we investigated the effects of prolonged exposure of control slices to O-Mg++ perfusate. Under Mg++ perfusate only 6% of all discharges originated in the Pc. In contrast, after aprox. 4 hours under O-Mg++ perfusate, 82% of all discharges originated in the Pc & led those in the PRc. When returned to Mg++ perfusate for a minimum of 1 hr, 100% of all discharges now originated in the Pc. Results of the study demonstrate that prolonged exposure of control slices to O-Mg++ perfusate results in an increase of discharges originating in the Pc & a re-organization of the relationship between the PRc & Pc which is identical to that seen in slices taken from electrically kindled rats.

284.9

MAGNESIUM REMOVAL INDUCES HYPERACTIVITY AND NEURONAL DEGENERATION IN VITRO. K. Rose*, C. W. Christine and D.W. Choi (SPON: E.S. Tecoma). Dept. of Neurology, Stanford Univ. Med. Sch., Stanford CA 94305.

Removal of extracellular Mg^{++} has been shown to induce epileptiform activity in hippocampal slices, and was recently observed by R.J. Miller and colleagues to trigger NMDA receptor-mediated neuronal degeneration in hippocampal cell cultures (personal communication). We report here that removal of extracellular Mg^{++} for 24-72 hr produced extensive neuronal degeneration in murine cortical cell cultures. The glial layer was not damaged. This selective neuronal injury could be substantially attenuated by addition of either an NMDA antagonist (e.g., 50uM dextrorphan) or 3uM tetrodotoxin, to the bathing medium. Whole cell voltage clamp recording revealed that neurons perfused with Mg^{++}-free extracellular medium fired at substantially higher rates than when perfused with the same medium containing 1 mM Mg^{++}. Dextrorphan was also effective in quelling this neuronal hyperactivity induced by Mg^{++} removal.

These observations support the idea that Mg^{++} removal is sufficient to trigger NMDA receptor-mediated neuronal degeneration in vitro, most likely by simultaneously increasing neuronal activity and reducing blockade of the NMDA channel. Mg^{++} removal may be a useful method of modeling epileptic brain injury in vitro.

284.10

HIGH [Ca++]o AND LOW [K+]o SUPPRESS INTERICTAL SPIKES AND PROMOTE ELECTROGRAPHIC SEIZURES IN SLICES OF RAT HIPPOCAMPUS-ENTORHINAL CORTEX IN LOW Mg++ MEDIUM. H. Kojima*, A.C. Bragdon and W.A. Wilson. Depts. of Medicine (Neurology) and Pharmacology, Duke University and VA Medical Centers, Durham, NC 27710.

In vitro models exhibiting electrographic seizures (ES) provide an opportunity to study physiological factors regulating epileptiform activity. Brain slices of hippocampus and entorhinal cortex (HC/EC) exhibit ES when first exposed to "0-Mg" (no added Mg) medium. These ES are eventually suppressed by discharges resembling interictal spikes (IIS). We hypothesized that, in this model, lowering K+ and raising Ca++ in the medium would promote ES by suppressing IIS.

Extracellular recordings were made in area CA3 of HC/EC slices from adult male Sprague-Dawley rats. Standard medium had 3.3 mM K+, 1.8 mM Ca++ and 1.2 mM Mg++. In 0-Mg medium, slices exhibited ES with a rapid-firing (2-12 Hz) "tonic" phase of simple population bursts, followed by a slower-firing (0.2-2 Hz) "clonic" phase of complex bursts. In all slices, ES gave way to IIS after 30-120 min in 0-Mg medium. 20 slices were then studied at a fixed Ca++ of 2, 2.5, 3, 3.5 or 4 mM in progressively lower K+. By 1.5 mM K+, ES had reappeared in all slices; all ES had only a tonic phase; ES duration correlated with Ca++ (confirmed in 11 more slices). In 1.5 mM K+, IIS were suppressed completely in 3-4 mM Ca++ but, unexpectedly, occurred along with ES in 2-3 mM Ca++.

Lowering K+ promoted ES and, with high Ca++, suppressed IIS in the 0-Mg seizure model. However, ES and IIS were not completely mutually exclusive. One possible explanation for this semi-independence may be: IIS originate in HC whereas the ES arise in EC; HC and EC are influenced separately by Ca++ and K+; and the end result reflects a balance between effects on the two areas.

These results contrast sharply with results in two other in vitro seizure models in which seizures were induced by low Ca++ and/or high K+, and suggest the roles played by Ca++ and K+ in epilepsy are more complex than previously thought.

284.11

MODEL OF HIGH POTASSIUM INDUCED SYNCHRONIZED BURSTS IN THE RAT HIPPOCAMPAL SLICE: POSSIBLE ROLE OF SPONTANEOUS EPSPs IN INITIATION. Roger D. Traub, Nancy L. Chamberlin and Raymond Dingledine. IBM Watson Res. Ctr., Yorktown Heights, NY 10598 & Dept. of Pharmacology, Univ. of N. Carolina, Chapel Hill, NC 27514.

High extracellular K^+ (7 to 10 mM) induces repeating synchronized epileptiform bursts in the CA3 region of rat hippocampal slices [Rutecki et al., *J. Neurophysiol.* **54** (1985) 1363]. High K bursting differs from picrotoxin (PTX)-induced synchrony: (i) pyramidal cells in high K do not generate intrinsic bursts; (ii) high K does not block Cl^- IPSP conductance, but rather moves E_{IPSP} toward resting potential. A model of PTX synchrony has been proposed [Traub, Miles and Wong. *J. Neurophysiol.* **58** (1987) 739] in which bursting spreads via excitatory collaterals between disinhibited, intrinsically bursting pyramidal cells; the model requires (and it has been shown) that bursting be able to spread from one cell to connected cells. We report here that, with modifications dictated by experiments, a similar model can account for high K bursting. We used 9,900 cells, none intrinsically bursting; E_{IPSP} was set at resting potential. Each pyramidal cell receives spontaneous EPSPs with mean interval 150 ms or more, as observed in high K. Firing in one cell does *not* elicit firing in a connected cell. Synchrony develops when synaptic noise generates, in concert with intrinsic cellular depolarization, a threshold number of firing cells. Spread of firing then occurs along excitatory collaterals; spread is cooperative rather than the chain reaction of the PTX model. Our model agrees with these data: (i) a subthreshold excitatory buildup occurs in many cells just prior to a synchronized event; (ii) the interburst interval is regulated largely by a K-dependent AHP; (iii) blockade of IPSPs leads to more intense, less frequent bursts; (iv) total block of EPSPs abolishes synchronized bursts. That the same network accounts for two different types of synchronized bursting supports the basic model.

284.12

PHARMACOLOGICAL STUDY OF THE CESIUM-EVOKED SPONTANEOUS DISCHARGES IN DISINHIBITED RAT NEOCORTEX MAINTAINED "IN VITRO". M.Avoli & G.G.C.Hwa. MNI, McGill Univ., Montreal, Que., Canada.

Several K^+ channel blockers are used as convulsants in experimental studies. Here, we report that in rat neocortical slices perfused with 50 µM bicuculline methiodide (BMI), application of Cs^+ (2-3mM) increased the stimulus-induced burst duration (>500%), and concurrently triggered spontaneous discharges (0.06-0.21 Hz) that were not observed in BMI alone. Morphologically, there was no discernible difference between the evoked and the spontaneous discharges (1-4s). Manipulation of the membrane potentials indicated that both types of activities were network in origin. While the NMDA antagonist CPP reversibly reduced the rate of occurence of the spontaneous discharges by 13±8.9% (n=12), the change was not significant (T-test, $p>0.1$). Furthermore, preliminary data indicate that the non-NMDA antagonist CNQX appeared to depress or abolish the spontaneous activties. Thus, the epileptiform discharges described in the present experiments may represent a seizure model that is mediated primarily by non-NMDA receptors.

284.13

SEIZURE SUSCEPTIBILITY IN HIPPOCAMPAL SLICES: RELATION TO REGIONAL VARIATION IN EXTRACELLULAR SPACE. S.F. Traynelis, C.J. McBain* and R. Dingledine. Department of Pharmacology, University of North Carolina, Chapel Hill, NC 27599.

Electrographic seizures arise spontaneously in the CA1 region but not in CA3 or dentate gyrus of rat hippocampal slices perfused with elevated [K]o. Such seizures are readily blocked by hyperosmotic media, suggesting a role for K-induced restrictions of extracellular space in seizure initiation. To determine if variations in extracellular volume fraction (Vo) might account for the different susceptibility of these hippocampal subregions, we measured the diffusion profile of impermeant ions with the Nicholson-Phillips method to estimate Vo. In each region of the hippocampal slice the diffusion profile of iontophoretically introduced tetramethylammonium (TMA) measured with an ion-sensitive electrode was consistent with the laws of macroscopic diffusion. TMA had minimal effect on population spikes at the concentrations attained (2mM). Vo measured in 11 slices was 12% in CA1 stratum pyramidale (SP), 19% in CA3 SP and 18% in the granule cell layer. Vo was higher in stratum radiatum than in SP in both CA1 and CA3. The tortuosity, or mean increase in path length of a diffusing particle, was the same in all three regions (range 1.67-1.89). These data are consistent with the proposal that regional differences in extracellular volume could contribute to the different behavior of CA3 and CA1 neurones during electrographic seizures.

284.14

SPONTANEOUS SPIKE AND WAVE DISCHARGES IN RATS: ONTOGENY AND EFFECTS OF THE EXPOSURE TO DIFFERENT ENVIRONMENTS. M.Buonamici*,R.Maj*,M.A.Cervini*,P.Cassutti*and A.C.Rossi R&D.,Farmitalia Carlo Erba-Erbamont Group, C.N.S. Line, 20014 Nerviano, Italy.

The so called Spontaneous Spike and Wave Discharges (SSWDs) have been described as genetically determined in Wistar rats, but we have observed that they occur with much higher frequency also in Long Evans (LE) rats. To assess the relevance of genetic versus environmental factors in the genesis of SSWDs we exposed LE rats to "normal" or highly "enriched" environmental conditions from day 43 to day 180 after birth. Fifty-five rats in "normal" laboratory conditions were singly housed in standard cages, whereas the fifty-five rats in "enriched" environment underwent twice daily various standardized manipulation (for a total of 25 different "games"). EEG from chronically implanted electrodes were recorded for 1h, each session and then analyzed by computer for measurement of the number of bursts and percentage of time occupied by SSWDs (100%=60 min). No differences were observed between the two groups in both variables under observation. This suggests that different environments do not modulate SSWDs and assigns a more determinant role to genetic factors in the genesis of SSWDs. As expected at the end of the experiment the rats kept in "enriched" conditions performed better in a simple learning test (repeated hot-plate).

284.15

METAPHIT. AN ISOTHIOCYANATE ANALOG OF PCP, INDUCES AUDIOGENIC SEIZURES IN MICE. E.A. Debler[1], M.N. Lipovac*[2], A. Lajtha[1], B.V. Zlokovic*[2], A.E. Jacobson*[3], K.C. Rice[3], and M.E.A. Reith[1]. [1]Ctr. Neurochem., NKI, Ward's Is., NY, NY 10035; [2]Inst. Med. Physiol., Belgrade U., Belgrade, Yugoslavia; [3]NIDDKD, NIH, Bethesda, MD 20892.

Phencyclidine (PCP), a psychotomimetic drug that produces a psychosis resembling schizophrenia, has both convulsant and anticonvulsant activity (Hayes and Balster, Eur. J. Pharm., 117:121, 1985). Many PCP related effects have been shown to be mediated by the PCP recognition site that modulates the action of excitatory amino acids at the N-methyl-D-aspartate (NMDA) receptor. In in vitro binding studies, metaphit (MET) has been found to irreversibly bind to the PCP receptor (Rafferty et al., FEBS Lett., 181:318, 1985). In the present study we demonstrate that MET (administered either retro-orbitally [r.o.] or i.v., 20 mg/kg) induces audiogenic seizures in BALB/cBy mice. The most severe clonic/tonic seizures occur 18 to 24 h after MET administration. After 48 h the incidence of the seizure episodes begin to diminish. These audiogenic seizures can be prevented by the administration of either PCP (i.p., 7 mg/kg) or MK-801 (i.p., 0.5 mg/kg) 24 h after MET and 30 min prior to audio stimulation. PCP itself (r.o., 1, 5, 10 mg/kg) failed to elicit sound dependent seizures 24 h after administration. These MET-induced seizures may be due to a modulation of the PCP recognition site by MET which results in an enhanced probability that the PCP/NMDA ion channels are open.

OPIATES, ENDORPHINS AND ENKEPHALINS: ANATOMY AND CHEMISTRY I

285.1

CHANGES IN HYPOTHALAMIC MET-ENKEPHALIN IMMUNOREACTIVITY IN THE LACTATING RAT. M.D. Fitzsimmons and G.E. Hoffman. Dept of Physiology, Univ. of Pittsburgh, Pgh PA 15261

Opiate agonists increase serum prolactin levels. A leading hypothesis for the mechanism of action postulates that these agonists decrease the tonic inhibitory tone of hypothalamic dopamine neurons in the arcuate region, the A12 cell group. Our double labeling immunocytochemical studies have demonstrated a significant innervation of A12 neurons (more than 60% of the perikarya counted) by dynorphin axons. Modest but statistically significant increases in contact frequency occur during diestrus and proestrus. Nevertheless, differences in prolactin levels during pregnancy and lactation are not reflected by changes in dynorphin interactions with dopamine perikarya.

In the course of these experiments, two significant changes in hypothalamic Met-enkephalin (mEnk) immunoreactivity emerged. Lactating rats showed a striking increase in the amount of mEnk immunoreactivity in the median eminence relative to the other groups examined. In addition, mEnk staining frequently colocalized with A12 neurons (as indicated by tyrosine hydroxylase immunoreactivity). The appearance of mEnk immunoreactivity in A12 perikarya without colchicine pretreatment suggests significantly increased mEnk synthesis in these dopaminergic neurons.

These results are consistent with the existence of two kinds of influence of opioid peptides on prolactin regulation: dynorphin innervation of dopamine neurons may account for changes in prolactin release during the estrus cycle and mEnk released from the median eminence may stimulate prolactin release during lactation. The mechanism of mEnk stimulation of prolactin during lactation remains unclear. Leading hypotheses include: 1) direct stimulation of lactotrophs by opioid peptides; 2) opioid peptide interference with dopaminergic inhibition of prolactin at the level of the pituitary; 3) paracrine effects of mEnk at the median eminence on other trophic influences over anterior pituitary function.

285.2

DIFFERENTIAL EXPRESSION OF PREPROENKEPHALIN AND PREPRODYNORPHIN mRNAs IN THE RAT BRAIN. Yasuhiro Morita, Takashi Hironaka*, Jian-Hua Zhang*, Masaya Tohyama*, and Shiro Nakagawa*. Dept. of Anatomy, Kagoshima Univ. Med. Sch., Kagoshima, and Osaka Univ. Med. Sch., Osaka, Japan

Proenkephalin and prodynorphin are opioid precursor proteins which yield various forms of opioid peptides following posttranslational proteolitic processing. Proenkephalin- and prodynorphin-derived peptides were localized immunologically in a variety of brain tissues, but there were substantial discrepancies about localization of cell bodies. In the present study, neurons containing preproenkephalin(PPE)- or preprodynorphin(PPD)-mRNA were examined in the rat brain by in situ hybridization.

A large number of neurons containing PPE-mRNAs were distributed in various regions of the rat brain, while neurons with PPD-mRNA were primarily localized in the forebrain tissues. Neuronal population containing PPE- or PPD-mRNA were clearly separated in the rat brain tissues: e.g., different layers in the parietal cortex, discrete cell populations in the hyppocampal formation, and caudate-putamen, paraventricular, ventromedial hypothalamic, and solitary nuclei.

285.3

IMMUNOCYTOCHEMICAL LOCALIZATION OF β-ENDORPHIN CONTAINING NEURONS IN THE OVINE HYPOTHALAMUS. R.L. Goodman and C.S. Whisnant*. Physiology Department, West Virginia University, Morgantown, WV 26506

Endogenous opioids, including β-endorphin (END), have been implicated in the control of gonadotropin secretion in breeding season ewes, but little is known about the anatomy of these neural systems in this species. In this study, we identified END-containing neurons in the ovine hypothalamus using immunocytochemistry. Breeding season ewes (n=4) were anesthetized and their heads perfused with saline followed by Zamboni's fixative. A block of tissue containing the preoptic area (POA) and medial basal hypothalamus (MBH) was dissected, embedded, and 70 μm coronal sections cut with a vibrotome. Free floating sections (490 μm apart) were stained using the avidin-biotin-HRP procedure with a primary antiserum against END. Preincubation of antiserum with END (2 μg/ml) abolished all staining. Nerve fibers stained for END were observed in the median eminence and scattered throughout the medial hypothalamus and POA. END-containing cell bodies were localized in the MBH, but were not limited to the arcuate (ARC) nucleus. Just posterior to the optic chiasm, perikerya were in the periventricular area at the bottom of the third ventricle (3V). More caudally, cell bodies were concentrated in the ARC, but were also observed laterally and ventrally (in the ME). A high concentration of perikerya were visible in the posterior MBH around the ventrolateral border of the mammillary recess of 3V.

285.4

SIGMA RECEPTOR BLOCKADE BY BMY 14802 INVERSELY AFFECTS ENKEPHALINERGIC AND TACHYKININ NEURONS IN THE BRAIN OF THE RAT. J. A. Angulo, J. L. Cadet°, and B. S. McEwen °Columbia University, Dept. of Neurology, N.Y. and The Rockefeller University, Lab. of Neuroendocrinology, N.Y.

Typical neuroleptics inversely affect striatal proenkephalin and protachykinin mRNA biosynthesis in rat brain. These effects are attributed to the anti-dopaminergic activity of neuroleptic drugs. In the present study we determined proenkephalin and protachykinin mRNA levels in the rostral, medial, and caudal aspects of the caudate nucleus of the rat by in situ hybridization histochemistry in controls and in animals treated for 14 days with the potential neuroleptic drug BMY 14802 (15 mg/kg, i.p.). Proenkephalin mRNA levels were increased relative to control values by 207, 188, and 194% and protachykinin mRNA levels decreased to 83, 72, and 90% in the rostral, medial, and caudal aspects of the caudate-putamen, respectively. Similar effects of proenkephalin and protachykinin mRNA levels were observed in the nucleus accumbens. Unlike commonly used neuroleptic drugs, BMY 14802 lacks anti-dopaminergic activity but displays potent antagonistic activity at the haloperidol-sensitive sigma receptor site. The data suggest that sigma receptor activity, directly or indirectly, affects proenkephalin and protachykinin mRNA expression in striatal and nucleus accumbens neurons.

285.5

OPIOID RECEPTOR DISTRIBUTION IN RAT BRAIN USING IMMUNOHISTOCHEMISTRY. C.J.C.Boersma*,C.Gramsch**, A.Herz**,and F.W.Van Leeuwen* (SPON: ENA). Neth. Inst.for Brain Res.,Amsterdam,The Netherlands*; Max Planck Inst. für Psych.,Martinsried,F.R.G.**.

We are currently studying the distribution of opioid receptors by means of a monoclonal antibody (anti-Id-14). This antibody was raised using the anti-idiotype procedure (Gramsch et al., J. Biol.Chem., 263:5853). For immunohistochemistry, rats were perfused with 4% formaldehyde and post-fixed for 2 hrs, followed by an ABC staining method. We demonstrated opioid receptor immunoreactivity distributed in the following structures: paraventricular and supraoptic nuclei, stria terminalis, claustrum, central amygdala, caudate putamen, suprachiasmatic and paraventricular thalamic nuclei, lateral habenula, arcuate nucleus and median eminence. This distribution of opioid receptor immunoreactivity is similar to that previously described, utilizing autoradiography (Mansour et al.,J.Neurosci., 7:2445). The present results are in agreement with data indicating that anti-Id-14 primarily recognizes the μ and δ opioid receptors, whereas it has a low affinity for the κ subtype (A.Hassan, personal communication).

285.6

MONOCLONAL ANTIBODIES REVEAL ENKEPHALIN PRECURSOR FORMS IN CEREBELLAR ASTROCYTES AND NEURONS B.A. Spruce*, R. Curtis*, G.P. Wilkin*, and D.M. Glover*. (SPON:J.Brockes). Biochemistry Dept., Imperial College,London,SW7 2AZ,U.K.

We have generated 15 monoclonal antibodies to human proenkephalin-b-galactosidase fusion proteins synthesised in *E. coli* which recognise the unprocessed enkephalin precursor in stable transformants of PC12 cells. Several antibodies stain Golgi cells, deep cerebellar nuclei, and a subpopulation of Purkinje cells in the young adult rat cerebellum. In addition, 6 antibodies, which recognise different domains of preproenkephalin, stain a subpopulationof both grey and white matter astrocytes. One monoclonal which is predominantly directed to high molecular weight cleavage products rather than the intact precursor, stains neurons only and not astrocytes. This suggests a predominance of unprocessed proenkephalin in the astrocytes. Immunostaining using a metenkephalin antibody reveals staining of the outermost pial layer only. In situ hybridisation experiments, using an antisense RNA probe, combined with a glial fibrillary acidic protein immunostain, support the findings. Our results are consistent with a previous report of low levels of enkephalin peptides in the cerebellum,despite levels of mRNA comparable to those in the striatum, a region rich in enkephalin peptides. They indicate that processing of proenkephalinin the cerebellum is markedly less than in other regions of brain. Our results are also consistent with previous findings of proenkephalin mRNA in cultured astrocytes,and of unprocessed proenkephalinin the C6 rat glioma cell line, which could indicate a contribution of astrocytes to the neuroendocrine network.

285.7

AUTORADIOGRAPHY OF ^{3}H-ETHYLKETOCYCLAZOCINE BINDING SITES: SODIUM EFFECT. **D.D. Conrad and G.F. Wooten. Dept. of Neurology, University of Virginia School of Medicine, Charlottesville, VA. 22908.**

We have examined the effect of sodium (Na) on the distribution of ^{3}H-ethylketocyclazocine (EKC) binding sites in rat brain with respect to putative areas of high density kappa receptors, specifically the paraventricular thalamic nucleus and periventricular hypothalamic nucleus (J. Neurosci. 7(8): 2445-2464, 1987). Twenty micron thick frozen sections were incubated with ^{3}H-EKC at concentrations three times the K_d to define total binding in either Tris base 50 mM or K_2HPO_4 30 mM with NaCl 100 mM buffer. ^{3}H-EKC binding in the presence of 10 μM ketocyclazocine defined non-specific binding. The sections were then processed for autoradiography. Ten anatomic areas were analyzed by quantitative autoradiography using the DUMAS Image Analysis System. There was no significant difference in binding between the two buffer systems in striatal patches, nucleus accumbens, striatal matrix, medial amygdala, presubiculum, medial geniculate, superior colliculus or cortical amygdala. There was however, a 100% increase in binding in the paraventricular thalamic nucleus and a 174% increase in the periventricular hypothalamic nucleus (p<.01 and .05, respectively) in brain slices incubated in Na buffer when compared to incubation in Tris buffer. These results suggest that Na selectively enhances ^{3}H-EKC binding in areas of the brain previously demonstrated to be rich in putative kappa receptors.

285.8

(+) and (-)-Cyclofoxy: Application to the Quantitation of Opiate Receptors In Vivo. A. H. Newman,[1] N. L. Ostrowski,[2] R. Cohen,[2] M. A. Channing,[3] K. C. Rice.[4] [1]Walter Reed Army Institute of Research, Washington D. C. 20307-5100, [2] NIMH, [3]Clinical Center, NIH, [4]NIDDK, NIH, Bethesda, MD. 20892

(-)Cyclofoxy, a 6β-fluoro-derivative of the opiate antagonist naltrexone, was prepared as a model opiate ligand potentially suitable for positron emission tomography (PET). In vivo and in vitro autoradiography using (-)[^{3}H]cyclofoxy demonstrated that this ligand labeled a population of opiate receptors in the brain that was identical to those labeled by [^{3}H]naloxone. (-)[^{18}F]cyclofoxy has now enabled the visualization of opiate receptors in humans, using PET. However, an important issue in the successful application of this ligand to study the human opiate receptor-endorphin system is the necessary capability of quantitating occupied opiate receptors. Such quantitation is essential in detecting small but significant differences in the receptor density and/or endogenous ligand concentration between clinically normal and abnormal human subjects. Opiates with the naturally occuring (-) stereochemistry demonstrate a 10,000-fold stereoselectivity in binding to classical opiate receptors suggesting that (+)[^{18}F]cyclofoxy could serve as an ideal control for only opiate receptors occupied by (-)[^{18}F]cyclofoxy by enabling accurate quantitation of nonspecific binding. Synthesis of these ligands and in vivo autoradiography using (+) and (-)[^{3}H]cyclofoxy will be described.

285.9

ELECTRON MICROSCOPIC LOCALIZATION OF KAPPA OPIOID RECEPTORS IN GUINEA-PIG NEOSTRIATUM. C. Jomary*, J.E. Gairin*, J. Cros* and A. Beaudet (SPON:G. Karpati). Mont.Neurol.Inst., 3801 University Montreal, H3A 2B4, Canada and *LPTF, 205 Rte de Narbonne, 31077 Toulouse Cedex, France.

The distribution of kappa opioid receptors was examined by light and electron microscopic radioautography in sections of guinea-pig neostriatum labelled with the selective mono-iodinated probe, [125-I-Tyr^{1},D-Pro^{10}]dynorphin (1-11) (125-I-DPDYN). Pilots studies indicated that prefixation of the brain with 0.5% glutaraldehyde modified neither the affinity nor the capacity of 125-I-DPDYN binding to striatal sections. 125-I-DPDYN binding was also found to be pseudo-irreversible, allowing for post-fixation, defatting and liquid emulsion processing of the tissue without redistribution of the radioligand. In the light microscope, 125-I-DPDYN-labelled sites were distributed uniformly in the ventrolateral aspect of the neostriatum, but showed patches of high binding in the dorsomedial tier. At high magnification, the label was mostly confined to the neuropil, sparing both neuronal perikarya and myelinated fibbers bundles. In the electron microscope, the labelled binding sites were almost exclusively associated with the plasma membranes of neuronal and/or glial elements. Most of the label was detected over axo-dendritic interfaces, many of which exhibited a synaptic specialization in the plane of section. These results indicate that in the guinea-pig neostriatum, kappa opioid receptors are mainly membrane-bound, and often associated with axo-dendritic synapses.

285.10

(+) AND (-)-CYCLOFOXY: RADIOLABELED OPIATE ENANTIOMERS ALLOW VISUALIZATION OF SPECIFIC AND NON-SPECIFIC LIGAND BINDING IN VIVO. N.L. Ostrowski, A. Hauck-Newman, K.C. Rice and R.M. Cohen. Section on Clinical Brain Imaging, Lab. of Cerebral Metabolism, NIMH and Lab. of Medicinal Chemistry, NIDDK, NIH, Bethesda, MD 20892

(-)Cyclofoxy (CF; Burke, et al., 1985) has high affinity for opiate receptors in vivo, no pharmacological activity in tracer doses and no metabolites are detectable in brain. We investigated whether the inactive enantiomer of CF, [^{3}H](+)CF (Hauck-Newman, et al., 1989; this meeting) could be used as an in vivo measure of non-specific brain accumulation. Comparisons of tritiated (+) and (-)-CF using brain to cerebellar ratios, brain to plasma ratios, brain and arterial microdialysates and autoradiographic data strongly indicate that the distribution of [^{3}H](+)CF is unrelated to, and independent of, the distribution of opiate receptors. This is the first demonstration that an enantiomeric pair of ligands can be used to differentiate specific from non-specific labeling of receptors in vivo.

285.11

DISTRIBUTION OF MU, DELTA, AND KAPPA OPIOID RECEPTOR SUBTYPES IN THE RAT SPINAL CORD. M.A. Romagnano, E.K. Richfield, L.S. Brady, and R.W. Hamill. Neurology Dept. Monroe Community Hospital/Univ. of Rochester School of Medicine and Dentistry, Rochester, N.Y. 14603 and Unit on Functional Neuroanatomy, NIMH, Bethesda, MD. 20892

The distribution of the mu (μ), delta (δ), and kappa (κ) opioid receptor subtypes were examined throughout spinal cord levels C8-S2-4 in the rat. Fresh frozen horizontal serial sections were incubated with [^{3}H]D-ala^{2}-N-methyl-phe^{4},gly^{5}-ol-enkephalin (DAGO) or [^{3}H]D-ala^{2}-D-leu^{5}-enkephalin (DADLE) to label μ and δ receptors, respectively. In order to study κ receptors, sections were incubated with [^{3}H]bremazocine in the presence of unlabeled DAGO and DADLE. Adjacent slides were coincubated with excess unlabeled competitors to determine nonspecific binding. Following incubation, slides were apposed to LKB Ultrofilm-^{3}H or coated with Kodak NTB-2 emulsion and allowed to expose for 1-2 months.

In the thoracic cord, specific dense μ and κ binding was found in laminae I and II. Moderate κ binding was present throughout the dorsal horn and in laminae VII while moderate-dense κ binding was found in laminae X in the dorsal commissural nucleus. In the sacral cord, dense μ and κ binding was observed in laminae I and II. Moderate κ binding was present throughout the rest of dorsal horn. In the dorsal commissural nucleus at sacral levels moderate μ and κ binding was found. These data establishing the presence of different opioid receptor subtypes in several spinal cord laminae support the involvement of opioid peptides in sensory and autonomic regulation.

TROPHIC AGENTS III

286.1

NERVE AND GLIAL CELL LINES SECRETE MITOGENS FOR GLIAL PROGENITOR CELLS. J.M.Levine and F.Stincone*,Dept. of Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook,NY,11794

The development of O2A progenitor cells, the precursors of some astrocytes and oligodendrocytes, is regulated by environmental factors. For example,platlet-derived growth factor(PDGF) is a mitogen for glial progenitor cells and ciliary neurotrophic factor(CNTF) can initiate astrocyte differentiation. To characterize potential novel factors that might effect the development and differentiation of glial progenitor cells, we assayed the trophic effects of serum-free medium that had been conditioned by several neuronal and glial cell lines.

Post-natal rat optic nerve cells were grown in conditioned medium(CM) for 3 days, pulse labeled with 3H-thymidine and immunofluorescently stained with antibodies that identify progenitor cells, astrocytes and oligodendrocytes.The CMs were also tested for the stimulation of DNA synthesis in NIH/3T3 cells which respond to PDGF and CHO cells which lack a PDGF receptor.

Medium conditioned by 3 neuronal cell lines(B103,B50 and B35) stimulated DNA synthesis in progenitor cells,3T3 cells and CHO cells. Medium conditioned by PC12 cells and N2A neuroblastoma cells did not stimulate progenitor cells to divide however, the number of oligodendrocytes that developed in the cultures was reduced when compared to control cultures grown in defined medium.PC12 CM stimulated the division of CHO and 3T3 cells and N2A CM increased cell division only in CHO cells. Among the glial cell lines tested,C6,B92 and B82 cells secreted a mitogen for progenitor cells. The effects of these media on 3T3 and CHO cells was variable; B82 CM stimulated both CHO and 3T3 cells,C6 CM stimulated only CHO cells and B92 CM stimulated 3T3 cells but not CHO cells. In no cases were oligodendrocytes stimulated to divide.

These data demonstrate that neuronal and glial cell lines are valuable sources of trophic activities. The CMs that stimulate O2A progenitor cell division may contain a PDGF-like molecule.

286.2

LONG TERM SURVIVAL OF PRIMARY CULTURE OF ADRENAL CELLS FROM ADULT RATS: IMMUNOHISTOCHEMICAL CHARACTERIZATION. K.Shimoda , M. Poltorak and H.-Y.T. Yang. NIMH, Neuroscience Center at St. Elizabeths, Washington D.C. 20032.

Previously we have developed a primary culture of medullary cells from adrenals of adult rats. These cells can survive for a very long term of culture, (5 months or longer). To determine whether chromaffin cells survive, the cells were cultured for two months and characterized immunohistochemically with various antisera after being stimulated with or without NGF. Without NGF, few outgrowth of neurite was observed but tyrosine hydroxylase and NPY were detected in cell bodies. With NGF, extensive NPY positive neurites were observed. The results clearly indicate the survival of chromaffin cells which are responsive to NGF. NGF also enhanced Thy-1 suface expression molecules and glyoxylic acid induced fluorescence on the cells and neurites further indicating the responsiveness of the cells to trophic agent. The reason(s) for the long term survival of the chromaffin cell cultured in this study is still unclear. Interestingly, we observed that the 2 month old cultured cells can extend neurites spontaneously if culture media were not changed for a week. The result seems to suggest that trophic material may be produced and accumulated in the culture medium of the adrenal medullary cells.

286.3

USE OF EMBRYONIC BRAIN SLICES TO ASSAY POTENTIAL NEUROTROPHIC FACTORS

Kathy V. Callison and Jill M. Roberts-Lewis. Cephalon, Inc., West Chester, PA 19380.

Because of the need to establish a high-throughput assay with maximum sensitivity to potential neurotrophic factors, we have developed a method which examines the induction of ornithine decarboxylase in embryonic brain tissue slices.

Pooled brains from E16 rat fetuses were minced thoroughly with a razor blade in Earle's Balanced Salt Solution and brought to a final concentration of approximately 100 mg wet weight/ml. Fifty ul aliqouts of this mince were added to 400 ul of Earles in each well of a 24-well tissue culture plate. The covered 24-well plate(s) were placed into a tissue culture incubator (37^{o}C, 10 % CO_2). Following a one hour equilibration period, asparagine dissolved in 50 ul Earle's was added to the wells (9 mM final) and allowed to incubate for another 5 hours. The tissue suspension was then removed from each well and briefly centrifuged. The pellets were assayed for ornithine decarboxylase activity using a radiometric ornithine decarboxylase microassay. Asparagine elicited a 10-fold induction of ornithine decarboxylase in fetal brain tissue under these incubation conditions.

The novel use of a tissue culture incubator to maintain brain slices for a prolonged period of time may have broad applicability for a number of neurochemical assays using brain slices, such as neurotransmitter uptake and release, or genome activation studies.

286.4

PREPARATION OF HIGH YIELD HIGHLY PURIFIED NEURONAL CULTURES FROM RAT CNS.

M.E. Blake, J.F. McKelvy and J. Chen, (SPON: D.E. Frail) Neuroscience Research Division, Pharmaceutical Discovery, Abbott Laboratories, Abbott Park, IL 60064

Highly pure primary cultures of CNS neurons, obtainable reproducibly and in high yield, represent an important tool for studying many aspects of neuronal function *in vitro*. We have developed a useful new approach which gives rise to such cultures. For example, rat embryonic cerebral cortices (E15) were dissociated by passage through fired pasteur pipets, followed by Percoll gradient centrifugation. Live cells were plated onto poly-D-lysine treated cover glasses, 35 mm dishes, and multi-well plates in alphidicolin containing media. Approximately, $3x10^8$ cells could be obtained from 30 cortices. After three days in culture, process outgrowth had proceeded to a point where extensive arborization and dense interconnecting networks of processes could be seen, with a remarkable uniformity of distribution of cell bodies. Immunocytochemical staining with neuronal specific neurofilament and GFAP antibodies revealed that the culture was at least 98% neuronal cells. These cultures are being used to explore growth factor actions; e.g. FGF and PDGF were found to enhance neurite outgrowth as well as neuronal maintenance.

286.5

A RE-DESIGNED INTRAVENTRICULAR INFUSION DEVICE PREVENTS PUMP TOXIN INDUCED BRAIN LESIONS.

H.L. Vahlsing*, T. Hagg, M. Manthorpe, A. Dekker*, M. Manley*, S. Varon. [SPON : M.E. Baker] Dept. Biology, M-001, UCSD, La Jolla, CA 92093.

We had previously introduced improvements in a device for continuous infusion of NGF or other substances into the lateral ventricle of adult rats which addressed, among others, the problem of infusion-related lesions in the tissue around the cannula tip. The severity of the lesion could be limited by decreasing the infusion rate suggesting either a mechanical or a toxic origin. To evaluate the latter possibility, sterile phosphate buffered saline (PBS) was introduced into the different components of the cannula device and incubated sterilely in vitro for 2-14 days at 37°C. This "conditioned" PBS was added in sequential two-fold dilutions to cultures of chick neurons, rat astroglial cells, and mouse fibroblasts and the cultures examined 24 hr later for cell survival. Toxic activity was found only in fluid taken from the Alzet 2002 mini-osmotic pump, its half-maximal activity seen between 100 and 500 fold dilution.

In a re-designed infusion device the cannula was connected to the pump by a coil of siliconized vinyl tubing. Pump-derived toxins are prevented from reaching the brain by an air or mineral oil spacer which separates the infusate in the coil from the fluid in the pump. With the new device infusion-related brain lesions were no longer seen even after infusions lasting a full month or delivered directly into the brain parenchyma. Additional advantages are i) the effects of the infused agent are not complicated by the presence of toxins, ii) the volume of infusate in the coil can be varied according to the infusion duration, an important point for treatments with scarce or expensive agents, iii) coil and spacer material can be adapted to fit properties of different agents, iv) different agents can be administered sequentially by alternating infusate and spacers in the coil, v) the volume of actually infused fluid can be calculated from its advancement in the coil. Supported by grants BNS-88-08285 (NSF) and NS-16349, NS-25011 (NINCDS).

286.6

NERVE GROWTH FACTOR RECEPTOR IN NEURAL TUMORS.

Alonzo H. Ross[1], David L. Baker[2*], Balaka Chattopadhyay[1*], Jie Chen[1*], David Pleasure[2], Usha Reddy[2], Amit Roy[1*], and Gita Venkatakrishnan[1*]. [1]Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545; [2]Childrens Hospital of Philadephia, PA 19104.

A 75,000-dalton glycoprotein (gp75) has been identified as nerve growth factor receptor (NGF-R). Using flow cytometry, Western blotting, and Northern blotting, we have surveyed a series a human neural tumor cell lines for gp75 expression. All 5 neuroepithelioma cell lines tested expressed gp75 but only 9 of 17 neuroblastoma cell lines tested expressed gp75. One of 4 medulloblastoma cell lines tested expressed gp75. NGF-induced neurite extension was observed for 3 neuroblastoma cell lines all of which lack amplified N-myc oncogene.

Two neuroblastoma cell lines apparently lacking gp75 expression were infected with a defective retrovirus encoding gp75, and one was found to express high-affinity NGF-R and to up-regulate fos expression in response to NGF. The other infected neuroblastoma cell line expressed only low-affinity NGF-R and lacked a fos response.

286.7

SYNTHESIS OF A NERVE GROWTH FACTOR-LIKE MOLECULE BY GRANULOMAS.

G. W. Varilek*, J. V. Weinstock* AND N. J. Pantazis, Departments of Anatomy and Internal Medicine, Univ. Of Iowa Medical College, Iowa City, IA 52242.

NGF is critical in the maintenance and development of specific neuronal cell types, but it is unclear whether NGF functions outside of the nervous system. The possibility that NGF is involved in the immune system was examined in this study. Our goal was to determine whether granulomas isolated from mice infected with the parasite, Schistosoma mansoni, synthesize NGF. These granulomas are t-cell mediated inflammatory lesions composed primarily of macrophages, eosinophils, lymphocytes and fibroblasts. METHODS: Granulomas were isolated aseptically from 10-wk infected mice. NGF bioactivity was assayed by neurite outgrowth from pheochromocytoma (PC12) cells. Granulomas were either co-cultured with PC12 cells or conditioned medium (CM) was collected from granuloma cultures and this CM was tested on PC12 cells. NGF concentrations were also determined by radioimmunoassay (RIA). RESULTS: In co-culture experiments, granulomas induced neurite outgrowth from PC12. In addition, granuloma CM stimulated neurite outgrowth. The RIA detected immunoreactive NGF in granuloma homogenates. The displacement curve generated by the granuloma "NGF" paralleled the standard curve derived with NGF purified from mouse submandibular gland. CONCLUSIONS: Biological and immunological assays determined that granulomas, isolated from mice infected with Schistosoma mansoni, synthesize an NGF-like molecule. The cell type responsible for the synthesis of NGF as well as the role of NGF in the immune system are under investigation. Supported by NFIC, NIH: AM34986, AM38327, GM28644.

286.8

TEMPORAL CHANGES IN NGF RECEPTOR LABELING IN PRIMARY CULTURES OF RHESUS AND RAT ADRENAL CHROMAFFIN CELLS.

M. A. Herman*[†] and P. Claude*. Wisconsin Regional Primate Research Center, [†]Dept. of Physiology and *Neuroscience Training Program, UW-Madison 53715.

Nerve growth factor (NGF) promotes the outgrowth of neurites from adrenal chromaffin cells, but little is known about NGF receptor (NGFR) deployment during this response. We examined changes in immunostaining for NGFR in chromaffin cells cultured for four weeks in the presence or absence of NGF, with or without dexamethasone (dex), which inhibits neuritic outgrowth from these cells (Unsicker et al. 1978). As previously described, differentially plated adrenal chromaffin cells from rhesus monkeys of various ages (Lillien and Claude, 1985a), or from 8 day old rats (Lillien and Claude, 1985b), were grown on collagen-coated glass coverslips in 25 ng/ml 7S NGF, 15 μM dex, NGF plus dex, or control medium. 1, 2, 3 or 4 weeks after plating, cultures were immunostained with monoclonal antibodies directed against NGFR (NGFR5 for rhesus or 192 for rat) and scored for percent of cells with neurites and percent of labeled cells with and without neurites. **Results.** The percentage of rhesus cells labeled for NGFR increased with time in culture in all conditions. However, cells were not uniformly labeled; not all neurite-bearing cells were labeled, and some cells without neurites were labeled. Different intensities of labeling occurred in all conditions, with dex-treated cells staining the most intensely at all time points. The intensity of labeling dramatically decreased with time in NGF alone. A slight decrease in intensity was seen in NGF plus dex and in control medium, while there was no change in dex alone. These results suggest that culturing *per se* may cause chromaffin cells to upregulate NGFR, that NGF may downregulate its own receptors, and that dex may antagonize this NGF-dependent downregulation of NGFR. Preliminary experiments using cells from young rats yielded similar results. (We thank C.A. Schulz for technical help and M. Bothwell for monoclonal antibodies. Supported by NSF BNS-8616958 to P.C. and NIH RR00167 to the WRPRC.)

286.9

EFFECTS OF NERVE GROWTH FACTOR AND ITS ANTISERUM ON SYMPATHETIC GANGLION CELLS IN YOUNG ADULT AND AGED MICE. K.G. Ruit[1], P.A. Osborne*[2], R.E. Schmidt[3], E.M.Johnson, Jr.[2], and W.D. Snider[1]. Depts. Of Neurology[1], Pharmacology[2], and Pathology[3], Washington Univ. Sch. Med., St. Louis, MO 63110.

The extent to which neurons in mature animals depend on neurotrophic molecules remains controversial. We have therefore investigated the effects of prolonged systemic injections of NGF and its antiserum on the survival and morphology of sympathetic ganglion cells in young adult and aged mice.

Treatment with guinea pig anti-mouse NGF led to substantial cell death in the superior cervical ganglion, as well as to retraction of dendritic arborizations. Neuronal counts showed that 32% of neurons had died after 1 month of treatment. Intracellular staining revealed that cross-sectional somatic area was reduced by 54% and that the total length of dendritic processes was reduced by 33%. After 3 months of treatment up to 60% of neurons had died. When a similar protocol was employed in aged animals (22 months old), cell loss in the superior cervical ganglion was 31% after one month of treatment.

To determine whether NGF stimulates dendritic growth in maturity, adult animals were treated systemically with 5-10 mg/kg of NGF for 2-4 weeks. Despite the fact that tyrosine hydroxylase activity increased in treated ganglia, this protocol resulted in little change in sympathetic ganglion cell morphology. Preliminary data suggest that total dendritic length was increased by only 14%.

Our results in anti-sera treated animals show that even in maturity sympathetic ganglion cells remain dependent on NGF for survival and maintenance of dendritic arborizations. Interestingly, administration of NGF to adult animals, even in very large doses, appears to have only a modest effect on ganglion cell morphology.

286.10

STRIVING FOR AN ANIMAL MODEL OF ALZHEIMER'S DISEASE: GENETIC CONSTRUCTION AND EXPRESSION OF A FAMILY OF TOXINS TARGETED TO NGF RECEPTORS. P.A. Schimke*, L Penchuk*, T. Jerome*, J.R. Muprhy* and G. Heinrich. Biomolecular Med., Boston University Medical Center, Boston, MA 02118

In Alzheimer's disease, a severe cholinergic deficit involving neurons that bear NGF receptors occurs. Induction of a corresponding deficit in animals would advance the understanding of the mechanisms of Alzheimer's disease, but specific cholinergic toxins are not available. To develop such toxins, we took advantage of previous work, in which fusion toxins were produced by replacing the sequence encoding the binding domain of diphtheria toxin (DT) or Pseudomonas exotoxin A (PEA) with sequences encoding alpha-MSH (PNAS 83:8258, 1986) and IL-2 (Protein Eng. 1:493,1987, PNAS 84:4538, 1987), which were specifically cytotoxic for cells bearing the targeted receptors. DT-NGF constructs, containing or lacking signal sequence, have NGF sequences extended from the 3' end of DT, therefore, the fusion junction occurs at the N-terminus of NGF. In NGF-PEA constructs, the preproNGF sequence is extended from the 5' end of PEA, with the fusion junction at the C-terminus of NGF. These fusion toxins are suitable for bacterial and mammalian cell expression. For mammalian expression, DT-resistant COS cells were selected in 5 uM DT, and transfected with fusion toxin expression vectors to study the resultant products and their effect on PC12 cells. Supported by ACS grant PF2973(PAS), ADRDA grant PRG88-055 (GH).

286.11

NERVE GROWTH FACTOR (NGF) AND ITS RECEPTOR IN ALZHEIMER'S AND PARKINSON'S DISEASE. N. J. Pantazis, S. Ning*, D. R. Brady and E. J. Mufson. Anatomy, Univ. of Iowa Medical College, Iowa City, IA 52242 and Institute for Biogerontology Research, Sun City, AZ. 85372

NGF is required for maintenance and development of sympathetic and sensory neurons of the peripheral nervous system and cholinergic (Ch) neurons present in the basal forebrain of the central nervous system. These Ch neurons express NGF receptor (NGFR), provide the major cholinergic innervation to neocortex and hippocampus, and degenerate in Alzheimer's disease (AD) and in Parkinson's disease with dementia (PKD). Since NGF is involved in maintaining Ch neurons, our hypothesis was that concentrations of either NGF and/or NGFR are reduced in AD and PKD, resulting in loss of basal forebrain Ch/NGFR neurons.

Brain samples were obtained postmortem (2.5 - 5 hr delay) and frozen in liquid nitrogen. Samples of basal forebrain (BF; nucleus basalis), hippocampus (HP) and motor cortex (MC) were homogenized and NGF concentrations were determined by radioimmunoassay. NGFR was determined by NGF immunoprecipitation. When AD or PKD samples were compared to unafflicted controls, there were no differences in NGF or NGFR concentrations in the brain areas studied. The lack of a difference in NGF suggests that the source of NGF is not altered in these disease processes. Since many NGFR neurons in the basal forebrain degenerate in AD and PKD, it is surprising that NGFR expression is not altered. Possibly, surviving NGFR neurons increase their expression of NGFR. Although the role of NGF in AD and PKD remains to be elucidated, loss of NGFR neurons in these diseases does not appear to be directly linked to reduced NGF or NGFR concentrations. (Support: American Health Assistance Foundation)

286.12

RE-EXPRESSION OF NERVE GROWTH FACTOR RECEPTOR IN ADULT BRAINSTEM MOTOR NEURONS AFTER AXONAL INJURY. S.J. Wood* and M.V. Sofroniew. (SPON: G. Ugolini) Department of Anatomy, University of Cambridge, U.K.

Nerve growth factor (NGF) is a trophic factor essential for the development and maintenance of sympathetic and some sensory neurons. NGF is thought not to be a neurotrophic factor for motor neurons, since experiments similar to those used on sympathetic and sensory neurons show no effects. Nevertheless, motor neurons transiently express NGF receptors (NGFr) and retrogradely transport 125-I labelled NGF during development (from E15 to P6) (Yan et al. *Neuron* 1:335). We report here, that some motor neurons re-express NGFr following axonal injury in the form of crush or transection.

The hypoglossal (Hg) and vagus (Vg) nerves were exposed unilaterally in the neck of young adult rats, and were either crushed, or transected and ligated. After varying survival times, animals were perfused and the brainstems immunohistochemically stained for NGFr using monoclonal antibody MC-192.

In contrast to neonatal animals (P1-P3), normal adult rats showed no staining of Hg or Vg motor neurons for NGFr. 7 days after a crush injury, many, but not all, Hg or Vg neurons stained positively for NGFr. Staining had declined by 14d after nerve crush in the Hg, and by 28d in the Vg. After axotomy and ligation, similar numbers of neurons were positively stained for NGFr in the Vg, but far fewer in the Hg; these were found at later survival times (>14d) and were observed in some neurons up to 100d after injury. While the function of NGFr in motor neurons remains obscure, re-expression of these receptors after axonal injury suggests they may be involved in similar events during development and the reaction of these neurons to injury.

286.13

ULTRASTRUCTURAL EVIDENCE FOR NERVE GROWTH FACTOR-MEDIATED ENHANCED SURVIVAL OF DEVELOPING CENTRAL CHOLINERGIC NEURONS IN REAGGREGATE CELL CULTURE. B.H. Wainer[a], S.D. Price[a*], S.G. Nelson[a*] and W.C. Mobley[b]. The University of Chicago[a], Chicago, IL, 60637 and The University of California[b], San Francisco, 94143.

Nerve Growth Factor (NGF) exerts trophic effects on cholinergic neurons of the basal forebrain. We previously reported that while NGF increases the number of developing cholinergic neurons in reaggregate cultures of the fetal septal region (Hsiang et al. Neuroscience 29: 209-223, 1989), the results of ultrastuctural analysis suggested that this effect was more likely to result from increased expression of the histochemical cholinergic marker, acetylcholinesterase (AChE), than from enhanced cholinergic cell survival (Wainer et al. Soc Neurosci Abs.14: 36. 1988). In the present experiments the effects of NGF treatment were examined in reaggregate co-cultures of embryonic day 15 septal cells and cerebellar cells, which normally do not support cholinergic cell survival. The analysis consisted of ultrastuctural quantitation of healthy versus degenerating AChE-positive cells in septal-cerebellar (S-Cb), and septal cerebellar + 20ng/ml NGF (S-Cb +NGF) co-aggregates following both 15 and 21 days of culture. Preliminary results of the analysis obtained thus far are as follows: In three S-Cb culture flasks at 15 days, ultrastructural analysis yielded a mean ± SE of 156 ±46 healthy; 35 ± 7 dying or 79 ± 7% intact cells. In three S-Cb culture flasks at 21 days, results yield 160 ± 55 healthy, 48 ± 4 dying or 73 ± 6% intact AchE-positive cells. In S-Cb + NGF cultures, three flasks at 15 days yielded 236 ± 52 healthy, 16 ± 5 dying or 95 ± 1% intact cholinergic cells. At 21 days, analysis of three flasks yielded 215 ± 73 healthy, 12 ± 2 dying or 94 ± 2% intact. These preliminary results suggest that when developing septal cholinergic neurons are cultured at lower effective density with cells that normally do not support their survival (ie. nontarget cerebellar cells) then the presence of NGF can enhance their survival. Supported by NS-25787.

286.14

CULTURED RAT HIPPOCAMPAL ASTROCYTES SECRETE NERVE GROWTH FACTOR IN VITRO, BUT DO NOT SUPPORT AXOTOMIZED CHOLINERGIC NEURONS IN VIVO. K. Yoshida, A.M. Fagan, and F.H. Gage. Dept. of Neurosciences, University of California-San Diego, La Jolla, CA 92093.

Two-site enzyme immunoassay was used to determine the secretion of Nerve Growth Factor (NGF) from cultured cells. Astrocyte rich cultures (GFAP-positive cells >90%) were obtained after 2 passages from neonatal rat hippocampal formations. Pure fibroblast cultures isolated from primary brain cell cultures were also examined. Cells were grown on poly-L-lysine coated 24-well culture plates with DME containing 10% fetal calf serum. After reaching confluence, the cells were incubated with 1 ml/well culture media as described above for 24 hrs, and NGF content in the culture media was measured. The results clearly show that hippo-campal astrocytes secrete NGF at the rate of 40-70 pg/24 hr/well (~ 4 x 10^5 cells per well). On the contrary, NGF levels in brain derived fibroblast conditioned medium were lower than the detection limit (10 pg/ml). The cultured hippo-campal astrocytes (1 x 10^5 cells/animal) were grafted into rat brains with unilateral fimbria fornix lesions, but astrocytes did not protect the medial septum cholinergic neurons from retrograde degeneration. We are presently investigating mechanisms for regulation of NGF secretion in primary astrocytes.

286.15

INTRACEREBROVENTRICULAR NGF INFUSIONS PREVENT RETROGRADE CHOLINERGIC NEURON DEGENERATION IN THE PRIMATE. M.H. Tuszynski, D.G. Amaral, H.-S. U, K. Yoshida, F.H. Gage. Depts. of Neurosciences and Neurosurgery, University of California-San Diego, La Jolla, California, 92037, and Salk Institute for Biological Studies, La Jolla, CA. 92037.

Cholinergic neurons of the adult rat basal forebrain undergo retrograde degeneration when deprived of target-derived nerve growth factor (NGF). Primate basal forebrain cholinergic neurons possess NGF receptors and may therefore also require NGF. To test this hypothesis, Macaca fascicularis monkeys underwent unilateral lesions of the fornix. Control animals received 4 week infusions of artificial CSF while experimental animals received mouse 2.5S ß-NGF, 180 ug/ml. All animals had complete unilateral interruption of the fornix as judged by extensive depletion of acteylcholinesterase and cholineacetyltranferase (ChAT) staining in the hippocampal formation ipsilateral to the lesion. Control animals had an average 50% loss of ChAT-immunoreactive (IR) neurons in the medial septal nucleus ipsilateral to the fornix lesion. Animals that received NGF had only a 24% ChAT-IR cell loss compared to the unlesioned contralateral side, and fewer of the remaining neurons were atrophic. NGF receptor-IR staining demonstrated similar changes. Thus, NGF treatment sustains some basal forebrain neurons that may otherwise undergo retrograde cell death. This may have implications for the treatment of neurodegenerative disorders characterized in part by cholinergic cell dysfunction, such as Alzheimer's disease.

286.16

STRESS DOWN REGULATES RAT BRAIN NGF RECEPTORS G. Taglialatela*, P.J. Foreman, M.T. Ramacci*, L. Angelucci* & J.R. Perez-Polo. SPON:(R. Stach) HBC&G Dept., Univ. of Texas Medical Branch, Galveston, TX, 77550.

The highest concentrations of nerve growth factor receptors (NGFR) in rodent brain are in basal forebrain area (BFA), hippocampus (H) and cortex (Cx,) all related to limbic function that regulates the stress response. To determine the effect of stress on NGF binding, we exposed rats to one hour of cold stress for five days. There was a 50% reduction in NGF binding in BFA, H, Cx but no significant change in cerebellum (Cb).

Decrements in NGF binding in aged rodent CNS can be ameliorated by acetyl-l-carnitine (ALC) (Angelucci et al, 1988). In ALC-treated rats, the stress induced reduction of NGF binding was abolished in H and Cx, but not in BFA. In unstressed ALC-treated rats there was a decline in NGF binding in H, but not in BFA, Cx or Cb. We are currently studying NGFR mRNA expression following stress exposure and ALC-treatment. Supported in part by NINDS, CNR & Sigma-Tau Co.

286.17

NERVE GROWTH FACTOR STIMULATES BASAL FOREBRAIN CHOLINERGIC NEURONAL DIFFERENTIATION IN TRISOMY 16 MICE. P. Corsi*, J.E. Sweeney and J.T. Coyle (SPON: M. Saltarelli) Depts. of Neuroscience and Psychiatry, The Johns Hopkins School of Medicine, Balto. MD 21205.

Since Down Syndrome (DS) results in Alzheimer's Disease (AD) pathology by the third decade, we have examined the differentiation of the basal forebrain cholinergic neurons (BFCN from mice with trisomy (Ts) of chromosome 16 (MMU16), a murine genetic model for DS. Basal forebrain dissected from 15 day gestational fetuses (Ts16 and euploid littermates) was disaggregated with papain and grown in MMEM containing 10% heat inactivated horse serum on polylysine coated multiwell plastic plates. Choline acetyltransferase (ChAT) immunoreactivity was visualized with antiserum, and its activity was measured enzymatically. After 10 days in culture, euploid BFCN exhibited extensive neurite extension whereas Ts16 BFCN had scanty perikaryal cytoplasm and sparse, short neurites. Exposure to β-nerve growth factor (NGF; 100 ng/ml) for 10 days resulted in a marked stimulation of neurite elaboration in the Ts16 BFCN but not in controls. Although, in the absence of NGF, ChAT was significantly lower in Ts16 cultures as compared to controls, NGF stimulated a greater increase of ChAT activity in Ts16 cultures. Preliminary results suggest that the increased NGF dependency of Ts16 BFCN persists in cells grown in completely defined medium. These findings indicate that BFCN from Ts16 may be more dependent upon NGF than those from littermate controls and suggest a mechanism of BFCN vulnerability in DS.

286.18

EXPRESSION OF NERVE GROWTH FACTOR RECEPTOR MESSENGER RNA IS DEVELOPMENTALLY REGULATED AND INCREASED AFTER AXOTOMY IN RAT SPINAL CORD MOTONEURONS. L. Olson[1], P. Ernfors*[2], A. Henschen*[1], M. Bygdeman*[3], C. Wetmore[1], M. Eriksdotter-Nilsson*[1], T. Ebendal*[4], H. Persson*[2]. Depts. of [1]Histology & Neurobiology and [2]Molecular Neurobiology, Karolinska Institute, S-104 01 Stockholm; [3]Dept. of Obst. & Gyn., Karolinska Hospital, S-104 01 Stockholm; [2]Biomedicum, Uppsala Univ., S-751 23 Uppsala, Sweden.

High levels of nerve growth factor receptor (NGF-R) mRNA in rat spinal cord motoneurons have been detected during the period of naturally occurring cell death (E13-14) with in situ hybridization and RNA-blot analysis. This expression is sustained, but reduced, during the time of synapse formation, and appears to be greatly reduced in the adult spinal cord. Comparably high hybridization of NGF-R mRNA was also found in human fetal anterior horn cells. Unilateral crush lesion of the adult rat sciatic nerve resulted in an 8-fold increase in NGF-R mRNA in spinal cord motoneurons 3 days after lesion compared to the non-lesioned side. Induction of NGF-R mRNA in spinal motoneurons was even more pronounced 7 and 14 days after the lesion, reaching levels 12 times higher on the lesioned side. However, 6 weeks after unilateral lesion, when the motor function of the leg was largely restored, the level of NGF-R mRNA decreased to a level similar to the unlesioned side. Studies in progress, including the administration of NGF or NGF-producing cell lines to the lesioned sciatic nerve, will further characterize the regulation of the NGF-R mRNA as well as the expression of NGF-R protein. We suggest that the NGF-R mediates a trophic or axonal guidance function for developing and regenerating spinal cord motoneurons.

286.19

SOMATOFUGAL AXONAL ATROPHY IN INTACT ADULT SENSORY NEURONS FOLLOWING INJECTION OF NERVE GROWTH FACTOR (NGF) ANTISERUM. S.F. Matheson*[1], B.G. Gold[1] and W.C. Mobley[2] (SPON: J. Falk). [1]Rutgers Univ. College of Pharmacy, Piscataway, NJ 08854 and [2]Dept. of Neurology, Univ. of California, San Francisco, CA 94143.

Induction of the neuronal perikaryal response to axonal injury appears to involve loss of a retrogradely transported trophic factor, perhaps originating in target tissue. Somatofugal axonal atrophy is a readily quantifiable component of the axon reaction. Recent studies have demonstrated that application of NGF to a proximal nerve stump reduces the degree of axonal atrophy in some sensory fibers in the dorsal root ganglia (DRG). In the present study, we asked whether NGF functions to regulate axonal caliber in intact adult neurons. Four week old rats were injected daily with antiserum to ß-NGF (0.5 ul/g body wt, for 11 days) in the footpad; age-matched controls were injected without antisera. Numbers of small and large neuronal perikarya in the L5 DRG demonstrating eccentric nuclei increased 2- and 3.6-fold, respectively, in rats given NGF antiserum. At this level, axonal calibers of sensory fibers were smaller and fibers less circular in shape compared to controls; motor fibers in the proximal L5 ventral root were unchanged. The results suggest that, in some sensory neurons, NGF regulates axonal caliber and that its loss initiates some features of the axon reaction. (Supported by NS 26265).

286.20

Fimbria-fornix transections influence NGF-receptor gene expression in the rat basal forebrain. J.E. Springer[1], M.E. Lewis[2], E. Robbins[2], S. Meyer[2],and F. Baldino, Jr.[2]. [1]Dept. of Neurology, Hahnemann Institute of Neuroscience, Philadelphia, PA 19102-1192, and [2]Cephalon Inc., 145 Brandywine Parkway, West Chester, PA 19380.

Several lines of evidence support the involvement of nerve growth factor (NGF) as a trophic substrate for basal forebrain cholinergic neurons in the central nervous system. However, the physiological events associated with the trophic effects of NGF are not well understood. It is known that the trophic actions of NGF are initiated by and require a receptor-coupling event. We have studied the effects of NGF removal on NGF receptor (NGFR) mRNA expression in a well defined cholinergic system, the rat septo-hippocampal pathway. Rats received a unilateral fimbria fornix transection (FXT) and were sacrificed 4 hr, 24 hr, 4 days, or 21 days following surgery. This process results in the loss and/or dysfunction of the majority of cholinergic neurons in the ipsilateral medial septal and vertical limb of the diagonal band (MS/VDB), and is presumably due to the loss of target-derived NGF. A NGFR cDNA (a gift from E. Shooter) was used to generate ^{35}S-labeled RNA probes for in situ hybridization histochemistry. Cells in the MS/VDB expressing NGFR mRNA were localized in a pattern similar to immunocytochemical studies using choline acetyltransferase and NGFR antibodies. In the ipsilateral MS/VDB, there was an apparent decrease in NGFR mRNA expression without cell loss at 4 hr following FXT. At all other times analyzed, the number of cells expressing the NGFR mRNA was significantly decreased. In addition, the level of NGFR mRNA expressed in the remaining cells in the ipsilateral MS/VDB was much less than the intact cells in the contralateral side. This observation could be due to a number of factors including; 1) remaining cells may have intact collaterals that transport NGF through a pathway not damaged by the transection, 2) remaining cells are able to acquire an alternate source of NGF, and 3) cells in the ipsilateral MS/VDB are not lost, but rather are functioning at some lower homeostatic level. Supported by the ADRDA and BRSG 1-S07RR07241-01.

287.1

DIRECT AND INDIRECT TROPHIC ACTIONS OF bFGF, EGF, TGF-a AND INSULIN ON RAT SEPTAL AND MESENCEPHALIC NEURONS IN CULTURE; LACK OF EFFECT OF GLIA DERIVED NEXIN. B. Knusel, P.P. Michel, C. Bakhit and F. Hefti (SPON: R. Clark), Andrus Gerontology Center, Univ. of Southern California, Los Angeles, CA 90089 and Genentech, Inc., South San Francisco, CA 94080.

This study is aimed at characterizing effects of various trophic factors in cultures of embryonic rat septum and mesencephalon. bFGF and insulin increased the activity of choline acetyltransferase (ChAT) in septal, and the uptake of dopamine (DA) in mesencephalic cultures. EGF and TGF-a failed to act on ChAT but stimulated DA uptake. Preventing cell proliferation by arabinoside-C (ara-C) did not affect the bFGF mediated ChAT increase in septal cultures, but abolished the stimulatory action of bFGF, EGF or TGF-a on DA uptake in mesencephalic cultures. The action of insulin in septal and mesencephalic cultures was only slightly reduced by ara-C. Glia derived nexin (GDN) failed to affect the appearance of our cultures and did not enhance biochemical activities in septal or mesencephalic cultures, and, therefore, is not a likely candidate to affect the development of cholinergic and dopaminergic neurons in the brain.

287.2

INSULIN-LIKE GROWTH FACTOR-I ENHANCES TYROSINE HYDROXYLASE ACTIVITY IN CULTURED BOVINE CHROMAFFIN CELLS. M. K. Dahmer and R. L. Perlman. Departments of Pharmacological Physiological Sciences and Pediatrics, The University of Chicago, Chicago, IL 60637.

We have previously reported that bovine chromaffin cells have receptors for insulin-like growth factor-I (IGF-I) and that IGF-I enhances secretagogue-stimulated Ca^{2+} uptake and catecholamine secretion in these cells. In this study we examined the effect of IGF-I on tyrosine hydroxylase activity in chromaffin cells. We cultured cells for 3 days in serum-free medium in the absence or presence of 10 nM IGF-I and then assayed tyrosine hydroxylase activity by measuring dopa synthesis in cells incubated in medium containing 0.1 mM tyrosine and 0.15 mM brocresine, a dopa decarboxylase inhibitor. Dopa synthesis was stimulated by incubating the cells in medium containing elevated K^+ (55 mM). High K^+-stimulated dopa synthesis was 30-150% higher in IGF-I treated cells than in untreated cells. This effect of IGF-I was time dependent, requiring at least 3 days to become maximal. High K^+-stimulated tyrosine hydroxylase activity was dependent on extracellular Ca^{2+} in both untreated and IGF-I treated cells. The enhanced high K^+-stimulated tyrosine hydroxylase activity in IGF-I treated cells may be explained, at least in part, by increased Ca^{2+} influx into the cells. (Supported by NIH grants HD-04583,HD-09402 and HL-29025 and NSF grant BNS-8820342.)

287.3

INSULIN-LIKE GROWTH FACTOR-I STIMULATES TYROSINE PHOSPHORYLATION IN BOVINE CHROMAFFIN CELLS. A.L. Cahill and R.L. Perlman Depts. of Pediatrics and Pharmacological and Physiological Sciences, University of Chicago, Chicago, IL 60637

Previous studies in our laboratory have shown that insulin-like growth factor-I (IGF-I) receptors are present on bovine chromaffin cells and that these receptors possess tyrosyl kinase activity against exogenous substrates (Dahmer, Li & Perlman, J. Neurochem. in press, 1989). In the present study we have used antiphosphotyrosine antibodies on Western blots to identify chromaffin cell proteins whose phosphotyrosine content is increased by acute treatment of the cells with IGF-I. Unstimulated chromaffin cells had three major phosphotyrosine containing proteins with approximate Mr's of 122, 71, and 35 kD. When chromaffin cells were treated with IGF-I the phosphotyrosine content of the 122 kD protein was increased and three additional phosphotyrosine containing proteins became apparent: a doublet of 183 and 167 kD, and a band at 96 kD. The 96 kD protein may be the ß-subunit of the IGF-I receptor which is known to be autophosphorylated on tyrosine residues. The effects of IGF-I were maximal at 10 nM and were rapid. Maximal effects of 10 nM IGF-I occurred within 1-2 min, but were detectable after as long as 60 min. (Supported by NIH grants HD04583 and HL29025.)

287.4

REGULATION OF TYROSINE PHOSPHORYLATION BY INSULIN AND IGF-I IN CULTURED FETAL NEURONS. K.A. Kenner* and K.A. Heidenreich Dept.of Medicine, UCSD, La Jolla, CA 92093

Tyrosine kinases are abundant in brain and thought to play a role in neuronal function, but little is known about their identity or regulation. In this study we examined the effects of various growth factors on tyrosine phosphorylation in cultured fetal chick neurons. Phosphotyrosine proteins were detected by immunoblot analysis using antisera against phosphotyrosine. Under basal conditions 3 separate antisera detected 5 major phosphoproteins (174 kDa, 139 kDa,115 kDa, 103 kDa, and 44 kDa) and 9 minor phosphoproteins (150 kDa, 128 kDa, 92 kDa, 87 kDa, 58 kDa, 51 kDa, 39 kDa, 37 kDa, and 35 kDa). Detection of the proteins was prevented by coincubation of the blots with antisera plus 2mM phosphotyrosine, but not by coincubation with 2mM phosphoserine or 2mM phosphothreonine. Insulin or IGF-I increased the phosphorylation of an 87 kDa membrane protein (tentatively identified as the beta-subunits of insulin and IGF-I receptors, respectively). Both peptides enhanced the phosphorylation of pp 87 within 30 sec and achieved maximal levels within 1 min of exposure. Phosphorylation was retained for 30 min (the maximum duration of exposure performed). Comparison of the dose-response curves for insulin and IGF-I suggest that each peptide stimulates autophosphorylation of its own receptor beta-subunit. The maximal response obtained with IGF-I was about 10x higher than that obtained with insulin (6 fold), consistent with the larger number of IGF-I receptors in these cells. EGF and NGF had no effect on neuronal tyrosine phosphorylation. These results demonstrate that both insulin and IGF-I receptors are active tyrosine kinases in fetal neurons and raise the possibility that the growth promoting effects of these peptides may depend on an activated receptor kinase.

287.5

COMPARATIVE EFFECTS OF CERTAIN NEURONAL LESIONS ON GROWTH FACTOR AND INTERLEUKIN-2 RECEPTORS IN RAT BRAIN. J.-G. Chabot, D.M. Araujo, P.A. Lapchak, L. Gewis and R. Quirion. Douglas Hospital Research Centre, Dept. of Psychiatry, McGill University, Montreal, Quebec, Canada.

Receptors for epidermal growth factor (EGF), insulin-like growth factor-I (IGF-I) and interleukin-2 (IL-2) have recently been demonstrated in the mammalian central nervous system (CNS). The fact that the expression of certain growth factors and lymphokines is increased after brain injury prompted us to examine the effect of various CNS lesions on EGF, IGF-I and IL-2 binding sites. Adult male albino rats were subjected to unilateral stereotaxic chemical lesions of either the striatum (kainic acid), hippocampus (kainic acid), and subtantia nigra (SN) (6-OHDA) or a mechanical lesion of the fimbria-fornix. 7 to 21 days after the surgery, brains were processed for *in vitro* receptor autoradiography as previously described. Under our incubation conditions, no significant modification in the density of [^{125}I]EGF binding sites was observed in any lesioned brain. An increase in the levels of [^{125}I]IGF-I binding was observed in the striatum and hippocampus after kainic acid lesion while no modification was observed in these areas after SN lesion and fimbriaectomy. The density of [^{125}I]IL-2 binding was also increased following a chemical lesion of the hippocampus. These results indicate that the expression of EGF, IGF-I and IL-2 binding sites are differentially modulated following the lesion of a given neuronal pathway. (Supported by MRCC and FRSQ, Canada).

287.6

EPIDERMAL GROWTH FACTOR IS NEUROTROPHIC IN RAT EMBRYO MESENCEPHALIC PRIMARY CULTURE. D. Casper, M. Blum and C. Mytilineou Fishberg Research Center in Neurobiology and Department of Neurology, Mount Sinai Medical Center, New York, NY 10029.

Epidermal growth factor (EGF), originally described to exert its effects on epithelial- and mesenchymal-derived tissue, has also been implicated as a neurotrophic factor. The presence of EGF-like immunoreactivity and EGF receptor in the basal ganglia have led us to examine the potential role of this factor on neurons of the midbrain, which receive afferent input from and project to these EGF-containing areas. Primary cultures of rat embryonic mesencephalon from several ages of gestation were established in a chemically defined medium. Cell survival, neuronal morphology and glial cell proliferation were monitored in the presence and absence of EGF by measuring cell number, DNA content, and using immunocytochemistry with neuronal- and glial-specific antibodies. EGF receptors were detected by ^{125}I-EGF binding. Neurotransmitter-specific high affinity uptake systems were measured by inhibitable uptake of radiolabelled dopamine (DA), GABA and serotonin (5-HT). Preliminary results indicate that EGF treatment (at 10ng/ml medium) has effects on both neuronal and glial populations, dependent on expression of the EGF receptors in the cultures. Over the course of the experiment (15 days), there was a 3-4 fold increase in the protein content of the EGF treated and control cultures respectively, suggestive of either cell division or morphological differentiation. GFAP and neurofilament immunocytochemistry demonstrated increases in the number of glia and neurons, indicating that EGF is a mitogen. DA uptake increased up to 10-fold in EGF-treated cultrues, whereas 5-HT uptake increased only 1.5 fold and changes in GABA uptake were only detected in younger embryos under certain conditions. Tyrosine hydroxylase immunohistochemistry indicated that the increase in DA uptake is due to quantitative and qualitative changes in dopaminergic neurons, i.e. increases in cell number and process outgrowth. These neurons however, comprise a small percent of neuronal elements in EGF-treated cultures, suggesting that the mitogenic properties of EGF are exerted either on a population of neuronal precursors not yet expressing markers of neurochemical diffferentiation, or on additional neurotransmitter-synthesizing systems.

287.7

INTACT SMOOTH MUSCLE PREPARATIONS SECRETE TROPHIC FACTORS THAT SUPPORT DISSOCIATED CILIARY NEURONS IN VITRO. L.A.F. Wentzek, C.W. Bowers, E.D. Oliva*, G. Pilar. Dept. Physiol. & Neurobiol., Univ. of Connecticut, Storrs, CT 06269.

The chick ciliary ganglion is divided into two neuron populations, one (choroid neurons) innervating smooth muscle and one (ciliary neurons) innervating striated muscle. Previous studies have established that during development the survival of both types of neurons depends on their target tissues. Although dissociated striated muscles secrete a factor that supports both neuron populations, and extracts of smooth muscle targets contain a factor (CNTF) that supports both neuron populations, attempts to demonstrate that such a factor is secreted from dissociated smooth muscle have failed (Creedon & Tuttle, J. Neurosci. 8: 3100 (1988)).

We demonstrate that when the normally uninnervated avian amnion (a smooth muscle-epithelial preparation) is cultured intact in a defined medium (N2), it secretes a factor that supports greater than 50% of the dissociated ciliary neurons for at least 5 days in defined N2 medium. When the normal target for choroid neurons (the choroid coat of the eye) is cultured intact, it too secretes a trophic factor for ciliary ganglion neurons. Studies are in progress to determine the maximum survival attainable with these secreted factors, their molecular identity, and the reasons for the apparent discrepancies between the trophic effects of intact and dissociated smooth muscle tissues.

Supported by NIH grant #NS 10338.

287.8

BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF): CDNA CLONING AND SEQUENCING J. Leibrock (SPON: Y.-A. Barde). Dept. of Neurochemistry Max-Planck-Inst. 8033-Martinsried FRG

BDNF is a protein that has been purified from pig brain using its ability to support the survival of embryonic neurons in culture. It has been defined as a very rare (purification factor: several million-fold), basic (pI= 10.0) and small (MW=12,300 d) protein. Microsequencing of BDNF (F. Lottspeich, Martinsried) allowed the synthesis of oligonucleotides that were used to isolate cDNA clones. Sequencing of these clones revealed that BDNF is synthesized as a large precursor, pre-pro-BDNF, a protein that consists of 252 amino acids. Mature BDNF corresponds to the last 119 amino acids. The most striking result of the sequence analysis of BDNF is its distinct relatedness to the protein nerve growth factor (NGF). Southern blot analysis reveals that there is only one gene coding for BDNF. A mouse NGF probe used on the same blot reveals that the pig NGF gene is located on DNA fragments of different sizes. Thus, it appears that BNDF and NGF, 2 proteins allowing the survival of (for the most part) different embryonic neurons, are structurally related and coded by different genes.

287.9

NEURONAL SURVIVAL IS PROMOTED BY THE OPENING OF VOLTAGE-GATED SODIUM CHANNELS. F. Collins, J. D. Lile*, and D. J. Smith*, Synergen, Inc., 1885 33rd St., Boulder, Colorado 80301.

The survival of chick embryo ciliary, sympathetic, and sensory ganglia neurons by elevated extracellular potassium requires neuronal depolarization and the opening of L-type voltage-gated calcium channels (Soc. Neurosci. Abs. (1988) 14:368 and Collins and Lile (1989) Brain Res., in press). One would anticipate that other depolarizing agents would also promote neuronal survival by the same means. We have found that veratridine and batrachotoxin, which depolarize neurons by opening voltage-gated sodium channels, also promote survival of ciliary, sympathetic, and sensory ganglia neurons in vitro to approximately the same extent as elevated potassium or the protein neurotrophic factors NGF, bFGF, and CNTF. Neuronal survival promoted by veratridine or batrachotoxin is inhibited by tetrodotoxin, suggesting that these agents act by opening voltage-gated sodium channels. Elevated potassium, which depolarizes by another mechanism, is insensitive to inhibition by tetrodotoxin. Potassium-mediated neuronal survival depends on the opening of neuronal voltage-gated calcium channels: survival is strongly potentiated by voltage-gated calcium channel agonists, such as Bay K 8644, and strongly inhibited by calcium channel antagonists, such as nitrendipine, verapamil, and diltiazem. In striking contrast, these agents have little or no effect on neuronal survival promoted by veratridine or batrachotoxin. These results suggest that although the three depolarizing agents potassium, veratridine, and batrachotoxin all promote extensive neuronal survival, veratridine and batrachotoxin-mediated survival, unlike potassium-mediated survival, does not depend on the opening of voltage-gated calcium channels.

287.10

OLIGODENDROGLIAL SURVIVAL FACTOR FROM PRIMARY HEART CULTURE. A.L. Gard, G.E. Gonye* and S.E. Pfeiffer. Dept. of Microbiology, University of Connecticut School of Medicine, Farmington, CT 06032.

We have observed that ≥ 75% of OL progenitors bearing the O4 surface antigen die within 2-3 days when cultured in modified N2 medium (mN2) following their immunoselection from dissociated postnatal rat telencephalon (Gard, A.L. and Pfeiffer, S.E., *Development*, 106: in press). Working under the hypothesis that specific growth factors present in the developing brain are absent from this culture system, conditioned media from primary cultures of rat neural cell types (Type I astrocytes, cerebellar interneurons, meninges, brain macrophages), newborn skin, heart tissue and 3T3 cells were assayed for activity promoting survival and process regeneration of freshly isolated O4+ progenitors. Target cells were seeded at low density (1.5×10^4 cells cm^{-2}) as microcultures on a polyornithine substratum in mN2 containing 0.25% (v/v) heat-inactivated fetal calf serum and 5-50% (v/v) conditioned mN2 medium (CM). Dose-dependent survival was observed only in CM from dissociated newborn heart (HCM), which promoted up to 80% survival of seeded cells at 3 days in culture; the cells remained non-proliferative and 90% were O4+ by immunofluorescence. A substantially weaker effect (10-20% survival) occurred with 3T3 cell and brain macrophage CM. By 16 hr in culture HCM markedly stimulated process outgrowth from progenitor perikarya. HCM pre-treated with heat (100°C, 10 min) or trypsin did not facilitate process outgrowth or subsequent survival. To test for the specificty of HCM, neuron-enriched cultures were prepared from dissociated 7-8 day-old postnatal rat cerebella in mN2 ± 33%(v/v) HCM. Although >95% of the seeded cells died within 7 days in both treatment groups, a minor population of OL produced myelin-like membrane and was selectively spared in the HCM-treated cultures.

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287.11

CHOLINERGIC DEVELOPMENT OF SPINAL MOTOR NEURONS BUT NOT SPINAL AUTONOMIC NEURONS IS REGULATED BY A FACTOR (MANS) ISOLATED FROM SPINAL CORD MEMBRANES. D. Lombard-Golly*, V. Wong and J.A. Kessler (SPON: K.Spiegel). Dept. of Neurology, Albert Einstein College of Medicine, Bronx, New York 10461.

Neurotransmitter expression is influenced by neuronal interactions with the cellular environment and particularly by contact between cells. We have been examining the effects of cell-cell contact on the development of 2 major populations of cholinergic neurons isolated from ventral (motor neuron enriched) and mediodorsal (autonomic neuron enriched) spinal cord (s.c.). In cultures of ventral s.c., levels of choline acetyltransferase (CAT), were stimulated by increased plating density. At all densities tested, CAT activity decreased during the first week *in vitro* and then increased over the subsequent three weeks. Our laboratory has partially purified a factor (MANS) from s.c. membranes which reproduces the effects of cell-cell contact on sympathetic neurons in culture (Wong and Kessler, 1987). Treatment of ventral s.c. cultures with MANS prevented the decrease in CAT activity normally observed during the first week. Further, MANS treatment resulted in elevation in CAT activity which persisted during the entire 4 week culture period. MANS treatment did not increase the number of cholinergic neurons visualized by CAT immunocytochemistry, suggesting that the factor increased CAT activity per neuron rather than increasing neuronal survival. CAT activity was regulated differently in the mediodorsal s.c. cultures. First, increased plating density did not elevate CAT activity. Second, MANS treatment resulted in only a small initial elevation in CAT activity which did not persist beyond the second week. Our observations suggest that different mechanisms regulate the development of cholinergic motor neurons and autonomic neurons in the s.c.. In motor neurons, cholinergic development is stimulated by cell-cell contact, and this stimulatory effect may be mediated by MANS.

287.12

SOMATOSTATIN EXPRESSION IN CULTURED CILIARY GANGLION NEURONS IS REGULATED BY TARGET DERIVED MACROMOLECULES. **J.N.Coulombe and R.Nishi, Dept. of Cell Biology and Anatomy, Oregon Health Sciences University, Portland, Oregon. 97201**

The avian ciliary ganglion contains two distinct populations of neurons: ciliary neurons which innervate the striated muscle of the iris and ciliary body; and choroid neurons which innervate vascular smooth muscle in the choroid layer of the eye. Although both types of neurons utilize acetylcholine as a neurotransmitter, only the choroid neurons employ the neuropeptide somatostatin as a co-transmitter. We previously found that induction and maintenance of somatostatin in cultured ciliary ganglion neurons (CGNs) depended upon the prescence of cells from the choroid layer. Moreover, somatostatin expression in these neurons was regulated independently of cholinergic differentiation. Here we report that the somatostatin stimulating activity (SSA) present in choroid cell cultures is also found when the choroid cells are lysed with water. This result indicates that SSA does not depend upon the neuron interacting with live target cells and suggests that SSA may be a membrane or matrix attached molecule(s). On the other hand, SSA is also released into the culture medium by choroid cells and can be concentrated by a filter which retains molecules greater than 10kD. In cultures of E8 CGNs, concentrated choroid cell conditioned medium can induce somatostatin expression in more than 75% of the neurons. Further biochemical characterization of SSA is in progress.

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287.13

CONDITIONED MEDIUM FROM ACTIVATED SPLENOCYTES STIMULATES SUBSTANCE P (SP) IN SUPERIOR CERVICAL GANGLIA. G. M. Jonakait and S. Schotland*, Dept. of Biological Sciences, Rutgers University, Newark, N.J. 07102

Conditioned medium from con-A-stimulated rat splenocytes (Rat T-cell Polyclone, Collaborative Research; PC) causes a dramatic, dose-dependent increase in levels of SP in cultures of rat neonatal superior cervical ganglia (SCG). The effect is somewhat specific for SP since the activity of tyrosine hydroxylase, the rate-limiting enzyme in catecholamine biosynthesis, is unaffected. Antibodies to nerve growth factor (anti-NGF; from Dr. Michael Coughlin, McMaster University) do not alter the increase, suggesting 1) that PC can substitute for NGF and 2) that NGF is not involved in mediating the action of PC.

In order to begin to identify active molecule(s), we grew SCG cultures in rat interleukin-2 (IL-2) partially purified from PC. The purification involved ammonium sulfate fractionation and DEAE column chromatography (Collab. Res.). Concentrations equivalent to 5 U/ml of IL-2 mimicked a 5% solution of PC. Higher doses stimulated SP further. However, concentrations up to 20 U/ml of human recombinant IL-2 failed to mimic the effects of 5% PC. While IL-1a increased SP, even 20 U/ml did not mimic the effect of PC. Moreover, the inclusion of anti-NGF eliminated a significant proportion of the increase, suggesting an NGF requirement for IL-1a different from that seen with PC. Purified goat antibodies against IL-1a (a gift from Hoffmann-La Roche) actually increased the efficacy of PC slightly. Further characterizations are in progress.

287.14

ELUCIDATION OF THE PRIMARY STRUCTURE OF CILIARY NEUROTROPHIC FACTOR (CNTF). M. Sendtner*(Spon: H. Thoenen), Dept. of Neurochemistry, Max-Planck-Institute, D-8033 Planegg-Martinsried, FRG.

CNTF was originally characterized as a target-derived neurotrophic factor supporting the survival of chick ciliary ganglionic neurons in vitro. However, it showed a broad spectrum of additional activities including the survival of chick sensory and sympathetic neurons, the shifting of rat optic nerve O-2A progenitor cells towards type 2 astrocytes and inducing cholinergic properties in sympathetic neurons. In view of these diverse activities of CNTF it was mandatory to elucidate its structure in order to approach the physiological and possibly pathophysiological functions of CNTF. The initial steps of CNTF purification were performed according to Manthorpe et al. (Brain Res. 357, 282 (1986)) followed by further HPLC purification suitable for microsequencing after BrCN and tryptic cleavage. Antibodies raised against corresponding synthetic peptides precipitated both purified CNTF and CNTF-activity of crude tissue extracts. In Western blots of rat sciatic nerve and type I astrocytes a single band at the position of purified CNTF was stained. Nucleotide sequence information obtained from cDNA and genomic clones confirmed determined peptide sequences.

287.15

FIBROBLAST GROWTH FACTOR ENHANCES (^{3}H)NOREPINEPHRINE RELEASE AND EXPRESSION OF GAP-43 mRNA IN PC12 CELLS A. A. Lettes, B. Costello*, and J. A. Freeman, Dept. of Cell Biology, Vanderbilt University, Nashville, TN 37232.

In the presence of nerve growth factor (NGF), rat PC12 pheochromocytoma cells undergo differentiation which includes neurite extension and enhanced release of catecholamines by cholinergic agonists. Fibroblast growth factor (FGF) has been proposed to mimic the actions of NGF in PC12 cells. We compared the actions of NGF and FGF on (^{3}H)norepinephrine (NE) release, neurite extension, and induction of GAP-43 mRNA. GAP-43, a growth associated protein, has been proposed to be essential for axonal outgrowth during neuronal development and regeneration.

PC12 cells treated with either NGF or FGF (bovine pituitary) for 3 days released 2-3 fold greater amount of (^{3}H)NE compared to controls when stimulated with 0.5 mM carbachol. These cells were grown in 70% well conditioned medium which maximizes the efficacy of FGF. We failed to demonstrate an effect of FGF on neurite growth even after treatment for 7 days. The induction of GAP-43 message by FGF closely resembled that by NGF.

Our results suggest that (1) FGF can promote functional differentiation even under conditions which do not promote axonal growth, and (2) GAP-43 expression is not related to the extent of neurite growth but rather to the pharmacological differentiation of PC12 cells.

287.16

DEVELOPMENT OF TRANSFORMING GROWTH FACTOR ALPHA IMMUNOREACTIVITY IN RAT BRAIN. S.E. Loughlin, J.A. Noriega*, G. Lee* F.M. Leslie, C.M. Annis and J.H. Fallon, Depts. of Anatomy and Neurobiology and Pharmacology, University of California, Irvine CA 92717.

Transforming growth factor alpha (TGFa) is present in the adult rodent brain. Immunocytochemical techniques determined whether the appearance of this growth factor is correlated with developmental events in rat brain. Three antisera were used which were raised against defined sequences of the precursor, including TGFa 137-151,137-159, and 153-159. In adult brain, all three antisera produced similar patterns of staining in a subpopulation of glial fibrillary acidic protein (GFAP)-like immunoreactive (LI) astrocytes. In developing brain, TGFa-LI was also present in a subpopulation of glia. While many GFAP-LI profiles were present at postnatal day (PND) 0, TGFa-LI glia were restricted to dorsal medial septum. By PND 4, corpus callosum, deep cortex and hippocampus were stained. TGFA-LI was observed in cerebellum by PND 10. Even by PND 36, some regions did not exhibit the same number of TGFA-LI profiles as the adult brain, especially in cortex and striatum. The presence of large numbers of TGFa-LI glia in developing fiber tracts is consistent with a role in the development and maintenance of axonal connections. Since it is a neural mitogen, it is surprising that, at all ages studied, no TGFa-LI was observed in ventricular germinal zones. However, the presence of TGFa-LI in hippocampal and cerebellar germinal zones is consistent with a role in postmigrational mitogenesis. Studies on prenatal brains are currently in progress. Supported by NS 15321 and NS 19319.

287.17

THE DISTRIBUTION OF BASIC FIBROBLAST GROWTH FACTOR mRNA IN THE ADULT RAT BRAIN. A.M.Gonzalez*, N.Emoto*, P.Walicke+, S. Shimasaki* and A. Baird* Molecular and Cellular Growth Biology, The Whittier Institute, La Jolla, CA 92037. +Department of Neuroscience, University of California, San Diego, La Jolla, CA 92037.

The distribution of basic fibroblast growth factor (FGF) mRNA in the adult rat brain was studied by *in situ* hybridization, Northern blot analysis and immunohistochemistry. A single hybridizing mRNA of 6Kb length was clearly detected in the total RNA prepared from hypothalamus, cortex, hippocampus and pons. In contrast, a similar band was barely detectable in pituitary and cerebellum. A series of studies using cells in culture established that *in vitro*, the mRNA is expressed in astrocytes rather than neurons. However, *in situ* hybridization of the rat brain using an anti-sense cRNA probe for rat basic FGF identified a distinct population of neurons in the field CA1 and CA2 of hippocampus, indusium griseum, fasciola cinerea and medial (cingulate) cortex. Thus, there exist discrete loci of basic FGF synthesis in the adult rat brain. These results were supported by the immunolocalization of basic FGF using specific polyclonal antibodies. Basic FGF was localized in perikaria, axons and dendrites of several populations of cells in the hippocampus as well as in cells in the cortex and hypothalamus.

The results establish a wide distribution of basic FGF in the rat brain and suggest that basic FGF may play a particularly important role in the hippocampus.

287.18

IMMUNOLOGICAL LOCALIZATION AND CHARACTERIZATION OF BASIC FGF IN THE ADRENAL GLAND. C.Grothe*, R. Westermann*, and K. Unsicker (SPON: H. Reitböck). Dept. of Anatomy and Cell Biology, Univ. of Marburg, F.R.G:

FGF is a small heparin-binding multifunctional protein. Biological functions include angiogenesis and mitogenicity for many mesoterm-derived cells. In addition, a neurotrophic role of bFGF for several types of neuron has recently been established. Basic FGF has been purified from bovine adrenal gland. Using an antiserum against the full length bFGF we studied its precise distribution, cellular and subcellular localization as well as biochemical characterization in the bovine adrenal gland. Basic FGF-like immunoreactivity was found in the steroidogenic cells of the cortical zona glomerulosa and in the medullary chromaffin cells. Immunoelectron-microscopy revealed a vesicular localization of bFGF in chromaffin cells. Immunochemical analysis using bFGF-enriched fractions (ammoniumsulfate precipitation, anti-bFGF affinity chromatography) revealed the presence of the typical 16-18 KD bFGF band in adrenal extracts of cortex, medulla, and chromaffin cell vesicle content. In addition, immunoreactive material of higher molecular weight (24, 30-33, 46 KD) could be identified. In conclusion, bFGF is distributed in a cell type-specific pattern in the adrenal gland. The location of bFGF in chromaffin vesicles suggests release by exocytosis.

287.19

BASIC FGF AMELIORATES MORPHOLOGICAL AND CHEMICAL DEFICITS IN THE MPTP-MODEL OF PARKINSON'S DISEASE. D. Otto* and K. Unsicker. Dept. of Anatomy and Cell Biology, Univ. of Marburg, F.R.G.

Curative effects of adrenal chromaffin grafts to the Parkinson brain have been suspected to be due to the release of trophic material rather than dopamine. Our group has recently shown that bFGF which can act as a potent neurotrophic factor in vitro and in vivo, is contained in chromaffin cells. Consequently, we have investigated whether bFGF (4 µg), unilaterally administered in gel foam to the striatum of MPTP-treated C57-BL/6 mice, may promote morphological and chemical recovery. Treatment with MPTP (3 x 30 mg/kg on 3 consecutive days) caused an 80 % reduction of dopamine (DA) and virtually complete disappearance of TH-immunoreactive fibers after two weeks in both striata of mice carrying a cytochrome C implant in the right striatum. Replacing cyto C by bFGF led to the reappearance of TH-fibers on the ipsilateral side and diminished the reduction of DA to 55 % on the ipsilateral and 68 % on the contralateral side. Determinations of TH-activity and TH-immunoblotting suggested that bFGF nearly fully reversed the 50 % loss of TH-activity on either side, but restored TH-protein more on the ipsi- than on the contralateral side. Our results suggest that bFGF can mimic the beneficial effects of chromaffin cell grafts to the Parkinsonian brain.

287.20

BASIC FIBROBLAST GROWTH FACTOR PROMOTES THE OUTGROWTH OF FETAL DOPAMINERGIC CELLS TRANSPLANTED TO THE DENERVATED CAUDATE-PUTAMEN OF THE ADULT RAT. H.W.M. Steinbusch, R.J. Vermeulen*, and J.A.D.M. Tonnaer. Dept. Pharmacology, Free University, Amsterdam, The Netherlands.

Basic fibroblast growth factor (bFGF) has been identified as trophic factor for fetal CNS cells. It has shown in vitro a positive effect on the survival of fetal astrocytes and in vivo a preserving effect on damaged adult septal cells. The working mechanism of bFGF is possibly related to a heparin receptor. In our study we have transplanted fetal (E 15) dopaminergic cells to the rat caudate-putamen, which was 6-OHDA denervated 14 days prior to the implantation. The cell suspension was mixed either with bFGF, bFGF and heparin or vehicle. After a survival of 6 weeks the animals were processed for dopamine immunocyto-chemistry. In all three groups of rats dopaminergic transplants survived. bFGF alone has only a very limited positive effect on the outgrowth and number of surviving fetal dopaminergic cells. However, the combination of bFGF with heparin showed a nearly threefold increase in the density of the outgrowing dopaminergic fibers, as noticed semi-quantitatively. In addition the transplants were more extensive and contained more dopaminergic neurons. The outgrowth of these transplants seemed to be more diffuse and extend over a longer distance. These results demonstrate that bFGF in combination with heparin is likely a trophic factor for fetal dopaminergic neurons. (bFGF was supplied by Calbio, USA)

NEUROPEPTIDES AND BEHAVIOR

288.1

GALANIN FRAGMENTS RETAIN BIOLOGICAL ACTIVITY ON DELAYED ALTERNATION IN RATS. J.N.Crawley, T.A.Podruchny*, M.Austin, S.DeMesquita, B.Martin*, G.Fisone*[1], T.Bartfai*[1]. Unit on Behavioral Neuropharmacology, Clinical Neuroscience Branch, NIMH, Bethesda, MD 20892, and [1]Department of Biochemistry, University of Stockholm, Stockholm, Sweden.

Galanin (GAL) is a 29-amino acid neuropeptide which coexists with acetylcholine (ACH) in medial septal neurons projecting to the ventral hippocampus of the rat (Melander et al, Brain Res. 360:130-138, 1985). GAL inhibits the release of ACH (Fisone et al, PNAS 84:7339-7343, 1987), inhibits carbachol-stimulated phosphatidyl inositol turnover (Palazzi et al, Europ. J. Pharmacol. 148:479-480, 1988), and inhibits the ability of ACH to improve working memory in rats with basal forebrain lesions (Mastropaolo et al, PNAS 85:9841-9845, 1988). To identify the minimum sequence of amino acids containing the biological activity of GAL in rat brain, fragments of GAL 1-29 were tested on the food-reinforced t-maze delayed alternation paradigm, in rats with ibotenic acid lesions of the nucleus basalis of Meynert-medial septal area. Sequences 1-16, 17-23, and 24-29 were enzymatically cleaved from GAL 1-29; sequences 12-29, 18-29, and 21-29 were prepared by solid phase synthesis. Behavioral scores after intraventricular microinjection with equimolar concentrations of each fragment, in combination with ACH, indicated that C-terminal fragments actively inhibited ACH on delayed alternation in lesioned rats. Short sequences of C-terminal amino acids may be sufficient to convey the biological activity of GAL, and may be useful substrates for modifications to confer galanin antagonist activity.

288.2

MALE-FEMALE DIFFERENCES IN GRF-INDUCED FEEDING. P.R. Dickson and F.J. Vaccarino, Dept. of Psychology, Univ Toronto, Ontario, Canada, M5S 1A1.

We have previously shown that intracerebroventricular (icv) injection of growth hormone releasing factor (GRF) increases food intake in male rats. In males, GRF interacts with somatostatin to release growth hormone in regular 3 hour pulses; this pattern does not hold for females. In order to examine the possibility that males and females also differ with regard to central GRF actions, females were tested for GRF-induced feeding.

Food (Purina Rat Chow) and water intake of female Wistar rats with cannula implants aimed at the lateral ventricles were measured for a 90 minute period on 5 consecutive days following 60 minutes exposure to fresh food. On subsequent days, consumption was measured for the 90 minutes following icv GRF treatment. Vaginal smears were taken following manipulations every day. The following doses were tested in random order: 0.0, 10.0, 20.0, and 40.0 pmoles. rhGRF was dissolved in a 0.01% ascorbic acid vehicle and administered in a 2.0 ul volume over 1 minute. 1 no-drug day separated drug tests.

GRF had no consistent effect on food or water intake in female rats. Further, there was no clear relationship between response to GRF and phase of estrus cycle. This can be taken as tentative evidence for a sex difference in GRF effects on food intake. It remains to be seen how sex steroids may influence the GRF effects on food intake in both male and female rats.

This research was supported by a NSERC grant to F.J.V.

288.3

STRUCTURE-ACTIVITY RELATIONSHIP OF BOMBESIN(BBS)-RELATED PEPTIDES AS EXAMINED BY BEHAVIORAL IN VIVO STUDY. A.Masui*, T.Itoshima*, K.Tsunashima* and N.Kato (SPON:K.Satoh) Dept.of Psychiatry, Shiga Univ.Med.Sci.,Otsu 520-21 and Musashi Hosp. N.C.N.P., Tokyo 187, Japan.

BBS, a tetradecapeptide originally isolated from amphibian skin, has potent biological effects such as hypothermia and excessive grooming. In the present study, the contents of immunoreactive gastrin-releasing peptide (IR-GRP) in rat central nervous system and the behavioral effects of icv administered BBS-related peptides were examined. IR-GRP was widely distributed, but not BBS, in discrete brain regions. BBS elicited intense and long-lasting scratching behavior in a dose-dependent manner. Naturally occurring peptides such as neuromedin B and GRP-10 seemed to be short-acting. Leu-phyllolitorin was as potent as BBS and acted for a longer period than Phe-phyllolitorin. DesTrp anlogues seemed to have no biological potencies and the preliminary study indicates that pretreatment with these peptides appeared to inhibit phyllolitorin-induced scratching behavior. The possibility of antagonizing action of desTrp analogues is currently examined in terms of other behavioral indexes and compared with the BBS antagonists recently reported.

288.4

MICROINFUSION OF BOMBESIN INTO THE PREOPTIC AREA PRODUCES HYPOTHERMIA IN INSULIN TREATED RATS. A.M. Babcock*, and C. Barton* (SPON: R.R. Yeoman). Dept. of Psychology, Univ. of South Alabama, Mobile, AL 36688.

Bombesin (BBS) produces hypothermia in food-deprived, but not food-sated rats at normal ambient temperatures (Calisher et al., Neuropharm. 21:1059, '82). The role of food deprivation in this response has not been evaluated. The present experiment examined the effects of BBS microinfusion into the preoptic area of the anterior hypothalamus (POA) on food intake and core body temperature (T_b) under conditions of food satiation, food deprivation (18hrs), and insulin pretreatment (Regular Iletin I, 10U/kg). Bombesin (50 ng/.25 µl) produced a significant fall in T_b for food-deprived and insulin treated rats. However, no hypothermia was observed in food-sated rats. Bombesin also significantly reduced feeding in food-deprived rats. Our findings suggest that the POA is a sensitive region for BBS-induced hypothermia in food-deprived rats and that factors associated with the fasting state are important in the production of this response.

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288.5

CYSTEAMINE-INDUCED SOMATOSTATIN DEPLETION AND WORKING MEMORY DEFICITS IN RATS.

G.R. Sessions, E. Demetriades*, L.L. Leber* and G.F. Koob. Walter Reed Army Institute of Research, Washington, D.C. 20307, U.S. Air Force Academy, Colorado Springs, CO 80840 and Scripps Clinic & Research Foundation, La Jolla, CA 92037.

Recent research revealing somatostatin depletions in the brains of patients with Alzheimer's Disease has focused interest in the functional role of this neuropeptide in learning and memory *(Roberts, et al., Nature, 1985, 314:92)*. Cysteamine HCl has been shown to temporarily deplete brain somatostatin in rats when administered intraventricularly (ICV) *(Bakhit, et al., 1983, Reg. Peptides, 6:169)*, and has been used in an experimental model for the study of the functions of the peptide in the mediation of behavior. The present study investigated the effects of cysteamine-induced depletions of central somatostatin on performance of an eight arm radial maze memory task in rats.

Animals received four daily post-trial ICV injections of cysteamine (250 µg, N=9) or saline (N=6) following extensive training on the task, then their performance was followed as they recovered from the effects of drug treatments. The drug-injected animals showed a small decrement in Working Memory without impairment of Reference Memory, and only while they received the drug. The alterations and recovery in Working Memory demonstrated by the injected animals followed closely the known course of somatostatin depletion after ICV injections of this drug. The results suggest that somatostatin depletion may produce selective deficits in neural processes underlying Working Memory.

288.6

LOW DOSES OF CENTRALLY ADMINISTERED SOMATOSTATIN INCREASES FOOD INTAKE IN RATS.

D. Feifel and F.J. Vaccarino. Department of Psychology, University of Toronto, Toronto, Canada, M5S 1A1.

Peripherally administered somatostatin (SRIF) has been consistently reported to attenuate feeding. The literature on centrally administered SRIF, however, is equivocal with some investigators citing a facilitation and others an attenuation of feeding.

To clarify the central feeding effects, if any, of SRIF, male Wistar rats were injected with 0, 0.4, 4.0 and 40 picomole SRIF in a 2µl vehicle (0.01% ascorbic acid) solution. The injections were administered via a chronically implanted cannula aimed at the lateral ventricle.

One hour post-injection measurements indicated that food intake was significantly increased with 0.4 and 4.0 picomole doses of SRIF. None of the doses tested had an effect on 24 hour intake. As well, locomotor activity, as measured by photocell activity cages, was unaltered. These results indicate that, in contrast to its peripheral anorexic effects, SRIF, in low doses, may act centrally to potentiate feeding.

This research was supported by a NSERC grant to FJV.

288.7

EFFECTS OF NEUROMEDIN B-32, NEUROMEDIN B-10 AND BOMBESIN ON INGESTIVE AND GROOMING BEHAVIORS.

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The functional significance of neuromedin B-10 (NB-10) and Neuromedin B-32 (NB-32), the newly isolated mammalian peptides, remains to be elucidated. This study comparatively assessed the behavioral effects of centrally administered NB-10 (0.31-6.2 nmol), NB-32 (0.062-3.1 nmol) and the amphibian structural analogue bombesin (BN; 0.0062-3.1 nmol). Rats implanted with 3rd ventricular cannulae were entrained on a schedule of 20 hr water deprivation. Following peptide administration, wet mash test meal and water intake as well as grooming behaviors were monitored. BN dose-dependently suppressed food and water intake and elicited intense grooming. The order of potency was grooming > anoretic > antidipsogenic. The order of relative effectiveness (potency and efficacy) on all the end points was: BN > NB-32 > NB-10. As compared to BN, NB-32 was 5X less potent for grooming effects and 6-10X less potent in suppressing ingestive behaviors. NB-10 was an order of magnitude less potent than NB-32. Thus in mammals, NB-32 and NB-10 may play a role in central mechanisms of grooming and ingestive behaviors. (Supported by NSERC).

288.8

NEUROBEHAVIORAL EFFECTS OF CALCITONIN GENE-RELATED PEPTIDE.

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CGRP-like immunoreactivity and putative receptors for the peptide have been reported to be widely distributed in brain of several animal species, including man, suggesting that CGRP might be implicated in a variety of neurophysiological processes. The purpose of the present study was to examine the profile of the neurobehavioral effects of CGRP following central administration. More specifically, the effects of various doses (0.312 -20.0µg) of human CGRP injected into the lateral ventricle of rats on spontaneous activity, muscular tone, body temperature, nociception, food and water intakes, as well as its potential for inducing catalepsy, were examined. hCGRP produced significant increases in body temperature, decreases in activity, nociception and muscular tone, and induced a strong catalepsy in animals. Threshold doses for these effects were : 0.625, 0.625, 5.0, 20.0 and 0.625 µg, respectively. *Ad libitum* food and water intakes were not affected by the peptide but, starting at a dose of 5.0 µg, both were significantly decreased in 22 hr food deprived animals. In a first structure-activity study, we have found that the pre-injection of 10 µg of the C-terminal fragment CGRP(8-37) significantly inhibited the hypophagic and hypodipsic, but not the hyperthermic effects of the same dose of hCGRP. A comparison of our dose effect relationships and our initial structure-activity study suggest that a heterogeneity of hCGRP central receptors might underlie various neurobehavioral effects of the peptide.

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288.9

LOCAL INFUSION OF TRH OR CGRP IN THE CENTRAL NUCLEUS OF THE AMYGDALA ENHANCES ACOUSTIC STARTLE.

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The central nucleus of the amygdala (ACE) has been shown to be critical for fear-potentiation of acoustic startle as well as the delayed increase in startle observed after footshock. Because ACE has high receptor densities for both Thyrotropin releasing hormone (TRH) and Calcitonin gene related peptide (CGRP) and because local infusion of these peptides in the ACE have been shown to produce behavioral responses similar to amygdala stimulation, the present study evaluated the effect of local infusion of TRH and CGRP in the ACE on the acoustic startle reflex.

All drugs were infused bilaterally in the ACE of freely moving rats. Acoustic startle was measured 15 min prior to infusion and for 90 min immediately following it. TRH (2, 4 or 7 µg /0.5 µl), the TRH analog RX77368 (.01, .02 or .04 µg / 0.5 µl) and CGRP (2 µg /0.5µl) were used. Startle was markedly increased following administration of 7 but not 2 or 4 µg of TRH. This increase did not appear until 30-40 min after injection of the drug. This delay is probably not due to diffusion of the drug to a distant site because intracerebroventricular infusion of 40 µg/10µl TRH had no effect on acoustic startle. Administration of .04 but not .01 or .02 µg of RX77368 markedly enhanced startle with a delay of 30-40 min. Preliminary data suggest that 2µg CGRP also facilitates startle with a similar delay.

The delayed enhancement of startle following administration of TRH or CGRP is similar to the delayed increase in startle seen following footshock. It is thus possible that TRH and/or CGRP are released into the amygdala following footshock. Activation of the amygdala may represent an unconditioned response to shock relevant to fear conditioning and TRH and CGRP may be the neurotransmitters necessary for this unconditioned effect.

288.10

THE EFFECT OF NEUROPEPTIDES IN ANIMAL MODELS FOR DEPRESSION INVOLVES DOPAMINE TRANSMISSION IN THE BRAIN.

F. Drago, F. Spadaro* and U. Scapagnini*. Inst. of Pharmacology, Univ. of Catania Medical Sch., Italy.

TRH and prolactin (PRL) stimulate various behaviors in the rat. In two animal models for depression, the despair test (DT) and the chronic unpredictable stress (CUS) procedure, TRH exerted "antidepressant" activity both after peripheral and icv administration, and potentiated the effect of desipramine in the DT. This effect was mimicked by the analogue RGH-2202, which is devoted of thyreotropic actions, but not by TSH, T3 or T4. The treatment with the D1 and D2 receptor antagonists, SCH23390 and sulpiride, inhibited the "antidepressant" effect of TRH. No change was found after prazosine or methysergide treatments.

Prolactin failed to exhibit a direct "antidepressant" effect in young rats but potentiated that of desipramine in the DT and CUS procedure. A clear effect, however, was found in old rats that show a spontaneous "depressive" actitude. Icv injection of rat PRL or endogenous hyperprolactinemia, as raised by pituitary homografts, were merely equipotent in this respect. Again, the block of dopamine receptors reduced this effect.

288.11

SIMILAR FEEDING RESPONSES FOLLOWING THE INTRAHYPOTHALAMIC INJECTION OF NEUROPEPTIDE Y (NPY) AND ITS HOMOLOGUES. T. Foley-Nelson, A. Balasubramaniam*, S. Sheriff*, J.E. Fischer* and W.T. Chance. Dept. of Surgery, University of Cincinnati Med. Ctr. and VAH, Cincinnati, Ohio 45267.

Although several peptides affect feeding, NPY appears to be the most potent enhancer of feeding yet described. To determine the generality of this feeding response, we assessed intake of rat chow for 1 hr following the injection into the paraventricular hypothalamic area of 2 μg NPY or the same dose of 3 NPY homologues found in fish pancreas: anglerfish peptide Y (aPY-NH_2), salmon pancreatic polypeptide (sPP) and daddy sculpin pancreatic polypeptide (dsPP). As a comparison, all rats (n=18) were tested initially for feeding following control injections (1 μl) of artificial CSF followed 60 min later by 2 μg norepinephrine (NE). The following week CSF injections were again given, with half of the rats being treated with NPY and the remaining rats receiving aPY-NH_2. Two days later, food intake was again assessed following the injection of sPP and dsPP counterbalanced across the previous treatments. As seen in the

CSF	NE	NPY	aPY-NH_2	sPP	dsPP
0.4±0.2	2.6±0.4	5.6±1.0*	6.2±0.6*	8.0±0.9*	8.6±1.1*

table, food intake following NPY was twice as great as that following NE (*$p<0.01$). Feeding to aPY-NH_2, sPP and dsPP was equipotent to NPY, suggesting a generality of feeding across these homologous peptides. The results show that homologous peptides are able to exhibit NPY-like feeding, possibly by interacting with NPY receptors in hypothalamus.

288.12

PERTUSSIS TOXIN INHIBITS NEUROPEPTIDE Y INDUCED FEEDING IN RATS. A. Balasubramaniam*, S. Sheriff*, T. Foley-Nelson, J.E. Fischer* and W.T. Chance. Dept. of Surgery, Univ. of Cincinnati Med. Ctr. and VAH, Cincinnati, OH 45267.

Neuropeptide Y (NPY) is one of the most potent stimulants of feeding behavior. Rats with 24 ga cannulae implanted into the paraventricular hypothalamic area (PVH) were used to investigate the mechanisms involved in the NPY-induced feeding. Consistent with previous reports, injection of NPY (2 μg in 1 μl of artificial CSF) into PVH significantly increased cumulative food intake over 1, 2 and 4 h periods. Injection of pertussis toxin (PT) (2 μg in 1 μl) had no immediate effect on basal or NPY-induced feeding. However, 4 days after PT injection, food intake following injection of NPY (2 μg) was reduced to control level and ad lib. feeding was decreased 18%. In vitro investigations revealed that isoproterenol-stimulated cAMP production in hypothalamus of control rats was inhibited by NPY. In PT-treated rats, however, no inhibition of cAMP production by NPY was observed.

Cumulative Food Intake (g ± SEM; *$p<0.01$ vs Control)

	Control	NPY	PT (Basal)	PT + NPY
1 h	1.9 ± 0.7	7.6 ± 0.7*	0.6 ± 0.5	0.8 ± 0.5
2 h	2.2 ± 0.7	9.8 ± 0.7*	0.6 ± 0.5	2.7 ± 1.1
4 h	2.4 ± 0.7	10.8 ± 0.7*	2.0 ± 0.7	2.5 ± 1.5

These results suggest that cAMP may mediate NPY-induced feeding and that a PT-sensitive G protein may be involved in this signal transduction.

288.13

BILATERAL ENUCLEATION CAUSES A DECREASE IN SUBSTANCE P IMMUNOREACTIVITY IN THE MATING BEHAVIOR PATHWAY OF THE MALE GOLDEN HAMSTER

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Mating behavior of the male Golden Hamster (*Mesocricetus auratus*) is mediated by three critical areas in the chemosensory pathway; Medial Nucleus of the Amygdala (M), Bed Nucleus of the Stria Terminalis (BNST), and the Medial Preoptic Area (MPOA). Neurons in these areas actively accumulate gonadal steroids. We have recently demonstrated that, 1) neurons in M, MPOA, and BNST display substance P immunoreactivity (SPIR), and 2) SPIR is decreased in castrated animals and restored with testosterone treatment. Mating behavior in the hamster is affected by photoperiod, therefore, we examined the effects of a short photoperiod on SPIR by bilateral enucleation.

Adult, male Golden Hamsters were either, 1) enucleated (n=6), 2) enucleated, castrated and treated with testosterone (n=6), 3) castrated and treated with testosterone (n=6), or 4) intact controls (n=6). After seven to ten weeks, each hamster was injected with colchicine into the lateral ventricle, and 48 hours later, perfused with 2% paraformaldehyde and 0.25% benzoquinone. Free floating sections (40μm) were processed for SPIR using the indirect method of Sternberger.

All groups showed similar SPIR in MPOA. Compared to intact controls, enucleated animals showed testicular regression and few, if any, SP containing neurons in BNST and M. Testosterone-treated castrates and testosterone-treated, enucleated castrates did not significantly differ from intacts for testicular regression or SP containing neurons in M and BNST. These results suggest that bilateral enucleation causes a decrease in SPIR in M and BNST that seems to be mediated by testosterone.

288.14

MOTIVATION AND ATTENTIONAL MEASURES FROM A TEMPERATURE REINFORCED CUED DISCRIMINATION TASK IN RATS. C.W. Simpson*, G.E. Regch* (SPON.: D. Justesen). Sch. Basic Life Sci., Univ. MO-KC, Kansas City, MO, 64108

Rats under cold stress are known to maintain core temperature physiologically and behaviorally. Rats were trained to a cued discrimination task for infra-red heat reinforcement (CD/IR) in a -4°C ambient temperature (Ta) environment. CD/IR was run in an interactive computer driven program that permitted the differential measurement of response parameters. To the best of the authors' knowledge this is the first report in which CD/IR has been used to show differential change in motivation and attention. The data show 1) discrimination of correct cues, 2) increased motivation at lowered colonic temperatures (Tc), and 3) PGE2-elicited increased attention shown by a decrease error rate. Discrimination of cues was shown by increases in % correct responding from a low point of 69% at the beginning of training to a final criterion of >85%. Rats trained to the 85% criterion showed consistent performance, ie. number, rates (bar press/min), and %, of responding between correct cue, inappropriate cue, and intertrial interval. Increased motivation was indicated by a comparable increase in all parameters when Tc was decreased below 38°C down to 30.5°C. Rats at Tc values of 26.5° to 30°C had a significant decrease in all parameters consistent with inability to perform in the task. Performance following injection of 1pg PGE2 into the MAHPOA showed 1) increased total, i.e. correct and incorrect, responding at Tc's <38°C and 2) a differential decrease of incorrect responding at Tc's >38° C. The data indicate that with the CD/IR paradim, motivation and attention were changed by central PGE2 microinjection. (Supported by USAFOSR-87-0297.)

NEURAL-IMMUNE INTERACTIONS III

289.1

NEUROPEPTIDES AND THE IMMUNE SYSTEM: PEPTIDERGIC NERVE FIBRES AND RECEPTOR BINDING SITES LOCALIZED IN THE BURSA OF FABRICIUS AND THYMUS. C.B. Lacey, V.S. Seybold, and R.P. Elde. Dept. of Cell Biology and Neuroanatomy, University of Minnesota, Minneapolis, MN 55455.

Several peptides have been implicated in the communication between the nervous and immune systems. In previous studies we have shown a sparse but generalized distribution of peptidergic innervation in the **bursa of Fabricius**, a dorsal diverticulum of the caudalmost portion of the GI tract, which is recognized as the site of B-cell development and differentiation in avian species. In the present study we noted numerous peptidergic nerve fibres in a region located close to the burso-cloacal duct. This area is believed to be functionally distinct and has been referred to as the diffusely infiltrated area (DIA). Vasoactive intestinal peptide (VIP)-, calcitonin gene related protein (CGRP)-, and substance P (SP)- immunoreactive (ir) fibres were localized in the DIA. Furthermore, VIP-ir cell bodies were observed within the outer smooth muscle surrounding the bursa, the DIA, and the burso-cloacal stalk. Immunoreactive nerve fibres were also demonstrated in both avian and mammalian **thymus**. VIP-, CGRP-, SP-, NPY-, and DβH- immunoreactive fibres were localized to blood vessels, interlobular connective tissue and the parenchyma of both thymic cortex and medulla in the rat and with less frequency in the chick.

In addition to peptidergic fibres, we have begun to investigate the presence of receptor binding sites in both lymphoid organs in the chick. *In vitro* autoradiographic localization of ^{125}I-VIP demonstrated specific binding associated with vascular elements in the interfollicular (bursa) and interlobular and trabecular (thymus) regions as well as in the DIA and the outer muscular layers of the bursa and the medullary regions of the thymus.

These results suggest that peptidergic nerve fibres and receptors may participate in the modulation of the immune response in lymphoid tissues responsible for the development of immunocompetency in both avian and mammalian species.

289.2

INNERVATION OF THE SPLEEN IN THE RAT: ABSENCE OF AFFERENT INNERVATION. D. M. Nance and J.Burns*. Dept. of Anatomy, Dalhousie University, Halifax, Nova Scotia, B3H 4H7, Can.

Catecholamine fibers in the spleen of the rat are believed to be an important source of modulation of the immune system. The presence of afferent feedback from the spleen has not been systematically investigated. We examined whether the spleen receives afferent innervation and assessed the sources of efferent innervation to the spleen. The tracers fluoro-gold (FGo), fast blue (FB), WGA-HRP and HRP were used. The effects of cutting the splenic nerve on labeling of ganglion cell bodies and on catecholamine fibers in the spleen were assessed. The results showed that the celiac-mesenteric plexus provides a major efferent input to the spleen. Also, thoracic sympathetic chain ganglia provide an additional efferent supply to the spleen. Cutting of the splenic nerve prevented labeling of cell bodies in the sympathetic ganglia and eliminated catecholamine fibers in the spleen. In terms of afferent labeling, the results with FGo indicated that there were no cell bodies labeled in any sensory ganglia following splenic injections. Injections of WGA-HRP and exposing the cut end of the splenic nerve to HRP substantiated the observations based upon FGo. In contrast, FB produced faintly labeled cell bodies in the dorsal root ganglia irrespective of their spinal levels and this was not eliminated by cutting the splenic nerve. FB produced false positive labeling of afferent cell bodies following splenic injections. Thus, the spleen receives virtually no afferent supply but rather, receives an extensive efferent innervation from both the celiac-mesenteric and sympathetic chain ganglia. The effects of nerve section indicate that all of the efferent fibers to the spleen travel in the splenic nerve. Supported by MRC of Canada.

289.3

INTERLEUKIN-1 IMMUNOREACTIVE NERVE FIBRES IN THE RAT BONE AND ADJOINING TISSUES. A. Bjurholm*, A. Kreicbergs*& M. Schultzberg, Dept. of Orthopaedic Surgery, Karolinska Hospital, Dept. of Pathology, Karolinska Institute, Huddinge Hospital, Stockholm , Sweden.

The 17kD protein interleukin-1 (IL-1) was isolated from human monocytes and recognized as an endogenous pyrogene and lymphocyte activating factor. Among a number of actions IL-1 has been implicated in the regulation of bone physiology and in the pathophysiology of rheumatoid arthritis. The present study was carried out to analyze the occurrence of IL-1 in intraosseal nerves, since IL-1 like immunoreactivity was previously demonstrated in nerves in many peripheral organs.Substance P-, CGRP-, NPY- and VIP-immunoreactive and noradrenergic nerves have previously been shown to occur in the rat femur and tibia. The rats were perfused with para-formaldehyde and picric acid, and the bones, freed from muscles, were demineralized for approximately 3 weeks in buffered ethylene-diaminotetraacetic acid (4%). Following soaking in 10% sucrose, the tissue was cut and processed for indirect immunohistochemistry using an antiserum raised against a synthetic peptide corresponding to the amino acid sequence 169-194 of the murine IL-1alpha precursor protein. IL-1 immunoreactive fibres were observed almost exclusively in connection to blood vessels. A few fibres were seen along periosteal blood vessels, but the majority of the IL-1 immunoreactive fibres occurred in the bone marrow of the epiphysis. The latter fibres were localized around blood vessels in the marrow, and sometimes in connection to the epiphyseal plate. The IL-1 immunoreactive fibres were more sparse in the diaphysis, and none could be seen in the bone or cartilage tissue itself. Blood vessels surrounded by IL-1 immunoreactive fibres could also be seen in the synovial membrane.

In conclusion, IL-1 immunoreactive nerve fibres were found in the rat femur, tibia and knee joint, predominantly around blood vessels, and were most numerous in the epiphysis. The importance of this source of IL-1 in normal bone physiology and in the pathophysiology of bone and joint disease remains to be elucidated. The distribution pattern was similar to that of NPY- and TH-positive fibres suggesting coexistence of the two proteins in noradrenergic fibres.

289.4

SUBSTANCE P (SP) AND CALCITONIN GENE-RELATED PEPTIDE (CGRP) INNERVATION OF THE RAT SPLEEN. D. Lorton, D.L. Bellinger, S.Y. Felten, and D.L. Felten. Department Neurobiology & Anatomy, University of Rochester School of Medicine, Rochester, NY 14642.

Numerous investigators have demonstrated measurable levels of SP in 1° and 2° immune organs, SP receptors on immune cells, and alteration in a variety of immune parameters following administration of SP agents. Further, CGRP is co-localized within SP nerve fibers in gut-associated lymphoid tissue and other peripheral organs. In the present study, we investigated the distribution of SP+ and CGRP+ nerve fibers in the spleen of adult male Fischer 344 rats with immunocytochemistry. SP+ varicose plexuses entered the spleen with the splenic artery in the hilar region. The fibers distributed mainly along trabecular systems and venous sinuses. A few fine fibers extended away from the trabeculae and venous sinuses into the outer zone of the periarteriolar lymphatic sheath (PALS). These fibers were associated with vascular plexuses in the marginal zone (MZ); a few varicose fibers extended into the parenchyma of the MZ. An occasional fiber wandered through the PALS in sites where T cells predominate. Numerous individual linear SP+ fibers were observed in the red pulp. Few, if any, SP+ profiles were associated with the capsule or the central arteriolar system of the white pulp.

CGRP+ profiles showed a similar density and pattern of distribution in the spleen. CGRP+ immunoreactivity was found in varicose fibers along trabeculae, venous sinuses, MZ, PALS, and red pulp. The similarity in the innervation of SP+ and CGRP+ fibers support the hypothesis that these peptides may be co-localized in the spleen. The presence of SP+ and CGRP+ immunoreactivity in discrete compartments of the spleen, coupled with the presence of SP receptors on lymphocytes and evidence for a modulatory role for SP in both cell-mediated and humoral immunity, suggest that neurally derived SP in the spleen may act as a neurotransmitter with immune cells as targets. Whether or not these SP+ and CGRP+ fibers are sensory axons remains to be demonstrated. (Supported by R37 MH4207, R01 NS25223, and N00014-84-K-0488 from ONR)

289.5

SUBSTANCE P (SP) INNERVATION OF THE RAT THYMUS. S.Y. Felten, D.L. Lorton, D.L. Felten. Department of Neurobiology and Anatomy, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642.

Experimental evidence suggest that SP modulates both cellular and humoral immune responses through interactions with specific receptors on lymphocytes, and measurable levels of SP have been reported in both 1° and 2° immune organs. We undertook the present study to look for a possible neural source of SP in the thymus that could provide neuromodulator signals for such interactions in vivo in this organ. SP innervation of the thymus in adult male Sprague Dawley rats was examined using immunocytochemistry. SP+ immunoreactivity was present in fine varicose neural-like profiles localized in specific regions of the thymus. SP+ varicose fibers were associated with the septa of interlobular connective tissue, and appeared to be present in regions where high densities of mast cells were observed. From the interlobular septa, SP+ fibers were observed entering the thymic cortex, where immature thymocytes reside. In the medullary region, numerous tangles of fine delicate SP+ fibers which resembled sensory nerve endings were noted. We are undertaking double label studies to ascertain the cellular associations of these fibers. We do not yet know whether these SP+ fibers are sensory or autonomic: however, regardless of the source of these fibers, it is likely that they can release SP and make it available for interactions with lymphocytes or accessory cells possessing receptors for SP. If the SP+ nerve are sensory axons, then an additional role can be hypothesized for possible immune-neural signaling using primary sensory axons for communication into the CNS. (Supported by R37 MH 4207, R01 NS25223, and N00014-84-K-0488 from ONR)

289.6

EFFECTS OF AGE ON SUBSTANCE P (SP)+ NERVE FIBERS IN THE SPLEEN OF FISCHER 344 RATS. D.L. Bellinger, D. Lorton, S.Y. Felten, and D.L. Felten. Department of Neurobiology & Anatomy, University of Rochester, School of Medicine, Rochester, NY 14642.

Immunocytochemistry was used to demonstrate SP+ nerve fibers in the spleen of Fischer 344 rats at 3, 8, 12, 17, 21, and 27 months of age. In young adult rats, SP+ nerve fibers entered the spleen with the vasculature, and penetrated the parenchyma as vascular and trabecular plexuses. Within the spleen, SP+ nerve fibers were found in nerve bundles along the large venous sinuses, and extended from these sinuses along trabeculae. Varicose SP+ profiles exited the venous plexuses into the surrounding red pulp and were present abundantly in this compartment. SP+ fibers were not observed around the central artery of the white pulp; however, occasional varicose linear fibers were found in the periarteriolar lymphatic sheath. In addition, SP+ vascular plexuses and long linear fibers were present in the marginal zone.

With normal aging, no apparent loss in the density of SP innervation of the spleen was observed; however, the distribution of SP+ nerve fibers was altered. SP+ nerve fibers retained their compartmentation, but appeared to regress toward the hilar region of the spleen, the site of entry of these nerve fibers. By 17 months of age, a progressive gradient loss of SP+ nerve fibers was observed with increasing distance from the hilus, continiuing thoughout 27 months of age. The presence of SP-containing nerve fibers in the spleen, coupled with other reports of chemical measurements of SP, the presence of SP receptors on immunocytes, and functional studies demonstrating alterations in immune parameters to administered SP and denervation with capsaicin, suggest that SP may modulate the immune system through direct "hardwired" communication channels from the central nervous system. Age-associated regression and redistribution of SP+ nerve fibers resembles some aspects of age-related NA and NPY+ fiber changes, suggesting that degenerattive or compensatory mechanisms may contribute to gradual alterations in the microenvironment of the spleen that accompany loss of immune competence. Supported by R37 MH4207, R01 NS25223, and N00014-84-K-0488 from ONR.

289.7

AGE-RELATED ALTERATIONS IN THE DISTRIBUTION OF NEUROPEPTIDE Y (NPY)-POSITIVE NERVE FIBERS IN THE RAT SPLEEN. D.L. Felten, D.L. Bellinger, and S.Y. Felten. Department of Neurobiology & Anatomy, University of Rochester School of Medicine, Rochester, NY 14642.

With light microscopic immunocytochemistry (ICC), NPY+ nerve fibers distribute in the rat spleen with a pattern remarkable similar to that of noradrenergic (NA) sympathetic innervation (Olschowka etal. 1988, Soc. Neurosci. Abstr. 14: 1288). Preliminary studies indicate that NPY may co-localize within NA nerve fibers in the spleen. Recent studies from our laboratory have reported that NA innervation in the spleen declines with age (S. Felten etal., 1987 Neurobiol Aging 8: 159-165). In the present study, we investigated whether a similar decline in splenic innervation occurs with NPY+ terminals.

ICC was used to demonstrate age-related changes in NPY+ nerve fibers in the spleen of F344 rats at 3, 8, 12, 17, 21, and 27 months of age. In young adult rats, an abundance of NPY+ nerve fibers distributed to specific compartments of the spleen. NPY innervation resembled the distribution pattern of splenic NA nerve fibers, but appeared to be slightly more robust. NPY innervation of the spleen remained constant through 12 months of age. By 17 months of age, a decline in density of NPY+ nerve fibers was apparent, and continued to diminish through 27 months of age. The depletion of NPY+ nerve fibers occurred with the same general pattern and time course observed for NA innervation; however, the loss of NPY+ nerve fibers did not appear to be as severe. These findings suggest that NPY co-localizes within NA nerve fibers that distribute to the spleen of F344 rats, and also may be present in non-NA nerve fibers. Further, based on previous findings that NPY can potentiate NE responses in some vascular beds (Beckner and Farrar), we suggest that NPY may interact with NE on the surface of immune cell targets to possibly potentiate the NA signal. Age-related loss in NPY+ and NA nerve fibers in the rat spleen may play a role in the decline of immunocompetence that occurs with age, or may occur secondary to age-related change in the immune system. Support by R37 MH4207, R01 NS25223, and N00014-84-K-0488 from ONR.

289.8

NEUROPEPTIDE-Y INVOLVEMENT IN NEURAL-IMMUNE INTERACTIONS IN THE RAT SPLEEN. T.A. Romano, J.A. Olschowka, S.Y. Felten and D.L. Felten. Dept. of Neurobiology and Anatomy, University of Rochester School of Medicine, Rochester, NY 14642.

It has been demonstrated at the light microscopic (LM) and electron microscopic (EM) levels that noradrenergic nerve fibers in addition to innervating the splenic capsule, trabeculae, and vasculature, enter into specific immune compartments of the spleen, contacting specific lymphoid cells. In addition to norepinephrine (NE) containing nerve terminals, there is evidence demonstrating neuropeptide-Y (NPY) containing nerve fibers innervating immune compartments of the spleen, such as the periarteriolar lymphatic sheath where contacts with T lymphocytes are probable, and the marginal sinus where contacts with B lymphocytes or macrophages may occur.

Evidence from our laboratory suggests that NE and NPY are colocalized in the same nerve fibers in the rat spleen. Chemical sympathectomy induced by 6-hydroxydopamine injections in rats results in destruction of noradrenergic nerve terminals, and hence noradrenergic denervation of the spleen. Immunocytochemistry was performed to determine the presence of NE and NPY after chemical sympathectomy. Not only were NE containing nerves destroyed, but NPY-positive nerve fibers were no longer present after sympathetic denervation, suggesting possible colocalization of NE and NPY. Colocalization of NE and NPY is currently being investigated with double labeling immunocytochemistry at the LM and EM level. Furthermore, the localization of the NPY receptor in spleen is being investigated.

These results suggest that in addition to norepinephrine's role in modulating or regulating immune phenomena, NPY may have a direct effect on the immune cells of the spleen and other lymphoid organs, or alternatively may have an indirect effect on immune activity by modulating and regulating norepinephrine. Supported by PHS NS24761, RO1 NS25223 and N00014-84-K-0488 from ONR.

289.9

THE IMMUNE RESPONSE EVOKES CHANGES IN LUTEINIZING HORMONE-RELEASING HORMONE RECEPTORS. M.C. Morale*, V. Guarcello*, F. Raiti*, G. Palumbo* and B. Marchetti*. (SPON: C. Bard). Dept. of Pharmacology, School of Medicine, University of Catania, 95125 Italy.

Evidence for the existence of a bidirectional network between neuroendocrine and immune systems involving the hypothalamic-pituitary-gonadal-thymic axis, is well documented and recent evidence indicates that the hypothalamic neuropeptide luteinizing hormone-releasing hormone (LHRH), is involved in this circuitry. In the present study we have investigated whether the immune response can evoke changes in LHRH receptor (R) function both in immune and neuroendocrine cells. Groups of rats were then immunized with sheep red blood cells (10 x 10^6, i.p.) and boosted again 7 days later. Rats were killed at different time intervals and the number of plaque-forming cells was measured in the spleen. Present results show that the antigen challenge is accompanied by a marked increase in the number of LHRH-R within the thymus gland. Furthermore, significant changes in LHRH-R were observed in neuroendocrine cells, indicating a reciprocal modulation of immune and reproductive axes, with LHRH serving as channel of communication.

289.10

EXPRESSION OF INTERLEUKIN-1 AND I-ASSOCIATED ANTIGEN IN RAT BRAIN. J.M. Dopp and J.A. Olschowka. Depts. of Microbiology and Immunology, and of Neurobiology and Anatomy, University of Rochester School of Medicine, Rochester, NY 14642.

Microglial cells and astrocytes have been shown to secrete interleukin-1 (IL-1) in vitro when cultured with lipopolysaccharide (LPS) and/or gamma-interferon (IFNγ) (Giulian, et al., 1986, J. Exp. Med., 164). The purpose of the present experiment was to determine if LPS and/or IFNγ injected into the brain could attract and stimulate endogenous phagocytic cells.

Male Sprague-Dawley rats were anesthetized and given 100-250 nl microinjections of IFNγ (1 U/nl) , LPS (2 ng/nl), an IFNγ /LPS mixture, or 9% saline into caudate putamen or hippocampus. Two to four days later, rats were anesthetized and perfused with 4% paraformaldehyde (PF) (for light-microscopy), or 4% PF plus 2.5% acrolein (for electron-microscopy). Brains were removed, cut into 30-µm sections, and stained immunocytochemically for IL-1ß, major histocompatibility (MHC) class II I-associated (Ia) antigen, glial fibrillary acidic protein (GFAP), or MAC-1.

Light microscopy results indicated that IFNγ or LPS alone induced minimal IL-1ß or Ia expression, and that this expression was enhanced by physical injury to the cortex during surgery. In contrast, the IFNγ /LPS mixture induced pronounced expression of IL-1ß and Ia regardless of cortical injury. Cell morphology indicated that the stained cells were microglia or astrocytes. Collectively the results indicate that expression of IL-1ß and Ia by these cells during the initiation of an immune response parallels that seen in peripheral macrophages. Supported by PHS NS24761.

289.11

INTERLEUKIN 1β-LIKE IMMUNOREACTIVE INNERVATION IN THE HUMAN CENTRAL NERVOUS SYSTEM. C.D. Breder and C.B. Saper. Depts. of Pharm. & Physiol. Sci., Neurology and Comm. on Neurobiology, The University of Chicago, Chicago, IL 60637.

Interleukin-1β is a cytokine secreted by macrophages in response to infection. It is thought to mediate several components of the febrile response. We have used immunohistochemistry to examine the hypothesis that, like other peripheral hormones, neurons in the brain may use IL1β as a neuromodulator in the central control of the immune system. We stained sections through six human basal forebrains and brainstems with polyclonal antisera raised against recombinant human (rH)-IL1β. Staining with these antisera was blocked with rH-IL1β but not with rH-IL1α, nor was it affected by preincubation with several neuropeptides found in the hypothalamus. IL1β-like immunoreactive (-ir) cell bodies were mainly found in the hypothalamus in the arcuate nucleus and retrochiasmatic area. IL1β-ir fibers were widely distributed in the hypothalamus, including the paraventricular, dorsomedial, ventromedial and arcuate nuclei and the medial preoptic and lateral hypothalamic areas. IL1β-ir fibers were also prominent in the central nucleus of the amygdala, the bed nucleus of the stria terminalis, and in the midline thalamic nuclei. In the brainstem, IL1β-ir innervation was observed in the periaqueductal gray matter, the locus coeruleus, the parabrachial nucleus, and in the nucleus of the solitary tract, amongst the cell bodies of the A2 noradrenergic neurons. IL1β-ir innervation of the brainstem and basal forebrain may participate in autonomic, endocrine and behavioral responses that underlie the febrile response.

HYPOTHALAMIC-PITUITARY-ADRENAL REGULATION I

290.1

DIURNAL GLUCOCORTICOID RECEPTOR BINDING AND CORTISOL SECRETION IN POST-TRAUMATIC STRESS DISORDER. R. Yehuda,* M.T. Lowy, S.M. Southwick,* D. Shaffer, * and E.L. Giller. VAMC-Yale University, West Haven, CT 06516 and Case Western Reserve University, Cleveland, Ohio 44106.

A dysregulation of the HPA axis in chronic post-traumatic stress disorder (PTSD) is suggested by our previous work showing lower mean 24-hr urinary cortisol excretion in combat veterans with PTSD compared to other psychiatric patients and normal controls. To further characterize HPA abnormalities in PTSD, basal AM and PM plasma cortisol and lymphocyte glucocorticoid receptor (GR) concentrations were measured in 10 PTSD patients and 10 normal controls. Both groups showed the normal AM to PM decline in plasma cortisol. However, the decline was significantly steeper in the PTSD group. Patients started out with a 20% higher mean cortisol concentration at 8:00 AM which fell to 25% below controls by 4:00 PM. GR concentrations also decreased significantly from AM to PM in both groups. Paralleling the cortisol findings, PTSD patients showed a steeper decline in GR number from AM to PM. In this case, AM GR number was 65% higher ($p<.05$) and remained 15% higher by the PM in the PTSD group. GR number was also positively correlated with overall depression and anxiety (assessed by Hamilton scales) as well as with PTSD symptoms measured by the Figley. The results further support the notion of a dysregulation of the HPA axis in PTSD.
Supported by Veteran Administration funds.

290.2

Effects of photoperiod on brain glucocorticoid receptors and the stress response in the golden hamster (Mesocricetus auratus). L. Krey*, E. Ronchi*, R.L. Spencer, A. Danielsson, B. McEwen. The Rockefeller University, New York, NY 10021

Hippocampal and hypothalamic Type I glucocorticoid receptor levels increase when male golden hamsters are maintained in short daylengths. Receptors were quantified with ^{3}H-cortisol single point binding assay on tissues from acutely adrenalectomized intact and castrated T-replaced male hamsters exposed to a long-day (LD 14:10) or a short day (LD 10:14) photoperiod for 4-8 weeks. The increase in receptor binding was associated with an elevation in Type I receptor steady state mRNA levels as measured by in-situ hybridization with the recently characterized mineralocorticoid receptor cDNA (Arriza et al., Science 237:268,1987). These changes in receptor biosynthesis occured along with modifications of the stress response to 1min exposure to ether. Serum glucocorticoid levels, as measured by RIA, showed that short day hamsters recover more efficiently, since they "reset" serum corticosterone/cortisol secretion to basal values at 60min after stress. However, in hamsters exposed to long photoperiods serum cortisol levels were still increased at 60min. The changes in the secretory dynamics of the brain-adrenal axis and the increase in receptor biosynthesis may be of adaptative value in a photoperiodic species like the hamster, presumably to shut down metabolic rate during torpor and hibernation.

290.3

ONTOGENY OF MINERALCORTICOID (MR) AND GLUCOCORTICOID RECEPTOR (GR) GENE EXPRESSION IN THE RAT BRAIN J.A.M. van Eekelen*, M.C. Bohn# and E.R. de Kloet (SPON: Dr. H. Rigter) * Rudolf Magnus Institute, University of Utrecht, Vondellaan 6, 3521 GD Utrecht, The Netherlands; # Dep. of Neurobiol. and Anat., Univ. of Rochester, Rochester NY, USA.

The cellular localization of MR and GR-mRNA in the developing rat brain was studied by in situ hybridization. The 35S-labeled cRNA, as well as cDNA-probes, were transcribed from either a 513 bp cDNA fragment encoding for the rat brain MR or a 500 bp cDNA fragment encoding for the rat liver GR (courtesy of J.L. Arriza and K.R. Yamamoto resp.). At all ages (pnd 2-16), MR-mRNA was mainly restricted to and evenly distributed over the hippocampal CA1-4 cell fields, the dentate gyrus (DG) and the pyriform cortex, which is in agreement with radioligand binding to MR during ontogeny. Hybridization to GR-mRNA was distributed more widespread. Moreover, with maturation, the pattern of GR-gene expression in hippocampus and hypothalamus changed, parelleling the developmental pattern of GR-immunoreactivity in CA1-2 neurons, DG and specific hypothalamic nuclei (PVN; Van Eekelen et al., B.Res. 436, 120, 1987/J.Neurosc.Res. 21, 88, 1988; Rosenfeld et al., Dev.B.Res. 42, 119, 1988).

290.4

EFFECTS OF HIPPOCAMPAL TYPE 1 AND TYPE 2 GLUCOCORTICOID RECEPTOR ANTAGONISTS ON ACTH LEVELS IN THE PM. M. Bradbury* and M. Dallman* (SPON: J. LaVail) Dept. of Neuroscience, Univ. of California, San Francisco, CA 94143

The hippocampus contains high concentrations of both type 1 and type 2 glucocorticoid receptors (GCR). Their effects on the hypothalamic-pituitary-adrenal cortical (HPA) axis in the PM have not been examined directly. To address this question, 26 gauge cannulae containing either GCR antagonists or cholesterol as a control were implanted into the anterior portion of the the hippocampus in 250-300 gram male rats. All rats were adrenalectomized and given subcutaneous pellets delivering plasma corticosterone doses of 3 to 6 ug/dl. Animals with cholesterol implants had ACTH levels in the PM similar to those in animals with no head surgery. Implants containing RU 38486, a type 2 GCR antagonist, dramatically increased the ACTH levels in the PM. The type 1 GCR antagonist, RU 26752, had a similar, although weaker effect. Effective implants were those penetrating the CA1 layer of the hippocampus. Preliminary diffusion experiments suggest that the spread of the steroids is restricted to the area immediately surrounding the cannulae. The data demonstrate the limbic system is involved in or may initiate negative feedback control of the HPA axis at the peak of the system's circadian rhythm in a GC dependent manner.

290.5

CHRONIC BUT NOT ACUTE IMIPRAMINE ADMINISTRATION DECREASES HIPPOCAMPAL TYPE I AND II CORTICOSTEROID RECEPTORS. M.T. Lowy and H.Y. Meltzer, Department of Psychiatry, Case Western Reserve University, Cleveland, Ohio 44106.

Depression is associated with increased cortisol secretion and alterations in lymphocyte glucocorticoid receptor regulation. Antidepressants may normalize some of the cortisol abnormalities in depressed patients. The present study assessed the effect of chronic and acute administration of the antidepressant drug, imipramine, on type I (mineralocorticoid) and type II (glucocorticoid) corticosteroid receptors in the hippocampus as well as type II receptors in other tissues. Chronic administration of imipramine (10 mg/kg x 2 x 14 days) significantly decreased both type I and II hippocampal corticosteroid receptors. Chronic imipramine had no effect on type II receptors in other brain regions such as the frontal cortex, hypothalamus or striatum. Acute imipramine administration (10 mg/kg x 2 x 1 day) had no effect on type I or II hippocampal corticosteroid receptors. The imipramine-induced decrease in hippocampal corticosteroid receptors may serve to minimize the effects of elevated glucocorticoid levels.

290.6

AGE-RELATED CHANGES IN TYPE II GLUCOCORTICOID RECEPTOR mRNA LEVELS IN PITUITARY AND SELECTED BRAIN REGIONS IN THE RAT. A. Peiffer, N. Barden & M.J. Meaney. Ontogénèse et Génétique Moléculaires, Laval Univ. Hospital Research Ctr., Ste-Foy, Québec G1V 4G2 and Douglas Hospital Research Ctr., McGill Univ. Montreal, H4H 1R3, Canada.

We have measured type II glucocorticoid receptor (GCR) mRNA and GCR binding in pituitary and brain of the aging rat to determine whether changes in receptor binding with age are reflected in altered gene expression for the GCR mRNA. Anterior pituitary, hypothalamus, amygdala, hippocampus and frontal cortex were dissected from intact Long Evans rats 6, 12, or 24 months of age. Type II, GCR binding was measured using [^{3}H]RU 28362. Total RNA was isolated by the guanidium isothiocyanate method and purified on CsCl gradients followed by phenol/chloroform extraction. Northern blots were hybridized with [^{32}P]-labelled Riboprobe corresponding to a 2.2Kb fragment of GCR mRNA, quantified by densitometry, and the data expressed as a ratio of ß-actin mRNA.

By 24 months there was a highly significant (40-50%) decrease in Bmax for [^{3}H] RU 28362 binding and a moderate (20-30%) decrease as early as 12 months in hippocampus. No other region examined showed any significant variation in [^{3}H] RU 28362 binding with age. Type II GCR mRNA was significantly elevated in all the regions at 12 months of age. At 24 months of age, GCR mRNA levels decreased in most regions and were not different from levels in 6 month-old animals. In the hippocampus, type II GCR mRNA levels also increased at 12 months of age, but, at 24 months of age and consistent with [^{3}H] RU 28362 binding, GCR mRNA levels were significantly decreased (40% and 74%, respectively) in comparison to both 6- and 12-month old animals.

In hippocampus, the loss of type II GCRs may involve a multi-step process, with compensatory increases in receptor mRNA levels at 12 months of age and decreases in both the receptor and its mRNA by the 24th month of life.

290.7

ESTROGEN MEDIATES SEX DIFFERENCES IN ADRENAL CORTICAL RESPONSES TO STRESS. A.A. Granberry*, B.N. Bunnell, E.H. Mougey and J.L. Meyerhoff. Dept. Psychology, Univ. GA, Athens, GA 30602 and Dept. Medical Neurosciences, Walter Reed Army Inst. Research, Washington, DC 20307

Several reports have indicated that corticosterone (CS) levels are higher in female than in male rats subjected to stress. To begin an examination of the role of estrogen in mediating such differences, we compared plasma CS and ACTH in males and females following acute exposure to stress. The subjects were 18 intact males, 18 intact females tested on their proestrous day, 18 ovariectomized (OVEX) females given 50µg estradiol benzoate in oil for 5 days before stress and 18 OVEX females that received only oil. Six rats in each treatment received 15 min of intermittent tailshock while they were restrained in small shock chambers, 6 were restrained but not shocked and 6 were home cage controls.

CS values were consistently higher in females than males across hormone treatments and stressor conditions. Females receiving tailshock did not differ as a function of hormone treatment, but under restraint stress, OVEX rats given estradiol were significantly higher than OVEX rats given oil. Restrained, intact females were intermediate. ACTH was highly variable, suggesting the presence of a complex interaction between stressor quality and hormone condition. We conclude that estrogen modulates the CS response to stress, possibly by acting directly on the adrenal cortex.

290.8

SEX DIFFERENCE IN TYPE I CORTICOID RECEPTORS IN RAT HYPOTHALAMUS. B.B. Turner and M.S. Ansari*. Dept. of Physiology, College of Medicine, East Tenn. State Univ., Johnson City, TN 37614.

We previously reported a sex difference in receptor affinity and number of the Type I corticoid receptor in rat hippocampus (Neurosci. Abstr. 14:443, 1988) and estrogen down-regulation of glucocorticoid receptor number in pituitary (Endocr. Soc. Abstr. 269, 1988). In the hippocampus, tissue from males exhibits greater affinity and receptor number. Here we ask whether a sex difference also exists in the hypothalamus with respect to affinity and receptor number.

In saturation plots with 3H-RU 28362 and 3H-DEX, males had greater affinity for the Type I receptor (0.70 $\pm$ 0.11 nM vs. 1.79 $\pm$ 0.24 nM), but the difference in receptor number was not significant; no sex differences were found in the binding of the Type II receptor. A series of single point studies also failed to demonstrate that the somewhat greater number of the Type I and Type II receptors found in males was significant. However, in vivo nuclear retention studies of 3H-corticosterone support the existence of functional differences in the occupation of Type I receptor system; at low dose (3 nmol/kg), but not at high dose (67 nmol/kg), retention of radioactivity was greater in males (3.26 $\pm$ 0.78 vs. 1.06 $\pm$ 0.37 nmols/mg protein, $p < 0.05$). These results suggest that in the female hypothalamus, they Type I system has less affinity than the male, and hence may not be fully occupied at all times. Supported by NIH grant NS-22158.

290.9

PRESENCE OF 11B-HYDROXYSTEROID DEHYDROGENASE IN THE HIPPOCAMPUS OF THE RAT. R.R. Sakai*, V. Lakshmi*, C. Monder*and B. S. McEwen. (SPON. L.J. Stein) Rockefeller Univ., Lab of Neuroendocrinology and The Population Council. New York, NY. 10021.

Corticosteroids circulate at levels 10^3-fold greater than aldosterone. Although they may cause local displacement of aldosterone from mineralocorticoid (type I) receptors, they do not do so in all tissues because 11B-hydroxysteroid dehydrogenase (11-DH) oxidizes cortisol (F) and corticosterone (B) to biologically inert 11-oxo metabolites which cannot compete with aldosterone for its receptor. We determined if this mechanism, which has been described in the kidney, applies to brain. Immunocytochemical analysis in adult brains showed 11-DH positive cells which correlated with the distribution of type I receptors. Highest 11-DH was found in hippocampus; lower levels were detected in other regions. The results were corroborated by enzyme activity using B as substrate in rat brain. No activity was found with F. These observations suggest a mechanism for specific aldosterone effects on brain physiology and behavior. Supported by grants DK 37094, MH 43787 and NS 08537.

290.10

CHARACTERIZATION OF GLUCOCORTICOID-INDUCED DECREASE IN GLUCOSE TRANSPORT IN FETAL RAT GLIAL CULTURES. H. Horner, C. Virgin*, R. Sapolsky. Dept. Biol. Sci. Stanford Univ., Stanford, CA 94305

Glucocorticoids (GCs), the adrenal steroids secreted during stress, impair the capacity of hippocampal neurons to survive hypoxia-ischemia, seizure and hypoglycemia. The cause of this effect is not known, but appears to involve energetic disruption, since the endangering effects of GCs can be reversed by supplementing the hippocampus with various energy substrates. As a possible mechanism, GCs inhibit glucose transport (GT) in peripheral tissues; we have found that GCs decrease GT in mixed cultures of neurons and glia from fetal rat hippocampus. In this study, we characterize a GC-induced inhibition of GT in hippocampal glia, since energy-dependent glial function (uptake of excitatory amino acids, of protons, etc.) can modulate neuronal function during neurological crises.

Hippocampi from Day-18 fetuses were dissected and dispersed. Cells were grown in flasks without poly-D-lysine pre-coating, and plated onto 48-well cluster dishes at 3 weeks of age; under such conditions, cultures are < 5% neuronal, as determined immunocytochemically. The specific transport of 14C-2-deoxyglucose was assessed 3 minutes after incubation with the sugar. 24-hour pretreatment with either corticosterone (CORT) or dexamethasone inhibited GT. Minimal effective doses were 1 uM and 10 nM, respectively, and maximal inhibition was approximately 15% ($p < .005$ in both cases). A minimum of 4 hours of steroid incubation was needed for the effect. Neither testosterone nor progesterone (1 uM, 24 hrs) were effective, whereas an equivalent treatment with estrogen caused a 15% increase ($p < .005$) on GT. CORT had no effect on GT in cultured glia from cortex or cerebellum, but caused a 40% ($p < .001$) inhibition in hypothalamus. The functional consequences of GT inhibition of these magnitudes in hippocampal and hypothalamic glia are under study. Supported by NIH RO1 AG06633.

290.11

SEROTONIN (5-HT) INCREASES GLUCOCORTICOID, TYPE II RECEPTOR BINDING CAPACITY IN CULTURED HIPPOCAMPAL CELLS. J.B. Mitchell, W. Rowe*, and M.J. Meaney. Douglas Hospital Research Center, McGill University, Montreal, Quebec, CANADA, H4H 1R3.

The hippocampus has been well established as a principle target region for the adrenal glucocorticoids (GCs), and corticoid activity at this site serves both neuroendocrine and behavioral functions. Thus, GC receptor regulation within the hippocampus is of considerable interest. In the work presented here, we have described the potent effects of serotonin (5-HT) on GC receptor binding in dispersed hippocampal cell cultures.

Primary cultures of dispersed hippocampal neurons from fetal (f19) rats were established and type II, GC receptor binding measured using [3H]RU 28362 as radioligand. Type II, GC receptor binding in hippocampal cells was dramatically increased (~ 190%) by nanomolar concentrations of 5-HT (ED50 = 4.3 nM). The effect of 10 nM 5-HT on type II, GC receptor binding was completely blocked by the 5-HT2 antagonists ketanserin and mianserin, and partially mimicked by the 5-HT2 agonist, ±DOI ((±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane). There was no effect of agonists or antagonists of the 5-HT1 and 5-HT3 binding sites.

The change in type II, GC receptor binding required 4 days of exposure to 10 nM 5-HT, indicating that the effect probably involves regulation of protein synthesis. Interestingly, changes in type II, GC receptor binding persisted 1 week following the removal of 5-HT, with no decrease in the magnitude of the effect. Preliminary data also suggest that the effect of 5-HT is selective for the type II, GC receptor binding site, since [3H]aldosterone, a ligand for the type I, mineralocorticoid-like receptor, was not affected by similar exposure to 5-HT.

These data suggest that variations in 5-HT activity might influence type II, GC receptor binding in the hippocampus and that this effect involves, at least in part, 5-HT2 receptor sites. The temporal characteristics of this effect indicate that 5-HT might be involved in the induction of type II, GC receptors during early periods of postnatal development.

290.12

GLUCOCORTICOID RECEPTOR(GR) EXPRESSION IN PRIMARY HIPPOCAMPAL NEURONAL CULTURES. G. Bing, U. Vielkind*, and M.C. Bohn. Department of Neurobiology and Anatomy, University of Rochester Medical Center, Rochester, NY 14642.

Hippocampus is a major target tissue for glucocorticoid action in the central nervous system. The actions of glucocorticoids are mediated by binding to specific intracellular receptors. Previous studies have demonstrated high levels of GR and GR mRNA in the rat hippocampus *in vivo*. The present study was undertaken to determine whether glucocorticoid receptors are expressed in rat hippocampal neurons grown in serum-free, and defined conditions.

Hippocampus from embryonic day 18-19 rat were dissociated, cultured in modified MEM plus 10% horse serum for 4 hrs. and then, grown in serum-free medium for 7 days. Various serum-free media were used including medium conditioned by C6 glioma cells or astrocytes from hippocampus or cerebrum. Defined media were modified MEM-N1 and DMEM-F12-N1. Neurons survived well in glia-conditioned media, and in modified MEM-N1, but most neurons died in DMEM-F12-N1. Immunocytochemistry using a monoclonal antibody against the rat liver GR, combined with an antibody against neuron specific enolase (NSE), demonstrated the presence of GR in the majority of pyramidal shaped neurons. GR immunoreactivity was predominantly nuclear, although light cytoplasmic staining was observed. This primary hippocampal neuronal culture serves as an excellent system to study GR regulation at the cellular and molecular levels.

Supported by NIH grant NS20832.

290.13

GLIAL CELLS EXPRESS GLUCOCORTICOID RECEPTORS. U. Vielkind[1*], A. Walencewicz[2*], J. M.Levine[2] and M.C. Bohn[1]. [1]Dept. of Neurobiology and Anatomy, Univ. of Rochester Medical Center, Rochester, N.Y. and [2] Dept. of Neurobiology and Behavior, State Univ. of New York, Stony Brook, N.Y.

The effects of glucocorticoids on gene expression are mediated via cytoplasmic glucocorticoid receptors (GR) which are translocated to the nucleus in the presence of steroid. In the nervous system, glucocorticoids are known to be involved in the regulation of neuropeptide and neurotransmitter-related genes, and presence of GRs has been demonstrated in neurons and adrenal chromaffin cells. Glucocorticoids also affect gliogenesis and regulate the expression of glial-specific enzymes. To determine whether specific types of glial cells express GRs, mixed glial cultures from rat cerebrum and cerebellum, as well as purified astrocytes and oligodendrocytes, were stained with a monoclonal antibody raised against the rat liver GR and double stained for glial-specific markers.

Astrocytes, both Type 1 and 2, contained GR-immunoreactivity (IR). Distinct granular staining was observed in the nucleus in the presence of dexamethasone, while light staining was observed in both the nucleus and cytoplasm in the absence of steroid. Nuclear GR-IR was also observed in oligodendrocyte nuclei, however the staining was less intense than in astrocytes. Glial cell lines, C6 glioma and JScl1 Schwannoma, also had GR-IR in nuclei. These studies demonstrate that all classes of glial cells express GRs which can translocate to the nucleus, suggesting that glia are a major target for glucocorticoid action in the nervous system.

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290.14

IN VITRO BINDING OF ^{3}H-ALDOSTERONE IN BRAIN SECTIONS. B.G. Yongue, H. Coirini, & B.S. McEwen, N.Y.S. Psychiatric Inst., Columbia Univ., & Rockefeller Univ. New York, 10032

Receptor autoradiography provides anatomical resolution and precise binding localization. Type I corticosteroid receptors have been labelled in vitro, in slide mounted brain sections, using ^{3}H-corticosterone (CORT; Sarrieu, et al, 1985). However, the determinants of type I receptor specificity for either endogenous ligand, aldosterone (ALD) or CORT, are not clear. Hence, the need to develop brain ALD autoradiography. Sections (12um) of a tissue mash of hippocampus from 3-day-ADX rats were incubated (room temp) in 50mM TRIS pH 7.4 buffer containing 2mM EGTA, 6mM molybdate, 5mM ATP, 10mM dithiothreitol, 5% glycerol, and 0.5uM RU28362. Non-specific was assessed using 2.5uM cold CORT. Different washing conditions were tested by varying either buffers or the number and duration of the washes. Specific binding was reduced by inclusion of DTT or ATP and also by more than two washes or more than 6 min total wash duration. Two three-min washes with TEM buffer provided the best ratio total/non-specific binding. Highest specific binding was found at 30 to 60 min of incubation. Concentrations of ^{3}H-ALD from 0.2-10nM produced binding saturation between 2.5 and 10nM, with a K_d=1.2nM estimated by Scatchard analysis. This technique may provide insights into differential regulation of ^{3}H-ALD binding to type I receptors by ALD and CORT as previously found in this lab, using cytosolic assays of tissue punches (Coirini, 1988, SN Abst, 528.15). (Supported by MH43787)

291.1

THE ORGANUM VASCULOSUM LAMINAE TERMINALIS (OVLT) IS CRITICAL FOR FEVER INDUCED IN GUINEA PIGS BY BLOOD-BORNE CYTOKINES. C.M. Blatteis, N. Quan, and R.D. Howell.* Dept. of Physiol. Biophys., Univ. of Tenn., Memphis, TN 38163.

The peripheral injection of various cytokines produces fever, a centrally mediated effect. We have suggested previously that the OVLT may be a site where cytokines could interact with the brain, because its electrolytic ablation prevents the febrile response to ip-injected lipopolysaccharide (LPS). We report now that such a lesion also attenuates the fevers produced by iv-injected LPS (2 ug/kg), recombinant human interleukin-1β (IL1, 100 ng/kg), tumor necrosis factor-α (TNF, 20 ug/kg), and interferonα_2 (IFN, 1x10^7 U/kg). IL1 and IFN evoked unimodal fevers in intact animals but no response in lesioned animals 11 d post-op. LPS and TNF caused bimodal febrile rises before lesion; after lesion, the first rise was unchanged, but the second was suppressed. These data suggest that the first peak of LPS fever may be mediated by TNF and the second by IL1/IFN, but the integrity of the OVLT is pivotal for the full febrile response to circulating pyrogens. (Supported by NS 22716.)

291.2

EFFECTS OF MICROIONTOPHORETICALLY APPLIED INTERLEUKIN-1 AND NOREPINEPHERINE ON SINGLE UNIT ACTIVITY RECORDED FROM THE RAT HYPOTHALAMUS IN VIVO. W. McVaugh* and N. Dafny. Dept Neurobiology and Anatomy, Univ. Texas Med. Sch. at Houston, TX 77225.

Interleukin-1 (IL-1), in addition to its role as a regulator of immune function, has been implicated in the modulation of neuroendocrine processes. The present study was designed to explore the effects of iontophoretically applied IL-1 on the electrical activity of single neurons recorded from the anterior hypothalamus/periventricular area in the anesthetized rat. Male Sprague Dawley rats (280-320g) were anesthetized with urethane (1.2g/kg bw ip) and placed in a stereotaxic instrument. The skull was bared, a 5mm hole drilled in the cranium and the dura reflected. IL-1 and norepinepherine (NE) were iontophoresed onto individual neurons in the hypothalamus by means of a multibarrel micropipette assembly with a recording microelectrode attached. Neuronal activity was measured as spikes per unit time. Preliminary results indicate that the percentage of neurons responding to IL-1 with a change from baseline activity increased with dose (44, 50 and 70%, respectively) and that roughly half the neurons were excited while half were inhibited. NE resulted in between 50% and 60% of the neurons showing a change from baseline for all three doses administered. As the dose of NE increased, so did the number of responders demonstrating an inhibition of firing. The intermediate doses of IL-1 and NE, when applied comcomitantly, resulted in the majority (79%) of the neurons showing a change in activity with 82% of those responders being excited and 18% inhibited. These results suggest that IL-1 may exhibit dualistic effects: i.e. direct effects on hypothalamic neurons and modulatory properties affecting noradrenergic regulation.

291.3

EFFECTS OF THE NEUROPEPTIDE, α-MSH (1-13), ON DELAYED-TYPE HYPERSENSITIVITY REACTION IN MICE. M.E.Hiltz* and J.M.Lipton. (SPON:W.G.Clark) Dept. of Physiology, U.T. Southwestern Medical Center, Dallas, Texas 75235

The neuropeptide α-melanocyte stimulating hormone, α-MSH (1-13), has antipyretic and anti-inflammatory activities. α-MSH (1-13) as well as a C-terminal fragment of α-MSH [α-MSH (11-13)] was recently found to inhibit the acute inflammatory reaction induced by picryl chloride solution; however, the influence of α-MSH peptides on cell-mediated host responses, e.g. delayed-type hypersensitivity, is unknown.

To assess the effect of α-MSH on such a response, mice were sensitized with 0.5% 1-fluoro -2,4-dinitro-benzene (DNFB) solution. The ears were challenged 96 hrs later with a 0.15% DNFB solution to induce a delayed-type hypersensitivity reaction. The thickness of both ears was measured initially and at 24, 48 and 72 hrs after challenge to quantitate the degree of swelling. α-MSH (1-13) (30 μg/mouse) or saline was injected i.p. 3 hrs prior to the 24-hour ear measurement. There was a significant ($p < 0.001$) inhibition of swelling at 24 hr in animals treated with α-MSH, when compared with controls, but not at 48 and 72 hr after challenge. The results indicate that α-MSH, in addition to inhibiting fever and acute inflammation, can inhibit delayed-type hypersensitivity reactions. Funded by Texas Advanced Technology Program.

291.4

EFFECTS OF BROMOCRIPTINE AND HALOPERIDOL ON IMMUNE FUNCTION AND TUMORIGENESIS. J.L. Bussiere*, J.H. Exon* and C.A. Johnston (SPON: R.C. Speth). Dept. Veterinary Science, University of Idaho, Moscow, ID 83843 and College of Pharmacy, Washington State University, Pullman, WA 99164.

The effects of prolactin (PRL) levels on various immune parameters were determined using the dopamine agonist, bromocriptine (BCR) and antagonist haloperidol (HALO) in male Sprague-Dawley rats. Natural killer cell activity, delayed-type hypersensitivity, antibody production, and interleukin 2 (IL2), interferon and prostaglandin E2 (PGE2) production were assessed following two weeks of daily s.c. injections of BCR or HALO. Both HALO and BCR increased natural killer activity. However, IL2 and PGE2 production were differentially affected by HALO or BCR treatment: BCR increased both IL2 and PGE2, while HALO decreased IL2 and had no effect on PGE2. The effect of decreased PRL (with BCR) was then determined in a chemical-induced tumor model using 3-methylcholanthrene, which causes the formation of an in situ fibrosarcoma. Daily injections of BCR during the first two weeks following exposure to the carcinogen resulted in a significant increase in tumor incidence and decrease in tumor latency. The data demonstrate the potential importance of the anterior pituitary hormones in immune homeostasis and tumorigenic response to chemical carcinogens.

291.5

LESIONS OF THE NUCLEUS BASALIS MAGNOCELLULARIS AND THE MEDIAL SEPTUM: EFFECTS ON IMMUNE AND ENDOCRINE FUNCTIONS. W. Mayo, J. Cherkaoui, K.W. Kelley, R. Dantzer, P.J. Neveu, P. Mormède, M. Le Moal and H. Simon. INSERM U.259, Domaine de Carreire, 33077 Bordeaux Cedex FRANCE.Dept. Animal Sciences, University of Illinois, Urbana, IL61801, USA.

A large body of literature has demonstrated the important role of neural and endocrine influences in regulating immune functions. The effects of selective lesions (quisqualic acid -15μg/0.6μl) of two major cholinergic pathways of the central nervous system, the nucleus basalis magnocellularis (NBM)- cortical pathway and the septo-hippocampal pathway were examined on immune and endocrine functions. These two cholinergic pathways are severely damaged in some neurodegenerative diseases, particularly in the senile dementia of the Alzheimer type.

The lesion of the NBM induced an important increase of T-lymphocyte proliferation in response to mitogens (a two fold increase as compared to control, $p<0.01$) and an increase in interleukine-2 production. However antibody production was not modified. In contrast, lesion of the septal area led to a depression of the lymphoproliferation. No differences between experimental groups was observed in the plasma levels of stress hormones (ACTH, corticosteroids, prolactin).

These preliminary results clearly show that the cholinergic system has modulatory influences on T-lymphocyte functions. Characterization of both brain structures involvement in immunoregulatory functions and immunological targets of cholinergic influence are currently under studies in relation to immunodeficiencies in Alzheimer's disease.

291.6

TISSUE BIOGENIC AMINE CONCENTRATIONS FOLLOWING ENDOTOXIN ADMINISTRATION AND NEUROTRANSMITTER ALTERATION. H.M. Brown-Borg and F.W. Edens*. Dept. of Poultry Science, North Carolina State Univ., Raleigh, NC 27695-7635.

Catecholaminergic and serotonergic systems have been implicated in the modulation of immunity and disease. The effects of drug-induced sympathectomy and neutrotransmitter injection on monoamine and corticosterone (CORT) levels following endotoxin (E) injection are reported. In each of 3 studies, 3-wk-old chicks (Gallus domesticus) were divided into groups: control (C); drug [reserpine (R), propranolol (P), norepinephrine (NE), serotonin (5HT)]. In study 1, R (0.25 mg/kg, i.v.) was administered 16 and 12 hr before E injection (100 ug/kg 00127:B8 E. coli). In study 2, P (30 mg/kg, i.a.) was given 10 and 1 hr prior to E administration. In study 3, 30 min. prior to sampling, NE (.25 mg/kg, i.v.) or 5HT (.50 mg/kg, i.v.) was administered. Control groups in each study were bled and tissues (spleen and left ventricle) removed at time of E injection. Three hr post E injection control and treated birds were sampled. In study 1, C birds exhibited lower splenic NE levels when compared to E and RE groups (79.4+5.3 vs 114+10.4 and 148.64+10.24 ng/g; $P<.05$, respectively). Birds treated with P had increased splenic NE levels compared to C but lower levels than birds in the PE group ($P<.01$). Plasma CORT increased both the E and drug-E groups over that of controls in all 3 studies ($P<.05$). Our data indicate that peripheral neuronal activity is altered in this model of acute bacterial infection in Aves.

291.7

A MODEL FOR THE BEHAVIORAL EFFECTS OF INTERFERON: MOUSE INTERFERON ALPHA IN DBA/2J MICE Mark Segall* and Linda Crnic, Univ. of Colorado School of Medicine, Denver, CO 80262. (SPON: M. Reite)

Clinical use of interferon causes fatigue, anorexia, and impaired cognition. DBA/2J mice and mouse interferon-alpha (IFN-α; Lee Biomolecular) were used to model these effects. EXP.1 To determine the time course and effective dose, male mice, continually housed in the test chamber, were monitored 24 hours a day. Stable baselines were established between i.p. injections of 400, 800, and 1600 U/gm of mouse IFN-α. The 1600 U/gm dose decreased horizontal activity for 8 hours, and food pellets obtained for6 hours, after injection. All doses decreased the number of water licks 2 to 6 hours after injection. EXP.2 Interferon-induced nausea is not the likely cause for the decreased food consumpton: a 1600 U/gm injection of mouse IFN-α did not elicit a conditioned taste aversion. EXP.3 Short term studies compared types of interferon and routes of injection. As in Exp.1, i.p. injections of 1600 U/gm of mouse IFN-α decreased horizontal activity and food pellets obtained while i.p. injections of mock interferon or human IFN-α, or s.c. injections of mouse IFN-α had no effect. Thus the decreased activity and food intake in mice, seen only with mouse IFN-α, parallels the effects of interferon in humans. Therefore it is important to use species-appropriate interferon in an animal model. Supported by MH44453, MH09718, and MH00621.

291.8

CORRELATION OF TRH STIMULATION OF IN VITRO PRODUCTION OF TSH BY LYMPHOCYTES WITH IN VIVO TRH PITUITARY TESTING. W. J. Meyer, III, D.V. Harbour, R. Gardner, A. Wassef, and E. M. Smith, Dept. of Psychiatry and Behavioral Sciences, University of Texas Medical Branch, Galveston, TX 77550.

We examined the correlation between the pituitary TSH production after standard in vivo stimulation TRH testing and the mononuclear leukocyte's TSH production after in vitro TRH exposure. Twenty euthyroid individuals were given 200 ug TRH IV in vivo, and plasma TSH was measured over 60 min. The TSH response to TRH of freshly isolated mononuclear leukocytes from each individual was measured in vitro by incubating them with either plain media or TRH for 48 hours. The cell supernatants were analyzed by RIA for TSH. The fourteen individuals with a normal (peak TSH value > 10 uIU/ml) in vivo TRH test had a mean in vitro leukocyte TSH difference of 0.7±0.6 uIU/ml, whereas the 6 individuals with a blunted in vivo test had a significantly less in vitro TSH difference of 0.0±0.2 uIU/ml, ($p < 0.006$). Individuals with a blunted in vivo test had a normal leukocyte receptor Kd but lower Bmax (44±42 vs. 89±89 fmoles/mg membrane protein). Blunted pituitary response to TRH is correlated with decreased leukocyte TSH in vitro response to TRH and decreased TRH leukocyte receptor number. The mononuclear TSH production offers an enticing model of pituitary hormone secretion control and may provide insight into pituitary function abnormalities associated with psychiatric disease.

291.9

INFLUENCE OF THE CORPUS CALLOSUM ON IMMUNE FUNCTION

E. Fride*, P. Skolnick and P.K. Arora*. Lab of Neuroscience, NIDDK/NIH, Bethesda, MD 20892, USA.

Recent data from our laboratory indicate that communication between the left and right cerebral hemispheres may influence immune function (Fride et al. subm.). Nearly 70% of 129/J mice and 30% of BALB/c mice either lack or possess only a rudimentary Corpus Callosum (CC). In this study, several immune parameters (mixed lymphocyte reaction, MLR; cytotoxic T-lymphocyte activity, CTL; mitogen-stimulated T and B cell proliferation, MS) were measured and compared with control mice (CC^+). Degree of behavioral asymmetry was determined using a paw-preference test (Collins 1985). Callosal presence was scored under a microscope using toluidine blue stain. In both BALB/c and 129/J strains, CC^+ mice had a higher degree of paw preference than CC^- mice. The no. of spleen cells was lower in CC^- mice of both strains. CTL, MLR and MS were all significantly lower in CC^- than in CC^+ BALB/c mice.

BALB/c	CTL	MLR	ConA	PHA	LPS
	(% Lysis)		(Stimulation Index)		
CC+ (7)	81±5.2	2.7±.4	18.0±3.1	11±1.6	65±15.7
CC- (8)	65±3.0*	1.3±.1*	6.6±1.3*	6± .6*	29± 7.0*

(*p<.05)

The influence of the CC on immune function in 129/J mice is under investigation. These data suggest that inter-hemispheric communication is important in modulating the immune response.

291.10

EFFECTS OF THE PLATELET ACTIVATING FACTOR RECEPTOR ANTAGONIST BN-52021 ON CRH AND ADRENOCORTICOTROPIN SECRETION IN THE RAT.

R. Bernardini, A.E. Calogero+, Y.H. Ehrlich^, T.C. Kamilaris*, S.J. Listwak, G.P. Chrousos+ and P.W. Gold. Clinical Neuroendocrinology Branch, NIMH, and +Developmental Endocrinology Branch, NICHD, NIH, Bethesda, MD 20892; ^Developmental Neuroscience, CUNY, Staten Island, NY 10131.

Platelet activating factor (PAF), a naturally occurring alkyl-ether phospholipid, and a major participant in the anaphylactic/immune response, strongly stimulates the rat hypothalamic-pituitary-adrenal (HPA) axis in vivo. This effect appears to be mediated by direct stimulation of both hypothalamic CRH and, to a lesser extent, pituitary ACTH secretion. To examine the specificity of the PAF effect on the HPA axis we investigated the ability of a specific PAF receptor antagonist, the Ginkgo Biloba derivative BN 52021 (BN), to antagonize this effect. BN (10 mg/kg i.p.) injected 30 min prior to PAF was able to prevent the increase in plasma ACTH levels induced by PAF (500 ng/100 g of body weight i.v.). In addition, the effect of PAF on hypothalamic CRH secretion in vitro was inhibited by pretreatment of the tissue culture system with 1 μM BN, confirming the specificity of the response.

Our data suggest that PAF and its receptors may play a role in the activation of the hypothalamic-pituitary-adrenal axis during the defense response. Thus, PAF may represent another biochemical link between the immune and the endocrine systems.

291.11

PHARMACOLOGICAL MODULATION OF BETA-ENDORPHIN IN LYMPHOCYTES. A.E.Panerai and P.Sacerdote Dept. Pharmacology, Univ.of Milano, Milano, 20129 Italy.

Beta-endorphin (BE) is present in unstimulated human and rat lymphocytes. In order to investigate whether BE could be pharmacologically modulated similary to what observed for the hypotalamic or pituitary peptide, we treated rats and human subjects with drugs affecting the dopaminergic, serotoninergic and GABAergic systems. The results obtained show that both in rats and humans, lymphocyte BE was depressed after acute and/or chronic treatment with dopaminergic, GABAergic and anti-serotoninergic compounds. Consistently with this results, treatment with antidopaminrgic and serotoninergic drugs increased the lymphocyte concentrations of BE. These data show that: 1) BE in lymphocytes can be pharmacologically modulated; 2) lymphocyte BE seems to be modulated similarly to hypotalamic and intermediate pituitary BE, and not like the anterior pituitary peptide; 3) therapy with psychoactive drugs might affect the Immune System through changes in neuropeptides.

291.12

AGE AND CIRCADIAN CHANGES OF BETA-ENDORPHIN IN LYMPHOCYTES. P.Sacerdote and A.E.Panerai Dept. Pharmacology, Univ. of Milano, Milano, 20129, Italy.

Beta-endorphin (BE) concentrations were measured in lymphocytes from 6 and 18 month old rats, normal volunteers aging 20-30, 31-50, 51-70, 71-99 years, and at different times during the day in volunteers in the 20-30 and 51-70 years range. BE was significantly higher in 6 vs 18 month old rats. Similarly, BE concentrations increased from 20±3 pg/10^6 cells in the youngest group and remained higher up to the oldest group. In the youngest group, but not in the older, BE showed a circadian rhithm with nadir at A.M. and maximal concentrations at 1 P.M.. HPLC showed that most of BE in human lymphocytes is the N-acetylated sequence, at difference with what observed in plasma. These results show that 1) BE is present in unstimulated human and rat lymphocytes and concentrations are age related; 2) BE in human lymphocytes has a circadian rhithm; 3) N-acetyl-BE is the prevalent BE in lymphocytes.

291.13

EFFECTS OF ETHANOL ON TUMOR GROWTH AND NK CELL CYTOTOXICITY. R. Yirmiya, S. Ben-Eliyahu, R. P. Gale, Y. Shavit, H. Weiner, J. C. Liebeskind and A. N. Taylor. UCLA and West LA VAMC/Brentwood Division, Los Angeles, CA 90024.

The effects of chronic and acute ethanol administration on metastatic tumor growth were studied *in vivo*, using the MAD-B106 tumor cell line, previously shown to be controlled by natural killer (NK) cells. Additionally, the cytotoxic activity of NK cells against this tumor was measured *in vitro*. In the chronic experiments, Fischer 344 rats were fed a liquid diet containing ethanol or a control diet. After three weeks on the diet, animals were injected with MAD-B106 tumor cells. Two weeks later, lungs were removed and surface metastases were counted. In a second experiment, animals were sacrificed after three weeks on the diet and their splenic NK cell cytotoxic activity against MAD-B106 cells was assayed. In the acute experiments, animals were injected with either saline or ethanol (1.5-3.5 g/kg). One hour later, animals were either injected with MAD-B106 tumor cells and surface lung metastases were counted twelve days later, or sacrificed in order to assess the cytotoxic activity of their splenic NK cells. In another study, splenic and MAD-B106 cells were incubated in solutions containing various concentrations of ethanol. Ethanol treatment significantly increased the number of metastases in both the chronic and the acute study. However, in neither condition was there an effect of ethanol on NK cell cytotoxicity. In contrast, incubation of effector and target cells in physiologically relevant concentrations of ethanol significantly suppressed NK cell cytotoxicity. These results suggest that ethanol increases tumor growth, possibly by a direct effect on the interaction between NK and tumor cells. Supported by grants NS 07628 (J.C.L.), AA 06744 and VA Medical Research Service (A.N.T).

291.14

CENTRAL CATECHOLAMINE VARIATIONS ASSOCIATED WITH ANTIGEN ADMINISTRATION. S. Zalcman*, N. Shanks and H. Anisman. Dept Psychology, Carleton University, Ottawa, Ontario K1S 5B6, Canada.

Administration of sheep red blood cells (SRBC; 10^6 cells, ip) to CD-1 mice reduced norepinephrine (NE) concentrations and increased the accumulation of the NE metabolite, MHPG, in hypothalamus, hippocampus and locus coeruleus. Likewise, decreased dopamine (DA) levels and increased accumulation of the DA metabolite, DOPAC, were evident in the nucleus accumbens, while nigrostriatal DA activity was unaffected. These effects corresponded with the peak immune response to SRBC (i.e., 4 days after inoculation). At other intervals (1, 2, 3, 5, 6 days) amine changes were minimal, except for NE reductions in the locus coeruleus, which were also evident 3 days after immunization. These findings are consistent with the proposition that central amine variations may be related to immune activity. Since stressors provoke a similar neurochemical profile, the possibility exists that the antigen-induced immunological changes are interpreted as one component of a stress response.

291.15

EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS: EFFECT OF INTRACISTERNAL INJECTION OF COMPOUND 48/80 E.L. Orr, F.L. Jackson*, N.C. Stanley*. (SPON. D. Barker) Dept. of Anatomy. Texas College of Osteopathic Medicine. Ft. Worth, TX 76107.

Mast cells have been shown to release bioactive compounds which mediate increased vascular permeability and cellular inflammation in response to various immune or non-immune stimuli. A similar release of these compounds can be induced by treatment with Compound 48/80, a recognized mast cell degranulating agent. The development and expression of autoimmunity relative to the nervous system requires that specific autoimmune mediators exit the blood vascular system and enter the cerebrospinal fuid (CSF). Experimental autoimmune encephalomyelitis (EAE) is a model for CNS associated autoimmune disease (eg., multiple sclerosis). Our laboratory uses a recurrent model for EAE based on the ability of female Lewis rats to exhibit predictable primary and secondary occurrences of disease. To examine the hypothesis that nervous system-associated mast cells may modulate the disease process in animals with EAE, the following experimental paradigm was employed. Adult female Lewis rats were inoculated to induce EAE and assessed for behavioral symptoms beginning on day 7 postinoculation (pi). Immediately prior to the initial onset of the disease, animals were injected intracisternally with Compound 48/80 (10μg/20μl/5min). Control animals received vehicle alone. Our results indicate Compound 48/80 caused a significant reduction in the severity of the secondary occurrence of EAE with a lesser reduction observed in the primary occurrence of EAE relative to controls. Similar treatment prior to the secondary occurrence of EAE failed to elicit a similar effect indicating a functional difference in sensitivity to mast cell manipulations between the two occurrences. Based on the response of the animals to Compound 48/80, mast cells either directly or indirectly via other CNS tissues are likely able to regulate the disease process. Supported by a Grant from the NMSS #PP0053.

291.16

GLIOSIS IN DISEASES WITH NEURODEGENERATION AND IMMUNE SUPPRESSION. W.S.T. Griffin, L.C. Stanley*, J.S. Johnson* and R.C. Woody*. Departments of Pediatrics and Anatomy, UAMS, Little Rock, AR 72205.

Neurodegeneration can result from a variety of insults to the brain; apparently, without regard to the cause, gliosis ensues. In addition, several neurodegenerative diseases are associated with depressed immunity and have decreased expression of immune-response-generating, macrophage-derived interleukin-1 (IL-1) perhaps via induction of corticotrophin releasing factor (CRF). Moreover, with trauma-induced neurodegeneration, increases in glia-derived IL-1 can stimulate astrogliosis. To test the hypothesis that the gliosis of human neurodegenerative diseases of diverse cause is associated with increased expression of glial IL-1, we examined formalin-fixed, paraffin-embedded postmortem temporal lobe sections from 10 Alzheimer's (AD), 8 Down's, 6 AIDS, and age-matched-controls using antibodies to GFAP, S100, IL-1, and α/β-tubulin. Reactive astrocytes filled with GFAP and S100 immunoreactive product were present in each disease as were reactive glia filled with IL-1 immunoreactive product. The levels of IL-1, S100, and GFAP, but not tubulin, were elevated in homogenates of AD temporal lobe compared to controls. Therefore, although macrophage-derived IL-1 is decreased in the diseases studied, we conclude that elevation of glial-derived IL-1 may induce astrogliosis and contribute to immune suppression if glial IL-1, like macrophage IL-1, can stimulate CRF release.

291.17

ANALYSIS OF IMMUNOGLOBULIN A (IgA) IN CEREBROSPINAL FLUID (CSF) OF PATIENTS WITH NEUROINFLAMMATORY DISEASE A.H. Woo*, P.M. Knopf*, and H.F. Cserr (SPON: J.T. Parmelee) Physiology & Biophysics, Brown Univ., Providence, RI 02912

Elevated levels of CSF IgA appear in several neuro-inflammatory diseases and cannot be solely attributed to serum leakage across damaged blood-brain barrier (BBB) membranes. IgA is secreted across mucosal membranes by a specific receptor called secretory component (SC) which is found in high concentrations in mucus, colostrum, etc. If SC were detected in patient CSF, it would provide evidence for a secretory immune system in the brain.

CSF and sera from patients with amyotrophic lateral sclerosis, CNS Lupus, encephalitis, Guillain-Barré, meningitis, and multiple sclerosis were analyzed for IgA and SC using an Enzyme Immunoassay. Albumin (Alb) concentrations were determined by Radial Immunodiffusion. Normal and Parkinson's disease patients were used as negative controls. Purified colostrum SC, bile, and saliva were used as positive controls. An IgA index was used to evaluate CSF IgA with respect to BBB integrity where Index = [CSF IgA/serum IgA] / [CSF Alb/serum Alb]. 11 of 21 neuroinflammatory patients exhibited an elevated IgA index (>log mean + 2 SD of controls) but no CSF SC was detected. There does not appear to be a secretory IgA immune system in the CNS. Supported by NS-11050.

291.18

IMMUNOLOGICAL DYSFUNCTION IN GENETICALLY EPILEPSY-PRONE RATS. R.R.R. Rowland*, D.Y. Tso-Olivas*, S. Tokuda*, G.K. Weiss* and D.D. Savage. (SPON: E.H. Uhlenhuth). Depts. Microbiology, Physiology and Pharmacology, Univ. New Mexico Sch. Med., Albuquerque, NM, 87131.

Lymphocyte function has been shown to be regulated by a variety of lymphokines, hormones and neurotransmitters. The ability of the central nervous system and the endocrine system to regulate the immune system predicts that developmental defects in neuroendocrine function could produce alterations in the immune system. Genetically Epilepsy-Prone (GEPR-9) rats have been inbred for a genetic predisposition for acoustic stimulus-induced seizures. These rats possess deficits in brain norepinephrine and serum thyroid and growth hormones. Given these neuroendocrine deficits, we investigated immune function in GEPR rats.

Adult GEPR-9 and non-epileptic control Sprague-Dawley rats were immunized with sheep erythrocytes (SE), a T cell dependent antigen. Spleens were removed on day 5 or 7 and the anti-SE plaque-forming cell (PFC) responses were measured. The direct PFC response (IgM, day 5) of the GEPR group was 30% of control and the indirect PFC response (day 7, IgG) was 13% of control, indicating significant immunosuppression in the GEPR rat. Immunocompetence of GEPR rats was further evaluated by stimulating spleenocytes *in vitro* with concanavilin A (Con A) and pokeweed (PWM) mitogens. GEPR rats exhibited a 6 fold greater response than controls when stimulated with 5 μg/ml Con A, a T cell mitogen. The GEPR response to 5 μg/ml of PWM, a T cell-dependent B cell mitogen produced a proliferative response that was 19 times greater than controls. In contrast to the PFC data, the mitogen data suggest *immunoenhancement* in GEPR rats, presenting an interesting paradox. The inability of a hyperresponsive T cell population to participate in the response to a specific antigen challenge (*e.g.* SE) suggests a breakdown in the normal modulation of T cell function. The specific cause of this immune dysfunction is not known, but may be due to the inability of neuroendocrine systems in GEPR rats to modulate T cell activity. (Supported by NS24008 and RR08139)

291.19

REGIONAL CEREBRAL CORTICAL DEFICITS IN THE IMMUNE-DEFICIENT MOUSE. G.O. Gaufo*, S. Cronin*, and M.C. Diamond. Depart. of Physiology-Anatomy, Univ. of Calfornia, Berkeley, CA 94720.

The purpose of our investigation is to identify the cerebral cortical representation of an important aspect of the immune system, the T-cell mediated immunity. Bizière et al. (*Neurosci. Abstr.*, 6:31, 1980) found that ablation of the left dorsal and lateral aspects of the frontal, parietal, and occipital cortices resulted in decreased T-cell and NK activities as compared to ablation of the same areas on the corresponding hemisphere. This experiment inspired us to examine the thickness of the cortex of the genetically athymic nude mouse, a T-cell deficient animal. Among thickness measurements in frontal, somatosensory, and occipital areas, the frontal and lateral parietal cortices showed the greatest deficiencies. Surprisingly, in many other areas, the thickness of the cerebral cortex of the nude mouse did not differ significantly from the BALB/c mouse, an immune-competent animal. In addition, we found a significant reduction in the number of oligodendrocytes per unit area in left area 18 of the cerebral cortex compared to the immune-competent mouse (Diamond et al., *Exp. Neurol.*, 92:311, 1986).

We are presently investigating the effects of neonatal thymectomy on development of the cerebral cortex in the BALB/c mouse. By removing the thymus neonatally, we deprive the animal of the primary lymphoid organ responsible for producing T-cells. At 2 days of age, male and female BALB/c mice are assigned to one of three conditions: (i) thymectomy (ii) sham-operated and (iii) non-operated. There are 12 mice per group. Animals are placed back with their lactating mothers and weaned at 21 days of age. On day 41, the mice are anesthesized, perfused and brains removed. Twenty micra, transverse, frozen sections are taken of the frontal, somatosensory, and occipital cortex. On thionine stained sections, cortical thickness measurements of 9 different regions are made on microslide projected images (22.5X). In addition, the cortical cell morphology is analyzed in these brain sections.

Our hypothesis is that reduction of the source of T-cell production might localize a specific cortical region related to the control of T-cell mediated events. This approach refines the work of other investigators who created widespread lesions of the cortex and found modulatory effects on the immune system as a result.

291.20

DRAINAGE OF INTERSTITIAL FLUID (ISF) INTO DEEP CERVICAL LYMPH FROM DIFFERENT REGIONS OF RABBIT BRAIN. S.Yamada* and H.F.Cserr. Physiology and Biophysics, Brown University, Providence, RI 02912.

50% of ISF from rabbit caudate nucleus (CN) drains into cervical lymph. The route involves flow via perivascular spaces, along olfactory nerve sheaths, and into nasal submucosa (Bradbury et al., Am.J.Physiol., 240:F329, 1981). This study examines ISF flow in anesthetized rabbits from two additional brain regions, internal capsule (IC) and midbrain (MB). Lymph from both jugular lymph trunks was collected for a period of 2 to 5 hours, between 1/2 and 25 hours after microinjection of ^{125}I-albumin into brain. ISF drainage rates ($\mu l \cdot g\ brain^{-1} \cdot min^{-1}$), estimated from rates of tracer efflux from brain, were 0.10 and 0.17 for IC and MB as compared to 0.11 for CN. High tracer activity was associated with the ipsilateral middle cerebral artery after forebrain injection (IC or CN) and with the ipsilateral superior cerebellar artery after MB injection, consistent with efflux via perivascular spaces surrounding arteries supplying the injection site. Tracer drained preferentially to ipsilateral lymph and nodes after forebrain injection in contrast to lack of laterality after MB injection, suggesting complete mixing of ISF draining from ipsilateral and contralateral MB. Drainage into lymph accounted for 25% of tracer cleared from IC and 34% from MB. Supported by NIH Grant NS-11050.

NEUROENDOCRINE REGULATION: PROLACTIN

292.1

INFLUENCE OF SEROTONIN (5HT) ON THE DIURNAL AND NOCTURNAL SURGE OF PROLACTIN (PRL) IN THE PREGNANT RAT. A. Mistry* and J.L.Voogt. Dept of Physiology, Univ of Kansas Med Ctr, Kansas City, KS 66103.

5HT has been implicated in PRL control during various physiological states. This study evaluated the role of 5HT in the nocturnal and diurnal surges of PRL seen during early pregnancy. On day 8 of pregnancy, blood samples were withdrawn between midnight and 600h from previously implanted intracarotid catheters. Rats were sacrificed by decapitation, brains were frozen on dry ice and stored at -70C until later determination of biogenic amines using HPLC with electrochemical detection. Administration of parachlorophenylalanine (PCPA 250mg/kg ip), an inhibitor of 5HT synthesis did not affect the nocturnal PRL surge although it reduced 5HT content in the medial basal hypothalamus (MBH) to 20% of controls. PCPA also reduced dopamine (DA) but to a much lesser extent. Ketanserin (Ket 10mg/kg ip) and LY-53857 (5mg/kg ip) two selective 5HT2 receptor blockers were effective in greatly reducing the nocturnal PRL surge when injected at midnight. It is not clear whether the failure of PCPA to block the PRL surge is due to its inhibitory effect on DA or whether Ket and LY-53857 have effects separate from their 5HT2 antagonism. To test whether 5HT may have some role in the diurnal surge (Endocrinol 124:878,1989) rats were decapitated at 1800h on day 7 of pregnancy. Pretreatment with PCPA (24h) or Ket (2h) or LY-53857 (2h) greatly reduced plasma PRL levels to 3-7% of controls. Similar to results in the nocturnal study, PCPA greatly reduced MBH content of 5HT and also reduced DA. It is concluded that 5HT plays a significant role in the diurnal PRL surge during pregnancy. Its role during the nocturnal surge is unclear because of the contradictory effects of PCPA and the receptor blockers. (Supported by grant HD 24190)

292.2

SEX-RELATED ALTERATIONS OF TYROSINE HYDROXYLASE (TH) ACTIVITY AND IMMUNOSTAINING IN THE STALK-MEDIAN EMINENCE (SME) AFTER NEONATAL MONOSODIUM GLUTAMATE (MSG) TREATMENT. L. A. Arbogast and J. L. Voogt, Physiology Dept., Kansas Univ. Med. Ctr., Kansas City, KS 66103.

Neonatal MSG-treatment causes a loss of hypothalamic dopamine (DA) perikarya. The TH-containing nerve terminals are located in the SME. DA serves as a PRL inhibiting hormone. The aims of this study were: 1) to evaluate MSG toxicity on the amount and activity of TH in the SME of male and female rats, and 2) to relate changes in DA neuronal function to serum prolactin (PRL) levels. Male and female rats were injected with 4 mg/kg MSG or 10% NaCl (controls) on days 2,4,6,8 and 10 of age. Females were ovariectomized on day 45 and all rats were used on days 60-80. The TH protein was evaluated immunocytochemically in coronal brain sections using an avidin-biotin immunoperoxidase method. The number of hypothalamic DA perikarya was reduced in both males and females after MSG-treatment as compared to controls. TH quantity in the SME was less in male vs. female controls, but was markedly reduced in females and only modestly reduced in males after MSG-treatment. *In vitro* TH activity was assessed by incubating hypothalamic explants with brocresine, a decarboxylase inhibitor. Dihydroxy phenylalanine (DOPA) accumulation in the SME was measured by HPLC with electrochemical detection. TH activity was 3-fold higher in female than male controls. TH activity in the SME was 5-fold greater in control than MSG-treated females, but was unchanged in males. Serum PRL levels were 4-fold higher in male than female controls, but was not altered by MSG treatment in either sex. Conclusions: 1) the effects of MSG toxicity on the postnatal development of hypothalamic DA neurons is more profound in female than male rats, and 2) alterations in DA neuronal function are not always related inversely to circulating PRL levels. Supported by NIH grant HD 24190.

292.3

CONTRIBUTION OF ARACHIDONIC ACID METABOLITES TO BASAL AND TRH-INDUCED PROLACTIN RELEASE. M.P. Junier*, J.M. Israel*, F. Dray, J.D. Vincent, INSERM U176 BORDEAUX, INSERM U207, Institut Pasteur, PARIS, FRANCE

The contribution of arachidonic acid (AA) metabolites to basal and TRH-induced release of Prolactin (Prl) was investigated using perifusion of enriched cultures of lactotroph cells derived from pituitary glands of lactating female rats . Inhibition of AA lipoxygenase (LPX) and epoxygenase pathways by 10 uM Nordihydroguaiaretic acid (NDGA) decreased Prl release to ~35% of control values. In contrast, 10uM indomethacin, an inhibitor of AA cyclooxygenase pathway, was without effect. Of the LPX metabolites tested only 15-HPETE and 15-HETE induced Prl release with a maximal effect at 10-7 and 10-6 M respectively. In the presence of 10uM NDGA the absolute amount of Prl released by TRH was markedly decreased. However, because NDGA decreased basal Prl release, the relative increase induced by TRH over basal Prl levels was not markedly different between the two groups (% of Prl increased by TRH / % of Prl increased by TRH+NDGA = 0.81+0.07). In contrast, inhibition of the protein kinases A and G by H8 (10 uM) failed to alter basal Prl release but strongly inhibited the effect of TRH (% of Prl increased by TRH / % of Prl increased by TRH+H8 = 0.58+0.10). These results suggest that : a) AA LPX metabolites are involved in the maintenance of basal Prl release from enriched lactotroph cells in culture, b) the availability of TRH to stimulate Prl release is mainly independant of LPX activity, c) TRH-induced Prl release involves the participation of protein kinases A and/or G.

292.4

STIMULATION OF GH AND PRL SECRETION AND GENE EXPRESSION BY GH-RH IN A HUMAN PITUITARY CELL LINE. L. Dufy-Barbe*, D.W. Schmid* and B. Dufy., Lab. Neurophysiologie, CNRS UA 1200, Université de Bordeaux II, F-33076 Bordeaux Cedex, France.

From a somatotropic tumor, we have isolated human pituitary adenoma cells which behave *in vitro* as a cell line and could be maintained in culture up to passage 25. Immunocytochemical identification revealed that it was multihormonal and contained prolactin (PRL), growth hormone (GH), and the glycoprotein α chain immunoreactive cells. Measurement of basal hormone secretion showed that basal PRL and GH release declined rapidly with time in culture after passage 5 but still remained detectable.

The effects of exposure to 5 nmol/l GH-RH in a chemically defined medium significantly increased the secretion of GH (2.1 ng GH/96h/10^5 cells vs 0.6 in controls) and PRL (parallel increase). In addition, GH-RH stimulated protein synthesis significantly (6.1 µg/well vs 3.7 in controls).

In order to demonstrate GH-RH effects on gene expression of GH and PRL, Northern blot hybridization with RNA isolated from control and GH-RH treated cells was carried out. Synthetic oligonucleotide probes corresponding to different parts of the coding region for GH (Seeburg et al. 1977) and PRL (Martial et al. 1977) were used. These probes detected a single RNA species the size of which corresponded to published values (Murdoch et al. 1983). Moreover, we could detect differences in RNA abundance related to the GH-RH treatment.
This GH-RH responsive human pituitary cell line is presently used for the study of human GH and PRL gene expression.

292.5

EFFECT OF PROLACTIN ON THE NUMBER OF TUBEROINFUNDIBULAR DOPAMINE NEURONS IN AMES DWARF MICE. W.W.Morgan and K.C.Besch*,Univ.Texas Hlth.Sci.Ctr.,San Antonio,TX 78284.

Ames dwarf mice do not produce growth hormone (GH) or prolactin (PRL), and dopamine (DA) is virtually absent in the median eminence (ME). PRL replacement partially restores DA synthesis but not DA levels in the ME suggesting that the tuberoinfundibular dopaminergic (TIDA) neurons are very underdeveloped in Ames dwarfs. An antibody to tyrosine hydroxylase (TH; Eugene Tech) and an advin-biotin-peroxidase procedure were used to immunohistochemically determine if the number of TIDA neurons is also reduced in Ames dwarfs. TH-immunoreactive neurons were marked fewer in number in the arcuate nuclei but not the substantia nigra of Ames dwarfs compared to phenotypically normal mice of the same strain. Replacement of PRL by the implantation of a normal female pituitary under the kidney capsule or by the administration of ovine PRL (250ug/day) for two weeks increased the number of TH-positive neurons in the arcuate nuclei of male or female dwarfs to a level comparable to that seen in normal mice. Sham surgery or the administration of the sodium bicarbonate vehicle were ineffective. These data suggest that Ames dwarfs possess a nearly normal number of TIDA neurons, but most are very inactive due to the absence of circulating PRL. Supported by NIH # 22141.

292.6

HYPERPROLACTINEMIA (HyperPRL) INCREASES PROOPIOMELANOCORTIN GENE EXPRESSION IN THE ARCUATE NUCLEUS OF OVARIECTOMIZED RATS. K. Scarbrough[1], R.A. Steiner[2] and P.M. Wise[1], [1]Dept. Physiology, University of Maryland School of Medicine, Baltimore, MD 21201 and [2]Dept. Physiology and Biophysics University of Washington, Seattle, WA 98195.

HyperPRL can suppress LH release in ovariectomized (OVX) rats through hypothalamic mechanisms. To determine whether hyperPRL inhibits LH secretion by influencing opiate tone, we measured proopiomelanocortin (POMC) gene expression in the arcuate nucleus of OVX and OVX prolactin-treated rats. Rats were OVX and 4 days later half the rats received a subcutaneous injection of ovine prolactin (4 mg/kg body weight) every 8 hours for 2 days. Controls received an injection of vehicle (0.01M $NaHCO_3$, pH 8.6) at these times. The rats were killed one hour after the last injection, brains were removed and frozen at -70°C. Trunk blood was assayed for LH by RIA. A ^{35}S-labeled riboprobe complementary to POMC mRNA was prepared according to the methods of Chowen-Breed et al. (Endocrinol. 124:1697, 1989). 20μm sections were fixed in 4% paraformaldehyde, washed, acetylated and hybridized to the riboprobe at 45°C. After RNase treatment, stringent wash, and dehydration, slides were first exposed to x-ray film and then dipped in emulsion. Both film and emulsion-coated slides were developed using conventional photographic methods. Experimental conditions were optimized for probe concentration and exposure to film and emulsion. Films and slides were analyzed using a computerized image analysis system. HyperPRL significantly suppresses serum LH (OVX: 5.64 ± 0.60 HyperPRL: 3.80 ± 0.44 ng/ml). HyperPRL rats show a significant ($p < .05$) increase in POMC mRNA expression in the arcuate nucleus when compared to OVX controls. These data suggest that hyperPRL may increase the activity of opiatergic neurons in the arcuate nucleus and thereby inhibit LH secretion.

292.7

DIHYDROTESTOSTERONE (DHT) INHIBITS ESTROGEN (E) INDUCED HYPERPROLACTINEMIA IN THE MALE RAT. P.C. Doherty, and A.J. Carrillo, Dept. of Anatomy, N.E. Ohio Univ. Coll. of Med., Rootstown, OH 44272.

Prolonged treatment with E causes pituitary cell hypertrophy, hyperplasia and hypersecretion of prolactin (PRL). Similar treatment with testosterone (T) does not. The present experiments were performed to determine if coincubation of in situ and ectopic pituitaries in vivo with both DHT and E, the principal metabolites of T, could inhibit E-induced increases in pituitary weight and serum PRL. Adult male Fischer 344 rats were castrated and treated with subcutaneous implants of crystalline T, DHT, E, or E+DHT. Two additional groups were given four ectopic pituitary grafts and steroid capsules containing E or E+DHT. Six weeks later, the animals were sacrificed, the pituitaries were

	E	DHT	E+DHT	T
pit. wt.(mg)	22.0± 2.0	7.0±0.2	16.0±1.0	9.0± 0.4
prl (ng/ml)	212.7±102.0	16.2±3.2	12.7±3.2	45.8±24.0

removed and weighed, and trunk blood was collected for the PRL RIA. The E-induced increase in pituitary weight was inhibited by treatment with DHT but not to the levels seen in T-treated animals. In contrast, serum PRL levels were greatly inhibited in the E+DHT group. No inhibitory effects of DHT were observed in pituitary grafted male rats (1480±290 vs 1280±178 ng/ml). These results suggest that DHT can inhibit the effects of E but that hypothalamic input is necessary for these effects to occur. In addition, the differential effects of E+DHT on serum PRL levels and pituitary weight suggest that the primary mode of DHT action is on secretion. (Supported by BMRS Grant No. S07RR05806-06 and a Grant from the United Way of Central Stark County.)

292.8

DIRECT FEEDBACK ACTIONS OF GONADAL STEROIDS ON IN VIVO LH SECRETION BY THE ISOLATED PITUITARY GLAND: EFFECTS OF HYPERPROLACTINEMIA F.J. Strobl*, J.M. Meredith*, and J.E. Levine (SPON: B. Menco). Dept. of Neurobiology & Physiology, Northwestern University, Evanston, IL 60208

We investigated whether hyperprolactinemia modulates the feedback actions of gonadal steroids exerted on the anterior pituitary (AP). Hypophysectomized, ovx rats (n=20) received 2 AP under the kidney capsule. Each rat was then fitted with an atrial catheter for infusion of LHRH (250ng/5min/h) and blood sampling. Blood samples were obtained at 2h intervals from 1600h on Day 5 of LHRH infusions to 2400h on Day 6. Rats received 1 Silastic capsule containing estradiol (E) (150μg/ml) at 1800h on Day 5 and 2 capsules containing oil or progesterone (P) (50mg/ml) at 1200h on Day 6. To prevent AP transplant-induced hyperprolactinemia a group of steroid-treated rats received the dopamine agonist CB-154 daily (1mg sc). Plasma LH and PRL levels were determined by RIA. LH levels in all control rats decreased from 0.79±0.09 to 0.36±0.03 ng/ml ($p < .01$) within 4h following E treatment. Thereafter, LH levels increased to 1.23±0.11 ng/ml by 2000h on Day 6. P had no additional effect on LH secretion ($p > .05$). Although CB-154 did not alter the pattern of LH responses to steroid treatment, it enhanced overall mean levels of LH by 48% ($p < .01$). These data suggest that hyperprolactinemia results in continued inhibition of LH secretion by direct actions on the AP, and that this action appears to be independent of the direct feedback effects of gonadal steroids.

292.9

THE PROLACTIN SECRETORY RESPONSE TO IMMOBILIZATION STRESS AND SEROTONERGIC STIMULATION DURING LACTATION. P. Callahan, Leslie Besecke, J. Janik, and J. Rabii. Zoology Dept., Miami University, Oxford, OH 45056 and Department of Biological Sciences, Rutgers University, Piscataway, NJ 08855.

We have previously reported an altered sensitivity in the prolactin secretory response to morphine and methadone during lactation. The purpose of this study was to determine the sensitivity of the lactating model to other prolactin secretory stimuli, namely immobilization stress and serotonergic stimulation. The immobilization stress was chosen since it has been reported to increase prolactin levels by decreasing tuberoinfundibular dopaminergic (TIDA) neuronal activity. The effects of serotonergic stimulation were also determined since serotonin appears to be involved in the suckling induced prolactin increase.

Non-lactating and lactating female rats (day 6-10 post-partum) were implanted with chronic jugular cannula 24 hours prior to the experiment. On the day of the experiment, pups were removed from the postpartum females prior to any treatment or drug administration. Animals were subjected to 10 minutes of immobilization stress. Serotonergic stimulation was accomplished by administering fluoxetine (FLX; 10 mg/kg, ip) followed one hour later by 5-hydroxytryptophan (5HTP; 30 mg/kg, ip).

The lactating female rats did not exhibit a prolactin secretory response to either of these stimuli. This was in marked contrast to the effects in the non-lactating female which clearly showed a significant increase in circulating levels of prolactin. These results support the notion that the lactating female exhibits an altered sensitivity to prolactin secretory stimuli. The mechanism(s) for this altered sensitivity is not known, however, we have previously reported a lack of sensitivity of the TIDA neurons to morphine induced inhibition (Callahan, et al., Life Sci., 43:49 '88). This may be one possible mechanism since TIDA neuronal inhibition seems to be required for a prolactin secretory response during lactation (Grosvenor, et al., Endo, 106:481, '80).

292.10

OPIOID NEURONS SYNAPSE ON TUBEROINFUNDIBULAR DOPAMINE NEURONS IN THE ARCUATE NUCLEUS OF JUVENILE MONKEYS. P.C. Goldsmith, J.E. Boggan* and K.K. Thind. Reproductive Endocrinology Center and Dept. of Ob/Gyn & Repro. Sci., Univ. Calif., Sch. Med., San Francisco, CA 94143.

Opioid (OP) neurons in the arcuate nucleus (ARC) synaptically inhibit nearby gonadotropin-releasing hormone (GnRH) neurons in monkeys (Thind and Goldsmith, *Neuroendocrinology* 47:203, 1988). Corticotropin-releasing factor neurons innervate periventricular dopamine (DA) neurons but not ARC OP neurons (Thind and Goldsmith, *Neuroendocrinology*, in press). To determine how these systems might interact, we investigated whether DA and OP elements form synapses in the cynomolgus monkey ARC. Tuberoinfundibular (TI) neurons in 4 juveniles were retrogradely labeled from the median eminence prior to aldehyde perfusion (see *Soc. Neurosci. Abstr.* 14:439, 1988). Frontal vibratome sections were immunostained for tyrosine hydroxylase with PAP for DA neurons, and for adrenocorticotropin with 15 nm gold for OP neurons. In the ARC, 63% of the DA neurons, but none of the OP neurons, were TI. Many DA neurons were contacted by OP axons, in agreement with results in the rat (Morel and Pelletier, *Peptides* 7:1197, 1986). In contrast to that report, symmetrical synapses were found between OP axon terminals and TI DA cell bodies. Small clear synaptic vesicles but no dense OP secretory granules were present at presynaptic sites, perhaps indicating tonic neurosecretion. The results suggest that OP neurons inhibit TI DA neurons in the ARC, where OP can act to increase prolactin secretion. Since both OP and DA neurons are steroid responsive, their interaction represents a sensitive control point in the neuroendocrine regulation of prolactin and perhaps also GnRH secretion in primates. (Supported by NIH HD10907).

292.11

ACETYLCHOLINE, MORPHINE, SEROTONIN AND DOPAMINE REGULATE THE IN VIVO RELEASE OF PROLACTIN THROUGH A COMMON PATHWAY.

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Nicotinic cholinergic, opiate and serotonergic agonists as well as dopaminergic antagonists release pituitary prolactin. The present studies were undertaken to explore the anatomical relationship and interdependence of the synapses utilized by such drugs. Consequently, we have characterized a common pathway employed by nicotine, morphine, 8-hydroxy dipropylaminotetralin (8-OH-DPAT) and haloperidol to release prolactin in conscious, freely moving, male rats implanted with jugular cannulae 24 hr prior to experimentation. In saline pretreated rats, the sequential administration (30 - 60 min apart) of nicotine (100 μg/kg), morphine (3 mg/kg), 8-OH-DPAT (100 μg/kg) and haloperidol (0.5 mg/kg) resulted in significant increases (3 - 10-fold) in plasma prolactin levels. Mecamylamine (3 mg/kg) antagonized the nicotine induced prolactin release only. Naltrexone (4 mg/kg) prevented the release by morphine and by nicotine, but not by 8-OH-DPAT or haloperidol. Methysergide (5 mg/kg) blocked the prolactin releasing effects of nicotine, morphine and 8-OH-DPAT but not haloperidol. Finally, bromocriptine (0.5 mg/kg) blocked the prolactin increases induced by all three agonists as well as by haloperidol. These data indicate that a poly-synaptic pathway regulating the in vivo release of prolactin is subserved, in series, by nicotinic cholinergic, opiate, serotonin and dopamine receptors, respectively.

292.12

SEROTONIN-1A RECEPTORS MEDIATE PROLACTIN RELEASE IN THE RAT.

B.A. Hulihan-Giblin[1]*, C.M. Flores1*, M.D. Lumpkin[2]*, and K.J. Kellar[1] (SPON: A.R. Raines). Dept. of Pharmacology[1] and Physiology[2], Georgetown Univ. School of Medicine, Washington, D.C. 20007.

Serotonin (5-HT) agonists stimulate prolactin secretion, probably by acting within the basal hypothalamus to influence the activity of tuberoinfundibular dopamine neurons. The type of 5-HT receptor involved in mediating prolactin release is not yet resolved. Although the 5-HT-1A agonist 8-OHDPAT has been found to stimulate prolactin release in some studies, other studies have found it to be ineffective or that the effect is not dose-related. We have examined the effect of 8-OHDPAT on prolactin release in rats in which an indwelling jugular cannula allowed intravenous injection of drugs and multiple sampling of blood over time. The effect of 8-OHDPAT (100 μg/kg) on prolactin release is rapid and relatively brief, with prolactin levels peaking at 8 times baseline within 10 min and returning to baseline within 40 min. 8-OHDPAT is a potent releaser of prolactin, with an ED_{50} of 20 μg/kg. The effect of 8-OHDPAT is completely blocked by pretreatment with methysergide 1 hr before. Another 5-HT-1A agonist, 5-methylurapidil is also a potent releaser of prolactin, reinforcing the idea that these responses are mediated by 5-HT-1A receptors. The prolactin response to 8-OHDPAT appears to desensitize rapidly; and 90 min after a single injection of 8-OHDPAT, the response to a second injection is diminished. The ED_{50} for this desensitization by 8-OHDPAT is approximately 200 μg/kg. This desensitization by 8-OHDPAT appears to be specific because the prolactin response to thyrotropin releasing hormone is unaffected by prior injection of 8-OHDPAT.

292.13

SUCKLING AND 5-HYDROXY-L-TRYPTOPHAN STIMULATE GROWTH HORMONE SECRETION VIA CHOLINERGIC MECHANISMS IN THE NEONATAL RAT.

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The present experiments investigated the mechanisms underlying the high circulating growth hormone (GH) levels occurring in neonatal rats. Separation of 2-day-old rat pups from their mothers decreased, while a subsequent suckling period increased, serum GH levels in the pups. The serotonin antagonist cyproheptadine (1 or 10 mg/kg, s.c.) decreased serum GH values in separated pups (i.e. decreased basal secretion), but failed to inhibit the suckling-induced release of GH. Similarly, the serotonin precursor 5-Hydroxy-L-Tryptophan (5-HTP, 5 mg/kg, s.c.) increased serum GH levels in separated pups, and further stimulated the GH release induced by suckling. Atropin (10 mg/kg, s.c.), a blocker of the cholinergic muscarinic receptors, decreased serum GH levels in separated pups, and prevented the suckling-induced release of GH. Further, atropin prevented the GH-releasing effect of 5-HTP in separated pups. The alpha 2 receptor blocker yohimbine (10 mg/kg, s.c.) did not reduce serum GH values in separated pups, yet it prevented the suckling-induced release of GH. Rat GH-releasing hormone (rGHRH) did not stimulate GH secretion either in separated or in suckled rat pups. These findings suggest that 1) the mechanisms stimulating GH secretion by suckling or by 5-HTP, are arranged in a parallel (rather than serial) fashion, 2) while serotonin regulates basal secretion, the alpha 2 system mediates the suckling-induced release of GH, 3) both suckling and 5-HTP stimulate GH secretion via cholinergic mechanism(s), and 4) neither suckling nor 5-HTP induce GH release via rGHRH. (Supported by HD-04358 to CEG).

292.14

EFFECT OF CENTRAL INJECTION OF CRF ON CORTISOL AND PROLACTIN SECRETION IN THE SHEEP.

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Stress evokes a response characterized by activation of the hypothalamo-pituitary-adrenal axis with release of CRF and/or AVP, ACTH and cortisol. As well as its role in stimulating ACTH release from the pituitary, there is evidence that CRF can act via central pathway(s) to modify other neuroendocrine systems. The following studies were therefore undertaken to investigate whether central administration of CRF can alter the secretion of two hormones under different regulatory control and known to be released during stress in the sheep, namely cortisol and prolactin.

Scottish Blackface ewes were ovariectomized and implanted with a cannula directed towards the third cerebral ventricle. Plasma cortisol and prolactin concentrations were measured in 20min blood samples taken over a 12h sampling period. Intracerebroventricular injection of saline (50μl) or CRF (0.12nmol and 1.2nmol) was made at 4h and naloxone injected intravenously at 4h, 5.5h, 7h and 8.5h as required.

Injection of CRF (1.2nmol) into the third ventricle resulted in a significant increase in both plasma cortisol and prolactin concentrations. At the lower dose, CRF (0.12nmol) increased cortisol levels by enhancing pulsatility, but had less consistent effects on prolactin secretion. Intravenous injection of naloxone blocked the CRF-induced increase in prolactin but did not alter the ability of CRF to increase plasma cortisol. Neither saline nor naloxone alone affected plasma cortisol or prolactin levels.

In conclusion, central administration of CRF increases both cortisol and prolactin secretion in the sheep. In the case of prolactin, this increase was reversed by naloxone, suggesting a central action mediated by endogenous opioids. With respect to cortisol, it is possible that centrally injected CRF acted directly on the pituitary gland following leakage of the higher dose into the pituitary portal circulation. However, the lower dose of CRF increased pulsatile secretion of cortisol, providing evidence for a central component in the response. At present, the relative contribution of each remains to be investigated. In addition, these data demonstrate that basal cortisol and prolactin secretion in the ovariectomized ewe is not under tonic opioid inhibition.

292.15

CENTRAL INVOLVEMENT OF EXOGENOUS AND ENDOGENOUS CRF IN REGULATING PROLACTIN SECRETION IN THE SHEEP.

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Central administration of CRF to sheep stimulates the release of prolactin from the pituitary gland. This effect of CRF is prevented by the opioid antagonist naloxone, suggesting that the stimulatory effect of CRF on prolactin secretion is mediated by endogenous opioid peptides and is central in origin. Several observations indicate that central CRF may participate as a neurotransmitter in the brain regulating neuroendocrine responses to stress. The following studies were undertaken in sheep to investigate further this hypothesis using prolactin, a hormone which is released during stress in this species. Specifically, the effect of a CRF antagonist (αCRF9-41) on prolactin secretion was investigated under basal and stimulated conditions.

Scottish Blackface ewes were ovariectomized and implanted with a cannulae directed towards the third cerebral ventricle. Half of the sheep were implanted s/c with silastic implants containing estradiol. Plasma prolactin was measured in either 10 or 20min blood samples taken over an 8-11h sampling period. Intracerebroventricular injection of sterile saline (50μl), CRF (1.2 nmol) or αCRF9-41 (15.5 nmol) was made 4h into the sampling period. Introduction of a ram was used as a stimulus to evoke prolactin release where nesessary.

Injection of CRF (1.2 nmol) evoked a significant release of prolactin which was antagonized by prior treatment with αCRF9-41 (15.5 nmol). In contrast, injection of saline or αCRF9-41 alone did not alter prolactin secretion. Introduction of a ram evoked an increase in plasma prolactin to about 3x control levels. When introduction of the ram was preceded by a central injection of αCRF9-41 (15.5 nmol), the elevation in prolactin was reduced in 5/6 of the animals when compared with the saline controls.

In conclusion, these data indicate that central CRF releases prolactin via a central mechanism involving CRF receptors. However, under basal conditions there is no tonic stimulatory control by endogenous CRF of prolactin secretion. Under stimulated conditions (ie. in the presence of a ram), there is evidence to suggest that endogenous central CRF may be responsible for the elevation in plasma prolactin.

292.16

PITUITARY AND HYPOTHALAMIC ACTIONS OF ADENOSINE ON PROLACTIN SECRETION.

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This study examined the effects of the purine nucleoside adenosine on prolactin (PRL) secretion both at the level of the anterior pituitary and hypothalamus. Adenosine is present in the hypothalamus and affects cellular processes through interaction with two classes of plasma membrane receptors which are coupled to adenylate cyclase. Anterior pituitaries from male rats were dispersed and cultured for 48 hours. The cultured cells (150,000/well) were first preincubated then subjected to a two-hour incubation containing medium plus adenosine or specific receptor agonists. Adenosine ($5x10^{-5}$M), phenylisopropyladenosine (PIA, 10^{-5}M), or N-ethylcarboxyamide adenosine (NECA, 10^{-5}M) did not influence PRL secretion. However, the addition of adenosine deaminase (AD, 0.3-0.5 U/ml) to the medium increased PRL significantly. AD is responsible for metabolizing adenosine; the results suggest that pituitary cells release adenosine which tonically inhibits PRL secretion. Administration of PIA (10^{-5}-10^{-10}M) with 0.33 U/ml AD reduced PRL levels significantly. Combining dopamine (DA, 10^{-7}M) with PIA or NECA also resulted in inhibition of PRL greater than with DA alone. In contrast, intracerebral infusion of adenosine (100-200 nmoles) or PIA (10-50 nmole) stimulated PRL release in unanesthetized females. NECA or PIA (2.5 nmole) increased PRL 10 fold 15 min. after infusion into the third ventricle, an effect blocked by theophylline (25 nmole). LH levels measured in all animals were unaffected. These results support a biphasic action for adenosine on PRL secretion, a central stimulatory effect in the hypothalamus and an inhibitory, possible paracrine, effect directly on the pituitary lactotroph.

292.17

LACTATIONAL INFERTILITY IS SHORTENED BY A BRIEF INTERRUPTION IN NURSING IN ADOLESCENT MONKEYS. M.E Wilson* & T.P. Gordon* (SPON: F.A. King). Yerkes Primate Res. Ctr., Emory Univ., Atlanta, GA 30322

Lactational infertility is prolonged in adolescent rhesus monkeys (Macaca mulatta) following first parturition due to an apparent increased sensitivity to nursing (Biol. Reprod.,38:163, 1988). This study tested the hypothesis that a brief interruption in nursing shortens the period of lactational infertility in adolescent mothers. Nursing was prevented for a two week period by placing a primate vest on a female, preventing nursing but allowing other mother-infant contact. After nursing restriction, infants nursed ad libitum. Nursing restriction occurred when infants were actively suckling but able to maintain normal growth by eating solid food (140 d postpartum). Following the nursing restriction, total time infants spent nursing did not differ between groups: adolescent nursing restricted (AP: n=4), adolescent nursing unrestricted (AU: n=5), multiparous nursing restricted (MP: n=6), and multiparous nursing unrestricted (MU: n=6). Prior to the manipulation, serum estradiol (E2) was similar (~20 pg/ml) in all mothers, whereas following nursing restriction levels were lower ($p<.05$) in AU (17 ± 5 pg/ml) compared to AR (39 ± 10 pg/ml) who were similar to both MR (42 ± 7 pg/ml) and MU mothers (45 ± 11 pg/ml). Differences in E2 were associated with increased luteinizing hormone (LH) pulse amplitude. The interval from parturition to ovulation was shorter ($p<.05$) in AR (186 ± 13 d), MU (193 ± 4 d), and MR (183 ± 8 d) vs AU mothers (226 ± 15 d). These data indicate that adolescent rhesus monkeys are exquisitely sensitive to the suppression of reproduction by nursing, as a brief removal of the nursing stimulus advanced postpartum ovulation in adolescent but not adult mothers. This enhanced sensitivity may be due to the inhibitory effects of suckling on a developing neuroendocrine sytem. Supported by HD18120 & RR00165.

292.18

AN ENDOGENOUS STIMULATORY RHYTHM REGULATES PROLACTIN RELEASE IN LACTATING RATS. B.J. Arey*, B. Kanyicska* and M.E. Freeman. Dept. of Biological Sciences, Florida State Univ., Tallahassee, FL 32306.

Prolactin (PRL) secretion in female rats is regulated by an endogenous stimulatory rhythm that corresponds to the periods of mating-induced surges of PRL (Arey et al, Endocrinology 1989 124:119). Oxytocin (OT), vasoactive intestinal peptide and serotonin are hypothalamic stimulatory regulators of this endogenous rhythm (Arey and Freeman, Endocrinology 1989 124:878). In this study we investigated the role of this endogenous rhythm in lactating rats. On the day following birth, litters were adjusted to eight pups. On day 9 of lactation dams were fitted with chronic, indwelling intraatrial cannulae. The following day, pups were separated from their mothers for six hours before being reunited with their dams at either 0300, 1200 or 1700 h. Blood samples were taken from the atrial cannulae just prior to pup separation, and again at 0 (immediately before reuniting pups), 15, 30, 60 and 120 min following reuniting pups and dams. Serum samples were assayed for PRL, OT and growth hormone (GH) by RIA. Regardless of the time of suckling, 0-time samples contained significantly less PRL and OT as compared to their pre-separation levels. GH levels were not significantly different between 0-time and pre-separation levels. In all rats studied, PRL and OT concentrations were increased within 15 min of reuniting pups. Dams suckled at either 0300, or 1700 h demonstrated significantly greater PRL secretory profiles than rats suckled at 1200 h. Rats suckled at 0300 h and 1700 h had approximately a 40-fold maximum increase in PRL levels, whereas rats suckled at 1200 h showed only a 20-fold increase. OT levels rose steadily throughout the entire blood sampling period to an approximate 2-fold increase in OT by 120 min. However, there was no significant difference in secretory profiles of OT between time periods tested. GH levels also increased steadily throughout the blood sampling period and achieved approximately a 2-fold increase in concentration as compared to 0-time. There was no difference in secretory profiles of GH between time periods tested. These results suggest that the endogenous stimulatory rhythm regulating PRL release in ovariectomized rats, is also present in the lactating rat. (NIH, HD-11669).

292.19

EVIDENCE THAT VASOACTIVE INTESTINAL PEPTIDE (VIP) ANGIOTENSIN II (AII) AND LHRH MODULATE THE RELEASE OF PROLACTIN INDUCED BY DOPAMINE BLOCKADE. T. Inoue* and A. Negro-Vilar (SPON: M. Ching) Reprod. Neuroendoc. Sect., LMIN, NIEHS, NIH, Research Triangle Park, NC 27709.

The peptides VIP, AII and LHRH are among a number of substances that can stimulate prolactin (PRL) secretion by neuroendocrine, paracrine and/or autocrine mechanisms. We and others have shown that the stimulatory effect on PRL release of endogenous factors can best be observed when the tonic dopaminergic inhibitory activity is eliminated. In this study, we evaluated the PRL response to domperidone (DOM), a dopamine receptor antagonist, in male rats that were pretreated with specific receptor antagonists for the above peptides. Prior to DOM injection (0.1 mg/kg, i.v.) groups of male rats received one of the following: VIP antagonist ([4Cl-D-Ph^6-Leu^{17}]VIP); AII antagonist (Sarthran); LHRH antagonist ([D-pGlu^1, D-Phe^2, D-Trp^{3,6}]LHRH); oxytocin antagonist ([d(Ch$_2$)$_5$,Tyr(OMe)^{2}Orn^8]vasotocin) or saline vehicle given by constant infusion for 60 minutes. Blockade of VIP receptors reduced the DOM-induced PRL release in a dose-dependent manner. Blockade of AII and LHRH receptors also significantly attenuated the release of PRL after DOM. Oxytocin antagonist or LHRH agonist infusion had no effect on the stimulated PRL release. None of the analogs affected basal PRL levels. These results suggest that VIP, AII and LHRH are involved in the modulation of PRL secretion occurring after the removal of dopamine inhibition.

292.20

IN VIVO STUDIES ON THE PARACRINE ACTIONS OF PITUITARY ANGIOTENSIN II (AII) IN STIMULATING PROLACTIN (PRL) RELEASE FROM THE ANTERIOR PITUITARY GLAND OF RATS. M.K. Steele, L.S. Myers*. Physiology Dept., Univ. of Calif., San Francisco, CA 94143

In rats, pituitary gonadotrophs contain AII, and AII receptors are found on lactotrophs and corticotrophs. *In vitro*, both LHRH and AII stimulate PRL and ACTH release. The present studies investigated whether LHRH released pituitary AII to affect PRL and/or ACTH secretion in conscious rats. In male rats, intravenous LHRH (IV, 100 ng) stimulated LH but failed to alter PRL and ACTH secretion (Ganong and Shackelford, unpublished data). However, in ovariectomized rats treated with estradiol and progesterone, LHRH increased both LH and PRL release. The PRL response to LHRH was reduced by saralasin (12 μg/kg/min IV) and abolished by sarthran (5 μg/kg/min IV) infusion. In proestrous rats, neither saralasin nor sarthran had any effect upon the midcycle PRL surge. In addition, in other female rats, saralasin did not affect the increase in LH or PRL induced by 10 min of restraint stress. Taken together, these data show that exogenous LHRH stimulates PRL by releasing AII from the gonadotrophs. However, under physiological conditions where LH and PRL (and presumably endogenous LHRH) are elevated, pituitary AII does not appear to be involved in the PRL rise.
(Supported by NIH grants HL29714 and HD18020).

BIOLOGICAL RHYTHMS AND SLEEP: OTHER II

293.1

ABLATION OF THE SUPRACHIASMATIC NUCLEI DISRUPTS HIBERNATION AND BODY MASS RHYTHMS OF GROUND SQUIRRELS IN THE COLD. J. Dark, T.S. Kilduff, H.C. Heller* and I. Zucker, Dept. of Psychology, Univ. of California, Berkeley, CA 94720, and Dept. of Biological Sci., Stanford Univ., Stanford, CA 94305

Circannual rhythms of hibernation and body mass were studied in golden-mantled ground squirrels maintained in an LD 12:12 photoperiod at 6°C. Hibernation rhythms were disrupted by complete or partial suprachiasmatic nuclei (SCN) lesions. Several animals progressed through 4 hibernation cycles over the course of 2 years. One squirrel underwent two unusually long hibernation seasons, and another failed to hibernate for almost 2 years. Squirrels without SCN lesions had normal hibernation rhythms with a period of 11.5 ± 0.3 months. Hibernation normally coincides with the weight loss phase of the cycle but this association was absent in animals with SCN lesions. The SCN may be essential for normal circannual organization of hibernation and body mass rhythms at low temperatures.

Supported by NIH Grants HD-14595 & NS-10367.

293.2

BRAIN PROTEIN SYNTHESIS IN GROUND SQUIRREL BRAIN DURING HIBERNATION AND EUTHERMIA. T. S. Kilduff, R. Castro*, W.C. Dement, and H.C. Heller*. Depts of Psychiatry and Biol. Sci., Stanford University, Stanford, CA 94305.

Changes in arousal state such as sleep and hibernation may be mediated by production of endogenous substances which facilitate the state transition. To evaluate whether change in the proteins synthesized occur in the hibernating brain, in vivo labelling and detection of incorporated amino acids by autoradiography was utilized. Ground squirrels (Citellus lateralis), chronically implanted with cannulae in the left lateral ventricle, were injected with 0.5mCi of [^{35}S] methionine in a volume of 2.0ul while euthermic (T_b = 37°) or hibernating (T_b < 12°C) in a cold room. The label was allowed to incubate for 2hr in the euthermic animals and 2d in the hibernating animals. At the end of the incubation period, squirrels were sacrificed by pentobarbital overdose, the brain removed and dissected into 13 subregions, and proteins extracted. Proteins were separated by 2-dimensional gel electrophoresis and the gels exposed to X-ray film. In this procedure, three or four brain regions per animal (hypothalamus, basal forebrain, septum and striatum) incorporated enough [^{35}S] methionine to provide an adequate autoradiographic exposure in 3 weeks or less. Initial results suggest a surprisingly high number of proteins being synthesized during hibernation.
(Supported in part by the Upjohn Company).

293.3

THE EFFECTS OF TEMPERATURE AND pH ON THE FIRING RATE OF SUPRACHIASMATIC (SCN) NEURONS IN HIBERNATORS AND NON-HIBERNATORS. J.D. Miller, V.H. Cao*, and H.C. Heller*. Dept. of Biological Sciences, Stanford University, Stanford, CA 94305-5020

The firing rates of SCN and non-SCN neurons from rats, euthermic ground squirrels, and hibernating ground squirrels were observed in the hypothalamic slice preparation. Bath temperature was varied between 10°C and 37°C and pH was continually monitored. Multiple regression analysis was performed to examine the contributions of temperature and pH to changes in firing rate. Standardized thermal coefficients, firing cutoff temperatures, and Q_{10}'s were calculated. Preliminary results indicate that the average Q_{10} for SCN cells in hibernating squirrels was significantly greater ($p < .01$) than that for euthermic squirrels or rats (which did not differ significantly from a Q_{10} of 2.0). Temperature accounted for at least twice as much of the variance in rate as did the effects of pH. The results are consistent with the hypothesis that the circadian clock may be greatly slowed in hibernation, possibly accounting for the multi-day temporal organization of hibernation bouts.

293.4

TRANSPLANTATION OF FETAL SCN INTO 3RD VENTRICLE OF ADULT HAMSTER SPARES DISRUPTION OF CIRCADIAN LOCOMOTOR RHYTHM FOLLOWING SCN LESION. A. Philpot*, M-T. Romero and R. Silver, Barnard College of Columbia University,New York, NY 10027.

We previously reported that transplants of fetal SCN into the 3rd ventricle of SCN-lesioned adult hamsters restores circadian locomotor rhythmicity (Lehman et al.,J. Neurosc 7:1626,1987). In the present study we report that if the host adult SCN is lesioned about 40 days following transplantation of fetal SCN into the 3rd ventricle, there is no disruption of circadian locomotor rhythmicity. Immunohistochemical verification of VIP- and NP-like immunoreactivity verified SCN transplantation and extent of lesion. The results suggest that donor SCN contributes to circadian rhythmicity prior to or immediately upon lesioning of the endogenous SCN. Given the evidence that fetal donor tissue requires a denervated target to establish synaptic connections, the results imply a non-synaptic mechanism mediating maintenance of locomotor rhythmicity following SCN lesions in these grafted animals and raises the possibility that a similar mechanism occurs in the SCN *in situ*.

293.5

SUPRACHIASMATIC NUCLEUS (SCN) TRANSPLANTS: COMPUTERIZED IMAGE ANALYSIS OF NEUROPEPTIDE DISTRIBUTION. J. Buggy, J. Ding, and P.J. DeCoursey*. Depts. of Physiology and Biology, Univ. of South Carolina, Columbia, S.C. 29208.

In transplants of fetal hypothalamic tissue, neuropeptide immunohistochemistry (IHC) characteristic of the normal SCN is observed. To better understand why these transplants to arrhythmic SCN-lesioned hosts restore free-running circadian rhythm in some instances but not others, computerized image analysis was used to analyze neuropeptide distribution. Videoscans of IHC sections stained sequentially for neuropeptide Y (Y), neurophysin (N), vasoactive intestinal polypeptide (V), and somatostatin (S) were fiducially aligned, digitized, and stored as image files. Areas outside the transplant were masked and a pixel intensity threshold selected for presence or absence of (IHC) stain. The percent of transplant area with IHC stain was determined individually for each neuropeptide and then, using superimposed images, for areas of neuropeptide overlap. VIP and N IHC in transplants were clustered as plexus aggregates, adjacent or with considerable overlap; S IHC was less clustered but often adjacent to N IHC. In transplants to lateral ventricle which did not restore rhythmicity, Y IHC was extensive and diffuse except for conspicuous absence in V and N aggregates, suggesting that normal Y innervation to SCN from geniculate leaflet did not occur. Circadian transplant function may require appropriate reinnervation with host brain which becomes less probable at transplant sites distant from the hypothalamus.

293.6

CONTROL OF PHASE AND LATENCY TO RECOVER CIRCADIAN LOCOMOTOR RHYTHMICITY FOLLOWING TRANSPLANTATION OF FETAL SCN INTO LESIONED ADULT HAMSTERS. M-T. Romero and R. Silver. Barnard College of Columbia University, New York, New York, 10027

Transplant of fetal hypothalamic tissue into SCN-lesioned (SCN-X) hamsters is followed by recovery of circadian locomotor rhythmicity with latencies ranging from 1-10 (X= 3.7) weeks (Lehman et al J. Neurosci., 7:1626, 1987). In the present study we gave melatonin (M) injections (75ug SC in sesame oil) at two different phase points. M treatment entrained the phase of the recovered free-running locomotor response and shortened the latency to restore rhythmicity following transplantation of fetal SCN into the 3rd ventricle of adult hamsters. Control animals including 1) SCN intact adult hamsters, 2) SCN-X adult hamsters, and 3) SCN-X hamsters bearing implants that did not restore rhythmicity, showed no entrainment to M injections. The results suggest that exogenously administered M can synchronize oscillators within the CNS resulting in rapid reinstatement of locomotor rhythmicity following transplantation of fetal SCN in adult lesioned hosts.

293.7

GONADAL RESPONSES TO PROGRAMMED MELATONIN INFUSIONS IN THE SYRIAN HAMSTER: EFFECTS OF INFUSION FREQUENCY AND SUPRACHIASMATIC LESIONS. E.Maywood*, R.Buttery*, G.Vance*, J.Herbert*, M.H.Hastings* (SPON:J.T.Clark). Dept. of Anatomy, Univ. of Cambridge, CB2 3DY, U.K.

The pineal melatonin signal mediates the effects of photoperiodic stimuli upon the neuroendocrine axis. The brain is able to identify and time the duration of its nocturnal exposure to melatonin although the location and mechanisms of action of the timer are unknown. The circadian system may provide a neural substrate for this timing process; the central oscillators of the suprachiasmatic nuclei (SCN) bind melatonin and regulate other neuroendocrine responses to timed hormonal stimuli, e.g. the LH surge. The involvement of the circadian system in reading the melatonin signal was tested in two ways. Pinealectomized Syrian hamsters received 35 programmed infusions of saline vehicle (SAL) or melatonin (MEL, 250 ng in 500 µl) via subcutaneous cannulae. The infusions varied in duration (4h or 10h) or frequency (20h to 48h). In a second experiment, animals received partial or complete lesions of the SCN, and were then given 35, 10 h infusions at a frequency of 24h. Paired testes weights (PTW) were recorded as an index of gonadal response.

Infusions of SAL or MEL for 4h daily had no effect on gonadal condition (PTW, Sal 2.93+0.23, Mel 4h 3.56+0.14g). MEL infusions for 10h induced significant gonadal involution (1.32+0.25). SAL infusions delivered every 20h or 25h had no effect (3.05+0.17) as did infusions of MEL for 10h every 28h and 48h (3.35+0.2; 3.20+0.3). However, MEL every 25, 23 or 20h induced gonadal regression (1.63+0.19; 0.75+0.48; 0.28+0.05), indicating that non-24h signals can be read by the brain. MEL induced gonadal atrophy in animals bearing partial (SAL 3.00+0.17, MEL 1.14+0.26) or complete SCN lesions (SAL 3.35+0.16; MEL 1.27+0.35). These data suggest that the photoperiodic gonadal response to MEL is not dependent upon the circadian clock of the SCN.

293.8

INTRACEREBRAL MELATONIN ENTRAINS RAT RUNNING WHEEL ACTIVITY Karen L. Brugge,* Harry Klemfuss, Daniel F. Kripke* SPON J. Christian Gillin, San Diego VAMC and M-003, UCSD, San Diego, CA 92093

Melatonin may entrain hormonal and behavioral rhythms by a direct action on the suprachiasmatic nucleus (SCN). Daily systemic melatonin injections entrain activity rhythms in rodents. This effect can be abolished by SCN lesions. We attempted to extend the results of Cassone's group (1986) by injecting melatonin intracerebrally rather than systemically to determine its entrainment effects on running wheel activity (RW).

Rats with cannulae placed into the region of the SCN were initially entrained to a L:D 12:12 cycle (lights off at 6 pm), and upon commencement of daily microinjections at 7-7:30 pm of melatonin (500 ng melatonin in 0.2 u L) or vehicle, the L:D schedule was changed to continuous red dim light (RR). RW activity was monitored. Four control rats receiving vehicle all free ran, whereas 6 of 9 rats receiving melatonin were synchronized with activity onset at the time of injection ($p<.05$). These results support the hypothesis that melatonin entrains RW activity by a direct effect on the SCN.

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293.9

MELATONIN DECREASES THE REENTRAINMENT RATE OF LOCOMOTOR ACTIVITY FOLLOWING AN 8 HR ADVANCE OF THE LIGHT/DARK (LD) CYCLE IN C3H/HeN MOUSE. J. M. Fang* and M. L. Dubocovich (SPON: S.F. Holloway). Dept. Pharmacol., Northwestern Univ. Med. Sch., Chicago, IL 60611.

Melatonin receptor sites have been localized in the suprachiasmatic nucleus of the mammalian hypothalamus, which is considered to be the central component of circadian organization. The aim of this study was to determine the role of melatonin in the reentrainment of the circadian rhythm of locomotor activity in the C3H/HeN mice after a phase shifts of the LD cycle. Baseline data on locomotor activity of C3H/HeN mice (5-6 week) were recorded for 2 weeks in a 12:12 hr LD cycle. An 8-hr advance of the LD cycle was applied by advancing dark onset. Vehicle or melatonin (10 mg/kg, i.p.) were injected for a total of two week just before the time of the pre-phase shift dark onset. A significant difference in the latency to reach reentrainment between vehicle-injected [7.66±1.08 (6) days] and melatonin-injected mice [12.6±1.77 (5) days, $p<0.05$] was observed suggesting that melatonin decreases the rate of reentrainment following an 8-hr advance of the LD cycle. The involvement of brain melatonin receptor sites modulating the circadian rhythm of locomotor activity will be further investigated by administration of luzindole (N-0774), a competitive melatonin receptor antagonist. Supported by MH 42922.

293.10

IN VIVO MICRODIALYSIS OF AMINO ACIDS IN THE SUPRACHIASMATIC NUCLEI. M.A. Rea, J.L. Blank*, S. Ferreira*, D. Terrian and J.D. Glass*., USAF Sch. of Aerospace Med., Brooks AFB, TX 78235, and Dept.Biol.Sci., Kent State Univ., Kent, OH 44242

Microdialysis probes were stereotaxically implanted in the suprachiasmatic nuclei (SCN) of male Siberian hamsters maintained under LD 16:8. Dialysate was collected every 20 min for 24-26 hrs and assayed for amino acids by HPLC. Pronounced diurnal rhythms were observed in glutamate (GLU) content of dialysates from two animals whose probes were located along the lateral border of the SCN. GLU rose during the latter half of the dark phase to peak within 2 hours after lights-on. Peak values were 1.56 and 0.87 nmol/ml and represent increases of 164% and 168%, respectively, over the mean concentration for the total sampling period. In one animal, a similar rhythm in glycine content was observed which peaked at lights-on. These rhythms were absent in a third animal whose probe was located 0.8 mm lateral to the SCN. Glutamine content fell steadily thoughout the collection period in dialysates from all animals. Aspartate content did not show rhythmicity and GABA was not detected. Supported by AFOSR-2312W6 (MAR) and BRSG SO7-RR-07208 (JDG).

293.11

N-ACETYLASPARTYLGLUTAMATE: A TRANSMITTER CANDIDATE FOR THE RETINOHYPOTHALAMIC TRACT? J.R.Moffett, L.Williamson, +M.Palkovits, and M.A.Namboodiri. Dept. Biology, Georgetown Univ., Washington, DC 20057 and +Lab. Cell Biology, NIMH, NIH, Bethesda, MD 20892.

The retinohypothalamic tract (RHT) is the neural pathway mediating the photic entrainment of circadian rhythms in mammals. The neurotransmitters which operate in this projection system have not yet been determined. Utilizing affinity purified antisera to N-acetylaspartylglutamate (NAAG), a neuron specific dipeptide which may act as an excitatory neurotransmitter, we have examined the levels of this peptide in the retinohypothalamic projection system. NAAG was found to be localized extensively in the RHT and all of its target zones including the suprachiasmatic nuclei. Optic nerve transections resulted in significant reductions in NAAG immunoreactivity in the suprachiasmatic nuclei and supraoptic nuclei. In addition, transection of the optic nerve resulted in a 55% decrease in NAAG levels in the suprachiasmatic nuclei as measured in tissue micropunches by a soluble RIA utilizing the same affinity purified antibodies (assay specificity: IC50 for NAAG = 2.5 nM, IC50 for NAA = 100 uM; assay sensitivity: 1-2 pg/assay). To date, no saturable high affinity binding sites for NAAG have been found in the nervous system raising the possibility that NAAG derived glutamate may be the neuroactive agent binding to postsynaptic receptors following release and enzymatic breakdown of the peptide within the synaptic cleft. The data presented are consistent with the idea that NAAG, or NAAG derived glutamate as opposed to free vesicular glutamate, may act as one of the neurotransmitters involved in retinohypothalamic communication. (Work supported by NIH Grant DK 37024).

293.12

NEUROTENSIN: LOCALIZATION IN THE HUMAN SUPRACHIASMATIC NUCLEUS. **E.T. Koh*, J. C. King, B. Tate-Ostroff, H.E. Albers and E. G. Stopa, (Spon: Lester Adelman) Dept. of Anesthes., U. Mass. Med. Ctr, Worcester, MA; Depts. of Anat. and Cell Bio, and Neurosurg., Tufts U. Sch. of Med, Boston, MA; Mailman Res. Ctr., McLean Hosp, Belmont MA; Dept. of Bio, Georgia State U., Atlanta, GA; and Dept. of Path, SUNY Health Sci. Ctr, Syracuse NY.**

The distribution of neurotensin-like immunoreactivity (N-LI) was examined in the human suprachiasmatic region.

Human hypothalami(n=12) were fixed by immersion in either 5% Acrolein (12-14 hours) or 10% NBF (7-10 days). The immunocytochemical procedures were performed on unmounted serial coronal sections (40-80 um) using a modified PAP technique.

Analysis of the distribution of reaction product observed by light microscopy was accomplished using Dr. Dean Hillman's Cellmate system. Comparisons made between computer-generated maps of alternate sections stained with Nissl, vasopressin antiserum, vasoactive intestinal peptide antiserum and neurotensin antiserum, confirmed that a large subpopulation of N-LI-containing cell bodies was present within the human SCN.

These data indicate that neurotensin-containing neurons are a major constituent of the human SCN and suggest that neurotensin may be an important mediator of human circadian rhythmicity. **(AG00295, AG02126, 1S10RR03442)**

293.13

PEPTIDE CO-LOCALIZATION: FUNCTIONAL SIGNIFICANCE WITHIN THE CIRCADIAN TIMING SYSTEM. H.E. Albers, S.Y. Liou* & R.T. Zoeller. Lab. Neuroendocrinol. & Behav., Depts. Biol. & Psychol., Georgia State Univ., Atlanta, GA 30303 & Dept. Anat., Univ. Missouri Med. Sch., Columbia, MS 65212.

Neurons within the suprachiasmatic nucleus (SCN) that contain both vasoactive intestinal peptide (VIP) and peptide histidine isoleucine (PHI) have been implicated in the control of circadian rhythms. VIP/PHI SCN neurons may also contain a third biologically active peptide, gastrin releasing peptide (GRP). The present study localized the mRNA encoding VIP/PHI and GRP within the SCN and examined the SCN function of these peptides. In situ hybridization studies revealed that VIP/PHI and GRP mRNA distribution within the SCN was nearly identical. Combined microinjection of VIP, PHI and GRP into the SCN region (N=39) phase delayed the circadian locomotor rhythm by >1.5 hrs when administered around the time of activity onset, but had no effect on circadian phase at other times of the circadian cycle. In the hypothalamic slice, VIP, PHI and GRP provided individually (10^{-7} M) produced a small excitatory response in SCN single units. In contrast, when all three peptides were provided together (10^{-7} M) a four fold greater excitation was observed. VIP, PHI and GRP appear to be co-localized in the SCN, and this co-localization may be functionally significant in the control of circadian rhythms. (Supported by ONR N00014-89-J-1640).

293.14

RUBIDIUM FUSES SPLIT CIRCADIAN RHYTHMS IN HAMSTERS. J.D. Hallonquist and N. Mrosovsky. Depts. of Psychiatry & Zoology, U. of Toronto, Toronto, Ont. M5S 1A1, Canada.

Abnormal circadian rhythms are reported in patients with mood disorders. Demonstration that an antidepressant normalizes atypical circadian function in animals would suggest that its clinical efficacy might reflect a similar mechanism. When hamsters are housed in bright constant light, the normally intact portion of a free-running circadian rhythm may split into 2 components which then stabilize at an abnormal phase relationship 12hr apart. Spontaneous fusing of split rhythms is extremely rare.

Male hamsters with split circadian activity rhythms were used as an assay for the chronotypic properties of the experimental antidepressant rubidium (Rb). The Rb group had RbCl (100mM) added to the drinking water for a period of 10wk, after which normal water was returned. The Control group received only normal water. Seven of the 12 hamsters that received Rb showed fusing of split rhythms, vs. none of the 7 Control hamsters ($p = 0.016$, Fisher Exact).

Rubidium may normalize atypical coupling between circadian rhythms by acting directly on the suprachiasmatic nuclei (SCN) or by reducing photic input to the SCN via the geniculo-hypothalamic tract. This first demonstration that an antidepressant drug can normalize a circadian anomaly in animals is consistent with a circadian dysfunction hypothesis of the etiology of some mood disorders.

293.15

EFFECTS OF ALCOHOL AND TRIAZOLAM ON THE CIRCADIAN ACTIVITY RHYTHM OF THE GOLDEN HAMSTER. J.E.Joy and F.W.Turek. Dept. Neurobiology and Physiology, Northwestern University, Evanston, IL 60208.

Several reports in the literature have suggested that chronic ethanol can alter circadian rhythmicity in animals. In hamsters, single injections of the benzodiazepine, triazolam, cause phase advances or phase delays, depending on the time of injection. Since many of the neuropharmacological effects of ethanol appear to be mediated through the GABA-Benzodiazepine receptor complex, we decided to explore the effects of ethanol on circadian activity rhythms in the golden hamster, and further whether ethanol treatment influences the effect of triazolam on circadian rhythms. In Expt I, adult male hamsters were given 25% ethanol in their drinking water. All animals showed an increase in the period of their free-running activity rhythm (mean increase = 0.11 h +_ 0.03; n=8). Control animals, provided with tap water, did not show a significant increase in period (mean increase = 0.02 h + _ 0.02; n=11). In Expt II, a separate group of male hamsters was given 25% ethanol for 10d, after which they were injected with a sub-maximal dose of triazolam (0.5 mg/animal) at times that typically cause phase advances (CT 6) or at times that cause phase delays (CT 21). Chronic ethanol treatment lowered the sensitivity of the animals to triazolam for both injection times. This pattern is consistent with cross-tolerance observed in the behavioral effects of ethanol and benzodiazepines.

293.16

DIURNAL VARIATION IN BRAIN SEROTONIN IS DRIVEN BY THE PHOTIC CYCLE AND IS NOT CIRCADIAN IN NATURE. J.S. Ferraro and R.W. Steger*. Physiology Dept, Southern IL Univ, Sch of Medicine, Carbondale, IL 62901.

In an effort to determine the driving force of the diurnal variation of serotonin (5-HT) in the brain, over 500 Syrian hamsters were exposed to long photoperiods (LD14:10), short photoperiods (LD10:14), constant dark (DD) or constant light (LL) for 12 weeks. Hamster in LD conditions were sacrificed at three hour intervals; those in constant conditions were sacrificed around the clock. The circadian time (CT) of tissue collection, in the animals in constant conditions, was determined from the onset of locomotor activity (defined as CT12; the beginning of the subjective night). Following decapitation, the brains were removed and the median eminence were rapidly dissected free and frozen on dry ice. The remaining brain was also frozen and stored at -70°C. Within several weeks, the brains were allowed to thaw partially and the medial basal hypothalamus (MBH); anterior hypothalamus (AH) and olfactory bulbs (OB) were dissected from the brain and weighed. Tissue samples were homogenized and N-methylserotonin was added to each sample as an internal standard with which procedure-related amine losses could be estimated. 5-HT levels were determined by HPLC with electrochemical detection. In LD14:10 and LD10:14, 5-HT levels displayed significant diurnal variation in the MBH, AH and OB (ANOVA; $P<0.05$). The sine waves of the 5-HT rhythm were of similar amplitude and phase with relation to lights on (i.e., high 5-HT content during the day and low content at night, with a sharp rise occurring just after lights on). This variation, however, was not apparent in animals exposed to DD or LL; 5-HT content did not display a significant diurnal oscillation. Since 5-HT failed to oscillate in the absence of environmental time cues, the rhythm must be driven by the environment and not an internal circadian clock. Supported by NIH grant NS23128 (JSF) and NSF grant DCB-8619702 (RWS).

293.17

SEROTONIN AGONISTS ALTER PHOTIC RESPONSES OF NEURONS IN THE DORSAL AND VENTRAL LATERAL GENICULATE NUCLEI OF HAMSTERS. D.X. Zhang and B. Rusak, Dept. of Psychology, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4J1.

Neurons in portions of the lateral geniculate nucleus (LGN) receive a retinal projection and project in turn to the suprachiasmatic nucleus, which functions as a pacemaker in the circadian system. This mechanism contributes to the regulation of circadian rhythms by photic cues. The LGN also receives a serotonergic projection from the raphe nuclei; we asked whether serotonergic agonists would affect the responses of LGN neurons to retinal illumination.

Neural activity was recorded using a multibarrel micropipette in the LGN of urethane-anesthetized Syrian hamsters. Responses to long light pulses were recorded extracellularly from single neurons in the dorsal and ventral LGN (including the intergeniculate leaflet) under baseline conditions and while serotonin (20 mM, 20-40 nA, 20-40 sec) or the serotonin type 1A receptor agonist, 8-OH-DPAT (2 mM), was iontophoresed onto the cell. Most cells showed reduced responsiveness to retinal illumination during drug applications. Some cells were also suppressed by the drug in darkness, but others that showed reduced responses to photic cues showed no change, or even an increase in baseline rates. The results indicate that the effects of serotonin agonists on LGN neurons are complex, and that the serotonergic raphe projection may modulate photic responses relevant to rhythm entrainment.

293.18

ORGANIZATION OF THE CAT INTERGENICULATE LEAFLET DEMONSTRATED BY NEUROPEPTIDE Y-IMMUNOREACTIVITY. J.C. Speh and R.Y. Moore, Depts. of Neurology and Neurobiology, SUNY, Stony Brook, NY 11794.

The intergeniculate leaflet (IGL) in rodents is a lateral geniculate component which receives a distinctive pattern of retinal innervation by retinal afferents (Hickey and Spear, 1976) and contains a population of neuropeptide Y-immunoreactive (NPY+) neurons. These neurons project via the geniculohypothalamic tract (GHT) to the suprachiasmatic nucleus of the hypothalamus and participate in circadian rhythm regulation. The terminals of the GHT have been demonstrated in the cat (Cassone et al, 1988) but the location and organization of the IGL has not been reported. In this study the IGL was demonstrated using NPY immunohistochemistry. NPY+ neurons are present in the lateral geniculate complex and adjacent thalamus. As the VLG forms rostrally, NPY+ neurons and axons are in the medial portion of the VLG adjacent to the DLG. A few NPY+ cells extend along the DLG border and the border of the VLG with the optic tract. These NPY+ cells are present in essentially the same location throughout most of the extent of the VLG. The major component of the IGL, however, is represented by a long band of neurons that extends from the medial VLG dorsally and rostrally along the lateral border of the diencephalon, medial to the optic tract and lateral to the internal capsule, extending into the zona incerta. Thus, the cat IGL is organized in a pattern that is very similar to rodents. Supported by NIH grant NS-16304.

293.19

PREVENTION OF LIGHT-INDUCED DISSOCIATED WHEEL-RUNNING ACTIVITY BY IGL LESIONS OF CHRONIC CLORGYLINE-TREATED SYRIAN HAMSTERS W. Duncan*, E. Sutin, W. Orem*, B. Gao*, P.G. Sokolove, T.A. Wehr* (SPON: O. Takahashi) Clinical Psychobiology Branch, NIMH, Bethesda, Md. 20892 & Dept. of Biological Sciences, U. of Maryland, Baltimore, Md.

In humans, chronic treatment with the antidepressant drug clorgyline (CLG), a type A monoamine oxidase inhibitor (MAOI), often results in a temporal disorganization of the sleep-wake cycle. We previously observed dissociated patterns of wheel-running in clorgyline-treated hamsters housed in continuous light (LL) but not in saline-treated controls. In order to identify the visual pathway that contributes to this light-induced disruption of running activity, CLG-treated hamsters were given sham (n=8) or bilateral (n=4) intergeniculate leaflet lesions (IGLx). Saline-treated (SAL) hamsters received sham (n=4) or bilateral (n=4) IGLx. All hamsters were tested in LL (40 μW cm-2). Two CLG-treated hamsters received complete IGLx as documented by the absence of immunohistochemical staining for the presence of neuropeptide Y (NPY) cell bodies in the IGL. These two animals exhibited intact patterns of wheel-running with tau=24 hours. Two CLG-treated hamsters received partial IGLx as evidenced by NPY-immunoreactivity in the IGL. One partially lesioned, CLG-treated animal exhibited an intact pattern of wheel-running; the other exhibited rhythm dissociation. All hamsters that received sham+CLG-treatment exhibited dissociated wheel-running. All sham+SAL-treated hamsters exhibited intact wheel-running patterns with tau>24 hours. All IGLx+SAL-treated hamsters received complete IGL lesions; these animals exhibited intact running patterns with tau=24 hours. The failure to dissociate seen in CLG-treated IGLx hamsters housed in LL suggests the retinogeniculohypothalamic tract (RGHT) participates in the dissociation response. We speculate that some of the sleep-wake cycle disturbances reported in depressed humans treated with MAOIs may be related to the interaction of MAOIs and light within or post-synaptic to the RGHT.

293.20

ALTERED PROTEIN EXPRESSION IN PERIOD MUTANT HAMSTERS. G.Johnson[1], J.E.Joy[1], M.R.Ralph[2], M.Menaker[2], and C.Merril[1]. [1]NIMH-St.Elizabeth's Hospital, Washington D.C. 20032, and [2]Dept.Biology, Univ. of Virginia, Charlottesville, VA 22901.

A genetic mutation that dramatically shortens the period of the circadian locomotor rhythm of golden hamsters has recently been reported (Ralph,M.R. and Menaker,M. Science 241:1225,1988). Wild-type animals have rhythms whose freerunning periods average about 24 hours, animals heterozygous for the mutant trait have periods of about 22 hours, while homozygous animals have rhythms with periods close to 20 hours. The pattern of inheritance of this mutation suggests that it has occurred at a single, autosomal locus.

Using high resolution two-dimensional gel electrophoresis, we have asked whether the expression of the mutant allele can be detected at the protein level. We have discovered a protein (Mr ~40 kd, pI ~6.5) that is present in brain samples of wild-type hamsters and undetectable in mutant animals. This protein is found in SCN tissue, as well as tissue from other areas of brain in wild-type hamsters. We are now in a position to characterize this protein, which may provide a new, molecular approach to the study of mammalian rhythmicity. (Supported in part my MH09483 to MRR and HD13162 to MM.)

294.1

AGE AT HEAD INJURY AND AGE AT FOLLOW-UP TESTING INDEPENDENTLY PREDICT LATER COGNITIVE DECLINE. T. J. Rosen* and S. Corkin (SPON: J. H. Growdon). Dept. of Brain and Cognitive Sciences, and Clinical Research Center, Mass. Institute of Technology, Cambridge, MA 02139.

Veterans of World War II with penetrating head injury (HI) incurred in young adulthood showed increased cognitive decline in later years relative to subjects with peripheral nerve injury (Corkin et al., in press). Aging increased exacerbated decline, but the prior paper did not determine whether the controlling factor was age at HI (range 18 to 33 years) or age at follow-up testing (range 54 to 72 years). The present report used multivariate analyses of the HI subjects' data ($N = 57$) to separate the consequences of age at injury from those of age at follow-up. A procedure analogous to factor rotation transformed the original, highly correlated age variables ($r = .90$) to new variables with a substantially lower correlation ($r = .26$). Multiple linear regression analyses predicting exacerbated decline from the new variables revealed that greater age at injury and greater age at follow-up testing separately contributed to exacerbated decline on a measure of general intelligence and on a block counting subtest. The association between greater age at injury and cognitive decline suggests that the characteristics of the brain at the time of injury affect repair processes, and that limited restorative processes in subjects who were older at injury make them more vulnerable to degeneration with the onset of old age. The association between greater age at follow-up testing and cognitive decline suggests that cognitive decline is a specific response to aging rather than a general response to the passage of time since the injury occurred.

294.2

IMPAIRED BODY PARTS LOCALIZATION WITH PRESERVED BODY REFERENCE SYSTEM A. Sirigu, J. Grafman, K. Bressler, T. Sunderland, (Spon. J. Grafman). Cognitive Neuroscience Unit, NINDS, NIH, Bethesda, MD 20892.

We studied body schema disturbances in a 62-year-old woman with diagnosis of Alzheimer's disease. She was severely impaired in tasks requiring her (1) to point at parts of her own body on verbal command, even though she correctly named the same parts when pointed at by the Examiner (Ex), or (2) to point on Ex's body (or a picture, or a doll) the corresponding part touched by the Ex on the patient's body. Performances were nearly normal in variations of these tasks, in which small objects attached to the patient's and Ex's bodies served as pointing targets. When tested for recall several days later, she pointed at the exact position of the objects. Yet, when we again attached the objects on her body, while requiring her to ignore their presence and point at different body parts, she performed very poorly.

The fact that the same point in "body space" is localized correctly when identified as an external object and erroneously when identified as a body part contradicts the idea of the body schema as a unitary function. Learning the position of objects on the body surface requires an intact body coordinate system on which to map this information. Why then, can't such a body reference system be used to localize the position of its component body parts? The paradox raised by this dissociation suggests that different processing and/or representational systems, with possibly distinct neural correlates, may contribute to orientation towards one's own body.

294.3

TEMPORAL ORDER INFORMATION IN NORMAL SUBJECTS AND PATIENTS WITH DEMENTIA OF THE ALZHEIMER'S TYPE. J. Madsen* and R.P. Kesner (SPON: B. Grosser). Department of Psychology, University of Utah, Salt Lake City, Utah 84112.

The purpose of the study was to evaluate the efficacy of temporal processing in college students, normal, older adults and older adults with mild to moderate dementia. A test for order memory was administered on a MacIntosh computer. There were 56 trials with two phases within each trial. During the study phase, a series of eight X's appeared on the screen. During the test phase, two X's from the study phase sequence appear simultaneously in their original locations. The subjects were asked to make a recency judgment. On a random basis the number of items between test items (lag) was varied. Lags from 0-6 were selected for the test. Results indicate that college students performance improves as lag increases. The performance of older adults is similar but slightly less accurate. Mildly demented subjects performed at chance levels for all lags but 5 and 6. Moderately demented subjects were chance levels for all lags. It is believed that at the time of the study phase, contextual cues are not encoded with much flexibility by the mildly demented subjects, hence, their inability to discriminate between contexts at the time of the test except for the longest lags.

294.4

FROM COVERT TO OVERT RECOGNITION OF FACES IN A PROSOPAGNOSIC PATIENT. J. Sergent & M. Poncet*. Montreal Neurological Institute, Montreal, Canada, and Hôpital La Timone, Marseille, France.

Prosopagnosia is an inability to recognize known persons as a result of a failure to access relevant memories through the inspection of their faces. The nature of this disturbance, and the fate of memories related to faces, were investigated in a long-standing prosopagnosic woman. An examination of her perceptual capacities indicated no major impairment at maching faces, including different views of the same faces, and multidimensional scaling analysis showed that she could achieve configurational representations of faces as do normal subjects. In spite of her inability to evoke memories of persons from their faces, she displayed clear evidence that she could use pertinent memories related to these faces in tasks that did not explicitly call for their evocation. This covert recognition of overtly unrecognized faces was observed in perceptual, learning, and cued-recognition tasks. Because these findings suggest preserved semantic knowledge about unrecognized faces, attempts were made at surmounting this blocked activation of pertinent memories. Some of these attempts were successful in inducing the patient into experiencing a sense of familiarity with the faces and identifying them on her own, for the first time since the onset of her illness more than 15 years ago. These findings suggest that (1) failure to recognize faces may occur in spite normal configurational encoding of facial representations, (2) covert recognition of overtly unrecognized faces requires the integrity of perceptual processes, (3) prosopagnosia may occur despite preserved knowledge related to faces, (4) this patient's failure of overt face recognition reflects a deficit in activating these pertinent memories about faces which thus remain meaningless.

294.5

A Self-Rating Scale for Both Poles of Affective Illness. Edward Dewees*, Mark Bauer, Paul Crits-Christoph*, William Ball, Thomas McAllister*, Jon Cacciola*, Peter Whybrow, Peter Alahi*. Dept. Psychiatry, Univ. Pennsylvania, Philadelphia, PA 19104

While many self-rating scales exist for the assessment of depressive symptoms, to our knowledge none have been well validated for the assessment of manic and hypomanic symptoms. The Internal State Scale (ISS) consists of 18 self-report visual analogue scale items based on the premise that a high energy, or activated, state comprises the core symptoms of mania, regardless of dominant subjective mood. The ISS has been given to 14 manics (M), 12 depressives (D), and 12 controls (C). Items were divided a priori into 3 subscales which exhibited good inter-item reliability (alpha=0.81-0.90) in M,D, and C, and good test-retest reliability in C. The mania subscale differs as expected across groups (M>D=C) and is well correlated with the clinician-administered Young Mania Scale (r= 0.62). The well-being subscale differs as expected across groups (D<M=C) and is well correlated with the Hamilton Depression Scale (r= -0.79). Discriminant function analysis using these two scales correctly classifies over 80% of M, D, and C.

294.6

PSYCHOTIC SYMPTOMS WITH LEFT HEMISPHERIC AND BITEMPORAL DYSFUNCTION IN EPILEPTICS. D. Lacroix and J.M. Saint-Hilaire*. Département de Psychiatrie et Service de Neurologie, Hôpital Notre-Dame, Université de Montréal, Montréal, Canada, H2L 4M1.

The object of this study was to determine if the presence of psychotic symptoms could be correlated to a specific localization of an epileptogenic focus (EF), of interictal spiking activity and of slow wave activity in epileptic patients who underwent EEG assessment of seizures using depth electrodes or prolonged scalp recordings. Psychotic symptoms were quantified using a modified Brief Psychiatric Rating Scale with the rater blind to the localization of dysfunctions.

Preliminary results indicate increased psychotic symptoms: 1- if slow wave or interictal spiking activity was localized in the left frontotemporal, temporal or bitemporal regions as opposed to the right hemisphere; 2- if the EF or slow wave activity was localized exclusively in the left hemisphere and 3- if the EF was localized in the left amygdala, hippocampus and adjacent T2 temporal cortex as opposed to an EF localized in the other brain areas, including a bitemporal EF. These results support the hypothesis of a left hemispheric and/or bitemporal dysfunction in psychosis.

294.7

TEMPORAL ORDERING DEFICITS IN SCHIZOPHRENIA. B. L. Schwartz*, L. H. Deutsch*, C. Cohen*, and S. I. Deutsch* (SPON:E. Parker). Psychiatry, Veterans Administration Medical Center, Washington, D.C. 20422

Several lines of evidence suggest that cognitive deficits in schizophrenia are associated with abnormalities in the frontal lobe. This study used the recency judgement task, which has been found to be sensitive to frontal lobe dysfunction, to compare 16 schizophrenic patients and 16 normal controls. Each subject viewed a list of word pairs. In the recency judgement task, the subject was shown a test pair containing two previously presented words and was asked to choose which word was presented more recently. In addition, recognition memory was tested with pairs containing one old word and one new word. Schizophrenic patients were impaired on the recency judgement task, but not on the recognition task. In particular, patients showed deficits in judging the recency of words that occurred on the immediately preceding trial, whereas they were not impaired in recognizing these words. A selective deficit in memory for temporal ordering may provide further evidence of frontal lobe dysfunction in schizophrenia.

294.8

D-AMPHETAMINE IMPROVES MENTAL PERFORMANCE & RAISES BODY TEMPERATURE AFTER 48 HOURS WITHOUT SLEEP. H. Sing*, D. Thorne*, M. Thomas*, N. Shepanek*, M. Rankin*, C. Galinski*, A. Schelling*, D. Penetar*, J. Fertig, P. Newhouse, & G. Belenky. Dept. of Behavioral Biology, Walter Reed Army Institute of Research, Washington, DC 20307-5100.

Thirty-six normal male volunteer humans subjects were given placebo, 5, 10, or 20 mg d-amphetamine sulfate after 48 hours of total sleep deprivation. d-Amphetamine produced dose-dependent increases in alertness (Multiple Sleep Latency Test & Stanford Sleepiness Scale), mental performance (serial add & subtract, choice reaction time, logical reasoning) and mood (Profile of Mood States). The high dose (20 mg) returned these parameters to baseline values. d-Amphetamine also produced a dose-dependent increase in body temperature. At the high dose, temperature significantly exceeded baseline values. d-Amphetamine's normalization of performance during sleep deprivation may be accompanied by hypermetabolism.

294.9

SHORT TERM CHANGES IN THE NEUROMAGNETIC EVOKED FIELD. G. W. Lewis, L. J. Trejo,* M. Inlow,* & M. H. Blankenship.* Navy Personnel Research and Development Center, San Diego, CA 92152.

We have been using neuromagnetic visual evoked fields (VEF) to assess brain processing with the goal of improved prediction of job performance. As compared to visual evoked potentials, short-term changes in the VEF are not clearly understood. We find that changes in average VEF amplitude occur within the first 18 trials of a series. Using a single channel DC SQUID system, VEF recordings were obtained from 15 male subjects (aged 21 +/- 2 yrs) at sites O1 and O2 (10/20 system). Checkerboard stimulus luminance averaged 34 nits, subtended 5 degrees and had spatial frequency of 1.3 c/degree. VEFs were averaged in 3 blocks of 6 epochs each for a total of 18. Inter-stimulus interval averaged about 1.75 sec. The VEF data were divided into 10 post-stimulus windows of 50 msec width, rms amplitude obtained, and submitted to analyses of variance. Substantial differences were observed between the first block and the last two blocks of trials. Site differences were significant during the first block, but were not significant during the second and third blocks. Differences between windows were significant during the first and second blocks, but were not significant during the third block. Variability was lowest in windows 3 and 4 (100-200 msec), where field reversals are often observed with these stimuli, and was highest later in the VEF (> 350 msec). These VEF changes, occurring over a few seconds, may reflect short term brain adaptive changes such as habituation, or may infer decay of an orienting response. This abstract does not necessarily reflect the views of the Navy Department.

294.10

EFFECTS OF VOLUME AND LOCUS OF BRAIN LESIONS ON NEUROPSYCHOLOGICAL PERFORMANCE IN HUMANS. E. Turkheimer*, R. A. Yeo*, Presty, S. K. and E. D. Bigler*. Dept. of Psychology, University of Virginia, Charlottesville, VA 22903.

In a common research design in human neuropsychology, brain-damaged patients are grouped according to location of lesion, and performance on a measure of functional deficit is compared among groups. Although it is widely recognized that lesion volume is an important determinant of severity of deficit, the absence of a model of how lesion volume combines with lesion locus to produce deficits has inhibited the development of methodological and statistical procedures for studying naturally occurring lesions in humans. We propose such a unified model, which predicts that the basic manifestation of localization of function is as a statistical interaction between lesion location and volume in the prediction of neuropsychological deficit. The model is then applied the neuropsychological performance of a sample of patients with naturally occurring unilateral lesions. Results demonstrate that the interaction between lesion volume and location is more important that the main effect of either in the determination of degree of deficit.

294.11

REACTION TIMES DIFFER WHEN HUMANS AND MONKEYS MAKE HAND MOVEMENTS IN RESPONSE TO VISUAL AS COMPARED TO VIBRATORY CUES. V. D. Douglas*, C. A. McCandlish and R. J. Nelson (SPON: M.E. Michel). Dept. of Anatomy and Neurobiology, College of Medicine, University of Tennessee, Memphis, 875 Monroe Avenue, Memphis, TN 38163.

Reaction times were determined for Rhesus monkeys and human subjects who made identical wrist flexion and extension movements in response to either vibratory or visual cues. Vibratory cues consisted of palmar vibration (low-amplitude sine wave at 27, 57 or 127Hz) delivered to the control handle of the apparatus. Changes in the display that signaled the wrist position served as visual cues. Visual and vibratory-cued trials were presented randomly in blocks of ten trials requiring the same movement in response to the sensory cues. Human subjects initiated movements approximately 50-80 ms sooner in response to vibratory as compared to visual cues (Vib. Mean RTs= 187-294ms; Visual Mean RTs=212-345ms). The differences in reaction times for the same movements to these cues increased with subject age. These differences were correlated with improvements by the older subjects during vibratory-cued trials, as measured by a decrease in the reaction times, whereas the reactions times for visually cued trials remained consistent as a function of subject age. Monkeys also showed differences in reaction times for vibratory as compared to visually-cued trials, which were 80-100 ms less for the former (Vib. Mean RTs= 186-264ms; Visual Mean RTs=269-360ms).

These findings of the normal reaction times for movements made in response to different sensory stimuli provide a framework that may be used to assess the extent of motor dysfunctions in human subjects and primates. As well, these data suggest that vibratory stimuli may be optimally used in sensory processing, especially in instances when movements must be made as quickly as possible or when auditory or visual cues may interfere with ongoing communication or attentive visual fixation. This study met NIH animal utilization guidelines.

Supported by USAF GR AFOSR 88-0179 to RJN.

294.12

THE QUADRIMENTAL BRAIN MODEL AS A NEUROSCIENCE WORKING HYPOTHESIS. B.E.Morton. Dept. of Biochem. and Biophys., Univ. of Hawaii Sch. of Med., Honolulu, HI 96822.

This model extends MacLean's Triune Brain concept to produce a framework for structure-activity explanations accounting for human behaviors previously not understood.

THE REPTILIAN SYSTEM (includes striatum, cerebellum, stem, autonomic system, site of Id): Maintains internal homeostasis by using body to conquer territory for food, shelter, and reproduction. Striatum does same-difference matching of sensory data with cerebellar memory to identify self and external objects. Maximizes immediate survival.

THE LIMBIC SYSTEM (includes emotions, site of Ego): Searches memory for the survival outcome of earlier-similar encounter, concludes same will repeat, produces approach-avoidance emotion motivators and adaptive responses.

LEFT HEMISPHERIC SYSTEM (site of usual consciousness and Intellect): Does serial, reductionistic, abstract data analysis upon which "intelligence", language, science, and civilization depend. Mostly unaware of mental activities of the three other brain elements, rationalizes their behavior as if they were its own. It can confabulate.

RIGHT HEMISPHERIC SYSTEM (includes Superego): Makes parallel, space-time global projections of worst-best survival outcomes. These produce insight and creativity. It values cooperation and deferred rewards. From these come conscience, morality, and wisdom. In conflict with immediate, selfish reptilian drives within or from others.

294.13

RELATIONSHIP BETWEEN SERIAL AND PARALLEL VISUAL SEARCH AND TARGET DISCRIMINABILITY. B. S. Oken and P. Stern*. Dept. of Neurology, Oregon Health Sciences University, Portland, OR 97201.

Prior research has posited 2 models of visual search; a preattentive, parallel and a more attention-requiring, serial process (Treisman, A., Psych. Rev., 95:15-48, 1988; Julesz, B., Trends Neurosci., 7:41-45, 1984). The factors that determine which of the 2 processes predominates in a given visual search task were examined in a series of experiments. Two-choice reaction times were obtained following presentation of several series of visual stimuli to 24 subjects. The subjects were instructed to determine whether or not a given target image was present on a screen that contained 2, 6, 10, 20, or 30 distractor images. Target discriminability was operationally defined by creating distractors that differed by varying degrees from the targets in either color or shape. In some series, the target was a unique conjunction of the features.

Response accuracies did not vary significantly across tasks. In easy target discrimination series, the reaction time to detect the presence of a target did not increase with increasing numbers of distractor stimuli. The slope of the reaction time - number of distractors correlation gradually increased as the target-distractor discrimination was made more difficult. The slopes of the correlations between the reaction time to detection of no-target condition (distractors only) and the number of distractors were consistently greater than those in the target present condition, but similarly varied with difficulty. Lastly, the slopes were modifiable by task repetition. It is postulated that the major determinant of which visual search process predodominates in a given task relates to the difficulty of the target discrimination.

294.14

NEUROPSYCHOLOGICAL DIFFERENCES BETWEEN HOMOSEXUAL MALES AND HETEROSEXUAL MALES AND FEMALES. C.M. McCormick and S.F. Witelson, Dept. of Psychology and Dept. of Psychiatry McMaster University, Hamilton, Canada, L8S 4K1.

Studies of certain clinical populations exposed to atypical levels of prenatal sex hormones indicate these people to show atypical patterns of cerebral asymmetry, cognitive skills, and sexual orientation (e.g., higher prevalence of left-hand preference and homosexuality in CAH females). In a study of homosexuals, we reported a higher prevalence of left-hand preference in homosexual females and a trend in homosexual males compared to the general population (McCormick et al., 1987, Soc Neur, 13). We suggested an association among prenatal sex hormones, sexual orientation, and cerebral asymmetry. In the present study, we investigated other neuropsychological measures in homosexual males compared to matched groups of heterosexual males and females. Subjects completed tests of spatial and verbal ability, and a dichotic test of hemispheric asymmetry. Homosexual males showed significant differences on cognitive tests compared to heterosexual males, and their mean scores typically fell between those of heterosexual males and females. On the dichotic test, homosexual males of each hand preference group showed a different pattern of asymmetry. However, in each case the homosexual males were reversed compared to the heterosexual males. We interpret the associations among sexual orientation, cognitive skills, and cerebral asymmetry in terms of sexual differentiation of the brain.

294.15

THREE-DIMENSIONAL RECONSTRUCTION OF THE HUMAN BRAIN. J.S. George, P.S. Jackson,* D.M. Ranken * and E.R. Flynn. Neuromagnetism Laboratory, Life Sciences and Physics Divisions, MS M882, Los Alamos National Laboratory, Los Alamos, NM, 87545.

We have developed procedures for 3-dimensional reconstruction of the human brain from magnetic resonance imaging (MRI) data. The basic structure of the model is a discrete Cartesian space. Logical resolution within the space is typically 32 planes of 256x256 elements (i.e. the resolution of an MRI tomographic series). The thickness of a plane is set equal to the MRI slice interval. 12 or 16 bit data provided by the MRI system is first compressed to 8 bits per pixel for display and may be further compressed to 4 bits (16 grey levels). This allows a representation of the original image data to be stored within a single byte volume element (voxel) while allowing 16 anatomical tag values for the voxel. Voxels are classified using image processing routines. A seed point and tag value identifying a structure are specified by the user, and the system tags all contiguous points satisfying intensity or contrast criteria. Our algorithms extend this 2-D "flood" procedure throughout the volumetric data structure. For 3-D rendering of an anatomical unit, the system can interrogate the model to identify all voxels on the surface of a designated structure. Shaded 3-D images can be calculated from any viewing angle, using microcomputer resident software or high performance hardware systems. The use of a volumetric model will allow neuroelectric source models to incorporate realistic head geometry and model fitting procedures to employ anatomically constrained source configurations.

294.16

AUTOMATED PERFORMANCE TEST SYSTEM (APTS): ASSESSMENT OF ENVIRONMENTAL HAZARDS ON HUMAN BRAIN FUNCTION. W. Dunlap†, R. Kennedy*, N. Lane*, J. Turnage*, and C. Latimer. Essex Corporation, Orlando, FL 30803.

Recent efforts have focused on development of computer-based neurobehavioral tests for assessment of the effects of environmental hazards on sensory, cognitive, and neuromuscular performance in man and in animal models. The neuropsychological test battery developed for assessment of human brain function has been designated as "Automated Performance Test System" (APTS) and includes: (1) visual pattern comparison, (2) visual search and code substitution, (3) associative and visual-spatial recognition; manikin spatial transformation, (4) short-term memory, (5) grammatical reasoning, and (6) preferred and nonpreferred hand finger tapping. The specific aims of this study were to examine the effects of various concentrations of ethanol, radiation-chemotherapy, and doses of different drugs on neurobehavioral performance. Significant dose and treatment dependent differential decrements were observed in visual, perceptual information processing, memory, reasoning and neuromuscular performance. The neuropsychological assessment indicated that the transient, subtle, and differential decrements of environmental treatments on neurobehavioral performance can be evaluated by APTS with precision, reliability, and reproducibility.

294.17

EFFECTS OF ATTENTION ON THE DETECTION OF VIBROTACTILE STIMULI IN MAN. L.J. Post* and C.E. Chapman. Centre de recherche en sciences neurologiques and Ecole de réadaptation, Université de Montréal, Montréal, Canada.

It has long been known that attention exerts a significant effect on the perception of visual and auditory stimuli. When attention is directed towards a particular stimulus modality, detection abilities are enhanced and reaction times (RTs) are reduced. As little is known regarding the effects of attention on the perception of somatosensory stimuli, this study was conducted to determine the magnitude of effect that attention exerts on the ability to detect vibrotactile, as compared to visual, stimuli.

Subjects (N=11) observed a panel containing 3 LED arrays. In a correctly signalled trial, the illumination of the green or red array indicated to which modality (vibrotactile or visual) subjects should direct their attention in the upcoming trial. A "no" signal trial occurred when both indicator arrays were illuminated, instructing subjects to divide their attention equally between the two modalities. A few incorrectly signalled trials were also presented. Subjects were instructed to respond, with a foot pedal press, as quickly as possible to the stimulus. The vibrotactile stimulus consisted of any of 3 suprathreshold intensities, delivered via an Optacon, to the right index finger; the illumination of the third LED array served as the visual stimulus.

Pooled data indicated that for both vibrotactile and visual stimuli, RTs were significantly shorter when the subject's attention was directed to the correct modality ($p<0.01$). In addition, RTs were significantly longer with weak, as compared to strong, vibrotactile stimuli ($p<0.01$). These findings suggest that detection abilities are improved when attention is directed to the somatosensory stimulus and that the influence of attention is greater on weak, than strong, somatosensory stimuli. Supported by the Canadian MRC and the FRSQ.

294.18

ANALYSIS OF FACTORS UNDERLYING UNILATERAL SPATIAL NEGLECT (USN). C.M. Butter, N. Kirsch* and G. Reeves*. Depts. of Psychology and Physical Medicine and Rehabilitation, University of Michigan, Ann Arbor, MI 48109.

Two factors thought to contribute to USN are deficient orienting to the neglected side and overactive orienting to the unneglected side due to disinhibition of the intact cerebral hemisphere. The contribution of the first factor was demonstrated by the finding that neglect shown by patients with right cerebral infarcts in a line-bisection task was consistently reduced by left-sided dynamic stimuli. We chose these stimuli because they activate the right superior colliculus, a component of the orienting system that is intact in these patients. Left-sided dynamic stimuli were more effective in reducing left USN than were the same stimuli presented in the center of the display (to control for their general alerting effects) or left-sided static stimuli. Furthermore, left-sided dynamic stimuli were as effective in reducing neglect in patients with left hemianopia as in those without this condition. The role of the second factor was shown by the beneficial effect on line-bisection of patching the right eye of patients with left USN, a procedure that blocks direct retinal input to the superior colliculus of the intact (left) hemisphere. This benefit was less consistent and smaller than that of dynamic left-sided stimuli. Combining the two procedures led to greater benefits than either procedure alone; thus, both deficient leftward orienting and overactivity of the left hemisphere appear to contribute to left USN.

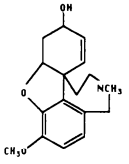
295.1

THE EFFECTS OF GALANTHAMINE ANALOGUES ON CORTICAL ACETYLCHOLINESTERASE (AChE) ACTIVITY.

J. E. Sweeney, S-H Yeop*, M.M. Joullié* and J. T. Coyle. Depts. of Neuroscience and Pharmacology. Johns Hopkins Univ., Baltimore, MD 21205 and Dept. of Chemistry. Univ. of Pennsylvania, Philadelphia, PA 19104.

We have previously shown that galanthamine attenuates performance deficits in basal forebrain lesioned mice, inhibits AChE in cortical homogenates with an IC_{50} of 410 nM and increases cortical acetylcholine levels by 55% one hour after i.p. injections (Sweeney, J.E. *Pharmacol. Biochem. Behav.*, in press). Using structure-activity relationships, we now demonstrate which portions of the molecule are critical for galanthamine to bind competitively to the AChE molecule. Several structural analogues inhibit cortical AChE more potently than galanthamine.

GALANTHAMINE



Alterations of the allylic hydroxyl group result in a severe decrease in the drug's ability to inhibit cortical AChE. Conversion of the hydroxyl group to an acetoxy function, to a butyl or naphthyl carbamate group, or to a semicarbazone moiety (after oxidizing the hydroxyl to a ketone) severely reduces, by 1-2 orders of magnitude, the inhibitory activities of these compounds as compared to galanthamine. O-demethyl galanthamine is a considerably more potent inhibitor of cortical AChE than galanthamine with an IC_{50} of 80 nM. Benzyl bromide and methiodide salts of galanthamine are slightly more potent inhibitors of AChE than galanthamine.

These data indicate that the hydroxyl, tertiary N, and O-methyl are critical for binding to AChE. Furthermore, these findings have implications for the design of drugs which may be used for the treatment of Alzheimer's disease. Testing the behavioral effects of several of these analogues is currently in progress.

295.2

SPATIAL WORKING MEMORY ENHANCEMENT BY NICOTINE OF AGED LONG EVANS RATS IN THE T-MAZE

E. Cregan*, J.M. Ordy, E. Palmer, J. Blosser, T. Wengenack*, and G. Thomas, Fisons Pharmaceuticals, Rochester, NY 14623; Univ. Roch, Rochester, NY 14642.

Degeneration of basal forebrain cholinergic neurons has been correlated with memory dysfunction in senile dementia of Alzheimer's type, SDAT. Thus far, primary focus has been on loss of muscarinic receptors. Recent binding studies have shown loss of cortical and hippocampal nicotinic receptors in SDAT. Other studies have shown nicotine enhancement of memory dysfunction in SDAT and in monkeys. Nicotine enhancement of memory dysfunction has not been reported in aged animal models of SDAT. Specific aims of this study were to evaluate the effects of 0.1 and 0.4 mg.kg, s.c., of nicotine on spatial working memory and performance of 36-42 mo. old Long Evans (LE) rats in the T-maze. Compared to middle aged 24 mo. old LE rats, there was a significant impairment of memory of aged LE rats. Compared to saline, 0.1 and 0.4 mg/kg of nicotine significantly improved spatial working memory of aged LE rats, without effects on start, choice, and goal speed performance in the T-maze. The results indicated that nicotine enhanced memory impairments in aged LE rats serving as animal models of SDAT.

295.3

CHARACTERIZATION OF NOVEL MUSCARINIC AGONISTS, 1-OXA-8-AZASPIRO[4.5]DECANE DERIVATIVES IN BIOCHEMICAL AND BEHAVIORAL STUDIES.

F.Wanibuchi, S.Usuda, T.Konishi, M.Harada, M.Terai, K.Hidaka, S.Tsukamoto*and T.Tamura(SPON:K.Inoue) Central Res. Labs., Yamanouchi Pharmaceutical Co.,Ltd., Miyukigaoka 21 Tsukuba-city, Ibaraki 305, Japan.

A presynaptic cholinergic hypofunction with no change in the number of postsynaptic M_1 receptors (R) in Alzheimer's disease suggests that M_1 agonists are possible therapeutic agents. We synthesized novel muscarinic agonists, 1-oxa-8-azaspiro[4.5]decane derivatives, YM796 (2,8-dimethyl-3-methylene) and YM954 (2-ethyl-8-methyl-3-oxo). These compounds inhibited ^{3}H-pirenzepine binding to rat cerebral cortical membranes like putative M_1 agonists, RS86 and AF102B in the micromolar range and weakly inhibited ^{3}H-QNB binding to cerebellar membranes. YM796, YM954 and RS86 stimulated phosphoinositide (PI) hydrolysis in hippocampal miniprisms which is mainly linked to M_1-R but AF102B did not. YM796 (0.031 mg/kg,po) and YM954 (0.016 mg/kg,po) reversed the cognitive impairment in nucleus basalis magnocellularis-lesioned rats in a passive avoidance task, being more effective than RS86 and AF102B. YM796 was weaker than YM954 and RS86 in the induction of mouse tremor which are mainly mediated by M_2-R. These results show that YM796, YM954 and RS86 have M_1 agonistic activity and that YM796 has relatively weak M_2 agonistic activity. We are now carrying out further studies with (-)- and (+)-isomers of YM796.

295.4

EFFECTS OF TACRINE ON COGNITIVE PERFORMANCE IN MICE AND MONKEYS.

E. Schwam, G.P. Vincent, L. Rumennik and J. Sepinwall. Department of Neurobiology and Obesity Research, Hoffmann-La Roche Inc., Nutley, N.J. 07110.

Tacrine (THA), an anticholinesterase, is in clinical trials in Alzheimer's Disease. It enhanced cognitive performance in rodents (Fitten et al., In: Cur. Res. Alz. Ther. Ed.: Gaicobini and Becker, 1988) but produced equivocal effects in monkeys (Bartus and Dean, ibid.). In our Morris water maze procedure in mice, THA significantly improved acquisition by decreasing the latency to find a hidden platform (0.003-0.1 mg/kg, ip and 0.3-3 mg/kg, po using a 30 and 60 min pretreatment times (pts), respectively). The peak active doses of 0.01 mg/kg, ip and 1 mg/kg, po enhanced acquisition from 5-60 min and 5-90 min, respectively, but not at longer pts. In a subchronic study, a dose inactive after acute administration (0.001 mg/kg, ip) enhanced acquisition after 3 days of administration, with a duration of action of at least 48 hours. When proximal maze cues were removed and only distal room cues were available, the acquisition deficit exhibited by C57BL/10 mice was reversed by a dose of 1 mg/kg, po. In squirrel monkeys in repeated acquisition and short-term memory (delayed match-to-sample) procedures, 0.03-3 mg/kg, po, 20 min pt, failed to enhance performance, with deficits on some measures evident at 1 and 3 mg/kg. In conclusion, the cognition-enhancing effects of THA appear to be species and/or task specific.

295.5

NICOTINIC ENHANCEMENT OF DELAYED MATCHING BY MONKEYS IS SPECIFIC TO THE MOST DIFFICULT PROBLEMS.

W.J. Jackson and J.J. Buccafusco. Depts. Pharmacology & Toxicology and Physiology & Endocrinology, Medical College of Georgia and Veterans Administration Medical Center., Augusta, GA 30912

We previously reported that nicotine enhanced a delayed matching task specifically during trials involving long delays which were near the limit of performance capabilities in monkeys (Life Sci 43:277, 1988). The present study extends these observations by examining additional facets of matching performance. For example, we have demonstrated that in addition to length of delay, stimulus variables also contribute to the difficulty of individual trials. For each monkey, the order of difficulty represented by various stimulus configurations was determined. We observed the nicotine-mediated (2.5-10 μg/kg, i.m., 15 min prior to test session) enhancement to be greatest for those trials in which the longest delay and the most difficult stimulus configurations were coincident. The enhancement for these most difficult trials exceeded the enhancement associated with the overall session by a factor of almost 5. Nicotine also lengthened the latency of the choice responses, perhaps indicating that the monkeys were more "deliberate" about their choices when influenced by nicotine. This nicotine-induced apparent memory enhancement was still evident in several animals when tested on the day following the injection. These results support the concept of pharmacological exploitation of central nicotinic receptors in diseases involving memory deficits, e.g., Alzheimer's Disease. Supported by the Smokeless Tobacco Research Council.

295.6

FACILITATION OF HIPPOCAMPAL ACETYLCHOLINE SYNTHESIS BY GLUCOSE: CONTRIBUTION TO THE ACTION OF GLUCOSE ON MEMORY.

C. MESSIER, T. DURKIN, O. MRABET and C. DESTRADE. Lab. de Psychophysiologie, U.A. CNRS n° 339, Université de Bordeaux I, 33405 Talence CEDEX, FRANCE.

The effect of a 3 g/kg glucose injection on the velocity of the sodium-dependent high affinity choline uptake mechanism in the hippocampus was measured in resting mice or in mice immediately after training in an operant bar pressing task. Glucose did not significantly change high affinity choline uptake in resting animals. High affinity choline uptake in the hippocampus was increased by training in the operant bar pressing task. Glucose significantly reduced the increase of high affinity choline uptake observed in the trained animals. Similarly, a 3 g/kg glucose injection also reduced the high affinity choline uptake increase observed in animals injected with 1 mg/kg scopolamine. Finally, a 3 g/kg glucose injection significantly attenuated the amnesia produced by a post-training 1 mg/kg scopolamine injection in mice trained for an operant bar pressing task. These results provide additional evidence for an action of glucose on hippocampal cholinergic activity under conditions of high ACh demand. This action may be mediated via an increase in acetyl coenzyme A availability, one of the precursors of acetylcholine. This facilitative effect of glucose on hippocampal acetylcholine synthesis may constitute the physiological basis for its facilitative action on memory and its attenuation of scopolamine amnesia.

295.7

HOE 065, A COMPOUND STRUCTURALLY RELATED TO ACE INHIBITORS INCREASES ACETYLCHOLINE METABOLISM IN RAT BRAIN. G. Wiemer*, R. Becker*, H.J. Gerhards*, F.J. Hock and H. Urbach* (SPON: R. Hess). HOECHST AG, P.O.B. 80 03 20, 6230 Frankfurt/M. 80, FRG.

Previously we have reported that the lipophilic inhibitor of the dipeptidyl-carboxypeptidase (ACE) Hoe 288 improves cognitive functions in rodents. This effect is accompanied by an increased metabolism of acetylcholine (ACh; Usinger, P., Drug Develop. Res., 14: 315, 1988).

Although Hoe 065 - a prodrug of the ACE inhibitor Hoe 498 (Ramipril) - exerts no significant influence on plasma ACE or blood pressure in SHR the compound beneficially modifies performance in mice and rats as well as increases cholinergic transmission in different brain areas of the rat. 0.03 - 30 mg/kg i.p. or p.o. of Hoe 065 acutely caused a fall in brain ACh, increased the capacity of the high affinity choline uptake and the activity of the choline acetyltransferase without influencing the activity of the cholinesterase. An elevation of brain cyclic GMP was concurrently observed.

It is concluded that these effects are not necessarily related to an inhibition of the degradation of angiotensin I as can be assumed from the action of ACE inhibitors.

295.8

BEHAVIORAL EFFECTS OF HOE 065, A COMPOUND STRUCTURALLY RELATED TO ACE-INHIBITORS. F.J. Hock, H.J. Gerhards*, G. Wiemer*, W. Rüger* and H. Urbach*. HOECHST AG, P.O.B. 80 03 20, D-6230 Frankfurt/M. 80, FRG.

Over the past decade a large number of peptides has been detected in the CNS with putative function in motivational, memory and learning processes. Since the activity of dipeptidyl carboxypeptidase EC 3.4.15.1 (angiotensin converting enzyme, ACE) was found to be elevated in several brain regions of Alzheimer's disease patients we tested the behavioral effects in mice and rats of a compound which is structurally related to ACE-inhibitors. The new compound Hoe 065; n-octyl 2-/N-/(S)-1-ethoxycarbonyl-3-phenylpropyl/-L-alanyl/-(1S, 3S, 5S)-2-azabicyclo-/3.3.0/octane-3-carboxylate maleate salt was found to be highly potent in several behavioral tests.

In the up-hill avoidance test with rats Hoe 065 caused a significant enhancement of the up-hill response after scopolamine treatment. The reduction of the step-through latency after scopolamine in an inhibitory avoidance test with mice was tested. Hoe 065 caused a significant enhancement of the step-through latency with a minimal effective dose of 0.03 mg/kg i.p. In the 8-arm radial maze rats were required to find food pellets. Hoe 065 antagonized the impairment after scopolamine treatment significantly.

295.9

CHOLINERGIC MANIPULATIONS AND PASSIVE AVOIDANCE IN THE RAT: STATE DEPENDENCY AND ACQUISITION TO CRITERION. W. Jeffrey Wilson & Jennifer C. Hall. Dept. of Psychological Sciences, Indiana - Purdue University, Fort Wayne, IN 46805, & Dept. of Psychology, Emory University, Atlanta, GA 30322, USA.

In studies of the role of central cholinergic systems in learning and memory the effects of cholinergic manipulations (either pharmacological or surgical) on passive avoidance tasks are often assessed. Unfortunately, these studies rarely train subjects to criterion, so it is never clear whether or not learning has occurred during acquisition, and these studies rarely control for state-dependent (S-D) learning, so specific effects of the manipulations on memory storage or retention/recall cannot be ascertained.

In two experiments, rats were trained to criterion in a passive avoidance task, then were tested for retention 24 h later. In Experiment One, scopolamine (1 mg/kg) disrupted both the acquisition and retention/recall of the task. Initial step-through and escape latencies in the training session were unaffected, and S-D learning was not present. In Experiment Two, rats with lesions of the n. basalis magnocellularis (nbM) were compared to sham-operated controls, and the effects of pilocarpine (3 mg/kg) in conjunction with a peripheral muscarinic blocker (methyl scopolamine 1 mg/kg) were assessed. The lesion had no effect on the acquisition or retention/recall of the task, or on escape latency. nbM rats were slower to cross in training, especially when given pilocarpine. In both the nbM and sham rats, pilocarpine disrupted acquisition and retention/recall of the passive avoidance task, and it delayed entry to the dark side during training. An interaction between drug conditions in training and test revealed the presence of S-D learning in this experiment.

Although the nbM lesion had no effect, both drug manipulations disrupted acquisition (assessed via trials to criterion) and performance of the task. State-dependent learning was apparent in the effects of pilocarpine. [Supported in part by Purdue XL Grant to W. J. Wilson.]

295.10

MUSCARINIC CHOLINERGIC SYSTEMS & REPRESENTATIONAL MEMORY. W.S. Messer, Jr., J. Stibbe* and M. Bohnett*. Medicinal and Biological Chemistry, College of Pharmacy, Univ. of Toledo, 2801 W. Bancroft, Toledo, OH 43606

Representational memory is a form of working memory indicated when animals make discrminations based on cues not present at the time of choice. The role of muscarinic receptors in representational memory was examined using the selective antagonists pirenzepine and AF-DX 116. Rats were trained to perform a paired-run, alternation task in a T-maze to measure representational memory. Once animals learned the task, cannulae were implanted stereotaxically under general anesthesia for injections into the hippocampus. Following recovery from surgery, animals were retested in the maze to measure baseline performance and exhibited no impairment due to the surgical procedures. Bilateral injections of antagonists in 1 µl of saline were administered via cannulae 20 min. prior to testing. Rats receiving pirenzepine exibited an impairment of performance as measured by a reduction in the percentage of correct choices (79.2 ± 2.4 % with 10 µg; 70.8 ± 3.1 % with 70 µg). Animals receiving AF-DX 116 did not exhibit a significant impairment at any dose tested. The highest dose of AF-DX 116 (70 µg) produced a slight decrease in the percentage of correct choices (83.3 ± 0.0 %). The data suggest that M_1 receptors are involved in representational memory.

Dispositional memory is indicated when animals make discriminations based on cues present at the time of choice. To determine the role of cholinergic systems in both types of memory, animals were trained to perform two tasks in a modified T-maze. To measure dispositional memory, animals were required to perform a tactile discrimination in the stem of the maze in addition to the alternation task described above. Once animals learned both tasks they were injected bilaterally in the hippocampus with either saline or AF64A, a cholinergic neurotoxin. Testing was resumed one week following surgery. Rats receiving either saline or 1 nmole of AF64A were not impaired on either task, while animals receiving 2 nmoles of AF64A were impaired initially on both tasks. Animals with AF64A lesions showed a recovery of dispositional memory by the third session but still showed an impairment of representational memory. Animals with lesions were injected with pilocarpine to determine if muscarinc agonists could reverse the representational memory deficit. Pilocarpine (3.0 mg/kg) reversed the impairment associated with administration of AF64A, providing further support for the hypothesis that muscarinic receptors play an important role in representational memory. Supported by NS 23929 and NS 25765.

295.11

DISSOCIATIVE EFFECTS OF DIFFERENT TYPES OF BASAL FOREBRAIN LESIONS ON CORTICAL AChE-POSITIVE FIBER STAIN AND CYTOCHROME OXIDASE ACTIVITY. M. Sarter and P. Dudchenko*. Dept. of Psychology, The Ohio State University, Columbus, OH 43210.

Recent evidence suggests that the behavioral impairments produced by excitotoxic lesions of the basal forebrain are not primarily due to cortical cholinergic denervation. Robbins et al. (Neurosci., 28:337, 1989) reported a dissociation between the behavioral and the anticholinergic effects of quisqualate and ibotenate induced lesions.

The effects of different types of unilateral basal forebrain lesions (ibotenate, quisqualate, colchicine) on cortical acetylcholinesterase (AchE) positive fiber stain and cytochrome oxidase activity were compared in parallel sections. The rank ordering in terms of AchE-fiber loss was quisqualate > colchicine > ibotenate; however, in terms of cytochrome oxidase activity the reversed order was found. These data suggest that the reduction of cortical metabolic activity following ibotenate lesions is not primarily due to the loss of cholinergic input. From a behavioral point of view, cytochrome oxidase seems to represent a more valid histological correlate of the cortical effects of basal forebrain lesions than the loss of cholinergic activity.

296.1

EFFECTS OF CHOLINERGIC BLOCKADE IN THE AMYGDALA ON INHIBITORY AVOIDANCE AND REINFORCED ALTERNATION. A.H. Nagahara* and J.L. McGaugh. Department of Psychobiology and Center for the Neurobiology of Learning and Memory, Univ. California, Irvine, CA 92717.

Lesions of the nucleus basalis magnocellularis (NBM) in rats produce memory impairments on a wide variety of tasks. Such deficits have generally been interpreted as due to the loss of the cholinergic projection to cortex. However, NBM also sends a major cholinergic projection to the amygdala. We examined the role of this projection in memory by infusing scopolamine into the basolateral amygdala prior to or following training on two tasks sensitive to NBM lesions.

Sprague-Dawley rats were trained on an inhibitory avoidance task (IA). Each animal received an intra-amygdala injection, through implanted cannulae, of buffer or scopolamine (0.03, 0.1, or 1.0 μg/0.5 μl) either 5 min-pretraining or immediately posttraining. Retention was tested 48 hr after training. One week later, a subgroup of these animals was tested on a reinforced alternation (RA) task (15 consecutive trials, ITI=30s). Animals were tested under both conditions. Half received pretesting intra-amygdala injection of buffer on day 1 and scopolamine (0.1 μg/0.5 μl) on day 2, and the other half received the reverse order.

In the IA task, 0.1 μg scopolamine administered pretraining significantly impaired performance ($p<.01$); posttraining scopolamine injections did not. This finding suggests that the cholinergic system in the amygdala plays a role in the acquisition of the IA task. However, no effect on the RA task was found: The amygdala cholinergic system appears not to be essential for performance on this task. These data suggest that cholinergic blockade in the amygdala produces memory deficits on certain tasks and that some of the memory deficits observed in NBM-lesioned animals may be due to disruption of this NBM projection.

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296.2

CHOLINERGIC-DOPAMINERGIC INTERACTIONS IN RADIAL-ARM MAZE PERFORMANCE: A LESION STUDY. S.R. McGurk, E.D. Levin, and L.L. Butcher. Dept. of Psychology, UCLA, Los Angeles, CA 90024-1563.

Accurate performance on the radial-arm maze is dependent on the integrity of nicotinic cholinergic, muscarinic cholinergic, and dopaminergic neurotransmitter systems, and is determined by complex interactions of these systems. It has been suggested that the basal nuclear complex is critical for the modulation of learning and memory.

We have examined the effects of lesions of the medial cholinergic pathway, arising from the basal nuclear complex and projecting to the cingulate and medial occipital cortices, on maze performance in rats. In Experiment I, we found that knife-cut lesions of the medial cholinergic pathway, but not similar lesions causing cortical damage but sparing this pathway, caused a robust but temporary disruption in radial-arm maze performance. In Experiment II, the effect of brain lesions on maze performance was eliminated by daily administration of the cholinergic agonist drug arecoline, suggesting that the performance deficit is due specifically to the loss of cholinergic innervation. In Experiment III, memory impairment caused by lesions of the medial cholinergic pathway were abolished by daily treatment with a D1 agonist (SKF 38393), diminished by daily treatment with a D2 agonist (LY 171555) or a D1 antagonist (SCH 23390), and not affected by a D2 antagonist (raclopride). Taken together, these data demonstrate the importance of the medial cholinergic pathway in maze performance and extend our previous observations that performance deficits due to cholinergic dysfunction can be reversed by modulation of dopaminergic systems. (NIH grant 10928 to L.L.B.)

296.3

CONCURRENT MUSCARINIC AND BETA-ADRENERGIC BLOCKADE IMPAIRS INHIBITORY (PASSIVE) AVOIDANCE AND MORRIS WATER MAZE PERFORMANCE. T. M. Gill*, M. W. Decker, and J. L. McGaugh (SPON: S. F. Zornetzer). Center for the Neurobiology of Learning and Memory and Dept. of Psychobiology, Univ. California, Irvine. CA 92717.

Cholinergic and noradrenergic (NA) deterioration is found in aging and Alzheimer's disease. Because experimental cholinergic disruption impairs performance on a variety of memory tasks but NA-depletion does not, cholinergic dysfunction is typically assigned a greater role in age-related memory decline than is NA dysfunction. However, NA-depletion enhances the disruptive effects of muscarinic cholinergic blockade on the radial maze (Decker and Gallagher, Br. Res., 417:59, 1987). The current study extends this finding by examining the effects of muscarinic cholinergic and ß-adrenergic blockade on two additional tasks.

Pretraining administration of either propranolol HCl (10 mg/kg ip) or scopolamine HBr (0.3 and 1.0 mg/kg ip) to rats had no significant effect on retention of step-through inhibitory avoidance training (0.5 mA, 2 s footshock) at a 1 day delay. However, concurrent administration of this dose of propranolol with either dose of scopolamine produced a profound amnesia ($p<.01$). Similarly, pretraining administration of either 10 mg/kg propranolol or 0.3 mg/kg scopolamine did not affect acquisition of the Morris water maze, but concurrent administration of these drugs increased escape latencies during training ($p<.001$) and impaired performance during a free swim probe trial conducted at the conclusion of training.

Thus, cholinergic/noradrenergic interaction is found in a variety of behavioral tasks and ß-adrenergic mechanisms play an important role in this interaction, suggesting a potential role for NA dysfunction in age-related memory decline.

Support: NIA Fellowship AG05446 (MWD), NIMH Grant MH12526 (JLM), ONR Contract N00014-87-K-0518 (JLM).

296.4

LONG TERM SCOPOLAMINE TREATMENT DURING THE POSTNATAL PERIOD IMPAIRS SHORT-TERM MEMORY PROCESSES BUT NOT PLACE LEARNING IN YOUNG RATS. R. Paylor and J.W. Rudy, Psychology Dept., Colorado University, Boulder, CO 80309.

Long term scopolamine treatment causes an increase in the number of hippocampal muscarinic receptor binding sites in both neonatal and adult rats (J. Ben-Barak & Y. Dudai, Brain Res., 193, 309-313, 1980). Currently, however, it is not known what impact muscarinic receptor upregulation has on an animal's behavior. In the present study we looked at the effects of long term scopolamine treatment on short-term memory and place learning processes in the young rat.

Rat pups were injected once a day for 19 days starting at 1 day old. Forty-eight hours after the last injection subjects were tested on one of two tasks. The two tasks selected are known to be sensitive to hippocampal damage and cholinergic manipulations. In one group, subject's place learning processes were tested by training them to locate a hidden platform in the Morris water task. In the second group subject's short-term memory processes were tested by training them to solve a delayed conditional spatial alternation task in a water T-maze. The scopolamine treatment did not effect performance on the place-learning task, but significantly impaired subject's ability to learn the delayed conditional spatial alternation task. Additional experiments will be directed at understanding the nature of the behavioral impairment.

296.5

MICROINJECTIONS OF SCOPOLAMINE AND SULPIRIDE INTO RAT AMYGDALA OR THALAMUS: EFFECTS ON DELAYED NON-MATCHING TO SAMPLE AND VISUAL DISCRIMINATION IN A T-MAZE. G.N.O.Brito, S.P.Silva*,and L.S.O.Brito*. Lab.Neuropsicol.Exp.,Setor de Neurociencias,Univ. Fed.Fluminense, Niteroi, RJ, Brasil.

Recently, we demonstrated that microinjections of scopolamine(SCO), but not sulpiride(SUL), into prelimbic cortex interfered with the performance of delayed nonmatching to sample(DNMS).Injections of SCO or SUL had no effect on visual discrimination(VD). A large body of data indicate close anatomical links between prelimbic cortex, thalamus and amygdala. In the present study, rats were implanted with cannulae in either the amygdala(AMY) or thalamus(THA), and they were tested on either a DNMS or VD task in a T-maze after bilateral 1.0 ul injections of SCO(4.0 and 7.5 ug/ul) and SUL (10 ug/ul). The results demonstrated that the higher SCO dose injected into AMY or THA impaired the performance of DNMS and VD, whereas the lower dose interfered with the performance of DNMS only. Injections of SUL had no effect on performance of either task. We conclude that:(i)cholinergic, but not dopaminergic, mechanisms in AMY and THA are involved in memory processes; (ii)performance of a working/representational memory task(DNMS) is differentially sensitive to SCO injections in AMY and THA. Research supported by CNPq and FINEP.

296.6

THE IMPAIRMENT OF RADIAL-ARM MAZE CHOICE ACCURACY BY COMBINED MUSCARINIC AND NICOTINIC BLOCKADE AND ITS REVERSAL BY A D2 AGONIST. E.D. Levin, J.E. Rose*, S.R. McGurk and L.L. Butcher. Dept of Psychology, University of California, Los Angeles, CA 90024 and Dept of Psychiatry, Duke University, Durham, NC 27705.

Choice accuracy performance in the radial-arm maze is dependent upon both nicotinic and muscarinic acetylcholine receptors. In our previous study nicotinic and muscarinic blockers acted in at least an additive manner in disrupting radial-arm maze choice accuracy. In this study, it was found in rats (N=13) that mecamylamine (2.5 mg/kg) and scopolamine (0.04 mg/kg) provided a combined blockade which was greater than additive. The combination of subthreshold doses of these drugs caused a pronounced impairment. Previous studies have shown that the impairment caused by nicotinic blockade is reversed by the dopamine D2 agonist LY 171555 and that the impairment caused by muscarinic blockade is reversed by the D1 antagonist SCH 23390. In the current study LY 171555 (0.01 mg/kg) significantly ($p<.05$) attenuated the cognitive deficit caused by combined nicotinic and muscarinic blockade, however, SCH 23390 (0.01 mg/kg) was not found to be effective. Nicotinic and muscarinic blockade were found to have synergistic effects in producing cognitive impairment in the radial-arm maze. This deficit was treatable with the D2 agonist, LY 171555. This research was supported by NIH grants DA 02665 and NS 10928.

296.7

THE EFFECTS OF SCOPOLAMINE, METHYLSCOPOLAMINE, CHLORDIAZEPOXIDE AND D-AMPHETAMINE ON DELAYED MATCHING-TO-POSITION PERFORMANCE OF MALE AND FEMALE RATS. F. van Haaren[1], A. van Hest* and R.P.W. Heinsbroek* [1] Dept. of Psychol, Univ. of Florida, Gainesville, FL 32611 and Netherlands Institute for Brain Research, Amsterdam, The Netherlands.

Previous studies have shown that SCOP may differentially affect behavior in intact male and female rats (van Haaren, et al. Pharmacol Biochem Behav, 1989). The present experiment was designed to investigate whether or not SCOP treatment differentially affect memory functioning. Other pharmacological challenges were included to determine the specificity of the anticholinergic effects. Male and female rats were trained in a delayed matching to position procedure (delay 1, 5, 10 or 20 s) and treated (mg/kg) with SCOP, METHSCOP (0, .08, .16, .32), CDP (0, 2.5, 5, 10) and d-AMPH (0, .4, .8, 1.6). SCOP and d-AMPH disrupted matching behavior, CDP and METHSCOP did not. d-AMPH, but not SCOP disrupted matching more in females than in males. SCOP and d-AMPH produced a differential increase in the number of trials not initiated; attentional factors may play a role in disrupting matching behavior.

296.8

ANTAGONISM BY ATROPINE, SCOPOLAMINE, AND APROPHEN OF PHYSOSTIGMINE EFFECTS ON RATS' PERFORMANCE OF A RADIAL-MAZE LEARNING TASK. J. R. Leu, T. F. Elsmore, S. A. McBride. Division of Neuropsychiatry, Walter Reed Army Institute of Research, Washington, D.C. 20307-5100.

Physostigmine (0.4 mg/kg) reduced the number of pellets earned in a 5-minute session by about 90%. Three anticholinergic drugs, atropine, scopolamine, and aprophen, all provided some degree of antagonism. Aprophen was the most effective, restoring performance to greater than 80% of control at doses of 3.2 and 6.4 mg/kg. Both atropine and scopolamine restored performance to only 45% of control at doses of 6.4 and 0.2 mg/kg, respectively. While the degree of antagonism produced by scopolamine was not as great as that of aprophen, it was somewhat effective over a wide range of doses.

296.9

MECAMYLAMINE ALTERS DYNAMIC COMPONENTS OF CENTRAL CHOLINERGIC SYSTEMS IN RAT BRAIN IN A DOSE PRODUCING LEARNING IMPAIRMENT. K. Elrod and J.J. Buccafusco. Dept. Pharmacology and Toxicology, Med. Coll. of GA and Vet. Adm. Med. Ctr, Augusta, GA 30912.

We previously demonstrated impairment of passive avoidance performance in rats following administration of the central nicotinic antagonist mecamylamine (M) but not the peripheral nicotinic antagonist hexamethonium (H). The present studies examined the effects of M and H on the dynamics of the cholinergic system in rat brain. Animals were pretreated s.c. with the behaviorally-deleterious dose of M in the rat (25mg/kg), a lower, behaviorally-ineffective dose of M (5mg/kg), H (25mg/kg) or saline prior to i.v. pulse, tracer injection of ^{3}H-choline. Pretreatment with M (25mg/kg) resulted in marked reductions of 38%-61% in newly formed ^{3}H-Ach in all 5 brain areas studied (frontal, limbic and parietal cortices, hippocampus and striatum). M (25mg/kg) did not, however, alter endogenous steady-state Ach levels. Estimates of Ach turnover revealed significant reductions of 59% and 64% in parietal cortex and hippocampus, respectively, of animals pretreated with M (25mg/kg). As with the behavioral studies, H or M (5mg/kg) was without effect on the same biochemical parameters. These drug-induced biochemical alterations may subserve the behavioral impairment observed following M (25mg/kg) administration. These results lend support to the hypothesis that presynaptic nicotinic receptors mediate a positive feedback mechanism for Ach release in that the centrally-acting antagonist produced a decrease in the release of Ach or firing rate of apparently tonically active central cholinergic neurons. Supported by the Smokeless Tobacco Research Council.

296.10

EFFECTS OF SCOPOLAMINE AND PYRITHIAMINE ON ALTERNATION BEHAVIOR IN RATS. A. S. Powers, D. V. Gutierrez,* & J. A. Hirsch.*(1) St. John's University, New York, NY 11439.

Hirsch & Gibson (Biochem. Pharmacol., 33, 1984) found that pyrithiamine (PY), a thiamine antagonist, stimulated the release of acetylcholine from rat cerebral cortex in vitro. The present experiment investigated the effects of the drug in vivo on an operant single alternation task in rats and compared its effects to those of scopolamine (SC), a muscarinic receptor blocker that is known to impair mnemonic processes.

Rats were trained in an operant chamber to alternate between two levers. Twenty reinforcements were given per day with an intertrial interval of 4s. Self-correction was allowed after an error. Errors per day were recorded.

After training to asymptote, the rats were given a series of intraperitoneal injections of PY (0.5 mg/kg) and SC (0.2 mg/kg), alone and in combination. Between injections, they were run without drug for at least 3 days. The sequence of injections was as follows: PY, PY, PY, SC, PY+SC, SC, SC, SC, PY, PY+SC. The injections were given 0.5 hr before testing.

Data were analyzed as difference scores between the previous drug-free day and the drug day. Although SC significantly disrupted performance ($p<.05$), PY had no significant effect. The results provide no evidence for a facilitatory effect of acute pyrithiamine on memory. (1)Present affiliation: Long Island Univ., Brooklyn, NY.

296.11

DIFFERENTIAL EFFECTS OF SCOPOLAMINE INJECTED INTO NEOSTRIATUM ON ACQUISITION, CONSOLIDATION AND RETRIEVAL OF CONDITIONED EMOTIONAL RESPONSES Marc D. Viaud and Norman M. White, Department of Psychology, McGill University, 1205 Dr. Penfield Ave., Montreal, Quebec, Canada, H3A 1B1

In previously published work we demonstrated that post-training micro-injections of amphetamine into the ventrolateral (VL) caudate nucleus improved retention of a conditioned emotional response (CER) with an olfactory conditioned stimulus (CS), but had no effect on retention of a CER with a visual CS; injections into the posteroventral (PV) caudate improved retention of a CER with a visual CS, but not with an olfactory CS. In the present experiment, using the same paradigm, we investigated the effects of the anti-cholinergic, scopolamine (1ug in 5uL), micro-injected 10 min before training (acquisition), 30 sec after training (consolidation) or 10 min before testing (retrieval). Compared to saline-injected controls, scopolamine prevented both acquisition and retrieval, but facilitated consolidation. All three effects were specific as to the relationship between injection site and CS modality. These findings are consistent with the hypothesis that the origin of the cortico-striatal input to areas of the dorsolateral striatum determines the content of the sensori-motor memory mediated in each area. The data are also consistent with other data suggesting that cholinergic function in the dorsolateral striatum is involved in the memory functions of this part of the brain.

296.12

RETROGRADE AMNESIA INDUCED BY SCOPOLAMINE: PRTOTECTIVE EFFECT PRODUCED BY OVERTRAINING. M. Durán*, and R. A. Prado-Alcalá. Psychophysiol. Lab., Anáhuac Univ. and Physiol. Dept., Med. Sch., Natl. Univ. of México, México, D. F.

Disruption of cholinergic activity of the striatum produces retrograde amnesia. When animals are submitted to overtraining such disruption is innocuous. Systemic application of anticholinergics also produces amnesia. We determined whether overtraining also has a protective effect against amnesia. Rats were trained, in one trial, to avoid the darker compartment of a two-compartment box; retention was tested 24 hr later. In Exp. I, a footshock of 3.0 mA was used, and the rats were injected (I.P., 5 min. posttraining) with 0, 2, 4, 8 or 12 mg/kg of scopolamine (SCOP). There was a dose-dependent amnesic state induced by SCOP. In Exp. II, rats were trained with 6.0 or 9.0 mA; there were three groups for each intensity: untreated, injected with 8 and with 12 mg/kg of SCOP. None of the groups showed amnesia. These results indicate that a) acetylcholine is involved in consolidation of normal training and b) acetylcholine is not involved in the consolidation of overtrained tasks. Supported by Fundación Miguel Alemán, A.C.

296.13

COMPARATIVE EFFECTS OF SCOPOLAMINE TREATMENT AND FIMBRIA-FORNIX LESIONS ON MAZE PERFORMANCE IN RATS. J.C.Cassel*, C. Kelche* and B.E. Will. (SPON: J. Hirsch). D.N.B.C., Centre de Neurochimie du C.N.R.S., 12, rue Goethe, F-67084 Strasbourg Cedex, France.

Intact Long Evans rats were trained in a 8-arm radial maze and subsequently tested under systemic treatment with physostigmine (0.05mg/kg; I.p.), scopolamine methylbromide (Mbr) and scopolamine hydrobromide (Hbr; 0.5mg/kg). The effects of these drugs were compared to those of aspirative lesions of the fimbria-fornix pathways at 3 postoperative delays in different rats: 3, 7 and 12 months.

At each of these delays (1) rats with lesions showed impaired performance during predrug trials, (2) physostigmine had no effect in any group, (3) scopolamine HBr impaired performances of intact rats in a manner which closely paralleled the lesion-induced behavioral effects (errors, arms before first error, strategies). This impairment was not dependent on the strategies used by rats.

These results further support the involvement of cholinergic processes in spatial memory and suggest that scopolamine-induced disruption of central cholinergic functions may mimic the effects of fimbria-fornix lesions, at least under our test conditions.

296.14

THE EFFECTS OF SCOPOLAMINE AND FORNIX TRANSECTION ON ACTIVITY, EXPLORATION, EXTINCTION AND NAVIGATION IN RATS. K. Swanson* and B. Osborne (SPON: T. Root). Department of Psychology, Middlebury College, Middlebury, VT 05753.

Cholinergic manipulation disrupts many aspects of memory function and hippocampal damage results in a variety of deficits including increased activity and altered exploration, a greater tendency to exhibit previously effective behaviors and cognitive disruptions. A relationship between hippocampal lesions and cholinergic manipulation has been postulated. These experiments investigated the effects of both fornix transections and cholinergic blockade by examining three tasks; extinction of rewarded behavior, activity in an open environment, and the Morris swimming maze. Both cholinergic manipulation and fornix transection had behavioral effects across tasks. However, cholinergic manipulation was also effective in rats with fornix transection. From the results of these experiments, one would have to conclude that the two syndromes are different -- one dealing with hippocampal deficits and a broader one affecting memory and cognitive processing in general.

296.15

RHESUS MONKEY PERFORMANCE IN AN OPERANT TEST BATTERY (OTB): EFFECTS OF ATROPINE. Merle G. Paule and Michael P. Gillam.* Div. Reprod. & Dev. Tox., National Center for Toxicological Research, Jefferson, AR 72079-9502.

The involvement of the cholinergic system in a series of operant behaviors was studied using atropine sulfate (ATR, 0.01-0.56 mg/kg). Tasks included in the multiple schedule OTB and their durations were: Progressive Ratio (PR) 10 min, Conditioned Position Responding (CPR) 5 min, Incremental Repeated Acquisition (IRA) 35 min, Delayed Matching-To-Sample (DMTS) 30 min, and Temporal Response Differentiation (TRD) 20 min. These tasks are thought to engender responding associated with motivation, color and position discrimination, learning, short-term memory and attention, and time perception, respectively. Sessions lasted 50 min and began 15 min after iv ATR or saline. Endpoints monitored included response rates (rr), accuracies (acc), and percent task completed (ptc). Significant effects on IRA were noted at 0.01 mg/kg (increased rr) followed by a significant decrease in rr at ≥0.03 mg/kg. CPR ptc was significantly decreased at ≥0.03 mg/kg. DMTS acc, TRD ptc, and PR rr were all significantly decreased at ≥0.10 mg/kg. Thus, the sensitivities of these tasks to ATR were IRA>CPR>TRD=DMTS=PR. Of the 5 behaviors monitored, those thought to be associated with learning processes are the most sensitive to muscarinic cholinergic manipulation.

296.16

THE EFFECTS OF EXCITOTOXIC LESIONS OF THE MEDIAL SEPTAL NUCLEUS ON CONDITIONAL VISUAL DISCRIMINATION IN RATS. H.M.Marston* and T.W.Robbins. (SPON: Brain Research Association) Department Experimental Psychology, University of Cambridge, Cambridge, CB2 3EB. UK.

The effects of quisqualic acid lesions to the septum were examined on acquisition and performance of an instrumental conditional visual discrimination in rats.

Lesions were made by infusing either 0.5ul of the excitotoxin (0.12M) or vehicle at four sites bilaterally. *Post mortem* biochemical and histological analyses revealed reductions in the level of choline acetyltransferase activity of approximately 60% in the hippocampal formation and 50% in the cingulate cortex. Reductions in the degree of acetylcholinesterase staining limited to these structures were also observed.

The behavioural paradigm involved a discrimination between fast and slow flashing lights. Following presentation of a stimulus two levers were introduced into the operant chamber, a response had then to be made either to the left or right, depending upon the frequency of the stimulus. Once an asymptotic level of performance had been attained, the animals were challenged with manipulations of the inter-trial interval and stimulus frequency and duration. In addition the effects of scopolamine(0.19, 0.38 mg/kg) methylscopolamine(0.24 mg/kg), physostigmine(0.05, 0.1 ,0.5 mg/kg), nicotine(0.08 ,0.23 mg/kg), d-amphetamine(0.4, 0.8 mg/kg) and chlordiazepoxide(5.0, 10.0 mg/kg) were investigated.

The lesion produced significant deficits on both the acquisition and performance of the task. The deficits in performance were ameliorated by lengthening the stimulus duration. In addition, the lesions attenuated the deleterious effect of scopolamine.

These results are compared with the lack of behavioural effects of excitotoxic lesions of the cholinergic projection to the anterodorsal frontal cortex on this task. A role for the cholinergic projections of the septum in the mediation of conditional instrumental learning is suggested, possibly as a result of disruption to attentional processes.

296.17

AF102B, A NOVEL M1 AGONIST, ENHANCED LEARNING AND MEMORY WITH A LONG DURATION OF ACTION. J. Sepinwall, G.P. Vincent and L. Rumennik. Neurobiology and Obesity Research, Hoffmann-La Roche Inc., Nutley N.J. 07110.

The cognitive deficit in Alzheimer's disease is associated with a presynaptic cholinergic deficit. Traditional muscarinic agonists lack receptor specificity and exhibit short durations of action (Bartus et al., In: Treat. Devel. Strat. For Alz's. Dis. Ed.: Crook et al., 1986), suggesting the need for a selective, long acting M1 agonist. AF102B (AF) in the Morris water maze improved acquisition in C57BL/10 mice by decreasing the latency to find the hidden platform (0.1 to 1.0 mg/kg, po, 60 min pretreatment [pt]). The peak active dose, 1.0 mg/kg, enhanced acquisition at 60 min and from 3 hr to 5 days pt following a single injection. It was inactive at 90 min thus suggesting a biphasic action. In another study, an inactive low dose after acute administration (0.03 mg/kg, po, 60 min pt) enhanced acquisition after a single injection with activity ranging from 3 hrs to 5 days pt. At shorter and longer pt times it was inactive. In squirrel monkeys AF improved the accuracy of retention on a delayed match-to-sample procedure by 8 and 10% (0.03 and 0.3 mg/kg, ig, 20 min pt, respectively). Thus AF enhanced cognition in both mice and monkeys with a duration of action longer than that reported for traditional muscarinic agonists.

296.18

EFFECT OF DM9384 ON DISCRIMINATION AVOIDANCE LEARNING DEFICIT INDUCED BY IBOTENIC ACID LESION OF THE FRONTAL CORTEX IN RATS. C. Hara and N. Ogawa. Dept. of Pharmacology, Ehime Univ. Sch. of Med., Ehime-ken 791-02, Japan.

Ibotenic acid (IBA) lesions of the dorsolateral frontal cortex (DFC) and medial prefrontal cortex (MPC) having cholinergic input from the nucleus basalis of Meynert (NBM) impaired retention of discrimination avoidance learning (DAL) similar to NBM lesion (Hara, C., Soc. Neurosci. Abstr., 14(2), 1224, 1988). In this study, effects of DM9384 (Daiichi Seiyaku Co.), a new pyrrolidone derivative, and tetrahydroaminoacridine (THA), a cholinesterase inhibitor, on the impairment were examined. Male Wistar strain rats (10 weeks old) which acquired DAL were injected IBA (7.5 μg) into the DFC, MPC or NBM under pentobarbital anesthesia. The rats which revealed the impairment of retention on Day 14 after the surgery were daily administered DM9384 (10 mg/kg, p.o.) or THA (0.5 mg/kg, i.p.) for 1 week. In the results, DM9384 and THA improved the impairment. Since DM9384 activates choline acetyltransferase and improves learning deficit induced by GABA antagonists, the learning deficit induced by the frontal cortex lesion may be involved in cholinergic as well as GABAergic neural systems.

296.19

NEONATAL INTRAVENTRICULAR INJECTION OF AF64A IMPAIRS PLACE LEARNING IN THE MORRIS WATER MAZE. J.N. Armstrong, S.J.E. Murtha, and B.A. Pappas. Unit for Behavioral Medicine and Pharmacology, Department of Psychology, Carleton University, Ottawa, Canada K1S 5B6.

Previous studies have demonstrated that intraventricular (i.v.) injections of AF64A into the adult rat impairs acquisition of place learning in the Morris Water Maze. In the present experiment, bilateral i.v. injection of 2 nmol/ul/side (but not 0.5 or 0.1 nmol) AF64A into Long-Evans rats 48-72 h after birth impaired acquisition of place learning when these animals were tested at 35 days of age. No group differences were found for cued navigation, suggesting that the impairment was specific to their ability to utilize external spatial cues rather than a primary performance deficit. The 2 nmol-injected rats were also more active than controls during exposure to the elevated plus-maze. However, they displayed avoidance of the open arm suggesting normal ability to process spatial cues. Neonatal i.v. injection of AF64A did not affect cortical or hippocampal choline acetyltransferase activity. The behavioral effects probably reflect the non-specific pathology caused by this drug.

TRANSMITTERS IN INVERTEBRATES IV

297.1

FMRFa INHIBITS THE AFTERDISCHARGE IN THE BAG CELL NEURONS OF APLYSIA. T.E.Fisher and L.K.Kaczmarek. Dept. of Pharmacology, Yale University Sch. Med., New Haven, CT 06510.

The bag cell neurons of Aplysia can be made to undergo a long-lasting afterdischarge of repetitive firing (mean of 25 minutes) by a brief electrical stimulation of an input from the head ganglia. An elevation of intracellular cyclic AMP contributes to this change in excitability. In this series of experiments we have examined the effects of phe-met-arg-phe-amide (FMRFa), a putative neurotransmitter in Aplysia, on the afterdischarge. Bath application of 10μM FMRFa rapidly suppresses firing during an afterdischarge (usually within 2-3 minutes) without otherwise altering membrane potential. This effect is reversible; when FMRFa is removed from the bath, the afterdischarge re-initiates either spontaneously or after brief electrical stimulation. Preincubation with FMRFa usually does not prevent an afterdischarge but results in a greatly reduced duration (1-5 minutes). Inhibition by FMRFa was not prevented by 8-parachlorophenylthio-cyclic AMP (pcpt-cyclic AMP; 500μM), forskolin/theophylline (50μM/1 mM) or tetraethylammonium ions (100mM). Experiments in single bag cell neurons in primary culture have identified two responses to FMRFa. Application of FMRFa elicits a rapid hyperpolarization of up to 20mV, likely due to the activation of a potassium current. This effect is prevented by pcpt-cyclic AMP, which may explain why it is not seen in afterdischarging cells. Under voltage clamp FMRFa also induces a modest decrease (10-30%) in voltage activated calcium currents. This decrease may be at least partly responsible for the suppression of the afterdischarge by FMRFa.

297.2

ACTIONS OF PEDAL PEPTIDE ON NEURON L5 IN *APLYSIA*. W. L. Pearson* & P. E. Lloyd, (SPON: R. McCrea) Comm. on Neurobiol. & Dept. of Pharmacol. & Physiol. Sci., Univ. of Chicago, Chicago, IL 60637.

Pedal peptide (Pep) is a neuropeptide synthesized predominantly in the pedal ganglia of *Aplysia*. Pep-immunoreactive varicosities have been observed throughout the periphery and the central nervous system, particularly on the left upper quadrant (LUQ) abdominal neurons. We have studied the effects of Pep on these cells, with emphasis on cell L5, using voltage clamp and current clamp techniques.

Under current clamp, bath application of Pep (10 μM) depolarizes LUQ cells as much as 20 mV and alters their firing pattern, eliciting tonic firing or bursting with shortened interburst intervals and decreased after-hyperpolarizations. Under voltage clamp, Pep evokes a net inward current associated with a conductance decrease. As measured at the peak of the net inward current, current-voltage (I-V) curves generated by digital subtraction of current records show that bath applied Pep in normal artificial sea water (ASW) produces an inward current that decreases near the potassium equilibrium potential, E_K. In low Ca^{++} and Ca^{++}-free, Co^{++}-containing ASW, this inward current is reduced. Altering the K^+ concentration of the bath shifts the I-V curve along the voltage axis in accordance with the shift in E_K. The Pep-evoked current can also be decreased by adding TEA to the bath or by filling the cell with Cs^+. These results suggest that Pep depolarizes LUQ neurons, particularly L5, by decreasing at least one K^+ conductance that is normally present in these cells at their resting potential or within their pacemaking voltage range. Supported by NS 23569.

297.3

INVOLVEMENT OF PEP-CONTAINING NEURONS IN LOCOMOTION IN *APLYSIA*. J. D. Hall* and P. E. Lloyd. (SPON: L. Minor). Dept. Pharm. Physiol. Sci. & Comm. on Neurobiol. Univ. Chicago, Chicago, IL 60637.

Pedal peptide (Pep) is a recently characterized very abundant neuropeptide in Aplysia. It is found throughout the CNS but is particularly concentrated in a band of large neurons in the pedal ganglia. Pep is transported in large amounts via peripheral nerves from the pedal ganglia to the foot. When exogenous Pep is applied to the foot via a cannulated artery, contractions of the foot produced by brief stimulation of a pedal nerve are modulated. Specifically, the amplitude and relaxation rate of each contraction increases during Pep application. These effects are seen with a threshold of ~10^{-8} M Pep.

A split-foot preparation was used to study the activity of Pep-neurons during locomotion and other behaviors. Pep-neurons have a low (~1 spike/sec) tonic activity when preparations are quiescent. During spontaneous or stimulus-induced locomotion, Pep-neurons fire more rapidly (3-4 spikes/sec), and in bursts which often coincide with the relaxation of each step. Also, Pep-neurons are strongly inhibited during sustained contractions of the foot.

The identity of Pep-neurons was confirmed after each experiment by microdissection and RIA. Pep-neurons in the pedal ganglia range from 100-200 μm in diameter (60-150 g animals) and contain from 0.8 to 3.8 pmol of Pep-like immunoreactivity. Since Pep modulates foot contractions and Pep-neurons are activated during locomotion, we propose that Pep has a modulatory role in peripheral regulation of foot muscle during locomotion. Supported by NIH NS23569.

297.4

IDENTIFICATION AND CHARACTERIZATION OF PUTATIVE DOPAMINE-CONTAINING NEURONS IMPLICATED IN THE CONTROL OF FEEDING BEHAVIOR IN *APLYSIA*. S. C. Rosen*, T. Teyke*, K. R. Weiss and I. Kupfermann. Cntr. Neurobiol. & Behav., Columbia Univ. and NYS Psychiatric. Inst. New York, N.Y. 10032

Application of 10^{-3} M dopamine (DA) to preparations consisting of isolated cerebral and buccal ganglia elicits a buccal motor program (BMP). In order to determine whether the DA effect can be accounted for by the actions of DA neurons, candidate neurons in the buccal and cerebral ganglia were impaled with electrodes for intracellular recording and stimulation. The neurons were subsequently marked with rhodamine and treated by the FaGlu method to reveal possible dopamine-like (DA-I) histofluorescence. A pair of bilaterally symmetrical DA-I buccal cells and a pair of DA-I cerebral cells were found to drive several cycles of a BMP. The buccal cells make direct connections to buccal motor neurons (e.g. B15s) and their activity is recruited into the BMP driven by the cerebral command element CBI-2. The cerebral DA-I cells are the previously identified CBI-1 cerebral-buccal interneurons. These cells are excited by mechanoafferent neurons in the cerebral ganglion (ICBMs) and provide direct inputs to numerous neurons in the buccal ganglion. The CBI-1s were found to receive strong excitatory input associated with movements of the lips or buccal mass. When the cerebral and buccal ganglia were isolated in separate chambers, a robust BMP was only elicited by application of the DA to the cerebral ganglion, and when the buccal mass was kept intact the evoked movements resembled swallowing. These data suggest that dopaminergic afferent input to the cerebral ganglion may elicit swallowing-like behavior, and that the dopaminergic CBI-1s may convey proprioceptive signals that modulate ingestive responses.

297.5

GLUTAMATE AS A PUTATIVE NEUROTRANSMITTER OF IDENTIFIED CEREBRAL GANGLION NEURONS OF THE SNAIL HELISOMA. R.L. Ridgway, J.E. Richmond, K. McKenney, K. Lukowiak, and A.G.M. Bulloch. Dept. of Medical Physiology, Univ. of Calgary, Calgary, Alberta, Canada T2N 4N1.

Glutamate has been shown to play a role in promoting neurite outgrowth of adult neurons of Helisoma trivolvis (Bulloch, J. Neurosci. Res., in press). The mechanism underlying this effect is unknown. We have sought here to determine if specific neurons of Helisoma use glutamate as a neurotransmitter. Autoradiographic visualization of ^{3}H-glutamate uptake in the CNS of Helisoma reveals labeled neurons in all central ganglia except the pedals. Of note is the labeling of two neurosecretory cell types of the cerebral ganglia: 1) the Mediodorsal Cells (MDCs), and 2) the Canopy Cells (CCs). Initial experiments using both HPLC analysis and antibodies specific to glutamate confirm that glutamate is localized in these cells. In addition, since these two cell types contain a somatostatin-like substance having putative neurotrophic properties (Bulloch, Brain Res. 412:6-17, 1987), we examined whether glutamate might feedback onto these cells to change their activity state. When glutamate is bath applied (at 300μM) or pressure-ejected (at 0.5M) onto MDCs of isolated ganglia, a 20-25 mV depolarization from resting potential is observed, implying that glutamate receptors are present. We conclude that glutamate is present in these identified cells and may function as an autoactive neurotransmitter.

297.6

ANALYSIS OF OCTOPAMINE-INDUCED INHIBITION IN AN IDENTIFIED EFFECTOR NEURON OF HELISOMA. F.H.Bahls Dept. of Physiology and Biophysics and Div. of Neurology,School of Medicine,University of Washington,Seattle, WA 98195

Patterned neuronal activity in the buccal ganglion of Helisoma can be modulated by a variety of neuroactive substances. Most recent investigations of neuromodulation in this system have focused on the effects of neurotransmitters on the interneuronal subunits responsible for generating patterned output (eg. Murphy,et al, Soc Neurosci Abs, 1988). However, many neurotransmitters also have direct modulatory actions on the effector neurons of the buccal ganglion. I have examined the response of an identified effector neuron (B5) to octopamine. Iontophoresis or bath perfusion with octopamine produces a sustained 15-20mV hyperpolarization, associated with an increase in input conductance. The minimal concentration of octopamine required to produce a detectable response is 10-7M. Prolonged exposure to octopamine produces no detectable desensitization. The reversal potential in normal saline is -84 (+/-15)mV. Doubling external potassium shifts the reversal potential to -69(+/-18)mV, while halving external potassium shifts the reversal potential to -102(+/-12)mV. The hyperpolarization is decreased in the presence of low concentrations of 4-AP (50μM). These results suggest that the hyperpolarization is due to an increase in potassium conductance. The response of B5 to octopamine is similar to that previously described to glutamate (Jones,et al, Brain Res 437:56-68,1987). The interaction between the responses produced by octopamine, glutamate and other neurotransmitters in B5 is currently under investigation. (Supported by NIH NS01167-01A1)

297.7

AN IDENTIFIED "DOPAMINERGIC" BUCCAL INTERNEURON ACTIVATES PATTERNED MOTOR ACTIVITY IN HELISOMA. E.M. McLean*, B. Behjatnia* and A.D. Murphy (SPON: M. Binder) Dept. of Biol. Sci., Univ. of Illinois at Chicago, Chicago, Illinois 60607.

The catecholamines, dopamine and octopamine, modulate the feeding central pattern generator (CPG) of Helisoma. The CPG consists of three subunits (S1-S3) which are independent interneuronal oscillators. Three octopamine immuno-reactive neurons (antibody provided by E. Kravitz and D. Valentine) were localized near the buccal commissure, in a similar position to the S3 interneuron, N3a. Approximately 50 "dopaminergic" neurons were localized in each of the paired buccal ganglia with a glutaraldehyde/formaldehyde histochemical technique. In some systems this technique produces yellow fluorescence in dopaminergic neurons and green fluorescence in serotonergic neurons. However, in Helisoma, this technique causes identified dopaminergic neurons to fluoresce but identified serotonergic neurons do not. The dopaminergic neuron N1a has been identified by combining electrophysiological recordings with co-staining of the soma by injection of the procion dye Reactive Red 4 (Sigma), and histochemical staining of the same preparation. Depolarization of neuron N1a or bath application of 10^{-6} M dopamine initiates the standard pattern of rhythmic activity (S1-S2-S3) in buccal neurons. Intracellular Lucifer Yellow injection revealed that N1a is a true buccal interneuron with a single axon that projects to the contralateral buccal ganglion and no processes occur in buccal nerve roots.
(Supported by NIH Grant 1 RO1 NS26145-01 to A.D.M.)

297.8

MODULATION OF CROP AND ESOPHAGEAL ACTIVITY BY THE FEEDING MOTOR PROGRAM, SCPS AND BUCCAL NEURON B1 IN THE TERRESTRIAL SLUG, Limax maximus. I.G. Welsford and D. J. Prior. Department of Pharmacology, Univ. of Pennsylvania School of Medicine, Philadelphia 19104-6084 and Dept. of Biological Sciences Northern Arizona Univ.,Flagstaff 86011-5640.

Activation of the feeding motor program (FMP) in semi-intact preparations of the terrestrial slug, Limax maximus, initiated rapid, high frequency contractions of the crop and esophagus. The FMP-induced increase in esophageal activity was mediated via the gastric nerves, while those of the crop were mediated via nerves N11 and N9. Application of SCP_A at concentrations of at least 10^{-7} M and SCP_B at concentrations of at least 10^{-9} M, similarly initiated rapid, high frequency crop and esophageal contractions. Unilateral stimulation of buccal neuron B1, which contains SCP-like immunoreactive material, initiated rapid high amplitude contractions of crop (but not esophagus) in a frequency-dependent manner (threshold 3-5 Hz). B1-induced increases in crop contractile activity were mediated via nerve N9. These data suggest that B1 may mediate FMP-induced alterations in crop activity in L. maximus, possibly via release of SCPs. Supported by Sigma Xi (IGW).

297.9

A PHYSIOLOGICAL AND IMMUNOCYTOCHEMICAL STUDY OF INNERVATION OF THE PENIAL COMPLEX IN THE SNAIL MELAMPUS BIDENTATUS. S.B. Moffett, Marine Biomedical Institute, Galveston, TX 77550.

Two clusters of neurons innervate the penial complex (sheath, penis, efferent duct and retractor muscles): one cluster is in the right cerebral ganglion and the other is in the right pedal ganglion. The transmitters utilized by cerebral neurons are not known, but serotonin-like and CARP (catch relaxing peptide)-like immunofluorescences are colocalized in the pedal neurons. A muscle preparation consisting of efferent duct, penis and sheath responds to brief stimulation of the penial nerve with twitch contractions. These increase in amplitude with increasing voltage until a plateau is reached and then, as the smaller pedal neurons are recruited, decrease to a stable lower plateau. Stimulation of the cerebropedal connective activates pedal neurons alone, producing a transient contraction sometimes followed by reduction in resting tension. The penial complex is also innervated by axons with buccalin-like and FMRFamide-like immunofluorescence and by peripheral neurons that exhibit CARP-like immunoreactivity. The muscle preparation responds to acetylcholine or FMRFamide with a prolonged contraction which is relaxed by serotonin. Effects of the other neuropeptides are under investigation. (Supported by NIH NS22896 to S.B.M. and NS27314 to J.E. Blankenship)

297.10

THE DISTRIBUTION OF SCP PEPTIDES IN MOLLUSCS. D.A. Price, D.M. Reed*, K.E. Doble*, T.D. Lee*,† and M.J. Greenberg. Whitney Lab, St. Augustine, FL 32086. †Div. of Immunol., Beckman Res. Inst. of the City of Hope, Duarte, CA 91010.

We have purified SCPs from various molluscan species using HPLC and RIA, and characterized them by FAB mass spectroscopy and, in some cases, micro-sequencing. We find that the SCPs are a fairly diverse group of peptides. For example, we found and sequenced an SCP analog from a clam (AMSFYFPRMamide) which had only its C-terminal tetrapeptide in common with the known SCPs. We have been unable to find any SCP-like immunoreactivity in chitons, so their SCPs may not react with our antisera. Within the gastropods all of the SCPs we have sequenced so far have a common heptapeptide core YLAFPRMamide, except for the mesogastropod Littorina irrorata which has an Ile for Leu substitution (SQPYIAFPRMamide). We found NYLAFPRMamide in one neogastropod, Thais haemastoma, but not in another, Busycon contrarium. One SCP, SGYLAFPRMamide, appears to be common to all pulmonates, but others are restricted to certain species. For example, SCP_B itself (MNYLAFPRMamide, identical to an SCP from Aplysia) is present in some (e.g., Helix, Siphonaria), but not others. Lymnaea has an 1120 dalton peptide with a blocked N-terminal which is absent from both Physa and Helisoma, and SQGYLAFPRMamide is found in both Biomphalaria glabrata and Helisoma. We are just starting to investigate the physiological significance of these structural variations.

297.11

EFFERENT NEUROTRANSMITTERS ONTO THE HAIR CELLS IN THE STATOCYST OF THE SQUID, *ALLOTEUTHIS SUBULATA*. R. Williamson.* (SPON: European Neuroscience Association) The Marine Laboratory, Citadel Hill, Plymouth PL1 2PB, U.K.

The squid statocyst has a sophisticated angular acceleration receptor system containing a strip of polarised secondary hair cells which make synaptic contact with underlying afferent neurones. The hair cells receive a very large efferent innervation which has been shown by histological methods to consist of cholinergic and catecholaminergic endings. Experiments were undertaken to establish whether the efferents had an effect on the statocyst output and if cholinergic and catecholaminergic agents were involved.

Electrical stimulation of the efferents was found to be able to decrease or increase the level of activity from the statocyst afferents. Bath application of gallamine could block the efferent induced decrease in afferent activity while phentolamine or haloperidol could block the efferent induced increase in activity. The spontaneous afferent activity could be decreased by bath applied acetylcholine and increased by dopamine or noradrenaline. Intracellular recordings from the secondary hair cells have indicated that they receive both inhibitory and excitatory efferent inputs and that, as with the afferent activity, the inhibitory input can be blocked by gallamine and the excitatory input by haloperidol.

These results support the view that the hair cells of the squid statocyst receive an efferent inhibitory input mediated by acetylcholine and an excitatory input which is catecholaminergic.

R.W. is a Wellcome Trust Senior Fellow.

297.12

LOCALIZATION AND CHARACTERIZATION OF 125I-α-BUNGAROTOXIN BINDING SITES IN COCKROACH BRAIN. G.L. Orr, N. Orr, R.M. Hollingworth*. Pesticide Res. Ctr., Mich. St. U., E. Lansing, MI 48824

Acetylcholine is an important neurotransmitter in the insect central nervous system and functional nicotinic acetylcholine receptors (nAChRs) in these tissues can be readily studied using α-bungarotoxin (α-BGT) as a receptor ligand. Nicotinic AChRs in cockroach brain membranes were characterized using ^{125}I-BGT in a filter binding assay and localized autoradiographically in 8 µm brain sections. Binding was saturable at α-BGT concentrations above 2 nM and Scatchard analysis of this data yields a linear plot with a K_d of 1.08 nM and a B_{max} of 8926 fmoles/mg protein with a Hill coefficient of 0.9. Displacement studies confirm the nicotinic nature of this site and demonstrate the high affinity of the Delphinium alkaloid, methyllycaconitine, and the antihelmintics, morantel and pyrantel. Kappa-bungarotoxin (k-BGT) displaces α-BGT in a biphasic manner and analyses of these data indicates that k-BGT is displacing ^{125}I-BGT from two sites which bind α-BGT with equal affinity. Autoradiographic studies show binding to be restricted mainly to neuropile regions of the brain and to be displaced by compounds showing high affinity in the binding assay.

297.13

CHARACTERIZATION OF A POTENT AGONIST OF OCTOPAMINE-SENSITIVE ADENYLATE CYCLASE. N. Orr, G.L. Orr, R.M. Hollingworth*. Dept. Zoology/Neurosci. and Pesticide Res. Ctr., Michigan St. Univ., E. Lansing, MI, 48824.

Octopamine (OA), a major biogenic amine in invertebtates, has been shown to function as a neuromodulator, neurotransmitter and a neurohormone. Many of these actions are mediated through the activation of octopamine-sensitive adenylate cyclase (OA-S-AC). The clonidine analogue, XAMI, was evaluated for effects on OA-S-AC in crude membrane preparations of neural and non-neural tissues in the cockroach, Periplaneta americana, and the tobacco hornworm, Manduca sexta. In cockroach nerve cord, XAMI was found to be a partial agonist with a V_{max} 87.9% of OA and a K_a= 36 nM which was 140 times less that that of OA. Additivity studies suggest that at a maximally stimulating concentration of 1 µM, XAMI interacts primarily with the OA-S-AC. The antagonist profile for XAMI mimics that of OA with mianserin being the best antagonist, followed by the α-adrenergic antagonist phentolamine. The β-adrenergic antagonist propranolol was ineffective. These data suggest that XAMI and related high-affinity analogues may be good ligands for studying the OA receptor and could have potential pesticidal activities with low vertebrate toxicity.

297.14

ISOLATION OF A PUTATIVE OCTOPAMINE RECEPTOR PROTEIN. James A. Nathanson and Edward J. Hunnicutt. Dept. of Neurology, Harvard Medical School, and Neuropharmacology Lab., Massachusetts General Hospital, Boston, MA 02114.

Despite recent advances in the isolation and cloning of vertebrate neurotransmitter receptors, little progress has been made in the isolation of invertebrate amine neurotransmitter receptor proteins. In the case of octopamine (OA) (thought by some to be the invertebrate analog to norepinephrine and epinephrine) progress has been hampered by the lack of high affinity or irreversible OA ligands. We now describe the isolation of a putative OA receptor protein from the firefly, Photinus pyralis using NC-5Z, a novel photoaffinity ligand for OA receptors. Under reversible conditions, NC-5Z stimulates light emission from intact firefly tails and, in membranes, activates OA-sensitive adenylate cyclase with a potency approximately 50-100 fold that of OA. Activation is non-additive with that due to OA and is blocked specifically by OA antagonists. When photolyzed with UV, NC-5Z irreversibly activates light organ adenylate cyclase, and such activation can be prevented by preincubation with an excess of OA. SDS-polyacrylamide electrophoresis and autoradiography of NC-5Z-labeled membranes reveals a 72±2 kD glycoprotein, the labeling of which can be displaced by mianserin and other OA antagonists. The identification of this putative OA receptor proteins may be useful in molecular biological studies of OA receptors.

297.15

5,7-DIHYDROXYTRYPTAMINE AFFECTS FEEDING BEHAVIOUR, SEROTONIN-LIKE IMMUNOREACTIVITY AND SEROTONIN CONTENT OF SELECTED TISSUES IN *RHODNIUS PROLIXUS*. H. COOK* and I. ORCHARD. Dept. of Zoology, Univ. of Toronto, Toronto, ON, Canada M5S 1A1.

5,7-dihydroxytryptamine (5,7-DHT), a neurotoxic analogue of serotonin, selectively lesions serotonergic neurons. In *Rhodnius prolixus*, 5,7-DHT treatment (1 µl of 10^{-3} M injected 24 hr previously) led to the enhancement of serotonin-like immunoreactive staining in the cell bodies and axons of 5 dorsal, unpaired, median (DUM) neurons in the mesothoracic ganglionic mass (MTGM). These neurons appear to be the major contributers to the serotonin-like immunoreactive neurohaemal areas of the ten abdominal nerves. The toxin-induced enhancement of immunoreactivity in the cell bodies and axons was accompanied by a severe depletion in the staining intensity of the neurohaemal areas. The serotonin-like immunoreactivity covering the salivary gland ducts also suffered a severe decrease in staining intensity. 5,7-DHT treatment resulted in a significant decrease in the serotonin content of the salivary glands and abdominal nerves, as measured by HPLC. No significant changes were noted in the brain, subesophageal ganglion, prothoracic ganglion or MTGM. In additon, bugs treated with 5,7-DHT ingested a blood meal which was significantly smaller than that of controls. These findings suggest that the depletion of peripheral serotonergic areas impairs feeding in *Rhodnius*.

297.16

EVIDENCE FOR NICOTINIC CHOLINERGIC AND FMRF-AMIDERGIC RECEPTORS IN BODY WALL MUSCULATURE OF A NEMERTINE WORM, *CEREBRATULUS LACTEUS*. W. R. Kem. Department of Pharmacology and Therapeutics, University of Florida College of Medicine, Gainesville, FL 32610.

Nemertines are a phylum of unsegmented carnivorous worms thought to have evolved from a turbellarian ancestor. The responsiveness of *Cerebratulus* longitudinal body wall muscle strips lacking nerve plexus to cholinergic compounds and various neuropeptides was investigated. ACh, nicotine, and several other nicotinoid compounds contracted the muscle, whereas four muscarinic agonists were without effect. Various nicotinic receptor antagonists, except for α-bungarotoxin, inhibited carbachol contractures, while muscarinic antagonists were inactive. The muscle was not irreversibly affected by either bromoacetylcholine or N-maleimidophenyltrimethylammonium after exposure to disulfide bond reducing agents. Histrionicotoxin did not block the carbachol response.

The contractural activity of a variety of FMRFa analogs was also tested. Although extensions at the N-terminus were tolerated without significant loss of activity, alteration of the RFa C-terminus always greatly reduced activity relative to FMRFa. Only one analog, FIRFamide, was more (3x) active than FMRFa. In collaboration with D. Price, two HPLC fractions immunoreactive with FMRFa antibody, but possessing different retention times, were detected in *Cerebratulus* extracts. This initial investigation indicates the presence of nicotinic cholinergic and FMRFa-like peptide receptors in longitudinal muscles of nemertines, a lower invertebrate phylum.

297.17

SEROTONIN REPRODUCES ASPECTS OF THE ALTERED BEHAVIOR INDUCED BY THE PARASITE *POLYMORPHUS PARADOXUS* (ACANTHOCEPHALA) IN *GAMMARUS LACUSTRIS* (CRUSTACEA). S.M. Helluy* and J.C. Holmes* (SPON: S. Wiener). Dept. of Zoology, Univ. of Alberta, Edmonton, Alberta, Canada, T6G 2E9.

Some larval helminths alter the behavior of their invertebrate intermediate hosts by changing the responses of the hosts to environmental stimuli. *P. paradoxus* induces in its intermediate host *G. lacustris* a shift in habitat towards zones of higher illumination and an altered escape behavior. Upon mechanical disturbance infected gammarids escape towards a light source, then cling firmly to any material and remain immobile in a flexed posture from minutes to hours. Uninfected gammarids escape away from a light source and do not cling (Bethel, W. M., and Holmes, J. C., *J. Parasitol.* 59:945, 1973).

Uninfected gammarids injected with serotonin (1 to 20 µg/50 mg) (but not other biogenic amines or GABA) responded to mechanical stimulation by clinging, but the response lasted for a shorter time than in infected gammarids. Octopamine (5, 10 µg/50 mg) suppressed the clinging response in infected gammarids for several hours. Also, serotonin elicited a photopositive behavior in uninfected gammarids in the hour following treatment, overriding the normal photic behavior of the animals. Octopamine did not affect the photic behavior of infected gammarids. The opposite actions of serotonin and octopamine on the clinging behavior of gammarids shared similarities with those obtained on the posture of lobsters and crayfish (Livingstone, M. S., et al., *Science*, 208:76, 1980), and on the escape behavior of crayfish (Glanzman, D. L., and Krasne, F. B., *J. Neurosci.*, 11:2263, 1983). (Supported by NSERC A-1444)

297.18

OBSERVATIONS ON THE MECHANISM OF THE STIFFENING ACTION OF ACh ON THE LIGAMENT OF THE SEA URCHIN SPINE. M. Morales*, J. Del Castillo* & D. S. Smith*. (Spon. C. Zuazaga). Institute of Neurobiol., UPR-MSC, 201 Blvd. del Valle, San Juan, Puerto Rico 00901 and Dept. of Zoology, U. of Oxford, Oxford OX1 3PS, U.K.

The spine-test articulation of the sea urchin is surrounded by a capsule or ligament which, in addition to collagen bundles, contains neurosecretory processes and thin muscle fibers. This structure exhibits rapid and reversible changes in viscosity under nervous control. Acetylcholine (ACh) (1-10 uM) stiffens the ligament rapidly. We tested the effect of ACh on: a) ligaments treated with saponin (0.1 mg/ml, 1 hour); b) ligaments immersed in sea water made 3 X hypertonic by the addition of sucrose, c) ligaments treated with Na azide and dinitrophenol [2 mM] and d) on frozen and thawed ligaments. As none of these treatments blocked the ACh effect, we concluded this is not likely to involve either cells or cellular membranes.

Calcium proved to be essential for the effect of ACh. Ca^{2+} ions in the medium could be replaced by Sr^{2+}, but the ACh effect was blocked by Mg, Ba, Co, Cd and La. In addition, the Ca channel blockers verapamil, bepridil and nifedipin [2 mM] prevented the effect of ACh. (Supported by NIH Grants No. NS-07464 and NS-14938).

PEPTIDES: ANATOMICAL LOCALIZATION III

298.1

ULTRASTRUCTURAL LOCALIZATION OF ENKEPHALINASE (E.C.3-4-24-11) IN RAT NEOSTRIATUM. D. Marcel*, H. Pollard*, P. Verroust*, J.C. Schwartz* and A. Beaudet (SPON: B.E. Jones). Neuroanatomy Laboratory, Montreal Neurological Institute, Montreal, Quebec, H3A 2B4, Canada.

The peptide degrading enzyme "enkephalinase" (enkase) was localized by light and electron microscopic immunocytochemistry in sections of rat neostriatum using a specific monoclonal antibody. The antibody was visualized using either radioautographic or PAP techniques. In the light microscope, both methods revealed a heterogeneous immunolabeling pattern characterized by the presence of dense patches prominent against a lighter matrix. These patches were concentrated in the dorsal aspect of the neostriatum and matched patches of mu opioid receptors detected by radioautography in adjacent sections. In the electron microscope, both techniques showed an ubiquitous distribution of the enzyme along neuronal plasma membranes. Immunolabeling was found with comparable frequencies along perikarya, dendrites, myelinated and unmyelinated axons and axon terminals and was most intense at the interface between two labeled elements. In rare instances, oligodendrocytes and thin glial leaflets also exhibited weak surface immunoreactivity. No obvious concentration of immunoreactive material was apparent at the level of synaptic junctions. The similarity between light and electron microscopic distributions of enkase and mu opioid receptor labeling supports a role of enkase in the inactivation of opioid peptides.

298.2

THE COEXISTENCE OF METHIONINE ENKEPHALIN- AND TYROSINE HYDROXYLASE-LIKE IMMUNOREACTIVITY IN COERULOSPINAL COMPLEX NEURONS: A THREE-COLOR IMMUNOFLUORESCENCE STUDY. H. Zhuo*, S.J. Fung, V.K. Reddy* and C.D. Barnes. Dept. of VCAPP, Washington State University, Pullman, WA 99164-6520

Previous studies have revealed the presence of spinally projecting methionine enkephalin- and tyrosine hydroxylase-containing neurons in the dorsolateral pontine tegmentum of the cat. Using a combined retrograde fast blue and immunofluorescence technique, the present study was designed to reveal the coexistence of methionine enkephalin and tyrosine hydroxylase in coerulospinal complex neurons.

In anesthetized cats, 5% fast blue was injected bilaterally into the lumbar spinal cord. After a two-week survival period, the animal received a colchicine injection intraventricularly 24 hr before sacrifice. The pontine brainstem was sectioned coronally at 20 um, and sections were first incubated with rabbit anti-methionine enkephalin and mouse anti-tyrosine hydroxylase antisera, and then reacted with anti-mouse IgG conjugated FITC and anti-rabbit IgG conjugated rhodamine, using the method of Staines et al. (J. Histochem. Cytochem. 36:145-151, 1988).

Under fluorescence microscopy, within one section, three-color fluorescence-labeled neurons were seen in the nucleus locus coeruleus, nucleus subcoeruleus, Kolliker-Fuse nucleus, and the medial and lateral parabrachial areas. The distribution of variously (triple-, double- and single-) labeled neurons of the dorsolateral pontine tegmentum were determined and morphometric measurements were performed. (Supported by NIH grant NS24388).

298.3

LOCALIZATION OF NEURONAL mRNA BY IN SITU HYBRIDIZATION USING A NON-RADIOACTIVE DETECTION METHOD. D.S. Grega, T.J. Cavanagh*, S. Grimme*, R. Martin, M. Lewis, E. Robbins* and F. Baldino, Jr. Boehringer Mannheim Corp., Indianapolis, IN. and Cephalon, Inc., West Chester, PA.

In situ hybridization (ISH) is a powerful tool for localizing mRNA's in specific cells. This is extremely valuable in studying the nervous system, where the presence of a specific cellular mRNA is determined in the context of the cell's unique location and function. This report describes a non-radioactive ELISA-based method for detecting digoxigenin-labeled oligonucleotide probes. Probe labeling procedures, determination of residue tail length, application of several probes to neuronal mRNA's in rat brain sections and a comparison with immunohistochemical detection for the end product is presented.

Oligonucleotide probes (20 to 40 base length) were 3'-end labeled with digoxigenin-labeled deoxyuridine triphosphate (Dig-dUTP) using terminal transferase. Tailing procedure modifications and tail length were assessed. Tail length varied between two and four Dig-labeled residues. Dig-labeled oligonucleotide probes were used to localize mRNA for arginine vasopressin, prosomatostatin, tyrosine hydroxylase and POMC in cryostat sections from appropriate brain regions using an alkaline phosphatase antibody-conjugate and subsequent color reaction. Immunohistochemical (IHC) detection of enzyme or neuropeptide was compared with ISH for the respective message.

Advantages of non-radioactive vs. radioactive detection methods for ISH include: rapid localization of mRNA's (days vs. weeks), increased cellular resolution, increased safety and ready comparison with conventional IHC.

298.4

VASOPRESSIN IMMUNOREACTIVITY IN THE BRAINS OF SEVERAL HAMSTER GENERA. T.P. Goodness, G.J. De Vries, C.F. Ferris, Neuroscience and Behavior, Univ. of Massachusetts, Amherst, Dept. of Physiology, Univ. of Massachusetts, Worcester.

Previous studies have shown similar patterns of vasopressin immunoreactivity (VP-IR) in the brains of rats, mice, gerbils, guinea pigs, and European hamsters. In all of these species, VP-containing neurons can be found in the paraventricular, supraoptic, suprachiasmatic, and medial amygdaloid nuclei, and in the bed nucleus of the stria terminalis . The VP content of the latter two nuclei is steroid dependent. In the rat, VP content decreases following castration and in European hamsters, VP content varies seasonally with testosterone levels. We compared the pattern of VP-IR in the Chinese hamsters (Cricetelus griseus), Armenian hamsters (Cricetelus migratorius), Djungarian hamster (Phodopus sungorus), and Syrian hamsters (Mesocricetus auratus). Although the pattern of VP-IR in the brains of Chinese, Armenian, and Djungarians was similar to that of other rodents, the steroid sensitive neurons were absent in the Syrian hamster. (Supported by NSF grant BNS-8809799 to GJD and NIH grant NS-23557 to CFF.)

298.5

COMPUTER-AIDED RECONSTRUCTION OF THE HYPOTHALAMO-NEUROHYPOPHYSIAL SYSTEM IN THE GOLDEN HAMSTER: EVIDENCE OF POPULATIONS OF NON-PROJECTING VASOPRESSIN MAGNOCELLULAR NEURONS AS REVEALED BY DOUBLE-STAINING. P.D. Mahoney*, E.T. Koh*, and C.F. Ferris. (Spon. A. Tischler) Dept. of Physiology, Univ. of Massachusetts Medical Center, Worcester, MA 01655.

In this study, we have mapped the hypothalamo-neurohypophysial system of the male Golden hamster, generating a 3-dimensional computer representation of the system in both a rotatable wire-frame model and a shaded and smoothed solid model. Three hamsters were micro-injected with HRP into the neurohypophysis, and 24 h later the hamsters were anesthetized and perfused, and their brains removed and sectioned (50 µm). The sections were first processed for HRP with TMB followed by nickel-conjugated DAB, and subsequently stained for vasopressin (AVP) using PAP with DAB. These double-stained sections were mapped using the PC-AT based software program Cellmate® (R&M Biometrics, Nashville, Tennessee). The average number of retrogradely labeled neurons, i.e. those projecting to the neurohypophysis, was 5773 ± 533. However, an average of 1110 ± 363 cells were immunoreactive to AVP but devoid of HRP. These neurons that did not project to the neurohypophysis were localized primarily in the medial supraoptic nucleus, paraventricular nucleus, and the nucleus circularis at the level of the anterior hypothalamus approximately 1000 µm caudal to the caudal border of the anterior commissure. Based on the distribution and localization of the non-projecting populations, it is speculated that these neurons may provide neurotransmitter for AVP-dependent flank marking in the Golden hamster. *This work was supported by NIH grant #NS23657.*

298.6

THE EXPRESSION OF MUTANT VASOPRESSIN PEPTIDE INCREASES THROUGHOUT LIFE IN BRATTLEBORO RATS. H.A. Griffioen*, F.W. van Leeuwen*, E.M. v.d. Beek* and R. Ivell**. (SPON: ENA) *Neth. Inst. for Brain Res., Amsterdam, The Netherlands, **Inst. for Horm. and Fert. Res., Hamburg, F.R.G.

The homozygous diabetes insipidus (HO-DI) Brattleboro rat is unable to synthetize a normal vasopressin (VP) precursor. A deletion in exon B of the VP gene results in a shift of the VP-mRNA reading-frame. As a consequence, a different non-glycosalated C-terminal mutant peptide is formed, which cannot be packaged in granules. An antiserum raised against the last fourteen amino acids of the mutant pwptide (CP-14) stained the supraoptic nucleus (SON) (e.g. Guldenaar et al., Cell Tiss. Res. 244: 431, 1986).

In the present study life-span changes in CP-14 immunoreactivity in the SON of male and female HO-DI rats are reported. Contrary to the report of Guldenaar et al., CP-14 immunoreactivity was also found in the PVN. During life the amount of CP-14 immunoreactivity in the SON slowly increases. In 16-day- and 6-week-old rats hardly any CP-14 immunoreactivity could be seen. This immunoreactivity slowly increased in 12- and 30-week-old HO-DI rats. In 44-, 52- and 83-week-old HO-DI rats a very intense staining is present. No sex differences were observed. The results indicate that during life the expression of the mutant VP-gene in HO-DI rats increases. Alternatively, the turn-over rate of m-RNA could be decreased.

298.7

EFFECTS OF COLCHICINE ON CGRP mRNA AND CGRP CONTENT IN RAT BRAIN STEM MOTONEURONS. M. Rethelyi*, C.B. Metz*, P. Petrusz* and P.K. Lund* (SPON: M.J. Sedivec). Depts. of Physiology and Cell Biology & Anatomy, Univ. of North Carolina, Chapel Hill, NC 27599.

In situ hybridization histochemistry and immunocytochemistry were used on adjacent sections to study the effect of colchicine on the expression of mRNA encoding CGRP in brain stem motoneurons. Vibratome sections were prepared from the brain stem of rats which received icv injections of colchicine (2x50 ug) 24 and 48 hours before sacrifice. Saline-injected and intact rats served as controls. Two oligomer probes of 23 and 32 bases in length were end-labeled with 32P. Autoradiography was performed on hybridized sections. - In the nucleus ambiguus and in the nuclei of the XIIth and VIIth nerves hybridization signal and immunoreaction (both of which were present in intact and saline injected controls) increased after colchicine injection. Hybridization signal and peptide immunoreaction could be detected in the neurons in the motor nuclei of the IIIrd, IVth, Vth and VIth nerves only after colchicine injection. (Supported by grants NS23804 and NS14899 from NINCDS, NIH.)

298.8

REGIONAL DISTRIBUTION AND CHARACTERIZATION OF THE BRAIN-GUT PEPTIDE: CALCITONIN GENE RELATED PEPTIDE (CGRP) IN PIG. D.R. Roddy*, L.D. Aimone, T.L. Yaksh, V.L.W. Go*. (SPON: J.A. Gilbert). Mayo Clinic, Rochester, MN 55905, Univ. of CA, San Diego, LaJolla, CA 92093, and Univ. of CA, Los Angeles, Los Angeles, CA 90024.

The presence of CGRP has been noted in tissues of the digestive, respiratory, urogenital, cardiovascular and central nervous systems where it appears by immunohistochemistry to be co-contained with tachykinins in primary sensory neurons (Neurosci 23:693, 1987). We used a specific radioimmunoassay (RIA) for CGRP to corroborate some of these findings. Tissues of gut, brain and spinal cord of pig (n=4) were dissected immediately postmortem, extracted in 0.1 N HCl and assayed by RIA. CRGP was least abundant in pig gut, where highest concentrations were noted in the small intestine (24 ng/gm). In brain, 115 ng/gm of CGRP was found in anterior hypothalamus and lateral medulla, 96 ng/gm in mammary body and 60 ng/gm in medial thalamus and substantia nigra. Notable amounts of CGRP were found throughout the dorsal horn of spinal cord (77-166 ng/gm) as well as in dorsal root ganglia, trigeminal ganglia and sciatic nerves (108-168 ng/gm). Thus, presence of CGRP in primary sensory neurons is confirmed by RIA.

298.9

CGRP PRIMARY AFFERENTS ARE PRESYNAPTIC TO GABA TERMINALS IN PRIMATE DORSAL HORN: A POST-EMBEDDING IMMUNOGOLD STUDY. Elizabeth Hayes* and Susan M. Carlton (SPON: J. Kitay). Marine Biomedical Institute, University of Texas Medical Branch, Galveston, TX 77550.

A considerable amount of evidence supports the role of γ-aminobutyric acid (GABA) mediating primary afferent depolarization. This modulation is accomplished through axo-axonic interactions. Much of the anatomical data supporting this theory is based on degeneration studies following dorsal rhizotomy. It is difficult, however, to determine by structural criteria if a degenerating profile is a presynaptic or postsynaptic element. Therefore, in the following study, the interaction of GABA with calcitonin gene-related peptide (CGRP) containing primary afferents was analyzed at the electron microscopic level using the PAP immunostaining method combined with post-embedding immunogold labeling. Monkeys were perfused with mixed aldehydes and lumbar sections (25µm) immunostained for GABA using the PAP method. Following flat embedding in plastic, ultrathin sections were collected on formvar coated nickel slot grids. Osmicated sections were etched with $NaOI_4$ and $NaBH_4$ and immunostained for CGRP with 15nm IgG-gold (Janssen). In laminae I and II, GABA axon terminals, dendrites and cell bodies were observed postsynaptic to large glomerular type CGRP terminals. These findings challenge previous data which suggested GABA was presynaptic to primary afferents. (Supported by NIH grant NS 11255.)

298.10

DYNORPHIN A (1-8) PROFILES ARE POSTSYNAPTIC TO CALCITONIN GENE-RELATED PEPTIDE (CGRP) CONTAINING PRIMARY AFFERENTS IN MONKEY DORSAL HORN: A POST-EMBEDDING IMMUNOGOLD STUDY. Susan M. Carlton and Elizabeth Hayes*, Marine Biomedical Institute, University of Texas Medical Branch, Galveston, TX 77550

The upregulation of dynorphin (DYN) message and DYN peptide has been demonstrated in the dorsal horn following painful manipulations. It is hypothesized that increased DYN levels are a direct result of increased primary afferent input. In the present study we investigated the synaptic interactions between CGRP containing primary afferents and DYN A (1-8) profiles in the dorsal horn of Macaca fascicularis.

Monkeys were perfused with mixed aldehydes. Free floating lumbar sections (25µm) were immunostained for DYN using the PAP method, osmicated and then flat embedded in plastic. Ultrathin sections were collected on formvar coated nickel slot grids, etched with $NaOI_4$ and $NaBH_4$ and immunostained for CGRP with 15nm IgG-gold (Janssen). In laminae I and II, numerous CGRP-DYN interactions were observed with the CGRP terminal presynaptic to DYN containing cell bodies, dendrites and terminals. We hypothesize that CGRP effects both the manufacture and synaptic release of DYN A (1-8) in the dorsal horn. Furthermore, this structural relationship may play an important role in the processing of noxious input at the spinal cord level. Supported by grants from NIH (NS 11255) and the Bristol Myers Co.

298.11

THE DEVELOPMENT OF CHOLECYSTOKININ IN THE INTERPEDUNCULAR NUCLEUS OF RATS. M.P. Joyce* and G.A. Barr. Biopsychology Doctoral Program, Dept. of Psychology, Hunter College, CUNY, New York, N.Y. 10021 and Department of Psychiatry, Albert Einstein College of Medicine, Bronx, New York 10461.

The interpeduncular nucleus (IPN) contains distinctive subnuclei distinguished by their neurotransmitter content and synaptic and cellular morphology. The octapeptide, cholecystokinin (CCK) has been observed to be confined to well defined areas of the IPN. The presence of fiber staining with the absence of cell body labeling, indicates that the CCK content of the IPN is from un defined extrinsic sources. In order to elucidate the development of the distribution of IPN afferents, changes in the staining characteristics of CCK were studied in rat pups aged 1 to 35 days. Immunocytochemistry was performed with the Vectastain ABC Kit (Vector Labs). The primary antibody against CCK (Incstar) was diluted to 1:20,000. In the adult rat, substantial labeling is observed in the rostral, dorsal, lateral, and dorsal lateral subnuclei as other studies indicate. Staining is present but sparse in the central and ovoid areas of the rostral subnucleus. As development proceeds, staining in the rostral, central and dorsal subnuclei becomes more intense. In the lateral and dorsal lateral subnulcei, high staining densities are observed as early as 7 days of age with minor changes in density but not pattern of staining occurring thereafter. By 35 days of age, the localized areas of relatively dense fiber labeling described above is observed.

298.12

NEUROPHYSIN EXISTS IN A DIFFERENT CONFORMATIONAL FORM IN PARVICELLULAR NEURONS OF RAT HYPOTHALAMUS. G. Nilaver, L.C. Rosenbaum*, H.H.M. Van Tol*, and E.A. Neuwelt*. Depts. of Neurol., Biochem., Neurosurg., & Vollum Inst. Adv. Biomed. Res., Oregon Hlth. Sci. Univ., Portland, OR 97201.

Neurophysins (NPs) are carrier proteins associated with oxytocin (OT) and vasopressin (VP) neurons of the hypothalamus. We have previously demonstrated that a monoclonal antibody (mAb L6) raised to a surface antigen of human lung adenocarcinoma recognizes a cytoplasmic epitope in OT and VP neurons, identified as NP by immunohistochemistry and Western analysis (Neurology, 38:392,1988). We now report on the distribution of L6 immunoreactivity in control and adrenalectomized (ADX) rats, and in rats following intracerebroventricular injection of colchicine (COL) and tunicamycin (TX). Analysis of coronal sections (100 μm) of control rat hypothalamus demonstrated NP immunoreactivity to be exclusively confined to magnocellular OT and VP neurons and their projections to neural lobe. No staining was noted in the suprachiasmatic nucleus (SCN), the parvicellular paraventricular nucleus (p-PVN), or its projections to median eminence-zona externa (ZE-ME) and extrahypothalamic brain sites. The persistent absence of staining in p-PVN or ZE-ME of ADX rats, and in p-PVN and SCN of COL and TX rats suggests the existence of NP in a unique conformational form in these regions that is not recognized by mAb L6. (supported by PHS grants NIDDKD-37205 & NCI-31770; mAb L6 was provided by Drs. Hellström, ONCOGEN)

298.13

INNERVATION OF RAT HYPOTHALAMIC PARAVENTRICULAR (PVN) TRH-SYNTHESIZING NEURONS BY IMMUNOREACTIVE NEUROTENSIN. R. Toni, I.M.Jackson, S.E. Leeman, R.M.Lechan. Divs. of Endo., New England Med Ctr. Boston, MA 02111, R.I. Hosp., Providence, R.I. 02902 and Dept. of Physiology, Univ. Mass. Med. Sch., Worcester, MA 01605

Neurotensin (NT) exerts a number of effects in the CNS and when administered intraventricularly in the rat, inhibits TSH secretion from the anterior pituitary (Endocrinology,103,1903,1978).In addition,NT-immunoreactive (IR) neurons and fibers are richly distributed throughout parvocellular subdivisions of the rat PVN. To determine whether NT-IR axon terminals directly innervate TRH tuberoinfundibular (TI) neurons in the PVN, we performed double-immunolabeling studies at light and ultrastructural levels using antisera recognizing NT (HC#8, dil. 1:800) and a N-terminal sequence of the TRH precursor, preproTRH 25-50 (pYE-27, dil. 1:500)), as a marker of TRH neurons. After perfusion fixation of the rat brain, tissues were sectioned through the PVN and processed by the ABC technique using DAB as a chromogen. In the first step NT was identified by silver-gold or nickel ammonium sulfate to yeld a black or blue reaction product and in a sequential step, proTRH was identified using DAB alone or streptavidin-gold particles. NT-IR fibers were observed closely apposed to TRH-synthesizing neurons in anterior and periventricular parvocellular subdivisions of the PVN and to establish axo-somatic and axo-dendritic contacts, suggestive of functional synapses. Few NT-IR boutons were also observed presynaptic to unlabeled dendrites and juxtaposed to blood vessels. These data provide morphologic evidence to suggest a neurotensinergic innervation of TRH-synthesizing neurons in the PVN and indicate that at least some neurons of the TRH TI system may be under direct regulation by this neuropeptide.PHS 3F05TWO3924,grants NIH DK 37021 and CNR

298.14

GALANIN IMMUNOREACTIVITY IN PARASYMPATHETIC POSTGANGLIONIC NEURONS IN RAT SALIVARY GLANDS. F.A. Frierson*, L.M. Konopka*, and C.J. Forehand (SPON: I.J. Young). Department of Anatomy and Neurobiology, University of Vermont College of Medicine, Burlington, VT 05405.

Neuropeptides coexist with classical transmitters in a variety of autonomic postganglionic neurons. For example, acetylcholine and functionally active vasoactive intestinal peptide (VIP) are colocalized in postganglionic neurons innervating cat salivary glands (Lundberg, J.M., et al., PNAS 77:1651, 1980). We have examined the distribution and source of galanin-like immunoreactive nerves in rat salivary glands.

Galanin-like immunoreactive fibers were observed throughout the submandibular, sublingual, and parotid glands in association with both inter- and intralobular ducts, and surrounding acini in the sublingual gland. The source of galanin-like fibers in the submandibular and sublingual glands was determined by denervation experiments. The glands were sympathectomized by severing the postganglionic branches from the superior cervical ganglion where they enter the glands. With sympathectomy, tyrosine hydroxylase immunoreactivity markedly decreased with no appreciable change in galanin-like immunoreactivity. Parasympathectomy, performed by severing postganglionic nerves from submandibular ganglia, resulted in a marked decrease in both VIP and galanin-like immunoreactivity. Moreover, galanin-like immunoreactivity was observed in submandibular ganglion cells.

These results indicate that galanin-like innervation of rat salivary glands is derived from parasympathetic nerves to the glands. Physiologic responses of individual gland cells to galanin application are described in the accompanying abstract (Forehand, C.J. and Konopka, L.M., Soc. Neurosci. Abstr. 15, 1989).

Supported by the American Heart Association and PHS grants KO401344 and RO125973.

298.15

RAT SUBLINGUAL AND SUBMANDIBULAR GLAND CELLS DIFFER IN THEIR RESPONSES TO BOTH GALANIN AND BETHANECHOL. C.J. Forehand and L.M. Konopka. Department of Anatomy and Neurobiology, University of Vermont College of Medicine, Burlington, VT 05405.

Rat sublingual glands contain largely mucous acini while rat submandibular gland acini are seromucous. In the previous abstract (Frierson, F.A., et al., Soc. Neurosci. Abstr. 15, 1989), we reported that a galanin-like peptide was present in the parasympathetic innervation to these glands, and that the acini of the sublingual, but not the submandibular, gland received dense galanin-like innervation. We have thus asked whether cells in these glands differ in their electrophysiologic responses to galanin and/or the muscarinic agonist, bethanechol.

Individual gland cells were impaled under visual control in wholemount preparations and maintained under single electrode current clamp. Resting membrane potentials ranged from -30 to -66 mv. Galanin (0.1mM) and bethanechol (10mM) were applied locally by pressure ejection. Agonist induced voltage shifts varied between the two glands: Most sublingual gland cells responded to bethanechol with biphasic voltage shifts, i.e., a short-lasting depolarization followed by a long-lasting hyperpolarization; a predominantly long-lasting depolarizing response was observed in the submandibular gland cells. Cells that responded to bethanechol with a biphasic response also responded to galanin. These results indicate a functionally different effect of the cholinergic and peptidergic inputs into the sublingual and submandibular salivary glands.

Supported by the American Heart Association and PHS grants K0401344 and R0125973.

298.16

GALANIN IMMUNOREACTIVE NEURONS IN THE HUMAN HYPOTHALAMUS. W.P. Gai*, L.B. Geffen*, and W.W. Blessing. Centre for Neuroscience, Flinders University of South Austrsalia, Bedford Park, SA 5042.

The localization of galanin (GA) immunoreactive neurons has been investigated by immunohistochemistry in the human hypothalamus. The coexistence of GA with arginine vasopressin (AVP), oxytocin (OXY) and tyrosine hydroxylase (TH) has also been examined in the supraoptic (SON) and paraventricular nuclei (PVN), in adjacent paraffin sections.

Somata and fibers positive for GA were found throughout the human hypothalamus. Major populations of GA-ir somata were seen in the preoptic area, intermediate, dorsal part of suprachiasmatic, SON, PVN, arcuate, dorsal and ventral part of the posterior hypothalamic, and the supramammillary nuclei. Scattered positive neurons were observed in the lateral part of the dorsomedial hypothalamic nucleus, lateral hypothalamic area and zona incerta. The number of GA-ir neurons in the intermediate nucleus was 10700; in the SON, 55900; and in the PVN, 43300. GA-ir fibers were more widely distributed than cell bodies and invaded most hypothalamic nuclei. The lateral tuberal nuclei and the SON (except the dorsomedial part) contained few GA-ir fibers. The mammillary complex contained almost no positive fibers. GA-ir axons were concenrated in the ventromedial part of the arcuate nucleus and formed a bundle travelling down the infundibular stem. In the median eminence the vascular plexus was wrapped by GA-ir fiber networks.

Neurons containing both GA and AVP were very common in the SON and also occurred in the PVN. These two nuclei also contained neurons positive for GA and OXY. Neurons positive for both GA and TH were rare.

The distribution of GA-ir neurons in the hypothalamus provides anatomical evidence for this neuropeptide being involved in endocrine and autonomic functions.

298.17

GALANIN/CHOLINERGIC INTERACTIONS IN THE HUMAN BRAIN: AUTORADIOGRAPHIC STUDIES OF GALANIN mRNA AND BINDING SITES USING in situ HYBRIDIZATION AND RECEPTOR AUTORADIOGRAPHY. J.M. Palacios[1], C. Bonnefond[1]*, G. Mengod[1]*, A. Probst[2]*, and P.H. Kelly[1]. 1) Precl. Res., Sandoz Ltd, 4002 Basle, 2) Dept.Neuropath., Inst.Pathol. Univ.Basle, 4000 Basle, Switzerland

Galanin, a 29 amino acid peptide, has been reported to colocalize with acetylcholine in certain regions of the rat brain. We have examined galanin-acetylcholine interactions in the human brain by using in situ hybridization, with synthetic oligonucleotides, and receptor autoradiography with ^{125}I-galanin. In both rat and human brain we observed high levels of galanin mRNA containing cells in several nuclei of the hypothalamus. However, in neither species were significant levels of galanin mRNA seen in the nucleus basalis of Meynert (nbM). Particularly high levels of galanin receptor binding sites were observed in the hypothalamus and some brainstem and bulbar nuclei. Neocortical areas were enriched in galanin binding, while intermediate levels were observed in the basal ganglia, the hippocampal formation and the nbM. In preliminary studies on the levels of ^{125}I-galanin binding in the nbM from senile dementia patients we did not observe significant differences in comparison with control cases. Unilateral lesions of the nbM in the rat also did not result in asymmetries of ^{125}I-galanin binding in any of the brain regions examined. These results are not consistent with a major direct anatomical interaction between galanin and acetylcholine in the human nbM.

AUDITORY SYSTEM: COCHLEAR NUCLEUS

299.1

ELECTRON MICROSCOPY OF THE 8TH NERVE AFTER IT'S TRAUMA IN THE RED-EARED TURTLE. R. H. Browner AND M. Nirenberg. Dept. Cell Biology and Anatomy, New York Med. Coll., Valhalla, N.Y. 10595.

Turtles were anesthetized with Sodium Brevital, placed on a stereotaxic apparatus and maintained with Halothane, Oxygen and Nitrous Oxide. The 8th nerves were crushed. The bony openings were closed with dental cement and the animals allowed to survive for 45, 50, and 80 days. Unoperated animals were used to determine the nerve's normal morphology. All animals were overdosed with Nembutal, the plastrons drilled open and the hearts exposed. The animals were transcardiaclly perfused with Reptilian Ringers and then 4% paraformaldehyde, 5% glutaraldehyde and 4% sucrose in phosphate buffer (0.15M, pH 7.4). The skulls were opened the heads were placed in the fixative overnight. The brainstem, 8th nerve and inner ears were removed, processed and embedded in Epon for EM analysis. Control 8th nerve fibers were myelinated and had normal elements. 8th nerve fibers in 50 and 80 day survivors were thinner with less myelin and fewer filaments. In both instances the 8th nerve were central nervous system tissue. (The Deafness Foundation).

299.2

AXONAL TRAJECTORIES OF TYPE-II SPIRAL GANGLION CELLS FROM VARIOUS COCHLEAR REGIONS IN MICE A.M. Berglund[1] and M.C. Brown[2]. Depts. of Anatomy and Cellular Biology[1], Cellular and Molecular Physiology and Otolaryngology[2], Harvard Medical School, Boston MA 02115; Eaton-Peabody Lab, Mass. Eye and Ear Infirmary, Boston MA 02114.

Most spiral ganglion neurons (type-I cells) provide the afferent pathway from inner hair cells, but a small population (5%; type-II cells) link outer hair cells with the cochlear nucleus (CN). Thin, unmyelinated axons of type-II cells from the basal region of the cochlea project to the CN, often forming terminal swellings in granule-cell regions (Brown et al., J. Comp. Neurol. *278*:581-590, '88). Our present objective was to determine if type-II axons from middle and apical cochlear regions shared these features. Extracellular HRP was deposited in the various turns of the spiral ganglion and the tissue was processed with DAB. Type-II axons (diameters < 1 um) from apical and middle regions were traced (9 fully and 17 partially) from their entrance in the nerve root to their terminals throughout the CN. These axons were compared with 6 basal axons of the previous study. Labeled axons from all cochlear regions followed labeled type-I axons and bifurcated in a cochleotopic manner, giving off ascending and descending branches. All type-II axons displayed many *en passant* (mean = 86), but only a few (mean = 5) terminal swellings on the ascending and descending branches. The type-II swellings were generally similar in morphology, regardless of their CN location and cochlear origin. Descending branches from all cochlear regions produced terminal swellings (20 of 37 total) in granule-cell regions. Unlike ascending branches from basal-region axons, however, those from apical-region axons produced terminal swellings primarily (37 of 39) near type-I terminals in magnocellular regions. We conclude: (1) Cochleotopic mapping of type-I and type-II axons onto the CN is similar for the bifurcation point and the general trajectory of the ascending branch; (2) Type-II axons, particularly descending branches, differ from type-I axons by their projection into CN granule-cell regions.

299.3

ACTIVATION OF THE AUDITORY BRAINSTEM OF DEAFENED GUINEA PIGS WITH COCHLEAR STIMULATION: A [14C]-2-DEOXYGLUCOSE STUDY. D.R. Schwartz*, J. Schacht, R.A. Altschuler, K. Frey and J. Miller. Kresge Hearing Research Institute, University of Michigan, Ann Arbor, MI 48109.

Profoundly deaf patients with remaining eighth nerve fibers are potential candidates for a cochlear prosthesis. However, after prolonged deafness, such implants have had variable success. The present study in guinea pigs used the 2-deoxyglucose (2DG) method to evaluate changes in the metabolic activity of the auditory brainstem with deafness and to determine the extent of reactivation with cochlear prosthetic stimulation.

Animals were deafened with kanamycin and ethacrynic acid. The following groups were compared: 1) unstimulated normal controls 2) sound-stimulated normal controls 3) unstimulated deafened animals 4) electrically stimulated (intrascalar electrode) deafened animals. The auditory brainstem (cochlear nucleus, superior olivary complex, lateral lamniscus and inferior colliculus) was evaluated.

In animals deafened for four weeks, auditory brainstem 2DG levels were reduced as compared to the sound-stimulated and unstimulated normal controls. Metabolic activity in the auditory brainstem was greatly increased with prosthetic stimulation but remained significantly below the level of either control group. These data show that auditory brainstem nuclei can be activated by cochlear prosthetic stimulation after 4 weeks of deafness. Future studies will examine the response after longer periods of deafness and with prolonged stimulation.

supported by NIH grants NS05785 & NS21440

299.4

AUDITORY NERVE PROJECTIONS IN THE POSTNATAL OPOSSUM. F.H. Willard, Dept. of Anatomy, Univ. of New England, Biddeford Maine, 04005

The projection of auditory nerve fibers into the cochlear nucleus of the opossum, Monodelphis domesticus was investigated using the orthograde transport of HRP. These animals are born 14 days after conception. Opossums ranging from postnatal day 13 through 21 received placements of the HRP chips into the cochlea. All animals had cartilaginous temporal bones and ossicles as well as an epithelial plug in the external meatus. Tissue, sectioned frozen, was processed with BDHCl. Fibers, labelled with HRP, could be followed out of the cochlea, along the auditory nerve and into the cochlear nucleus. These fibers were arranged in a series of vertically oriented lamina extending throughout the rostrocaudal dimension of the cochlear nucleus. A topographic relationship was evident: placements in the apical region of the cochlea labelled fibers in the lateral aspect of the nucleus, while basal placements labelled medial fibers. Even at PND-13 labelled auditory nerve fibers were arranged in narrow lamina and exhibited little side branching. Evidently an adult-like, restricted distribution of auditory nerve fibers is established very early in development.

299.5

NEONATAL CONDUCTIVE HEARING LOSS AFFECTS COCHLEAR NUCLEI IN ALTRICIAL RODENTS BUT NOT IN PRECOCIAL RODENTS. D.B. Webster and N.A. O'Connell. Kresge Hearing Research Laboratory, Dept. of Otolaryngology, LSU Medical Center, New Orleans, LA 70112.

Unilateral conductive hearing losses of about 50 dB were initiated at 1 to 3 days of age by surgical removal of the external auditory meatus in CBA/J mice, cotton rats, spiny mice, and guinea pigs. At puberty, the animals were sacrificed with an overdose of anesthesia. Ears and brainstems were prepared for light microscopy and the cochlear nuclei were quantitatively studied. In CBA/J mice and cotton rats, who develop hearing postnatally, the volume of their ventral cochlear nuclei (VCN) and the size of VCN neurons were significantly smaller ($P<0.05$) on the experimental side than on the control side. In spiny mice and guinea pigs, who develop hearing prenatally, the volume of their VCN and size of VCN neurons were not significantly different ($P>0.05$) between experimental and control sides. The precocial rodents develop hearing in utero--essentially with a conductive loss--whereas the altricial rodents develop hearing postnatally with no conductive loss. Assuming that primitive mammals were altricial, the ability to develop normal cochlear nuclei with a conductive loss would be a derived character. [Supported by NIH grant NS 19238 and Kam's Fund.]

299.6

AGING AND THE MORPHOLOGY OF THE OCTOPUS CELL AREA OF THE COCHLEAR NUCLEUS IN C57BL/6J AND CBA MICE. J. F. Willott, L. S. Bross*, K. Jennings*, and J. Kelley*. Dept. Psychol., Northern Illinois Univ., DeKalb, IL 60115.

In C57 mice, which display progressive sensorineural cochlear pathology during middle age, volume of the octopus cell area (OCA) declines after 12-mo. of age, plummeting by 50% at 30 mo.; the number of neurons decreases, and the density of glial cells increases. Octopus cell size decreases only in the oldest mice. In CBA mice, which display minor sensorineural pathology late in life, a reduction in OCA volume and neuron density and increased glial density are dramatic after 24-mo.; octopus cell size is reduced by 24-mo. Golgi material indicates age-related changes including discontinuous dendrites, diminished size of arbors, and loss of some primary dendrites. However, normal-appearing octopus cells are found in extremely old mice of either strain.

While there are some differences in the profiles of age-related changes in the OCA between strains, the chronic sensorineural pathology in C57 mice does not appear to exacerbate the changes associated with aging.

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299.7

MORPHOMETRIC ANALYSIS OF DEVELOPING ORIENTED DENDRITIC FIELDS IN THE DORSAL COCHLEAR NUCLEUS OF THE HAMSTER. L. Schweitzer. Dept. of Anatomical Sciences and Neurobiology, University of Louisville, Louisville, KY 40292.

We have demonstrated previously that the apical dendritic fields of fusiform cells in the dorsal cochlear nucleus become oriented parallel to the cochleotopic planes in the young hamster after the onset of hearing (Schweitzer & Cant, Neuroscience, 1985). These findings were based on comparisons between cells from different animals cut in different planes of section. With the use of a three-dimensional reconstruction system designed to analyze branching structures (Eutectic Electronics, Inc.), it has been possible to extend these initial observations by rotating the image of the cells and by analyzing each individual cell in different planes. In addition, detailed morphometric analyses of the dendrites of hamsters of various ages were performed in order to study the growth which underlies the formation of the oriented dendritic fields. The data indicate there is no change in the number of primary dendrites as a function of age. However, the dendrites do get longer in the dimension parallel to the cochleotopic planes as new branches are added to the existing dendrites. The distance between branch points remains remarkably constant with age.

These data from the normal hamster will be compared to the dendritic growth patterns in the neonatally-deafened hamster in which oriented dendritic fields fail to develop.

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299.8

GLYCINE IMMUNOREACTIVITY IN THE COCHLEAR NUCLEUS: POSTEMBEDDING LIGHT AND ELECTRON MICROSCOPIC IMMUNOCYTOCHEMISTRY. J.M. Juiz, R.H. Helfert, J.M. Bonneau*, R.J. Wenthold+ and R.A. Altschuler. Kresge Hearing Research Institute, Univ. of Michigan, Ann Arbor, MI, 48109; +LMO, NIDCD, NIH.

An abundance of evidence supports a role for glycine as an inhibitory neurotransmitter in the cochlear nucleus (CN). To accurately identify glycine-like immunoreactivity in cells, fibers and terminals of the guinea pig CN, we used postembedding immunocytochemical techniques at both the light and electron microscopy levels.

Semithin and thin sections from aldehyde-fixed, plastic embedded CN were incubated with an affinity purified polyclonal rabbit antisera raised against glycine. The immunoreactivity was visualized using immunoperoxidase (for semithin sections) and immunogold (for ultrathin sections) techniques.

Immunoreactive cell bodies were found in all three subdivisions of the CN, with the greatest numbers located in the dorsal CN. These cells are currently being characterized ultrastructurally. Glycine immunoreactive terminals were abundant and contacted every major cell type in the CN. Two types of immunoreactive terminals have been identified, so far. The one more frequently observed contained oval/pleomorphic synaptic vesicles, while the other type possessed flat vesicles. Studies are in progress to further characterize these terminals with regard to shape, size, distribution and co-containment with other neurotransmitters.

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299.9

CAVITATION IN THE ADULT GERBIL VENTRAL COCHLEAR NUCLEUS IS REVERSED BY COCHLEAR ABLATION. L. M. Kitzes and H. C. Moore* Depts. of Anatomy and Neurobiology and Surgery-Otolaryngology, University of California Irvine, Irvine, Ca 92717.

Round cavities are easily detectable in the ventral cochlear nucleus of the gerbil (Meriones unguiculatus) after 3 mo of age and increase in number and size thereafter. They have been taken as indicative of a neurodegenerative process (McGinn and Faddis, Hear. Res. 1987, 31: 235-244; Ostapoff and Morest, Hear. Res. 1989, 37: 141-162). McGinn and Faddis suggested that the initial formation of cavities was linked to afferent activity because they were reduced in gerbils subjected to a conductive hearing loss from 12 days of age.

To determine whether the appearance of cavities was reversible, the right cochlea of gerbils older than 7 mo of age was destroyed by inserting a probe through the round window. Following a survival period, the animals were sacrificed and the brainstem sectioned (15 or 30 μ thick) in the coronal plane. Alternate sections were stained with both thionin and eosin to visualize neuronal somata, cavities, and blood vessels. Cavities within the anteroventral (AVCN) and posteroventral (PVCN) cochlear nucleus were counted.

After 24 hr survival, the average number of cavities in the right PVCN and AVCN was reduced to 76% and 71%, respectively, of the numbers observed on the unablated side. After a survival period of 3 days, these percentages fell to 23% and 32%, respectively. The dimensions of PVCN and AVCN on the ablated and control sides did not differ significantly at this time. The relative number of cavities on the ablated side continued to decrease, although at a slower rate, after survival periods of 5, 7, and 11 days. These data suggest that the presence of cavities in the adult gerbil VCN depends upon the integrity of the primary afferent supply to this structure and that the maintenance of the cavities in the normal gerbil is a dynamic and reversible process. Whether the critical variable is pre- or postsynaptic remains to be determined.

Supported by NS-17596.

299.10

NUMBER OF LESIONS IN MONGOLIAN GERBIL COCHLEAR NUCLEUS CAN BE PREDICTED FROM ANIMAL'S AUDITORY EXPERIENCE. K.D. Statler*, S. C. Chamberlain, R. L. Smith*, & N. B. Slepecky*, Institute for Sensory Research and Department of Bioengineering, Syracuse University, Syracuse, NY 13244

Under normal conditions, aging Mongolian gerbils develop an increasing number of microcystic lesions in the cochlear nuclei. The lesions form a contiguous area in the posteroventral cochlear nucleus and adjacent granular layer. The anteroventral and dorsal cochlear nuclei are spared. Ostapoff and Morest suggested that the lesions represent a progressive neurodegenerative disorder, possibly hereditary (Hearing Research, 37:141-162). McGinn and Faddis showed that auditory experience can affect the number of lesions that develop (Hearing Research, 31:235-244). By correlating the noise spectrum of the animals' environment with the tonotopic map of the cochlear nucleus, we have been able to show a relationship between the total driven afferent input and the number of lesions that develop. This has led to a predictive relationship between an animal's auditory history and the number of lesions occurring in its cochlear nuclei.

We have tested this relationship in two ways. We raised animals in a low noise environment different from that used to obtain the relationship. The number of lesions that developed in these animals was within 10% of our prediction and 20% lower than developed in the original environment. We have also raised animals from birth in a very low noise environment. We predict no lesions should develop until about age 50 days. Periodic samples up to age 39 days showed that no lesions developed, whereas gerbils raised in our normal environment develop about 25 lesions by age 38 days. If this correlation between the auditory experience of the animal and the number of lesions occurring in the cochlear nucleus holds, we may be able to use lesions as an activity marker in the posteroventral cochlear nucleus.

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299.11

VOLTAGE-CLAMP STUDIES OF ISOLATED VENTRAL COCHLEAR NUCLEUS NEURONS. P.B. Manis and S.O. Marx*, Dept. Otolaryngology-HNS, Johns Hopkins Univ. Sch. Med., Baltimore, MD 21205.

Neurons of the ventral cochlear nucleus (VCN) display a variety of responses to acoustic stimuli. Some features of these responses have been correlated with non-linearities in the membrane current-voltage relationship (Oertel, J. Neurosci. 3: 2043, 1983). We have investigated the nature of these nonlinearities in current and voltage-clamp recordings from neurons acutely isolated from the guinea pig VCN. In current clamp, isolated cells exhibited 2 types of responses (termed type I and type II) similar to those reported by Oertel (83). Type I cells discharged trains of action potentials in response to depolarizing current injections whereas type II cells discharged only 1-2 spikes and showed strong outward rectification above rest.

Outward currents evoked by voltage steps from -68 mV were studied in voltage clamp. A non-inactivating outward current in type I cells was activated near -20 mV (half-activation -5 mV). This current was blocked by 20 mM TEA and slightly reduced by 4 mM 4-AP. A non-inactivating outward current in type II cells was activated at -64 mV (half-activation -29 mV). This current was slightly reduced by 20 mM TEA, and could be substantially reduced by 4 mM 4-AP. Other currents, including a sustained inward current, a transient outward current, and a TEA and 4-AP resistant sustained outward current, were also observed in both cell classes. These results indicate that the outward rectification in type II VCN cells (presumed bushy cells) is mediated by a non-inactivating low threshold 4-AP sensitive outward conductance with rapid activation and deactivation kinetics. (Supported by Deafness Research Foundation and NIH T32 NS07283.)

299.12

3-DIMENSIONAL FREQUENCY MAPPING IN THE CAT DORSAL COCHLEAR NUCLEUS. G.A. Spirou*, B. May*, and D.K. Ryugo. Depts. Biomedical Engineering and Otolaryngology-HNS, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

We have been investigating the frequency representation of the dorsal cochlear nucleus (DCN) in decerebrate cats using single and multiple unit recordings. Characteristic frequencies (CFs) were determined in 100-200 μm steps in electrode penetrations oriented parallel to the strial axis and distributed in the caudal 2/3 of the nucleus. CFs were plotted onto histologically-reconstructed electrode tracks and analyzed using a computer-aided imaging system.

Typical tracks began in the 40-50 kHz region of the nucleus and terminated in the 0.5-1 kHz region. There was a systematic progression of CFs from high to low, with type II, III, and IV units interdigitated throughout. Frequency versus distance may be represented by two logarithmic functions, one with slope 1.08 ± 0.16 mm/decade at low frequencies and the other with slope 4.99 ± 1.5 mm/decade at high frequencies; the intersection occurred at 13.7 ± 4.4 kHz. The frequency organization of the nucleus is distinct from that of the cochlea in that there is an expanded representation of high CFs in the DCN.

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299.13

DEVELOPMENT OF DYNAMIC RESPONSE PROPERTIES OF COCHLEAR NUCLEAR NEURONS IN KITTENS. E.J. Walsh, J. Fitzakerley and J. McGee*. Depts. of Surgery and Pharmacology, Southern Illinois Univ. Sch. of Med., Springfield, IL 62794-9230.

It is widely accepted that dynamic response features of auditory neurons are important determinants of intensity coding in adults (Smith and Brachman, 1980; Moller, 1976). We have previously shown that the dynamic response ranges of cochlear nuclear (CN) neurons in kittens to long-duration stimuli are smaller than those observed in adult cats, suggesting that immature animals have a restricted ability to establish neural codes for intensity discrimination. We have extended the investigation of the development of intensity-dependent response properties of single CN neurons by examining dynamic range differences between the "onset" response (first 5 msec) and the "sustained" response (steady-state) to acoustic stimulation. Clear differences in dynamic response properties for both epochs were observed for each of the principal CN response types. Maximum driven discharge rates and sensitivities of neurons to changes in stimulus intensity (rate-intensity slopes) were higher during the onset response compared to the sustained response, regardless of age. Maximum driven rates generally increased during the first two weeks of development for both portions of the response. In contrast, rate-intensity slopes associated with onset responses decreased during development, while slopes for sustained responses increased. The development of dynamic response characteristics may reflect the final stages of neuronal differentiation, particularly with regard to membrane receptors. These results will be discussed in light of intensity-dependent responses of CN neurons during the microionophoresis of neuroactive amino acids. (Supported by NIH grant NS21171).

299.14

DISTRIBUTION OF PARVALBUMIN AND CALBINDIN IN THE AUDITORY SYSTEM OF BATS. K. Braun[1], M. Vater[2]*. [1]Otolaryngol. Dept., Univ. Wash., Seattle, WA. [2]Inst. Zool., Univ. Munich, FRG.

The distribution of the two neuron-specific Ca-binding proteins parvalbumin (PV) and calbindin-D-28K (CaBP) has been compared immunocytochemically in the auditory system of Rhinolophus rouxii. Similar to previous findings in the avian auditory system (Braun et al 1985 Cell Tissue Res 240,101) the neuron populations containing either protein show either a partially overlapping or a complementary spatial distribution, depending on the nucleus. In the cochlear nucleus all subdivisions contain PV+ cells, whereas only few CaBP+ cells are found in DCNd and DCNv. All nuclei of the sup. olivary and periolivary complex contain PV+ cells, whereas CaBP-immunoreactivity is found only in a rostral stripe of cells in LSO. In the lat. lemniscus many PV+ cells occur in VNLL, INLL and DNLL, but DNLL and INLL are void of CaBP+ cells. In the IC the central nucleus contains a heterogenous population of PV+ cells, while CaBP+ cells are found mostly in the dorso-medial and ventro-medial region. A subpopulation of large PV+ cells is clearly CaBP-. In the deep layers of the sup. colliculus many PV+ cells but only few CaBP+ cells are observed. In the MGB PV+ cells and neuropil are abundant in all subdivisions and show a clear regional specificity. All subdivisions of MGB contain numerous CaBP+ somata, in the dorsal MGB a subpopulation of medium to large oval cells are CaBP+ and PV-.
Supported by DFG, grant Br 950/2-1 and SFB 204.

299.15

AUDITORY BRAINSTEM IN THE MOLE (Mogera): ANATOMICAL BASIS FOR POSSIBLE HEARING SPECIALIZATION OF THE UNDERGROUND DWELLER. M. Kudo*, Y. Nakamura, H. Tokuno*, Y. Kitao* and T. Morilzumi*. Department of Anatomy, School of Medicine, Kanazawa University, Kanazawa 920, Japan.

The cyto-, myelo- and chemo-architectures of the mole auditory nuclei were studied in Nissl, myelin and acetylcholinesterase stained materials, and then the origins of the ascending afferents to the inferior colliculus (IC) were identified using retrograde transport of wheatgerm agglutinin conjugated to horseradish peroxidase (WGA-HRP) injected into the unilateral IC. In all surgical procedures, moles were anesthetized with sodium pentobarbital and chloral hydrate. 1) In the cochlear nuclei, granule celled fields are very large in both the ventral (VCN) and dorsal (DCN) nuclei. Among several populations of neurons, fusiform cells in the DCN, multipolar cells in the VCN and DCN, and small spherical cells in the VCN project to the IC directly. 2) In the superior olive, the medial nucleus (MSO) is well developed in comparison with that in other similar body-sized mammals. Most strikingly, the projection from the MSO to the IC is bilateral in the mole. 3) The nuclei of the lateral lemniscus show a great development and differentiation of the dorsal (DLL), intermediate (ILL) and ventral (VLL) nuclei. Particularly the large ILL is comparable to that in the mustache bat.

300.1

INHIBITORY PATHWAY TO THE LATERAL SUPERIOR OLIVE FROM THE CONTRALATERAL VENTRAL COCHLEAR NUCLEUS STUDIED IN MOUSE BRAIN SLICE. S.H.WU* (SPON: T.P.Feng).Dept. of Cellular and Molecular Neurobiol., Shanghai Institute of Physiol., Shanghai, 200031 China

To study synaptic connection between the contralateral ventral cochlear nucleus(VCN) and lateral superior olive(LSO), intracellular recordings were made from neurons in the LSO and medial nucleus of trapezoid body(MNTB) of mouse brain slice. Neurons in the LSO usually give IPSPs with synaptic delays of 1.2-1.3 ms to electrical stimulation of the contralateral trapezoid body(TB). As the stimulating electrodes moved to the ipsilateral MNTB, the delays of the IPSPs shortened to about 0.7 ms. To the same stimulation neurons in the MNTB only give EPSPs with synaptic delays of 0.4-0.6 ms. These results indicated that the MNTB neurons are inhibitory neurons in the pathway between the contralateral VCN and LSO. Injection of a depolarizing current in the MNTB neurons elicited a single action potential and much smaller voltage change than a hyperpolarizing current of the same magnitude. The MNTB cells with such electrical properties seem to be well suitable for securing a one-to-one synaptic transmission.

300.2

A NOVEL PROJECTION OF THE VENTRAL NUCLEUS OF THE TRAPEZOID BODY IN THE RAT. W.B. WARR and K.M. SPANGLER. Center for Hearing Research, Boys Town National Institute, and Department of Anatomy, Creighton University School of Medicine, Omaha, NE 68131.

The ventral nucleus of the trapezoid body (VNTB) is a heterogeneous cell group situated within the ventral acoustic stria of most mammals. Various of its constituent neurons are known to have reciprocal connections with the ventral cochlear nuclei and the inferior colliculus, or to project to the cochlea by way of the olivocochlear bundle. In addition, experiments in the cat (Spangler et al., *J. Comp. Neurol.* 238:249-261, 1985) reveal a collateral projection from VNTB to parts of the periolivary zone which contain lateral olivocochlear neurons, but none to the lateral superior olivary nucleus (LSO) itself. Unfortunately, the methods were not capable of demonstrating inputs to lateral olivocochlear neurons. In the present study in rats, anterograde tract-tracing methods (tritiated leucine, PHAL and WGA-HRP injections into the VNTB) and retrograde methods (fluorogold and HRP injections into the LSO) revealed, among others, an unexpectedly strong, topographically organized, bilateral projection to the LSO proper, as well as to the lateral nucleus of the trapezoid body. Since in the rat, lateral olivocochlear neurons are situated clearly within the borders of the LSO, this novel, intrinsic projection may indeed represent inputs to lateral olivocochlear neurons.

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300.3

DENDRITIC SPECIALIZATIONS IN THE MEDIAL AND LATERAL OLIVARY SYSTEM. J. K. Moore and M. Sakuma*. Depts. of Anatomical Sciences and Psychology, SUNY at Stony Brook, Stony Brook, NY 11794.

The medial and lateral olivary nuclei are composed of "bushy" or tufted neurons. Their dendritic morphology has been studied in the guinea pig by Golgi impregnation and by retrograde transport of a highly diffusable tracer (fluorogold) injected into the inferior colliculus. Both the Golgi technique and fluorogold cell labeling show that each stem dendrite terminates in a spray of thin, tortuous, varicose dendritic branches. The terminal dendritic tufts are identical to those of the cochlear nucleus bushy neurons which innervate the olivary nuclei. In both the medial and lateral nuclei, terminal tufts are intertwined with those of neighboring neurons, forming a shell of fine dendritic branches at the periphery of the nucleus. Electron microscopy of the peripheral dendritic zone reveals a meshwork of fine dendrites which are not contacted by synaptic boutons and which form numerous points of direct membrane apposition with surrounding dendrites. No gap junctions or other membrane specializations have been seen at these points of dendrodendritic apposition. The terminal dendritic tufts may be involved in some form of cell-cell coupling, but to date there is no evidence for either chemical or electrical synaptic junctions.

300.4

EXPERIMENTALLY INDUCED PROJECTIONS FROM THE VENTRAL COCHLEAR NUCLEUS OCCUR BEFORE THE ONSET OF HEARING. J. Kil*, F. A. RUSSELL* and L.M. KITZES (SPON: K. Spangler.) Dept. of Anatomy and Neurobiology, Univ. of California Irvine, Irvine, Ca 92717.

We have demonstrated previously that destruction of one cochlea in the neonatal gerbil (*Meriones unguiculatus*) induces the formation of new efferent projections from the ventral cochlear nucleus (VCN) on the non-ablated side to the superior olivary complex (SOC) bilaterally. These projections have been observed several months after the ablation using standard HRP procedures. We have used the fluorescent tracer Di-I to examine the developmental time course of these induced projections.

Age-graded series of normal gerbils and gerbils whose left cochlea was destroyed by aspiration at two days of age were sacrificed and perfused with saline followed by aldehyde fixatives. After post-fixation, a small crystal of Di-I was inserted into the right VCN. Two months later, 30 μm thick coronal sections of the brainstem were examined under a fluorescent microscope.

At 6 days of age efferent projections of the VCN are essentially adult-like. Heavy labeling occurs in the ipsi-LSO, along the lateral border of the ipsi-MSO, medial border of the contra-MSO, and in the dorsal hilus of the contra-LSO. This pattern is severely altered in neonatally ablated gerbils sacrificed at 14 days of age. In these animals, in addition to the normally observed efferent pattern, heavy labeling is observed on both sides of each MSO and within the primary limbs of the contra-LSO. In neonatally ablated animals sacrificed at 7 days of age, in addition to the normally observed efferent pattern, individual fibers, some with growth cones, are evident at or approaching the medial border of the ipsi-MSO and the lateral border of the contra-MSO. More extensive labeling within the contra-LSO is unclear at this time. As the onset of hearing in this species occurs at about day 12 or 13, it is evident that induction of these pathways does not depend upon acoustically driven, afferent activity.

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300.5

COLLATERAL AXONS OF THE MEDIAL NUCLEUS OF THE TRAPEZOID BODY. N. Kuwabara, R.A. DiCaprio & J.M. Zook. Dept. of Zoological & Biomedical Sciences & OU-COM, Ohio University, Athens, OH 45701.

All mammals examined have a set of large diameter axons which arise from cells of the ventral cochlear nucleus, cross the midline, and end as calyces of Held upon principal cells of the medial nucleus of the trapezoid body (MNTB). The calyces are specialized terminals, each enveloping the soma of a single principal cell. The principal cells give off a large axon which projects to the lateral superior olive. In addition, each calyciferous axon and principal cell axon have many small, collateral branches, but the projections of these collaterals have not been well described.

We have characterized these collateral axons by intracellular labeling of calyciferous axons and principal cells with a tissue slice preparation of the auditory brainstem using two bat species: *Pteronotus parnellii*, *Eptesicus fuscus* and the mouse: *Mus musculus*. The collateral branches of calyciferous axons and principal cells have very similar projections, with branches to each, or most, of the following cell groups: the ventromedial and dorsomedial periolivary nuclei, the ventral and lateral nuclei of the trapezoid body, the medial olivocochlear bundle cell group and the ventral nucleus of the lateral lemniscus.

In a few cases, by injecting the dye Lucifer Yellow, we have been able to label both a single calyciferous axon and the principal cell that is immediately post-synaptic to the calyx. In these cases, two labeled collaterals, one from the calyciferous axon and one from the associated principal cell, were found to converge upon a single cell within one of the target nuclei.

Supported by NIH Grant NS26304 and by OU-COM.

300.6

MATCHING OF EXCITATION AND INHIBITION IN THE LATERAL SUPERIOR OLIVARY NUCLEUS OF THE F344 RAT. Finlayson, P.G., Yuscius, S.D.* and Caspary, D.M. Depts. of Pharmacology and Surgery, Southern Illinois University School of Medicine, Springfield, Illinois 62794-9230.

Age-related cell loss in structures which project to rat lateral superior olivary neurons (LSO) may be asymmetrical (Casey and Feldman, '82), suggesting a greater functional loss of glycinergic inhibition in LSO principal cells in aged animals. Recordings from LSO principal cells displaying EI (Excitation to ipsilateral stimuli / Inihibition by contralateral stimuli) responses were examined quantitatively in young adult (3 month) F344 rats. Measures of frequency matching and relative magnitude of excitation and inhibition were obtained by examining excitatory and inhibitory response areas, and families of rate-intensity (RI) curves. Seventy-six percent (32/42) of LSO neurons studied exhibited matched response areas including similar excitatory and inhibitory bandwidths and best frequencies. Excitatory and inhibitory thresholds were within 10dB in the majority of neurons. Families of RI curves were normalized by calculating interaural intensity differences (IID) and percent inhibition of ipsilaterally evoked activity. IID functions displayed a 10%-20% change in contralateral inhibition per 10 dB change in interauaral intensity. Binaural inhibition was maximal when the contralateral intensity was 10-40dB more intense than the excitatory stimulus. Twenty percent of rat LSO cells did not exhibit matched EI frequencies but exhibited inhibition at frequencies 1-2 octaves above the excitatory region. The present findings will enable data from the F344 rat LSO neurons to be compared with similar data obtained from cat and gerbil and also provide a baseline for studies of normal and pathologic changes in this species. (Supported by NIH grant NS15640, MRC of Canada, and SIU-SM CRC funds)

300.7

PHYSIOLOGY AND ANATOMY OF PRINCIPAL CELLS IN THE CAT MNTB. P.H. Smith, P.X. Joris*, M.I. Banks* and T.C.T. Yin. Dept. of Neurophysiology, Univ. of Wisconsin, Madison.

We have recorded from principal cells of the cat medial nucleus of the trapezoid body (MNTB) using either metal electrodes or HRP-filled glass microelectrodes. These cells are believed to relay inhibitory input from the contralateral side to binaural cells in the LSO. We assumed that extracellular complex spike waveforms with a prominent prepotential recorded with metal microelectrodes were from principal cells. Prepotentials are thought to reflect synaptic potentials generated by the large calyces of Held arising from globular bushy cell axons originating in the contralateral AVCN. Prepotentials were never seen with high impedance glass electrodes where verification of recordings from principal cells was made by HRP injection and parent cell identification. Principal cells with low characteristic frequencies (CFs) showed good phase-locking. Those with high CFs usually showed primary-like with notch (PLn) response histograms. However, the response histograms of some cells were not PLn even at high stimulus intensities. There was a noticeable dip in the sustained portion of some of the responses. Mean first spike latencies of MNTB cells were slightly longer than the latencies typical for their major input, globular bushy cells axons with comparable CFs. In some instances the prepotential of the complex spike was absent when the spike occurred between stimulus presentations. The dendritic trees were not well filled in any of our axonally injected cells. Where distinguishable, the number of primary dendrites (2 or 3) corresponded to the number reported by others. The cell bodies were oval with asymmetric nuclei. The axon arose from the soma and headed laterally, passing close to or directly thru the MSO to innervate the appropriate frequency region of ipsilateral LSO. Some collaterals arising from the axon innervated the ipsilateral MSO, ipsilateral VNLL and the ipsilateral VNTB/VMPO region. At the EM level, terminals of MNTB axons contained non-round vesicles and could provide these regions with a source for contralateral inhibitory input.

300.8

SUPERIOR OLIVARY COMPLEX CYTOLOGY AND AUDITORY BRAIN STEM RESPONSES FOLLOWING NEONATAL COCHLEAR REMOVAL IN THE FERRET. David R. Moore and Lindsay M. Aitkin *, University Laboratory of Physiology, Parks Road, Oxford OX1 3PT, U.K.

Neonatal, unilateral cochlear lesions produce a sequence of degenerative and regenerative changes among neurons of the cochlear nucleus (CN) and their target nuclei, the superior olivary complex (SOC) and inferior colliculus (IC). A rapid degeneration of the CN on the lesioned side is followed by a gradual reinnervation of the IC and SOC by CN neurons on the unlesioned side. We have examined second order transneuronal degeneration in the SOC and obtained an overview of the functional consequences of cochlear removal at several levels of the auditory system. Cochlear removals were performed at postnatal day (P)5, P25, P40 or P180. Auditory brain stem responses (ABRs) were recorded at least weekly during the following three months. Brain tissue was paraffin-embedded, frontally sectioned at 10um and Nissl stained. ABRs to click stimuli first appeared at P28. Thresholds improved rapidly, reaching adult levels by P40. Neonatally lesioned animals underwent a second phase of ABR change, from P40 to P80, during which thresholds improved by around 10dB. Maximum sensitivity during this phase transferred from waves I and II to waves III and IV of the ABR. Cochlear removal at P5 produced marked degeneration (35% neuron loss) in the ipsilateral lateral superior olive, but not in other SOC nuclei. Removal at older ages did not markedly affect SOC cytology.

300.9

GENERATION OF AN AUDITORY SPACE MAP IN THE FERRET SUPERIOR COLLICULUS REQUIRES THE PRESENCE OF MONAURAL LOCALIZATION CUES A.J. King and S. Carlile*, University Laboratory of Physiology, Parks Road, Oxford OX1 3PT, U.K.

Spatially tuned auditory receptive fields of deep layer superior colliculus (SC) neurons are derived from a combination of monaural and binaural cues. To assess the capacity of the brain to synthesize a map of auditory space in the absence of normal monaural cues, we examined single unit responses from the SC of adult ferrets subjected to bilateral pinna removal at 28 days of age. The animals were anesthetized, paralysed and placed in an anechoic room, where recordings were made from units throughout the rostrocaudal extent of the SC. Within 10 dB of threshold, some units were sharply tuned around the contralateral interaural axis, while others responded equally well to noise bursts throughout this hemifield or exhibited a broad preference for frontally placed sounds. The receptive fields expanded with increasing sound intensity, becoming unselective for sounds in the contralateral and/or ipsilateral hemifields. A free-field impulse response analysis showed that the azimuthal location of the acoustic axis of the ear was constant from 1-28 kHz. In contrast to normal animals, azimuth spectral transformations were symmetrical, and therefore ambiguous, about the interaural axis. The lack of spatial tuning at high intensities suggests that, under these conditions, a map of auditory space based on binaural interactions has not been formed.

300.10

PARALLEL MONAURAL PATHWAYS TO THE MIDBRAIN IN AN ECHO-LOCATING BAT: THE VENTRAL LATERAL LEMNISCAL COMPLEX (VLL). E. Covey and J.H. Casseday, Department of Surgery, Duke University Medical Center, Durham, North Carolina 27710.

In echolocating bats, the VLL is hypertrophied and differentiated into three major cell groups. The cyto-architecture and connections of these cell groups suggest that VLL may be a complex system of parallel monaural pathways to the midbrain. We recorded the responses to sound of single neurons in the VLL of Eptesicus fuscus. The results confirm that VLL consists only of monaural pathways. Neurons in each division have very different response properties as would be expected if each cell group performs some different transformation of the auditory signal. These differences include: 1) breadth of tuning and 2) temporal pattern of response. For example, cells in one division (the columnar nucleus) are broadly tuned to frequency and respond with a single spike. These spikes are precisely locked to the stimulus onset; their latency is nearly identical for each stimulus presentation, with standard deviations as low as 0.02 ms. In addition, their latency remains virtually constant even in the face of large variations in stimulus intensity and frequency. Such "constant latency" neurons in the VLL could provide a precise marker of stimulus onset to higher levels involved with temporal analysis and thus represent an early stage in the neural circuitry for behavioral tasks such as echo-ranging. [Supported by NIH grant NS 21748]

300.11

AXONAL DOMAINS WITHIN THE DORSAL NUCLEUS OF THE LATERAL LEMNISCUS. Craig K. Henkel, Department of Anatomy, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27103

Experiments were carried out to determine the organization and interrelationships of afferent axonal arbors within isofrequency domains in the dorsal nucleus of the lateral lemniscus. To stain axonal arbors PHA-L was injected into the superior olivary complex in cats anesthetized with sodium pentobarbital. The animals were allowed to survive for 5-7 days. Serially spaced, 50 µm thick sections were collected and the tissue processed for immunostaining with the ABC method (Vector). The results show that projections from the medial superior olivary nucleus to the dorsal nucleus of the lateral lemniscus have a very specific arborization pattern. First, the thickness of the arbor (perpendicular to the cochleotopic axis) varies but consists of a single tier. Then, secondly, the arbors end in isolated territories that are separated from one another. Finally, all of the axons reconstructed thus far arise as collaterals of thick axonal trunks that enter the central nucleus of the inferior colliculus where they also arborize extensively. The results suggest that the horizontal tiers of afferent fibers in the dorsal nucleus of the lateral lemniscus are a mosaic of restricted axonal arbors rather than uniform sheets from divergent axonal arbors.

300.12

RESPONSE PROPERTIES OF SINGLE NEURONS WITHIN CLUSTERS IN INFERIOR COLLICULUS AND AUDITORY CORTEX. G.K. Hui*, J.M. Cassady* and N.M. Weinberger (SPON: J.H. Ashe). Centr. Neurobiol. Learning and Memory and Dept. Psychobiology, Univ. of Calif., Irvine, California, 92717.

"Cluster" recordings ("multiple unit activity"), consisting of the discharges of several neurons, are widely employed. However, the extent to which individual cells within a cluster have the same response characteristics has received little study.

In barbiturate-anesthetized guinea pigs, the responses of clusters to contralateral tone stimulation were obtained using epoxy-insulated tungsten microelectrodes (tips 1-3 microns, impedances 1-2 megohms). On-line separation of single unit waveforms was achieved for 2-4 neurons per cluster (central nucleus of inferior colliculus [ICc]: 16 clusters, 38 cells; primary auditory cortex [ACx]: 25 clusters, 60 cells) using a computer algorithm that included waveform confidence limits. Response characteristics included best frequency (BF), bandwidth, intensity type (monotonic or non-monotonic) and discharge pattern. For both the ICc and ACx, differences in one or more characteristics were found in approximately 1/3 to 3/4 of the clusters, including BFs differing by at least 0.5 octaves.

These findings indicate that cluster recordings frequently consist of the discharges of single neurons for which one or more response characteristics differ. The possibility of such heterogeneity should be considered in the interpretation of neuronal cluster recordings.

Supported by the Office of Naval Research (NMW).

300.13

NARROW BANDPASS FILTER NEURONS IN THE INFERIOR COLLICULUS OF AN FM BAT. J.H. Casseday, B.R. Johnson* and E. Covey. Depts. of Neurobiology and Surgery, Duke University, Durham, NC 27710

Neurons selectively tuned to a narrow frequency band (filter neurons) have been found in the central auditory system of bats that use constant frequency (CF) echolocation calls; however, filter neurons have not previously been reported in bats that use frequency modulated (FM) calls. We recorded responses from single neurons in the inferior colliculus (IC) of the big brown bat, Eptesicus fuscus. This bat navigates and locates prey using an FM echolocation call that sweeps from about 80 kHz to about 20 kHz. The majority of neurons in the IC respond vigorously to pure tones within this range and have frequency tuning curves that broaden as the sound intensity is increased. However, one population of neurons respond only to a very narrow band of frequencies regardless of stimulus intensity. For example, for most filter neurons the Q_{10dB} and Q_{40dB} were virtually identical, about 40. Although each filter neuron is selective for a narrow frequency band, different neurons have slightly different best frequencies (BF), but all are between 23 and 29 kHz. This is in contrast to CF bats where all filter neurons have about the same BF. Filter neurons in Eptesicus are located in a sheet about 700 um thick covering the dorsal and lateral surface. [Supported by NIH Grant NS-21748].

300.14

DIFFERENTIAL TIMETABLE OF PROJECTIONS INTO THE DEVELOPING INFERIOR COLLICULUS IN RAT. B. Maxwell* and J.R. Coleman (SPON: M. Welsh). Depts. of Psychology and Physiology, Univ. South Carolina, Columbia, SC 29208.

The sources of input to the inferior colliculus (IC) from the brainstem and cortex were examined in the postnatal S-D rat. Colliculi were iontophoretically injected with horseradish peroxidase (HRP) or a lectin conjugate in rat pups beginning at birth.

By postnatal day (PND) 7 all basic adult patterns of ascending and descending projections to IC are intact. At this age robust label appears in neurons of the cochlear nuclei, superior olive complex, lemniscal nuclei and auditory cortex. At PND 0 no label typically appears in the lateral superior olive (LSO), but at PND 1 the contralateral LSO is labelled along more medial sectors corresponding to the earliest generated LSO cells; few labelled neurons appear in the ipsilateral LSO. Heavy label subsequently appears throughout the contralateral LSO and moderate label in the ipsilateral LSO. Neurons from the cochlear nuclei, medial superior olive and superior paraolivary nucleus are retrogradely labelled from birth. The relative percentage of ipsilateral to contralateral labelled neurons in the dorsal cochlear nucleus increases after birth. Input from pyramidal cells of auditory cortex to the IC does not appear until after PND 3. Therefore, neurons from different levels of the auditory system neuraxis differentially invade the IC during postnatal life, although all input from auditory pathway structures clearly precedes functional responses to acoustic stimuli.

300.15

NEURAL GROWTH IN THE MEDIAL GENICULATE BODY OF THE POSTNATAL RAT. J.R. Coleman, W.J. Clerici, B. Maxwell* and M.C. Zrull*. Depts. of Psychology and Physiology, Univ. South Carolina, Columbia, SC 29208 and Neurotoxicology Progr., Johns Hopkins Univ., Baltimore, MD 21205.

The rat medial geniculate body (MGB) is subdivisible into ventral (MGv), dorsal (MGd) and medial (MGm) compartments and component nuclei. Neurons of these MGB sectors were studied in a series of Sprague-Dawley rats from birth to 80 days of age using thionin and rapid Golgi material.

At birth the nuclei of the MGB are discernable with MGv relatively larger than MGd and all cells small and darkly staining in thionin material. Golgi stains in newborn animals show substantial dendritic growth in cells in the MGv ventral nucleus and MGd dorsal nucleus when collicular afferents are just invading the MGB. Therefore, initial dendritic and somal growth of MGB neurons must largely be independent of ascending synaptic input. Somatic growth in neurons of all divisions of the MGB most sharply increases from postnatal days 3 to 7 which also parallels maximal postnatal expansion of auditory cortex. A second growth spurt in geniculate cells appears between postnatal days 11 and 16 at hearing onset. Neuron density most rapidly declines in geniculate nuclei from birth to postnatal day 7. These results suggest that epigenetic influences play the initial role in development of auditory forebrain structures, while further developmental events occur after onset of function. (Supported by NIH NS-20785).

300.16

ADULT-LIKE STRUCTURE OF FETAL TECTUM GRAFTED TO INFERIOR COLLICULUS. M.C. Zrull* and J.R. Coleman (SPON: S.J. Kelly). Dept. of Psychology, Univ. South Carolina, Columbia, SC 29208.

For comparison to suspension grafts, whole fetal tecta were grafted into the inferior colliculus (IC) of adult rats. Whole tecta were dissected from anesthetized Long Evans rat fetuses at 17 days of gestation (E17), bisected, trimmed to include the caudal half, and unilaterally injected to a lesion site in IC of adult conspecifics.

At 1 month post-implant, compared to suspension grafts, whole grafts were healthier with neurons exhibiting morphology and organization like the undamaged host IC. Dark stained multipolar and principal cells were more densely packed and often larger than host IC cells. Large grafted principal and multipolar neurons were sometimes aligned in rows similar to laminar gradients of corresponding cells in normal adult IC, although not always dorsomedial to ventrolateral due to graft orientation. Usually one interface between graft and host tissue was found across a border of small multipolar and glial cells.

Whole E17 tectal grafts were more viable, showed more mature features, and seemed more likely to aid repair of host IC than suspension grafts. (Supported by the Deafness Research Foundation).

300.17

GLUTAMATE AND ASPARTATE: LOCALIZATION AND COLOCALIZATION IN THE RAT INFERIOR COLLICULUS. M.R. Eyerly*, J.R. Coleman, M.C. Zrull* and A.J. McDonald (SPON: J.Freeman). Depts. of Psychology, Anatomy, and Physiology, Univ. South Carolina, Columbia, SC 29208.

The excitatory amino acids glutamate and aspartate have the same high-affinity uptake systems, but the distribution of these transmitter candidates according to neuron class may not be identical. The present study focuses upon morphological classification of glutamate and aspartate immunoreactive neurons of the midbrain inferior colliculus in S-D rats using the avidin-biotin procedure (Vector Labs.). Amino acids were colocalized by reaction of alternate sections in separate antibodies or in the same sections reacted sequentially by DAB and BDHC. Cells were categorized according to perikaryl shape and the digitized somal areas statistically analyzed with SAS.

In the central nucleus of inferior colliculus principal cells (usually ovoid) were immunoreactive for glutamate and aspartate in separate sections. The glutamate-reactive principal cells were statistically larger as a group than aspartate-reactive cells. Furthermore, neurons that colocalized glutamate and aspartate were typically among the largest of the principal cell population. Large multipolar cells were immunoreactive for glutamate, but not for aspartate. These results show that glutamate and aspartate immunoreactive neurons of the central nucleus of inferior colliculus constitute both separate and overlapping cell populations.

300.18

A NOVEL COINCIDENCE DETECTION MECHANISM BASED ON A NEURAL COMPARTMENTAL MODEL. W.E. Sullivan. Dept. of Biology, Princeton Univ., Princeton, N.J. 08544

A compartmental model was used to explore how intrinsic cellular properties can affect neural computations. Coincidence detection, in which spike output requires coincident synaptic input is thought to be involved in binaural time comparison. Conventional models assume rapidly decaying, near threshold synaptic inputs from each source, so that simultaneous arrival is needed to exceed spike threshold. Rapidly decaying inhibitory inputs, timed to occur 180 degrees out of phase with excitation may also contribute by suppressing non-synchronous excitatory inputs.

An alternative mechanism, based on the non-linear properties of spike initiation is suggested by simulations using the Hodgkin-Huxley model. The model contains passive electrical compartments designed to simulate soma and dendritic membrane and an active, spike initiating compartment. A low ratio of voltage sensitive sodium to potassium channels and a large conductance between active and passive compartments, produces models in which steady excitation at or near the soma causes a transient response for all suprathreshold stimuli. In the model, the lack of sustained firing is due to insufficient post-spike hyperpolarization under the influence of steady depolarizing current, which leads to insufficient removal of sodium channel inactivation.

This intrinsic transient response suggests a new mechanism for coincidence detection. The model can be driven by trains of excitatory pulses, but stops firing when excitation is constant. Thus, if two inputs are out of phase, so that e.p.s.ps from one source fill the gaps between those from the other, a steady input results and no output is produced. Since this is due to a refractory effect, inhibitory synapses can enhance the response by helping to remove sodium channel inactivation. In fact, the model can be driven by pulsatile inhibition combined with steady excitation, and also works well if pulsatile excitation is combined with steady inhibition. This is especially true for high frequency inputs where rapid recovery from spiking is needed. These results show how synaptic mechanisms may depend on intrinsic cellular properties in that both excitation and inhibition can contribute to either response enhancement or supression depending on the temporal context of their actions.

300.19

EFFECTS OF UNILATERAL INFERIOR COLLICULUS LESIONS IN THE FERRET ON MINIMUM AUDIBLE ANGLES FOR MIDLINE AND LATERAL FIELD SOUND LOCALIZATION. G.L. Kavanagh* and J.B. Kelly. Laboratory for Sensory Neuroscience, Carleton University, Ottawa, Canada, K1S 5B6.

Unilateral ablation of auditory cortex in the ferret results in an incapacity to localize brief sounds in the hemifield contralateral to the lesion. However, animals show no impairment in the ability to localize on midline and in the ipsilateral hemifield. In the present study the effects of inferior colliculus (IC) lesions were examined in eight ferrets to determine whether unilateral ablation of auditory midbrain results in a cortical pattern of sound localization deficits. The tectum was exposed by removing the dorso-posterior tip of visual cortex and the IC was aspirated. Minimum audible angles (MAA's) were obtained around 0°, -60° and +60° azimuth. Results indicate that unilateral ablation of the IC produces a profound contralateral sound localization deficit. With the largest lesions, postoperative performance was not above the level expected by chance, even with speakers separated to 60°. Also, animals showed substantial shifts in MAA's on midline and in the hemifield ipsilateral to the lesion. Deficits persisted despite repeated testing over several months. These results suggest that the representation of auditory space is not fully contralateralized at the level of the inferior colliculus.

300.20

DESCENDING TARGETS OF INFERIOR COLLICULUS EFFERENTS IN BUSH BABY (GALAGO CRASSICAUDATUS). G.C. Thompson and A.M. Thompson. Dept. of Otorhinolaryngology, Univ. of Oklahoma Health Sci. Cntr., Oklahoma City, OK 73190.

Efferent neuroanatomical projections descending from the bush baby inferior colliculus (IC) were labeled with the anterograde tracer, Phaseolus vulgaris leucoagglutinin (PHA-L). Small ionotophoretic injections were made into IC and, after survival times from 7-15 days, the animals were deeply anesthetized and intracardially perfused. Frozen brainstem sections (40 um thick) from the cochlear nucleus through IC were immunohistochemically processed with an antibody against PHA-L.

After injections into the central nucleus, PHA-L-labeled fibers could be observed streaming ventrally within the ipsilateral lateral lemniscus yielding numerous varicosities, presumptive terminal endings, throughout all three lemniscal nuclei. The labeled fibers continued ventrally and posteriorally and began to travel medially at the level of the pre-olivary region and entered the trapeziod body (TB). Some crossed within the dorsal half of the TB to the opposite side of the brainstem. Terminal varicosities and boutons, associated with the labeled fibers, were observed in the ventral nucleus of the trapezoid body (VTB) bilaterally. Additionally, some fibers continued to travel in the TB laterally and then dorsally to finally terminate within the anteroventral cochlear nucleus (AVCN).

Since the VTB is a source of input to the cochlea, our data suggests the existence of a two-synaptic pathway from the midbrain to the cochlea. Additionally, the presence of terminal endings within the AVCN suggests that the midbrain has the potential to modulate ascending auditory input via second order auditory neurons.

300.21

NEURONS OF ANTERIOR THALAMUS TRANSMIT SHORT LATENCY (S-L) AUDITORY SIGNALS SUPPORTING CONDITIONING OF A PAVLOVIAN EYEBLINK REFLEX IN CATS. C.D. Woody, E. Gruen*, V. Chizhevsky* and O. Melamed*. UCLA Med. Ctr., MRRC, BRI, Los Angeles, CA 90024.

Definition of primary (throughput) sensorimotor pathways underlying simple forms of conditioning has been a goal of research in this field. By defining such pathways a more complete understanding of the neural basis of conditioning can be obtained. In cats S-L eyeblink conditioning to a 70 db click CS obligatorily involves the motor cortex and facial nucleus, but not posterior cortical auditory areas. We therefore sought S-L unit activation in subcortical pathways that might support the S-L blink CR. Recordings (>80 units) from the parageniculate - lemniscal adjunct region failed to find such activity with onsets <10ms after the CS. Recordings from 200 anterior thalamic units (at the border of the reticular, lateral dorsal, ventroanterior, anteroventral, caudate and anterior intralaminar nuclei) found units activated at latencies 6-8 ms after the CS. The proportion of units responsive to the CS increased from 27% to 43% after conditioning ($p<.05$, Chi Sq 4.61). The CR formed by pairing click CS, glabella tap US and hypothalamic electrical stimulation (570-10ms ISI) was discriminatively elicited by the forward paired click CS as opposed to a backward paired hiss DS of comparable intensity, and so was the unit activity after conditioning. Cells of this region may transmit messages to the motor cortex supporting elicitation of the CR. (Supported by NS25510.)

CHEMICAL SENSES: PERIPHERAL OLFACTION

301.1

PATCH CLAMP STUDIES INDICATE THAT OLFACTORY RECEPTOR CELLS FROM IMPRINTED SALMON HAVE INCREASED RESPONSIVENESS TO AN IMPRINTED ODORANT. G. A. Nevitt. Dept. of Zoology, NJ-15, Univ. of WA, Seattle, WA 98195.

Coho smolts (*Oncorhynchus kisutch*) imprinted to the odorant phenyl ethyl alcohol (PEA), later specifically home to streams treated with PEA at concentrations as low as 10^{-7}M. I have used the cell attached patch clamp technique to show that ciliated olfactory receptor cells isolated from PEA imprinted fish respond to micromolar concentrations of PEA with a higher probability than cells isolated from nonimprinted salmon.

The cell attached mode was used to monitor cell responsiveness to PEA while minimizing disruption of the intracellular milieu required for odorant transduction. Brief (.5-2 sec) applications of PEA (10^{-6}M-10^{-8}M) were applied using a picospritzing pipet positioned far enough (~100 microns) from cilia to avoid cell movement during odorant application. Bursts of action potentials and/or rapidly activating bursts of inward current (5 pA) were observed in a significantly greater number of imprinted than nonimprinted cells (17/22 imprinted, 9/21 nonimprinted; $p<.05$). However, responsitivity of cells to a second, but behaviorally important odorant (L-serine) were not significantly different (10/18 imprinted, 13/19 nonimprinted; $p>.05$). For both odorants, cells responded in a dose dependant manner, but single channel activity was difficult to resolve even at nanomolar odorant application concentrations. These results suggest that exposure to PEA during smoltification may lead to selection of new PEA sensitive receptor cells, or alter sensitivity to PEA of preexisting cells. Supported by an NIH Neurobiology Training Grant and a Sigma XI Award to G. Nevitt, and by NIH grant HD17486 to W.J. Moody.

301.2

PATCH ELECTRODE STUDY OF FROG OLFACTORY RECEPTOR NEURON MEMBRANE PROPERTIES. R. Pun, S. Kleene* & R. Gesteland, Anatomy & Cell Biology, Univ. of Cincinnati Medical Center, OH 45267.

The effects of odors on the membrane properties of mechanically isolated receptor neurons were studied by whole cell tight-seal recording. Cells had a mean membrane potential of -36 mV (range -20 to -48 mV, n=9). At resting membrane potential, an off-spike followed a hyperpolarizing current pulse in most cells. The only spontaneous activity was long duration depolarizing shifts resembling Ca^{++} spikes (>200 msec) following stimulation. Cells were spritzed with a mixture of 10 odorous substances in Ringers each at a concentration of 1 μM. Four neurons responded unequivocally. Following a latency of up to 30 sec, hyperpolarization of the membrane (by current injection or spontaneously) was followed by oscillations which were enhanced by further hyperpolarization and which ceased with depolarization. Under voltage clamp conditions, a slow outward current was recorded after odor stimulation, which was followed by 1-2 sec duration spontaneous inward currents at regular intervals.

Current contributions of single cilia in isolated neurons were measured by drawing one of the several motile cilia into a small patch pipette and establishing a tight seal at the base of the cilium. With this cell-attached configuration and under voltage clamp conditions, cells were stimulated with 1-sec spritzes of the odor mixture. Most cells showed no response at any holding potential tested. In about 15% of the cells, stimulation resulted in current flow into the cilium. Current began to increase about 250 msec after stimulus onset, reached a peak in 100 msec and decayed in the next 2-10 sec. The maximum evoked current was 50 pA. Peak current amplitude and decay time increased with stimulus duration and when a hyperpolarizing potential was applied. In 4 out of 6 responding cells, no odorant-induced current was detectable in the absence of an applied potential.

Frog olfactory receptor membrane properties are apparently different from those measured in salamanders and mud-puppies. Supported by NIH NS23523 and NS23348.

301.3

OLFACTORY TRANSDUCTION IS MEDIATED BY THE DIRECT ACTION OF cAMP. S. Firestein and G.M. Shepherd. Section of Neuroanatomy, Yale University Medical School, New Haven CT.

The link between odor binding and ionic current generation in olfactory receptor neurons is presumed to be through a G-protein regulated second messenger cascade involving cAMP. However a direct measurement of an odor elicited current which is cAMP dependent has not been described. We now present such data as a direct demonstration of the role of cyclic nucleotides in olfactory transduction. Whole cell patch clamp recordings were obtained from isolated olfactory receptor neurons from tiger salamanders. Ionic currents elicited by brief pulses of odors were transformed from transient to sustained by IBMX, a phosphodiesterase inhibitor and by Forskolin, an adenylate cyclase activator. GTP-γ-S and GppNHp, both non-hydrolyzable GTP analogues, tonically activated the G-protein in a time dependent manner. The current was abolished by the GDP analogue GDP-β-S. The synthetic peptide analogue of the cAMP dependent protein kinase inhibitor (WIPTIDE) lengthened the response, suggesting that cAMP acts both directly on the channel and through a phosphorylation step affecting adaptation.

301.4

MOLECULAR CLONING AND LOCALIZATION OF NOVEL OLFACTORY-SPECIFIC ENZYMES POSSIBLY INVOLVED IN SIGNAL TERMINATION: A NEW ROLE FOR SUPPORTING CELLS. D. Lancet, J. Heldman*, M. Khen*, D. Lazard*, J. Lazarovits*, T. Margalit*, Y. Poria* and K. Zupko*. Department of Membrane Research, the Weizmann Institute of Science, Rehovot, Israel.

We recently cloned three enzymes that may play key roles in controlling olfactory signals. These are: (i) G_{s2} or G_{olf}, the olfactory-specific stimulatory GTP-binding protein, suggested to be important for chemosensory signal amplification; (ii) olfactory cytochrome P450 (P450olf1) and olfactory UDP glucuronosyl transferase (UDPGT), both likely to mediate olfactory signal termination. All three enzymes may serve as markers for specific cell types in olfactory tissue. We have produced antibodies against them, and are currently studying their tissue and subcellular localization. We hope to support our hypothesis that while signal reception and amplification occur in the most superficial layer of the chemosensory epithelium, i.e. the sensory neuronal cilia, enzymes that underlie odorant signal termination may be concentrated in a layer immediately below the cilia, i.e. the apical endoplasmic reticulum (ER) of the glia-like olfactory supporting (sustantacular) cells. We propose that olfactory cytochrome P450 and UDPGT in the highly enriched ER of supporting cells catalyse the biotransformation of lipophylic odorants to their respective membrane-impenetrable glucuronides. These undergo vectorial transport in secretory granules to the mucus layer at the epithelial surface, where they are cleared by mucociliary transport. Drug- or odorant-induced changes, or gentic differences, in the level of olfactory biotrans-formation enzymes could contribute to variations in olfacotry acuity.

301.5

OLFACTORY SENSORY NEURONS ARE TROPHICALLY DEPENDENT ON THE OLFACTORY BULB FOR THEIR PROLONGED SURVIVAL. J.E.Schwob and K.E.Szumowski*. Dept. Anatomy & Cell Biology, SUNY Health Science Center, Syracuse, NY 13210

Ablation of the olfactory bulb results in a chronic reduction in the number of mature olfactory sensory neurons (OSN's) on the operated side, despite some recovery from the acute effects of the lesion (Costanzo and Graziadei, 1983; Monti Graziadei, 1983). In rats studied one month or more after bulbectomy, TEM and SEM examination of the olfactory epithelium and a direct evaluation of the lifespan of OSN's were used to determine whether the olfactory bulb is required for prolonged OSN survival or promotes OSN differentiation without affecting lifespan. OSN's on the ablated side fail to elaborate the dense superficial ciliary mat characteristic of the normal epithelium, and most of the olfactory knobs bear no or only a few cilia. In addition, the fila olfactoria in the lamina propria contain large numbers of swollen and blown-out degenerating axons. An injection of bromodeoxyuridine (BrdU; 20 mg/kg, i.v.) was used to label newly postmitotic neurons on the chronically ablated and unoperated sides. The life cycle of these newly born neurons was assessed by sacrificing animals at 2 h, 5 d and 2 w after BrdU injection, and staining cryostat sections with anti-BrdU MAb. On the ablated side, basal cell proliferation is significantly stimulated by comparison with the unoperated side at 2 h after injection. At 5 d after BrdU, the number of labeled nuclei remains much higher on the ablated side, and the vast majority of the labeled cells are found in the neuronal zone of the epithelium. However, by 2 w, almost all of the labeled cells have disappeared from the ablated side, indicating that the life span of neurons born after bulbectomy is foreshortened. Therefore, in the absence of the olfactory bulb OSN's die before differentiating fully, i.e. they are trophically dependent on the bulb for prolonged survival. Supported by NIH NS19658 and NS 25958.

301.6

CHARACTERIZATION OF THE PRECURSOR POPULATION IN MOUSE OLFACTORY EPITHELIUM AFTER BULBECTOMY. M.A.Schwartz, D. M. Chikaraishi and J.S. Kauer. Neuroscience Program, Tufts NEMC, Boston, MA 02111.

The olfactory epithelium contains at least two types of basal cells; horizontal cells and more superficial globus cells, which may give rise to the sensory cells. Using immunocytochemistry and 3H-thymidine (TdR) autoradiography, we have studied how the mitotic index of these stem cell populations changes after turnover induced by bulbectomy (OBx). While both cell types show turnover as indicated by 3H-TdR uptake, cytokeratin antibody appears to label only the horizontal and not the globus basal cells. By determining the number of 3H labelled cytokeratin-positive and 3H labelled cytokeratin-negative cells at various intervals after OBx, the replacement elements for the olfactory receptor cells following induced turnover can be defined. Results indicate that approximately 90% of the 3H labelled cells 1-15 days after OBx are globus, cytokeratin-negative, cells. Although labelling is seen in the horizontal cells at all intervals after OBx, the number of labelled horizontal cells does not significantly change. Therefore, the increase in label index after OBx appears to be due to an increase in DNA synthesis in the globus basal cells which thus may be the replacement elements for the receptor cells during experimentally induced turnover. Supported by PHS Grants NS-20003, GM-33991 and the Dept. of Neurosurgery.

301.7

CULTURED RAT OLFACTORY NEURONS ARE EXCITABLE AND RESPOND TO ODORS. S.K. Pixley and R. Y. K. Pun. Dept. Anat. & Cell Biol. /Physiol. & Biophys., Univ. of Cincinnati Coll. Med., Cincinnati, OH 45267.

In order to study olfactory neurons and the poorly understood olfactory transduction mechanisms, we have developed dissociated, primary cell cultures of olfactory receptor neurons from nasal tissues of newborn Spraque-Dawley rats. An enriched culture medium increased neuron numbers by more than 20 fold. Neurons were immunostained for neuron-specific enolase, tau protein, synaptophysin and microtubule-associated protein 2. Neuron cell bodies averaged 10 um by 15 um, with two or more thin processes. Neurons could be found in 26 day old cultures, although at lower numbers than during the first 5-6 days. Electrophysiological studies performed on these cells after 3-5 days in culture, using the whole cell "tight seal" recording technique, indicated that they generate action potentials following stimulation. Some cells showed spontaneous inward currents. Application of an odorant stimulus resulted in depolarization or generation of an inward current. This is the first description of a monolayer culture system containing rat olfactory neurons which demonstrate biochemical and physiological maturity. (Supported by APA grant PB1-8803-1 (to SKP)).

301.8

CHARACTERIZATION OF PRIMARY CULTURES OF NEONATAL RAT OLFACTORY NEURONS. G.V. Ronnett, L.D. Hester* and S.H. Snyder. Dept. of Neuroscience, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

We have developed a method for culture of rat neonatal primary olfactory neurons (Pevsner, J.P. et al., Soc. for Neurosci. Abst., 424, 1988). Greater than 98% of the cells in these cultures stained positively for anti-olfactory marker protein (OMP), a generous gift from Dr. Frank Margolis. In addition the same percentage of cells stained positively with anti-vimentin antibody, neuron specific enolase and chromogranin. Cell cultures were negative for glial fibrillary acidic protein and S-100 protein. The specificity of staining in all cases was confirmed by Western Blot analysis.

A number of substrates were tested, including laminin, heparan sulfate, polyornithine, collagen, collagen type IV, fibronectin and polylysine. The substrate which provides the highest yield of bipolar cell attachment as well as maintenance of bipolar morphology is laminin. Within 4 days after plating, in serum-free medium, cells withdrew their processes, suggesting that further nutrients/growth factors are needed. Therefore, we have tested the effects of growth factors, including NGF, TGF, FGF, BDNF, insulin, IGF-I and IGF-II on the cutures. In addition, endogenous levels of cAMP are measured in absence and presence of odorants.

301.9

CHARACTERIZATION OF A CONTINUOUS CULTURE OF OLFACTORY CELL LINES. F.F.Borisy[1], G.V.Ronnett[1], R.Hen[2*], S.H.Snyder[1]. [1]Dept. of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21205, [2]Howard Hughes Medical Institute, Columbia University, New York, New York 10032.

Olfactory neuronal cell lines have been characterized immunocytochemically and levels of cyclic nucleotides have been studied in response to stimulation by a variety of odorants. Cell lines immortalized from olfactory epithelium have been grown continuously in culture for up to fifty generations. The cells stain immunopositive for 160 KD neurofilament, neuron specific enolase, 43 KD growth associated protein, and vimentin, but not for glial fibrillary acidic protein, S100, or keratin. Treatment with retinoic acid, dibutyryl cyclic AMP or isobutylmethylxanthine switches the cell morphology from epithelioid to neuronal. The treated cells have small refractile bodies and extended processes. Measurement of adenylate cyclase activity indicates a basal activity of 0.45 nmol/mg/min and a GTP S stimulated activity of 0.9 nmol/mg/min. Odorant stimulation produces a 20 - 30 per cent increase in adenylate cyclase activity.

301.10

OLFACTORY MECHANISMS: ROLE OF THE LATERAL NASAL GLAND. A.A. Khan and S.H. Snyder. Dept. of Neurosci., Johns Hopkins Univ. Sch. of Med. Balt., MD 21205.

The lateral nasal gland (LNG) is the major source of odorant binding protein (OBP) which may be a carrier for odorants. The protein has been shown to bind odorants of various structural classes such as pyrazines, terpenoids, and aromatics. OBP is significantly homologous to a superfamily of proteins that are believed to transport hydrophobic ligands. This homology has suggested the possibility of a receptor binding site for the complex of odorants and OBP. Sagittal sections of the removed gland and olfactory epithelium indicate novel aspects of glandular anatomy: multiple ducts projecting from the gland anteriorly and posteriorly; a projection from the gland to the tear ducts; and regions of glandular tissue confluency with olfactory epithelium. Preliminary autoradiographic studies, with metabolically labelled [^{35}S]-met OBP, show a specific localization of OBP binding to olfactory epithelium and not to adjacent brain regions. These preliminary results indicate the existence of a binding site in olfactory epithelium for OBP.

301.11

OLFACTORY MARKER PROTEIN IN A SECOND CELL TYPE IN THE RAT OLFACTORY EPITHELIUM. E.W. Johnson*, P.M. Eller*, Bruce W. Jafek*, and D.T. Moran* (SPON: A. Martin). RMTSC and Univ. Colorado Hlth. Sci. Cntr., Denver, CO 80262. Supported by NS20486.

Immunocytochemical localization of olfactory marker protein (OMP) has been a reliable technique for identifying receptor cells in the mammalian olfactory epithelium (OE). Recently, we've demonstrated ultrastructural immunocytochemical localization of OMP (AChemS-XI Abstract #127, 1989) in the receptor vesicles and cilia of the olfactory bipolar neurons. The same techniques, using anti-OMP (courtesy of F. Margolis) with subsequent peroxidase conjugation and a DAB reaction, are used in the current study. A previous electron microscopic study in our lab suggested that a second cell type (termed "microvillar cell") in the mammalian OE may also be a receptor cell (Moran et al., Brain Res. 253:39, 1982). In the present study, we describe OMP labeling in the microvillar cell of the rat septal OE, at the light and electron microscopic levels. These scarce cells, with a central nucleus in an oval soma close to the epithelial surface, have microvilli that extend into the nasal cavity. The cytoplasm surrounding the nucleus and at the apical surface is immunocytochemically labelled with anti-OMP. These observations support the suggestion that there are two distinct cell types in the mammalian OE that contain OMP.

301.12

DISSOCIATED MOUSE OLFACTORY BULB CELL CULTURES. L. Guo*, R.M. Thompson*, S.P. Fracek Jr. and R. Schafer. Department of Biological Sciences, University of North Texas, Denton, TX 76203.

As part of a project to develop an *in vitro* model of the olfactory system using co-cultures of olfactory neuroepithelium and bulb cells from embryonic mice, we have compared the morphologies of intact adult tissue to cultured cells using light microscopy, scanning and transmission electron microscopy.

Cultured olfactory bulb cells are very similar to those found in the adult bulb, with both neurons and glial cells present. Cultured cells characteristically form clusters - heterogenic clumps of neurons with many neurites and fascicles of neurites that project to adjacent clusters. Many individual neurons are also present outside the clusters. Neuronal soma are spherical, oval, or pyramidal in shape and vary from less than 10 µm to more that 25 µm in diameter. The nuclei of these cells are spherical or oval, with few clusters of marginal heterochromatin and one or two nucleoli. The perikaryon is rich in organelles, especially endoplasmic reticulum (ER), mitocondria (MI), free ribosomes and Golgi. Microtubules (MT) and microfilaments (MF) predominate in the neurites.

Neurites of cultured olfactory bulb cells are well developed and some are several millimeters long and form a dense interconnecting network, especially around cell clusters. The diameter of the neurites ranges from 0.5 µm to 4 µm; fascicles can be up to 20 µm in diameter. Many MT, MI, and RER are found in In dendrite-like neurites. In axon-like neurites, only MT and an occasional MI are found. Presumed growth cones are found at the tips of some of the neurites.

Synapses are very prominent in these cultures, especially in a zone surrounding cluster areas. The terminals are often slightly swollen and may contain MI, in addition to synaptic vesicles. The vesicles can be divided into two types: 1) small, spherical and electron lucent vesicles of about 50 nm in diameter and 2) larger, spherical and electron lucent vesicles of about 90 nm in diameter. The large vesicles are more variable in size and have an electron dense core about 45 nm in diameter. Synaptic clefts are about 25 to 35 nm wide and contain an electron dense material. This research is supported by NSF Grant BNS-8719319 (to SPF).

301.13

BIOCHEMICAL PROPERTIES OF SENSILLA SPECIFIC PROTEINS IN SAND SCORPIONS. **D.A. Balseco* and P.H. Brownell (Spon: G. Ciment). Department of Zoology, Oregon State University, Corvallis, OR 97331-2914.**

The pectines of scorpions are large, mid-ventral, sensory appendages comprised of cuticular peg sensilla that contact the substrate. Behavioral and morphological evidence indicate that the pectines are chemosensory structures. In reducing SDS-PAGE, low molecular weight proteins (14-18 kd) have been localized to the pectinal sensilla of the sand scorpion, *Paruroctonus mesaensis*. These sensillar specific proteins (SSP) have species and sex specific molecular masses and are rapidly turned over. Incorporation of ^{35}S-methionine into SSP occurs within minutes when incubated *in vivo* or *in vitro* and is more rapid in males. Control tissues do not contain proteins with these characteristics. In non-denaturing PAGE, SSP occur as dimers (26-32 kd). These proteins are not glycosylated, and are easily extracted under mild conditions. Extraction with Triton X-114 results in the separation of aqueous and detergent phases with the SSP occurring in the aqueous phase. These results imply that SSPs are not integral membrane proteins.

The SSPs have similarities with a family of hydrophobic molecule binding proteins (15 kd pheromone binding protein in sensilla trichoidea of saturnid moths and 19 kd olfactory binding protein in rat nasal epithelia). The SSP of scorpions may function similarly and we are investigating the possible role of SSP in pheromone reception. **Support: BSF grant BNS 87-00990; PHS grant 07-32 NH-10002; Grant-in-Aid of Research from Sigma Xi.**

301.14

DETECTORS OF MAJOR AND MINOR FEMALE PHEROMONE COMPONENTS ON THE ANTENNAE OF MALE REDBANDED LEAFROLLER MOTHS. R. Patrick Akers* and Robert J. O'Connell. Worcester Foundation for Experimental Biology, 222 Maple Ave., Shrewsbury, MA 01545.

The pheromones of many insects are now proving to be complex blends of organic compounds. The biologically active components of the sex attractant pheromone of the female redbanded leafroller moth includes seven different compounds. Olfactory receptor neurons responsive to the two most abundant pheromone components are routinely observed within the long trichoid sensilla found abundantly on the proximal margins of male antennal subsegments. Here, we sought to determine if receptor neurons to the other components of the pheromone could be found in a systematic survey of the whole antenna. Extracellular single-sensillum recordings were obtained from 113 long sensilla trichodea broadly distributed on the antennae in response to the seven components of the female sex pheromone, presented both singly and in combinations. All of these sensilla contained two receptor neurons. The neuron with the larger impulse was specialized for *Z*-11-tetradecenyl acetate (Z11-14:Ac) and the companion neuron with the smaller impulse was specialized for *E*11-14:Ac. None of the receptor neurons found in these sensilla were responsive to any of the other components of the pheromone. A search was then initiated among the other sensillar types on the antenna. Olfactory receptor neurons responsive to stimulation with two of the minor components, *E*- and Z9-12:Ac, were found in separate sensilla. Standard SEM observations indicate that these neurons are housed within a shorter, narrower type of sensillum trichodeum than that which houses the receptor neurons responsive to the two major components. These neurons account for <10% of the number found to be responsive to major components of the pheromone, although they have similar sensitivities for their most effective stimuli. Supported by NS 23946.

301.15

PATCH CLAMP ANALYSIS OF MALE *MANDUCA SEXTA* OLFACTORY RECEPTOR NEURONS IN PRIMARY CELL CULTURE. M. Stengl, F. Zufall*[,1], H. Hatt*[,1], J. Dudel*[,1], and J.G. Hildebrand. ARL Div. of Neurobiology, Univ. of Arizona, Tucson, AZ 85721, & [1]Tech. Univ. of Munich, FRG.

Sex pheromones released by female moths *Manduca sexta* are detected solely by male-specific olfactory receptor neurons (ORNs). To investigate the transduction mechanisms underlying pheromone detection, we have established a primary culture system of male pupal antennal cells (Stengl & Hildebrand, *Neurosci Abs* 14:379,1988). Patch clamp studies showed that after only 2 days *in vitro*, most ORN-like cells (with resting potentials of -40 to -80 mV) exhibit (in whole-cell patch clamp experiments) a non-inactivating, voltage-dependent, outwardly rectifying K^+ current when the voltage is stepped from -70 to 0 mV. After about 2 weeks *in vitro*, these cells express a fast TTX-blockable inward Na^+ current. In cell-attached patches (with 150 mM NaCl and 2 mM KCl in the pipette), at least 2 different types of Cs^+-blockable K^+ currents could be distinguished: (1) a non-inactivating, voltage-dependent, outwardly rectifying K^+ current, which could be blocked by ATP or cGMP applied from the outside as well as from the inside of the cells; and (2) a rapidly inactivating, voltage-dependent K^+ current. In inside-out patches, we found a rapidly flickering Ca^{++}-dependent K^+ current. After 18 days *in vitro*, cells showed (at resting potential in cell-attached patches, with 150 mM KCl in the pipette to mimic the receptor lymph), very few, briefly opening K^+ channels, whose currents reversed around 0 mV cell potential and open-time probability (OTP) increased with depolarization. If these cells were stimulated with the female pheromone blend (prepared by washing the gland with DMSO), some of their responses changed: channel openings of longer duration and higher OTP appeared, while the OTP of the smaller-amplitude, briefly opening channels decreased. We now seek to learn whether these responses to pheromone underlie the depolarizing receptor potentials in the male-specific olfactory receptor cells.

301.16

acj6, A NEW *DROSOPHILA* OLFACTORY BEHAVIOR MUTANT WITH A DECREASED AMPLITUDE ELECTROANTENNOGRAM. R.K. Ayer, Jr.* and J. R. Carlson* (SPON: C. Sahley). Department of Biology, Yale University, New Haven CT. 06511.

The electroantennogram (EAG) was used to monitor the peripheral steps of olfactory transduction in both wildtype and mutant *Drosophila melanogaster*. The EAG, thought to represent the summed odorant response of all cells in the fly's antenna, is extracellularly measured as the voltage difference between a saline filled reference electrode inserted into the head capsule and a similar recording electrode placed on the anterior surface of the fly's antenna. A reflexive olfactory behavior assay developed in this laboratory was used to isolate a group of x-linked, *abnormal chemosensory jump* (*acj*) mutants on the basis of a decreased jump response to vaporous pulses of the odorant ethyl acetate (EA). One of these, *acj6*, is shown to have significantly reduced amplitude EAGs over a wide range of concentrations of EA and benzaldehyde (BZ). EAG amplitude is reduced to about 30% of wildtype levels for pulses of EA at a 10,000-fold dilution or BZ at a 100,000-fold dilution. The *acj6* jump response is reduced for odorant pulses of BZ as well as EA. Qualitative behavioral and quantitative physiological evidence has been used to assess whether the *acj6* mutation affects many functions of the fly or is specific to olfaction. While the general activity level of the *acj6* fly in the culture vial seems somewhat depressed, mating behavior and viability levels of flies carrying the mutant chromosome are similar to wildtype. The *acj6* electroretinogram appears normal, suggesting wildtype visual function. Genetic mapping experiments, presently in progress, may be useful in determining if a single mutational event on the x-chromosome is responsible for the behavioral and physiological phenotypes of *acj6*. Supported by NIH grant GM-36862 to J.C.

301.17

ECTONUCLEOTIDASE ACTIVITIES ASSOCIATED WITH THE OLFACTORY ORGAN OF THE SPINY LOBSTER: EFFECTS ON THE DETECTION OF EXOGENOUS AMP, ADP, AND ATP. H.G. Trapido-Rosenthal, R.A. Gleeson*, S.L. Wachocki* and W.E.S. Carr*. The Whitney Laboratory, University of Florida, St. Augustine, FL 32086.

The olfactory organ of the Florida spiny lobster, Panulirus argus, consists of aesthetasc sensilla on the lateral branches of the antennules. Within each sensillum are the dendrites of several hundred chemosensory neurons including those that respond to exogenous AMP, ADP, or ATP. Ectonucleotidase activities that dephosphorylate these nucleotides are also associated with these sensilla. Dephosphorylation of each of the nucleotides can be described by Michaelis-Menten kinetics. Maximum rates of dephosphorylation are variable; rates are half-maximal at micromolar substrate concentrations. While the product of sensillar 5'-ectonucleotidase is the electrophysiologically inactive nucleoside adenosine, the products of ATPase and ADPase activities are ADP and AMP, respectively; these compounds are themselves stimulants of their own classes of receptor cells, and are substrates for their respective ectonucleotidases. In addition, ADP, the initial product of ATPase activity, inhibits both ATP receptor cells and AMP dephosphorylation. We use the kinetic parameters of sensillar ectonucleotidase activities to estimate the effects that these perireceptor events may have on the olfactory detection of purine nucleotides by the lobster. Supported by NSF grants BNS-8607513 and BBS-8712420.

301.18

HIGH-RESOLUTION ELECTROCHEMICAL ANALYSIS OF AQUATIC ODOR SIGNALS. P.A. Moore[1,*], J.M. Parrish[2], J. Atema[1] and G.A. Gerhardt[2]. Boston University Marine Program[1], Marine Biological Laboratory, Woods Hole, MA 02543 and Depts. of Psychiatry and Pharmacology[2], Univ. of Colorado Health Sci. Ctr., Denver, CO 80262.

Odor signals are shaped by molecular diffusion and turbulent dispersal in the marine environment, and serve as important sources of information for sensory and neural systems of aquatic animals. However, the precise temporal, spatial and concentration dynamics of such signals remains unclear. In the present study, high-speed (10-200 Hz) electrochemical recording techniques utilizing miniature (30-150 microns) carbon-based electrodes were used to characterize the chemical dynamics of aquatic odor signals using dopamine as the odor tracer. Flume studies showed that odor signals consisted of bursts of concentration peaks; peaks had total time courses of 2-4 seconds and varied in amplitude, frequency and slope when electrodes were moved away from the source. Recordings at different depths along the shaft of the aesthetasc hairs in the lateral antennule of the lobster, Homarus americanus, demonstrated a major difference in the temporal and concentration parameters of signals that reached the deeper portions of the receptor structure. However, under the short impulse flow conditions that characterize the lobsters flicking behavior (i.e. odor sampling) the signals showed equally sharp onset slopes at all depths. These studies have contributed to a more detailed understanding of the temporal dynamics of odor signals as they are perceived by chemoreceptor structures in marine animals.

CHEMICAL SENSES: TASTE AND CAROTID RECEPTORS

302.1

SOLITARY EPIDERMAL CHEMORECEPTORS IN ANAMNIOTE AQUATIC VERTEBRATES: THE STATE OF THE ART. K. Kotrschal* (SPON: W. Hahn). Zool. Inst., Univ. Salzburg, A-5020 Salzburg, Austria & U. Colo. Med. Sch., Dept. Cellular and Structural Biology, Denver, CO 80262

Solitary epidermal chemoreceptor cells (SEC) innervated by spinal or cranial nerves are distributed across the epidermis of many aquatic vertebrates. Although SEC's are present in the agnathans, and in gnathostome fishes as well as in anuran tadpoles, our knowledge of this widespread sensory system used to be very limited. Due to research models such as rocklings (Gadidae: Teleostei), sea robins (Triglidae: Teleostei) and cyprinids (Cyprinidae, Teleostei) and the application of TEM, SEM, conventional and tracer neuroanatomy as well as electrophysiology, our knowledge of the fine structure (Whitear, M. and K. Kotrschal, 1988), innervation and central representation (Kotrschal, K. and M. Whitear, 1988), as well as the function of this system (Silver, W.L. and T.E. Finger, 1984; Peters, R. et al., 1987) has improved. The contemporary state of the art in SEC research will be surveyed.

The densities, distribution and innervation of SEC as compared to taste buds was studied with light and scanning electron microscopy in a variety of cyprinids and catfish. The density of SEC in many species appears to be one or two orders of magnitude greater than that of chemosensory cells organized in taste buds. Thus the SEC system appears to be a widespread, major sensory system in aquatic animals rather than a specialized system occurring in only a limited number of species. We may assume that SEC comprise a bulk-water sampling system of unknown biological roles. Whether SEC represent the morphological substrate for the "common chemical sense" as described by Parker remains doubtful.

302.2

PHOTOAFFINITY LABELING OF ALANINE TASTE RECEPTORS IN CATFISH. **B.P. Bryant, P. Shah, and K. Leftheris, Monell Chemical Senses Center, Philadelphia, PA, 19104**

To facilitate the isolation and ultra-structural localization of the alanine taste receptor we synthesized a photolabile analogue of L-alanine, the 4-azidobenzyl ester (AzB-Ala). Without photoactivation, AzB-Ala specifically and reversibly inhibited the binding of L-(3H) alanine to plasma membranes from taste epithelium following pre-exposure to AzB-Ala. L-(3H)arginine binding was unaffected. Used as an adapting stimulus, AzB-Ala caused differential inhibition of taste neural responses (Ala,87%; Arg,34%). Initial attempts to label membranes revealed that alanine but not arginine binding activity was labile to UV (254 nm). Using a filtered 350 nm lamp, it was possible to irradiate membranes without loss of alanine binding in controls. Specific, irreversible inhibition of the binding of L-(3H) alanine but not L-(3H) arginine was observed. Although protection against photo-activated inhibition using excess alanine has not been achieved, labeling appears to be specific to the alanine receptive pathway.

Supported by NS-22620 and BRSG S07RR05825-06

302.3

INTRACELLULAR RECORDING FROM *NECTURUS* TASTE CELLS IN LINGUAL EPITHELIAL SLICES Douglas A. Ewald & Stephen D. Roper Dept. of Anat. & Neurobiology, Colorado State Univ., Ft. Collins CO 80523 and Rocky Mountain Taste and Smell Center, Denver CO 80262

Taste buds in *Necturus* contain three morphologically distinct cell types: dark and light receptor cells with apical ends extending to the surface of the lingual epithelium and basal cells which are located at the base of the taste bud. Receptor cells exhibit generator potentials and action potentials in response to tastants (*cf.* Kinnamon & Roper, J. Physiol. **383** 601, 1987 and Chem. Senses **13** 115, 1988). In ultrastructural studies, chemical synapses have been identified in the basal region of the taste bud between taste cells and sensory axons and also between adjacent taste cells (Delay & Roper, J. Comp. Neurol. **277** 268, 1988). We have used transverse slices of epithelium (200 um in thickness) that contain taste buds to probe the electrophysiological properties of both receptor and basal cells *in situ* and to investigate the hypothesis that there are synaptic interactions among cells in the taste bud. The apical extremities of receptor cells were depolarized by focal application of brief pulses of high K^+ solution (140 mM) delivered from a micropipette (20 um tip diam.). Intracellular recordings from the **apical** processes of receptor cells showed large depolarizations (20 - 40 mV) with short latencies (100 - 150 ms). Recordings in the **basal** region of the taste bud revealed two classes of responses: i) large depolarizations (<40 mV) with short latencies (<150 ms) presumably representing electrotonic conduction of receptor potentials from apical to basal ends of receptor cells, and ii) smaller depolarizations (5 - 20 mV) with longer latency (>150 ms) which might represent synaptic transmission from receptor to basal cells. We are testing the latter hypothesis electrophysiologically (*e.g.* with pharmacological agents and by voltage dependence) and anatomically (with Lucifer Yellow dye injection). Supported by AG06657.

302.4

IMMUNOCYTOCHEMISTRY OF PUTATIVE NEUROTRANSMITTERS IN *NECTURUS* TASTE BUDS. S. B. Jain* and S. D. Roper. Dept. of Anat. and Neurobiology., Colorado State Univ., Ft. Collins, CO 80523 and the Rocky Mountain Taste and Smell Center, Denver, CO 80262.

Synaptic contacts have been identified at the ultrastructural level between taste cells and gustatory nerve fibers, and between adjacent cells in *Necturus* taste buds (Delay & Roper, 1988, *J. Comp. Neurol.* **277**, 268). The neurotransmitters released at these synapses are not known. Electron micrographs of synapses reveal small spherical electron-lucent vesicles at some sites, and large dense-core vesicles at other sites. We report here that GABA-like and glutamate-like (henceforth, GABA and glutamate) immunoreactivity, studied at the light microscopic level, has been localized in taste buds, especially in sensory fibers innervating taste cells in *Necturus*. Lingual epithelium was dissected from the animal and fixed in formaldehyde-glutaraldehyde. Thick (20 microns) sections were cut with a cryostat and treated with primary anti-GABA-BSA (rabbit serum) or anti-glutamate-KLH (monoclonal) antibodies. Controls included: (1) treating tissues with pre-immune rabbit serum (i.e. devoid of anti-GABA); (2) preadsorbing with 0.5 mM GABA, GABA-KLH, or glutaraldehyde conjugates (with BSA) of the following amino acids: GABA, taurine, glycine, beta-alanine, l-glutamate, l-glutamine or l-aspartate. Secondary antibody and biotinylated peroxidase (ABC kits) were obtained from Vector Laboratories and Pel-Freez. Control tissues showed non-specific, background staining in some taste cells and in numerous non-taste epithelial cells. Tissues treated with anti-GABA or anti-glutamate antibody showed strong immuno-reactivity at the base of the taste bud, especially in nerve fibers inner-vating the taste buds. These findings suggest that GABA and glutamate may be neurotransmitters in taste buds in *Necturus*. Supported by NIH grants NS20486 and AG06657.

302.5

A METHOD FOR ISOLATING INDIVIDUAL MAMMALIAN TASTE CELLS. A.I. Spielman , J.F. MacDonald, M. W. Salter, I. Mody, J.G. Brand* and G. Whitney*. Monell Chem. Sens. Ctr. Philadelphia., PA 19104 & Dept. Physiol., Univ. Toronto, Toronto, Ont. M5T 2S8.

Delination of the cellular mechanisms of taste reception in mammals has been limited by the difficulty of making electrophysiological recordings from individual taste cells. We present a method for acute isolation and voltage-clamp recording of single mammalian taste receptors.

Tongues were dissected from adult mice (SWR & CF1) which were killed by cervical transection. Individual folliate and vallate papillae were punched out of each tongue and pre-incubated for 1 hr in a well oxygenated divalent cation free buffer (mM): $NaHCO_3$ 26, NaH_2PO_4 2.5, NaCl 65, KCl 20, D-glucose 20, & EDTA 1, maintained at pH 7.4 & 32°C. This was followed by a 30 min incubation in 1.5 mg/ml pronase and 1 mg/ml elastase. The cells were then mechanically dissociated as previously described for CNS neurons (Mody et al, *Neurosci. Lett.*, 96: 70, 1989). Individual taste cells from the suspension attached to the bottom of plastic culture dishes. Whole cell patch clamp recordings were then made using conventional techniques. These cells demonstrated both a voltage-dependent transient inward Na^+ current and a sustained outward K^+ current (Delayed Rectifier).

In order to examine the responsiveness of these cells to taste stimuli we applied denatonium benzoate, the most "bitter" compound yet identified, to individual voltage-clamped taste cells. Pressure applications of denatonium (10 μM) produced a potent blockade of the Delayed Rectifier current in these cells.

This work was supported by BRSG (AIS), MRC of Canada (JFM, MWS & IM), NIH (JGB) & NINCDS (GW).

302.6

EVIDENCE FOR INS(1,4,5)P_3 AS A SECOND MESSENGER IN RAT TASTE RECEPTOR CELL SIGNAL TRANSDUCTION. P.M. Hwang, A. Verma, D.S. Bredt, D. Verma and S.H. Snyder. Depts. of Neuroscience, Pharmacology and Molecular Sciences, Johns Hopkins Univ. Sch. of Medicine, Balt., MD 21205.

Denatonium, a potently bitter substance, has been shown to mobilize intracellular Ca^{++} in isolated rat circumvallate (CV) taste receptor cells (Akabas, M.H., et. al., Science, 242:1047, 1988). We examined the energy-dependent sequestration of $^{45}Ca^{++}$ into 10 um frozen sections of rat CV via autoradiography. Accumulated $^{45}Ca^{++}$ was selectively localized to circumvallate papillae, which mediate bitter taste.The Ca^{++} taken up was taste bud cell-specific as demonstrated by lack of uptake post-denervation. In the presence of 100 nM Ins(1,4,5)P_3 there was a significant reduction in the amount of Ca^{++} uptake. This effect was IP_3 isomer-specific: Ins(1,4,5)P_3 = Ins(2,4,5)P_3 >> Ins(1,3,4)P_3. Other inositol phosphates such as IP_1 or IP_2 had no effect. The effect of IP_3 on Ca^{++} uptake was reversed with 100 ug/ml heparin, a known antagonist of IP_3 receptors. In preliminary experiments denatonium caused a rapid increase in mass levels of IP_3 as assayed by the IP_3 radioreceptor assay.

302.7

INNERVATION-DEPENDENT EXPRESSION OF NEURAL-RELATED ANTIGENS IN RAT TASTE CELLS. D. V. Smith, R. A. Akeson, M. T. Shipley and T. L. Belecky*. Depts. Otolaryngol. & Maxillofac. Surg., Pediatrics, Anat. & Cell Biol., Univ. Cincinnati Coll. Med. & Children's Hosp., Cincinnati, OH 45267.

Mammalian taste receptors are modified epithelial cells which are continually replaced throughout life. These cells are arranged within taste buds that are trophically dependent upon their innervation by gustatory nerves. Previous studies have reported the presence of growth-dependent neural membrane proteins on rat taste cells. Monoclonal antibodies (Mabs) directed against several neural membrane-related molecules were used for immunocytochemical study of taste buds in the rat vallate papilla. Mabs 2B8, 3F4, and 9OE recognize either neuronal cell adhesion molecule (NCAM) polypeptides or carbohydrate groups common to NCAMs and other molecules. In adult normal rats, vallate taste buds contained immunoreactivity for 2B8, 3F4 and 9OE, with a pattern of staining specific to each Mab. Subsets of taste cells were stained with 3F4 and 9OE, whereas 2B8, which also stains rat olfactory receptor neurons, appeared to be associated with all of the cells within each taste bud. Bilateral transection of the glossopharyngeal nerve resulted in the complete elimination of taste cell immunoreactivity to each of these Mabs after seven days. Earlier studies have shown that all morphologically detectable taste buds degenerate within this time. Studies are underway to determine the time course of this decline in antigenic expression, its recovery following reinnervation, and the role(s) of these antigens in the normal development of taste cells.

Supported by NS23524 and NS23348.

302.8

CALCIUM DEPENDENT ATPASE STAINING OF NORMAL AND DENERVATED FUNGIFORM TASTE BUDS. M.A. Barry and L.D. Savoy*. Dept. of BioStructure and Function, Univ. of Connecticut Health Center, Farmington, CT 06032.

These experiments are part of a study on the fate of denervated taste buds and the source of new taste bud cells following reinnervation in fungiform papillae of the golden hamster. Vallate and foliate taste bud cells show intense calcium dependent ATPase (Ca^{2+}-ATPase) activity, but fungiform papillae have not been previously examined. Ca^{2+}-ATPase activity was revealed histochemically in 10-20 μm sections of the tongue. Denervation was accomplished by cutting and devitalizing the combined chorda tympani-lingual nerve unilaterally. The normal fungiform taste bud and the connective tissue core (including nerve fibers) show intense activity relative to the remainder of the fungiform papilla and the filiform papillae. At 7 and 20 days following denervation, the taste buds are smaller than normal but still stain strongly for Ca^{2+}-ATPase. By 20 days, there are also fewer taste buds than normal on the denervated side. These results confirm recent findings that denervated fungiform taste bud cells persist rather than completely degenerate as previously thought. In addition, at least one property of taste bud cells, elevated Ca^{2+}-ATPase activity, is retained following denervation. Studies are in progress to localize Ca^{2+}-ATPase at the electron microscopic level. NS16993.

302.9

STIMULATION OF THE GERBIL'S GUSTATORY RECEPTORS BY INTENSE NATURAL SWEETENERS. W. Jakinovich Jr., Dept. of Biological Sciences, Herbert H. Lehman College and the Graduate School, City University of New York, Bronx, NY 10468; N.P.D. Nanayakkara*, R.A. Hussain*, and A. Douglas Kinghorn*, Program for Collaborative Research in the Pharmaceutical Sciences, College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60612.

The purpose of this study was to determine if the gerbil responds to the INTENSE NATURAL SWEETENERS in the same manner as the human. The gerbil's chorda tympani nerve responses were obtained to each of the following sweeteners; hernandulcin (Hern), mogroside V (Mogro), periandrin III (Peri), rebaudioside A (Reb-A), stevioside (Stev) and sucrose (Suc). As stimuli, all of these compounds were more effective than Suc; Mogro>Stev=Reb-A>Peri>Hern>Suc. By comparison, in the human, the sweetness ranking of these compounds is Hern>Reb-A>Stev=Mogro>Peri>-Suc.

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302.10

K+ CHANNEL BLOCKERS ATTENUATE IXTH NERVE RESPONSE TO QHCL BUT NOT UREA. P. E. Scott and J. Farley. Princeton Univ., Prog. Behav. Neurosci., Princeton, NJ 08541 and Indiana Univ., Prog. Neurosci., Bloomington, IN 47405.

Since K+ channels have been implicated in taste transduction processes, we sought to determine if K+ channels were involved in the rat IXth nerve response to bitter compounds. Concentration-response relations for QHCl (0.0001 M - 0.1 M) and urea (0.3 M - 4.0 M) were evaluated before and after treatment of the tongue with either the K+ channel blocker tetraethylammonium (TEA; 0.1 M) or 4-aminopyridine (4-AP; 0.01 M). TEA clearly reduced the response to QHCl but had little effect on the response to urea. The response to urea was also unaffected by treatment of the tongue with 4-AP. These results suggest that TEA and QHCl act at similar sites on primary taste neurons, presumably K+ channels. The fact that neither TEA nor 4-AP affected the IXth nerve response to urea indicates that the urea response is not mediated by TEA- or 4-AP- sensitive K+ channels. These results support a role K+ channels in the transduction of bitter taste and further support multiple receptor mechanisms for bitter taste.

302.11

ACTIONS OF HODULCIN: DYNAMICS AND KINETICS OF SUPPRESSION OF TASTE RECEPTOR CELL RESPONSES TO SUCROSE. L.M.Kennedy and D.E. Kolodny*. Dept. of Biology and Neuroscience Program, Clark Univ., Worcester, MA 01610.

Hodulcin (from Hovenia dulcis) selectively suppresses sweetness perception in humans and behavioral and receptor cell responses to sucrose in flies (Kennedy et al., Chem. Senses 13, 1988, 529; Kolodny & Kennedy, Chem. Senses 13, 545). We have been studying the effects of various concentrations of purified hodulcin (HDE) on fly (Phormia regina) receptor cell action potential responses to sucrose (in 50 mM NaCl) to determine the mechanisms of action. The concentration-effects (C-E) curve for responses to sucrose 50 mM is bell-shaped with suppression beginning at 0.025% HDE ($p<0.012$) and peaking at 0.075% HDE ($p<0.001$). While there is a significant decrease in effectiveness at 0.1% HDE ($p<0.000$), a small suppression remains ($p=0.01$)(Kruskal-Wallis,Mann-Whitney). Nonlinear regression fits for responses to sucrose 10, 20, 40, 80, 160, and 320 mM to the Michaelis-Menten equation ($r^2=0.75$-0.95, $p<0.0001$) show a decrease in Vmax (10.8-8.9- 7.4) and an increase in Km (19.6- 34.1- 48.3) as HDE concentration increases (0- 0.03- 0.07%) ($p\leq0.04$)(ANOVA). This analysis indicates that HDE concentrations on the rising phase of the C-E curve suppress sucrose responses by both competitive and noncompetitive mechanisms. Preliminary data suggest that primarily the noncompetitive mechanism acts at higher HDE concentrations ($\geq$0.1%). (Supported by NIH NS24159 to L.M.K.).

302.12

EFFECTS OF HALOPERIDOL ON BASAL AND STIMULUS EVOKED CATECHOLAMINE RELEASE FROM RABBIT CAROTID BODY. A. Gomez-Niño*, B. Dinger, C. Gonzalez and S.J. Fidone. Dept. Physiol., Univ. Utah Sch. Med., Salt Lake City, UT 84108

Previous experiments have established that the synthesis and release of dopamine (DA) are activated by natural and pharmacological stimulation of the carotid body. These data have suggested an important role for DA in chemotransmission between type I cells and afferent nerve terminals, both of which have been shown to possess DA receptors. However, the precise role of DA in chemotransmission has been confused by pharmacological experiments which demonstrate that DA can both excite and inhibit carotid sinus nerve discharge. In the present study, ^{3}H-catecholamine (^{3}H-CA; synthesized from ^{3}H-tyrosine) release was studied in carotid bodies superfused in the presence or absence of the DA receptor antagonist haloperidol (10^{-5} M). The introduction of haloperidol to carotid bodies superfused in media equilibrated with 100% O_2 increased the basal ^{3}H-CA release by 47% ($p<0.025$). Likewise, the evoked ^{3}H-CA release (stimulus-control) in the presence of hypoxic media (10% O_2-equilibrated) was elevated 5.2-fold ($p<0.001$) by haloperidol. These data suggest that DA autoreceptors, previously demonstrated to be present on type I cells, can modulate the basal and stimulus-evoked release of DA from the carotid body. Supported by USPHS grants NS12636 and NS07938.

302.13

EFFECTS OF HYPOXIC STIMULATION ON CYCLIC NUCLEOTIDE CONTENT IN THE RABBIT CAROTID BODY. W.-J. Wang*, B. Dinger and S.J. Fidone. Dept. of Physiol., Univ. of Utah Sch. of Med., Salt Lake City, UT 84108.

We have initiated experiments designed to test the involvement of cyclic nucleotides in chemotransduction and chemotransmission in the arterial chemosensory tissue of the rabbit carotid body. Carotid bodies incubated in vitro in media equilibrated with 100% O_2 contained 5.4 ± 0.36 pmole/mg tissue ($\bar{X}$ ± SEM) of cAMP and 0.068 ± 0.007 pmole/mg tissue of cGMP (RIA measurements). Exposure to incubation media equilibrated with 5% O_2 (10 min) elevated the cAMP content (19.6 ± 3.9 pmole/mg tissue; $p<0.0005$) and depressed the level of cGMP (0.046 ± 0.005; $p<0.025$). Direct activation of adenylate cyclase by forskolin (10 µM; 10 min) increased the cAMP content by 47-fold, but decreased the amount of cGMP in the tissue by 49% ($p<0.01$), suggesting a reciprocal relationship between the two cyclic nucleotides. Incubation of carotid bodies in zero Ca^{++} media to prevent neurotransmitter feedback effects on adenylate cyclase resulted in increased levels of cAMP (13.2 ± 3.3 pmole/mg tissue) under resting conditions (100% O_2-media). Furthermore, hypoxic stimulation (5% O_2-media) in zero Ca^{++} media evoked an additional increase in the cAMP content (29.9 ± 7.3 pmole/mg tissue; $p<0.05$). Thus, processes independent of neurotransmitter action may initiate the generation of cAMP. Supported by USPHS grants NS12636 and NS07938.

302.14

IMMUNOCYTOCHEMICAL STUDIES OF CYCLIC GMP IN THE NORMOXIC AND HYPOXIC RAT CAROTID BODY. Z.-Z. Wang, B. Dinger, S.J. Fidone and L.J. Stensaas. Dept. of Physiol., Univ. of Utah Sch. of Med., Salt Lake City, UT 84108

The cellular mechanism of chemosensory transduction in the carotid body remains obscure. The present study was undertaken to localize cGMP in the rat carotid body exposed in vitro to: 1) normoxic or hypoxic conditions, 2) to iso-osmolar 100 mM potassium, or 3) an activator of guanylate cyclase (sodium nitroprusside, SNP). Polyclonal antibodies to cGMP (obtained from Dr. De Vente) were used with the avidin-biotin peroxidase technique to immunostain frozen sections. Cyclic GMP immunoreactivity was present in about half of the type I cells of the rat carotid body in 100% O_2-equilibrated media. A dramatic increase in the number of cGMP-positive type I cells occurred following incubation in 100 mM K^+. Hypoxic stimulation (5% O_2, 10 min), and hypoxia combined with 100 mM K^+ greatly reduced both the number and intensity of cGMP immunostaining in type I cells. SNP (1.0 mM, 10 min) resulted in an elevation of cGMP, but mostly in vascular elements of the carotid body, and this effect was potentiated by hypoxic stimulation. These data indicate that cGMP may be involved in the transduction process by type I cells of the rat carotid body; they also provide evidence that cGMP-producing systems in type I cells and vascular elements are differentially sensitive to hypoxia and SNP. Supported by USPHS Grants NS12636 and NS07938.

302.15

EFFECTS OF HYPOXIA INDUCED BY Na-DITHIONITE ON GLOMUS CELLS IN CULTURE. L. Pang* and C. Eyzaguirre. Dept. Physiol. Univ. Utah Sch. Med., Salt Lake City, UT 84108.

The carotid body removed from 50-70 gm anesthetized rats was shredded in ice-cold Ham's F-12 saturated with O_2. The pieces were triturated with pipettes and plated on cover-glasses coated with polylysine (100 mg/l). After the cells attached to the glass (3-4 hrs), sodium selenite (1 mM), insulin (80 U/l) and $CaCl_2$ (100 mg/l) were added to the medium for incubation at 37° with 5% CO_2 in air. After 2-7 days the cultures were bathed in flowing rat saline (pH 7.43 at 31°) equilibrated with air, viewed with an inverted phase contrast microscope and impaled with microelectrodes for recording membrane potential (E_m) and input resistance (R_o). Glomus cell clusters (n=67) and isolated cells (n=34) were used. Cell diameter was 8.6±0.2 μ (SEM) and E_m -24.3±0.5 mv in both instances. R_o was 242.3±35.1 MΩ in clusters but 154.7±25.6 MΩ in isolated cells. After the controls, the preparation was bathed for 1-2 min with saline containing 1.25 mM Na-dithionite to lower saline PO_2 to 12 torr. Cells in clusters depolarized to -20.6±0.7 mV and R_o decreased to 160±33.7 MΩ. However, isolated cells hyperpolarized to -34.7±36.6 mV and R_o increased to 198± 36.6 MΩ. Differences may be due to destruction of enveloping sustentacular cells and/or intercellular communications in isolated cells. After fixation in 0.5% paraformaldehyde and 2% glyoxylic acid, glomus cells showed typical yellow-green fluorescence. Supported by grants AHA 860662, NS05666 and NS07938.

SPINAL CORD

303.1

PROPERTIES OF DORSAL HORN NEURONS STUDIED INTRACELLULARLY AND THEIR RELATIONSHIP TO CHANGES SEEN AFTER LESIONS. L.M. Pubols, R.S. Dow Neurol. Sci. Inst., Good Samaritan Hosp. & Med. Ctr., Portland, OR 97210.

Previous work (L. Pubols et al., J. Neurophysiol. 60:1253, 1988) revealed that chronic dorsolateral funiculus (DLF) lesions increase the percentage of dorsal horn neurons responsive to sural nerve stimulation. The present study was designed to explore the hypothesis that strengthening of subliminal inputs is responsible for this change. To accomplish this, the response properties of 35 cat L_6 and L_7 dorsal horn cells were studied intracellularly, and the lesion data were reexamined in light of the findings.

Results of this study support the stated hypothesis in two ways: 1) Within broad limits the percentage of cells giving only EPSPs to sural nerve stimulation matched the postlesion increase in the percent of cells giving impulses to this stimulus. 2) Cells giving only EPSPs to sural nerve stimulation were predominantly those that had low threshold receptive fields on the proximal hindlimb. The postlesion increase in cells responding with impulses to sural nerve stimulation occurred primarily within this subpopulation.

No purely subliminal cutaneous receptive fields (RFs) were observed using natural stimuli. However, subliminal responses were commonly observed within the supraliminal RFs of these cells. (Support:NIH,NS19523)

303.2

WITHDRAWN

303.3

DETERMINATION OF THE CENTRAL DELAY OF THE DORSAL ROOT REFLEX IN AN ISOLATED MAMMALIAN SPINAL CORD PREPARATION. J.BAGUST*, Y.CHEN* and G.A.KERKUT* (SPON: Brain Research Association), Dept. of Neurophysiology, Univ. of Southampton. U.K. SO9 3TU.

Dorsal root reflexes can be evoked in lumbar spinal roots by stimulation of adjacent dorsal roots or ascending branches of afferent fibres in the dorsal tracts of the cord. Changes in latency of the dorsal root reflex with distance between the site of stimulation and recording have been used to calculate the conduction velocity of those fibres responsible for evoking the reflex, and the central delay in the generation of the reflex. Isolated whole spinal cord preparations from adult golden hamsters were maintained at 25-27°C, and recordings made of evoked activity in lumbar dorsal roots following stimulation of dorsal tracts at 5mm intervals. The dorsal root response consisted of a directly conducted volley, followed by a synaptically generated reflex. Plots of distance against latency of these two components produced straight lines with similar gradients (mean conducted component 5.1 ±0.3m/s, reflex 4.5 ±0.2 m/s, n=7) but different intercepts on the time axis (mean 1.0 ±0.2ms for the conducted activity and 5.2 ±0.3ms for the reflex,n=7). This difference of 4.2ms represents the time for the generation of the dorsal root reflex within the cord, and indicates a polysynaptic connection between the stimulated fibres and those carrying the dorsal root reflex.

303.4

SERIAL-SECTION ELECTRON MICROSCOPE ANALYSIS OF AN ENTIRE Ia AFFERENT COLLATERAL IN THE CAT SPINAL CORD. M.J. Nicol* and B. Walmsley. Neural Research Laboratory, School of Anatomy, Univ. of N.S.W., Kensington, 2033, Australia

The present study was aimed at obtaining ultrastructural evidence concerning our previous proposal that there is considerable non-uniformity in the probability of quantal transmitter release among release sites arising from the same primary afferent fiber.

Serial-section electron microscopy was used to examine an entire HRP-labeled group Ia afferent collateral in Clarke's column of the cat spinal cord. A wide range of geometries and myelination patterns were found along the collateral, including: a) conventional nodes of Ranvier, b) nodes exhibiting a single synaptic bouton, c) nodes with two or more synaptic boutons connected by thin unmyelinated branches, d) terminal heminodes giving rise to one or more synaptic boutons separated by unmyelinated branches, e) complex arrangements of myelinated and unmyelinated branches giving rise to a number of boutons.

The afferent collateral gave rise to 35 synaptic boutons which contained synaptic specializations exhibiting a wide range of sizes and shapes, including perforations.

The geometry and myelination pattern of the presynaptic axon and its branches play an important role in determining the amplitude and duration of an action potential. The varied axonal geometries observed in the present study may contribute to non-uniformities in transmitter release from synaptic boutons along different branches. Morphological differences in the synaptic specializations located within synaptic boutons may also reflect intrinsic differences in the efficacy of transmitter release from different release sites.

303.5

SOMATOSENSORY INPUT FROM LUMBAR PARASPINAL TISSUES: ANATOMICAL TERMINATIONS AND NEURONAL RESPONSES TO MECHANICAL AND SYMPATHETIC STIMULI. W.J. Roberts, R.G. Gillette* & R.C. Kramis*, R.S.Dow Neurol. Sci. Inst., Good Samaritan Hosp. & Med. Ctr., Portland, OR 97209.

Chronic pain referred to deep tissues of the lower back is common in the human population. The physiological bases for low back pain are not well understood, partly because few studies have been done of somatosensory systems relating to paraspinal tissues. We have therefore undertaken studies in anesthetized cats - to anatomically determine the spinal projections from primary afferent fibers innervating paraspinal tissues and to record activity from dorsal horn neurons responsive to mechanical stimulation of paraspinal tissues.

Our preliminary studies using HRP labeling of populations of paraspinal afferent fibers indicate that: many afferents terminate bilaterally in lamina I, II, V & X; the rostro-caudal extent of the afferent terminals appears to be more extensive than that reported for afferents from the limbs; and a small percentage of these afferents have terminals in the cervical dorsal horn.

Preliminary recordings from dorsal horn neurons with paraspinal receptive fields have indicated that: many of the neurons receive convergent input from both skin and deep tissues; some have bilateral receptive fields; many have additional receptive fields in deep tissues of the hindlimb; and many are responsive to sympathetically-evoked afferent activity.

303.6

INTEGRATION OF THE SYNAPTIC POTENTIALS IN DORSAL HORN NEURONS EVOKED BY ELECTRICAL STIMULATION OF ADJACENT SPINAL ROOTS. S. Jeftinija, Department of Veterinary Anatomy, Iowa State University, Ames, Iowa 50011.

To study the integration of synaptic potentials in dorsal horn (DH) neurons, a horizontal spinal cord slice (400-500μm thick) and the functionally connected dorsal roots (DR) with the dorsal root ganglion (DRG) were prepared from 18-28 day old rats. Conventional intracellular recording from DH neurons using 3M K-acetate-filled electrodes was employed. Dorsal roots were electrically isolated from the spinal cord and stimulated with pulses of different duration and intensity.

Results are based on the recordings from 35 DH neurons located in the three most superficial laminae of the spinal DH. All of the neurons responded to the stimulation of both roots. In the majority of the cases the pattern of response of a particular neuron following the stimulation of one DR was identical or very similar to that following stimulation of the second DR. In a minority of cases, however, the response pattern evoked by stimulating one DR was different from that evoked by stimulating the neighboring DR. During the course of the experiments in which two adjacent DR were stimulated, the excitatory postsynaptic potentials (EPSP) recorded from single DH neuron did not summate. In several cases in which neuron responded differently to stimulation of the adjacent DR, a interaction was observed. An inhibitory postsynaptic potential evoked by stimulation of one DR induced a partial inhibition of excitatory transmission evoked by second DR stimulation. Supported by USDA Grant PL95-113.

303.7

PHYSIOLOGICAL STUDIES OF DORSAL HORN NEURONS IN LAMINAE I-IV OF ADULT CHICKENS. C. J. Woodbury* (SPON: A. Carlson), Dept. of Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook, N.Y. 11794.

The central projections of hindlimb cutaneous nerves of hatchling chickens were previously shown to form two somatotopic maps across the mediolateral axis of the superficial dorsal horn (DH) (Neurosci. Abst. 13:1387, 1987). The medial map fell within lamina III and the lateral map fell within lamina II of Brinkman and Martin (Br. Res. 56:43, 1973). These maps can be differentially labeled by choleragenoid- and WGA-HRP conjugates, respectively, which suggests that afferents segregate mediolaterally in chickens according to size and/or modality. In the present study I examined this possibility by investigating the conduction velocity (CV) and modality of cutaneous inputs to neurons in DH laminae I-IV of spinal, adult chickens anesthetized with α-chloralose. Glass micropipettes (2% Fast Green/2% NaCl) were used to record from units in segment LS8 that were activated by brief shocks (above C-fiber thresholds) applied to the posterior femoral cutaneous nerve. Multiunit recordings (1-2 MOhm electrodes) in lamina III-IV contained only a single, short latency response, regardless of stimulus intensity. Similar recordings in lamina I-II contained both a short and long latency component, the latter generated by afferents with a CV around 1 m/sec. Afferents to single neurons in lamina III-IV (12-18 MOhm electrodes) had CVs of 41 ± 16.6 m/sec, and most were briskly activated by light puffs of air across the receptive field (RF). In contrast, afferents to single neurons in lamina I-II had CVs of 10.8 ± 9.7 m/sec, and 24 % of these neurons were only activated by afferents with a CV below 2 m/sec. Further, most of the RFs of lamina I/II neurons could not be located by stimulating the skin with air or light touch; when the RF was found, a higher intensity stimulus (e.g., pressure or pinch) was usually required to activate the neuron. (Supported by an NIMH Predoctoral Training Grant, a Sigma Xi Grant-in-aid-of-research, and NSF and NIH grants to S. A. Scott.)

303.8

DORSAL HORN TERMINATION PATTERNS OF CUTANEOUS LOW THRESHOLD MECHANORECEPTORS. R. Sonty, J. Culberson, W. Gladfelter, R. Millecchia, and P. Brown. Physiology Dept., W. Va. Univ. Health Sci. Ctr., Morgantown, WV 26506.

A. G. Brown et al. (1981) reported that dorsal horn terminal distributions of hair afferents (HFA) and Pacinian corpuscles (PC) were rostrocaudally continuous; and rapidly adapting glabrous (RAG), slowly adapting type I (SA1), and slowly adapting type II (SA2) were rostrocaudally discontinuous. Only PCs and some SA1s and SA2s had projections ventral to lamina IV.

Cutaneous axons were impaled with horseradish peroxidase (HRP)-filled microelectrodes, and after determination of afferent type and receptive field location the axons were injected with HRP. The sample included 13 HFAs, 11 SA1s, 4 SA2s, 2 PCs, and 1 RAG. Although occasional rostrocaudal discontinuities were seen, they were irregular within axons and inconsistent across axons, and not correlated with afferent type. Projections ventral to lamina IV were seen in PC, SA1, SA2, and hair; these were generally wider than, and roughly in register mediolaterally and rostrocaudally with, more dorsal projections. The reasons for discrepancies between the two studies are not obvious.

303.9

CHARACTERIZATION OF SPINAL NEURONS CONTAINING A SELECTIVELY DISTRIBUTED NERVE TERMINAL PROTEIN. S.S. Kanekar, T.C. Ritchie and J.D. Coulter, Neuroscience Program and Dept. of Anatomy, The University of Iowa, Iowa City, IA.

NT75 is a neuron-specific 75kD protein recognized by the S-7B8 monoclonal antibody. NT75 is localized in select populations of nerve terminals and is not detectable in cell bodies and axons. The distribution of NT75 does not parallel that of any known neurochemical system, although this protein occurs in many putative excitatory amino acid pathways. In spinal cord, dense NT75 immunoreactivity in the superficial dorsal horn is contained in primary sensory nerve terminals. Nerve terminals of the corticospinal tract and other descending systems contribute to the moderate staining in the deeper dorsal horn and sparse staining in the ventral horn. The current study examines whether intrinsic spinal neurons contain NT75. After the lumbar cord is exposed to colchicine for 18-24 hrs, neurons of various morphological types and sizes within all spinal laminae exhibit NT75 labeling. Lamina I contains larger stained cells with horizontal processes, and small cells. Lamina II, however, contains only a few NT75-positive small cells. Large multipolar neurons containing NT75 are seen in the lateral spinal nucleus. Laminae III to VII contain numerous labeled small neurons, and a few larger neurons stain in the medial part of lamina VII. Laminae VIII and IX exhibit intensely labeled motorneurons, and a few small cells. Small NT75-positive neurons are clustered in Lamina X. The distribution of some NT75 cells suggests they may be ascending tract neurons, and dense NT75 staining occurs in the ventrobasal thalamus and the terminal fields of other ascending spinal systems. Further studies will characterize NT75 positive spinal neurons and will determine whether NT75 is a constituent of motor nerve terminals. Supported by NS23783.

303.10

SLOW EPSP IN CAT SPINAL NOCICEPTIVE NEURONES *IN VIVO* POTENTIATES VIBRATION-INDUCED INHIBITION: IMPLICATION OF NEUROKININS AND PURINES. Y. De Koninck and J.L. Henry. Departements of Physiology and Anaesthesia Research, McGill University, Montreal, Que.

We have suggested that exogenous application of tachykinins potentiates the purine-induced inhibition of dorsal horn neurones either by exogenous application of ATP or AMP (Salter & Henry, *Neurosci.*, 22:631, 1987) or by endogenous release in response to a peripherally applied vibration stimulus (Salter & Henry *Neurosci.*, 27:243, 1988; *Neurosci.*, 23:903,1987). In the present study we investigated the possibility that the endogenous release of a neurokinin may also potentiate the purine-mediated inhibition of dorsal horn neurones in the chloralose anaesthetised or decerebrated cat. Extracellularly, a prolonged excitation was obtained following a train (10-20Hz for 5-10s) of high intensity electrical stimulation to the afferent nerves. This excitation peaked 15-25s after the start of the train and decayed slowly over 1 to 6 min. The intensity-response relationship indicated the mediation of high threshold afferents in this response. Intracellular recording (KCl, K-Ac or K-CH_3SO_4) revealed a slow prolonged depolarization associated with an apparent increase in input resistance. This EPSP had the same time course as the excitation observed with extracellular recording and had similar characteristics to that reported by Urban & Randic (*Brain Res.*, 290:336, 1984) after a train stimulation of dorsal rootlets in the rat spinal slice and in which a neurokinin was implicated (Randic et al, *Brain Res.*, 383:15, 1986). Interaction between this slow EPSP and the inhibition obtained by peripheral vibration was studied. The inhibition was potentiated according to previously defined criteria (*Neurosci.*, 23:903,1987) when occurring during excitation following the train stimulation (n=7) as opposed to excitation by iontophoretic application of glutamate. In two cases, the response to vibration changed from excitation to inhibition during the post train EPSP. These results suggest that noxious stimulation induces the release of a neurokinin which may in turn increase the sensitivity of spinal nociceptive neurones to the purine-mediated, vibration-induced inhibition. Supported by MRC grant MA-5891 to JLH, YDK is a stagiaire de recherche of the FRSQ.

303.11

GLUTAMINASE IMMUNOREACTIVITY IN RAT SPINAL CORD. K.E. Miller, T. Kaneko, and A.T. Salvatierra. G.D.Searle R&D, Monsanto Co., St. Louis, MO 63198; Dept. Anatomy, Kyoto Univ., Kyoto 606, Japan; Dept. Neurol. Surg., Univ. Miami, Miami, FL 33136.

Glutamate is considered to be a neurotransmitter in sensory systems and glutamate-like immunoreactivity has been localized in the dorsal horn of spinal cord (Miller et al., Synapse 2:28,'88). To further understand the distribution of neurons that may use glutamate as a neurotransmitter, immunohistochemistry was performed on rat spinal cord using a monoclonal antibody against glutaminase, the enzyme that converts glutamine to glutamate (Kaneko et al., J.Neurosci. 7:302,'87). Rats (300g) were anesthetized and perfused with fixative. The spinal cords were dissected and frozen sectioned at 30um. The sections were processed for glutaminase immunoreactivity with avidin-biotin-peroxidase. Immunoreactive (IR) puncta were observed in the superficial dorsal horn. IR neurons were found most prominently in following locations: the marginal zone, lateral spinal nucleus, central gray region (lamina X), and intermediolateral cell column. These results support the hypothesis that glutamate is used as neurotransmitter in spinal sensory pathways. It also suggests that glutamate is important in regulating sympathetic activity. Supported by the Miami Project to Cure Paralysis.

303.12

INTRATHECAL GALANIN SELECTIVELY BLOCKS THE FACILITATORY EFFECT OF SUBSTANCE P AND CALCITONIN GENE-RELATED PEPTIDE ON THE SPINAL CORD. Z. Wiesenfeld-Hallin[1], X-J. Xu[1]*, M.J. Villar[2]* and T. Hökfelt[2]*, Karolinska Institute, Huddinge[1] and Stockholm[2], Sweden.

Galanin (GAL), substance P(SP) and calcitonin gene-related peptide (CGRP)-like immunoreactivities coexist in some primary afferents. We examined the effects of intrathecal (i.t.) application of these peptides on spinal flexor reflex excitability in decerebrate, spinalized, unanesthetized rats.

I.t. SP or CGRP caused a brief facilitation of the flexor reflex. GAL, which by itself facilitated the reflex at the dose employed, was applied 2-4 min prior to SP or CGRP and reduced or totally blocked the facilitation evoked by these neuropeptides. In contrast, the facilitatory effect of i.t. somatostatin or vasoactive intestinal polypeptide, which do not coexist with GAL in primary afferents was not reduced by the preadministration of GAL.

These results may clarify the mechanisms underlying our previous observation that i.t. GAL inhibits the facilitatory effect of C-afferent stimulation on spinal excitability. It is concluded that i.t. GAL may exert a selective postsynaptic inhibitory effect on the spinal cord by blocking the facilitation caused by SP and CGRP, neuropeptides released at the central terminals of C-afferents.

303.13

NEUROPEPTIDES IN THE RAT SPINAL CORD: LOCALIZATION OF BN/GRP- AND FLFQPQRF-AMIDE-LIKE IMMUNOREACTIVITIES IN NEURONS. L. Kivipelto and P. Panula. Dept.Anat., Univ.Helsinki, 00170 Helsinki, Finland.

GRP_{18-27} and FLFQPQRF-amide-like peptides are found in the superficial laminae of the rat spinal cord, where they are probably involved in sensory neurotransmission. Most of the GRP_{18-27} is derived from the spinal ganglia, while there is no evidence of the presence of FLFQPQRF-amide-like peptides in the spinal ganglia. Biochemical and immunohistochemical studies after lesions have suggested that both peptides may be localized in spinal cord neurons, but direct evidence is lacking.

Adult rats treated with intraspinal colchicine were perfused with 4o/o paraformaldehyde. Cryostat sections of the spinal cord were incubated with antisera against GRP_{18-27} and FLFQPQRF-amide and the PAP method was applied. Immunoreactive neurons were seen with both antisera in laminae I-II and X. Preadsorption of the antisera with corresponding peptides abolished the immunoreaction, while unrelated peptides did not affect the staining. The results suggest that neurons in laminae I-II and X give rise to those nerve fibers immunoreactive for these peptides that remain after rhizotomy and transsection of the spinal cord.

303.14

SPECIFIC ANTAGONISM OF THE ANTINOCICEPTIVE EFFECTS OF INTRATHECAL DPDPE BY NALTRINDOLE IN RATS. E.J. Drower[1], A. Stapelfeld*[1], M.F. Rafferty[1], D.L. Hammond[2], K.C. Rice[3], and B. DeCosta*[3]. [1]G.D. Searle & Co.,Skokie, IL 60077; [2]Dept. of Anesthesia & Critical Care, Univ. of Chicago, Chicago, IL 60637; and [3]NIDDK/NIH, Bethesda MD, 20892.

Portoghese et al. recently reported the discovery of Naltrindole (NTI),a potent and selective delta opioid receptor antagonist in smooth muscle assays (Eur. J. Pharm.(1988)146:185). Although the authors indicated that NTI also selectively antagonized the in vivo effects of a delta agonist (DSLET), no experimental details were presented. In this study, we have evaluated the ability of NTI to antagonize the effects of intrathecal (i.t.) DPDPE, a highly selective delta agonist, and morphine on tail flick latency. (TFL) in rats. Intrathecal administration of 10 ug DPDPE produced a significant increase in TFL which was maximal at 10 min and persisted for up to 90 min. Administration of NTI (0.01-10 ug i.t.) 10 min after DPDPE dose-dependently antagonized the effect of DPDPE. The 1 ug dose completely reversed TFL to control levels within 30 min. NTI at a dose of 30 ug had no effect alone, nor did it antagonize the antinociceptive actions of i.t. morphine (1 ug). These data support the in vitro finding that NTI is a potent and selective antagonist of delta receptor-mediated responses, and indicate that the antinociceptive effects of i.t. DPDPE result from activation of delta receptors in the spinal cord.

303.15

PHYSIOLOGY OF SINGLE SPINAL CORD SENSORY NEURONS RESPONDING TO PENILE STIMULATION IN THE RAT. R.D. Johnson. Dept. of Physiological Sciences, University of Florida, School of Veterinary Medicine, Gainesville, FL 32610.

Normal erectile and ejaculatory events in the male rat are dependent on intact sensory pathways from the penis. Because little is known about the physiology of single dorsal horn neurons responding primarily to low threshold tactile stimulation of the penis, this study was undertaken to find these neurons and describe some of their physiological attributes.

Male Sprague-Dawley rats were anesthetized and maintained with I.V. urethane for the duration of the acute experiments. The spinal cord was transected at T7-8 to remove supraspinal inhibition. Following a thoracolumbar laminectomy, tungsten microelectrodes were advanced into the spinal cord to obtain extracellular recordings from single neurons responding primarily to penile stimulation.

Single neurons were isolated from the medial portions of the dorsal horn and intermediate zone from the T13-L1 junction to the caudal L6 spinal cord segments. The neurons had penile receptive fields that were at least 5 times bigger than single primary afferent fields suggesting a convergent input. Most of the neurons responded vigorously to slight indentation (60 mg von Frey hair) or stretch of either the ventral glans midline (frenulum) and the distal prepuce or the distal glans (cup). Some of the spontaneously firing neurons exhibited convergent excitatory and inhibitory effects from noxious stimulation of the perineal region or thigh. Some of the low threshold penile neurons also responded to noxious pinch and thermal stimulation of the penis.

The results of this study indicate that information from low threshold penile mechanoreceptors is distributed to sensory neurons throughout the lumbar spinal cord and their response patterns suggest they may play a role in the penile reflexes of the rat.

303.16

APPARENT SELECTIVE ANESTHETIC EFFECTS ON SPINAL DORSAL HORN NEURONS IN PHYSIOLOGICALLY INTACT, AWAKE, DRUG-FREE CATS

J.G. Collins, H. Iwasaki,* Y. Saito*, Dept. of Anesth., Yale Univ. Sch. Med., New Haven, CT 06510

Tonic inhibition of spinal dorsal horn neurons is known to exist and is likely to be influenced by general anesthetics. This study is part of a long-range project designed to evaluate the pharmacology of anesthetic effects on spinal dorsal horn neurons.

Extracellular single unit activity was recorded from the spinal dorsal horn of physiologically intact, awake, drug-free cats (IACUC approved protocol). Following baseline studies, general anesthetics (ketamine, 10 mg/kg, propofol, 7.5 mg/kg) were administered intravenously and stimulus response properties were re-evaluated. Interactions with methysergide (non-specific 5HT antagonist) were also evaluated.

Previous work demonstrated significant but at times different effects of pentobarbital and methysergide on both noxiously and non-noxiously evoked activity. Ketamine, at the dose studied, produced no such changes, but did alter the ability of methysergide to produce observed changes. Propofol produced changes in responses to noxious and non-noxious stimulation similar to those caused by both pentobarbital and methysergide. Although the observed changes were similar following propofol and methysergide administration, the two drugs did not always produce similar changes in the same neurons. These studies demonstrate the complex effects of anesthetics on tonic inhibition of spinal dorsal horn neurons, both in the selectivity of individual agents and each agent's specificity for particular neurotransmitter systems.

Supported in part by NIH GM 29065

303.17

TUNING OF SPINAL NETWORKS TO TEMPORAL CHARACTERISTICS OF SPIKE TRAINS IN SINGLE PRIMARY AFFERENTS. H.R. Koerber, A.W. Seymour and L.M. Mendell, Dept. of Neurobiology and Behavior, SUNY, Stony Brook, NY 11794.

Single primary afferents were impaled in the L7 dorsal root ganglia of α-chloralose anesthetized cats. Spike trains were recorded in response to natural stimulation of the fiber's receptive field (RF). Two such trains were chosen as standard test trains to study the ability of different central networks to process physiologically realistic frequency modulated afferent activity. One train was recorded in a hair follicle afferent fiber in response to a brief puff of air applied to the RF and consisted of 6 APs. The second was recorded in a slowly adapting type 1 (SA1) afferent fiber in response to a stroke across the RF using a small probe and consisted of 9 APs. These spike trains were delivered to impaled primary afferents (1 train/2s) while recording the central response as either cord dorsum field potentials (CDPs: see Koerber and Mendell, J. Neurophysiol. 60), extracellular single unit activity or intracellular EPSPs and APs. CDP recordings reveal that dorsal horn networks responding to stimulation of Aβ rapidly adapting afferents (e.g. Aβ hair follicle and RA pad afferents) are, with the exception of Pacinian corpuscles (PCs), tuned to respond preferentially to the first AP or pair of APs (interval <5ms) of the spike train. PC and Aδ-Down hair follicle afferent activated networks are also tuned to short intervals but require greater numbers of APs to activate them; single shocks or long interval (50ms) pairs of shocks usually either failed to or only weakly activated these networks. SA afferent networks tend to reproduce the afferent activity spike for spike as do Aβ HTMR afferent-supplied networks. Activity recorded in single dorsal horn neurons (extracellular spikes; intracellular EPSPs and APs) in response to these same spike trains in Aβ hair follicle afferents and SAs are consistent with these findings using CDPs. Supported by NS 23725 (HRK) and NS 16996; NS 14899 (LMM).

303.18

CROSSED DORSAL HORN CELL DENDRITES. W. Gladfelter, R. Sonty, J. Culberson, R. Millecchia, R. Ammar, and P. Brown. Physiol. Dept., W. Va. Univ. Health Sci. Ctr., Morgantown, WV 26506.

Receptive fields (RFs) in lateral or medial dorsal horn often span the dorsal or ventral body midline. Dermatomes are unilateral, so crossed RF components must be mediated by crossed afferent projections, cell dendrites, or internuncials. We have found crossed afferents. This study tested for crossed dendrites.

In anesthetized cats, sacrocaudal cells were penetrated with horseradish peroxidase (HRP)-filled microelectrodes, their RFs were mapped, and HRP was injected. Sections were processed with cobalt-enhanced diaminobenzidene.

All three cells with RFs spanning the dorsal midline had crossed dendrites; the one cell with a RF spanning the ventral midline had none. Of three cells with dorsal RFs not spanning the midline, one had crossed dendrites; all three with uncrossed ventral RFs had crossed dendrites. The one cell with a lateral RF had no crossed dendrites. Thus, there was a high correlation between crossed or uncrossed dorsal RFs and crossed or uncrossed dendrites (five out six). However, there was a perfect negative correlation for the four cells with ventral RFs.

303.19

BULBOSPINAL SEROTONINERGIC PATHWAYS IN THE FROG, *Rana Pipiens*. H. Tan* and V. Miletic. Dept. Comp. Biosci., Sch. Vet. Med., Univ. of Wisconsin, Madison, WI 53706.

We combined retrograde tracing and rhodamine immunofluorescence to identify the origin of serotoninergic (5HT) neurons with descending projections to the spinal cord of the frog. After spinal injections of Fluoro-gold, retrogradely-labeled 5HT neurons were detected in the caudal part of the brainstem. These doubly-labeled cells were distributed along the midline throughout the rostrocaudal extent of the dorsal portion of the raphe nuclear region.

The fluorescent tracer 1,1'-dioctadecyl-3,3,3,'3'-tetramethyl-indocarbocyanine perchlorate (DiI) was then placed in the middle and rostral raphe nuclear area. Labeled fibers could be traced bilaterally in the lateral portion of the dorsal funiculus and the lateral and ventral funiculi. These fibers were seen terminating in the dorsal and ventral horns, as well as the intermediate grey matter. After placement of DiI in the caudal raphe area, labeled fibers were found only in the intermediate grey and ventral horn.

These findings suggest that the organization of bulbospinal 5HT pathways in amphibians is similar to that of mammals, and that an isolated frog spinal cord preparation could be a useful model for pharmacological and physiological studies of the mechanisms of 5HT action in the spinal cord. (Supported by NS21278).

303.20

ORGANISATION OF EXCITATORY AND INHIBITORY CUTANEOUS INPUT TO POSTSYNAPTIC DORSAL COLUMN NEURONES IN THE ANAESTHETISED CAT. C.Pover and A.Kitchin* Dept. Preclin.Vet.Sci., Univ. Edinburgh, Summerhall, Edinburgh, EH9 1QH, U.K.

Postsynaptic dorsal column (PSDC) neurones relay cutaneous afferent information from the dorsal horn to the dorsal column nuclei; they respond to different types of stimuli applied to glabrous and hairy skin and may have complex receptive fields (RF) on discontinuous areas from the toe to the thigh. They may also be inhibited by mechanical stimulation adjacent to, overlapping or surrounding the excitatory field. (Noble & Riddell, J.Phys., 396: 497, 1988). Similar cutaneous evoked inhibition of neurones in the spinocervical tract (SCT) a parallel system linking other dorsal horn neurones with the lateral cervical nucleus via the ipsilateral dorsolateral funiculus has been shown to arise from both inside and outside the receptive field following air-jet or piezo-electric mechanical stimulation (Brown et al, pers.comm.) We are testing PSDC neurones in the same way and preliminary results indicate that conditioning and test stimuli applied within the RF produce inhibition which is related to both the spatial and the time separation of the two stimuli. It therefore seems likely that at least two sorts of peripheral inhibition may modify the responses of SCT and PSDC neurones to excitatory input and this could represent a system for refining or eliminating excess information. Supported by the Medical Research Council.

303.21

THALAMICALLY PROJECTING CELLS OF THE LATERAL CERVICAL NUCLEUS IN MONKEY, M.V. SMITH*, G.L. STANLEY*, and A.V. APKARIAN, (SPON: R.T. STEVENS), Dept. of Neurosurg., SUNY Hlt. Sci. Ctr., Syracuse, NY 13210.

The thalamic projection from the primate lateral cervical nucleus (LCN) was studied using retrograde transport of horseradish peroxidase injected into the thalamus. Four macaque and two squirrel monkeys were used. Retrogradely labeled LCN cells were examined in spinal tissue reacted with tetramethyl benzidine.

The size of the thalamic projection from LCN was found to be about 500 cells, unilaterally. 80 to 90% of labeled LCN neurons were located in the first two spinal segments. In the medulla and C1 LCN cells formed a distinct group off the tip of the dorsal horn, in C2 they were separated into two or more groups, and in C3 the cells were oriented along the axis of the dorsal horn. In two animals, the distance of the shortest and longest perpendicular axes of individual labeled cells were measured. This analysis revealed that thalamically projecting LCN cells become smaller in size, and more oblong in shape in caudal segments ($p<0.05$). In one additional macaque, all LCN cells were counted using Nissl stain. The estimated number of LCN cells in this monkey was 2,640 on one side. The smaller number of thalamically projecting LCN cells as compared to the total size of LCN indicates that only a fraction of LCN cells project to the thalamus in monkey.

304.1

GONADAL STEROIDS MAINTAIN DENDRITIC MORPHOLOGY OF NEURONS IN THE MEDIAL NUCLEUS OF THE AMYGDALA IN MALE SYRIAN HAMSTERS. D. M. Gomez, S. W. Newman. Dept. Anatomy & Cell Biology, University of Michigan, Ann Arbor, MI 48109

The adult male Syrian hamster is dependent upon both environmental chemosensory and internal hormonal signals for normal reproductive behavior. Because the medial amygdaloid nucleus (M) is the first area in the chemosensory pathway that contains gonadal steroid accumulating cells, we hypothesized that gonadal steroids maintain the dendritic length and arborization of neurons in this area and thereby maintain the integrity of this behaviorally essential pathway. Our previous studies demonstrated that neurons in caudal, but not in rostral, M were altered by castration. In the present study, we quantitatively analyzed the dendrites of Golgi stained neurons in caudal M from adult male Syrian hamsters in the following six groups of animals: reproductively intact animals (n=12), castrated animals immediately implanted with a blank silastic capsule (n=12), or a capsule filled with either testosterone (T,n=13), dihydrotestosterone (DHT,n=13), estradiol (E,n=12), or two capsules, DHT and E (n=8). Animals were tested for copulatory behavior to assess the effectiveness of the capsules. All animals were perfused at 12 weeks postcastration and the brains were processed using a Ramon Moliner modification of the Golgi technique. Neurons were analyzed using a digitizing tablet linked to a computerized measurement program. Two measures were recorded for each neuron: total dendritic length (TDL) and highest dendritic branch (HDB). Mean values of the treatment groups were compared using ANOVA. Consistent with previous findings, neurons from castrated animals without hormonal replacement had fewer distal dendritic branches than intact animals ($p<0.01$). These neurons also had shorter TDL, although the difference was not statistically significant ($p<0.06$). Total dendritic length and the dendritic branching pattern of castrates treated with DHT were not different from castrates but were significantly different from intacts (TDL $p<0.02$; HDB $p<0.02$). Neurons from T, E and the DHT+E groups were not different from intacts. These findings suggest that neurons in caudal M do not maintain their normal dendritic structure after long term castration or in the presence of DHT alone, whereas the presence of T, E or both DHT and E maintains the normal dendritic morphology. (Supported by NIH, NS#20629 to SWN)

304.2

THE UPTAKE OF MEDROXYPROGESTERONE ACETATE BY THE PRIMATE BRAIN: IN VIVO COMPETITION STUDIES. R.W. Bonsall*, H.D. Rees and R.P. Michael, Department of Psychiatry, Emory University School of Medicine, Atlanta, Georgia 30322, and the Georgia Mental Health Institute, Atlanta, Georgia 30306.

Medroxyprogesterone acetate (MPA) is a synthetic progestin that has been used in the treatment of sex-offenders. It has androgen-depleting actions and reduces the sexual activity of male macaques. To investigate its sites of action and the receptor systems that might be involved, we castrated 8 adult male cynomolgus monkeys and administered i.m. 40 mg progesterone (P)(2 males), 10 mg dihydrotestosterone propionate (DHTP)(3 males) or oil vehicle (controls)(3 males). Twenty-four hours after castration, all males were injected i.v. with 5 mCi ^{3}H-MPA, and killed 60 min later. Left halves of the brains were processed for thaw-mount autoradiography and right halves were analyzed by HPLC to measure the uptake of ^{3}H-MPA in nuclear fractions from 14 different brain regions. In control males, there were labeled neurons in the ventromedial nucleus (n.), arcuate n., medial preoptic n. and anterior hypothalamic area. In P-pretreated males, percentages of labeled neurons in these regions were reduced to 2-31% of control values ($P<0.05$), but in DHTP-pretreated males, percentages were not significantly different from those in controls. Nuclear concentrations of ^{3}H-MPA measured by HPLC were highest in the hypothalamus, preoptic area and amygdala of controls. Pretreatments with P reduced these levels to 15-42% of control values ($P<0.01$), but DHTP pretreatments had no significant effect. Pretreatments did not alter the concentrations of ^{3}H-MPA in blood and in tissue supernatants. The effects of MPA in the primate brain may be mediated mainly by progestin-target neurons.

This work was supported by USPHS grant MH 19506 and by the Georgia Department of Human Resources.

304.3

A HORMONAL ROLE FOR A CHOLECYSTOKININ-LIKE PEPTIDE IN THE CONTROL OF THE GASTRIC MILL IN LOBSTER. G. Turrigiano* (SPON: A.I Selverston). Dept. of Biology, U.C.S.D, La Jolla, Ca 92093

The stomatogastric ganglion (STG) contains the gastric mill(GM) central pattern generator(CPG), which controls rhythmic contractions of the teeth. Previous work has shown that the stomatogastric nervous system contains a cholecystokinin(CCK)-like peptide, a CCK-like peptide can be released into the STG, and the output of the GM can be modulated by bath application of such peptides (Turrigiano, G., and A.I. Selverston, J. Neurosci, in press, 1989). The purpose of this study was to determine whether CCK-like peptides have a hormonal role in the control of the GM.

The pericardial plexus, a well-described crustacean neurosecretory organ, was found to be strongly immunopositive for CCK-like peptides. There were detectable levels of CCK-like peptides in the hemolymph, as determined by radioimmunoassay. When food-deprived lobsters were fed, there was on average a 4-fold elevation in the levels of CCK-like peptide in the hemolymph, with an average duration of 4 to 6 hours (n=7). The activity of the GM was monitored in freely moving lobsters using teflon-coated silver wires inserted into GM muscles; the GM was found to be strongly activated for the first 4 hours following ingestion, with a time-course similar to that of the elevation in levels of CCK-like peptides in the hemolymph. Finally, injections of CCK into the hemolymph were able to activate the GM (n=5). These data suggest that a CCK-like peptide in lobster has a hormonal role in the control of the gastric mill.

304.4

IMMUNOCYTOCHEMICAL CHARACTERIZATION OF PROJECTION - NEURONS IN THE VOCAL MOTOR SYSTEM OF CANARIES.
W.Zuschratter[1]*, M.Gahr[2], H.R. Güttinger[2], M.Sperl[1]*, H.Scheich[1].
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[2]Univ. Kaiserslautern, Biology Department, Kaiserslautern, FRG.

In songbirds high levels of the calcium-binding protein parvalbumin (PV) have been found in the telencephalic vocal motor nuclei HVc, RA, Area X and MAN (Braun et al, Cell Tissue Res., 240:101, 1985). A subset of these PV containing cells was identified as GABAergic neurons (Zuschratter et al, Neurosci.Suppl.22: S114, 1987). The present study was done to determine 1. whether PV immunoreactive cells in HVc are projecting neurons, for instance to Area x and 2. whether these cells contain the estrogen receptors described in HVc (Gahr et al, Brain Res., 402:173, 1987). Adult male canaries were injected in Area X with rhodamine containing latex microspheres. Four days later sagittal sections including the retrogradly labeled HVc neurons were processed with a parvalbumin antiserum, using the ABC-method and FITC as chromogen. Afterwards sections were incubated with a monoclonal antiserum against estrogen receptors using the PAP technique and DAB as chromogen. In HVc most cells filled with the retrograde tracer showed moderate immunoreactivity for the calcium-binding protein. A second class of PV neurons which exhibited strong PV immunoreactivity and may be GABAergic cells did not contain microspheres. Double labeling experiments with PV and estrogen antisera revealed that some of the moderately stained PV projection neurons contained estrogen receptors. In conclusion PV immunoreactive cells could be devided into subpopulations by double labeling techniques. Partial co-localization of PV-immunoreactivity with estrogen receptors in neurons points to a special Ca buffering mechanism in steroid induced processes. (SPON: B. Hose)
(Supported by DFG SCH 132/13-1 and DFG Gu 148/7-8)

304.5

FEMALE ZEBRA FINCHES GIVEN ONLY ESTROGENS PRODUCE MALE-LIKE VOCALIZATIONS. H.B. Simpson and D.S. Vicario. The Rockefeller University, New York, N.Y. 10021

Sex hormones administered early in development can have permanent effects on the behavior of both birds and mammals. We examined the changes estrogens alone can induce in the vocal behavior of female zebra finches. Song behavior is sexually dimorphic: females normally do not sing; adult males do and learn their song as juveniles. These behavioral differences are accompanied by differences in the neural structures serving vocal behavior.

Female zebra finches received estradiol-17β implants at three different ages: within the first week of hatching, at 20 days, or when adult (after 100 days). Between 100 and 150 days of age, we recorded vocalizations produced by treated females, focusing especially on song and the long call, a sexually dimorphic call.

We found that exogenous estrogens can cause female zebra finches to produce male-like song and male-like long calls, if given either in the first week after hatching or at 20 days of age. Some treated females produced song and long calls like those of normal adult males. Some learned from their male tutor. Others sang, but their song contained abnormal features. We correlated these behavioral changes with changes in the neural structures governing vocal behavior. Our data confirm earlier reports linking the masculinization of behavior to that of its neural substrate. These reports claimed that treated females sing only after receiving testosterone in adulthood. However, our data demonstrate that exogenous estrogens alone (either early or even at 20 days of age) can masculinize female vocal behavior. (NIH GMO-7739 and MH-40900)

304.6

SITES OF ACTION OF ANDROGEN AND ESTROGEN IN THE REGULATION OF MASCULINE SOCIOSEXUAL BEHAVIOR IN THE RAT. R.J. Barfield and J.A. Matochik. Dept. Biol. Sci. Rutgers Univ. New Brunswick, NJ, 08903.

It is well established that testosterone (T) acts in the brain in the regulation of male copulatory behavior; however, the precise identification of cellular targets for this action is lacking. In addition, although it is known that T regulates other aspects of sociosexual behavior such as signaling (scent marking and vocalization) and agonistic behavior, even less is known concerning the sites of hormonal action in these behaviors. In the present study, dilute estradiol (1-100 cholesterol) was used as a probe in the identification of sites of T action in the regulation of copulatory behavior in castrated males treated with systemic DHTP. In addition, we attempted to assess the role of the lateral septum and medial amygdala in the hormonal regulation of masculine copulatory behavior. Finally, we are currently attempting to clarify the role of androgen in the regulation of signaling behavior and to determine the location of target cells involved in this action. Results to date indicate that the rostral ventromedial preoptic area (and perhaps the diagonal band of Broca) are the sites upon which aromatized T acts. The central medial preoptic area does not appear to be particularly responsive. At present, a clear localization of the site of action of hormones in the regulation of other components of sociosexual behavior is not available.

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304.7

NEUROENDOCRINE MODULATION OF MALE COURTSHIP BEHAVIOR IN THE BLUE CRAB (Callinectes sapidus) Debbie Wood, Charles D. Derby[1], Georgia State University, Barbara Beltz[2], Wellesley College, Richard A. Gleeson, Whitney Marine Laboratory
(SPON: M.-N. Girardot)

The male blue crab produces a sterotyped courtship display in response to a pheromone produced by the female. This behavior consists of both postural and rhythmic components. This system provides a good model for the study of neuromodulation of simple motor systems because of the rhythmic aspect of the behavior. Bioassays of neuromodulators have shown that the monoamine dopamine and the neuropeptide proctolin are capable of eliciting the postural and rhythmic behavioral components respectively. We have localized by used of immunocytochemistry potential neurosecretory cells which have proctolin-like and dopamine-like immunoreactivity. We are currently undertaking electrophysiological studies to detect changes in motor neuronal output in the presence of proctolin and dopamine.

1
2 Partially Supported by Whitehall Foundation and NSF #BNS-8718938

304.8

STEROID MODULATION OF GABA RECEPTORS: NOVEL MECHANISM FOR REGULATION OF SEXUAL BEHAVIOR. M. Orchinik* and F.L. Moore. Oregon State Univ., Corvallis, OR 97331-2914

Previous studies reported that corticosterone (CS) and GABA rapidly inhibit amphibian (Taricha granulosa) sexual behaviors. We conducted radioligand binding studies using amphibian brain tissue, and have preliminary evidence that this rapid behavioral response to CS may reflect a direct modulation of the $GABA_A$ receptor. Binding of (^{35}S)-TBPS (specific for $GABA_A$ receptor) was inhibited by CS and related steroids. Conversely, specific binding of ^3H-CS to membranes was altered in opposite directions by GABA agonists and antagonists. We used in vitro receptor autoradiography to identify TBPS binding sites in an amphibian brain. The tectum and tegmentum contained the greatest density of putative $GABA_A$ receptors, with moderately high binding in lateral amygdala, striatum and medulla. TBPS binding in all brain regions was inhibited by steroids. Our findings suggest that CS may rapidly modulate behavior through the $GABA_A$ receptor, a non-genomic mechanism. (Supported by NSF Graduate Fellowship to MO and NIH Grant RO1-HD13508).

304.9

STEROID MODULATION OF GABA-INDUCED ANALGESIA. M.M. McCarthy, M. Caba, C. Beyer, B.R. Komisaruk. Institute of Animal Behavior, Rutgers University, Newark, NJ 07102.

GABA has been implicated in analgesia at the spinal cord level. Intrathecal (IT) infusion of GABA-A agonists increases pain thresholds whereas antagonists produce hyperalgesia. Steroid effects on GABA transmission are multiple and complex. We report here that steroids modulate nociception after IT infusion of the GABA-A agonist mucimol (MUS). A 1 ug dose of MUS given IT to ovx'd female rats has no effect on pain thresholds. Females in late estrus infused with the same dose of MUS are analgesic up to 30' post-infusion in the vocalization threshold to tail shock (VTTS) test ($p<.05$;MWU) but there is no effect on tail-flick latency (TFL). Females in late proestrus exhibited analgesia 10' after infusion of 1 ug MUS ($p<.01$;MWU) and females in diestrus were not analgesic at any time after 1 ug MUS 1T. Ovx'd females treated with EB (10ug for 2 days) and tested 40hr later exhibited analgesia after 1 ug MUS IT in the VTTS test. Ovx'd females injected with P (2mg) and infused with MUS 10' later exhibited analgesia in both the VTTS and TFL tests up to 30' after infusion ($P<.05$;MWU). Ovx'd females treated with EB+P (10ug 40hr prior; 1 mg 4hr prior) did not exhibit analgesia after 1 ug MUS IT. These data suggest E and P enhance GABA-induced analgesia thru different mechanisms which may be antagonistic. Supported by grant NIH1RO1NS 22948 (BRK) and Rutgers-CINVESTAV/CIRA Exchange Program.

304.10

EFFECTS OF CORTICOSTERONE IN THE FORCED SWIM TEST: EVIDENCE FOR EFFECTS ON CONSOLIDATION AND RETRIEVAL. L.J. Iny, J.B. Mitchell, S. Welner, and M.J. Meaney. Douglas Hospital Research Center, McGill University, Montreal, Quebec, CANADA, H4H 1R3.

Among the behaviors that have been reported to be affected by the presence or absence of corticosterone (B) is the amount of time that a rat is immobile in the Porsolt forced swim test. The Porsolt test, commonly used to screen antidepressants, consists of an initial 15 min forced swim and a 5 min test swim 24 h later. Rats spend progressively more time immobile during the 15 min training swim, and a high percentage of time immobile on the test.

Rats were tested in the Porsolt swim test 1 wk after adrenalectomy or sham adrenalectomy. Adrenalectomy significantly reduced time immobile during the test (from a mean of 206 s to 100 s), and this effect was completely reversed by B. In order to reverse the effect of adrenalectomy, it was necessary only to return plasma B levels to resting, basal levels; increasing plasma B to higher, stress levels did not further affect behavior. Adrenalectomized rats were also tested after administration of B before training, immediately after training, or prior to the test. Only B administration before, or immediately after the training session fully reversed the effect of adrenalectomy. When animals were tested repeatedly, at 1 wk intervals, it was found that if B had been present during training, performance depended, to some extent, on the presence of B during the test. That is, for animals trained with B, more time was spent immobile on tests with B than on tests without B. Adrenalectomized animals trained without B showed low levels of immobility on all tests.

In agreement with earlier studies (e.g. Jefferys et al., Eur. J. Pharmacol., 92:99, 1983), B appears to have a role in the incorporation or consolidation of information in the Porsolt swim test. The results of the experiments reported here indicate that B also plays some role in the manifestation, or retrieval of this information.

304.11

WKY RAT AS A MODEL FOR ANXIETY-INDUCED DEPRESSION. W.P. Paré* and E. Redei* (SPON: D. Brunswick), VA Medical Center, Perry Point, MD 21902 and U. Pennsylvania, Philadelphia, PA 19104

Wistar Kyoto (WKY) normotensive rats demonstrate a behavioral impairment in several behavioral tests, as well as an exaggerated plasma ACTH response to stress. In the open field test of emotionality, WKY's had longer response latency and lower ambulation scores as compared to spontaneously hypertensive rats, Fischer 344 and Wistar rats. Depressed ambulation by WKY's was also revealed in a learned helplessness paradigm; as compared to other strains, WKY's were significantly deficient in the acquisition of an avoidance/escape task following inescapable shock treatment the day before. Low open field ambulation and rearing scores and slow movement and freezing in the avoidance task suggested depressive behavior as a characteristic of WKY rats. WKY's were subsequently judged as more depressed in the Porsolt forced swim test. We hypothesized that the WKY's "depression" was anxiety-induced and reflected heightened activity of the hypothalamic-pituitary axis. Basal plasma ACTH levels did not differ between WKY's and Wistars, but the serial plasma ACTH response to 2-h restraint stress was significantly greater for WKY's. We propose that anxiety-induced depression is a characteristic of WKY rats and this strain is a valuable model for studying depression which may be induced by an exaggerated stress response.

304.12

CORTISOL IMPLANTS IN THE MEDIAL PREOPTIC-ANTERIOR HYPOTHALAMIC REGION (MPOA AH) INDUCE SUBMISSIVE BEHAVIORS IN GOLDEN HAMSTERS. D.M. Hayden-Hixson*[1] and C.F. Ferris[2] (SPON: M.K. Wolf). [1]Neurosci. & Beh. Prog., U. Mass., Amherst, MA 01003; [2]Dept. of Physiol., U. Mass. Med. Sch., Worcester, MA 01655.

Lesion, electrophysiological and glucocorticoid-implant studies have suggested involvement of the MPOA-AH in the expression of agonistic behaviors in a number of species. Also, recent immunocytochemical studies have described moderate to high densities of Type II glucocorticoid receptors in the hypothalamus, including the MPOA-AH. To examine the effects of elevated MPOA-AH cortisol levels on aggressive behaviors, male hamsters received 1mm (~25µg) implants of either hydrocortisone (N=30) or cholesterol (N=30) in the MPOA-AH.

In 15 minute paired-encounters, cortisol-treated animals were significantly ($p<.03$) more submissive than cholesterol-implanted controls. The effect was most pronounced 2 days postimplant, and disappeared by day 5. In contrast, cortisol- and cholesterol-treated animals were equally aggressive when juvenile males were placed in their home cages.

The results suggest cortisol inhibits the expression of aggressive behaviors in social, but not territorial, contexts through its action at specific sites in the medial preoptic-anterior hpothalamic region. (Work supported by NIH grant #NS23657).

304.13

DEXAMETHASONE INCREASES THE SPONTANEOUS YAWNING FREQUENCY IN MALE RATS. J.R. Eguibar *, A. Moyaho *, E. Zurita *, and B. Holmgren *. (SPON: R.M. Budelli). Depto. de Ciencias Fisiologicas, Instituto de Ciencias. Universidad Autonoma de Puebla, Mexico.

Yawning is an ubiquitous innate motor pattern among Vertebrates. It is well known that the administration of ACTH and related peptides into the brain ventricles produces a stretch-yawning syndrome (SYS) in different animal species . Although ACTH does not cross the blood-brain barrier, Gispen et al (1975) reported that a systemic injection of ACTH (0.5 mg/kg) in rats can induce yawning behavior, thus suggesting that a peripheral effect might be influencing yawning secondarily.

The purpose of these experiments was to explore the effect of dexamethasone (Dxm) on yawning behavior in adult male Sprague-Dawley rats. The animals were maintained under constant light and ad lib feeding to circumvent circadian variation of yawning. Intraperitoneal injection of Dxm (2-4 mg/rat) produced a significant increase in yawning from 6 h to about 24 h after administration ($p<0.05$, Mann-Whitney U test). Adrenalectomy was followed by a significant decrease in spontaneous yawning ($p< 0.05$). The administration of Dxm to adrenalectomized rats significantly increased their yawning rate ($p< 0.01$, Kruskal-Wallis test). These data suggest that glucocorticoids might exert a permissive effect on yawning, through central receptors participating in the regulation of this behavioral pattern.

This work was partially supported by a grant from SEP C88-01-0143 and a fellowship awarded to A. Moyaho by CECyT.

304.14

THE THYROID-ADRENAL AXIS AND THE ACQUISITION AND RETENTION OF THE IMMOBILE FLOATING RESPONSE. D. Jefferys and J.W. Funder. Medical Research Centre, Prince Henry's Hospital, Melbourne 3004, Australia.

We have previously shown that adrenalectomized (ADX), hypophysectomized (HPX) and ADX/HPX rats show indistinguishable levels of progressive immobility over a 15 min swimming test. Retested for 5 min 24 h later, HPX rats show immobility indistinguishable from control (~70%); ADX and ADX/HPX rats show only ~30% immobility, an effect reversed by glucocorticoids or kappa-selective opioids (Jefferys & Funder, Endocrinology, 121:1006, 1987). We have also shown that propylthiouracil (PTU) given daily for 21 days markedly inhibits the acquisition of the immobile response, which inhibition is reversed by T_4 given immediately before test (Jefferys & Funder, Soc. Neurosci., Abstract 41;11, 1988). We now report that T_4 given to PTU-treated rats up to 2 h after the initial test elevates immobility on retest to ~70%, further evidence for a role of T_4 in the retention, as well as the immediate performance, of the acquired response. Secondly, in a series of control studies, we have repeatedly shown that T_4 (15 µg) is equipotent with dexamethasone (6 µg) or ketocyclazocine (7.5 µg) in reversing the effect of adrenalectomy on retention of the behavioral response. The possible mechanisms, central or peripheral, of this unexpected effect of T_4 in adrenalectomized rats are currently under active investigation.

304.15

REM SLEEP IS INCREASED BY PROLACTIN, VIP, OR PHM TREATMENTS IN RABBITS. J.M. Krueger, F. Obal, Jr., M. Opp, A.B. Cady, and L. Johannsen. Dept. of Physiology and Biophysics, Univ. of Tennessee, Memphis, TN 38163.

These experiments were designed to study whether 1) vasoactive intestinal peptide (VIP) has effects on rabbit sleep similar to those reported for rats and cats; 2) peptide histidine methionine (PHM), a peptide structurally related to VIP, mimicks the somnogenic effects of VIP; and 3) prolactin (PRL), for which VIP and PHM act as releasing factors, has similar effects on sleep. Compared to control recordings, ICV injection of VIP or PHM (0.01, 0.1 or 1.0 nmol/kg) selectively increased rapid eye movement (REM) sleep during postinjection hours 2 to 6; the effects were not dose-dependent. A reduction in non-REM sleep was observed after the high dose of VIP and PHM. In response to SC injection of PRL (45 and 200 IU/kg), REM sleep increased in a dose-related manner. The results support the hypothesis that VIP is involved in the regulation of REM sleep. The VIP- and PHM-induced increases in REM sleep may be mediated via pituitary PRL release.

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304.16

CENTRAL PROLACTIN ADMINISTRATION STIMULATES MATERNAL BEHAVIOR IN NULLIPAROUS FEMALE RATS. R.S. Bridges, M. Numan[1] and P.M. Ronsheim*. Dept. Anatomy & Cellular Biology, Harvard Medical School, Boston, MA 02115; [1]Dept. Psychology, Boston College, Chestnut Hill, MA 02167.

Previous studies in female rats have demonstrated a role for prolactin (PRL) in the induction of maternal behavior (Science 227:782, 1985). In the present report, the effects of both intracerebroventricular (ICV) and local neural infusions of PRL were assessed in an attempt to identify possible sites of PRL action. Adult nulliparous rats were ovariectomized and given a pregnancy-like steroid regimen (Silastic capsules) of progesterone (Days 1-11) and estradiol (Days 11-17). Endogenous pituitary PRL secretion was suppressed by s.c. injections of bromocriptine (Days 11-17). In the first study, ovine (O)-PRL (50 ug) or vehicle was infused twice daily on Days 11 and 12 and once on Day 13. Behavioral testing was conducted daily from Day 12 through 17. PRL-treated rats became maternal to foster young significantly faster than did controls. When separate groups of similarly treated rats were injected systemically with this dose of PRL, maternal behavior was unaffected. In a final study, infusion of O-PRL (5 ug) bilaterally into the medial preoptic area (MPOA) also stimulated maternal behavior more rapidly. Thus, PRL appears to act in the CNS, one site being the MPOA, to stimulate maternal behavior.

Supported by NIH HD19789 and NIMH MH00536 [RSB].

304.17

HIGH DOSES OF TESTOSTERONE INCREASE INTROMISSION RATES IN HYPERPROLACTINEMIC MALE RATS. M.A. Mroczynski* and P.C. Doherty. Dept. of Anatomy, N.E. Ohio Univ. Coll. of Med., Rootstown, Ohio 44272.

Chronic elevation of serum prolactin (PRL) levels, hyperprolactinemia (hyperPRL), inhibits male sexual behavior, including aspects of both sexual arousal and erectile function. To determine if the inhibition of sexual arousal and erectile function occur independently, the effects of hyperPRL were examined in animals given graded doses of testosterone (T). Groups of sexually experienced Fischer 344 male rats were castrated and given Silastic implants of T (group 1, 7.5mm; group 2, 15mm; group 3, 30mm; and group 4, 60mm). Two weeks later half the animals of each group were given four ectopic pituitary grafts and the remainder received sham surgery. Four weekly tests of copulatory behavior followed with repeated sampling for determination of serum levels of LH, PRL and T. A significant dose response effect of T was noted on mounting rates in standard tests of copulatory behavior. Hyperprolactinemia led to reduced mounting rates but this was significant only at the intermediate doses. Testosterone increased intromission rates in hyperprolactinemic males, but only in animals given the 60mm capsules. No effects of T on intromission rates were observed in the sham-operated controls. In mounting tests after genital anesthetization, T increased mounting rates in a dose response manner in the sham animals, but not in the grafted males. These differential effects of T on sexual arousal and erectile function during hyperPRL suggests that each of these measures of male sexual function is affected independently by the hyperprolactinemic state. Supported by a Research Challenge Grant from the Ohio Board of Regents.

304.18

EFFECTS OF PROLACTIN MICROINJECTIONS IN THE PREOPTIC AREA AND VENTROMEDIAL HYPOTHALAMUS ON FOOD INTAKE AND GONADAL CONDITION IN DOVES. J.D. Buntin and K.T. Foreman*, Dept. of Biol. Sciences, Univ. of Wisconsin, Milwaukee, WI 53201.

In ring doves, intracerebroventricular (ICV) injections of ovine prolactin (PRL) profoundly suppress plasma LH levels and testes weight (J.Endocr.,188:33,1988) and markedly stimulate food intake (Horm. Behav.,19:188,1985). In addition, PRL receptors are concentrated in several regions of the dove brain that have been implicated in the regulation of feeding behavior or gonadal activity (Fechner & Buntin, Br. Res.,in press). This study explored the role of two of these regions, the preoptic area (POA) and ventromedial hypothalamus (VMN), in mediating PRL-induced hyperphagia and gonadal suppression. Twice-daily unilateral injections of 25ng (1.1pmole) ovine PRL into either the VMN or POA of male ring doves resulted in a significant elevation of body weight ($p<.001$) and average daily food intake ($p<.05$) over a 5 day treatment period (n=8-10/group). However, VMN-injected birds showed a greater hyperphagic response than POA-injected birds (28% vs 17%, increase, respectively; $p<.05$). No significant changes in feeding occurred after POA or VMN injections of vehicle. Unlike the effects of ICV injections, reductions in testes weight did not accompany the hyperphagia induced by PRL injection at either site. These results implicate the VMN and POA as sites of PRL action in promoting hyperphagia.

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304.19

ARE ALL AREAS OF THE SMALL INTESTINE ACTIVE IN INITIATING SATIETY OF SHAM-FEEDING RATS? V.E.Mendel and M.Palieschekey*. Dept. of Animal Physiology, Animal Science and Food Intake Lab.,Univ. of California, Davis,CA. 95616.

Cholecystokinin (CCK) has been identified by a number of laboratories as a possible mechanism for initiating satiety. Because the ileum of the rat does not contain CCK secreting cells infusion of nutrient into the ileum should not interrupt sham-feeding. In a preliminary study three male, Sprague-Dawley rats were surgically equipped with a stainless steel gastric cannula plus a silastic cannula in either the proximal duodenum, the jejunum, or the ileum. The animals were trained to drink a 20% (w/v) sucrose solution following a 16 hr. fast while housed in a restraining cage. Rats were allowed to sham-feed for 15 min before intestinal infusion began. Sucrose solution at 299.4 mOsm and 0.38 kcal/ml was infused at 0.3 ml/min (0.113 Kcal/min). The results are as follows.

	Duodenum	Jejunum	Ileum
Consumption (ml)			
Ga.Close	11.0 ±1.43	10.5 ±0.64	11.4 ±0.72
Sh-feed.	26.9 ±2.96	22.2 ±2.31	14.1 ±1.41
Infused	14.2	12.8 ±2.01	10.9 ±1.01

We conclude that all areas of the small intestine are active in initiating satiety and that satiety may not be initiated by CCK.

304.20

RECIPROCAL EFFECTS OF FASTING ON INSULIN BINDING IN DIFFERENT FRACTIONS OF RAT OLFACTORY BULB. J.L.Marks*, D. Porte Jr.*, and D.G.Baskin. Dept. Medicine, University of Washington, Seattle, WA,98195 and V.A. Medical Center, Seattle, WA, 98108.

Decreased insulin binding has been demonstrated in the plasma membrane (P2) fraction of olfactory bulbs(OB) from rats totally deprived of food, but not water, for 4 days (Marks and Eastman, Neurosci., in press, 1989). However, when quantitative autoradiography (QAR) was used to measure specific, OB insulin binding in fasted (n=4) vs. fed (n=4) animals, we found no change in insulin binding on the external plexiform layer: 142±20 vs. 146±17 or the granule cell layer: 54±9 vs. 61±15 dpm/sq mm (±SEM), even though these are the major sites of OB insulin receptors. This discrepancy was investigated by measuring specific insulin binding in brain homogenate, crude nuclear (P1), P2 and microsomal (P4) fractions prepared from the OB and amygdala of 3 day fasted (n=3) and free-feeding control (n=3) female rats. In both brain regions, the homogenate insulin binding, and in the amygdala, P1, P2 and P4 binding were similar in fed and fasted rats. However, in the OB, P2 insulin binding decreased from 49.0±2.1 to 41.1±1.5, P1 binding increased from 14.7±0.8 to 24.1±0.5 and P4 binding increased from 53.5±0.6 in the fed to 93.0±1.3 %/mg protein in the fasted rats, respectively(p values ≤ .05). The total insulin binding in the P1,P2 and P4 of fasted rats was added and was only 8% greater than that of fed controls.

We conclude that in the OB, total insulin binding is unaltered by a 3 day fast, when measured in tissue slices by QAR or in brain homogenates. However, there are reciprocal changes in binding in specific subcellular fractions, suggesting that insulin receptors may move between subcellular sites during food deprivation and this may be specific for the OB.

EXCITATORY AMINO ACIDS: EXCITOTOXICITY II

305.1

NMDA ANTAGONISTS INDUCE PATHOMORPHOLOGICAL CHANGES IN CEREBROCORTICAL NEURONS. J. Labruyere*, M.T. Price and J.W. Olney (SPON: A.I. Cohen) Washington University School of Medicine, St. Louis, MO.

Recent evidence implicating endogenous excitatory amino acids (EAA), such as glutamate and aspartate, in neurodegenerative disorders has stimulated interest in the use of EAA receptor antagonists as neuroprotective agents. Antagonists of the N-methyl-D-aspartate (NMDA) subclass of EAA receptor have received the greatest attention, especially non-competitive NMDA antagonists, such as PCP and MK-801, which freely penetrate blood brain barriers. Competitive NMDA antagonists, such as 2-amino-5-phosphonopentanoate (AP5) and 2-amino-7-phosphonoheptanoate (AP7), are also of potential interest, provided a means of increasing their blood brain barrier penetrance can be developed. In either case, a significant obstacle to the clinical use of these agents is their potential side effects. Recently, we found (Olney et al., Science, in press) that non-competitive NMDA antagonists (MK-801 > PCP > tiletamine > ketamine), when systemically administered in relatively low doses to adult rats, induce acute pathomorphological changes (extreme vacuolization of endoplasmic reticulum and dissolution of mitochondria) in large multipolar and pyramidal neurons (layers III and IV) of the posterior cingulate and retrosplenial cortices. To study whether this is an exclusive property of agents acting at the PCP site within the NMDA receptor channel, or might be reproduced by agents that block the NMDA receptor complex by other mechanisms, we micro-injected AP-5 and AP-7 unilaterally into a cerebrocortical locus immediately bordering on the posterior cingulate cortex. When thus injected, the corpus callosum and cingulum direct the spread of the compound toward both ipsilateral and contralateral cingulate neurons. We found that each agent induced PCP-like pathomorphological changes bilaterally in the same populations of cingulate neurons that are selectively affected by systemically administered PCP or MK-801. We conclude that if competitive NMDA antagonists could penetrate blood brain barriers as freely as non-competitive NMDA antagonists, they would mimic the latter in producing pathomorphological changes in cerebrocortical neurons when administered systemically. Supported by RSA MH 38894 (JWO) and DA 53568.

305.2

A COMPARISON OF THE EFFECTS OF MDL 27,266 WITH KNOWN NMDA ANTAGONISTS ON LONG TERM POTENTIATION IN THE IN VIVO AND IN VITRO RAT HIPPOCAMPUS . S.M. Sorensen, T.M. Humphreys*, and J.M. Zwolshen*, Merrell Dow Research Institute, Cincinnati, OH 45215.

MDL 27,266 is a novel triazole-3-one derivative which is being investigated as a broad spectrum anticonvulsant. It has been shown to protect against quinolinic acid seizures and hypoxic damage of the hippocampus in gerbils but it has no affinity for the NMDA receptor and only very poor affinity for the PCP binding site in vitro. In order to further characterize the activity of this compound we compared the activity of MDL 27,266 with MK-801 and CPP against long term potentiation (LTP) in the hippocampus; a phenomenon which has been shown to involve activation of the NMDA receptor. In the anesthetized rat MDL 27,266 (40 mg/kg) blocked LTP completely without altering the baseline population spike amplitude. This is in contrast to MK-801 (2 mg/kg i.p.) and CPP (10 mg/kg i.p.) which produced marked effects on basal population spike at doses which blocked LTP. Bath application of MDL 27,266 (100 uM) to the hippocampal slice caused a small decrement in the initial LTP which then further declined through the course of 60 minutes to 1/3 rd of control size. In similar experiments MK-801 (4 uM) completely blocked LTP and CPP (100 uM) had no effect on LTP. Implications of these differences in efficacy will be discussed.

305.3

CALCIUM-DEPENDENT AND -INDEPENDENT RELEASE OF ENDOGENOUS ADENOSINE FROM RAT CORTICAL SLICES BY K^+ AND EXCITATORY AMINO ACIDS T.D. White and K. Hoehn, Dalhousie University, Halifax, Nova Scotia, Canada, B3H 4H7

Adenosine, which inhibits neuronal firing and decreases glutamate release, may have a protective role in the CNS in situations of excess excitatory neurotransmission. Adenosine released from superfused rat cortical slices was measured by HPLC. When Ca^{2+} was omitted from the medium, K^+-evoked release of adenosine was decreased by about 50% whereas NMDA, kainate, and quisqualate-evoked release was not diminished, indicating Ca^{2+}-dependence of K^+ but not EAA-evoked adenosine release. In the absence of Ca^{2+}, adenosine release by K^+, kainate and quisqualate occurred earlier and was less sustained than control. In the presence of Ca^{2+}, adenosine release by a second K^+ or EAA stimulation was markedly diminished, suggesting depletion of a releasable pool of adenosine. Similarly, in the absence of Ca^{2+}, kainate-evoked release of adenosine was markedly diminished during a second stimulation. However, when Ca^{2+} was restored to slices previously stimulated in the absence of Ca^{2+}, release by a second exposure to K^+ or kainate was no different from initial control release. Restoration of Ca^{2+} did not restore quisqualate-evoked adenosine release and only partially restored NMDA-evoked release, possibly due to desensitization of these receptors. These findings suggest the existence of separate Ca^{2+}-dependent and -independent releasable pools of adenosine. (Supported by the MRC of Canada).

305.4

GLUTAMATE UPTAKE MEDIATES RELEASE OF ENDOGENOUS ADENOSINE FROM RAT CORTICAL SYNAPTOSOMES K. Hoehn and T.D. White, Dalhousie University, Halifax, Nova Scotia, Canada, B3H 4H7

We have previously shown that glutamate releases adenosine from rat cortical slices by acting at excitatory amino acid (EAA) receptors. To determine whether receptors mediating release are located on presynaptic terminals, we have studied release from cortical synaptosomes. Glutamate (30-1000μM) released adenosine from synaptosomes as detected by HPLC. Studies with EAA antagonists, DNQX, MK-801 and Mg^{2+}, and agonists, NMDA, kainate, and quisqualate, determined that glutamate-evoked release of adenosine from synaptosomes was not EAA receptor-mediated. Inhibition of glutamate uptake by dihydrokanate or replacement of Na^+ in the medium with Li+, choline, or sucrose blocked glutamate-evoked release of adenosine. Inhibition of ecto-5'-nucleotidase decreased adenosine release by glutamate, indicating that release was derived from extracellular metabolism of a released nucleotide. Glutamate did not release ATP or cAMP from synaptosomes, as determined by a firefly luciferin-luciferase assay and studies with IBMX respectively. Therefore, uptake of glutamate into presynaptic terminals releases a nucleotide (but not ATP or cAMP), which is converted extracellularly to adenosine. This adenosine, acting at A_1 adenosine receptors, may provide a negative feedback mechanism for decreasing glutamate release. (Supported by the MRC of Canada).

305.5

ADENOSINE-MEDIATED NEUROPROTECTION IN VITRO AND IN VIVO.
M.L. Weber, A.W. Probert, Jr., J.E. Goodrich*, M.A. Dominick* and F.W. Marcoux, Depts. of Pharmacology and Pathology and Experimental Toxicology, Parke-Davis Pharm. Res. Div., Warner-Lambert Co., Ann Arbor, MI 48105.

The neuromodulator and cerebrovasodilator, adenosine, has been proposed to be neuroprotective. We have studied adenosine agonists in vitro and in vivo in models of hypoxic/ischemic neuronal injury in which vasodilators would be unlikely to be neuroprotective. Cortical neuronal cultures from fetal rat brain were subjected to hypoxia. Hypoxia-induced, NMDA-mediated intraneuronal accumulation of $^{45}Ca^{++}$ was measured in the presence of vehicle or either cyclopentyladenosine (CPA) or cyclohexyladenosine (CHA). In cultures showing a comparable degree of control $^{45}Ca^{++}$ accumulation during 4 hrs of hypoxia CPA and CHA inhibited this response in a concentration-dependent manner (IC-50s - 0.65 and 0.24μM). Gerbils were subjected to 10 mins of bilateral carotid occlusion under brief ether anesthesia. Twenty-four hrs later, ischemia-induced, CA1 neuronal injury-mediated increases in exploratory locomotor activity (LMA) were measured. While 0.1mg/kg had no effect on the ischemia-induced 128% increase in LMA observed in vehicle pretreated control gerbils, 0.3mg/kg CHA reduced the LMA increase by 50%. These findings suggest direct neuroprotective effects for adenosine agonists. In focal cerebral ischemia vascular effects of adenosine agonists may enhance or limit direct neuroprotective effects.

305.6

PRECLINICAL ANTICONVULSANT, ANXIOLYTIC AND NEUROPROTECTIVE PROFILE OF 8319, A NON-COMPETITIVE NMDA ANTAGONIST.
D.K. Rush, J.C. Wilker*, C.A. Wilmot, R. Corbett*, R.W. Dunn*, K. Locke*, L.L. Martin*, K.A. Rudolphi*[1] & S. Fielding. Hoechst-Roussel Pharmaceuticals, Inc., Somerville, NJ 08876 & [1]Hoechst-Werk Albert, Wiesbaden, FRG.

8319, ((±)-2-Amino-N-ethyl-alpha-(3-methyl-2-thienyl) benzene-ethanamine •2HCl), is a novel compound with the profile of a non-competitive NMDA antagonist. The compound displaced ^{3}H-TCP with high affinity (IC_{50}=43 nM), but was inactive at the NMDA, benzodiazepine and GABA sites; in vivo, 8319 showed good efficacy as an anticonvulsant, anxiolytic, and potential neuroprotective agent. It blocked seizures induced by NMDLA, supramaximal electroshock, pentylenetetrazol (PTZ), picrotoxin and thiosemicarbazide with ED_{50}'s of 1-20 mg/kg ip. In traditional operant conflict and the social interaction and elevated plus maze assays, 8319 showed anxiolytic activity at doses of 1-5 mg/kg ip. As a neuroprotectant, 8319 (30-100 mg/kg sc) presented the death of dorsal hippocampal pyramidal cells induced by direct injection of 20 nmol NMDA. At 15 mg/kg ip, the compound was also effective against hippocampal neuronal necrosis induced via bilateral occlusion of the carotid arteries in gerbils. In summary, 8319 is a non-competitive NMDA antagonist with good anticonvulsant and anxiolytic activity and may possess neuroprotective properties useful in the treatment of brain ischemia.

305.7

7-CHLORO-KYNURENATE BLOCKS NMDA RECEPTOR-MEDIATED NEUROTOXICITY IN CORTICAL CELL CULTURES.
D.M. Hartley, H. Monyer, S.A. Colamarino* AND D.W. Choi. Dept. of Neurology, Stanford Univ. Med. Sch., Stanford, CA 94305.

Glycine strongly potentiates NMDA receptor-mediated neuroexcitation, and several investigators have reported that the glycine antagonist 7-chlorokynurenate (7-Cl Kyn) can block NMDA receptor-mediated neurotoxicity. We examined the neuroprotective actions of 7-Cl Kyn in murine neocortical cell cultures. Cultures exposed to 500 μM NMDA for 5 min in the absence of added glycine developed substantial neuronal cell loss over the next 24 h. The addition of 10 μM glycine did not increase submaximal neuronal injury, suggesting that endogenous glycine in the cultures was sufficient to achieve NMDA receptor saturation. Addition of 3-300 μM 7-Cl Kyn produced a concentration-dependent reduction in neuronal damage, with IC_{50} about 30 μM; some benefit was seen even if the drug was added after completion of the NMDA exposure. The protective effect of 100 uM 7-Cl Kyn was overcome by adding 10-1000 μM glycine, with ED_{50} about 100 μM. Furthermore, 10-300 μM 7-Cl-Kyn also produced concentration-dependent reduction in the neuronal damage induced by 4-60 min of combined glucose and oxygen deprivation. We concur with others that antagonists of the glycine receptor on the NMDA receptor complex may prove to be an effective means for combating stroke or other disease involving glutamate neurotoxicity.

305.8

BLOCK OF N-METHYL-D-ASPARTATE NEUROTOXICITY BY GLYCINE ANTAGONISTS IN CORTICAL CULTURES.
W.C. Zinkand*, J. Patel, and A.I. Salama (SPON: J.B. Malick). Dept. of Pharmacology, ICI Pharmaceuticals Group, ICI Americas Inc., Wilmington, DE 19897.

It is widely believed that the neurotoxic effect of glutamate, specifically as acting through the N-methyl-D-aspartate (NMDA) receptor subtype, causes the neuronal cell loss involved in many neurodegenerative diseases. Glycine, binding to strychnine-insensitive sites, is known to be a positive modulator of the NMDA responses in the CNS and is thought to be obligatory for these responses. We have shown that in primary cell culture from rat cerebral cortex, NMDA is a potent neurotoxin which is potentiated by glycine and D-serine. This toxic action is blocked by the putative glycine antagonists HA-966 and 7-chlorokynurenic acid even in the absence of added glycine. Block by these antagonists is reversed by adding glycine, unlike the block of NMDA toxicity by the NMDA antagonist 3-3(2-carboxypiperazine-4-yl)propyl-phosphonate (CPP), and the PCP analog MK-801.

305.9

DYNORPHIN-INDUCED NEUROTOXICITY IS BLOCKED BY PRE-TREATMENT OR POST-TREATMENT WITH MK-801.
Larry Isaac, Terri O'Malley*, Helen Ristic* and Peggy Stewart, Department of Pharmacology, Univ. of Ill. College Medicine, Chicago, 60612

Previously, we showed that dynorphin A (1-13)-induced neurotoxicity (DIN) in rats is mediated by the N-methyl-D-aspartate (NMDA) subclass of glutamate receptor (Brain Res. 443:329, 1988). The non-competitive NMDA antagonist, MK-801, blocks glutamate-induced calcium accumulation in rat cerebral cortical neurons in cell culture (Neurology and Neurobiology 46:563, 1988). Using MK-801 as a pre- and post-treatment to dynorphin injection in rats we determined whether the NMDA-associated ion channel participates in DIN and we estimated the time course of cell death. We pretreated rats, 30 min i.p., with 0.01-3 mg/kg MK-801 and we post-treated others, 15-90 min, with 3 mg/kg i.p. after dynorphin (25 nmoles i.t.).

Pretreatment with MK-801 provided a dose-dependent protection against DIN (loss of the tail-flick reflex) and at 1 mg/kg i.p. virtually prevented degeneration of spinal cord neurons assessed histologically 24 hrs later. Post-treatment with MK-801 at 15 min did not protect against DIN whereas 30 min post-treatment protected 50% of the rats against DIN. Post-treatment at 60 min protected 30% of the animals and 90 min post-treatment did not protect.

These data parallel our previous findings that DIN involves the NMDA supra molecular complex. In addition, neuroprotection by MK-801 suggests that dynorphin injection results in "opening" of the NMDA-associated ion channel which apparently permits a rapid and lethal accumulation of calcium into some neurons of the spinal cord central gray.

305.10

THE SELECTIVE κ OPIOID RECEPTOR AGONIST U50488H ATTENUATES NMDA INDUCED BRAIN INJURY IN THE NEONATAL RAT.
C.J.Hudson*, H.M.Scherch*, and E.D.Means. CNS Diseases Research, The Upjohn Company, Kalamazoo, MI 49001.

Enhanced activation of the NMDA-preferring glutamate receptor subtype seems to mediate hypoxic-ischemic brain injury in the immature brain. U50488H, a selective κ opioid receptor agonist, has been shown to exert cerebro-protective action, but has not been evaluated in a model of NMDA induced neuronal injury.

In this study, we examined the effects of U50488H on NMDA induced brain injury in the 7 day old rat pup. The injury was quantitated by assessing hemisphere weight reduction in pups that received unilateral NMDA injections. Rat pups (n=38) receiving peripallidal injection of NMDA (7.5 nmol/0.5 μl) showed a 17.5±0.7% reduction in the injected hemisphere dry weight compared to the non-injected hemisphere. U50488H (3, 10, 20 and 30 mg/kg, s.c.; 30" before, 1' after NMDA injection) significantly limited the hemisphere weight reduction at doses of 10,20 and 30 mg/kg, with maximal protection (50.4%, $p<.02$) at 20 mg/kg.

These results suggest that U50488H is effective in limiting NMDA mediated brain injury. Further work is needed to determine if this protective effect is due to antagonism of excitatory amino acids and/or induction of water diuresis with subsequent prevention of cerebral edema formation.

305.11

Depolarizing Conditions Protect Against Non-NMDA Mediated Glutamate Inhibition of Protein Synthesis in the Hippocampal Slice. J. J. Vornov* and J. T. Coyle (SPON: G. Capone), Depts. Neurology and Psychiatry, The Johns Hopkins School of Medicine, Baltimore, MD 21205

In some animal models of ischemia, NMDA receptor antagonists protect against neuronal degeneration, suggesting that glutamate causes injury by activating NMDA receptors. We have used inhibition of neuronal protein synthesis in the hippocampal slice as a method to localize acute, toxic effects of excitatory amino acids. Glutamate agonists at all three receptor subtypes (NMDA, quisqualate and kainate) produced a dose and time dependent inhibition of incorporation of TCA precipitable 3H-leucine into hippocampal slices. We confirmed that the effect is specific to neurons by autoradiography. NMDA was blocked by the specific antagonists MK-801 or ketamine and by the less selective antagonist kynurenate. Kynurenate antagonized the effects of quisqualate. In contrast to results reported in neuronal tissue culture, the inhibition caused by glutamate exposure was unaffected by antagonists.

Incubation of slices in high K^+ (120mM), low Na^+ (28 mM) medium produced a time-dependent decrease in protein synthesis which was partially mediated by NMDA receptor activation, since it was significantly decreased by simultaneous incubation with MK801. The effect of glutamate under depolarizing conditions was not significantly different from that in normal Krebs, but was significantly decreased by MK-801. There was no statistically significant effect of short exposures to glutamate when slices were depolarized in the presence of MK801. Substitution of choline for Na^+ had no effect on glutamate inhibition (choline 120mM, Na^+ 28 mM). When 60 mM KCl was added to normal Krebs without reducing Na^+, a small protection was observed. A larger protective effect was produced by veratridine (100μM), but still less than that of high K^+, low Na^+. It appears that under normal conditions, high concentrations of glutamate inhibit protein synthesis through non-NMDA sites not antagonized by high concentrations of kynurenate. When the Na^+ gradient is decreased, as occurs during energy failure, neurons appear sensitized to glutamate's NMDA effects and are at least partially protected against non-NMDA effects.

305.12

MORPHOLOGIC ALTERATIONS IN PRIMARY CULTURES OF RAT CEREBRAL ASTROCYTES AFTER EXPOSURE TO GLUTAMATE (GLU). L.J. Noble and P.H. Chan (SPON: R. Fishman). Dept. of Neurology, Univ. of California, San Francisco, CA 94143.

High extracellular concentrations of GLU, an excitatory neurotransmitter, are neurotoxic to the CNS. This toxicity was evaluated in astrocytes after exposure to 1mM GLU for 4 to 24 h. At the light microscopic level, GFAP-positive astrocytes in control cultures assumed varying, distinctive morphologies. The cytoskeleton of these cells was distributed in both a reticular pattern (usually associated with the soma) and in parallel arrays (identified primarily in processes). After exposure to GLU the heterogeneity of astrocytes was replaced by a predominance of cells in which the soma was swollen and angulated and processes appeared retracted. Cytoskeletal structures were not obvious and as a result the cytoplasm appeared homogenous. At the ultrastructural level, the normal compact appearance of the nucleolus was replaced by a structure which was irregular in shape and markedly dispersed, as evidenced by the prominence of intervening karyoplasm. Prominent dilated cisterns associated with endoplasmic reticulum contributed to the sparse appearance of the soma. In control cultures filaments in processes were distributed parallel to the axis. This linear organization was lost after exposure to GLU where filaments appeared disorganized and less numerous. Supported by NS23324 to LJN and NS14543 and 25372 to PHC.

305.13

REDOX STATE ALTERS NMDA RECEPTOR-MEDIATED NEUROTOXICITY. Daniel I. Levy and Stuart A. Lipton. Dept of Neurology, Children's Hosp & Harvard Med Sch, Boston MA 02115.

NMDA is lethal to postnatal rat retinal ganglion cells cultured in high Ca/low Mg medium (Hahn, Aizenman & Lipton *PNAS* 85:6556, 1988). Exposure to a reducing agent, such as dithiothreitol (DTT), selectively increases the electrophysiological response to NMDA in central neurons, including retinal ganglion cells; conversely, oxidizing agents reverse this effect (Aizenman, Lipton & Loring *Neuron* 2:1257, 1989). To investigate the effects of redox modulation on NMDA neurotoxicity, we studied retinal ganglion cell survival 24 hr after a 5-10 min exposure to DTT. Following dissociation, retinal cells were rinsed with control saline, DTT (0.5 - 2 mM), or DTT in the presence of the oxidizing agent DTNB (1 mM). Culture medium contained high Ca (10 mM), low Mg (50 μM), and micromolar concentrations of glutamate to induce neurotoxicity. To determine the degree of killing specifically related to activation of the NMDA receptor, APV (200 μM) was added to sibling cultures.

APV-preventable, glutamate-induced death was increased 70±9% with DTT treatment. This effect was totally blocked by the concomitant addition of DTNB. These findings suggest that the enhanced killing following reduction with DTT is mediated at the NMDA receptor site, and that the redox state of the NMDA receptor is crucial for the survival of neurons facing glutamate-related injury. Since a reducing state has been found in small and moderately-sized cerebral infarcts, these results may have implications for the treatment of stroke.

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305.14

TURTLE CORTICAL NEURONS IN DISSOCIATED CULTURE ARE RESISTANT TO GLUTAMATE NEUROTOXICITY. A.M.Wilson*, A.R.Kriegstein (SPON: S.Ryan). Neurology Dept., Stanford Med. Ctr., Stanford, CA 94305.

The cerebral cortex of the pond turtle is resistant to ischemic injury. Because glutamate (Glu) neurotoxicity is an important mechanism of cell death in animal models of cerebral ischemia, we evaluated the sensitivity of cultured turtle neurons to Glu. Cortical neurons from the turtle, P. scripta, were prepared by a modification of the technique of Choi, Maulucci-Gedde, and Kriegstein (1987). Neurons were dissociated from the cortex of Stage 21 embryos, a week after the expression of NMDA receptors, and maintained at 30°C. Neurons appeared morphologically mature after 25 ± 3 days. Whole cell patch clamp recording of mature neurons showed spontaneous excitatory synaptic activity. Cultures were exposed to 3 mM Glu for 5 min at 22°C, washed free of Glu, and incubated for 24 hr at 35°C. Control cultures were not exposed to Glu but otherwise treated the same. The extent of cell death, as assayed by cellular morphology and trypan blue exclusion, was identical in Glu-exposed and control cultures. By contrast, murine cortical cell cultures treated with 3 mM Glu and incubated at 35°C showed >90% cell death (H. Monyer, pers comm). The remarkable difference between chelonian and mammalian neurons in susceptibility to Glu neurotoxicity may reflect differences in the timing or magnitude of receptor expression, circuit properties in culture, or intracellular mechanisms.

305.15

GLUTAMATE STIMULATED Ca_i ELEVATION IN CULTURED NEURONS MEASURED WITH Fluo-3 IN A 96 WELL MICROTITER FLUORIMETER J. Drejer[1*], P. Wahl[1,2*], A. Schousboe[2*], T. Honoré[1*]
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Fluo-3 is a new calcium chelator, which is non-fluorescent but undergoes a 40-fold enhancement of fluorescense on calcium binding. Fluo-3 is excited at 506 nm permitting the use of conventional non-quartz equipment. In the present model we have used Fluo-3-AM loaded mouse cortical neurons cultured in 96-well microtiter plates for measuring Ca_i with the use of a standard microtiter Fluorimeter (Fluoscan II, Labsystems). This setup allows the simultaneous measurement of a high number of identical cultures and may be used as a high throughout screening model for testing effects of compounds influencing Ca_i. Exposure of cortical neuronal cultures to excitatory amino acids such as glutamate, NMDA, kainate, quisqualate and AMPA lead to an immediate increase in Ca_i from a resting level of 70 nM to a maximum of 200-300 nM. The effect of NMDA could be antagonized potently by D-APV and MK 801. Non-NMDA responses induced by kainate, AMPA or quisqualate were blocked by CNQX. Responses to glutamate could only be blocked partially with PCP (60% inhibition). Cells exposed to low concentrations of glutamate (<20 μM) for up to 20 min were able to restore normal Ca_i levels after exposure. However, exposure to high concentrations of glutamate (>300 μM) for only a few min lead to irreversible calcium overload.

305.16

POTENCY OF GLUTAMATE AS A NEUROTOXIN IS GREATLY ENHANCED IN ASTROCYTE-POOR CULTURES OF RAT CEREBRAL CORTEX P. A. Rosenberg and E. Aizenman. Dept. of Neurology, Children's Hospital, Harvard Medical School, Boston, MA 02115.

Glutamate neurotoxicity has been proposed as a cause of neuronal death in a variety of illnesses, including stroke. An important element of the glutamate hypothesis is that under conditions producing neuronal death, glutamate, or a related agonist, attain concentrations in the extracellular space exceeding the toxic threshold for neurons. Since the presence of astrocytes might be expected to have a significant effect on the dose-response relationship for glutamate neurotoxicity (Garthwaite, Br. J. Pharmacol. 85: 297, 1985), we attempted to determine the sensitivity of neurons to glutamate in relative isolation from glia in cortical cultures. Astrocyte-poor (AP) cultures, containing approximately 50% neurons, demonstrated an EC50 to glutamate of 1.9±0.6 μM (n=8). In contrast, astrocyte-rich (AR) cultures, containing 6% neurons and approximately 15 times the number of astrocytes as AP cultures, demonstrated an EC50 to glutamate of 194±43 μM (n=3). These results suggest that central neurons are vulnerable to glutamate neurotoxicity at concentrations comparable to those that have been measured in normal hippocampus, using microdialysis. This work was supported by NS00993 and #P30-HD18655.

305.17

ULTRASTRUCTURAL RELATIONSHIPS BETWEEN NEURONS AND GLIA MODULATING GLUTAMATE TOXICITY IN CORTICAL CULTURES. K.M. Harris, D. Rulf, P.A. Rosenberg. Dept. of Neurol. Children's Hosp., Harvard Med. Sch., Boston, MA 02115

In a related study, Rosenberg et al. (this meeting) have shown that neurons in astrocyte-rich (AR) cortical cultures are significantly less sensitive to glutamate toxicity than neurons in astrocyte-poor (AP) cultures (1/15 the number of nonneuronal cells). We have compared AP and AR cultures with electron microscopy. Cultures were thin sectioned perpendicularly to the substrate thus, containing the full depth of culture in each section. In AR cultures, we have observed 2-4 overlapping astrocytic processes at the media surface; dense staining material occludes the inter-process space. The neuronal processes and synapses reside beneath the astrocytic sheet and thus, may be shielded from glutamate in the media. In contrast, AP cultures show neuronal processes and synapses exposed directly to the media. Portions of the neuronal somas in both AP and AR cultures are at the media interface; in AR cultures no axo-somal synapses have been observed here. These results suggest: 1) that the physical isolation of neuronal processes from media glutamate may render neurons in AR cultures less vulnerable to glutamate toxicity; and 2) that the primary target for glutamate as a toxin is likely to be dendritic. Supported by NS21184, NS00993, P30-HD18655.

305.18

EXCITOTOXINS AND ANTIOXIDANTS IN REAGGREGATE CULTURES OF DEVELOPING RAT BRAIN. M. Halks-Miller, L. Toll, and M. F. Davies. SRI International, Menlo Park, CA 94025.

Reaggregate cultures of fetal rat brain contain neurons and glia that mature on schedule with their *in vivo* counterparts. Large aggregates can develop central neuronal degeneration. The relationship of this degeneration to the development of the NMDA-receptor in reaggregate cultures was examined by conducting ^{3}H-MK 801 saturation isotherms on *in vitro* days 8, 15, 19, and 23. Binding was greatest on days 8 and 15 (1,000 and 916 pmoles/mg protein), falling to 516 and 691 pmoles/mg protein on days 19 and 23 respectively. Addition of 500 µM NMDA to the cultures for one hour on day 14 *in vitro* caused an immediate swelling of cellular processes. One day later, approximately 50% of neurons were undergoing necrosis. By day 19 necrotic cells had been cleared by phagocytosis. The aggregates were reduced in volume and in protein content, but the remaining neurons appeared normal. This pattern of necrosis correlated with the release of the intracellular enzyme lactate dehydrogenase (LDH) into the culture medium. Peak levels of LDH were found 24 hr after exposure to NMDA. Both MK801 (10^{-5} M) and dextrorphan (10^{-4} M) prevented the release of LDH into the medium. Tocopherol, which has been shown to ameliorate neuronal necrosis in long-term cultures, had no effect in inhibiting the NMDA-induced release of LDH in short-term cultures.

305.19

MONOSIALOGANGLIOSIDES REDUCE NMDA NEUROTOXICITY IN NEONATAL RATS. M. Lipartiti*, S. Mazzari, A. Lazzaro*, R. Zanoni*, M.S. Seren* and A. Leon. Fidia Research Laboratories, 35031 Abano Terme, Italy.

We here report that systemic administration of monosialogangliosides is efficacious in limiting EAA-related neurotoxicity in vivo as already shown in vitro. Monosialogangliosides or saline were subcutaneously injected 1 hour prior and immediately following i.c.v. administration of NMDA (25 nmoles) in 7-day-old (P7) rats. At P12, the NMDA + saline treated group showed extensive brain damage as well as reduction in brain weight, whilst such neurotoxic effects were significantly reduced in the ganglioside treated groups. Interestingly, even though a similar effect was observed utilizing non-competitive NMDA antagonists, the monosialoganglioside effect, at least in vitro, occurs in the absence of any interference with specific EAA recognition sites or receptor operated calcium influx. This differentiates gangliosides from non-competitive NMDA antagonists. Furthermore, since EAA are most probably implicated in hypoxic-ischemic brain damage, the results here observed support the hypothesis that a similar effect underlies, at least in part, the ganglioside capability to ameliorate outcome following cerebral ischemia in adult rodents.

305.20

CONTINUOUS INTRATHECAL INFUSION OF THE GANGLIOSIDE GM-1 IN SHEEP. L.E. McCarthy* R.W. Colburn and D.W. Coombs. Dept. of Anesthesiology, Dartmouth Medical School, Hanover, NH 03756.

Implantable infusion pumps from 2 commercial sources (Shiley-Infusaid, Norwood, MA; Therex, Walpole, MA) are being used to administer intrathecal GM-1 or saline continuously to adult female sheep for up to 6 months. The main series of 11 animals is exposed for 12 weeks to 10 µg to 1 mg GM-1 per day, delivered via indwelling lumbar catheters. Infused volumes varied up to 3.5 ml/day. GM-1 infusions began 2 weeks after pump implantation or after complete recovery from surgical procedures. Animals were observed for signs of toxicity and spinal cords were harvested at term for histopathologic examination. Regardless of the dose of GM-1, no functional changes were observed which could be attributed to infusion. Histopathologic examinations of the spinal cords are currently underway. Thin layer chromatography of CSF samples showed that test animals had markedly elevated levels of GM-1 in lumbar and cisternal CSF samples compared to saline-infused controls. Measurements from one animal receiving 10 mg/day yielded concentrations of 120 and 12 µg/ml of CSF at the lumbar and cervical levels, respectively, suggesting that the CNS has the capacity for handling large amounts of GM-1. Pending histological examination, the chronic application of high concentrations of GM-1 directly to the lumbar cord, appears to be non-toxic.

EXCITATORY AMINO ACIDS: EXCITOTOXICITY III

306.1

AMINO-OXYACETIC ACID PRODUCES EXCITOTOXIC LESIONS IN THE RAT STRIATUM. E. Urbanska‡, C. Ikonomidou‡, M. Siekluckaŧ and W.A. Turski‡ (SPON: M.S. Shahid Salles). ‡Dept. of Pharmacology, Medical School, PL-20-090 Lublin, Poland.

Amino-oxyacetic acid (AOAA) was microinjected into the striatum of adult (over 90 d of age) and 6-d-old Wistar rats and the resulting neuropathology was examined after a survival period of 30 min -30 days. AOAA (0.1, 0.25 and 1 µmol) produced neuronal degeneration in the adult rat striatum in a dose-dependent manner. In 6-d-old rats, AOAA caused no striatal damage up to the dose of 1 µmol. Neuronal injury produced by AOAA displayed excitotoxic characteristics, i.e. acute swelling of dendrosomatic elements with relative sparing of presynaptic axon terminals and glial cells. Prominent gliotic reaction was observed in the injured striatum within 4-5 days after microinjection of AOAA. Activity of L-glutamate decarboxylase (GAD) was decreased in the lesioned striatum 14 d after injection of AOAA (0.25 or 1 µmol) by 18 and 50% respectively. The neurotoxic effect of AOAA (0.25 and 1 µmol) in the adult rat striatum could be prevented efficiently by 2-amino-7-phosphonoheptanoic acid (AP7; 0.25 µmol) or kynurenic acid (KYN; 0.5 µmol). AP7 and KYN also prevented reduction in striatal GAD activity. These findings suggest that AOAA causes excitotoxic lesions in the adult rat striatum by excessive activation of N-methyl-D-aspartate (NMDA) receptors. AOAA however does not activate NMDA receptors by itself. This becomes evident in the failure of AOAA to damage the 6-d-old rat striatum, which reportedly is more susceptible to the neurotoxic action of NMDA. The mechanism by which AOAA activates striatal NMDA receptors seems to depend on the presence of intact corticostriatal pathways, since cortical ablation abolishes the excitotoxic reaction of striatal tissue to AOAA (1 µmol). AOAA is known to inhibit KYN-synthesis and it is thus tempting to speculate that this mechanism might partly underlie its neurotoxic properties. It is remarkable that striatal neurotoxicity of AOAA increases with age. This makes the mechanism of AOAA neurotoxicity a potential candidate for the pathogenesis of Huntington's disease, which starts in adult life and has been proposed to base on an excitotoxic process. In AOAA (0.25 and 1 µmol) lesioned rats, apomorphine HCl (0.5 mg/kg) produced circling ipsilateral to the lesioned striatum. Bilateral striatal lesion with AOAA (0.25 and 1 µmol) reduced catalepsy response to haloperidol (2 mg/kg) and increased that to morphine HCl (15 mg/kg) or arecoline HBr (15 mg/kg). This behaviour is similar to that observed in previously described rodent models for Huntington's disease. Supported by the Polish Academy of Sciences.

306.2

AN INDIRECT PRESYNAPTIC GLUTAMATERGIC MECHANISM IS INVOLVED IN STRIATAL EXCITOTOXIN LESIONS MEDIATED BY NMDA, KAINATE AND QUISQUALATE RECEPTORS

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Removal of the corticostriatal glutamatergic pathway protects against excitotoxic neuronal degeneration produced by kainic acid (KA), quinolinic acid (QA) and N-methyl-D-aspartate (NMDA). Decortication may protect against striatal excitatory amino acid (EAA) mediated toxicity by preventing presynaptic release of glutamate from corticostriatal terminals. We investigated the role of presynaptic glutamatergic afferents (corticostriatal) in striatal excitotoxin lesions caused by agonists acting at the three subtypes of EAA receptors. Rats were decorticated 14 days prior to intrastriatal injections of either NMDA (200 nmol), QA (240 nmol), KA (7.5 nmol), quisqualic acid (QUIS; 1000 nmol), (RS)-2'-amino-3-hydroxy-5-methyl-isoxazole-4-proprionic acid (AMPA; 22.5 nmol) or ibotenic acid (IBO; 200 nmol). Control (corticate) rats also received an equimolar dose of one of the above EAA agonists. The lesions were evaluated quantitatively by measuring the striatal content of GABA, substance P, neuropeptide Y and somatostatin. Consistent with previous reports, decortication totally blocked the excitotoxic effects of NMDA, QA and KA. In addition, we found that decortication totally blocked AMPA and QUIS striatal lesions. However, IBO, an EAA with agonist properties at both NMDA and QUIS (inositol phosphate linked) receptors, produced lesions that could only partially be blocked by prior deafferentation. These findings are consistent with the hypothesis that EAA mediated presynaptic glutamate release is involved in striatal excitotoxin lesions *in vivo*. The fact that decortication only partially blocked IBO lesions suggests that activation of the inositol phosphate linked QUIS receptor may play a role in EAA induced neuronal death. Agonists acting at other EAA receptors may activate this receptor by the presynaptic release of glutamate.

306.3

REGIONAL BRAIN QUINOLINIC ACID LEVELS AND NEUROCHEMICAL MARKERS OF TOXICITY: EFFECTS OF ACUTE VS CHRONIC TRYPTOPHAN FEEDING

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Quinolinic acid (QA), a metabolite of tryptophan (TRYP), is markedly responsive to acute TRYP loading. As an agonist of the NMDA receptor, QA is implicated in the pathogenesis of several neurological disorders, including Huntington's Disease (HD). Studies have suggested that an alteration of metabolism of TRYP into QA may in part be responsible for HD.

We have examined the effects of chronic and acute TRYP feeding in rats on plasma TRYP levels, brain QA levels and neurochemical markers of excitotoxin induced toxicity. Rats were fed chow with either 0.3 % (normal), 1.5 %, 3.0 % or 5.0 % TRYP. At the end of either 3 days or 2 months, rats were sacrificed between 5-7 AM, plasma was collected and tissue sections from striatum, hippocampus and cortex were dissected and frozen. Plasma TRYP levels were measured using high performance liquid chromatography (HPLC) with UV detection. Regional brain content of QA was measured using gas chromatography with negative ionization mass spec. detection. Neurochemical markers of toxicity (somatostatin, neuropeptide Y, substance P and GABA) were measured by radioimmunoassay and HPLC with electrochemical detection.

After 3 days of TRYP feeding (5.0 % vs 0.3 %), brain QA levels were increased 5 fold. However, after 2 months of TRYP feeding, brain QA levels did not significantly differ among the different doses of dietary TRYP. Plasma TRYP levels did remain markedly elevated in a dose dependent manner. Also at 2 months no sign of neurodegeneration (as measured by neurochemical markers of toxicity) was observed in any brain region examined. These results suggest that under chronic conditions of TRYP loading QA synthesis downregulates and is no longer precursor responsive.

306.4

GM_1 GANGLIOSIDE PRETREATMENTS REDUCE QUINOLINIC ACID INDUCED STRIATAL DAMAGE. G. Lombardi[1]*, G. Toffano[2] and F. Moroni[1]. [1]Dept. of Pharmacology, Univ. of Florence, [2]Fidia Res. Lab., Abano Terme (Padova), Italy.

A systemic treatment with GM_1 ganglioside has previously been found to reduce experimentally induced neuronal damage in dopaminergic, cholinergic and serotoninergic neurons, provided that the lesion is in proximity to the affected cell bodies (see Lombardi, G. et al., *Neuropharmacology*, 27: 1085,1988). Quinolinic acid (QUIN) and other excitotoxins typically cause neuronal damage only when applied near the affected cell bodies. In order to verify whether or not systemic GM_1 ganglioside administration could act by reducing the effects of endogenous excitotoxins, rats were unilaterally injected with 250 nmoles of QUIN (injection coordinates: AP=0; L=3.4; H=-4.2 from bregma, Koenig-Klippel, 1963). QUIN induced striatal lesions were evaluated both histologicallly on Nissl stained section and biochemically by measuring the striatal activities of choline acetyltransferase (ChAT) and L-glutamic acid decarboxylase (GAD). The intrastriatal injection of QUIN reduced GAD activity in the striatum of the injection side (from 11.1±0.5 to 7.14 ±0.4 nmol /h/mg of tissue) and ChAT activity (from 7.02±0.3 to 4.51±0.3 µmol/h/mg of tissue) by approximately 40 %. No changes were observed in the controlateral side. A treatment with intraperitoneal GM_1 ganglioside (10 or 30 mg/Kg/day) started immediately after the excitotoxin injection failed to modify QUIN toxicity. However, when GM_1 ganglioside was administered for 3 days before surgery at a dose of 30 mg/kg/day and its administration was continued for at least 12 days a significant reduction in QUIN toxicity was observed. Lower doses were inactive. Thus, appropiate doses of GM_1 ganglioside may reduce the excitotoxic damage in gabaergic and cholinergic neurons.

306.5

EFFECTS OF CHRONIC INTRASTRIATAL INFUSION OF QUINOLINIC ACID. Z. Susel*, T.M. Engber, S. Kuo* and T.N. Chase (SPON: J.R. Walters). Exptl. Therapeutics Branch, NINDS, Bethesda, MD 20892.

Acute injection of the excitotoxin quinolinic acid into the rat striatum has been reported to produce a pattern of neuronal degeneration similar to that seen in post-mortem striatal tissue from patients with Huntington's disease. However, if an endogenous excitotoxin is involved in the pathogenesis of Huntingtons's disease, exposure to such a compound is likely to occur over a prolonged period of time. We studied the effect of chronic infusion of quinolinic acid into the rat striatum on striatal choline acetyltransferase (ChAT) activity, glutamic acid decarboxylase (GAD) acitivity and somatostatin content. Infusions were conducted for 14 days at a rate of 0.5 µl/hr via an Alzet osmotic pump (Model 2002) connected to a stainless steel cannula implanted in the striatum. Quinolinic acid (dissolved in artificial CSF, pH adjusted to 7.4) was administered at doses of 9, 90, or 900 nmol/day. Control animals received infusions of artificial CSF vehicle. After 14 days, rats were sacrificed and the striata dissected for measurement of ChAT and GAD activity. Infusion of quinolinic acid significantly depleted ChAT activity at all 3 doses (69% of control values at 9 nmol/day; 61% of control at 90 nmol/day; 8 % of control at 900 nmol/day). Quinolinic acid infusion also significantly reduced GAD activity (73% of control at 9 nmol/day; 71% of control at 90 nmol/day; 12% of control at 900 nmol/day). Preliminary data showed a decrease in somatostatin content due to quinolinic acid infusion (69% of control at 9 nmol/day, 71% at 90 nmol/day, 20% at 900 nmol/day), but this decrease was statistically significant only at the highest dose of quinolinic acid. These findings indicate that chronic infusion of a low dose of quinolinic acid can cause excitotoxic damage in the striatum and may provide support for excitotoxic models of neurodegenerative diseases such as Huntington's disease.

306.6

NEURODEGENERATIVE EFFECTS OF CHRONIC INFUSION OF QUINOLINIC ACID INTO RAT HIPPOCAMPUS AND STRIATUM. A.Vezzani, R.Serafini*, M.Rizzi* and R.Samanin* Istituto di Ricerche Farmacologiche "Mario Negri", Via Eritrea 62, Milano 20157 (Italy).

The effect of chronic infusion of quinolinic acid (QUIN) into rat hippocampus and striatum was examined at light microscopic level on 40 µm cresyl-violet stained brain sections. Continuous infusion was done at constant speed (0.5 µl/h) for one or two weeks by osmotic minipumps (14 days-Alzet Model 2002) implanted subcutaneously and connected to an injection needle inserted in the dorsal hippocampus or striatum. No build up of 3H-QUIN occurred in the tissue during infusion as assessed by measuring radioactivity at various times (3h-1 week) after minipump placement. One-week intrahippocampal infusion of 6 nmol/h QUIN but not of its inactive analog nicotinic acid (n=6) caused complete degeneration of all pyramidal and granule cells; 2.4 nmol/h QUIN selectively affected pyramidal cells (CA1, CA3-4) while granule cells were generally spared (n=8). Neuronal loss was limited to a spherical area with a radius of approxymately 1 mm. The neurodegenerative effects induced by 2.4 nmol/h QUIN were antagonized by coinfusion of 7.2 nmol/h kynurenic acid (n=6), a tryptophan metabolite with anti-excitotoxic properties. One and two weeks-infusion of 1.2 nmol/h QUIN did not induce neurodegeneration except occasional loss of CA4 cells (n=6). One week-intrastriatal infusion of 10 nmol/h QUIN revealed an area of pronounced neurodegeneration extending approxymately 1 mm around the injection track; 4 nmol/h QUIN infused for one week were ineffective while two weeks-infusion caused cell loss less severe than 10 nmol/h (n=6-8). Possible implications for the pathogenesis of human neurodegenerative disorders will be discussed.

306.7

IBOTENIC ACID IS SIMILAR TO N-METHYL-D-ASPARTATE IN CYTOTOXICITY BUT SIMILAR TO QUISQUALATE IN PHOSPHOINOSITIDE HYDROLYSIS ENHANCEMENT IN RAT CORTICAL CULTURES. J. Patel , W.C. Zinkand*, W.C. Moore*, and A.I. Salama. Dept. of Pharmacology, ICI Pharmaceuticals Group, ICI Americas Inc., Wilmington, DE 19897.

Ibotenic acid (IBO) has been reported to interact with several systems associated with excitatory amino acids throughout the mammalian central nervous system. These include, but are not limited to, excitotoxicity and enhancement of phosphoinositide (PI) hydrolysis. We report that in primary neurocortical cultures, IBO, like N-methyl-D-aspartate (NMDA) is a potent neurotoxin as measured by release of lactate dehydrogenase (LDH). However, unlike NMDA, it enhances PI hydrolysis in a manner similar to quisqualate. IBO cytotoxicity, like that of NMDA, is potentiated by glycine and blocked by MK-801. Like quisqualate, the enhancement of PI hydrolysis by IBO is not modulated by glycine or blocked by NMDA antagonists. In neurotoxicity, IBO is about five-fold less potent than NMDA and roughly equipotent to quisqualate. In enhancement of PI hydrolysis, IBO is one hundred-fold less potent than quisqualate.

306.8

REGIONAL EFFECTS OF THE CHRONIC ADMINISTRATION OF THE EXCITOTOXIN B-N-OXALYLAMINO-L-ALANINE (BOAA) ON DOPAMINE AND SEROTONIN METABOLISM IN RAT BRAIN. V. Jackson-Lewis, J.L. Cadet, F. Suber, S. Fahn. Dept. Neurology, Columbia Univ., NY, NY 10032.

B-N-Oxalylamino-L-Alanine (BOAA), a potent excitotoxin, causes stereotypic behaviors when given acutely. Some of these behaviors have been related to catecholamine (CA) and serotonin (5-HT) metabolism. We investigated the effects of BOAA on regional CA and 5-HT metabolism in rat brain.

Rats received saline or BOAA (50ug x 1 day or 25ug x 5 days) icv and were sacrificed at 24 or 96 hours after treatment. DA, DOPAC, HVA, 5-HT and 5-HIAA levels were determined in the caudate (CN), accumbens (NAc), hippocampus (HIP), frontal cortex (FC) and brainstem (BS). Treated animals showed significant decreases in DA and to a lesser extent DOPAC and HVA levels in CN and NAc. While a single dose of BOAA was effective in decreasing DA and its metabolites, multiple dosage had a far greater effect. 5-HT and 5-HIAA levels decreased in all areas studied, the most marked effect being in HIP and FC. Decrements in 5-HIAA paralleled that of 5-HT. Multiple dosage was more effective in HIP whereas single dosage was equally as effective as multiple doses in FC. These data suggest that the excitotoxin, BOAA, given acutely or chronically, causes marked alterations in regional CA and 5-HT metabolism.

306.9

BOAA ENHANCEMENT OF L-GLUTAMATE RELEASE FROM GUINEA PIG HIPPOCAMPAL MOSSY FIBER SYNAPTOSOMES. R.L. Gannon and D.M. Terrian. Clinical Sci. Div., USAF Sch. Aerospace Med., Brooks AFB, TX. 78235.

The plant-derived amino acids Beta-N-oxalylamino-L-alanine (BOAA) and Beta-N-methylamino-L-alanine (BMAA) have been suggested to be environmental neurotoxins. Both BOAA and BMAA have been reported to possess similar neurotoxic properties, and appear to activate postsynaptic non-N-methyl-D-aspartate (NMDA) and NMDA receptors, respectively. We report here the effects of BOAA and BMAA on the basal and stimulated release of L-glutamate (Glu) and Dynorphin (Dyn) A(1-8)LI from guinea pig hippocampal mossy fiber synaptosomes. Fifteen minute applications of high (200 uM) but not low (30 uM) concentrations of BOAA significantly increased the basal release of cytosolic L-Glu by over 20 pmoles/min/mg protein. In contrast, BMAA (up to 1 mM), was without effect on basal L-Glu release. Depolarization of synaptosomes by a 30 mM KCl-containing medium in the presence of 200 μM BOAA significantly increased the evoked Ca^{2+}-dependent L-Glu release from 96 ± 7 to 136 ± 9 pmoles/min/mg protein. Lower concentrations of BOAA (30 uM) and BMAA (up to 1 mM), had no effect on KCl-stimulated release. Neither BOAA nor BMAA affected the basal or evoked release of Dyn A(1-8)LI. AFOSR 2312W3.

306.10

ENVIRONMENTAL NEUROTOXINS: A ROLE FOR DOMOIC ACID. A. Novelli, J. Kispert*, A. Reilly* and V. Zitko*. Lab. of Biophysics, International School for Advanced Studies, 34014 Trieste, Italy; Lab. Mol. Biology, NINDS, NIH,Bethesda, MD 20892, USA; Marine Chem. Division, Biological Station,St. Andrews, N.B., E0G2X0, Canada.

During the period from mid-November to mid-December 1987, more than 150 Canadians suffered from acute intoxication after eating cultured blue mussels. Their symptoms included disorientation, confusion and memory loss. The mussels were found to contain high amounts of domoic acid (DA), a peculiar excitatory amino acid (EAA), expected to act similarly to kainate. We used rat cerebellar granule cells in primary culture as an experimental system for the characterization of biochemical and neurotoxicological properties of DA. Exposure of the neuronal culture, for 1 min., to increasing concentrations of DA, resulted in a dose-dependent stimulation of cGMP formation (ED_{50}=5μM). Maximal stimulation (>100 times over basal levels) was achieved with 10μM DA. DA effect on cGMP formation was not reduced by physiological concentrations of Mg^{++}(1mM), by the NMDA-receptor antagonist amino-phosphonovalerate (APV) (1mM) or by the NMDA-receptor-associated channel blocker MK-801(1μM) and was dependent upon extracellular calcium. DA (50μM) did not affect intracellular cAMP levels. Neurotoxicity by DA (1.5μM) occurred within 30 min. of exposure to the drug when the neurons were in conditions of reduced cellular energy levels. However, DA toxicity was not potentiated in the absence of Mg^{++}, as occurred for NMDA agonists, and was not antagonized by APV or MK-801. Quisqualic acid (100μM), an agonist at the NON-NMDA type of EAAR, selectively antagonized DA's biochemical and toxicological effects.

In conclusion, DA appears to exert its neurotoxicity through the activation of the NON-NMDA subtype of EAAR, which is also involved in the stimulation of the formation of cGMP as a specific second messenger .

306.11

PHARMACOLOGY OF DOMOIC ACID FOLLOWING SYSTEMIC ADMINISTRATION: COMPARISONS WITH OTHER EAA AGONISTS. R.A.R. Tasker*, S.M. Strain* and B.J. Connell* (SPON: J.W. Downie). Dept. of Anatomy & Physiology, Atl. Vet. Coll., Univ. of PEI, Charlottetown, PEI, Canada C1A 4P3.

In Dec. 1987 a number of people suffered seizures and memory loss after ingesting cultured mussels containing Domoic acid (DA). To study the pharmacology of DA, female CD-1 mice (20-25 g) were injected (i.p.) with serial dilutions of toxic mussel extracts. Dose-related behaviours were recorded and subsequent data were based on cumulative scores over 60 min. according to a 7 point rating scale. Injections of DA in water or in acid extracts from DA-free mussels produced parallel ($p > 0.05$) sigmoidal dose-response curves (ED50 = 4.0 and 4.9 mg/kg, respectively). Injections of Kainic acid produced identical dose-related behaviours and a parallel ($p > 0.05$) dose-response curve (ED50 = 32.0 mg/kg). Injections of various doses of N-methyl-d-aspartate or Quisqualic acid failed to produce equivalent behaviours. DA-induced behaviours were not significantly antagonized by injections of 200, 400 or 600 mg/kg Kynurenic acid (before, simultaneously or after DA) or by prior injection of 40, 80 or 120 mg/kg Glutamic acid diethyl ester (GDEE). We conclude that systemically-administered DA produces excitotoxicity via activation of kainate receptors, and is not antagonized by either Kynurenic acid or GDEE. Supported by NSERC Grant STRGP 036 to R.T.

306.12

NEUROTOXICITY OF DOMOIC ACID-CONTAMINATED SHELLFISH: REVERSAL BY KYNURENIC ACID. **G.B. Glavin, C. Pinsky* and R. Bose. Dept. Pharmacol. and Dept. Surgery, Fac. Med., Univ. Manitoba, Winnipeg, CANADA R3E 0W3.**

The potent neuroexcitant amino acid (NEAA), domoic acid (DA), has been implicated as the major neurotoxin involved in the recent outbreak of amnesic shellfish poisoning (ASP), characterized by severe gastrointestinal bleeding and ulceration and protracted CNS disorders such as seizures, coma and death. An aqueous acidic extract from poisonous Atlantic mussels was injected in mice, yielding an LD_{84} of 40 ml/kg. Body-scratching, clonic convulsions and death occurred. Autopsy revealed antral and duodenal ulcers and alkaline peritoneal ascites. Protein content of the ascites correlated positively with pH. Pure domoic acid (0.5-8.0 mg/kg) produced similar findings. The broad-spectrum NEAA antagonist, kynurenic acid (KYN), 300 mg/kg, protected powerfully and significantly against DA-induced neuropathy and gastropathy. In other experiments, KYN showed anti-ulcerogenic and gastric anti-secretory effects. Optimal protection by KYN occurred 60 and 75 minutes after DA treatment, suggesting that KYN may show a form of use-dependent antagonism. We suggest that KYN would likely provide a significant measure of protection against DA neuro- and gastro-toxicity seen in humans at approximately 20 h after ingestion of contaminated shellfish. **(Supported by the MRC and Alzheimer Society of Canada).**

306.13

EXCITATORY AMINO ACID ANTAGONISTS (EAAA) PROTECT AGAINST AGONIST-INDUCED WILD RUNNING (WR) IN MICE. **J.T. Simmonds*, T.L. Sailer*, AND R.R. Notvest** (Spon: K.L. Keim). Wyeth-Ayerst Research, Princeton, NJ 08543-8000

In a previous report, it was shown that infusion of kainate into the colliculi induces WR in mice (Frye, G.D., Neurosci Abs, 13:510, 1987). This study examined the utility of the WR response as a pharmacological model. Intracollicular injections (1nmole) of kainate, AMPA or NMDA resulted in a rapid onset (<1 min) of WR. To examine the pharmacology, EAAA were co-administered with the agonists, and considered to protect if the delay to WR was >5 min. Co-administration of MK-801 (50 nmoles) protected only against NMDA WR. AP7 (25 nmoles) protected against NMDA and kainate but not AMPA WR. FG9065 (6 nmoles), FG9041 (14 nmoles) and GAMS (20 nmoles) protected against kainate; greater concentrations (50 nmoles) also protected against AMPA and NMDA WR. With the exception of AP7, the efficacy of these EAAA in the WR models is in agreement with their receptor selectivity. These WR models will be used to evaluate other known and novel EAAA.

306.14

THE KAINATE-INDUCED RELEASE OF AMINO ACIDS FROM CEREBELLAR GRANULE CELLS IS PARTIALLY PREVENTED BY QUISQUALATE AND 100 mM SUCROSE P.P. McCaslin and T.G. Smith Dept. Pharmacol. and Toxicol., UMC, Jackson, MS, 39216

Kainate is neurotoxic for cerebellar granule cells in primary culture. We have found that 25 *u*M quisqualate will prevent the rapid toxicity induced by 100 *u*M Kainate. Similarly, protection occurs by making the extracellular fluid hyperosmolar with 100 mM sucrose. The kainate-induced secretion of the excitatory amino acids, aspartate and glutamate, as well as several other amino acids were measured in granule neurons 18-22 day *in vitro* and the effects of quisqualate and sucrose on this kainate-induced release were examined. Neurons were washed with a balanced salt solution containing the following mM concentrations: 154 NaCl, 5.6 KCl, 2.5 $CaCl_2$, 10 glucose and 5 HEPES (pH 7.4, 37°C). Cells were left in this buffer to equilibrate for from 10 to 20 minutes. The release of several amino acids was then determined by placing a 0.25 ml drop of buffer (containing varying concentrations of kainate, quisqualate and/or 100 mM sucrose) on slides. After 3 min, the solution was collected and the amino acids (aspartate, glutamate, serine, threonine, glycine, alanine and GABA) secreted into the buffer were determined by 2-dimensional thin layer chromatography. Results indicate that kainate induces a large, apparently nonselective and calcium-independent, increase in the release of amino acids from granule cells. A significant release of most amino acids occurred with 10 *u*M kainate. While quisqualate and 100 mM sucrose protect against toxicity, they only partially prevent the release of amino acids induced by kainate.

306.15

CATECHOLAMINE CONTENT IN RAT CEREBRAL CORTEX AFTER KAINIC ACID INJECTION. Tzen-Kwan Chang, May J. Hsu, Wan-Lin Yang and Albert Y. Sun, Molecular and Cellular Neurobiology group, Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan, ROC

Kainic acid (KA) is an excitotoxin that produces seizures by directly binding to glutamate receptors. We have used this potent glutamate agonist to study action of excitatory neurotransmitters and epileptogenesis. Sprague-Dawley rats (about 250 g. body weight) were given 24 mg of KA/Kg body wt. in buffered solution by intraperitoneal injection. Controls were given the same volume of vehicle solution. Rats were killed by decapitation at 3, 6 and 24 hours after injection and the cerebral cortices were dissected out for the analysis of norepinephrine (NE), dopamine (DA), adenine nucleotides and their metabolites. NE content decreased about 30% at 3 hrs after drug treatment and recovered to significantly higher than control level after 24 hrs while the DA content remained constant. Concomitantly, there was a decrease in ATP, ADP and AMP content while xanthine and hypoxanthine increased. We have also examined the high affinity uptake of NE by synaptosomes isolated from cerebral cortices of controls and drug-treated animals. There was a reduction in NE uptake activity at 3 hrs after KA treatment, and this reduction recovered after 24 hrs. It is postulated that KA causes excessive release of NE from noradrenergic neurons as well as reduction in NE-uptake. The reduction of NE uptake activity may be caused by damages in NEergic neurons due to the excitotoxic effect of KA.

306.16

RAT STRAIN DIFFERENCES IN KAINIC ACID NEUROTOXICITY II: DOSE RESPONSE. G.T. GOLDEN, G.G. SMITH*, J.H. KULP*, P.F. REYES and T.N. FERRARO. VA Medical Center, Coatesville, PA 19320 & Thomas Jefferson Med. Coll. Phila., PA.

It has been our observation as well as others that the use of kainic acid (KA), in particular as an animal model of status epilepticus, is complicated by excessive variability in the magnitude of the behavioral, EEG and neuropathological changes seen. Recently, a strain of rats has been reported (Neurosci Abstr, 1988, 14: 252) which reliably and consistently demonstrate electrographic and behavioral status epilepticus in response to a 10mg/kg sc injection of KA. In the present study we report dose response results in this strain (WF/NCR) and two other strains of rats.

Male WF/NCR (n=39), SD/BC (n=40) and LEH/CR (n=41) were administered sc, one of four doses of KA (6, 8, 10 or 12mg/kg) and were monitored for 240 min after KA injection. At doses greater than 6mg/kg, WF/NCR rats had the shortest, least variable latencies to onset of initial behavioral seizure and to behavioral status epilepticus. All WF/NCR rats exhibited behavioral seizures at 8, 10, and 12mg/kg doses of KA and 100% attained status epileticus at KA doses of 10 and 12mg/kg. All strains showed similar scores at the 6mg/kg dose, with the WF/NCR rats demonstrating the greatest dose dependent response in behavioral seizure score. WF/NCR rats had significantly more WDS at KA doses of 8, 10 and 12mg/kg than either SD/BC or LEH/CR rats. (Supported by VA funds)

306.17

Neurovisceral Toxic Syndrome (NVTS) from domoic acid-contaminated mussels: Toxicity profile, putative antidote and involvement of free radicals. R. Bose, G.B. Glavin and C. Pinsky*. **Depts. of Pharmacology & Therapeutics and Surgery, University of Manitoba, 770 Bannatyne Avenue, Winnipeg, Manitoba, Canada R3E 0W3.**

The neuroexcitotoxin, domoic acid, which is kainate-like but of higher potency, has been implicated in a recent outbreak of NVTS from PEI mussels characterized by gastrointestinal diatheses, persistent memory disturbances and other symptomatology. We have discerned higher sensitivity to domoic acid in infant Swiss-Webster mice than in adults, and have characterized a behaviorotoxic profile which might be utilized for commercial screening of mussel products for the presence of excitotoxin contamination. In testing putative antidotes against domoic acid toxicity in mice, kynurenic acid, a nonselective antagonist at excitatory amino acid receptors was the most effective. Neuroexcitants and free radicals both have been implicated in neurodegeneration associated with aging and its related disorders as well as that following cerebral ischemia. Acute pretreatment of mice with toxic mussel extract (1 and 2 mg per kg domoic acid equivalents), at 60 min prior to sacrifice, resulted in significantly increased superoxide dismutase activity in brain with an associated decrease in thiobarbituric acid reactive lipid peroxidation products. These suggest an initial compensating phase of increased free radical activity. Chronic treatment with the toxin and tissue sampling after longer delays might reveal a failure in the brain's limited capacity to scavenge free radicals, leading to neuronal damage. **Support:** *MRCC, Alzheimer Soc Can.*

306.18

ACUTE FUNCTIONAL ACTIVITY AFTER EXCITOTOXIC LESIONS OF THE NUCLEUS BASALIS MAGNOCELLULARIS. L. Telford, B.J. Frost and †R.J. Boegman. Departments of Psychology, and †Pharmacology and Toxicology, Queen's University, Kingston, Ontario, Canada, K7L 3N6.

The nucleus basalis magnocellularis (nbM) is the site of origin of a major cholinergic projection to the cerebral cortex. Using the [^{14}C]2-deoxyglucose (2-DG) method, we examined regional functional activity in the brains of Sprague-Dawley rats following unilateral quinolinic (QUIN) (60 nmol) and ibotenic acid (IBO) (10 nmol) injections into nbM (according to the coordinates of Paxinos and Watson, 1986). 2-DG (125 μCi/kg) was infused into a femoral vein 20 minutes after injection of the excitotoxin. A slight decrease in functional activity occurred in ipsilateral visual cortex (QUIN, -18%; IBO, -26%), motor cortex (QUIN, -16%; IBO, -38%), and somatosensory cortex (QUIN, -23%; IBO, -38%) relative to contralateral cortex. Regions near the injection site displayed substantial functional activation relative to the contralateral side in QUIN-lesioned rats [substantia inominata (SI), 259%; globus pallidus (GP), 421%; caudate-putamen (CPu), 75%]. This asymmetry was less apparent after IBO injections (SI, 21%; GP, 122%; CPu, -16%). Intense labelling occurred in the medial forebrain bundle (MFB), 330%, and substantia nigra (SN), 121% after IBO, but not QUIN lesions (MFB, 14%; SN, 54%). We have demonstrated acute reductions in functional activity in ipsilateral cortex similar to those observed after chronic lesions. Additionally, massive ipsilateral functional activation occurred in several forebrain structures involved in motor control, which may correlate with the profound behavioral activation observed after excitotoxic lesions. Supported by MRC grants (MA 7244, MA 9118).

306.19

ANTAGONISTIC PHARMACOLOGY OF GLUTAMATE NEUROTOXICITY IN INFANT RAT BRAIN. C. Ikonomidou, J. Labruyere*, A. Benz* and J. W. Olney, Department of Psychiatry, Washington University, Medical School, St. Louis, MO

The antagonistic pharmacology of glutamate(Glu) neurotoxicity was studied in the infant rat brain. Glu (300-700 nmol), N-methyl-D-aspartate (NMDA) (3-8 nmol), kainic acid (KA) (2-6 nmol) and quisqualate (QA) (10-40 nmol) were microinjected into the head of the caudate nucleus of 2-d-old rats and the resulting neuropathology was quantitatively examined after a survival period of 4 hrs. All four excitatory amino acids produced a dose-dependent excitotoxic reaction which involved the head and tail of the caudate nucleus, the cingulate, frontal and parietal neocortices, the septum, several thalamic nuclei and the hippocampus. The damage produced by NMDA was more severe than that produced by KA, although in adult brain at these same doses, NMDA would produce no damage and KA would be severely neurotoxic. This corroborates prior reports that the infant rat brain, compared to the adult, is hypersensitive to NMDA and hyposensitive to KA. Systemic administration of the non-competitive NMDA receptor antagonist MK-801 (5mg/kg) totally prevented the excitotoxic reaction to NMDA (4 nmol) and substantially reduced the reaction to KA (3nmol, QA (20 nmol) and Glu (700 nmol). Similar results were obtained with the competitive NMDA-receptor antagonist APH (10 nmol), when co-injected with each agonist into the head of the caudate nucleus. The ability of MK-801 and APH to protect against KA and QA toxicity was unexpected. A possible explanation would be that KA and QA damage infant brain indirectly by release of endogenous Glu which then exerts excitotoxic action at hypersensitive NMDA receptors. This may be analogous to hypoxic/ischemic brain damage in the infant rat which, because it is effectively blocked by MK-801, is presumably caused by accumulation of endogenous Glu at NMDA receptors. Based on these and related findings, we propose that there may be a period during human ontogenesis when NMDA receptors are hypersensitive, rendering neurons hypervulnerable to the excitotoxicity of endogenous Glu, and that during this period, relatively minor disturbances in Glu homeostasis (e.g., due to hypoxia/ischemia, hypoglycemia, epilepsy, etc.) might cause brain damage that NMDA antagonists can prevent. Supported by RSA MH 38894 (JWO) and HD 24237.

306.20

POTASSIUM CYANIDE-INDUCED RELEASE OF GLUTAMATE FROM BRAIN SLICES: IMPLICATIONS FOR TOXICITY. M.N. Patel*, B.K. Ardelt*, G.K.W. Yim and G.E. Isom*. Dept. of Pharmacol. & Toxicol., Sch. of Pharm., Purdue U., W. Lafayette, IN 47907.

KCN produces a rapid onset of convulsions, tremors and subsequent generation of brain lesions. These manifestations are similar to those produced following activation of the glutamate excitotoxic transmitter system by acute brain hypoxia. Hence the interaction of KCN with the brain glutamate system was studied. Endogenous glutamate release from mouse cortical and hippocampal slices was monitored continuously through an enzyme-linked assay by fluorescence spectroscopy. Both a Ca-dependent and independent release of glutamate was evoked by KCN and KCl. In Ca^{+2} media, KCN (2mM), caused a release of glutamate from cortical (0.460μM/g/min.) and from hippocampal slices (0.708μM/g/min.) In EGTA-media it released 0.621μM/g/min glutamate from cortical slices and 0.577μM/g/min glutamate from hippocampal slices. Diltiazem (10^{-5}M), a Ca^{+2} channel blocker inhibited the KCN-induced Ca-dependent release of glutamate. It is concluded that KCN activates the glutamate excitotransmitter system in part by a calcium dependent process and these effects may contribute to the central actions of KCN. Additionally, the method employed to measure glutamate release gives a quantitative assessment of the different endogenous pools of the neurotransmitter in brain slices. (Supported by PHS Grant ES04140).

307.1

CYSTEINE PROTECTS BASAL FOREBRAIN CHOLINERGIC NEURONS FROM IBOTENIC, BUT NOT QUISQUALIC, ACID. G.L. Wenk. Department of Psychology, The Johns Hopkins University, Baltimore, MD 21218.

Ibotenic acid (IBO) may inhibit the exchange of cysteine (CYS) and glutamate in the neuronal membrane. The lack of CYS within the neuron may decrease the formation of glutathione and lead to increased levels of free radicals. The toxicity of excitatory amino acids (EAAs), e.g. IBO and quisqualic (QUIS), may depend upon the formation of free radicals within the neuron. If IBO destroys cells by inhibition of the transfer of CYS into the cell, then excess amounts of extracellular CYS may inhibit the cytotoxicity of IBO. CYS may not prevent the cytotoxicity of other EAAs, e.g. QUIS, that do not affect the CYS-glutamate exchange system. IBO or QUIS, in the presence or absence of CYS, were injected into the basal forebrain of rats. Cortical choline acetyltransferase activity indicated the extent of cholinergic cell loss. The results show that CYS protects basal forebrain cholinergic cells from the cytotoxicity of IBO but not QUIS. These data indicate the extent to which a potential therapeutic intervention, CYS, can alleviate the biochemical and pathological results of excessive amounts of EAAs in the basal forebrain.

307.2

Immature Cortical Neurons in Culture are Uniquely Sensitive to Glutamate Toxicity by Inhibition of Cystine Uptake. T.H. Murphy, R.L. Schnaar, and J.T. Coyle Dept. of Neuroscience, The Johns Hopkins University School of Medicine, Baltimore MD 21205.

Using the N18-RE-105 neuroblastoma X retina cell line, we previously demonstrated a mechanism of Ca++-dependent quisqualate (Quis)-type Glu toxicity which involves the inhibition of high-affinity cystine (Cys) uptake leading to glutathione depletion and an accumulation of cellular oxidants (*Neuron* 1989 in press). Similarly, primary cultures of immature cortical neurons (E17; 48-72 h in culture) were killed when exposed to culture medium with reduced Cys, with 50% cell death at 10 μM. Methionine-free medium was nontoxic. Toxicity was apparent within 2-6 h of substituting with low Cys medium. Immunohistochemistry indicated that non-neuronal cells (20% of the population) were less sensitive to Cys depletion. At this developmental stage no cytotoxicity was apparent with continuous exposure to 1 mM NMDA, quinolinate, aspartate, kainate, AMPA, or D-Glu for 24 h (100 μM Cys). However, compounds which significantly inhibit neuronal Cys uptake: Quis, Glu, homocysteate, BOAA, and ibotenate, killed greater than 50% of the neurons present (0.1-1.0 mM). The toxic potencies of Glu, Quis, and homocysteate were inversely proportional to the concentration of Cys in the medium.

Autoradiography of [35S]Cys uptake indicated that neurons and glia transport cystine by different mechanisms. Neurons used a high affinity uptake system which was Quis-sensitive, whereas non-neuronal cells used a low affinity system which was Quis insensitive, but potently blocked by D-Asp and Glu.

Exposure to Glu and homocysteate resulted in a time dependent depletion of cellular glutathione, which rendered cells vulnerable to endogenous oxidants. The centrally acting antioxidant idebenone at (1-3 μM), and vitamin E (30 μM) completely blocked the toxicity attributed to 1 mM Glu exposure.

In immature primary cortical cultures which lack NMDA toxicity, Glu toxicity results from the inhibition of Cys uptake leading to oxidant stress. The marked dependence of immature neurons on the uptake of Cys for viability may suggest a new mechanism of perinatal brain damage related to Glu.

307.3

Actions of Excitatory Amino Acids on Levels of Met-Enkephalin in Rat Brain. B.B. Ruzicka and K. Jhamandas, Department of Pharmacology and Toxicology, Queen's University, Kingston, Ontario, K7L 3N6

To examine the sensitivity of striatopallidal enkephalinergic neurons to excitatory amino acids (EAA's), the effects of N-methyl-D-aspartate (NMDA) and quisqualic acid (QUIS) on the levels of methionine-enkephalin-like immunoreactivity (ME-i.r.) were evaluated following their focal injection into the right caudate-putamen of male rats. Levels of ME-i.r. were estimated by radioimmunoassay in the caudate-putamen and the globus pallidus 7 days after a single excitotoxin injection. NMDA (51-135 nmoles) produced no significant change in levels of ME-i.r. in the caudate-putamen, but it produced a dose-related (51-102 nmoles) increase in the ipsilateral and contralateral globus pallidus. The peak response, observed at 102 nmoles of NMDA, represented a 300% increase in ME-i.r. above baseline levels. QUIS produced a similar action and was more potent than NMDA. Thus, NMDA and QUIS elevated the levels of ME-i.r. in the terminal region of the enkephalinergic neurons. The functional significance of EAA-induced changes in the globus pallidus is currently under study, but it appears that the response characteristics of enkephalinergic neurons to EAA's differ from those of other striatal neurons.

(Supported by the Medical Research Council of Canada)

307.4

FURTHER EVIDENCE THAT ACTIVATION OF N-METHYL-D-ASPARTATE (NMDA) RECEPTORS CONTRIBUTES TO METHAMPHETAMINE (METH)-INDUCED DAMAGE TO NIGROSTRIATAL DOPAMINERGIC NEURONS IN MICE. P.K. Sonsalla, W.J. Nicklas, and R.E. Heikkila, Dept. of Neurology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854.

The administration of METH to mice results in extensive damage to nigrostriatal dopaminergic nerve terminals as is evidenced by marked decreases in tyrosine hydroxylase activity, the number of dopamine (DA) uptake sites, and in the content of DA and its metabolites in the neostriatum. We previously reported that noncompetitive NMDA receptor antagonists including MK-801, phencyclidine, and ketamine protect against METH-induced damage. We now report that the competitive NMDA receptor antagonists, NPC 12626 (150 mg/kg) and CGS 19755 (80 mg/kg) administered s.c. 15 min prior to each injection of METH (3 or 4 i.p. injections of 10 mg/kg given every 2 h) also provided substantial protection against these METH-induced changes. Moreover, the intrastriatal infusion of NMDA (0.01 to 0.1 μmoles in 1 μl) produced a dose-dependent decrease in neostriatal DA content. In addition, the intrastriatal infusion of NMDA (0.1 μmole) produced a decrease in DA content which was potentiated by the systemic administration of a dose of METH (single s.c.injection, 30 mg/kg) which by itself did not alter DA levels (50% depletion of DA in NMDA plus METH treated mice. These data provide additional evidence which support a role for excitatory amino acids in mediating the toxic actions of METH on dopaminergic neurons in mice.

307.5

EXCITOTOXICITY OF N-METHYL-D-ASPARTATE IS MODULATED BY NIGRO-STRIATAL DOPAMINERGIC FIBRES. A.G. Chapman, N. Durmuller*, G.J. Lees* and B.S. Meldrum. Dept. of Neurology, Inst. of Psychiatry, London, SE5 8AF, U.K.

The excitotoxic effect of intrastriatal injection of N-Methyl-D-aspartate (NMDA) 300 nmol in 1.0μl in Wistar rats (wt 400 g) was assessed after 4 days by measurement of glutamic acid decarboxylase (GAD) and choline acetyltransferase (CAT) activity in the striatum. In some rats the nigrostriatal dopaminergic system was lesioned unilaterally by injecting 6-hydroxydopamineHBr (6-OHDA), 36 nmol in 2.0 μl in the substantia nigra, 7 days prior to NMDA injection. Striatal GAD activity was reduced by 79% following NMDA in control rats. A comparable reduction occurred on the non-lesioned side in 6-OHDA injected rats. The fall was only 45% on the lesioned side ($p < 0.01$ vs non-lesioned side).

CAT activity fell 54% after NMDA in unlesioned rats, and by 52% on intact side and 30% on lesioned side in 6-OHDA rats.

The excitotoxicity of NMDA appears to be enhanced by the nigrostriatal pathway. Involvement of excitotoxic mechanisms in ischemic brain damage may explain the protective effect of nigrostriatal lesions in fore-brain ischemia (Globus M.Y.T. et al Neurosci Lett 80:251, 1987).

307.6

DOPAMINE-RELATED SUBSTANCE ACTS AS A GLUTAMATERGIC AGONIST. E. Aizenman, W.F. White, R.H. Loring, and P.A. Rosenberg. Department of Neurology, The Children's Hospital and Harvard Medical School, and Department of Pharmacology, Northeastern University, Boston, MA 02115.

A structure-activity study of putative excitatory amino acids agonists (Biscoe et al., *Br. J. Pharmacol.* 58: 373, 1976) showed that 2,4,5-trihydroxyphenylalanine (TOPA, 6-hydroxydopa) and 3,4-dihydroxyphenylalanine (DOPA) had excitatory effects on neurons. Since TOPA was found by Biscoe et al. to be the most potent substance tested among the dopamine-related compounds, we studied the glutamatergic properties of TOPA in a variety of systems. TOPA (50-300 μM) produced excitatory responses in both rat cortical neurons in culture and in the isolated chick retina preparation. Whole-cell voltage-clamped responses to 50 μM TOPA recorded in cortical neurons were almost totally blocked by 10 μM CNQX, but only partially inhibited by 200 μM DL-APV. In binding experiments using the high affinity NMDA antagonist ^{3}H-CGS-19755 as a ligand, the addition of TOPA produced a dose-dependent decrease in binding with an IC_{50} near 20 μM. Finally, a 24 hour exposure of rat cortical cultures to media containing >200 μM TOPA produced extensive neuronal death. These studies suggest that TOPA, or an oxidation product, is a potent glutamatergic agonist, and may be related to an endogenously occurring excitotoxin derived from dopa, dopamine, or a metabolite. Our findings may help explain results from previous reports suggesting a role for dopamine in neurotoxicity (Preston et al., *Brain Res.* 338: 243, 1985; Globus et al., *Neurosci. Lettr.* 80: 251, 1987; Sonsalla et al., *Science* 243: 398, 1989). Supported by #P-30-HD18655 and NS00993.

307.7

NMDA RESPONSES ON HIPPOCAMPAL NEURONS CHANGE AFTER EXPOSURE TO ANOXIA AND HYPOGLYCEMIA. N. Hori*, S. Miyahara*, N. Doe*, Y. Shinoda* and D.O. Carpenter, Kyushu University, Fukuoka, Japan and New York State Department of Health, Albany, NY 12237.

Two to three days after transient forebrain ischemia neurons of rat hippocampus show characteristic changes in response to synaptic activation. Responses become prolonged and of a bursting nature, changes which may be associated with delayed neuronal cell death. We have found similar changes in a more acute preparation. In hippocampal slices we have studied the effects of low O_2 (5%) on the response of CA1 neurons to CA3 stimulation. With this protocal most neurons show a transient hyperpolarization followed by depolarization. Upon return to normal O_2 after 10-12 min the membrane potential recovers but after about 20 min the response to synaptic activation becomes a prolonged burst. This bursting response but not the fast initial excitation is blocked by 10^{-4}M APV, and thus appears to involve NMDA receptors. In other experiments we have examined the effects of low O_2 on ionophoretic responses to quisqualate, GABA and NMDA. Over the same period of time after return to normal O_2 the peak response to a constant ionophoretic application of NMDA increased, often by a factor of two, while quisqualate and GABA responses and membrane resistance did not change. Similar results were obtained with hypoglycemia (3 mM glucose). This preparation may be a good model of delayed neuronal death, and the preliminary observations suggest that anoxic and hypoglycemic insults may cause significant changes in the nature of responses to NMDA.

307.8

PERTURBATIONS OF METABOLISM AND ACUTE EXCITOTOXICITY. G. Zeevalk, S. Olynyk* and W.J. Nicklas. Dept. of Neurology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854.

An ex vivo preparation of chick neural retina was used to evaluate the effects of chemically-induced hypoglycemia or hypoxia and its relationship to acute excitotoxicity. Metabolism was evaluated by incubation of embryonic day 13 retina for 30 min with either an inhibitor of glycolysis (Iodoacetate, IOA), or electron transport (Rotenone, KCN), and in the presence or absence of a direct Krebs cycle substrate (pyruvate). The effects of this treatment on tissue ATP and phosphocreatine (PCr) and on lactate were measured enzymatically. Acute excitotoxicity was assessed by histology, by amino acid release, and with the use of antagonists MK-801 and CNQX. Results showed that the embryonic chick retina was very sensitive to hypoglycemia, the consequences of which were a reduction of tissue PCr and ATP which corresponded with a neuropathology exhibiting excitotoxic properties, predominately, but not entirely involving the NMDA receptor. Conversely, neural retina were fairly resistant to electron transport inhibition; ATP and PCr were only slightly reduced and excitotoxic damage could not be elicited except when mM concentrations of KCN were used ($\geq$2.5). Further, the decline in ATP and PCr elicited by IOA could not be prevented by pyruvate addition, suggesting that the reason for resistance to electron transport inhibition is that high energy phosphates are maintained predominately by glycolysis in this embryonic tissue.

307.9

CORTICOSTERONE ENDANGERS HIPPOCAMPAL NEURONS VIA THE NMDA-RECEPTOR. M. Armanini, C. Hutchins*, B. Stein, R. Sapolsky. Dept. Biol. Sci., Stanford Univ., Stanford, CA 94305

Corticosterone (CORT) is the rat adrenal hormone released during stress. CORT increases hippocampal vulnerability to excitotoxins, hypoxia-ischemia and metabolic poisons. Since these insults appear to damage via glutamate release and NMDA receptor activation, we examined whether CORT's endangerment might also be via the NMDA receptor. We sought a hippocampal toxin which, itself, does not work via the NMDA receptor, but whose toxicity is exacerbated by CORT; we would then determine if the CORT-induced exacerbation could be subtracted out with an NMDA receptor antagonist.

Rat hippocampi were bilaterally infused with 3-acetylpyridine (3AP), an electron transport uncoupler; one side was co-infused with the NMDA receptor antagonist APV. Damage induced by 220 ug 3AP was blocked by 7.5 ug APV, implying a component of 3AP toxicity mediated by the NMDA receptor. This is likely due to the 3AP-induced energy failure disrupting glutamate reuptake, as shown for other forms of energy depletion. 160 ug 3AP caused damage which was not sensitive to APV. Thus, this dose was used for the subsequent CORT experiment.

Rats were adrenalectomized (AD)Xed) and kept CORT-free 1 week before and after 3AP infusion, or were injected daily with 5mg CORT. The latter produces sustained circulating CORT levels in the range of major stressors. As previously reported, 3AP caused 75% more hippocampal damage in CORT-treated than in ADX rats. However, this CORT exacerbation of damage was entirely eliminated by 60 ug APV. This suggests that CORT endangers hippocampal neurons either by increasing the amount of glutamate reaching the NMDA receptor, and/or by increasing the sensitivity of the post-synaptic neuron to the NMDA receptor activation. The ability of CORT to disrupt glucose transport in hippocampal neurons and glia could potentially bring about either of these routes of dysfunction. Supported by NIH RO1 AG06633.

307.10

CALCIUM(Ca^{2+}) REGULATES QUISQUALIC ACID(QA) COUPLED PHOSPHOINOSITIDE(PPI) TURNOVER IN N-METHYL-D-ASPARTATE (NMDA) LESIONED BRAIN OF PERINATAL RATS. C.-K. Chen, F.S. Silverstein, M.V. Johnston. University of Michigan, Ann Arbor, MI 48104 & Johns Hopkins Univ, Baltimore, MD 21205

We previously reported that in NMDA lesioned brain, QA stimulated PPI turnover was enhanced in hippocampus and striatum. To explore the biochemical mechanisms underlying this response, we examined the influence of extracellular calcium levels on QA coupled PPI hydrolysis.

In 7-d-old rats, anesthetized with ether, unilateral intrastriatal injections of NMDA(17nmole/0.5ul) were done; rats were sacrificed 3 d later. QA coupled ^{3}H-IP1 formation was measured in hippocampal and striatal tissue slices prepared from the injected(I) or contralateral(C) hemisphere. ^{3}H-IP1 release was assayed after incubating myo-2-^{3}H-inositol labelled brain slices with QA(10^{-5} M) or carbachol(Carb, 10^{-2} M) for 2 h, in the presence of lithium, in buffers containing Ca^{2+}(2.2mM), without Ca^{2+}-{Ca(-)} or Ca(-) plus EGTA(0.5mM). In the presence of Ca^{2+}, QA coupled ^{3}H-IP1 formation(as % of contralateral basal) was enhanced in lesioned hippocampus(I vs C: 1212±192 vs 516±88, $p<0.05$). Stimulation was further enhanced in the Ca(-) buffer in the injected tissue{Ca(-) vs Ca(+): 1858±140 vs 1212±192, $p<0.05$}. ^{3}H-IP1 release was decreased significantly in Ca(-) plus EGTA(462±69). A similar trend was observed in striatal tissue. In contrast, Carb stimulated PPI turnover was not enhanced in Ca(-) buffer.

Intracellular Ca^{2+} levels have not been measured in NMDA lesioned brain slices, but elevated levels might be expected. These data may reflect improved Ca^{2+} homeostasis in lesioned tissue in Ca(-) buffer or alternatively suggest suppression of release of endogenous neurotransmitters that inhibit QA coupled PPI turnover.

307.11

CALCIUM-INDEPENDENT, NMDA-MEDIATED INJURY IN THE HIPPOCAMPAL SLICE. R.A. Wallis*, M.D. Fairchild*, K.L. Panizzon*, C.G. Wasterlain (SPON:R. Nishimura). Basic Neurophys. Lab., VAMC, Sepulveda, CA 91343 and Depts. of Neurology & Pharmacology and Brain Res. Inst., UCLA Sch. of Med., Los Angeles, CA 90024.

NMDA excitotoxicity is thought to be mediated by the opening of receptor-operated ionic channels resulting in calcium influx. We investigated the ionic dependence of NMDA-mediated cell injury in rat hippocampal slices perfused with artificial cerebrospinal fluid (ACSF) containing (mM) $CaCl_2$, 2.4 or 0; glucose, 4; and saturated with 95%O_2/5%CO_2. The extent of injury was judged by measuring the amplitude of the orthodromic population spike evoked in CA1 by stimulation of Schaeffer collaterals. Exposure to 50 uM NMDA for 40 minutes resulted in the complete loss (0% mean recovery) of the population spike after wash with ACSF. The non-competitive NMDA blocker MK-801 (32 uM) administered prior to NMDA exposure resulted in 89% (SD 2.83) recovery. When NMDA exposure was given in media without added calcium, 30% (SD 6.36) recovery was observed. NMDA exposure with MK-801 in media without added calcium, yielded 87% (SD 7.57) recovery. These data suggest the existence of a calcium-independent component of NMDA-mediated excitotoxicity in the hippocampal slice.

Supported by the research service of the Veterans Administration and by research grant NS 13515 from NINDS.

307.12

TIME COURSE OF NMDA-INDUCED DAMAGE AND RESULTING INFLAMMATORY RESPONSE IN RAT HIPPOCAMPUS. P.C.Harrison*, G.J.Patel*, N.Haynes*, L.Andrews*, R.E.Donaghy*, and L. G.Letts* (SPON: R. Anderson). Boehringer Ingelheim Pharmaceuticals Inc., Ridgefield, CT. 06877.

Excitatory amino acids have been demonstrated to produce neuronal degeneration in mammalian CNS tissue (Foster et al., *Neuroscience Let.*, 76:307, 1987). Extent of neuronal damage and time course of the inflammatory response following unilateral stereotaxic administration of NMDA (20 nm) into rat hippocampus was examined. Neuronal damage alone was assessed in perfusion fixed, frozen, cresyl violet stained sections. Both neuronal damage and inflammation were assessed in immersion fixed, paraffin-embedded, H&E stained sections. In frozen sections, acute unilateral hippocampal necrosis of the CA 1 and CA 3 regions appeared at 6 hr and was maximal between 30-48 hr post-NMDA (n=4/time point). In paraffin sections, a similar course of neuronal damage was observed followed by a localized inflammatory response consisting of a rapid increase in number of macrophages, capillaries and astrocytes beginning at 48 hr and peaking between 48-96 hr post-NMDA (n=4-6/time point). Neutrophils appeared to be a minor component of the NMDA-induced inflammatory response in contrast to what is known in models of stroke and CNS ischemia. Thus, injection of NMDA into rat hippocampus elicits neuronal damage and an inflammatory response which may differ from that associated with stroke and other disease states.

307.13

SELECTIVE ANTAGONISM OF EXCITATORY AMINO ACID EFFECTS ON STARTLE AND FIGURE-8 MAZE ACTIVITY IN MICE. J. Tizzano, D. Helton*, R. Tamura*, and J. Buelke-Sam. Tox. Div., Lilly Research Labs, Eli Lilly & Co., Greenfield, IN 46140.

Previously, it has been shown that the excitatory amino acids, N-methyl-D-aspartate (NMDA), L-glutamic acid (GLU), and L-aspartic acid (ASP) exhibit proconvulsive activity in the mouse maximal electroshock model (MES). Pretreatment with either a competitive (AP-4) or noncompetitive (MK-801) antagonist blocked the proconvulsive actions of NMDA and ASP but enhanced GLU proconvulsive activity. This study evaluated the interactions of effects produced by NMDA (1.56, 3.12, 6.25 mg/kg), GLU (250, 500, 1000 mg/kg), ASP (250, 500, 1000 mg/kg), alone or in combination with AP-4 (6.25, 12.5 mg/kg) or MK-801 (0.01, 0.10 mg/kg) on 1-hr activity or auditory and tactile startle. Male CD-1 mice (10/group) were injected ip 15 min prior to testing. Startle was measured in automated SDI chambers. Each 50-trial startle session was initiated with a 5-min adaptation period at a background noise level of 70 dBA, followed by alternating 5-trial blocks of auditory (120 dBA noise) and tactile (20 psi air puff) stimuli presented at 8-sec intervals. Activity was not altered by treatment. NMDA, AP-4, MK-801, and NMDA+MK-801 did not alter startle amplitudes compared to controls. NMDA(1.56)+AP-4 decreased auditory and tactile startle. GLU and ASP alone produced dose-related decreases in startle; however, only the decreases produced by GLU(250) were reversed by pretreatment with AP-4 or MK-801.

307.14

CHANGES IN MUSCARINIC ACETYLCHOLINE RECEPTOR DIFFERENTIAL BINDING IN RAT BRAIN AFTER MONOSODIUM GLUTAMATE TREATMENT. C. Beas-Zárate*, R. Schliebs*, A. Morales-Villagrán* and A. Feria-Velasco. Fac. Ciencias, Univ. Guadalajara; Paul Flechsig Inst. for Brain Res., Karl Marx Univ., Leipzig, G.D.R.; and Unidad Inv. Biomed. Occte., I.M.S.S. Guadalajara, Jal. MEXICO.

Systemic administration of monosodium L-glutamate (MSG) to rats results in neuronal degeneration in various brain regions and induces convulsions which might provide a useful model for studying partial status epilepticus of multifocal origin and general convulsive disorders. In a previous work it was shown that MSG-induced convulsions affect catecholaminergic neurotransmission in both, cerebral cortex and caudate nucleus of the adult rat. The present work was designed to reveal a possible involvement also of cholinergic mechanisms in MSG-induced convulsions. Because neurotransmitter receptors respond highly sensitive to changed neuronal activity at the synaptic level, muscarinic acetylcholine receptor binding using ^{3}H-quinuclidinyl benzilate (^{3}H-QNB) as radioligand, was applied as a biochemical marker. ^{3}H-QNB binding was measured in frontal, motor, and visual cortex, in caudate nucleus (CN) and hippocampus of rats treated with MSG. No alterations in receptor binding in either of the regions studied were found in adult rats which received a single dose of MSG. Repeated administration of MSG to adult rats for 4 days resulted in slight decreases of ^{3}H-QNB binding in the frontal cortex and CN as compared to control values. In contrast, neonatal MSG treatment led to a considerable increase in ^{3}H-QNB binding in CN, already detectable at the age of 7 days, persisting until adulthood. Data suggest that MSG-induced convulsions in adult rats, hardly affect cholinergic transmission in cerebral cortex, CN or hippocampus, while neonatal application of MSG has striking effects on the development of the cholinergic system, at least in the CN.

307.15

MONOSODIUM GLUTAMATE TOXICITY IS MASKED BY "ENRICHED" HOUSING ENVIRONMENT. K. Fisher*, J. Kleim*, R. Lloyd* and M. J. Saari, Neuroscience Research Unit, Nipissing University College, North Bay, Ontario. P1B 8L7.

We have recently demonstrated that neonatal monosodium glutamate (MSG) administration to male rat pups causes a severe performance deficit in the Morris water maze (Soc. Neurosci. Abstr., 14(2), 885, 1988). We have also shown that this deficit is partially masked by an "enriched" post-weaning housing environment. In two separate experiments we examined whether pre-weaning handling, like "enriched" housing, also protects against MSG induced behaviour deficits. Enrichment was found to significantly improve performance of the MSG treated in the Barne's maze, a recently developed task of spatial learning for rats, while pre-weaning handling did not. The platform dominance task also revealed the same pattern of behavioural results. We conclude that, unlike an "enriched" post-weaning environment, pre-weaning handling does not mask behavioural deficits induced by neonatal MSG administration. Supported by the Ontario Mental Health Foundation.

307.16

EXCITOTOXIC AMINOACIDS TOXICITY TO NERVE TERMINALS: MORPHOLOGICAL VS FUNCTIONAL ASPECT OF DEGENERATION. T. Moriizumi*, M. Takada and T. Hattori, (SPON: N. Miki), Dept. of Anatomy, Univ. of Toronto, Toronto, Ontario, M5S 1A8, Canada.

Fine structural analysis of the rat striatum injected with various excitotoxins unequivocally revealed axonal/terminal degeneration far too serious to be attributed only to damage of interneuronal populations. This led us to reinvestigate the feasibility of the so called axon-or terminal-sparing properties of these excitotoxins. Four to 28 days after an injection of either kainic acid (5 nmol) or quinolinic acid (200 nmol) into the striatum, nigrostriatal dopamine terminals were examined at injection sites by tyrosine hydroxylase (TH) immunohistochemical and 5-hydroxydopamine (5-OHDA) uptake studies. Injections of either toxin produced marked dilations of intensely TH-positive terminals with tremendous amounts of vacuoles. One hour after an intrastriatal injection of 5-OHDA, many labeled vesicles were clearly detectable in terminals which underwent dark type degeneration. These results indicate that both synthetic and uptake machineries of neurotransmitters could be retained in fine structurally defined degenerating terminals, and biochemical and retrograde tracing studies alone are not sufficient enough to claim that axons and terminals can be spared by toxic effects of these excitotoxins. Supported by the MRC of Canada.

307.17

CHARACTERIZATION OF CELL DEGENERATION, BLOOD-BRAIN BARRIER DISRUPTION AND BRAIN GLYCOGEN DEPOSITION FOLLOWING INTRAPERITONEAL ADMINISTRATION OF KAINIC ACID. S.A.L. Bennett*, Wm. Staines[1], and D.C.S. Roberts (SPON: G. Fouriezos[2]) Department of Psychology, Carleton University, Ottawa, Ont., K1S-5B6; [1] Department of Anatomy, [2] Department of Psychology, University of Ottawa, Ottawa, Ont., K1H-8M5.

Neuronal degeneration, blood-brain barrier (BBB) disruption, and glycogen deposition were correlated with kainic acid-induced (KA; 10 mg/kg) seizure activity using immunohistochemical and histochemical staining methods. Animals exhibiting loss of postural control, rearing, foaming at the mouth, and generalized limbic seizures were perfused at 2, 3, 4, 5, 24, and 30 hrs following KA administration. Immunoreactivity for rat IgG, HNK-1, GFAP, and C-Fos, and histochemistry using cresyl violet, PAS-Dimedone, acridine orange, and cytochrome oxidase were carried out on adjacent sections. Chromatolysis and simple neuron atrophy were evident in pyramidal neurons of the hippocampal CA fields, select thalamic and hypothalamic nuclei, specific cortical layers, habenular nucleus, and entorhinal cortex. Neuronal degeneration, in some regions, was anticipated at early time periods by enhanced C-fos immunoreactivity. BBB disruption, determined by the extent of rat IgG leakage into brain tissue, was correlated with regions showing neurodegenerative damage but was also prominent in regions showing no neural lesions. Glycogen depositions were found to surround, but not overlap, regions of marked BBB disruption.

307.18

PROTECTION OF DENTATE HILAR CELLS FROM THE EFFECTS OF PROLONGED AFFERENT STIMULATION BY INTRACELLULAR INJECTION OF A CALCIUM CHELATOR. H.E. Scharfman and P.A. Schwartzkroin. Dept. of Neurological Surgery, RI-20, Univ. of Washington, Seattle, WA 98195.

Prolonged afferent stimulation of rat dentate gyrus *in vivo* leads to degeneration only of those cells that lack parvalbumin and calbindin (CaBP $D_{28}K$) (Olney *et al.*, 1983; Sloviter, 1983, 1987, 1989). To test the hypothesis that calcium binding proteins (CaBPs) play a protective role against the effects of prolonged stimulation, we have recorded intracellularly from cells that lack immunoreactivity for CaBPs (CaBP-negative cells) in the hilus of the fascia dentata of hippocampal slices with microelectrodes containing the calcium chelator BAPTA (200 mM in 4 M KCH_3COOH), and allowed BAPTA to diffuse into the cells. CaBP-negative cells impaled with electrodes without BAPTA showed electrophysiological signs of deterioration during prolonged stimulation, such as depolarization and decrease in input resistance. CaBP-negative cells that were impaled with BAPTA-containing electrodes did not show signs of deterioration. Electrophysiological deterioration of CaBP-negative cells was also prevented by raising extracellular [Mg^{2+}] to 2 mM or by perfusing slices with the NMDA receptor antagonist aminophosphonovaleric acid (DL-APV, 100 μM).

These observations suggest that regulation of intracellular levels of free calcium by CaBPs plays a crucial role in protecting cells from calcium-mediated damage, and that differential concentrations of intracellular CaBPs is one factor that determines selective vulnerability of subpopulations of mammalian CNS neurons. Critical rises in intracellular calcium appear to be mediated by NMDA receptors.

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307.19

ALTERATION OF ACTIVITY AND AMMONIA INHIBITION OF PHOSPHATE-ACTIVATED GLUTAMINASE FROM AGED RAT BRAIN. D.R. Wallace and R. Dawson Jr., College of Pharmacy, University of Florida, Gainesville, Florida 32610.

Phosphate-activated glutaminase (PAG: L-glutamine amidohydrolase, EC 3.5.1.2) is the enzyme responsible for the formation of glutamate and ammonia from the hydrolysis of glutamine. PAG is bound to the inner membrane of the mitochondrial matrix in nerve terminals and is enriched in synaptosomal fractions. The aim of this study was to examine possible age-related alterations in either the activity, or regulation, of PAG. The data indicate a trend towards increased activity in aged rats. Thirty month old rats displayed a higher Km (0.95±0.20 vs. 0.81±0.19mM)when compared to adults, and a higher Vmax (104.00±15.90 vs. 77.00±9.60 nMole/mg/min) when compared to adult rats. There was no age-related difference in phosphate activation, interference by aspartate, or pH optimum, although aged rats had a pH optimum slightly higher than adults. Ammonia concentrations of 100uM-5mM produced a significant ($p<0.02$) reduction in PAG activity in both age groups. At physiological (100-500uM) concentrations of ammonia there was a significant ($p<0.02$) attenuation of inhibition in aged rats when compared to adults. This change in ammonia regulation may be detrimental to the aging brain, resulting in increased production of glutamate and ammonia, both of which are neurotoxic in elevated concentrations.

307.20

U-54494A SELECTIVELY PROTECTS AGAINST KAINATE TOXICITY IN RAT CEREBELLAR GRANULAR CELLS. J.S. Althaus and P.F. VonVoigtlander. Central Nervous System Diseases Research, The Upjohn Company, Kalamazoo, MI 49001.

U-54494A (an analogue of the selective kappa opioid agonist U-50488H) is a selective anticonvulsant that lacks the analgesic, sedative and diuretic activity typical of kappa agonists. To understand its mechanism of action, U-54494A was tested as a protectant against excitotoxicity in rat cerebellar granular cells. The basic method used was reported previously (Novelli, A., Brain Research, 451:205, 1988), however we used the uptake of 3H-AIB (a selective system A amino acid uptake substrate) as a measure of cell viability instead of fluorescein diacetate staining. Dose-excitotoxic responses showed that glutamate, kainate and quisqualate were equipotent (LC50 = 3 μM) and that NMDA was 10 times less potent (LC50 = 30 μM). In this paradigm, U-54494A was found to be a selective antagonist of kainate toxicity. The selectivity profiles of other excitatory amino acid antagonists will be presented.

307.21

THE STEREOTAXIC INJECTION OF KAINIC ACID INTO THE STRIATUM OF RATS INDUCES UNCREASED PRODUCTION OF mRNA FOR HEAT SHOCK PROTEIN 70. J.B. Uney P.N. Leigh S. Thomas and B.H. Anderton. Department of Immunology, St George's Hospital Medical School, Cranmer Terrace, London. (SPON: BRA)

We have found that the injection of kainic acid into the striatum causes an increase in the synthesis in mRNA coding for heat shock protein 70 (Uney et al, FEBS Lett., 235:215). We have further shown that C1300 cultured neuroblastoma cells treated with Fe also show an increase in the levels of mRNA to the heat shock protein ubiquitin. This shows that degenerating neurones exhibit a stress response. Therefore the results may be seen to suggest that in human neurodegenerative diseases (eg.Parkinson's and Alzheimer's, where there is labelling of intracellular inclusions with antibodies to ubiquitin) free radical damage and production of heat shock proteins may play a part in the neurodegeneration process.

REGIONAL LOCALIZATION OF RECEPTORS AND NEUROTRANSMITTERS III

308.1

DISTRIBUTION OF GAMMA-AMINOBUTYRIC ACID-LIKE IMMUNOREACTIVE NEURONS IN THE MIDBRAIN PERIAQUEDUCTAL GREY OF THE RAT. J.C. Pearson and V.R. Roettger. Depts. of Anatomy and Physiology and Biophysics, Wright State University, Sch. of Med., Dayton, OH 45435.

Antiserum against GABA-glutaraldehyde-keyhole limpet hemocyanin was used to localize GABA-like immunoreactive (GABA-LI) neuronal somata in the midbrain periaqueductal grey (PAG) of the rat. Rats were intraventricularly colchicinized two days prior to perfusion with 4% paraformaldehyde and 0.5% glutaraldehyde in 0.1M phosphate buffer (pH 6.5). Vibratome sections (50um) were processed by the avidin-biotin-peroxidase method. GABA-LI somata show a heterogeneous distribution pattern within the subdivisions of the PAG. GABA-LI cells are concentrated in the ventral parts of the ventral lateral (VL) and medial (M) subdivisions of the PAG. The dorsal parts of the VL and M subdivisions contain only a few, scattered GABA-LI somata. GABA-LI cell bodies are present in the dorsal and dorsal lateral subdivisions of the PAG, but are fewer in number and more widely scattered when compared to GABA-LI neurons in ventral areas of the PAG. GABA-LI somata are present within the dorsal raphe (DR) and dorsal tegmental nuclei. Measurements of cell body area indicate that GABA-LI somata in the VL and DR are larger than GABA-LI somata in other PAG subdivisions.

308.2

IMMUNOGOLD LOCALIZATION OF PROBABLE GABA-B RECEPTOR SITES BY MONOCLONAL ANTI-BACLOFEN ANTIBODIES. G.R. Holstein, G.P.T. Martinelli*, E.D. Reis* and P. Pasik. Depts. of Neurology, Anatomy & Surgery, Mount Sinai Sch. Med., CUNY, New York, N.Y. 10029.

The immunolocalization of baclofen by the colloidal gold technique was utilized to identify the regional and ultrastructural distribution of potential GABA-B receptor sites. Mouse monoclonal antibodies were raised against the L-enantiomer of gamma-amino-beta-(p-chlorophenyl)butyric acid (baclofen) conjugated to keyhole limpet hemocyanin (KLH) by glutaraldehyde. Two culture supernatants and one ascites generated by monoclonal hybridomas were selected for (a) reactivity against baclofen conjugated to human albumin, (b) binding inhibitability by baclofen-HCl, and (c) lack of reactivity against GABA, histamine and monoethanolamine conjugates to bovine albumin. Pre- and post-embedding immunocytochemical studies were conducted on several structures of the rat brain. Experimental rats received an i.m. injection of the L-enantiomer of baclofen-HCl (5 mg/kg), while control rats received an equal volume of saline. Rats were sacrificed 90 min. later under ether anesthesia by cardiac perfusion. For pre-embedding studies, 50 μm vibratome sections were exposed to the monoclonal antibodies for 16 hr. The sections were further treated with biotinylated goat anti-mouse IgG preabsorbed with normal rat serum, followed by immunogold application using 1 nm gold particles and silver enhancement. Postembedding studies using 15 nm gold particles were performed on serial sections mounted on formvar-coated gold slot grids.

Electron microscopic observations suggest the presence of immunoreactive elements in discrete brain regions of the baclofen-injected rats. Distinct immunogold-labeled elements are present in the molecular layer of cerebellar cortex only in sections from the baclofen-injected animals. Identically processed sections from the uninjected animals showed no immunoreactivity. Also, sections from the baclofen-treated rats incubated in culture medium instead of primary antibody showed no immunostain. Preliminary observations in structures of the basal ganglia and thalamus suggest similar specificity.

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308.3

3D GLIOMA IMAGES USING PERIPHERAL BENZODIAZEPINES-IMPLICATIONS FOR PET

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Three dimensional comparison of peripheral benzodiazepine binding and histology in an experimental glial tumor, time course of binding and the effect on binding of labeling a positron emittor to the ligand were studied to evaluate their potential utility in positron emission tomography (PET). Time course studies of ^{3}H-flunitrazepam revealed high densities of peripheral benzodiazepine binding in C6 glioma with a constant tumor/cortex ratio within the first 60 min. Fluorodiazepam had similar ability as diazepam to displace ^{3}H-flunitrazepam binding *in vitro* and ^{3}H-diazepam demonstrated high specific bindings to tumors. However, 3-^{18}F-diazepam did not show high tumor binding. For three dimensional studies, images were generated from thionin stain sections and autoradiograms. ^{3}H-flunitrazepam, was superior in showing normal anatomical structure surrounding the tumor, whereas ^{3}H-PK11195 was superior in revealing high tumor/brain contrast. We conclude that specific peripheral-type ligands may prove more useful in PET in identifying the tumor boarders. However, positron labelling of these ligands can significantly alter binding characteristics, illustrating the necessity for *in vivo* animal binding studies prior to studies in man.

308.4

RADIOTRACER STUDIES IN HUMAN BRAIN WITH AN MRI-REGISTERED DUAL DETECTOR SYSTEM

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A dual-detector probe makes possible certain positron studies of CNS at much reduced radiation dose and cost. The device consists of two sodium iodide scintillators which detect positron annihilation by coincident detection of 511 keV gamma-ray pairs. The probe lacks the imaging capability of PET, providing instead, a composite measure of activity within a volume of brain tissue. The scintillators, placed on opposite sides of the head, delineate a 5 cm diameter cylinder through the brain. A method using multiple fields-of-view and simultaneous linear equations has been developed in order to increase the specificity of measurements. Studies of the correlation between probe measurements and PET ROI data in humans are currently being carried out in order to test the instrument. In these studies, subjects are scanned with the probe immediately following a PET scan. Multiple studies are performed on each subject with varied doses of neuroleptic drugs. Blockade of ^{11}C-N-methylspiperone binding is used as a measure of occupancy of D-2 receptors in the caudate nuclei by these drugs. Probe measurements of the degree of blockade in specific brain regions are found to be within 5% of PET measurements.

308.5

A MODIFIED EMULSION-COATED COVERSLIP TECHNIQUE FOR COMBINED RECEPTOR AUTORADIOGRAPHY AND HISTOCHEMICAL STAINING OF LARGE TISSUE SECTIONS.

Kenneth J. Rhodes and Douglas L. Rosene. Dept. of Anatomy, Boston Univ. Sch. Med., Boston, MA 02118.

Autoradiograms generated using tritium sensitive films (e.g. Ultrofilm) generally suffer from limited resolution due to the comparatively large size of their silver grains. Furthermore, since the physically separated autoradiogram and underlying tissue section must be manually aligned for analysis, it is difficult to maintain areal and laminar relationships. These two technical problems can be overcome with the coverslip technique (Young and Kuhar, Brain Res., 179:225, 1979) in which an emulsion-coated coverslip is superglued to one end of a slide. This "sandwich" must then be pried open to allow autoradiographic development and subsequent tissue staining. However, chemical reactions near the glued end of the slide are often uneven and exposing the unfixed tissue section to developer interferes with many histological procedures. Thus we modified this technique to obtain high resolution autoradiograms of whole monkey brain sections (50 x 50mm). Sections were cut from two unfixed rhesus monkey brains on a Hacker-Brights cryostat and thaw mounted onto subbed slides. Muscarinic and benzodiazepine receptors were labeled using standard procedures. Glass coverslips (73 x 47mm; Surgipath) were coated with Kodak NTB-2 emulsion and permanently coupled to the microscope slide using a rectangular piece of adhesive-coated 3 mil aluminum foil (Cole Parmer). Using this method the coverslip could swing 180° away from the slide, allowing the coverslip autoradiogram to be developed without having the tissue section contact the harsh photographic chemicals. Similarly, the underlying tissue sections can be fixed and then processed using standard histological and histochemical staining procedures. Subsequently, the autoradiogram is permanently and precisely re-apposed to the tissue with mounting medium. This technique optimizes both autoradiographic and histological procedures so that the distribution of receptor sites can be directly compared with many other markers. (Supported by NIH grants NS19416, AG04321 AND PO1AG00001).

308.6

DIFFERENTIAL QUENCHING BETWEEN FETAL AND ADULT BRAIN IN RECEPTOR AUTORADIOGRAPHY WITH TRITIUM LABELLING.

J.D.E.Barks*, J.Bertlik* (SPON: U.Tuor) Department of Pediatrics, Univ. of Toronto, Toronto General Hospital, The Hospital for Sick Children, Toronto, Ont. M5G 1X8.

The purposes of this study were: (a) determine the need for separate calibration of autoradiography (AR) standards for fetal brain (FB), and (b) determine if white-matter quenching is important in human FB. Pastes were made from (i) whole 20 day Wistar rat FB (ii) dissected adult Wistar rat brains and (iii) dissected fetal (19 wk) and premature infant (33 wk) human brain. Increasing amounts of [3H]-lysine were added to tissue aliquots which were frozen, sectioned and apposed to film. From each aliquot sections were weighed, solubilized and dpm/mg tissue measured.

For rat tissue there was quenching from fetal to adult grey matter (GM) to adult white matter (WM) in plots of optical density (OD) vs tissue concentration (TC), shown by decreasing slope of regression lines ($P<.001$ F vs GM, $P<.001$ GM vs WM). In human FB there was no evidence of quenching between GM and WM. Plots of OD vs TC for human fetal and premature infant brain were best fit by quadratic equations and their 95% confidence intervals overlapped.

Conclusion: (a) AR studies of receptor ontogeny must use age-appropriate standards before firm conclusions can be made about maturational decreases in regional receptor expression. (b) Unlike adult brain, WM quenching is not seen in fetal brain.

308.7

LOCALIZATION OF [^{3}H]-L-GLUTAMATE BINDING SITES IN GOLDFISH BRAIN.

R.E. Davis, G.R. Wilmot* and J.J. Cha. Mental Health Research Institute and Neuroscience Program, University of Michigan, Ann Arbor, MI 48104-1687 (USA).

Previous studies have found specific binding sites for excitatory amino acids in membrane fractions of goldfish brain (Francis, A *et al.* [1981] *Brain Res.* **216**, 375; Henley, JM and Oswald, RE [1988] *J. Neurosci.* 8, 2101). For a more detailed anatomical study, we used autoradiography to investigate the distribution of [^{3}H]-L-glutamate ([^{3}H]glu) binding sites in 20 µm frozen sections of goldfish brain.

Binding was most prominent in the molecular layer of the valvula of the cerebellum, nucleus torus lateralis, and the nucleus diffusus and nucleus centralis of the inferior lobe. Moderate binding occurred in the optic tectum, torus semicircularis, and dorsal tegmentum. N-methyl-D-aspartate decreased [^{3}H]glu binding in the torus semicircularis, dorsal tegmentum, and optic tectum other than the fascicular layer of the periventricular grey zone (PGZ-f). In contrast, both kainate and kynurenate blocked binding in PGZ-f but not other layers of the tectum. Kainate and kynurenate also decreased binding in nucleus torus lateralis, nucleus diffusus, and nucleus centralis. Quisqualate strongly displaced [^{3}H]glu binding in the valvula and to a lesser extent in the torus semicircularis, dorsal tegmentum, and layers of the optic tectum superficial to PGZ-f. The nonuniform distribution of specific subtypes of [^{3}H]glu binding sites suggests that excitatory amino acids are widespread neurotransmitters in goldfish brain. (Supported by NIH NRSA 5T32.)

308.8

QUANTITATIVE AUTORADIOGRAPHY REVEALS NMDA RECEPTOR-COUPLED CHANNELS ARE LOCATED POSTSYNAPTICALLY.

J.W. Bekenstein, E.W. Lothman, G.F. Wooten, and J.P. Bennett, Jr., Dept. Neurology, University of Virginia Health Sciences Center, Charlottesville, VA 22908.

In vitro quantitative autoradiography with [^{3}H]MK-801 was used to determine K_d and B_{max} values for the NMDA receptor-coupled channel in subregions of the rat hippocampal formation. A single form of the channel with an apparent K_d in the 15-20 nM range was found for [^{3}H]MK-801 binding in the presence of both 1 µM glutamate and 1 µM glycine. Specific binding was highest in the molecular layer of the dentate gyrus, followed by CA1 stratum radiatum and CA1 stratum oriens. Fewer binding sites were observed in the hilus of the dentate gyrus, cerebral cortex, CA1 stratum pyramidale, CA3 subregion (stratum oriens, stratum pyramidale, stratum radiatum), and thalamus. Selective destruction of dentate granule cells by colchicine microinjections reduced the number of [^{3}H]MK-801 binding sites by 40-60% in the molecular layer of the dentate, compared to intact tissue. [^{3}H]MK-801 binding did not change in other hippocampal subregions as a consequence of colchicine injection. Electrolytic entorhinal cortical lesions produced no changes in regional MK-801 binding site density in any of the regions under study. More detailed analysis revealed no change in the number of MK-801 binding sites per unit length of dendrite in the molecular layer of the dentate gyrus. These findings indicate that NMDA receptor-coupled channels are confined to a postsynaptic location in the adult rat hippocampus, with the highest concentration on dentate granule cells.

308.9

Distribution and ultrastructure of glycine-immunoreactive (GLY-ir) terminals in the rat dorsal vagal complex (DVC). L. Modarressi*, W.T. Talman and M.D. Cassell.* (Spon. H. Damasio) Depts. of Neurol. and Anat., Univ. of Iowa, Iowa City, IA 52242.

Microinjections of glycine into the rat DVC produce site-specific changes in blood pressure and heart rate. The distribution of GLY receptors in this region has been described, yet there is little information available concerning the regional distribution of endogenous GLY-containing terminals in the dorsal medulla. We have therefore examined the distribution and ultrastructure of GLY-terminals in the DVC using a specific anti-glycine antibody (gift of R. Wenthold, NINCDS). Serial 10 μm sections through the brainstem were obtained from 8 adult Sprague-Dawley rat brains fixed with 3% glutaraldehyde/0.5% paraformaldehyde. Binding of anti-glycine was detected using avidin-PAP immunocytochemistry. GLY-ir terminals were present in all subdivisions of the DVC except the dorsolateral part of the nucleus tractus solitarius (NTS) and the dorsal vagal motor nucleus. High densities of GLY-ir terminals were additionally present in the gracile, cuneate and hypoglossal nuclei. The highest densities of GLY-ir terminals in the DVC were found in the ventrolateral and intermediate subnuclei of the NTS, with low to moderate densities in the medial, commissural and interstitital subnuclei. Ultrastructurally GLY-ir terminals formed mainly axo-dendritic contacts, usually without pre- or post-synaptic densities. Two forms of GLY-ir terminals were observed: one type containing large spherical vesicles, the other containing densely packed pleomorphic vesicles. The presence of glycine terminals within the medial, ventrolateral and commissural subnuclei - all of which are related to cardiac, chemo- and baroreceptor afferents - is consistent with a role for glycine in medullary cardiovascular reflexes. (Support: VA Merit Review, HL32205, HL14388, NS24261).

LEARNING AND MEMORY: PHYSIOLOGY III

309.1

PINEAL AND LEARNING: EFFECTS OF BOTH IMPLANTATION AND REMOTION OF PINEAL GLAND ON PASSIVE AVOIDANCE TASK. G. G. Cobos-Zapiaín,* E. B. Naranjo-Rodríguez* and B. Barrera Mera. (SPON: Solano-Flores LP), ENEP. Iztacala, U.N.A.M. & Departamento de Fisiología, Facultad de Medicina,U.N.A.M. México, D. F.

This experiment examined the effect on memory of pnealectomy, and of a second pineal gland implanted on the brain. Under nembutal anesthesia rats, were surgically intervened. These animals were allowed 21 days to recover from the surgical procedures befores trainingwas initiated. A group of intact rats was also studied. Rats were trained with a footshock (0.2 in a 5 sec) in a one-trail inhibitory (passive) avoidance task. On a retention test 24 hours after training, animals with pinealectomy had retention performance which was not significantly different than that of control animals.

Rats with a second pineal gland implanted on the brain were impaired on retention performance as compared with the former groups (p's < 0.05). These findings indicate that implanting a second pineal gland interferes with memory processes.

309.2

CELIAC GANGLION LESIONS BLOCK THE MEMORY-IMPROVING EFFECTS OF POST-TRAINING GLUCOSE AND FOOT-SHOCK Norman M. White, Claude Messier and Barry Connell, Department of Psychology, McGill University, 1205 Dr. Penfield Ave, Montreal, Quebec, Canada, H3A 1B1

Previous experiments have demonstrated that post-training consumption of sucrose or injection of glucose (2g/Kg) improve retention in several memory tasks. The finding that fructose, a sugar that does not enter the brain, improves retention at the same doses as glucose suggests that this effect may be peripherally mediated. Previous experiments also suggest that the effect is not mediated by insulin or by the adrenal medulla. In the present experiments we found that lesions of the celiac ganglion, through which passes most of the sympathetic and parasympathetic innervation of the liver, eliminated the memory-improving action of post-training glucose injections on memory for a conditioned emotional response (CER). We have also shown that post-training foot-shock, a stressful event, one of the sequelae of which is an increase in blood glucose, improves memory. In the present experiment we found that lesions of the celiac ganglion also eliminate the effect of post-training foot-shock on memory for a CER. These findings suggest that some glucose-related function of the liver may initiate a neural signal which acts in some as yet unknown way on a memory substrate in the brain. This mechanism may mediate the effects on memory of a wide variety of normally occurring events.

309.3

AMYGDALA LESIONS PRODUCES DISRUPTION OF CONDITIONED TASTE AVERSIONS THROUGH DECREASED GASTRIC ACID CONCENTRATION. S. Alvarez*, G. Ortiz*, and F. Bermúdez-Rattoni (SPON: R. Salceda). Instituto de Fisiología Celular, UNAM, Apdo. Postal 70-600, 04510 México, D.F.

Several studies have shown, that permanent lesions of amygdala disrupt conditioned taste aversions (CTA) induced by a variety of unconditioned stimuli, such as lithium chloride and cupper sulfate. Other authors have reported that amygdala stimulation produces gastric ulcers and significant increments on gastric acid concentration (GAC). In the present study we assess the role that GAC by amygdala electrolytical lesions have on the acquisition of CTA. Two groups of rats; one with amygdala lesions and other of unoperated controls were trained in a CTA procedure. On the acquisition day animals received .1% of saccharin followed by i.p.injection LiCl(190 mg/kg). Two days later the saccharin was presented again and the volume consumption was measured. After the test trial animals were rapidly killed and the gastric pH was determined. Result showed that lesioned animals did not acquire the CTA and their GAC were significantly decreased as compared with the controls ($t=8.7; p < 0.001$). In addition, similar results were obtained when the GAC was decreased through an i.p. injection of 100 mg/kg of cimetidine (histamine H2 blocker receptor); i.e. blockade of CTA and enhanced gastric pH. These results suggest that the GAC plays an important role on the acquisition of CTA.

309.4

THE 5-HT_{1A} AGONIST, 8-OH-DPAT PREVENTS THE EXPRESSION BUT NOT THE DEVELOPMENT OF A CONDITIONED TASTE AVERSION. G.A. Hunter, G.P. Mark and B.G. Hoebel. Department of Psychology, Princeton University, Princeton, NJ 08544-1010

This experiment examined the involvement of serotonin (5-HT) in the development and expression of a conditioned taste aversion (CTA). Twenty-four male, Sprague-Dawley rats were subjected to a CTA paradigm in which the taste of 2.5 mM sodium saccharin (conditioned stimulus, CS) was paired with lithium chloride-induced malaise. Control animals received the same saccharin taste followed by saline injections. During conditioning, one group (A) was pretreated with 8-OH-DPAT (75 mg/kg, i.p.) 15 min prior to saccharin exposure and saline on the test day. A second group (B) received saline during conditioning and 8-OH-DPAT before testing. Development of the taste aversion was unaffected by 8-OH-DPAT pretreatment (group A). However, subjects in group B exhibited a pronounced attenuation of the CTA. The results suggest serotonergic involvement in the expression but not the learning of a conditioned taste aversion.

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309.5

CONDITIONED TASTE AVERSION REVERSES DOPAMINE RELEASE IN THE NUCLEUS ACCUMBENS. G.P. Mark, D.S. Blander* and B.G. Hoebel. Department of Psychology, Princeton University, Princeton, NJ 08544-1010

Extracellular dopamine (DA) as measured by microdialysis, has previously been shown to be increased in the nucleus accumbens (NAC) following ingestion of food, water or salt and by pharmacological means, e.g. local nicotine, cocaine and amphetamine administration. This suggests that accumbens DA release is related to the reward value of a stimulus. If so, stimuli with aversive qualities may attenuate DA release. To test this hypothesis, the microdialysis technique was used to determine the extent to which DA output in the NAC could be manipulated by development of a conditioned taste aversion (CTA) in which the taste of intraorally infused saccharin was paired with LiCl-induced malaise. Small but statistically reliable *increases* (26%, $p<0.05$) in DA were observed when animals tasted saccharin for the first time. After CTA, the same dose of infused saccharin caused a 50% *decrease* in accumbens DA ($p<0.05$). This effect was absent in pseudo-conditioned controls. These results suggest that the positive and negative reinforcement effects of a taste stimulus may involve increased and decreased mesolimbic DA output, respectively.

Supported by USPHS grant DA-03597.

309.6

CONDITIONED RESPONSES TO INSULIN IN MAN. G. Fehm-Wolfsdorf, U. Beermann*, H. L. Fehm*. Depts. of Medical Psychology and Internal Medicine I, University of Ulm, D 7900 Ulm, F.R.G.

Classically conditioned changes in blood glucose levels have been repeatedly demonstrated. Till now the direction of the conditioned response is not clarified. Flaherty et al. (Physiol. Behav., 33: 587, 1984) described conditioned hypoglycemia as well as hyperglycemia depending on the nature of the CS. Eikelboom & Stewart (Psychol. Rev.,. 89: 507, 1982) have argued that direct vs. indirect central nervous system effects of insulin could lead to opposite peripheral physiological changes, and the temporal relationships between the drug effects and CS exposure may be decisive for direction of CR. We studied 24 male volunters in a differential conditioning procedure. Injection of 0.04 I.U./kg body weight of insulin or saline served as the UCS, two different odours were the CS. Sequence and combinations of the different UCS and CS were totally counterbalanced across subjects during four days. Insulin injections provided marked hypoglycemia which, however, was not always noticed by the subject. During the course of hypoglycemia mood state and cognitive performance of the subjects were checked. Levels of endogenous free cortisol were repeatedly measured to rule out a conditioned stress response as a mediator of blood glucose changes. Blood glucose levels on test day five did ot differ between CS^+ and CS^- groups, i. e. no differential conditioning was obtained, possibly due to unclear temporal S-R relationships.

309.7

EXTINCTION OF A CLASSICALLY CONDITIONED ODOR PREFERENCE IN INFANT RATS: NEUROBEHAVIORAL CONSEQUENCES. Regina M. Sullivan and Donald A. Wilson. Developmental Psychobiology Laboratory, Dept. Psychology, University of Oklahoma, Norman OK 73019.

The preweanling rat olfactory bulb demonstrates a modified response to odors which have acquired value through classical conditioning. The present study examined how these learning-associated neural changes are influenced by extinction of the conditioned behavioral response.

Wistar pups received daily conditioning sessions from PN1-PN17. Experimental pups received daily 10 min, forward pairings of odor and tactile stimulation (Odor-Stroke). Control pups received daily random presentations of odor and stroking (Random) from PN1-PN17. On PN18-PN19, half of the pups from each training condition underwent extinction which consisted of 10 min exposures to odor without stroking, 3 times each day. ON PN20, all pups were tested for response in one of two tests: behavioral preference in a two-odor Y-maze or olfactory bulb 2-DG uptake in response to the conditioned odor.

The results of the behavior test suggest that odor preferences classically conditioned early in life can be extinquished. Work currently in progress is assessing the neural consequences in the olfactory bulb of behavioral extinction. (supported by NS26100 to RMS and BNS8606786 to DAW)

309.8

OLFACTORY CLASSICAL CONDITIONING IN INFANT RATS WITH MEDIAL FOREBRAIN BUNDLE STIMULATION AS UCS: NEUROBEHAVIORAL CONSEQUENCES. Donald A. Wilson and Regina M. Sullivan. Developmental Psychobiology Laboratory, Dept. Psychology, University of Oklahoma, Norman OK 73019.

Early olfactory learning can induce a long-term change in olfactory bulb function. A major difficulty in the search for neural mechanisms of this change has been the diverse nature of the UCS. The present study examined the possibility of avoiding this problem by using medial forebrain bundle (MFB) stimulation as the UCS.

On PN12, Wistar rat pups had bipolar stimulating electrodes chronically implanted unilaterally in the MFB, under Ketaset/Rompun anesthesia. One hour after recovery from anesthesia (3-4 hr post-implant), experimental pups received 50 forward pairings of odor (5 sec) and MFB stimulation (300 ms, 200 Hz), with an ITI of 30-60 sec. Control pups received 50 random presentations of odor and MFB stimulation. On PN13, pups were either given a two-odor choice test or tested for olfactory bulb 2-DG uptake in response to the conditioned odor.

The results of the behavior test suggest that an odor preference can be classically conditioned using MFB stimulation as the UCS. Work currently in progress will determine the effects of this conditioning on olfactory bulb function.

309.9

COMPARISON OF LEARNING ABILITIES IN INBRED AND RECOMBINANT INBRED STRAINS OF MICE. M. Le Moal, J.L. Martinez, Jr., C. Berman*, and G.F. Koob (SPON: R. Emeson). INSERM Unite 259, Bordeaux, France; University California, Berkeley, CA 94720; Research Institute of Scripps Clinic, La Jolla, CA 92037

Acquisition of a one way active avoidance and an operant appetitive task was characterized in inbred mice as a prelude to studying the molecular basis of learning. The current used as the unconditioned stimulus (UCS) for active avoidance was the amount needed to cause 75% of the mice to jump in a flinch/jump test. Forty-five trials were given over 5 days . The rank ordering of learning in inbred mice as measured by mean number of avoidances was as follows: 1) DBA/2J and C57L/J. 2) AKR/J, and 3) C57BL/6J. The performance of BXD recombinant inbred mice did not indicate that the differences observed between the C57BL/6J and the DBA/2J was due to a single-gene mode of inheritance. In the operant task, animals were required to make a spatially discriminated nose-poke for a food reward on a fixed ratio 2 schedule in a 10 min training session for 8 days. The total number of rewards received differed between the C57BL/6J and DBA/2J with the C57 being superior. Here, four recombinant strains (BXD-8, -15, -18, -24) were like the C57, while one (BXD-30) was like the DBA progenitor. This strain distribution pattern is consistent with a single-gene mode of inheritance. These strain differences may reflect basic differences in learning but differences in motivation cannot yet be ruled out as an explanation. (Supported by NS 20912).

309.10

SELECTIVE LOSS OF HIPPOCAMPAL CA1 CELLS CAUSES DEFICIT IN PLACE NAVIGATION BUT NOT IN T-MAZE ALTERNATION IN RATS

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It is not known whether selective damage to hippocampal CA1 field, a region particularly affected by cerebral ischemia, disrupts spatial and/or working memory. To get information on this issue, a selective loss of pyramidal CA1 cells was obtained by injecting bilaterally 5µ/1µl (30nM) quinolinic acid (QUIN) into the stratum radiatum of the dorsal hippocampus in rats. Ten days after QUIN injection, lesioned, sham operated and intact animals were required to learn the spatial location of a hidden platform in the Morris swim maze. Lesioned rats were impaired in learning the place task, particularly at the beginning of the training period. When lesioned animals reached an escape latency similar to controls (19 days post-lesion), they showed a severe impairment in the transfer test. Cue learning in the water maze was not significantly affected by QUIN treatment.

Separate groups of animals with QUIN induced-lesion of the pyramidal CA1 cells, tested ten days after surgery as in the previous experiments, showed no impairment in learning response alternation in a T-maze.

The results suggest that rats with selective loss of hippocampal CA1 cells are impaired in learning a spatial task but are able to learn a non-spatial test of working memory.

309.11

RADIAL-ARM MAZE DEFICITS IN RATS WITH VESTIBULAR DYSFUNCTION FOLLOWING INTRATYMPANIC INJECTIONS OF SODIUM ARSANILATE. E.L. Hargreaves and K.-P. Ossenkopp. Dept. Psychology, Univ. of Western Ontario, London, Ontario, CANADA N6A 5C2.

Vestibular dysfunction was induced in Long-Evans rats by intratympanic injections of sodium arsanilate (atoxyl). Following a 1 week recovery period the animals were tested for labyrinthine integrity and their spontaneous locomotor behavior was analyzed (See: Ossenkopp, Prkacin & Hargreaves, this Volume).

Following this procedure the animals were reduced to 85% of their normal body weight. They were then run on an eight-arm radial maze over a period of 10 days (1 trial/day). Each arm of the maze was baited with a single sunflower seed, such that an optimal foraging strategy would dictate every arm to be entered only once, during a single trial.

Results indicated that the animals with vestibular dysfunction exhibited specific maze-running patterns such as more sequential re-entry of arms ($p=.01$) and less response stereotypy (sequential entry of adjacent arms) ($p=.02$). They did not show improvements on the learning measure of number of arms entered to complete the maze. They did however, show improvements on the measure of running-time ($p=.03$). These improvements may be accounted for by a gradual decrease in stress related behaviors, which disinhibits a characteristic hyperactivity (See: Ossenkopp et al., Above). Overall the animals with vestibular dysfunction show a deficit in performing the radial-arm maze. (Supported by NSERC).

309.12

QUANTAL ANALYSIS OF SYNAPTIC STRENGTH AND SHORT-TERM FACILITATION AT HIPPOCAMPAL CA1 SYNAPSES T. C. Foster and B. L. McNaughton. Department of Psychology, University of Colorado, Boulder, CO 80303.

Intracellular EPSPs were evoked in CA1 pyramidal cells either by the discharge of isolated CA3 cell spikes (SP) or by minimal stimulation of Schaffer afferents (MS). Quantal parameters ($m,n, p, v1$) of the response distribution were compared for paired pulse facilitation. At least 150 response pairs (50 msec ISI) were collected and noise deconvolution used to find the optimal quantal parameters of the response distributions. Most (SP: 7/9; MS 14/14) could be described by Poisson or binomial statistics at $P < .01$. Mean EPSP size (SP: 218 mV; MS: 244 mV) and quantal parameters for SP and the MS conditioning responses were not significantly different (means SP: $m = 1.17$, $n = 5.1$, $p = 0.23$, $v1 = 225$ mV; MS: $m = 0.98$ $n = 5.6$, $p = 0.18$, $v1 = 274$ mV). Mean EPSP amplitude was significantly correlated with m values but not with $v1$. Statistical comparisons confirmed ($P < .01$) that MS facilitation (206 $\pm$ 64%) resulted from increased release probability ($p = 0.33 \pm 0.14$). We conclude that 1) responses to minimal stimulation are drawn from a population with the same characteristics as the spike triggered responses; 2) transmission at this synapse can be described by Poisson or binomial statistics; 3) the 5-fold range of differences in mean synaptic efficacy between synapses (under these conditions) is due to differences in m and not $v1$; 4) facilitation is largely due to increased probability of transmitter release. Supported by grants NS-08585A to TCF and NSF BNS-8617464 to BLM .

309.13

RECOVERY OF SPATIAL LEARNING FOLLOWING DECAY OF EXPERIMENTAL SATURATION OF LTE AT PERFORANT PATH SYNAPSES. C. A. Castro, L. H. Silbert*, B. L. McNaughton, and C. A. Barnes, Department of Psychology, University of Colorado, Boulder, CO 80309.

Long-term enhancement (LTE/LTP) of hippocampal synapses is viewed by many to represent the underlying mechanism for storage of spatial information. Saturation of the LTE mechanism at perforant path synapses was previously shown to be accompanied by a severe deficit in the acquisition of spatial information (McNaughton et al., J. Neurosci 6:563-571, 1986). While this was consistent with conceptual models of distributed associative memory (McNaughton and Morris, TINS 10:408-415, 1987), the caveat could still be raised that the behavioral effect may have been due to some secondary effect of the stimulation, rather than to the saturation of LTE *per se*. Experimentally-induced LTE in the fascia dentata is not permanent, but decays with a time constant of one to several weeks (depending on stimulus parameters and other variables). Thus, if the learning impairment is actually an effect of the LTE saturation, then spatial learning should recover as LTE decays. Two groups of animals were implanted bilaterally with perforant path stimulating electrodes and electrodes for recording the synaptic and postsynaptic field responses in FD. One group (HF) received 14 days of high-frequency stimulation which produced a 50% increase in the field EPSP and a 150% increase in the population spike. The other group (LF) received only low-frequency stimulation. Both groups were then tested in the Morris water-pool task. While the LF group was able to use spatial cues to locate the escape platform, the HF group was completely unable to do so, showing no differential quadrant search during probe trials in which the submerged platform was removed. Neither group was impaired in escaping to a visible platform. Following the recovery of the field EPSP and population spike back to baseline (17 days after the last HF stimulation), the HF group was now just as proficient in using spatial cues to locate the escape platform as the LF group. The responses of the LF group remained stationary throughout the decay of LTE in the HF group, indicating that the decay was not an artifact of deterioration of the recording situation. These findings support the view that it is indeed the saturation of the synaptic connections produced by LTE that interferes with the acquisition of spatial information. Supported by NS-20331, AG-03376.

309.14

DIFFERENTIAL TIMECOURSES OF EPSP AND POPULATION SPIKE GROWTH IN RAT FASCIA DENTATA RESULTING FROM SPATIALLY COMPLEX HOUSING. L. H. Silbert*, C. A. Castro, C. A. Barnes, and B. L. McNaughton (Spon: C. E. Dixon) Dept. Psychology, Univ Colorado Boulder, CO 80309.

Previous studies from this laboratory have demonstrated persistent changes in perforant path evoked field potentials in the fascia dentata resulting from prolonged exposure of normally cage-housed animals to complex spatial environments (Sharp et al , Br. Res.,1985; Sharp et al., Exp. Aging Res., 1987). A substantial increase in the population spike component developed gradually over several days of this treatment. Changes in the epsp component were smaller and apparently less reliable. The spike increments persisted, with gradual decay following termination of the enrichment treatment. In the present studies, we have used daily recording and a larger sample size to gain a better understanding of the quantitative relationship between the epsp and population spike changes. Nineteen hemispheres from 11 animals were studied in the complex housing group and compared to 9 hemispheres from 5 control animals. Responses were collected daily between 0900 and 1100. After 5 days of baseline recording, during which both groups remained singly cage-housed, the experimental group was transferred daily between the hours of 1700 and 0730 to large (0.8 m sq) plexiglas boxes filled with junk objects which were rearranged and/or changed daily. Both epsp and population spike exhibited significant progressive growth over the first five days, reaching asymptotes of 16% and 55%, respectively. Interestingly, over the next five days of complex housing, the epsp increments decayed back to baseline, whereas the spike increments persisted. This suggests that at least two independent physiological processes are activated by complex housing and reemphasizes the point that population spike measures alone are not good indices of *synaptic* plasticity. Supported by Grants AG-03376 and NS-20331.

309.15

LONG-TERM ENHANCEMENT DOES NOT PRODUCE A CHANGE IN THE SPONTANEOUS FIRING RATE OF DENTATE GYRUS BASKET AND GRANULE CELLS. L. L. Chen, B. L. McNaughton, and S. J. Y. Mizumori. Department of Psychology, University of Colorado, Boulder, CO 80309

The relationship between the spontaneous firing of dentate gyrus granule and basket cells and induction of long-term enhancement (LTE/LTP) was studied in Nembutal-anesthetized rats. Identification of basket and granule cells was based upon the paired-pulse synaptic inhibition test described by Mizumori et al. (1989). The main differences between electrophysiologically defined granule and basket cells were that the latter were not inhibited during inhibition of the population spike, were activated by perforant path stimulation at short latency and at intensities below the threshold for a population spike, discharged multiple spikes, and showed high spontaneous firing rates. Stereotrode recording (McNaughton et al, 1983) of spontaneous unit activity was collected for 10 min before and 10 min after high-frequency perforant path stimulation. Eleven granule cells and 24 basket cells were recorded from 22 rats. High-frequency stimulation of the perforant path increased the EPSP slope (33.7% $\pm$ 2.7%) and population spike amplitude. Of those tested, 11/21 basket cells and 5/6 of granule cells showed a reduced threshold to perforant-path activation following high frequency stimulation (in agreement with Buszaki et al.,1982). However, apart from an occasional very transient (<1 min) elevation, there was no statistically significant change in the mean spontaneous rate of either granule or basket cells measured in the presence of LTE. These apparently contradictory results lead to the suggestion that the entorhinal cortical layer II cells of origin of the perforant path have very low spontaneous rates under these conditions and contribute little to the average spontaneous activity of dentate neurons. It remains to be determined whether the same is the case in conscious animals. Supported by Grant NS-20331 to B. L. M. and AG-05375 to S. J. Y. M.

309.16

EFFECTS OF MEDIAL SEPTAL LESIONS ON EXPLORATION-RELATED SYNAPTIC EFFICACY INCREASES IN RAT FASCIA DENTATA. E. J. Green, B. L. McNaughton, and C. A. Barnes. Department of Psychology, University of Colorado, Boulder, CO 80309.

Exploratory behavior in rats is accompanied by several moderately persistent alterations in the components of perforant path evoked synaptic responses in the fascia dentata (Sharp et al.,1989; Green et. al, 1989). These include a substantial increase in population EPSPs, and a decrease in both the amplitude and onset latency of population spikes. These changes have a gradual onset and decay, and long outlast exploration and its associated hippocampal theta activity. Thus, they are distinguished from the momentary "gating" effects of behavioral state described by Winson and colleagues. Neither locomotion nor hippocampal theta activity is sufficient to produce these efficacy changes, an observation consistent with the possibility that the effects are related to alterations in spatial sensory input.

The foregoing data, together with evidence indicating that an intact septo-hippocampal pathway is necessary for efficient performance of certain spatial tasks, prompted us to assess exploration-related alterations in perforant path evoked synaptic responses prior and subsequent to lesioning the medial septum. Transportation of animals from their home environment to a different location was accompanied by: 1) an increase in the incidence of exploratory behavior (and EEG theta), 2) substantial (20-70%) elevation in the initial slope of the field EPSPs, and 3) reduction in spike amplitudes and latencies to spike onset. Electrolytic lesions of the medial septum/diagonal band sufficient to attenuate theta rhythm severely or to abolish it were made through an indwelling septal electrode. Preliminary results indicate that these lesions were not sufficient to prevent the exploration dependent response changes (peak EPSP growth pre-lesion: 44 $\pm$ 5.5 %, post-lesion: 35 $\pm$ 3.3%). These results further dissociate hippocampal theta activity from the synaptic alterations associated with exploration and suggest that the information critical for such changes does not depend exclusively upon neural pathways which originate in or pass through the medial septal region. Supported by F32 AG-05345 to E.J.G, BNS8617464 to B.L.M., and AG-03376 to C.A.B.

309.17

A COMPARISON OF PLACE AND CUE LEARNING FOLLOWING REVERSIBLE INACTIVATION OF THE MEDIAL SEPTUM. S. J. Y. Mizumori, G. M. Perez, M. C. Alvarado, C. A. Barnes, and B. L. McNaughton, Dept. Psych., Univ. Colo., Boulder, CO 80309.

Injection of a local anesthetic (tetracaine) into the medial septum produces a reversible working memory deficit in rats performing a radial maze task (Mizumori et al., *J. Neurosci.*, in press). During this period of behavioral impairment, the hippocampal θ-rhythm is abolished, CA3 place fields are disrupted, and the specificity of CA1 place fields remains unchanged. The purpose of the present experiment was to: 1) examine whether this behavioral deficit involved disruption of acquisition, maintenance, or retrieval aspects of spatial information processing, and 2) determine the extent to which the performance deficit was uniquely spatial.

Fischer 344 rats (9 mo old; n=8) were trained to perform one standard working memory trial daily on an 8-arm radial maze. A 30-min delay was interposed between choices 4 and 5. The first four (forced) choices comprised the sample phase of the experiment, while (free) choices made after the delay constituted test responses. When rats achieved criterion performance, bilateral hippocampal EEG electrodes and a medial septal guide cannula were implanted. Tetracaine injection into the septum (0.5 μL) reduced the θ-rhythm for about 20 min. When tetracaine was injected either before the sample phase or just before the test phase, a significant increase in errors was observed during the test phase ($p<.02$). Saline injections, or tetracaine given at the beginning of the delay, had no effect on choice accuracy. These findings indicate that normal cellular activity in the septohippocampal system is required for accurate acquisition and retrieval, but not maintenance, of spatial information. This suggests that this information is stored as a distribution of synaptic weights rather than as specific patterns of neural activity. The same rats were subsequently trained to perform a nonspatial task on the radial maze by selecting one of eight distinct (intramaze) cues presented simultaneously. The identity of the correct goal cue remained constant across days but varied in its spatial location from trial to trial. The rats performed four trials per day. Preliminary results (n=2, *thus far*) indicate that tetracaine-treated rats readily learn new nonspatial information when hippocampal θ is absent. That place, but apparently not cue, learning was disrupted by septal inactivation is consistent with the hypothesis that this treatment does produce a selective, rather than general, learning deficit. Supported by Grants AG-05375, AG-03376, and NS-20331.

309.18

A COMPARISON OF "THETA-ON" AND "THETA-OFF" CELLS RECORDED IN THE RAT HIPPOCAMPAL FORMATION DURING RADIAL MAZE PERFORMANCE. B. L. McNaughton, S. J. Y. Mizumori, C. A. Barnes, Dept. Psych, Univ Colorado, Boulder, CO 80309.

Most hippocampal formation single units in freely behaving rats fall into one of two categories (Ranck '73). CS cells have low average firing rates and sometimes fire complex spikes when the hippocampal EEG exhibits large irregular activity (LIA) associated with awake immobility. Theta (θ) cells fire simple spikes, have higher mean rates, and show substantially increased firing in phase with the EEG θ rhythm associated with Vanderwolf's Type I behaviors (e.g., walking, exploration). CS and θ cells almost certainly correspond to pyramidal and basket cells respectively (Fox and Ranck '75). CS cells are usually highly spatially selective (O'Keefe and Dostrovsky '71), whereas spatial selectivity of θ cells, while demonstrable, is marginal at best (McNaughton,Barnes and O'Keefe '83); Colom and Bland ('87) described, in urethane anaesthetized animals, a class of non-CS cell which was inactive in the presence of EEG θ and discharged continuously during LIA. They called these "θ-off" cells and used the term "θ-on" to refer to the classical "θ" cell. Others (e.g , Fox and Ranck '75, Buzsaki et al '83) have reported occasional encounters with such cells (which they called "anti-θ" cells) in conscious animals. We have studied the behavioral correlates of 14 θ-off cells encountered in CA1 (1), Hilus FD (4), Subiculum (6), and Entorhinal Cortex (3). Mean rates for θ-on and θ-off were 8.7 Hz and 6.5 Hz, respectively. Maximum rates were 114 Hz and 104 Hz, respectively. Some cells of *both* types showed 6-8 Hz modulation. Whereas firing rate for θ-on cells increased smoothly with running velocity, it decreased smoothly for θ-off cells. Whereas no θ-on cell exhibited clear spatial selectivity, two θ-off cells (in FD) did. When EEG θ rhythm was temporarily abolished by local injection of tetracaine into the medial septum (Mizumori et al '87), a θ-off cell was observed to fire continuously at high rate irrespective of behavior, with a pronounced 18-20 Hz rhythmic modulation. Under these circumstances, θ-on cells decrease their rates. Possible candidates for θ-off cells are the parvalbumen negative GABA interneurons (Kosaka et al '87) or those containing somatostatin (Kohler and Chan-Palay '82; Bakst et al '86). Supported by grants AG-05375, NS-20331, and AG-03376.

309.19

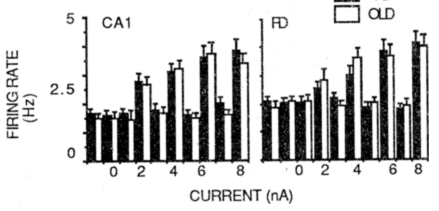
COMPACT SPATIAL REPRESENTATIONS ARE FORMED IN HIPPOCAMPUS BUT NOT PASSED BACK TO CORTEX. C. A. Barnes, B. L. McNaughton, S. J. Y. Mizumori, B. W. Leonard, L.- H. Lin*. Department of Psychology, University of Colorado, Boulder, CO 80309.

Pyramidal cells in the CA1 and CA3 subfields of the rodent hippocampus exhibit a remarkable selectivity for both location and (in most environments) orientation of the animal in space (O'Keefe, 1976). This study addresses the questions of whether this compact spatial representation is constructed in the hippocampus itself, and whether it is passed on to other cortical structures via the return pathway through the subiculum. Accordingly, single units were studied in 34 rats using the stereotrode method, in entorhinal cortex (n = 44), hippocampal subfields CA3 (n = 220) and CA1 (n = 121), and the subiculum (n = 194), while rats performed on a radial 8-arm maze for chocolate milk reinforcement. Based on a measure of the tendency of firing to be restricted to one maze arm, entorhinal units exhibited little if any spatial specificity (mean specificity index = 1.94; a score of 1 indicates no specificity by this measure). As found in many other studies, hippocampal cells were highly specific, with CA3 exhibiting significantly more specificity than CA1 cells (mean specificity index CA3 = 7.45, CA1 = 4.33). This strongly suggests that spatial specificity is constructed in hippocampus from relatively unspecific inputs (a finding supporting Quirk and Ranck, 1986, Soc. Neurosci. Abst). Surprisingly, subicular cells also exhibited little if any spatial specificity by this measure (mean = 1.53), although some indication of a dispersed but consistent specificity was found in some cells. This suggests that sparsely coded, highly localized spatial representations are necessary for some operations performed within the hippocampus itself (e.g., efficient event storage) and that the information is recoded into a much more distributed form before transmission back to other cortical areas. Supported by AG-03376 and NS-20331.

309.20

RESPONSIVENESS TO GLUTAMATE IS STABLE WITH AGE IN RAT HIPPOCAMPUS. G. Rao, C. A. Barnes, and B. L. McNaughton. Dept. of Psychology, Univ. of Colo., Boulder, CO 80309.

Age-related changes in rat hippocampal formation include a partial deafferentation of the dentate gyrus and a presumably compensatory increase in excitability of senescent hippocampal pyramidal and granule cells. Changes in basal neurotransmitter release and mitochondrial uptake have also been reported in aging brain. In this context, we investigated whether the sensitivity of hippocampal CA1 and dentate granule cells to glutamate applied iontophoretically near the cell body remains stable or changes with age. Slices were taken from a young (8 mo) and an old (27 mo) F344 rat each day (n=8 animals in each age group) to counterbalance any variability in the preparation. Recording pipettes (6-8 m tips) were filled with a .2 M glutamate in slice Ringer's solution. Units between 400-600 mV were recorded in stratum pyramidale and the granule cell layer (n=20: old CA1, 198 yg Ca1, 197 old FD, 193 yg FD). Glutamate application consisted of an ascending series of current pulses (10 sec off, 5 sec on) of amplitudes 0, 2, 4, 6, and 8 nA. The series was delivered 4 times, with a 30 second delay in between. No significant difference in unit firing rate to glutamate at any current amplitude was detected between the old and the young groups in either CA1 or FD. At least to the extent that it can be assessed at the level of the cell body, glutamate sensitivity appears to be neither enhanced nor compromised in senescent hippocampus. Supported by AG-03376 to C.A.B.

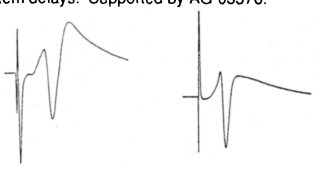


309.21

POST-MORTEM RECOVERY AND VIABILITY OF CENTRAL NERVOUS SYSTEM TISSUE SUBSEQUENTLY MAINTAINED IN VITRO. B.W. Leonard, C.A. Barnes, G. Rao, J. Meltzer, & B.L. McNaughton. Dept. of Psychology, Behavioral Neuroscience Area, Univ. Colorado, Boulder 80309.

The influence of post-mortem delay on the recovery and viability of in vitro brain slices has not been systematically studied. To explore the feasibility of gaining meaningful data about pre-mortem electrophysiological function from tissue that is not immediately available upon death, we have examined the effects of post-mortem delay on evoked field potentials in the in vitro rat hippocampal slice preparation. Brains were kept in situ at room temperature for delays of zero (n=1), 30 (n=7) or 120 min (n=4) before dissection and slicing of the tissue. Using standard in vitro conditions, tissue viability was assessed by recording Schaffer-collateral evoked field potentials in the CA1 pyramidal layer at 2, 4, and 8 hr after slicing. At 8 hr the percentage of slices displaying responses with the zero or 120 min post-mortem delay was 50% and 33%, respectively. At a fixed constant current stimulus the average spike amplitude of viable slices in these conditions was 16.6 mV and 8.2 mV, respectively. These results indicate that the processes responsible for the maintenance of electrochemical gradients across neuronal membranes can recover from a period of severe anoxia when the tissue is subsequently maintained in vitro , and suggests that meaningful information about synaptic and cellular function may be inferred from brain tissue available only at relatively long post-mortem delays. Supported by AG-03376.

Potentials recorded 8 hrs. after 30, or 120 min. post-mortem delays.



309.22

A SIMPLE MODEL FOR HIPPOCAMPAL PLACE CELLS IN WHICH DIRECTIONAL TUNING OF PLACE FIELDS DEPENDS ON ENVIRONMENTAL COMPLEXITY. W. E Skaggs and B. L. McNaughton, Dept Psychology, Univ Colorado, Boulder, CO 80309.

Many cells in the rat hippocampus are "place cells," firing preferentially when the animal is in a particular small portion of the space available to it. The "place fields" of these cells can appear on first exposure of the animal to the preferred location in an environment. They vary widely in size and shape, and in susceptibility to manipulations of the environment. We have programmed a simple computer model of place cells, using two layers of units. The units of the input layer form a value-coded representation of selected features of the environment as seen by the animal. The units of the second layer each receive randomly weighted excitation from the first layer, and are subject to a variable "inhibition" such that only the five receiving the most input become active (fewer if less than five exceed a predetermined threshold). These second layer units exhibit the sort of spatially localized firing characteristic of hippocampal place cells. As in the hippocampus, some units fail to be active at all in a given environment, and the sizes and shapes of the place fields vary widely.

With certain plausible but unproven assumptions, the model is capable of reproducing an apparently perplexing effect. On a radial eight-arm maze, the firing of place cells depends strongly on the direction the animal faces [McNaughton et al ,1983]; in a small grey cylinder in which the only "polarizing" cue is a single white card covering 100° on the wall the directional dependence of place fields is very much weaker [Bostock, Taube, and Muller, 1988]. Reasoning that the difference may arise from the greater complexity of the radial-maze environment, we set up the model so that the input layer contains 110 units, 22 associated with each of five "objects." The activity of each input unit depends either on the distance or on the direction from the animal to the associated object; also a unit becomes inactive if its object drops out of the animal's roughly 300° visual field. When only the two objects representing the edges of the cue card are present, the directional tuning of place cells is very broad. When all five objects are present and widely scattered, the directional dependence becomes much stronger. Although these results are preliminary, they do suggest that the differences in directional tuning of place cells in different environments may plausibly be explained in terms of environmental complexity without having to invoke the concept of place cells representing points in some abstract coordinate framework. Supported by The Sloan Foundation.

309.23

HEBBIAN SYNAPSES AS CROSS-CORRELATION FUNCTIONS IN DELAY LINE CIRCUITRY. D. C. Tam[1] and T. A. McMullen*[2]. Dept. of Physiology and Biophysics, University of California[1], Irvine, CA 92717 and Dept. of Biomedical Engineering, Boston University[2], Boston, MA 02215.

Hebbian learning has long been proposed to be a plausible mechanism for synaptic plasticity. Artificial neural networks using the Hebbian rule have exhibited some aspects of learning. Analysis of the mathematical operation performed by Hebbian synapses in such networks indicates that for continuous signals the synaptic weight after learning has occurred is a representation of the cross-correlation between the input and output signals. Therefore, during learning, the synapse computes the correlation between input and output signals by accumulating weight changes. When the input signal is time shifted by τ, along a delay line, the final weight of the synapse represents the value of the cross-correlation coefficient at lag time τ. When the input and output are discrete time series such as spike trains, the final synaptic weight represents the probability, estimated from the learning sequence, that the output neuron fires at time lag $\tau = k - \Delta t$ after the input neuron has fired. The final synaptic weight is given by:

$$w(k\Delta t, n\Delta t) = w(k\Delta t, 0) + \sum_{h=1}^{n} x(h\Delta t - k\Delta t)\, y(h\Delta t) \quad (1)$$

where $w(k\Delta t, n\Delta t)$ is the synaptic weight at lag time $k\Delta t$ after n learning steps, $y(h\Delta t)$ is the output at time $h\Delta t$ and $x(h\Delta t - k\Delta t)$ is the input, time shifted by $k\Delta t$. It can be shown that the correlation coefficient is given by:

$$R(k\Delta t) = \lim_{\Delta t \to 0} \lim_{n \to \infty} [w(k\Delta t, n\Delta t) - w(k\Delta t, 0)]/n. \quad (2)$$

Equation (1) can be generalized such that x and y are replaced by x_i and x_j, any two inputs to the same target neuron. The operation of correlation may occur during learning or development in structures with delay line architecture such as cerebellar cortex, hippocampus and dorsal cochlear nucleus.

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EPILEPSY: KINDLING II

310.1

MK-801 IS NEUROPROTECTIVE, BUT NOT ANTICONVULSIVE, DURING PILOCARPINE-FACILITATED STATUS EPILEPTICUS IN KINDLED RATS. G.M. Hudson* and G. G. Buterbaugh. (SPON: J.W. Ferkany). Department of Pharmacology and Toxicology, University of Maryland School of Pharmacy, Baltimore, MD 21201.

Male amygdala kindled rats were subjected to 3 hours of pilocarpine facilitated status epilepticus (SE). Rats were treated with MK-801 (1 mg/kg i.p.; N=7) or vehicle (V; N=5) after 10 min of SE. In V treated rats, power spectral analysis of neocortical EEG revealed a 60 - 65% decrease in mean frequency (MF) and 97% edge frequency (EF) after 3 hrs of SE. Two weeks later, severe bilateral neuropathology was found in thalamic and septal regions, substantia nigra reticulata (SNr), amygdala, posterior & ventral hippocampus and entorhinal, piriform and frontal cortices. In MK-801 treated rats, MF and EF decreased by 20% and 21%, respectively, during the 3 hrs of SE. Neuropathology was limited to thalamic regions and the SNr. Rats treated with MK-801 (0.5 mg/kg; N=3) showed additional mild damage within entorhinal and piriform cortices. In ventral hippocampal kindled rats (N=3), similar MK-801 protection against SE-induced brain damage was found in spite of high-frequency spiking activity induced by MK-801 in the ventral hippocampus. These results demonstrate neuroprotective effects of MK-801 during prolonged SE and suggest that seizure mechanisms may be dissociated from mechanisms of seizure-induced brain damage in some brain regions. (Supported by PHS Z SO7 RR 05770)

310.2

STATUS EPILEPTICUS INDUCED NEUROPATHOLOGY RELATED TO KINDLING SITE AND DURATION OF STATUS. G. G. Buterbaugh and G. M. Hudson*. Department of Pharmacology and Toxicology, University of Maryland School of Pharmacy, Baltimore, MD 21201.

Rats were kindled by daily stimulation of the piriform cortex, frontal neocortex, ventral hippocampus (VH) or dorsal hippocampus (DH) and subjected to 3 hours of pilocarpine facilitated status epilepticus (SE). Two weeks later, a bilateral pattern of damage was found within thalamic, septal, amygdala, substantia nigra reticulata (SNr), and posterior & ventral hippocampal regions. Mild to moderate bilateral cell loss was also observed within the entorhinal and piriform cortices. Rats kindled by amygdala (Am) stimulation and subjected to 2-3 hours of SE showed a similar qualitative regional pattern of damage with the exception of a more regionally extensive bilateral pattern of near complete degeneration of much of the entorhinal and piriform cortices. DH kindled rats surviving up to 6 hrs of SE showed damage similar to Am kindled rats after 2-3 hrs of SE. Moreover, four distinct kindling-site dependent patterns of SNr damage were observed ranging from blade necrosis (AM and DH) to "ring" necrosis (VH). These results suggest that the focal origin of kindled epileptogenesis is a determinant of susceptibility to seizure-induced damage within some brain regions. In particular, amygdala kindling results in a greater sensitivity to damage within paliolimbic structures. (Supported by PHS Z SO7 RR 05770 and NS 20670)

310.3

PHARMACOLOGICAL ALTERATION OF 2-DG UPTAKE DURING STATUS EPILEPTICUS. B.E. Jones and G.G. Buterbaugh. Department of Pharmacology and Toxicology, University of Maryland School of Pharmacy, Baltimore, MD 21201.

The uptake of 2-DG was used to study the relative sensitivity of brain regions to anticonvulsant drugs during pilocarpine facilitated status epilepticus (SE) in amygdala kindled rats. Pentobarbital (Pent) or diazepam (Dz) were administered after 10 min of SE, followed within 10 min by [^{14}C]2-DG (100 uCi/kg; iv); rats were decapitated 45 min later and brains rapidly removed and frozen sectioned for contact autoradiography. Pent (18 mg/kg; iv) produced marked behavioral depression and suppression of EEG discharge in the neocortex and hippocampus, but not the amygdala. Uptake of 2-DG was decreased in all regions except the amygdala and piriform and entorhinal cortices. Pent (9 mg/kg; iv) had little effect on behavior, EEG or 2-DG uptake. Dz (4 mg/kg; iv) resulted in marked behavioral depression and little change in EEG seizure discharge. However, 2-DG uptake was selectively and bilaterally decreased in parietal cortex and the entire extent of the hippocampal formation. Diazepam (1 mg/kg; iv) had little effect on behavior, EEG discharge or 2-DG uptake. These results indicate that the amygdala and piriform-entorhinal cortices are relatively resistant to drug effects during SE and suggest that 2-DG uptake may not be a reliable index of seizure activity. Additional drugs are being studied to further assess this possibility.

310.4

ROLE OF ADENOSINE IN LIMITING STATUS EPILEPTICUS ENTRY AND SEVERITY IN THE ELECTROGENIC LIMBIC STATUS MODEL. D.M. Treiman* and A. Handforth (Spon: K. Syndulko) VAMC Wadsworth, Dept. of Neurology, ULCA, Los Angeles, CA 90024

In a limbic model of status epilepticus (SE) amygdala stimulation induces continuous spiking associated with exploratory behavior. Functional mapping studies indicate that the anatomic substrate is usually unilateral extensive limbic (Handforth, A. and Ackermann, RF, Brain Res 460:94, 1988). We hypothesized that an endogenous adenosine mechanism limits SE entry and severity. Rats had intracerebral electrodes implanted in both amygdalae and epidural electrodes over frontal and parietal cortex. In blinded experiments saline, 2-chloroadenosine (2CA, 20mg/kg), or aminophylline (AP, 100 mg/kg), 7/group, was given IP 15 min prior to stimulation of left amygdala with successive 5 min sessions of current; 15 sessions without achieving 5 min of post-current spiking constituted failure to enter SE. All saline animals entered exploratory SE, whereas entry was blocked or delayed by 2CA. AP accelerated SE entry and the resulting SE was generalized convulsive. In preliminary experiments, these treatments were administered after subjects had already entered SE (3/group). Whereas saline subjects remained in exploratory SE, AP resulted in worsening to generalized convulsive SE, and 2CA resulted in sedation with partial or complete spiking suppression. These results support a role for adenosine in limiting status entry and severity.

310.5

STRUCTURAL DAMAGE OF THE BRAIN TRIGGERS KINDLING AND SPONTANEOUSLY RECURRENT SEIZURES. E.A. Cavalheiro†, J.P. Leite†, Z.A. Bortolotto†, W.A. Turski, C. Ikonomidou† and L. Turski. Department of Neurology and Neurosurgery, Laboratory of Experimental Neurology, Escola Paulista de Medicina, Sao Paulo, SP, Brazil and Department of Pharmacology, Medical School, Lublin, Poland.

Structural damage of the human brain may lead to chronic epilepsy in survivors. Epidemiologic analyses show that a considerable time-delay occurs between the exposition of the brain to injury and the appearance of seizures. Such seizures are usually partial or mixed in type, may develop at any age, and are difficult to treat. We now show that in adult male Wistar rats, 240-280 g in weight, subjected to structural damage of the brain induced by means of sustained convulsions triggered by systemic administration of a cholinergic agent pilocarpine HCl (380 mg/kg; ip), spontaneous seizures may develop after a mean latency of 14-15 days. The mean number of spontaneously recurrent convulsions remains constant for several months. The evolution of such seizures proceeds through several electrographic and behavioural stages resembling kindling. Kindling may be otherwise induced in rodents by repeated systemic administration of convulsants or by repeated electrical stimulation of sensitive brain regions. These observations demonstrate that structural damage of the brain may lead to spontaneously recurrent convulsions (chronic epilepsy) in the rat and that kindling may be engaged in the evolution of such a condition. This finding suggests that kindling mechanisms underlie the development of epileptic foci from structural brain lesions. Such mechanisms may be involved in the etiology of some forms of epilepsy in humans. Supported by CNPCT, FAPESP, FEP and Polish Academy of Sciences.

310.6

SPATIO-TEMPORAL MAPPING OF CORTICAL EEG DURING AMYGDALOID ELECTRICAL KINDLING IN THE CAT. R. Fernández-Mas* A. Fernández-Guardiola, A. Martínez*, L. Rocha* and R. Gutiérrez. Instituto Mexicano de Psiquiatría, México 14370 D.F.

Time- and frequency-domain topographic maps of the EEG activity of frontal, parietal, temporal and occipital cortices were daily computed during amygdaloid kindling. A 16 epidural electrode array (matrix) was used in 4 cats, two implanted in the left amygdala and two in the right one. The EEG maps were obtained using a specifically designed program (RBEAM). Sixteen-channel EEG ink recordings, the stimulated amygdala activity and the behavioral responses were sampled and stored. The frequency-domain (FFT) maps computed for specific high EEG frequency bands (8-16Hz) revealed a clear pattern of ipsilateral ectosylvian and contralateral frontal high spectral density areas during behavioral stages 2-4. Stages 5 and 6 were characterized by parieto-rolandic and ectosylvian contralateral propagation. The historial layout (time-domain mapping) of the cortical projection of isolated AM/AD spikes, that was computed for a specific AD latency and during different behavioral stages, also showed the contralateral propagation of ectosylvian ADs to the frontal lobe.

RBEAM allows the averaging of several AD maps during Kindling, showing the constancy of the contralateral frontal focus, expressed in terms of a standard deviation.

310.7

KINDLING ANTAGONISM IS AGE SPECIFIC. K. Haas* and E.F. Sperber (SPON: S.L. Moshé). Depts. of Neurology and Neuroscience, Albert Einstein College of Medicine, Bronx, NY

Burchfiel et al have demonstrated that, in adult rats, stimulations alternating between two limbic sites suppress the development of kindling in one or both sites. We report kindling antagonism is an age-specific phenomenom not present early in life.

Adult rats and 16 day old rats received alternating stimulations between amygdala and hippocampus. Another group of pups was stimulated at one site - either amygdala or hippocampus. Adults were stimulated three times daily while the pups were stimulated every 20 minutes.

Six adult rats kindled only from the amygdala. In two adults mutual suppression occured. The suppression of generalized motor components of kindled seizures was not due to lack of afterdischarges (ADs) since stimulation of the suppressed sites produced ADs as long as the ADs from kindled sites.

In pups alternating stimulations did not lead to suppression of kindled seizures from either site. Furthermore there was no difference in the total number of stimulations required for the completion of kindling compared to controls.

Our results in the adult continue the original observations of Burchfiel et al. The lack of kindling antagonism in rat pups may underly the propensity of the immature CNS to develop multifocal seizures.

310.8

SUBREGIONAL CHANGES IN HIPPOCAMPAL THYROTROPIN-RELEASING HORMONE (TRH) AFTER AMYGDALOID KINDLING IN RATS. S.M. Knoblach*, S. Durbin*, and M.J. Kubek. Indiana Univ. & VA Medical Centers, Indianapolis, IN 46223.

We have previously shown that hippocampal TRH levels are increased after kindling. To examine TRH increases at the subregional level, we kindled rats with a 200 ua, 1 msec pulse daily, until five successive Stage 5 seizures occurred. Later, animals were stimulated once and killed either 24 or 48 h afterward. Hippocampi were dissected into dorsal and ventral CA1, CA3, CA4/Hilus (CA4), Dentate (D) and Subiculum (S) subregions, frozen, extracted, and analyzed via specific TRH radioimmunoassay.

Baseline TRH content (pg/mg wet wt) in ventral subregions was: CA3, 4.17 +/-0.76; CA1, 3.24 +/-0.21; D, 1.93 +/-0.36; S, 1.66 +/-0.22; CA4, 1.39 +/-0.15. In dorsal subregions, TRH content was: CA3, 2.09 +/-0.37; CA1, 1.31 +/-0.24; D, 1.11 +/-0.16; S 0.95 +/-0.16; CA4, 3.57 +/-0.86. TRH levels 24 h after convulsion were significantly increased in ventral subregions: CA4 22x, D 9x, CA3 4x, CA1, S, 3x. TRH content was also significantly increased in dorsal subregions: D 18x, CA4 10x, CA3 8x, CA1 4x. Values for the 48 h timepoint were not significantly different from those at 24 h. This report is the first to show subregional changes in hippocampal TRH after kindled convulsion. We suggest that the large increase in CA4 and D represent changes in intrinsic TRH synthesis stimulated mainly by perforant path input, and note that the degree of TRH increase observed in the various subregions parallels the sequence of the hippocampal transsynaptic loop. Supported by VA and NIH NS-25661 (MJK).

310.9

EFFECT OF A 12 WEEK INTERVAL ON PERMANENCE OF 'PARTIAL' AND 'FULL' KINDLING. Z. Dennison, G.C. Teskey and D.P. Cain, Dept. Psychology, University of Western Ontario, London, Ontario, CANADA.

Kindling is considered a permanent change, and animals kindled to a stage 5 seizure are often referred to as 'fully' kindled, while animals kindled to a lesser stage are referred to as 'partially' kindled. We were interested in examining the implications of this distinction by comparing the permanence of electrographic and behavioral development due to amygdala kindling between rats kindled to stage 3 (unilateral convulsion) and rats kindled to a stage 5 (bilateral generalized convulsion).

Rats were implanted with bipolar stimulating-recording electrodes in the amygdala. After kindling to either stage 3 or stage 5, the rats were left in their home cages for 12 weeks and then restimulated. The preliminary results suggest the degree of permanence might not be as great as previously thought. Both duration of afterdischarge and behavioral stage attained had decayed, and a range of 2-7 restimulations were needed to restore the previous behavioral stage. Secondly, approximately equivalent numbers of restimulations between groups indicated that the degree of permanence was similar in 'fully' and 'partially' kindled animals. These terms may be misleading if they are interpreted to mean greater or lesser degrees of permanence.

310.10

SPONTANEOUS HIPPOCAMPAL INTERICTAL SPIKES INDUCED BY LOCAL KINDLING: RELATIONS TO SEIZURES AND PHYSIOLOGY. K. BOON*, E. SZCZUTKOWSKI* AND L. STAN LEUNG(SPON:S. BRUDZYNSKI). DEPT. CLIN. NEUROL. SCI. AND PHYSIOLOGY, UNIV. WESTERN ONTARIO, LONDON, CANADA N6A5A5.

Spontaneous interictal spikes (SISs) were recorded in the hippocampus in freely behaving rats following hippocampal CA1 kindling. SISs emerged after 2-10 daily kindling stimulations, and persisted until behavioral convulsions. However, seizures may be accompanied with a high or low SIS rate. After fully kindled, interruption of kindling for 4-8 days resulted in the decline of SIS rate to near-zero, but seizure susceptibility, as tested by the ability to evoke seizures, remained unchanged. The increase in SIS rate was time-locked to the tetanic stimulation or afterdischarge (AD), after which SIS rate declined with two time constants of approximately 80 min and 1.5 days. The long time constants suggest the participation of some form of long-term potentiation. Preliminary experiments suggest that the increase of SIS rate following an AD was resistant to intraventricular injection of 10-40ug of the NMDA antagonist APV. Supported by NS25383 and NSERC A1037.

310.11

HIPPOCAMPAL MOSSY FIBER ZINC IN WSP AND WSR MICE. D.J. Feller, J.C. Crabbe, D. Tso-Olivas*, C. Meshul, D.D. Savage, Oregon Health Science University and VA Medical Center, Portland, OR 97201, University of New Mexico School of Medicine, Albuquerque NM 87131

Alcohol withdrawal-seizure prone (WSP) and withdrawal-seizure resistant (WSR) mice have been selectively bred for differences in susceptibility to handling-induced convulsions (HIC) during withdrawal from chronic ethanol exposure. The basis for enhanced susceptibility to HIC in WSP mice is unknown. A deficit in hippocampal mossy fiber zinc (HMZ) has been observed in rats with a genetic predisposition to audiogenic seizures. Given this observation, HMZ was measured in naive WSP and WSR mice by quantitative histofluorescence using zinc-TSQ. HMZ was 72% lower in WSP than in WSR mice. Serum, hippocampal and whole brain zinc levels were identical for both lines suggesting that the decrease in mossy fiber zinc was not due to differences in zinc bioavailability. Ultrastructural analysis of mossy fibers in the CA3 region showed higher synaptic vesicle density in WSP compared to WSR mice. These data suggest that WSP mice may be producing more vesicles as a compensatory response to lower HMZ. The reduction in HMZ in WSP mice is a correlated response to selection, suggesting that the mossy fiber zinc deficit may contribute to seizure susceptibility in WSP mice. These studies were supported by Grants AA05828, AA06243, AA06498, NIDA Contract 271-87-8120, AA06548 and RR08139.

310.12

DECREASE IN HIPPOCAMPAL MOSSY FIBER ZINC IN RATS KINDLED TO SPONTANEOUS SEIZURES. DD Savage, D Corwin* and LL Paxton*, Dept. Pharmacol., Univ. New Mexico Sch. Med., Albuquerque, NM, 87131.

We have observed striking reductions in hippocampal formation (HPF) mossy fiber zinc in two different genetic models of epilepsy: 1) the Genetically Epilepsy-Prone (GEPR) rat and 2) the Alcohol Withdrawal Seizure Prone (WSP) mouse. This observation has prompted an investigation of mossy fiber zinc in other animal models of epilepsy. Using a computer-automated kindling system to facilitate the production of rats exhibiting spontaneous seizures, we tested the hypothesis that mossy fiber zinc is decreased in kindling-induced spontaneously epileptic rats.

Male Sprague-Dawley rats received angular bundle kindling stimulations (700 μampere biphasic square wave pulses at 60 Hz for 2 sec.) eight times a day at 3 hour intervals. Stimulations were discontinued after each rat had exhibited a minimum of five spontaneous forelimb clonic motor seizures. Four weeks later, each kindled rat and its matched unstimulated control were sacrificed and their brains frozen. Coronal sections of dorsal HPF and horizontal sections of ventral HPF were collected from ten epileptic and ten control rats. Quantitative zinc:TS-Q histofluorescence, expressed as femtograms zinc/100 μm^2, was measured in HPF CA_3 *stratum lucidum* and adjacent *stratum radiatum*. Specific mossy fiber zinc was defined as the difference in zinc:TS-Q fluorescence between these two regions. Specific mossy fiber zinc was reduced by 55% in dorsal HPF and 62% in ventral HPF of spontaneously epileptic rats compared to the unstimulated controls. This reduction parallels the mossy fiber zinc reductions observed in GEPR rats and WSP mice. This similarity in three different animal models of epilepsy suggests that a decrease in HPF mossy fiber zinc may be a contributing factor in the manifestation of a seizure prone state in epileptic rodents. Alternatively, the mossy fiber zinc reduction may be a consequence of spontaneous epileptic seizure activity. (Supported by NIH-NS23461).

310.13

EFFECT OF AMYGDALA KINDLING ON THE SEIZURE RESPONSE OF THE OLFACTORY BULB AND DORSAL HIPPOCAMPUS. M.E. Kelly, C. Dufresne* and D.C. McIntyre. Psychology Dept., Carleton Univ., Ottawa, Ontario, Canada K1S 5B6.

Previous amygdala kindling affected the dorsal hippocampus by raising its afterdischarge threshold (ADT) and/or shortening its AD duration. Such amygdala pretreatment had no effect on the olfactory bulb ADT, while enhancing its provoked AD and, in most cases, triggering stage-5 seizures. Subsequent 60 min stimulation of the kindled amygdala produced several generalized seizures but no status epilepticus. Twenty-four hours later, brief amygdala stimulation produced neither an electrographic nor behavioural response, while stimulation of the olfactory bulb produced only local AD. On the other hand, dorsal hippocampal stimulation showed increased responsiveness, reflected by lowered ADT's, longer AD durations and, in many cases, full generalized seizures. One week later, the amygdala re-acquired its stage-5 response, as did the olfactory bulb, while the hippocampus regressed to its previous short AD durations with no behavioural responses. These data suggest a facilitative relationship between the amygdala and olfactory bulb, possibly related to their shared neurology in the pyriform cortex. Conversely, a more antagonistic relationship between the amygdala and dorsal hippocampus is evident.

310.14

ASTROCYTIC RESPONSE TO IN VIVO KINDLING OF THE HIPPOCAMPUS. N. Hawrylak, F.-L. Chang, W.T. Greenough. Neur. & Beh. Biol. Prog., Depts. Psych. & Cell & Struc. Biol., Univ. IL, Champaign, IL 61820

Kindling of the dorsal hippocampus was employed to assess the possible morphological plasticity of astrocytes in response to epileptiform activity. Five pairs of adult rats with chronically implanted electrodes were stimulated twice a day via the Schaffer collateral pathway and responses monitored in the CA1 region. Kindling was performed with 2 msec pulses @ 60 Hz for 2 sec at a current intensity that initially induced at least 10 sec of afterdischarge (AD). Control animals were stimulated with parameters that did not induce AD (.2 msec pluses @ .2 Hz for 10 min.) at an intensity that elicited 50% of the maximal response. Following the induction of five stage 5 seizures, matched pairs were prepared for electron microscopy.

Astrocytic processes were identified in the middle third of the CA1 s. rad. 400 microns from the recording electrode. Kindled animals had a 45% increase in the volume fraction (v/v) of astrocytic processes compared to controls ($p < .01$). The increased astrocytic v/v may relate to either a general ionic, neurotransmitter or cellular metabolic adjusment or to synaptogenesis or other kindling phenomena. (Supported by Epilepsy Foundation of America and ONR N00014-89-J-1556)

310.15

THE LEVELS OF AMINO ACIDS DURING KINDLING DEVELOPMENT IN EIGHT REGIONS OF THE BRAIN M.Pless*, M.Pintor*, N.S.Nadi. (SPON:M.S.Kafka). Emory Un., Atlanta, Ga.,and and NINDS, Bethesda, Md. 20892

The levels of the putative transmitter amino acids, glutamate (GLU), aspartate (ASP), glycine (GLY), GABA and taurine (tau) were measured in microdissected hippocampus, temporal cortex, frontal cortex, entorhinal cortex, amygdala, thalamus and striatum in rats at stages i,ii,iii,iv and v of seizure development.The levels of the amino acids were compared to those measured in the same brain regions of sham operated but unkindled rats. In rats at stage i the levels of ASP, GLU, GABA and GLY were unchanged in all brain regions studied. In stages ii and iii the levels of GLU and ASP were significantly elevated when compared to controls (1.9 to 2.4X increased) in all regions except the entorhinal cortex. The levels of GABA were elevated in all regions examined 2X at stages ii, iii and iv. At stage v the levels of GLU, ASP, GABA were not significantly different from the controls. The levels of GLY and TAU were not altereed in any of the stages examined.

310.16

THE LEVELS OF SOMATOSTATIN IN EIGHT BRAIN REGIONS OF THE RAT DURING THE DEVELOPMENT OF KINDLING. N.S.Nadi, M.Pless* and M.Pintor*. NINDS, Bethesda, MD, and Emory University Atlanta, GA 30322.

The levels of the neuropeptide somatostatin (ST) were measured in microdissected hippocampus, temporal cortex (TC), frontal cortex (FC), entorhinal cortex (EC), occipital cortex (OC), amygdala, thalamus and striatum in rats at stages I,II,III,IV and V of the kindling development. The levels of ST were compared to those measured in the same brain regions of sham operated but unkindled rats. The levels of ST were decreased in all brain regions from rats in stages II,III (ranging from 25% to 80%) and V. The EC, TC and hippocampus, which are known to be involved in the origin and the spread of the epileptic discharge, showed the major decrease in the above stages. The stage IV where the epileptic and electrographic seizures start to evolve from partial to generalized, showed an increase of ST levels compared to the previous stages, becoming significantly higher than controls. The role of ST in the kindling development and the significance of its changes in the five stages will be discussed.

310.17

SECOND MESSENGER COUPLING CHANGES IN THE KINDLED RAT BRAIN. V.E.Chee* and N.S.Nadi.(SPON: W.W. Alberts) NINDS, NIH, Bethesda Md. 20892.

The exact nature of the chemical changes in the central nervous system leading to seizures is unknown. One possible mechanism could be the alteration in the coupling of receptors to second messenger systems such as cyclic AMP or inositol phosphate. We investigated the effect of norepinephrine (NE), N-methyl D-aspartate(NMDA) alone, in combination and in the presence or absence of glycine (GLY) on cyclic AMP and inositol phosphate formation in hippocampal (HP) and cortical slices (CX) from stage V amygdala kindled rats(K) and sham operated (S) rats. In the presence of NMDA and GLY the formation of inositol phosphate was significantly higher in the H of the K when compared to the S ($p<0.01$). In the presence of NE the levels of cyclic AMP were significantly more elevated in the H of the K when compared to the S ($p<0.001$). Furthermore in the K H NE potentiated the effect of NMDA on the formation of cyclic AMP. This interaction was not detected in the S or the CX of the K. The results suggest that region selective plastic changes do occur in the kindled brain.

310.18

TETRAPHENYLPHOSPHONIUM(TPP) UPTAKE IN RESPONSE TO EXCITATORY AMINO ACIDS IN THE KINDLED AND SHAM OPERATED RAT BRAIN. K. Wayns*, V.E.Chee* and N.S.Nadi.(SPON:W.H.Theodore). NINDS, NIH, Bethesda, Md. 20892.

The nature of the chemical changes leading to kindling is not clearly understood. One possible mechanism could be the altered response of membrane potentials to neurotransmitters and/or modulators. In order to study this question we prepared synaptoneurosomes(SN) from amygdala kindled (K) and control (C) rats. The SN were incubated with TPP in the presence of NMDA, glycine (GLY), norepinephrine (NE) and GABA (all at 1 uM). In the presence of NMDA the SN from the K rats were depolarized significantly more than the C (45±5.6 mV vs 57±3.2 mV). The hyperpolarization in the presence of GABA was not significantly different in the K vs the C rats. When SN were incubated in the presence of NMDA and GLY the depolarization in the K brain was also significantly more than the C (39±4.5 mV vs 55±3.9 mV). The presence of NE in the incubation medium potentiated the effect of NMDA. This potentiation was significantly greater in the K rats when compared to the C. These observations suggest the occurrence of plastic changes in neural networks in the kindled brain.

310.19

ALCOHOL CONSUMPTION IN KINDLED RATS. W.M. Burnham, A. Penushkovich*, G.A. Cottrell and M.A. Linseman. Dept. of Pharmacology, Univ. of Toronto and Addiction Research Foundation, Toronto, Canada M5S 2S1.

Numerous biochemical and electrophysiological changes have been reported in kindled rats, but few long-lasting changes in behavior. The present abstract reports a kindled/control difference in alcohol consumption which is observed as long as one month after the last kindled seizure. RVH rats were implanted with unilateral amygdaloid electrodes, and kindled daily to 5 Stage 5 seizures (1 sec, 60 Hz, 400 uA). Three days after the last seizure, they were introduced to alcohol (increasing concentrations up to 12%) in a "limited access" paradigm (access: 1 hr/day over a period of 6 wks). Initially, kindled and control subjects drank similar (low) levels of alcohol, but, over days, the control subjects drank increasingly more while the kindled subjects drank significantly less. Subsequent experiments showed that the kindled subjects did not differ in their physiological response to alcohol (hypothermia test), and that they actually drank more when a dextrose solution was offered. The data from the limited access experiment are similar to the effects of repeated ECS on alcohol consumption reported by Pinel and Mucha (Physiol. Behav., 15:585, 1975). Thus it appears that repeated seizures modify BOTH brain and behavior. (Supported by MRC MA5611 and the Addiction Research Council Of Canada)

310.20

KINDLING A PURE SLEEP EPILEPSY IN KITTENS. M.N. Shouse* (SPON: M.B. Sterman). VA Med. Ctr., Sepulveda, CA 91343 and Dept. of Anatomy and cell Biology, UCLA Med. Ctr., Los Angeles, CA 90024.

Secondary generalized temporal lobe epilepsy (TLE) is most common pure sleep epilepsy in humans. The mechanism for sleep epilepsy is obscure, in part because convincing animal models are unavailable. We describe the kindling of spontaneous sleep epilepsy in kittens and a possible brain stem mechanism.

Nine kittens, aged 2.5 to 6 months, and 12 adult cats, aged > 12 months, were kindled from amygdala. Kittens had higher initial afterdischarge (AD) thresholds (mean= 6.1 vs. 1.2 mA in adults), but seizure manifestations and kindling rates were identical to adults. Only the five youngest kittens (2.5 to 5 months) developed spontaneous seizures. Partial seizures could occur in any sleep or waking state, but all GTCs occurred in slow-wave-sleep (SWS), usually in the transition from SWS to rapid-eye-movement sleep (REMS). Threshold tests in older kittens and adults revealed maximum susceptibility to elicited GTCs in the transition from SWS to REMS.

The timing of spontaneous and evoked GTCs in kindled kittens and adult cats thus resembles human TLE. Moreover, electrolytic lesions of locus ceruleus complex increased REM sleep transitions and seizure susceptibility, suggesting that reduced norepinephrine could be a factor in pure sleep epilepsy.

NEURAL PLASTICITY IN ADULT ANIMALS: MONOAMINES AND ACH

311.1

PLASTICITY OF 5-HT INNERVATION OF THE DORSAL HORN IN THE RAT SPINAL CORD AFTER CAPSAICIN TREATMENT, AS SEEN WITH LIGHT- AND ELECTRON-MICROSCOPE IMMUNOCYTOCHEMISTRY. L. Marlier*, F. Sandillon*, N. Rajaofetra*, P. Poulat* and A. Privat* (SPON: M.C. Calvet). Lab. Neurobiologie du Développement, CNRS UPR41 INSERM U.249, EPHE, Inst. de Biologie, 34060 MONTPELLIER, FRANCE.

The dorsal horn (DH) of the rat spinal cord contains a high density of 5-HT immunoreactive profiles (5-HT IR). Sprague Dawley rats were treated neonatally with capsaicin, in order to specifically destroy C afferent fibers. They were sacrificed as adult in order to evaluate the influence of the lesion on serotonergic innervation of the dorsal horn. The efficiency of capsaicin treatment was monitored with immunodetection of calcitonin-gene-related-peptide (CGRP) and substance P on vibratome sections. In control animals, 5-HT IR is present in layer I, outer part of layer II (IIo) where most of the profiles are non-synaptic, layers III and IV, leaving a "clear band" corresponding to the inner part of layer II (IIi). This pattern is disrupted in capsaicin treated animals, with the invasion of layer IIi by 5-HT IR profiles which are found to establish axo-spinous synapses. In conclusion, the topography of 5-HT innervation in the rat DH is modified after neonatal capsaicin treatment. Moreover, the nature of the interactions is shifted from a non-synaptic to a synaptic one, indicating that the target exerts a determinant influence on the shaping of 5-HT afferents. (Supported by INSERM, IRME and DRET).

311.2

TRANSNEURONALLY-MEDIATED DOWN REGULATION OF OLFACTORY BULB DOPAMINE PHENOTYPE INVOLVES A LOSS OF TYROSINE HYDROXYLASE MESSENGER RNA. D.M. Stone, T. Wessel, H. Paivarinta, T.H. Joh and H. Baker. Lab of Mol. Neurobiol., Cornell Univ. Med. Coll., White Plains, N.Y. 10605.

Previous studies in this and other laboratories have demonstrated that the maintenance of tyrosine hydroxylase (TH) expression in dopaminergic neurons of the main olfactory bulb (OB) is dependent upon intact afferent input from the peripheral olfactory receptor epithelium. Thus, chemical or surgical deafferentation of the rat OB, or interruption of afferent activity via neonatal unilateral closure of the nares, results in a gradual loss of dopamine phenotype in a population of OB juxtaglomerular cells ipsilateral to the lesion. This loss of neurotransmitter phenotype is evidenced by a decrease in OB dopamine content, and a reduction in the activity of, and immunocytochemical staining for, TH, the rate-limiting dopamine biosynthetic enzyme. To further characterize this transneuronal effect, TH messenger RNA (mRNA) content was measured in OB from rats sacrificed 1 month after neonatal unilateral cauterization of the nares. In situ hybridization of an ^{35}S-labelled 33-base oligonucleotide complementary to rat TH mRNA revealed a > 50% reduction in signal intensity in the ipsilateral (odor-deprived) vs. contralateral (control) OB glomeruli. Similarly, northern blot analysis of OB poly (A)$^+$ RNA revealed a reduction in TH message level in the odor-deprived side when compared to the contralateral side. These results suggest that the loss of juxtaglomerular cell TH expression following interruption of afferent activity can be attributed to a decrease in the enzyme message level, possibly reflecting a transneuronally-mediated reduction in TH gene transcription. Supported by Grants # NS 23103 and MH 44043.

311.3

RECOVERY TO NORMAL OF PINEAL MELATONIN CONTENT AND URINARY MELATONIN METABOLITES FOLLOWING UNILATERAL DENERVATION BUT NOT UNILATERAL DECENTRALIZATION OF THE PINEAL GLAND R.E.Zigmond[1], R.L.Sherman*[3] and G.A.Kuchel*[1,2] Dept. Biol. Chem. & Mol. Pharm.[1], Division on Aging[2], Harvard Medical School, Boston, MA 02115; Section Analytical Biochem., NIMH[3]

The rat pineal gland receives bilateral innervation from the two superior cervical ganglia via the internal carotid nerves (ICN). The ganglia are in turn innervated by the cervical sympathetic trunks (CST). Sympathetic stimulation at night results in increased serotonin N-acetyltransferase (NAT) activity and consequently increased melatonin synthesis. We have previously found that after unilateral denervation (UnilCNX), but not after unilateral decentralization (UniCSTX), nighttime NAT activity returns to normal within 1.5 days.

Recovery of function was studied further by measuring pineal melatonin content and melatonin metabolites in the urine after such lesions. Following UnilCNX, nighttime melatonin content measured by HPLC with electrochemical detection was similar to controls (43.7 ± 4.5 pmole/mg) at 1.5, 14 and 21 days. However, following UniCSTX, pineal melatonin content was decreased at all 3 time points (37%, 61% and 47% of control respectively). 6-OH melatonin, the main metabolite of melatonin, was measured in the urine using gas chromatography-mass spectroscopy. Measurment of this metabolite in an animal before and after the lesions also indicated a rapid recovery of pineal function by the second night after UnilCNX. However, function was impaired for at least 12 days after a UniCSTX lesion. Thus functional recovery after sympathetic nerve lesions depends on the specific site of the lesion. Together with previous data, our results suggest that decentralized nerves can adversely impact on the efficacy of nearby intact nerves. (NS17512, MH00162 and a fellowship from the Brookdale Foundation).

311.4

REMODELING OF NORADRENERGIC INNERVATION IN RAT CEREBRAL CORTEX INDUCED BY REPEATED STRESS.
T. Sakaguchi* and S. Nakamura. Dept. of Physiol., Fac. of Med., Kanazawa Univ., Kanazawa 920, Japan.

We have presented electrophysiological evidence for terminal sprouting of locus coeruleus (LC) noradrenergic (NA) neurons in rat cerebral cortex following repeated mild stress, recently. We now report that the stress-induced change of cortical NA terminals may depend on the intensity of stress. Adult male Sprague-Dawley rats were used. The stress groups, restrained in a small cage for 1 or 6 hours daily, received the treatment for 1 or 2 weeks. The percentage of LC neurons activated antidromically by stimulation to a given brain site (projection index, P-index) was examined to assess the amount of LC projection to the brain site. The P-indices for 7 points covering nearly the entire cortex were determined under urethane anesthesia. The P-index for each cortical point did not differ between the control and 1-week stress groups. In the animals stressed for 1 hour daily for 2 weeks, the P-index for a point in the medial frontal cortex was significantly higher than that in the control. In contrast, in the 6-hour stressed animals for 2 weeks, the P-index for each cortical point appeared to decrease rather than increase. The stress treatment did not change the excitability of cortical LC terminal axons. These results suggest that the occurence of the morphological changs of LC neurons (i.e. either an increase or decrease in the density of cortical NA terminals) following repeated stress depends on the intensity of stress.

311.5

BEHAVIORAL RECOVERY AFTER UNILATERAL 6-OHDA LESION IN THE SUBSTANTIA NIGRA RELATED TO NIGRO-STRIATAL NEUROPLASTICITY.
S. Morgan*, G. Nomikos* and J.P. Huston (SPON: R. Schwarting). Inst. of Physiol. Psychology I, Univ. of Düsseldorf, Universitätsstr. 1, D-4000 Düsseldorf, FRG.

We looked at the relationship between behavioral recovery after unilateral 6-OHDA lesion of the substantia nigra (SN) and the number of cells in the SN labeled by HRP deposited in the caudateputamen (CPU). After unilateral injection of 6-OHDA into the SN rats were tested for asymmetrical behavior either only on the day after the lesion (group L1) or on the day after the lesion plus every alternate day for the following 15 days (group L15). HRP was deposited in the CPU ipsilateral to the lesion and the SN ipsilateral and contralateral to the lesion were examined for labeled cells. L15 animals that showed recovery from asymmetrical behavior had more cells than those which did not in contralateral and ipsilateral SN. Recovered rats in group L15 had more cells in the ipsilateral SN than group L1, while non-recovered animals did not differ from group L1. Thus, one correlate of recovery after a neurotoxic lesion of the SN is an increase in HRP uptake by cells which give rise to the nigrostriatal system.

Supported by DFG-grant Hu 306/6-1.

311.6

PLASTICITY IN TUBEROMAMMILLARY-STRIATAL AND NIGRO-STRIATAL PROJECTIONS RELATED TO RECOVERY FROM BEHAVIORAL ASYMMETRIES INDUCED BY HEMIVIBRISSOTOMY.
J.P. Huston, H.-T. Weiler* and H. Steiner*. Inst. of Physiol. Psychology I, Univ. of Düsseldorf, Universitätsstr. 1, D-4000 Düsseldorf, FRG.

Unilateral clipping of vibrissae (UCV) in rats induces an asymmetry in facial scanning which subsides after 3 days. We determined the time-course of neuronal plasticity in tuberomammillary-striatal (TMS) and nigro-striatal (NS) projections after UCV and compared it to behavioral recovery. After UCV for 1-3, or 4-20 days rats were injected with HRP into the striatum ipsilateral or contralateral to UCV. Labeled cells were counted in the caudal magnocellular and postmammillary caudal magnocellular subnuclei of the tuberomammillary nucleus and in the substantia nigra. In UCV4-20 rats an asymmetry in TMS projections from both subnuclei was found, with more HRP-labeling in the crossed and uncrossed projections to the CPU contralateral than ipsilateral to UCV. These asymmetries were in the same direction as those found in NS projections in UCV4-20 rats. UCV1-3 rats had no asymmetries in TMS projections, but in the NS system they exhibited an asymmetry opposite to that in the UCV4-20 rats. The changes in TMS+NS projections appeared when rats recovered from behavioral asymmetry, suggesting a functional role of these projections in behavioral recovery.

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311.7

DISTRIBUTION OF GAP-43 mRNA IN THE BRAINSTEM OF ADULT RATS, AS EVIDENCED BY IN SITU HYBRIDIZATION: LOCALIZATION WITHIN MONOAMINERGIC NEURONS
C.Bendotti and R.Samanin Dept. Neuropharmacology" Mario Negri" Institute, 20157Milano, Italy

GAP-43 is a nervous tissue specific phosphoprotein associated with development, regeneration, and functional modulation of synapses. Using the in situ hybridization of GAP-43 mRNA we examined the distribution of the sites of synthesis of this presynaptic phosphoprotein in the brainstem of adult rats. GAP-43 was selectively expressed in certain cell populations, with the highest hybridization signal detected at the level of the n. raphe dorsalis and substantia nigra compacta. An intense grain density was also observed within the neurons of the locus coeruleus , n. raphe medialis, ventral tegmental area, n.raphe pontis and, at a lower degree in the interpeduncular nucleus. No significant signal was detected at the level of the substantia nigra reticulata, red nucleus and nucleus peduncular pontine. Selective lesions of serotonergic neurons by I.C.V.injection of the neurotoxin 5,7-DHT significantly reduced the hybridization signal at the level of n. raphe dorsalis and medialis, demonstrating that GAP-43 transcript is expressed in serotonergic cells. A complete overlapping of the hybridization signal of tyrosine hydroxylase mRNA and GAP-43 mRNA was observed at the level of the substantia nigra of adjacent sections indicating the presence of this phosphoprotein in dopaminergic neurons.
These results suggest that this protein is synthesized within the serotonergic and catecholaminergic neurons of adult rat brain. It remains to be elucidated whether this phosphoprotein plays a role in the functional plasticity and/or signal trasduction of these neurotrasmitters.

311.8

METHYLAZOXY-METHANOL INDUCED MICROENCEPHALY: EVALUATION OF PROTEIN KINASE C MEDIATED PHOSPHORYLATION PROCESSES.
M. Di Luca*, M.P. Abbracchio*, M. Cimino* and F. Cattabeni Inst. Pharmacol. Sci., Sch. of Pharm., Univ. of Milano, 20133-Italy.

The administration of the antimitotic agent methylazoxy-methanol (MAM) to rats at day 15 of gestation results in a microencephaly in the offspring characterized by a consistent loss of interneurons in cortex, hippocampus and striatum. Previous studies demonstrated that the altered neuronal circuitry in this animal model results in active processes of neuronal reorganization and plasticity.

Since a family of phosphoproteins (GAPs, B50/F1), which show an apparent MW between 43 and 47 Kd and are selectively phosphorylated by PKC, has been implicated in neuronal adaptive changes, we have evaluated the phosphorylation state of a 47 Kd MW protein band in synaptic membrane extracts obtained from cortex and hippocampus of control and MAM-rats. This protein band showed a marked reduction of ^{32}P-phosphate incorporation in MAM-rats. This reduced *in vitro* incorporation might be indicative of an increased *in vivo* phosphorylation mediated by PKC: indeed PKC activity resulted markedly enhanced in MAM-rats.

These results suggest that MAM induced microencephaly might represent an useful experimental model to study the role of these phosphoproteins in neuronal adaptive events such as synaptic remodeling following toxic insults.

311.9

L-DOPA AND D1-SELECTIVE AGONISTS ACTIVATE C-FOS EXPRESSION IN THE STRIATUM OF 6-HYDROXYDOPAMINE-LESIONED RATS.
Harold A. Robertson and George S. Robertson, Department of Pharmacology, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4H7.

Following unilateral destruction of neurons in the substantia nigra with the neurotoxin 6-hydroxydopamine, rats given dopamine agonists turn towards the unlesioned side (contralaterally). Recently, we reported that stimulation of brain can lead to activation of the nuclear proto-oncogene c-fos (Dragunow, M. & Robertson, H.A., Nature, 329: 441, 1987). Fos activation may also be utilized as a high resolution metabolic marker for polysynaptic pathways (Hunt, S. P., Pini, A. & Evan, G., Nature, 328: 632, 1987). Accordingly, we have studied the effects of L-Dopa-induced rotational behaviour on the expression of c-fos-like immunoreactivity (IR) in rat brain. Here we report that L-Dopa-induced rotation is accompanied by the expression of c-fos. In lesioned animals receiving L-Dopa or a D1 selective agonist, there was strong contralateral rotation accompanied by activation of c-fos in the striatum. D-amphetamine produced ipsilateral rotation and a strong activation of c-fos in the unlesioned striatum. In rats given pentobarbital to prevent rotation, SKF 38393 still activates striatal c-fos. Moreover, the D2 selective agonist LY 171555 produced rotation but no activation of c-fos. Thus turning itself does not result in activation of c-fos. It is possible that fos activation may play a role in long-term changes such as "priming" and tardive dyskinesia.
(supported by the Parkinson Foundation of Canada).

311.10

THE NMDA ANTAGONIST MK-801 REVERSES D-AMPHETAMINE-INDUCED ACTIVATION OF THE PROTO-ONCOGENE C-FOS IN RAT STRIATUM.
Krista Johnson* and Harold A. Robertson. (SPON. J. Fisk). Dept. Pharmacol., Dalhousie Univ., Halifax, N.S., Canada B3H 4H7.

Excess excitatory amino acid activity is frequently associated with pathophysiological changes in brain. Methamphetamine produces degenerative changes in the striatal terminals of dopamine neurons and this damage is prevented by NMDA antagonists (Sonsalla, P. et al., Science 243:398, 1989). Recently, we found that L-Dopa induced c-fos in 6-OHDA lesioned rats (Robertson, G.S. et al, Eur. J. Pharmacol. 159: 99, 1989). We have also found that brain damage can induce the proto-oncogene c-fos (Dragunow, M., Robertson, H.A., Brain Res., 455:295, 1988) and this induction of c-fos can be reversed by NMDA antagonists. It seemed possible therefore that c-fos could play a role in NMDA-dependent methamphetamine-toxicity in striatum. D-amphetamine (5 mg/kg, i.p.) produced a "patchy" increase in c-fos protein throughout the striatum which, in medial regions, was reversed by pre-treatment with the NMDA antagonist MK-801 (3 mg/kg, i.p.). However, while reversing the effects in medial regions, rats which received both MK-801 and D-amphetamine exhibited a dramatic increase in c-fos protein immunoreactivity in the dorso-lateral striatum.
(supported by the MRC of Canada).

311.11

LESIONS OF THE PEDUNCULOPONTINE TEGMENTAL NUCLEUS PRODUCE LOSS OF BASAL FOREBRAIN CHOLINE ACETYLTRANSFERASE: EVIDENCE FOR CHOLINERGIC-CHOLINERGIC INTERACTIONS
Justin D. Oh*, Nancy J. Woolf, and Larry L. Butcher, Laboratory of Chemical Neuroanatomy, Dept. of Psychology, University of California, Los Angeles, CA 90024-1563, U.S.A.

The choline acetyltransferase (ChAT)-immunopositive neurons in the pedunculopontine tegmental nucleus were unilaterally lesioned in rats and the animals were sacrificed 3 days, 8 days, or 1 month later. Brain sections were processed immunohistochemically for ChAT and nerve growth factor receptor (NGFR). Although little or no immunocytochemical changes were seen 3 days following the ablation, a marked depletion of ChAT positive cells in various parts of the basal forebrain were observed ipsilateral to the lesion side at 8 days. The decreases were most apparent in the magnocellular preoptic area and rostral nucleus basalis and exhibited some topographic relationship to lesion placement. Staining for NGFR was still present in the ipsilateral cells, which in adjacent sections showed a loss of ChAT staining, suggesting that the decrease in ChAT-like immunoreactivity of basal forebrain cells in response to pedunculopontine lesions did not reflect neuronal degeneration. [Support: USPHS grant NS 10928 to L.L.B.]

311.12

THYROID HORMONE ALTERS CHOLINERGIC EXPRESSION IN ADULT BASAL FOREBRAIN.
Christopher W. Mathes*, Nancy J. Woolf, Charles Lee and Larry L. Butcher. (SPON: J. T. Beatty) Laboratory of Chemical Neuroanatomy, Dept. of Psychology, University of California, Los Angeles, CA 90024.

Chronic thyroid hormone treatment alters the morphology and histochemistry of choline acetyltransferase (ChAT) immunoreactive neurons in the basal forebrain during postnatal development [Gould et al., Dev. Brain Res. 46:297, 1989], but it has not been established if similar effects occur in the mature animal. In the present study, young adult rats (52 days) were made hyperthyroid by daily i.p. injection of L-thyroxine (2.5 µg/g) or hypothyroid by the addition of 0.4% propylthiouracil in the feed. One group of euthyroid controls received i.p. injections of saline and another no injections. All treatments lasted seven weeks. Tissue sections were immunohistochemically processed for ChAT, and morphologic analyses and cell counts were performed. Significantly more cells at selected rostral and caudal basal forebrain levels expressed ChAT in hyperthyroid rats ($\bar{X}$=509) than in hypothyroid ($\bar{X}$=356), vehicle injected euthyroid ($\bar{X}$=250), and non-treated euthyroid ($\bar{X}$=318) rats [$F(3,12)=6,33$, $p<0.01$]. Compared to neurons from euthyroid rats, ChAT positive cells in the basal forebrains of hyperthyroid and hypothyroid animals demonstrated aberrant morphologic profiles including hypertrophied or shrunken somata and alterations in cell shapes and dendritic patterns [Support: USPHS grant NS 10928 to L.L.B.]

311.13

CORTICAL DEVASCULARIZATION AND NGF TREATMENT AFFECT THE NUMBER OF AXO-SOMATIC SYNAPSES ON CHOLINERGIC NEURONS OF THE ADULT RAT NUCLEUS BASALIS MAGNOCELLULARIS.
M. Piotte, L. Garofalo* and A.C. Cuello. Dept. Pharmacology & Therapeutics, McGill Univ., Montreal (Quebec), Canada H3G 1Y6.

Cortical devascularization causes biochemical and morphological retrograde degenerative changes in cholinergic neurons of the rat nucleus basalis magnocellularis (NBM). In order to determine how this cortical lesion affects the synaptic input of these cells, choline acetyltransferase immunoreactive NBM neurons were examined at the ultrastructural level in intact and cortically devascularized adult rats. In animals examined 15 days after the lesion, the number of axo-somatic synapses was markedly higher than in controls. At 30 days post-lesion this number was still higher than in controls but lower than at 15 days post-lesion. When the neurotrophic factor NGF was administered intracerebroventricularly via an osmotic minipump (12 µg/day ; immediately after the lesion for a maximum of 7 days) the increase in the number of axo-somatic synapses seen at 15 days post-lesion was less pronounced than in lesioned untreated rats. The increase in axo-somatic synapses could be explained in part by the decrease in perikaryal volume resulting from the lesion. The contribution of cell shrinkage to the observed effect is now under investigation.

Supported by the MRC of Canada and the FRSQ

311.14

EFFECTS OF CARBAMAZEPINE ON RECOVERY AFTER CORTICAL DAMAGE.
R.L.Fulton* and T. Schallert (SPON: N. Lobaugh); Psychology Dept. & Institute for Neuroscience, U. of Texas, Austin, 78712.

Manipulation of gamma-aminobutyric acid (GABA) activity has been found to alter behavioral recovery after experimental cortical damage. We have found that diazepam, at anti-convulsant doses, can disrupt recovery following lesions to the anteromedial cortex (AMC). The purpose of this study was to assess whether carbamazepine, a commonly prescribed anti-convulsant, also disrupts recovery from cortical damage. Although the mechanisms of action of carbamazepine are not fully understood, it is not thought to work directly at GABA-ergic synapses. Male rats sustained unilateral electrolytic lesions to the AMC. Rats were injected for a 14 day period with carbamazepine (15mg/kg; anticonvulsant dose,ED-100) 3 times a day for 3 days post-operative and twice daily thereafter. Behavioral tests measuring sensorimotor asymmetries (bilateral tactile stimulation tests) were administered pre-operatively and for 28 days post-operatively. Both carbamazepine-treated and control rats exhibited a unilateral bias ipsilateral to the lesion immediately after surgery, and both groups showed near total recovery by day 10 to 14. Tentative conclusions are that carbamazepine does not disrupt recovery of function after AMC lesions.The data are consistent with the view that diazepam interferes with recovery via GABA-related mechanisms. (Supported by NIH grant NS 23964 awarded to T. Schallert.)

311.15

EFFECT OF DIAZEPAM ON CALCIUM UPTAKE IN STRIATAL SYNAPTOSOMES FOLLOWING UNILATERAL NEOCORTICAL DAMAGE. J.S. Sims, T. Schallert, R.D. Trent *, L.E. Shapiro*, and S.W. Leslie. Inst. for Neuroscience and Dept. of Psychology, Univ. of Texas at Austin, Austin, TX 78713.

Unilateral damage to the anteromedial cortex (AMC) produces a transient sensorimotor asymmetry quantified using a bilateral tactile stimulation test. Diazepam is capable of preventing this recovery. Evidence from behavioral, pharmacological, and metabolic studies suggests that changes occur in striatal function following cortical damage. Male Long-Evans rats sustained unilateral AMC lesions or sham surgery and were given daily doses of diazepam (5 mg/kg) or vehicle. Animals were sacrificed at 3, 7 or 14 days following surgery. Striatal synaptosomes were stimulated with potassium (30 mM; for 3 sec) and the entry of $^{45}Ca^{++}$ was measured. In addition, potassium-stimulated dopamine release and dopamine content were measured. When comparing the two striata of each animal, animals injected with diazepam had greater asymmetries in calcium influx relative to vehicle-injected rats. It is possible that the dysfunction in calcium entry was related to a change in the release of dopamine or other neurotransmitters in the striatum. Asymmetries in dopamine release will be discussed as related to the disruption of calcium uptake. Supported by NIH Grant NS-23964 to T. S. and NIAAA grants AA05809 and RSDA AA00044 to S.W.L. J.S. was supported by NIAAA training grant AA07471.

311.16

ALPHA-2-ADRENORECEPTOR-MEDIATED DECREASE IN GABA OUTFLOW IN BRAIN SLICES DURING MORPHINE TOLERANCE. L.Beani, C.Bianchi, L.Ferraro, M.Morari, M.Simonato, G.P.Spalluto and S.Tanganelli. Dept. of Pharmacology, University of Ferrara, Italy.

The cholinergic neurones projecting to the cerebral cortex are inhibited by norepinephrine (NE) through alpha-2-receptors (a-2-rec), whereas the intracortical gabaergic neurones are stimulated through alpha-1-receptors (a-1-rec). Deep plastic changes are produced by chronic morphine treatment. In fact, in morphine tolerant guinea-pigs NE stimulates the cholinergic neurones through a-1-rec and inhibits the gabaergic neurones through a-2-rec (JEPT,247: 294,1988). The inverted response of the cholinergic structures to NE and to alpha-agonists has been confirmed in cortical slices (J. Neurochem., in press).

This report shows that the intracortical gabaergic neurons, too, undergo inverted response to NE during morphine tolerance. Under these conditions, the spontaneous efflux of endogenous GABA from cortical slices is reduced by NE 30 uM and by clonidine 1 uM (ineffective in naive animals). This inhibition is prevented by idazoxan 0.1 uM. Conversely, phenylephrine (which normally increases GABA outflow) is ineffective.

In conclusion, the appearance of this novel, non physiological a-2-rec mediated inhibition versus GABA replaces the normal a-1-rec-mediated stimulation. Further esperiments have been undertaken in synaptosomes in order to identify the site (pre- or postsynaptic) of these effects.

Supported by C.N.R. and M.P.I. Grants.

OCULOMOTOR SYSTEM II

312.1

TRANSLATIONAL VESTIBULO-OCULAR REFLEX (TVOR) RESPONSES OF MONKEY ARE A LINEAR FUNCTION OF THE INVERSE OF THE VIEWING DISTANCE. U. Schwarz and F. A. Miles. Lab. Sensorimotor Research, National Eye Institute, Bethesda, MD 20892.

The TVOR was investigated in 4 rhesus monkeys using a sled that moved on a linear track and accelerated the animals along the inter-aural axis. Movements of both eyes were recorded with the electromagnetic search coil technique and accommodation was recorded with an infra-red optometer. Transient sinusoidal jerks (period, 200ms; amplitude, 630cm/s^2/s) were applied after the animal had satisfactorily fixated an LED (one of 5 targets at distances of 16-150cm). The LED was extinguished immediately prior to the onset of acceleration, leaving the room dark throughout the period of movement. (To preclude "learning", animals never experienced sled motion in the light and were never reinforced for their responses to the sled.) Dependence on proximity would be appropriate for the TVOR, and peak compensatory eye speed achieved within 250ms of the onset of sled motion was a linear function (coef. determination >0.9) of the inverse of viewing distance (diopters, D), with a mean slope of 1.4°/s/D (range, 1.0-2.0°/s/D) and a mean intercept of 1.2°/s (range, 0.5-2.2°/s). Full compensation would require a slope of 2.3°/s/D with an intercept of zero. From this it can be shown that the gain *(measured response/required response)* exceeded unity with the most distant LED (mean, 1.4) and fell short of unity with the nearest (mean, 0.7). Data from 2 monkeys indicate that responses could be increased by selectively increasing *either* vergence (using base-out prisms with the most distant LED) *or* accommodation (using base-in prisms with the nearest LED) and, for both animals, these increases in response were similar in the 2 cases. We conclude that TVOR responses are not solely a function of the vergence state, and that accommodation and/or other cues for absolute distance must also affect the dependence on proximity.

312.2

ARE THE TWO COMPONENTS OF THE PRIMATE OPTOKINETIC RESPONSE CONCERNED WITH TRANSLATIONAL AND ROTATIONAL DISTURBANCES OF GAZE? F. A. Miles, U. Schwarz and C. Busettini*. (SPON: D.L. Robinson). Lab. Sensorimotor Research., NEI, Bethesda, MD 20892.

Canal-ocular reflexes and otolith-ocular reflexes compensate for the rotational and translational components, respectively, of head movements. We suggest that any residual disturbances of gaze are dealt with by 2 independent visual tracking systems that correspond to the *direct* and *indirect* components of primate OKN: the *direct* dealing with the translational component of optic flow and the *indirect* with the rotational component. The *indirect* component is assumed to be phylogenetically older and mostly subcortical, while the *direct* component is assumed to be a cortical addition that deals with the translational problems of frontal eyed animals. A summary of the major differences:

ROTATIONAL MECHANISM	TRANSLATIONAL MECHANISM
1. *Indirect* component of OKN:	*Direct* component of OKN:
- Long time-constant.	- Short time-constant.
- Strong after-nystagmus.	- Weak after-nystagmus.
- Responds to low slip-speeds.	- Responds to high slip speeds.
- Strong naso-temporal asymmetry?	- Weak naso-temporal asymmetry?
2. Helps to stabilize gaze against rotational disturbances.	Helps to stabilize gaze against translational disturbances.
3. Back-up to canal-ocular reflex.	Back-up to otolith-ocular reflex.
4. Organized in canal planes.	Organized in otolith planes?
5. Cannot utilize motion parallax?	Can utilize motion parallax.
6. Insensitive to proximity & disparity?	Sensitive to proximity & disparity.
7. In all animals with mobile eyes?	In some frontal-eyed animals only?
8. Mainly a subcortical system?	A cortical system?

312.3

INITIAL OCULAR FOLLOWING RESPONSES OF MONKEY ARE A LINEAR FUNCTION OF THE INVERSE OF THE VIEWING DISTANCE. C. Busettini* and F. A. Miles. Lab. Sensorimotor Research, National Eye Institute, Bethesda, MD 20892.

The dependence of ocular following on viewing distance was investigated in 2 rhesus monkeys. Positions of both eyes were recorded with the electromagnetic search coil. Visual stimuli were random dot patterns subtending 40° back-projected onto a screen facing the animal and moved at constant speed for 100ms starting 30ms after a 10° rightward saccade into the center. Animals were neither trained to track these stimuli nor reinforced for doing so: our interest was solely in defining the basic linkage between the visual and oculomotor systems that we assume helps to stabilize gaze with respect to the surroundings. Six viewing distances were used (range, 20-150cm), the visual stimulus being adjusted for each so as to have constant size and speed for the animal. Response latencies were about 50ms and insensitive to viewing distance. Response amplitudes, as indicated by the maximum eye speed achieved within 100ms of the onset of the stimulus, were a linear function of the inverse of the viewing distance (coefficient of determination >0.95). When each animal's data were normalized with respect to that same animal's highest response and plotted against the viewing distance in diopters (D), the mean slope was 14.4%/D (range, 12.8-16.2%/D) and the mean intercept, 30% (range, 21-40%). This dependence on proximity is similar to that reported for the translational vestibulo-ocular reflex (TVOR) in the previous Abstract. We suggest that the ocular following system and TVOR are synergistic and share a pathway whose efficacy is modulated by absolute distance cues. We further suggest that the initial ocular following response corresponds to the *direct* component of OKN and provides visual back-up for the TVOR (otolith-ocular reflex), in contrast with the *indirect* component of OKN, which we suggest provides visual back-up for the rotational VOR (canal-ocular reflex).

312.4

Smooth Pursuit and Vestibulo-ocular Reflex Cancellation during Unpredictable Visual and Vestibular Stimulation K. E. Cullen, R. A. McCrea and T. Belton Comm. on Neurobiology, Univ. of Chicago

The ability of squirrel monkeys to fixate a target during passive, unpredictable vestibular stimulation by cancelling their vestibulo-ocular reflex (VORc) was compared to their ability to follow an unpredictable moving target by generating smooth pursuit eye movements. Two types of unpredictable stimuli were used: 1) a sudden, jerk-like acceleration of head or target or 2) pseudorandom movement of the target or the vestibular turntable.

The ability of the monkeys to fixate a head stationary target when the turntable was rapidly accelerated was considerably better than their ability to pursue a rapidly accelerating target when the turntable was stationary. When the target was unexpectedly accelerated, a smooth pursuit eye movement was evoked at a latency of 110 - 150 msec. but when the turntable was unexpectedly accelerated during VORc, the VOR evoked by the turntable 'jerk' was suppressed 20 - 40 msec after the turntable jerk. The amplitude of the VOR generated by the jerk varied as a function of the head acceleration prior to the jerk.

The ability of the monkeys to fixate a target during pseudorandom turntable movement was also considerably better than their ability to pursue a target that was moving pseudorandomly. Gaze velocity during VORc of pseudorandom vestibular stimuli matched head velocity closely in phase and amplitude when the highest frequency components of the stimulus were less than 2 Hz. When higher frequency components were included in the vestibular stimulus, the gaze velocity gain re head velocity decreased as a function of frequency, although the reduction in gain was much less than the gain reduction observed either during smooth pursuit of pseudorandom target movement or during VOR cancellation of predictable (sinusoidal) turntable movement.

These results suggest that an alternate mechanism exists for suppressing the VOR that does not depend on visual feedback of target motion. Since the VOR evoked by unexpected head movements varied as a function of head acceleration, the non-visual mechanism for VORc may utilize vestibular afferent information.

312.5

EFFECT OF VIEWING AN EARTH-STATIONARY SURROUND ON TORSIONAL POST-ROTATIONAL NYSTAGMUS IN HUMANS. S.H. Seidman*, W.P. Huebner, and R.J. Leigh, Ocular Motility Lab, Cleveland VA Medical Center, University Hospitals and Dept. Biomedical Engineering, Case Western Reserve University, Cleveland, OH 44106.

We measured the gain and time constant (T_{vor}) of the torsional vestibulo-ocular reflex (VOR) in three subjects (ages 22-41) who were rotated about an earth-vertical axis with their necks extended and faces supine. After a 60-second period of rotation in darkness at 100 deg/s, followed by a rapid brake to 0 deg/s, the post-rotational responses (PRRs) in darkness had a mean gain of 0.47 and a mean T_{vor} of 4.0 s. In further trials, a one-second room illumination, to reveal a stationary visual surround, was presented 2 s after the onset of the PRR and caused slow-phase eye velocity to drop rapidly to 0 deg/s. Within 0.5-1.5 s following the return to darkness, torsional eye velocity rose to a value similar to that occurring in PRRs for which there had been no visual exposure. These results support the hypotheses that for this head orientation (1) velocity storage for torsional vestibular nystagmus is weak or absent, and (2) there is a suppression/cancellation mechanism present in the torsional system which acts independently of velocity storage. (Supported by NIH grant EY06717 and Veterans Administration).

312.6

COMPARISON OF OCULAR TORSION IN PRIMATES DURING HORIZONTAL PURSUIT VERSUS VOR MEASURED BY SCANNING LASER OPHTHALMOSCOPY. D. Ott and R. Eckmiller. Dept. Biophysics, Div. Biocybernetics, University of Düsseldorf, F.R.G.

Torsional eye movements (about the line of sight) were studied in humans by analyzing the rotation of the ocular fundus pattern monitored by Scanning Laser Ophthalmoscopy (SLO) during horizontal pursuit and VOR: (1) Ocular torsion during sinusoidal horizontal pursuit (±5 deg, 0.1-1.0 Hz) was sinusoidally modulated (up to 8 deg peak-to-peak) with varying phase relationship re. horizontal eye position (varying from 30 deg lead to 15 deg lag), showing intorsion (extorsion) during temporal (nasal) eye movements. (2) Static fixation at various horizontal positions revealed no significant eye position dependant ocular torsion but occasional torsional saccades of varying amplitudes and durations. (3) During VOR-light (subject fixates a stationary visual target while being sinusoidally rotated about the vertical body axis, ±5 deg, 0.1-0.8 Hz) ocular torsion was also clearly modulated. (4) During VOR-suppression (subject and visual target rotate both in the same direction, ±5 deg, 0.1-0.8 Hz) the sinusoidal modulation of ocular torsion was very small if at all measurable. These findings suggest that ocular torsion serves the maintenance of vision not only during target movements (pursuit) but also during head movements (VOR) and is actively controlled by both visual and vestibular signals. (Supported by the DFG, SFB 200-B10)

312.7

ALTERATION OF LOAD CONDITIONS ON THE EYE DURING FIXATION AND TRAINED SACCADIC EYE MOVEMENTS J.G. McElligott, L.M.Eisenman, and C. M. Philips* (SPON: S. McElligott) Temple University School of Medicine, Dept. of Pharmacology and Physiology, Phila., PA 19140, and Thomas Jefferson School of Medicine, Dept. of Anatomy, Phila., PA. 19107

Various schemes have been developed to alter normal motor function as well as sensori-motor interaction during oculomotor behavior in order to investigate adaptive changes mediated by the central nervous system. These include tenectomy as well as alteration of vision during a variety of eye movements. However, these paradigms suffer from a number of deficiencies. They produce permanent changes that cannot be undone, or they alter only one aspect of the sensory feedback (e.g., only visual and not motor proprioceptive feedback) The procedure reported overcomes these difficulties by allowing a change in the loading conditions or a perturbation to the eye during normal eye movements without interfering with vision. The head of an animal is placed in field of a permanent magnet. A wire is threaded under the 4 ocular rectus muscles of a cat in the form of a coil. When a D.C. current is applied to the coil, a magnetic dipole is created that produces a force that tends to rotate and align the eye with the field of the permanent magnet. The direction and the magnitude of the force is related respectively to the direction and the magnitude of the current in the eye coil. With this schema, we have produced forces of several grams that can change the magnitude of a saccade during trained eye movements as well as displace the eye with respect to its conjugate partner. All these alterations were produced in a monocularly viewing animal. Eye movements in both eyes were measured by the standard electromagnetic ocular search coil technique. (Supported by a grant from NIH S07-RR05417)

312.8

CHANGES IN OCULAR ALIGNMENT AFTER SUSTAINED PASSIVE DISPLACEMENT OF ONE EYE. D.S. Zee, D. Nommay* and G.M. Gauthier*, Universite de Provence, Marseille, France

We measured eye alignment in two normal subjects before and after a sustained passive rotation of one eye. To quantify alignment we used the Lancaster test which gives relative eye position without disparity cues. Preadaptation measures were made after 10 min of monocular viewing. Subject 1 had a 1-2 deg exodeviation and subject 2 a 1-2.5 deg esodeviation.

Using a suction lens one eye was covered and pulled laterally 30 deg. After 10 min the lens was removed and alignment measured. For subject 1 alignment shifted in a divergent (exo) direction by 2-3 deg and then returned to baseline in 7-10 min. For subject 2 alignment also shifted by 2-3 deg in an exodirection but within 1-3 min changed direction to reach 4-5 deg of esodeviation. After 10 min alignment returned to normal.

These results indicate that after a passive rotation of one eye there is a change in ocular alignment that lasts for minutes. We suggest the sequence of alignment changes can not be accounted for by mechanical changes alone but that central mechanisms, based upon nonvisual orbital afference, are also important.

312.9

FLIGHT SIMULATOR SICKNESS: ADAPTATION EFFECTS. R.S. Kennedy, J.E. Fowlkes*, K.S.Berbaum*, G.O. Allgood*, and D.W. Gower*. Essex Corporation, Orlando, FL 32803.

Simulator sickness, like space motion sickness, is manifested by perceptual disturbances of visuomotor, vestibular, and neuroendocrine origin. Symptoms result from discordant visual, vestibular, and proprioceptive stimuli. With repeated exposure to a simulator, symptoms subside as neural comparisons of information from different sensory modalities are recalibrated. Symptoms may return during readaptation to the normal environment. Adaptation may reduce sickness which is experienced during simulated flight, but safety hazards may result as pilots readapt. Evidence for adverse readaptation effects include: 1) flashbacks, and 2) ataxia in aircrew after simulated flight. Pilots' retrospective accounts show that flashbacks are sudden, compelling experiences of self-motion. Ataxia was measured after simulator flights in aircrews using tests of standing and walking steadiness. In one study, 36 pilots were measured repeatedly across ten simulator flights. While there was a decrease in symptomatology experienced while in the simulator over the course of the ten flights, there was a systematic increase in postural instability upon egress from the simulator, suggesting readaptation. The paradox of favorable and unfavorable outcomes from adaptation is described using these and related data. In addition, the development of a biocybernetic device to predict in real time pilots' likelihood of developing simulator sickness is discussed.

312.10

EYE POSITION IS AFFECTED BY GRAVITY IN ANESTHETIZED, PARALYZED HUMANS. M. J. Steinbach and J. Lerman*, Depts of Ophthalmology & Anesthesiology, Hospital for Sick Children, Toronto, Canada M5G 1X8, and the York University Vision Group.

In order to determine if the the center of rotation of the eye coincides with its center of mass we have been photographing the eyes of patients undergoing surgery for non-paralytic strabismus. Patients were unpremedicated and anesthetized with IV thiopental, atropine and succinylcholine. After tracheal intubation, anesthesia was maintained with halothane, nitrous oxide, oxygen and atracurium, a non-depolarizing muscle relaxant. Five minutes after administration of the muscle relaxant and just prior to the onset of the surgery, the patients were photographed with the lids gently lifted and the head rotated so that the left ear, and then the right ear faced down, with this sequence repeated once again. The camera, an 8 mm video with close-up lens, was equippped with a plexiglas strut which allowed for accurate positioning of the camera in the patient's midline. The position of the eye was determined from the corneal reflex produced by the camera's spotlight. In 7 patients there was a change in corneal reflex which indicated that the patients' eyes were rotating upward (about 5 deg), against the direction of gravity, suggesting that the center of mass is behind the center of rotation. This finding complicates models of eye-head coordination because the position of the head with respect to gravity will alter the requirements of the extraocular muscles in maintaining gaze stability. It may also be a cause of the motion sickness astronauts frequently experience in microgravity.

312.11

3-DIMENSIONAL HUMAN EYE POSITIONS AS A FUNCTION OF HEAD ORIENTATION: D.Straumann*, J.van Opstal*, B.J.M.Hess*, V.Henn, K.Hepp*. Neurology Clinic, University Hospital, CH-8091 Zürich, Switzerland.

We measured human eye rotations in 3 dimensions while head position was systematically varied relative to the stereotaxically defined horizontal plane. Absolute rotational vectors of the viewing eye were measured with Robinson's 3D scleral induction coil (modified by Collewijn, manufactured by Scalar instruments). Typically, we recorded 2000 eye fixations in each subject for every given head position. For calibration we first determined in-vitro length and relative orientation of the two coil vectors in 3D space. For offset voltages observed in-vivo, correction was done by vertical fixation tasks in the sagittal plane, where we assumed absence of torsion. Periodical repetition of the in-vivo calibration checked for possible slips of the annulus. With the head erect, all eye positions could be described as rotations about axes lying in one plane, defined as Listings' plane, which reduces the degrees of freedom for eye fixations to a function of horizontal and vertical rotations. We are investigating to what extent eye rotations can still be described as lying in one plane when head position is varied relative to body and gravity, and whether this plane will stay head-fixed. Supported by: Swiss National Foundation for Scientific Research 3.503-0.86.

312.12

VERTICAL VISUAL-VESTIBULAR INTERACTION (VVI) INDUCED BY TELESCOPIC SPECTACLES (TS) IN HUMAN ADULTS. J. L. Demer and R. W. Baloh*. Jules Stein Eye Institute and Department of Neurology, UCLA Medical School, Los Angeles, CA. 90024.

Although TS are known to induce a strong, immediate VVI during horizontal head movements, the effects of these devices on the vertical vestibulo-ocular reflex (VOR) are poorly understood. We investigated the immediate effects of normal vision and vision with 4X binocular TS on the vertical VOR in 6 adults. Subjects were trained to make active, vertical head movements (amplitude 5-10 deg) synchronously with an audible tone modulated sinusoidally at 0.4 or 1.6 Hz. Gaze and head movements were recorded using magnetic search coils; eye position in the orbit was reconstructed by electronic subtraction. Smooth pursuit of a projected laser target was also tested.

In darkness, VOR gain (eye vel./head vel.) was directionally symmetric and ranged from 0.8-1.3 at both frequencies tested. During magnified vision, gain was closer to the ideal value of 1.0 than in darkness. During wearing of TS, there was considerable inter-individual variability in gain, but all subjects exhibited increases with TS to as much as 4.0 at 0.4 Hz, and some exhibited increases to as much as 2.5 at 1.6 Hz. Vertical smooth pursuit, however, was poor at these frequencies.

These data indicate the presence of robust vertical VVI at frequencies previously believed to be above the range of the smooth pursuit mechanism.

312.13

SACCADIC EYE MOVEMENTS AND RAPID ARM MOVEMENTS IN A DOUBLE-STEP TRACKING TASK. G.K.Kerr. Univ. Lab. of Physiology, Parks Road, Oxford OX1 3PT, U.K.

The interdependence of visual and limb movement control processes during goal-directed movements was examined in 7 subjects who used a manipulandum to track a target displayed on an oscilloscope. After a random foreperiod the target stepped to the right (visual angle 15°;elbow angle 45°) followed, on 60% of trials, by a second target step (visual angle 10°;elbow angle 30°) at one of 10 different inter-stimulus intervals. The second target step occurred in one direction only (left or right) on each day of testing.

When the second target step occurred 100ms or more prior to onset of the initial saccadic eye or elbow movement their amplitude of movement was able to be altered. However, there was an increased delay in producing a second movement. This appeared to result from the interruption of early stages of response programming arising from the incorporation of new visual information into initial amplitude computations. These stages involve a greater interdependence of visual and motor processes, possibly due to the utilisation of a common coordinate system.

312.14

EYELID MOVEMENTS WITH VERTICAL SACCADES IN HUMAN SUBJECTS. K.A. Manning, P.A. Sibony, & C. Evinger. Depts. of Neurobiology & Behavior and Ophthalmology, SUNY Stony Brook, NY 11794.

The upper eyelid follows the trajectory of the eye in a similar, but not idential fashion, during vertical saccades. The mechanisms underlying lid saccades, however, are poorly understood. We examined the lid kinematics (magnetic search coil method) and orbicularis oculi (OO) muscle activity (skin electrodes) of saccadic lid movements and blinks in 9 normal human subjects. Subjects executed 100 saccades (3-38 deg) and > 20 each of voluntary blinks and reflex blinks (supraorbital nerve stimulation).

For upward lid saccades, peak lid velocity increased linearly with amplitude at the same rate as the up-phase of the blink. This supports the notion that a single mechanism produces upward lid saccades and the blink up-phase. In contrast, the increase in velocity with amplitude of downward lid saccades plateaus and reaches only half of that seen with the blink down-phase. Thus, lid lowering with saccades and blinks employs different mechanisms. Further evidence of this difference occurs following lid injection of botulinum toxin (Manning et al. 1989). This treatment for facial spasms significantly reduces the velocity of the blink down-phase, but not that of downward lid saccades.

In most subjects, upward lid saccades lasted longer (sometimes twice as long) as downward lid saccades. Upward lid saccades often undershot, and down ward lid saccades frequently overshot final lid position. A small 50-100 msec increase in OO activity commonly occurs near the start of lid saccades in both directions. This tendency for OO activity to accompany gaze changes could contribute to the downward lid saccade overshoot, upward lid saccade undershoot, and the overall faster speed of downward lid saccades.

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312.15

NICOTINE TRANSIENTLY INCREASES THE LATENCY OF THE BLINK REFLEX. C. Evinger. Dept. Neurobiology & Behavior, SUNY, Stony Brook, NY 11794

Smoking a cigarette causes a transient, 50% increase in the latency of the blink reflex in humans (Evinger et al., 1989). This increased latency might result from the nicotine in cigarette smoke acting on nicotinic receptors in the trigeminal nuclei and medullary reticular formation and/or by binding to nicotinic receptors in the basal ganglia. Consistent with the latter possibility, Huntington's chorea causes a similar increase in blink latency. We investigated the effect of nicotine alone on blink latency and examined the relative contributions of the brainstem and more rostral regions to modification of blink reflex latency in the rat.

Air puffs or electrical stimulation of the cornea of urethane anesthetized rats elicited robust blinks. Monitoring the EMG activity of the lid closing orbicularis oculi muscle (OOemg) allowed measurement of blink onset and magnitude. Electrical stimulation of the cornea evoked a blink with a latency of 12 ms and a relatively constant magnitude over repeated trials. An I.V. injection of nicotine (.8 mg/kg), however, caused a transient (3-5 min) 15% increase in corneal blink reflex latency and a slightly larger blink. Air puffs evoked blinks with a latency of approximately 20 ms and injecting nicotine transiently increased (2-7 min) the latency of these blinks by 40%. These results support the conclusion of our human study that the nicotine in cigarette smoke alters the blink reflex. To investigate if the brainstem nicotinic receptors were responsible for this modification, precollicular decerebrate rats received IV injections of nicotine. In these animals, which exhibit robust blinks, nicotine failed to alter the OOemg latency. This result supports the hypothesis that nicotine transiently increases the blink reflex latency by its action on more rostral regions such as the basal ganglia. Supported by grant EY07391.

313.1

UNIT ACTIVITY IN THE PRIMATE POSTERIOR PARIETAL CORTEX DURING AN OCULOMOTOR DELAYED RESPONSE TASK. M. Chafee*, S. Funahashi, and P.S Goldman-Rakic (SPON: P. Rakic). Sec. of Neuroanatomy, Yale Univ. Sch. of Med., New Haven, CT 06510.]

Spatially-selective mnemonic activity recorded in the prefrontal cortex was recently shown to reflect the location of visual targets presented during an oculomotor delayed-response (ODR) task. (Funahashi et al. J. Neurophysiol. 61:331-349,1989). Although the prefrontal cortex (PFC) receives visual input from a number of cortical areas, the posterior parietal cortex (PPC) is a likely source of visuospatial information because of its known projection to prefrontal cortex and its involvement in visuospatial processing.

Unit activity was collected from the PPC during ODR performance. Of the 134 units collected from two hemispheres, 99 were related to the task, and were activated either during the cue (13%), delay (28%) or response (79%) periods. The directionality of these responses was described by a gaussian curve fit to the magnitude of the response observed in each cell as the target postion was varied. Roughly 1/2 of all cue, delay and saccade responses recorded in the PPC were directionally selective. Although prefrontal and posterior parietal neuronal populations appear grossly equivalent in that directionally-selective cue, delay and oculomotor units were encountered, visual activity appeared significantly earlier ($p < .01$) in the PPC (mean latency, 92 msec) than in the PFC (mean latency, 134 msec), consistant with a feedforward transmission of visual information during ODR performance. On the other hand, oculomotor activity collected from the two areas was remarkably similar: the majority of the responses were post-saccadic, and frequency distributions of saccade latencies ranged from 200 msec before to 350 msec following the eye movement with the modal latency value occuring between 0 and 50 msec after the initiation of the eye movement in both regions.

These data provide physiological evidence supporting the idea that the prefrontal and posterior parietal cortices are engaged in a common circuit dedicated to visuospatial memory. Supported by MH44866.

313.2

VISUAL AND MNEMONIC CODING IN THE PRIMATE PREFRONTAL CORTEX DURING OCULOMOTOR DELAYED-RESPONSE PERFORMANCE. S. Funahashi, C.J. Bruce, and P.S. Goldman-Rakic. Sec. of Neuroanatomy, Yale Univ. Sch. of Med., New Haven, CT 06510.

We have previously reported that many prefrontal (PF) neurons have "memory fields", i.e. show excitation or inhibition in the delay period for selected cue directions in an oculomotor delayed-response (ODR) task (Funahashi et al. J. Neurophysiol. 61:331-349, 1989). To understand how visual activity relates to mnemonic activity in PF cortex, in the present experiment we compared the activity of PF neurons during the delay period in the ODR task with their visual activation in the cue period of the ODR task.

Among 434 PF neurons studied, 74 had phasic visual response to the onset of the cue, 168 had delay activity, and among them, 43 displayed both activities. Of the PF neurons with visual responses, 96% showed directional selectivity; best directions for 66% of neurons were toward the contralateral visual field, and the remaining neurons had best directions in the ipsilateral field (20%) or along the vertical meridian (14%). Similarly, of PF neurons with delay activity, 77% showed directional selectivity, and among them, 62% had best directions toward the contralateral visual field. The average breadth of directional tuning for the visual and mnemonic cells were 46° and 43° respectively, and did not differ significantly.

Comparison of the directional selectivity of the visual response with that of the delay activity for the 43 neurons with both activities revealed positive correlations between the best directions (+0.69) and tuning specificity (+0.27).

These findings indicate that most particulars of the visual activity exhibited by PF neurons are consistent with a role in constructing "memory fields" which appear to hold visuospatial information "on-line" to direct subsequent behavior. This is of interest because many neurons with memory fields lack overt visual activity. Thus, the mnemonic process may involve several distinct types of neurons that communicate with each other locally, perhaps within a cortical column.

Supported by MH38546, MH00298, and EY04740.

313.3

CELLS IN PARIETAL CORTEX OF MACAQUES INVOLVED IN OCULAR FIXATION AND GOAL-DIRECTED MOVEMENTS.
M.P. GUICHARD*, J.P. JOSEPH, and M. ARZI INSERM U 94, 16 av. doyen LEPINE, 69500 BRON, FRANCE

We have studied the properties of cells recorded from the Intra-Parietal Sulcus (IPS), which are activated by both fixation of spatial targets and arm movement towards these targets.There were two targets (R and L separated by 70°) and four possible starting positions of the hands. The animal was fitted with EOG electrodes.

About half of the cells were activated by fixation of the ipsilateral and the other half by fixation of the contralateral target (spatial selectivity). For most cells, the intensity of the discharge during fixation did not depend on the arm used for the movement, but on the relative position or distance (before movement) between the starting point of the hands and the fixated target.

The same cells were also activated during arm movements. Activation induced by movement of the ipsilateral arm was weaker but comparable in all aspects to that induced by the contralateral arm. About half of the cells were activated with movements towards one target only (primarily towards the target ipsilateral to the recording site) whichever starting position was used. In these cells, spatial selectivity for movement and for fixation were for the same target. In the other half, spatial selectivity for movement was less pronounced than for fixation.

These data suggest that a population of cells in IPS is implicated in visual control of goal-directed movements.

313.4

POSTERIOR PARIETAL CORTEX, THE OCULOMOTOR NEAR RESPONSE AND SPATIAL CODING IN 3-D SPACE. JW Gnadt and LE Mays. Dept. of Physiological Optics and Neurobiology Research Center, Univ. of Alabama at Birmingham, Birmingham, AL 35294.

Single unit recordings were performed in posterior parietal cortex and superior temporal sulcus during near response behavior in trained Rhesus monkeys. Vergence demand, accommodative demand, binocular disparity, vergence response, accommodative response and their time derivatives were independently manipulated and measured. The following types of neurons were found:

1) *Disparity-sensitive visual tracking cells* were related to ongoing binocular disparity during visual tracking in depth. 2) *Velocity-sensitive motor cells* reflected the velocity of either ocular vergence or lens accommodation, independent of stimulus parameters. The cells responded during pursuit trials or for movements to a stepped target. In some cases, the activity led the movements; in other cases, it followed. 3) *Visual fixation cells* modulated their firing rate during changes in conjugate *and* disjunctive eye position. The cells' activity lagged eye position by about 100 ms and was unaffected by accommodation. The cells did not require a visual target during fixation. 4) *Eye-position-dependent visual cells*. Some visually responsive cells were modulated by the conjugate *and* disconjugate position of the eyes, even though the stimulus fell on the same positions on the two retinae. This suggested that some visual cells in parietal cortex are tuned in a three dimensional coordinate frame of reference.

Histological reconstruction has shown that these cell types exist in both the lateral bank of the intraparietal sulcus and in the medial bank of the superior temporal sulcus, two cortical areas which are interconnected anatomically. *(Supported by F32 EY06089 and T32 EY07033 to JWG, EY03463 to LEM and CORE EY03039.)*

313.5

COMPARISON OF CELL ACTIVITY IN CORTICAL AREAS 6, 4, and 5 RECORDED IN AN INSTRUCTED-DELAY TASK. D.J. Crammond and J.F.Kalaska, Centre de recherche en sciences neurologiques, Université de Montréal, Montréal, Québec, Canada. H3C 3J7.

253 arm-related cells were studied in 2 hemispheres of 1 monkey trained to make whole arm reaching movements in two-dimensions, during both an INSTRUCTED-DELAY and a CONTROL task described previously (Crammond and Kalaska 1988, Neurosci. Abstr. 14:343). In the DELAY task, the activity of 153 cells changed significantly after presentation of the CUE signal and varied with the direction of the intended movement. DELAY period activity was more common in area 6 (95/120 cells) than in area 5 (56/101 cells) and was infrequent in area 4 (2/32 cells). There were three major patterns of DELAY period activity (short-duration phasic bursts, sustained tonic rate changes and ramp changes). Tonic responses predominated in area 5 (74%), while phasic (41%) and ramp responses (37%) predominated in area 6, suggesting separate roles for the two areas in motor preparation. Latencies of cell activity in the DELAY period were similar in areas 5 and 6.

DELAY period activity predicted the directionality of cell activity in the CONTROL task. In the CONTROL task, 43/98 area 5 cells discharged before the first sign of EMG activity compared to 93/117 in area 6 and 22/30 in area 4. However, the mean pre-EMG latencies of these cells (-57 ms in area 6, -50 ms in area 4, -38 ms in area 5) were similar. Most area 5 and 6 cells which were active prior to EMG activity in the CONTROL task also showed DELAY period responses, but the majority of pre-EMG area 4 cells did not. This suggests that pre-EMG activity in area 4 was almost entirely related to movement execution. Many cells with phasic DELAY responses were less active after the GO signal in the DELAY task, than in the CONTROL task. Thus, pre-EMG activity in these cells could be dissociated into response components related to movement preparation and execution. (Supported by MRC Group Grant in neurological sciences).

313.6

RECIPROCAL TEMPORAL TRENDS OF SENSORY- AND MOTOR-COUPLING IN PREFRONTAL UNITS DURING VISUAL DELAY TASKS.
J. Quintana* and J.M. Fuster. Department of Psychiatry and Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

The purpose of this work is to determine the degrees of sensory- and motor-coupling of prefrontal units in the monkey engaged in visuo-motor performance with interposition of time between discrimination stimuli and discriminant responses. A delay task is used in which stimuli and responses are not only dissociated in time but in space: A brief and central color-light stimulus (1) is followed by a delay and a second stimulus (2) --a pair of white or color lights-- prompting the manual response to one side (right or left). The contingencies of the task are as follows:

Stimulus 1	Delay	Stimulus 2	Response direction
Yellow or Green	12 s	White lights	Right
Blue or Red	12 s	White lights	Left
Red or Green	12 s	Red and Green	Matching color (random side).

Thus, the color of the stimulus 1 predicts the response direction with different probabilities (Yellow, 100% right, 0% left; Blue, 0% right, 100% left; Red, 75% left, 25% right; Green, 25% left, 75% right). Some prefrontal units (8%) are coupled differentially to sensory information, and some (17%) to response direction. A third group of units are both sensory- and motor-coupled. Direction-related reactions may appear at any time after stimulus 1, as long as this stimulus provides unambiguous information about response direction, or after stimulus 2 in the absence of that information. Within and between units, the amplitude of direction-related reactions tends to increase with the proximity of the response, whereas that of sensory-related reactions tends to decrease after stimulus presentation. A few prefrontal units show selective activities in relation with certainty or with uncertainty of impending response direction. The existence of units coupled to such a variety of factors stresses the role of prefrontal cortex in cross-temporal integration of visuo-motor contingencies and behavioral planning.

313.7

FORELIMB MOVEMENTS WERE INDUCED BY BICUCULLINE INJECTION IN THE MONKEY PREFRONTAL AREA 8 AFTER A GO/NO-GO TASK WAS LEARNED. T. Oishi* and K. Kubota. Dept. of Neurophysiol., Primate Res. Inst., Kyoto Univ., Inuyama, Aichi 484, JAPAN.

In naive monkeys (Macaca mulatta, N=2), somatotopically organized movements were induced by local injection of bicuculline (BMI, 5-10 μg) in areas 4 and 6, and only eye movement (19 sites) and shoulder elevation (5 sites) were induced in area 8. In contrast, in monkeys well-trained for a GO/NO-GO task (N=4), BMI injection in area 8, in addition to eye and shoulder movements, induced forelimb movements (16 sites). To confirm this further, in the same sites of area 8 of the same monkeys (N=2, 19 sites), BMI effect was compared before and after the training. Only after the training, BMI injection induced contralateral forelimb movements, similar in temporal patterns to the lever release performance of the task. In ipsilateral area 8, no changes in BMI-induced movements were observed before and after the training. These data suggest that, during learning of the task, a structure responsible for learned forelimb movements may be formed within contralateral area 8 and that the GABA controls are involved in such a learning-associated structuring.

313.8

SPATIAL REPRESENTATION OF MOVEMENT DISTANCE AND DIRECTION IN THE PREMOTOR CORTEX. D. Karluk*, T.J. Ebner. Depts. Neurosurgery and Physiology, Neuroscience Grad. Prog., Univ. of MN, Mpls., MN 55455.

Our previous chronic unit recording studies described the existence of a "movement field" for premotor cortex cells, each neuron exhibiting a preferential movement direction and distance. This study examined whether these two movement parameters were topographically represented. Single neurons were recorded from the premotor cortex of Rhesus monkeys trained to move to targets within the horizontal plane. The targets were arranged in circles at 3 distances from a central start box. For each cell, the response profile was divided into a series of time intervals of the percent change in firing relative to background. The response profile for each cell was plotted for a specific target and time epoch relative to its location in the premotor cortex. Although preliminary, the results suggest that for the same direction, different populations of cells are recruited for different distances. In contrast, for a given distance roughly the same population is activated, the magnitude of the increase varying with direction. These findings suggest a premotor cortex topography in which the population activated varies with movement distance and the modulation within a population varying with direction. Supported by NSF-BNS-8707572.

313.9

ABNORMALITIES OF SUPPLEMENTARY MOTOR AREA (SMA) NEURONAL ACTIVITY IN MPTP PARKINSONISM. R.L. Watts, A.S. Mandir, Depts. of Neurology & Physiology, Emory Univ., Atlanta, GA 30322; E.B. Montgomery, Jr., Dept. of Neurology, Washington Univ., St. Louis, MO 63110.

We recorded single unit activity in the SMA of a macaque during self-initiated, rapid right wrist movements in the normal state and following induction of MPTP hemiparkinsonism. The animal was trained to "self"-initiate a flexion or extension movement following a visual instruction by at least 1.5 sec. In the normal state 51 task-related units were recorded in the left SMA: 40% were pre-movement "preparatory set" cells, 35% were "movement onset" related cells and the remaining 25% were best related to achievement of final target or receipt of a juice reward. A mild-moderate degree of right hemiparkinsonism was induced with 4 injections of 1.25-1.5 mg of MPTP into the left carotid artery over 5 weeks. Movement time remained significantly prolonged 6 months after MPTP ($p < .0001$; 477±29 msec vs. 315±17 in normal state), and electromyographic (EMG) activity disorganized in a pattern characteristic of parkinsonism. Following MPTP directional specificity of SMA neuronal activity was lost, and normal timing characteristics of SMA pre-movement "preparatory set" cells were greatly altered. Based on preliminary results, we conclude that abnormal "set" cell firing in SMA may play a role in defective movement initiation caused by parkinsonism.

313.10

CHANGES IN MOTOR CORTEX NEURONAL ACTIVITY ASSOCIATED WITH INCREASED REACTION TIME IN MPTP PARKINSONISM. A.S. Mandir, R.L. Watts, Depts. of Physiology and Neurology, Emory Univ. Sch. Med., Atlanta, GA 30322, and S.R. Buchholz*, E.B. Montgomery, Jr., Dept. Neurology, Washington Univ. Sch. Med., St. Louis, MO 63110.

We recorded motor cortex (MC) neuronal activity in macaques performing either self- or stimulus-initiated rapid wrist flexion and extension tasks. Preliminary data reflect 3 normal animals and 2 of the 3 following induction of MPTP parkinsonism. Neurons were judged best related to either "GO" signal ("GO" neurons) or movement onset ("MO" neurons) by criteria examining abruptness, magnitude and temporal consistency of changes of neuronal firing. The timing between behavioral events and onset of first significant neuronal activity change are reported [PRE/POST MPTP] for **9** pre- and **6** post-MPTP "GO" neurons and **23** pre- and **16** post-MPTP "MO" neurons: "GO" signal to movement onset (MO) [260/330; median values in msec], "GO" signal to "GO" neurons [80/140], "MO" neurons to MO [180/250], and EMG onset to MO [100/140]. EMG patterns were less organized following MPTP, and therefore inefficient in generating MO. We hypothesize that movement initiation is delayed in MPTP parkinsonism due to decreased MC responsiveness (possibly due to failed "set" function in supplementary motor area) and inefficient generation of EMG for MO.

313.11

LEARNING-DEPENDENT ACTIVITY IN PREMOTOR CORTEX OF RHESUS MONKEYS. A.R. Mitz, S.P. Wise, M. Godschalk, Laboratory of Neurophysiology, NIMH, Poolesville, MD 20837.

Single-unit activity was studied in the premotor cortex (PM) of two rhesus monkeys while they learned new visuomotor associations. On each trial, the animals were presented one of a set of 4 visual stimuli and used a joystick to respond either 'Left', 'Down', 'Right', or 'NoGo'. Each stimulus in the set was associated with one correct response. One to 3 s later the stimulus flashed to trigger the response. Only a correct response within 1 s was rewarded. A given stimulus was repeated after a failed trial until the monkey responded correctly. After all stimulus-response associations were learned for a given set of 4 stimuli (fewer than 1 error in 10 trials per stimulus), a new set of 4 stimuli was presented. Animals typically learned to criterion in 3 to 20 minutes (40 to 200 trials). Single-units were recorded during the entire learning process and during the presentation of a set of 4 well-known (overlearned) stimuli.

Neuronal activity patterns observed during the task were similar to those described previously, including movement-, set-, and signal-related activity. Response-specific activity was observed. Half (15/29, first monkey) of the neurons analyzed showed no change during the learning process: their activity during early trials, when the monkey could not know the proper response, was nearly identical to activity late in the learning process (as well as during trials with the corresponding overlearned stimuli). The other half (14/29) showed learning-dependent activity. These neurons typically showed disorganized unit discharge during the initial phase of learning, and well-organized firing later, when the stimulus-response association had been learned. This later, well-organized firing, was characteristic of the same unit during responses to the corresponding overlearned stimuli.

314.1

CODING OF TONGUE MOVEMENT DIRECTION BY NEURONES IN TONGUE REGION OF MOTOR CORTEX (MI) OF AWAKE MONKEYS (M. FASCICULARIS). G.M. Murray and B.J. Sessle. Fac. of Dentistry, Univ. of Toronto, Toronto, Ont., M5G 1G6.

Some neurones within limb MI change their activity with arm movement direction (Georgopoulos et al, Sci., 1986). The aim of this study was to see if neuronal activity in tongue MI varies with tongue movement direction. In 2 awake monkeys, extracellular recordings were made from 48 single tongue MI neurones firing in relation to the monkey's trained tongue protrusion task towards a force transducer. Each neurone's activity was recorded during 10 or more trials with the transducer fixed at each of 2 or 3 positions in front of the monkey's mouth: at 0^{o} or 30^{o} to left or right from the midline. The firing rates of 27 of the neurones (65%) varied significantly (ANOVA; $p<0.05$) with the direction of tongue protrusion; 24 of the 27 exhibited a single preferred direction for which firing rate was significantly greater than for any other direction. All 27 neurones were located at intracortical microstimulation-defined (≤ 20 μA) tongue MI sites; an intraoral mechanoreceptive field was identified for 14. Thus, many tongue MI neurones, some of which receive intraoral afferent inputs, show modulation of their activity in relation to the direction of a tongue protrusion movement. This suggests that, in accordance with recent findings in limb MI, neuronal population coding of movement direction may be a feature of tongue MI. Supported by Canadian MRC.

314.2

DISCHARGE PROPERTIES OF NEURONES WITHIN PRIMATE TONGUE SOMATOSENSORY CORTEX SI DURING TRAINED MOTOR TASKS. L.-D. Lin, G.M. Murray, E. Moustafa* and B.J. Sessle. Fac. Dentistry, Univ. of Toronto, Toronto, Ont., M5G 1G6.

Changes in activity of SI neurones during limb movements have been implicated in limb motor control mechanisms. The aim of this study was to see if neurones in tongue SI also alter their activity during orofacial movements. Extracellular single unit recordings were made in two awake monkeys (M. fascicularis) trained to perform tongue protrusion and jaw biting tasks. The activity of 17 of 40 neurones with a mechanoreceptive field on the dorsal surface of the tongue was analyzed during the trained tongue protrusion task. 15 of these neurones showed a significant change in activity during the task: 13 neurones showed an increase in activity and 2 a decrease in activity; 2 of the 15 neurones increased their activity before the onset of tongue muscle EMG activity associated with the task. Activity during the biting task was analyzed in 10 of the 15 neurones which altered their activity during the tongue task, but only 3 of these neurones showed a change in activity during the biting task. These data suggest that tongue SI neurones may selectively alter their activity in relation to different trained orofacial tasks(i.e., tongue protrusion vs biting) and different phases of the tasks, and that these activity patterns may be utilized in the sensorimotor integration of orofacial movements. Supported by Canadian MRC.

314.3

A MOVEMENT-SEQUENCE DELAY TASK FOR THE STUDY OF MOTOR CORTICAL ACTIVITY DURING ARM REACHING IN 3D SPACE TJ Gould and RE Kettner (SPON: GP Frommer) Department of Psychology, Program in Neural Science, Indiana University, Bloomington, Indiana 47405

Past studies in prefrontal, premotor, supplementary motor and primary motor cortex have established that single-neuron responses can be observed well before the initiation of simple movements. These early responses may be associated with the generation and/or storage of appropriate motor responses. The present work sought to develop a behavioral preparation which could be used to study the generation of a more complicated sequence of movements than has previously been studied. The task temporally separates neural responses related to sensory input, short-term "memory" storage, and movement output. It also allows the analysis of reproducible, multi-joint, arm movments which have reasonably complicated trajectories in time.

A rhesus monkey was trained to hold a center button when a cue light came on. While continuing to hold at the center button, two of four randomly selected target buttons were lit in sequence. A 2-3 s delay period, during which no target buttons were lit, was terminated by dimming the cue light. The monkey then moved from the center button to target button 1 to target button 2 and then back to target button 1 to receive a liquid reward. Behavior in this task was shaped slowly over a period of several months. Final performance was at the 82% correct level with 46% of the errors being center-button release errors. Behavioral analyses indicate stereotyped eye movements during this task with the eyes fixed upon the cue light during the delay period. Arm movements were rapid averaging 274 ms. Motor cortical activity recorded during performance in this task will be reported.

314.4

NEURAL MODEL OF MOTOR CORTICAL RESPONSES DURING REACHING IN 3D SPACE RE Kettner & TJ Gould, Department of Psychology, Program in Neural Science, Indiana University, Bloomington, Indiana 47405

In past work by Georgopoulos et al. [Science (1986) 233: 1416; J Neurosci 8: 2913] the response properties of neurons in the motor cortex of monkeys making arm movements in 3D space have been described. We find that a large class of multi-layered, linear, network models produce response properties which are very similar to those observed in the above mentioned recording studies. A specific model which works consists of a feed-forward network with three layers corresponding to: the retina, association cortex, and motor cortex. The neural population code proposed in the above mentioned studies is used to predict movement direction in 3D movement space from motor cortical responses. Neuron-like nodes in each layer receive feed-forward input from elements at the next lowest level, and send outputs to nodes in the next highest level that are linearly porportional to their summed inputs. It can be proven mathematically that this and other, linear, feed-forward models have the following properties:

1. Activity changes are maximal in a preferred direction.
2. Motor cortical tuning functions follow a cosine relationship.
3. The population code predicts movement direction well given certain statistical and uniformity conditions.
4. Sustained activity varies linearly with hold position.
5. The same activity changes occur for same-direction movements initiated from different points in space.
6. The population code predicts movement initiated at multiple points in space using a fixed set of parameters.

In sum, a class of linear models may provide a first step in the creation of more general models of motor cortex responses observed experimentally during arm reaching in 3D space.

314.5

RELATIONSHIPS BETWEEN PRIMATE CORTICOMOTONEURONAL CELLS AND THEIR TARGET FOREARM MOTOR UNITS DURING TORQUE TRACKING. P. A. Fortier, D. Flament and E. E. Fetz, Dept. Physiol. & Biophysics and Regional Primate Res. Center, Univ. of Washington, Seattle, WA 98195.

Previous studies have shown that primate corticomotoneuronal (CM) cells and single motor units (SMU) of forearm muscles can be classified according to their response patterns during ramp-and-hold wrist movements (e.g., phasic-tonic, tonic, decrementing, etc.), and by their discharge rates as a function of active torque. The present study sought to determine (a) the degree to which CM cells facilitate different SMUs in their target muscles and (b) whether particular types of CM cells and SMUs are preferentially interconnected. In a monkey performing an isometric torque tracking task about the wrist, CM cells were identified by the presence of post-spike facilitation of forearm multiunit EMGs and were recorded simultaneously with several SMUs. By leaving the electrode in place, the same CM cells could be recorded for several days with different SMUs. Cross-correlograms between CM cells and SMUs of facilitated muscles showed post-spike peaks for most, but not all SMUs of the target muscles. The proportion of SMUs facilitated by CM cells (71%) was comparable to the proportion (73%) reported by Mantel and Lemon (Neurosci. Lett. 77:113), but less that the proportion facilitated by single intracortical microstimuli (95%; Palmer and Fetz, J. Neurophysiol. 54:1194). In many cases single CM cells facilitated SMUs in different muscles, consistent with divergent effects. Preliminary results show that the cross-correlation peaks tend to be larger for CM-SMU pairs which had similar discharge patterns during the tracking task.

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314.6

MOTOR CORTICAL IMAGES OF SINUSOIDAL TRAJECTORIES. Andrew B. Schwartz and Bryan J. Anderson.* Barrow Neurological Inst., 350 W Thomas Rd., Phoenix, AZ 85013.

Monkeys were trained to draw with their fingers on a computer touch screen. Two tasks were performed as single units in the motor cortex were recorded extracellularly. The animals in the first task were required to move their index fingers directly from a center start position to one of eight radially oriented targets. The discharge rates of the active cells recorded in this task were found to be maximal in a single preferred direction and to fit a cosine function when plotted against the direction of movement. Sine waves of different amplitude and spatial frequency were presented to the animals in the second task. The animals were trained to smoothly trace, with their index fingers, the sinusoid from one end of the screen to the other. 'Population vectors' representing the combined activity of the many cells active in the motor cortex during this task were constructed. The weight of each cell's contribution in its preferred direction was determined by that cell's discharge rate at 20 msec intervals throughout the task and a population vector was calculated for each interval by summing all the cells' contributions. The direction of the population vectors changed throughout the sinusoidal task, closely matching the smoothly changing direction of the finger path. The 'image' formed by connecting the population vectors was that of the actual sinusoidal trajectory. (NIH- NS26375)

314.7

MOVEMENT TRAJECTORY OR GOAL REPRESENTATION IN PRIMATE PREMOTOR AND PRIMARY MOTOR CORTEX. S. Hocherman and S.P. Wise, Lab. Neurophysiol., NIMH, Poolesville, MD 20837

Neuronal activity was examined in areas 4 and 6 of two rhesus monkeys operantly conditioned to execute curved vs. straight arm-projection movements. Movements began from a common origin point under the chin and ended at one of three 36 mm wide targets: 0°, -30°, or +30° in front of the monkey. Three paths were used to approach each target: straight, curved convex to the right or curved convex to the left. After an initial hold time, an instruction stimulus (IS), consisting of 2-4 LEDs indicating the final target and the approach trajectory, was presented for 120 ms. A trigger stimulus (TS) followed 350-1000 ms after the IS. Movement was not constrained in time, but the chosen path had to be followed within ± 8 mm.

Of the 324 task-related units analyzed, 76 were located in area 4, 73 in ventrolateral area 6, 115 in dorsomedial area 6, and 60 in mesial area 6. Unit activity was found immediately after the IS, between the IS and TS, immediately before movement, and during other task epochs. For most units, activity modulation took place during more than one epoch. We hypothesized that if neurons represented spatial goals, we should observe similar activity for all movements to a given target. Similarly, if movement trajectory was represented, modulation should be observed in relation to given path types, regardless of target location. Neurons representing trajectory outnumbered those representing target position. Curved trajectories were found to be represented more often than straight ones.

314.8

EFFECTS OF MOTOR CORTEX INACTIVATION ON FORELIMB MOTOR CONTROL IN THE CAT. S.E. Cooper*, J. H. Martin, E. Sybirska, J. Brennan* & C. Ghez, Ctr. for Neurobiol & Behav, Columbia Univ and NYS Psych Inst, New York, NY, 10032.

The present study analyzes deficits in forelimb motor control resulting from inactivation of motor cortex (MCx). Deficts were assessed in a prehension and a step-tracking task. In the prehension task, cats reached to a baited target to grasp a morsel of food. In tracking, cats made adjustments of elbow torque to match abrupt changes in a target level indicated by a compensatory display (reaction time, RT, ~ 100 ms). Motor cortex was inactivated by microinjection of the short-acting local anesthetic lidocaine (10-30μg) or the long-acting GABA agonist muscimol (1-2μg). We measured changes in limb kinematics in prehension, and in RT and force trajectories in tracking.

Inactivation of the forelimb area of MCx resulted in the loss of placing and changes in posture characteristic of MCx lesions. In prehension, animals exhibited (1) hypometria, (2) impairment in the normally fluid sequence of elbow flexion and wrist extension in reaching, and (3) a reduced ability to use the digits in grasping. In tracking, responses were modestly hypometric in 12 of 14 experiments, while increases in RT occured in only 5 of 14 experiments and were quite small.

Our data on prehension suggest that the motor cortex plays a role in the independent spatial and temporal control of proximal and distal components of the motor response. In tracking, the lack of RT prolongation in the presence of impaired force production, taken together with our earlier finding that reversible inactivation of the red nucleus can prolong RT without impairing force production, supports the hypothesis that response initiation and amplitude control are mediated independently. (Supported by NS19205)

314.9

TIME-RELATED COGNITIVE EVENTS ENTRAIN NON-LINEARLY COUPLED RHYTHMS IN HUMAN EEG.

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When human subjects learn and attempt recall of the relative timing of acoustic rhythms their scalp-conducted EEG shows rhythmical activity around onset-time of the accented transient, which can account for the phenomena called sensory evoked potentials (EP). Selective frequency analysis of this activity in the time-domain shows it to consist of a subharmonic and higher harmonics of their predominant alpha rhythm, synchronized for sufficient successive trials to reach statistical significance of phase-angle and amplitude either for stimulated trials alone, for unstimulated ("silent recall") trials alone, or for both together. This global mixture of alpha sub- and higher-harmonics, which is reflected in the unanalyzed "vertex potential" of EP, is further evidence for non-linear coupling of oscillating neural networks seen in nonhuman brains [1]. 1. Boeijinga, P.H. & Lopes da Silva, F.H. Brain Res. 478 (1989) 257-268.

314.10

CEREBRAL POTENTIALS PRECEDING ORAL MOVEMENT. A. B. Wohlert* and C. R. Larson. Dept. of Communication Sciences and Disorders, Northwestern U., Evanston, IL 60208.

The "readiness potential" is an averaged EEG record which shows increasing negativity at vertex and sensorimotor scalp sites prior to voluntary limb movements. It becomes lateralized to the hemisphere contralateral to the movement, just before the movement's onset. In order to determine whether simple, midline oral movement engenders the same pattern, potentials preceding bilateral lip protrusion and right finger extension tasks were compared in 12 right-handed subjects. Prior to the finger movement, potentials were generally more negative over the dominant left hemisphere. Prior to the lip movement, there was no consistent pattern of lateralization, nor did the hemisphere showing greatest negativity always correspond to the side of the lip showing earliest onset or greatest amplitude of EMG activity. Results do not support left hemisphere dominance in the pre-movement control of coordinated midline oral gestures, at this simple level.

314.11

FOCAL STIMULATION OF CORTICOSPINAL NEURONS LOCATED DEEP IN PARASAGITTAL PRECENTRAL CORTEX WITH THE MAGNETIC COIL. Joan B. Cracco*, Roger Q. Cracco, Nassar F. Hassan*, Mahendra Somasundaram*, Vahe E. Amassian and Paul J. Maccabee* Departments of Neurology and Physiology, SUNY Health Science Center at Brooklyn, Brooklyn, N.Y. 11203.

Using ourselves as subjects, we have shown that lateral areas of the precentral motor cortex can be focally activated to elicit individual finger movements. For focal activation we used a small "figure of eight" stimulator composed of two magnetic coils, each 5.0 x 4.8 cm., optimal stimulation occurring under the junction. We also demonstrated that focal activation of precentral parasagittal cortex is possible. Movements limited to abduction or flexion of toes and plantar or dorsiflexion of the foot were elicited. Recordings of compound motor action potentials from muscles of the left leg showed that the threshold for activation for small foot muscles was lower than for more proximal muscles. Furthermore, at certain precentral parasagittal scalp locations the abductor hallucis could be activated nearly in isolation whereas at sites a short distance away only abductor digiti minimi and extensor digitorum brevis were activated. Thus focal activation of corticospinal neurons deep in parasagittal precentral cortex can be achieved with magnetic coil stimulation.

314.12

SPECTAMINE IMAGING OF CEREBRAL BLOOD FLOW DURING VOLUNTARY MOVEMENT AND FOCAL STIMULATION OF MOTOR CORTEX WITH MAGNETIC COIL. B. Shafran*, P.J. Maccabee*, V.E. Amassian, R.Q. Cracco, A. Strashun*, R. Vaquer*, R. Singman* (SPON: B.M. Altura). Dept. Neurol., Physiol. and Nuclear Med., SUNY Hlth Sc. Cntr, Bkln, NY 11203

Spectamine imaging (I^{123}) of cerebral activity was performed during voluntary finger flexion (VFF) and magnetic coil stimulation (MCS) of motor cortex resulting in contralateral finger flexion. A butterfly (MC) over motor cortex hand area was energized just above threshold to elicit the finger flexions. Movements occurred once/20s in both groups. Imaging revealed increased activity (13-19%) in motor and premotor cortex contralateral to VFF. With MCS, this difference was reduced (4%) or reversed i.e. motor cortex opposite the MCS side had higher scintillation counts (15%) than the stimulated cortex, but both were absolutely increased. Cerebellar hemispheres did not differ significantly with voluntary activity (1.7%), but was 18% greater contralateral to the side of MCS. Thus, activity in motor and premotor cortex increases contralateral to voluntary finger movement, but increases bilaterally in motor cortex with MCS. This bilaterality may reflect transcallosal responses. Cerebellar cortex contralateral to the side of MCS was selectively activated. Evidently, focal MCS may activate different pathways in human motor cortex.

314.13

FRONTAL CORTICAL CELLS OF RATS ARE ACTIVATED IN A DIVIDED ATTENTION TASK. K. Pang, D. Olton and H. Egeth. Dept. of Psychology, The Johns Hopkins University, Baltimore, MD 21218.

The frontal cortex (FC) is critically involved in divided attention. FC lesions in rats disrupt divided attention, while leaving focussed attention intact. In the present study, the activity of FC neurons was examined in a temporal discrimination task. Rats were trained to two stimuli (light and tone), each associated with a different fixed interval. Focussed attention trials (single stimulus presented) and divided attention trials (two stimuli presented) were given randomly within a session. Recording electrodes were implanted into the FC in trained rats. Extracellular recordings of FC cells were taken while the rat was performing the discrimination task. Neuronal activity was correlated with the behavioral performance. Some cells had divided attention correlates; these cells selectively increased their firing rate to the second stimulus in divided attention trials, but not to the same stimulus in focussed attention trials. Several firing patterns were observed, suggesting that FC neurons may code different aspects of divided attention. The remaining cells had more complicated firing patterns, which seem to involve motor responses; these cells increased their firing rate with increasing bar press rate in both focussed and divided attention trials. The data provide evidence that the frontal cortex is involved in both divided attention and motor responses.

314.14

NEUROSCIENCE IN CHICAGO 50 YEARS AGO: CONCEPTS AND CHARACTERS I. L.H. Marshall, Brain Research Institute, University of California, Los Angeles, CA 90024-1761

The decade of the 1930s was unusually productive in biomedical subfields related to neuroscience. Subdisciplines that already had a foundation, such as the behavioral aspects of cerebral localization and the conduction of the nerve impulse, were flourishing. Others were about to emerge, for example, the neuroendocrinology of the hypothalamus and the biomathematics of nerve nets that led to cybernetics. Looking at the scene in Chicago, we find in the 1930s a powerhouse of activity in neuroscience-related fields situated in three localities: Northwestern University Medical School's Institute of Neurology, the neurophysiology laboratory of the University of Illinois Neuropsychiatric Institute, and the University of Chicago's department of physiology. At Northwestern, Stephen Walter Ranson (1880-1942) had moved into the tower of a new building and revived the stereotaxic approach to pinpointing cerebral loci for stimulation, recording, or making lesions. The notable results were discoveries on posture control and hypothalamic influences on the pituitary gland concerning feeding, drinking, and other vital behaviors. At Illinois's INI Percival Bailey (1892-1973) had assembled a remarkable group led by Warren Sturgis McCullock (1898-1971). Their experimental work was a continuation of neuronography using strychnine to explore cerebral association pathways. The third center was at the University of Chicago.

314.15

NEUROSCIENCE IN CHICAGO 50 YEARS AGO: CONCEPTS AND CHARACTERS II. H.W. Magoun and L.H. Marshall, Brain Research Institute, University of California, Los Angeles, CA 90024-1761

The University of Chicago, along the Midway on the southside, was home to several groups working in neuroscience fields in the 1930s. Nathaniel Kleitman's research on sleep was in full swing, with graduate students as subjects. The "iron wire" model of nerve conduction devised by Ralph Staynor Willie (1875-1952) was a source of debate. The largest research group was the laboratory of Ralph Waldo Gerard (1900-1974) in the physiology department, in which several candidates for the doctor of medicine were taking an additional year and earning the Ph.D. degree. During the 1930s Gerard's laboratory published the first evoked potentials from the central nervous system and perfected the capillary pipette electrode. Gerard's support services were exemplenary: the biochemistry lab under a Russian emigree was a model of industry and cleanliness, and expert "electronikers" were available. Other departments, especially anatomy, had well established investigators in neuroscience. Heinrich Kluver and Paul Bucy were analysing the syndrome that bears their name, and Paul Weiss and Roger Sperry in zoology were looking at neuronal specificity and regeneration. The constellation of important concepts under investigation during the decade of the thirties placed Chicago among the top centers for neuroscience.

SUBCORTICAL VISUAL PATHWAYS III

315.1

DIFFERENCE IN THE PROCESSING OF MULTISENSORY AND MULTIPLE UNISENSORY CUES BY SINGLE SUPERIOR COLLICULUS NEURONS, M.A. Meredith, P.W. Archer and B.E. Stein, Deparments of Anatomy and Physiology, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA, 23298-0709.

When two contiguous stimuli from different sensory modalities (e.g. auditory and somatosensory) excite a superior colliculus (SC) neuron, their combination results in a dramatic response enhancement. The object of the present experiments was to determine whether this multisensory integration differs in any significant way from the integration that would result from the combination of stimuli within the same modality. Therefore, the activity evoked in SC neurons (n=22) by pairs of stimuli from within the same modality was compared to that elicited by pairs of stimuli from different modalities in chronically prepared, anesthetized cats.

Simultaneously presented unimodal stimulus pairs failed to evoke responses that exceeded the sum of the impulses evoked by these stimuli individually. Usually the combined response was significantly less than the sum of the two responses independently. These 'occlusive' effects were exhibited even when the stimuli were presented at wide temporal disparities (25 - 800 msec). These results contrasted sharply with the effects induced by combining excitatory stimuli from different modalities simultaneously and at these temporal disparities (see Meredith et al., J. Neurosci. 7:3215, 1987), indicating a substantial difference in the manner in which unimodal and multisensory information is integrated in the same SC neurons.

Supported by grant NS 22543

315.2

THE EFFECTS OF GABA AND GLUTAMATE ON MULTISENSORY SUPERIOR COLLICULUS NEURONS: AN IONTOPHORETIC STUDY

D.D. Dunning and B.E. Stein. Department of Physiology, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA, 23298.

We have begun investigating the neurochemical bases of multisensory integration in cat superior colliculus (SC) neurons by pharmacologically manipulating the responses of multisensory neurons. This was accomplished by presenting unimodal stimuli before, during and after the application of transmitter agonists and antagonists. Transmitter substances were applied by extracellular iontophoresis from multibarrel micropipettes and the responses of SC neurons were recorded from a carbon-fiber electrode in the central barrel.

Gamma-aminobutyric acid (GABA) and glutamic acid (GLU) affected all multisensory neurons tested: GABA (500mM) decreased spontaneous activity while GLU (500mM) increased spontaneous activity, regardless of the properties a given neuron exhibited. Responses were dose dependent, with the minimum effective dose in the 10-20nA range and the maximum effect at 50-100nA. GABA and GLU also affected responses to visual, auditory and somatosensory stimuli in the same fashion: GABA decreased evoked activity, while GLU increased evoked activity. The effects of GABA and GLU on both spontaneous and evoked activity were readily blocked by appropriate antagonists (bicuculline methochloride or methiodide for GABA; kynurenic acid or gamma-D-glutamyl glycine for GLU). However, when the natural sensory stimuli were presented without adding GLU, the neuronal responses they evoked were unchanged by administering GLU antagonists.

These results indicate that GABA and GLU probably function to modulate responses of multisensory SC neurons. An unexpected finding was that the quisqualate- and kainate-type GLU receptors do not appear to be involved in initiating the responses of these neurons to natural sensory stimuli.

Supported by NIH grant NS 22543

315.3

THE RETINOTECTAL W-CELL PROJECTION IN THE CAT: EVIDENCE FOR TOPOGRAPHIC VARIATIONS IN DENSITY D.M. Berson, J. Lu*, and J.J. Stein* Div. Biol. & Medicine, Brown Univ., Providence, RI 02912.

Local magnification in central visual maps is widely thought to reflect the intraretinal density of afferent ganglion cells. This may imply that retinal terminal arbors are uniformly spaced and that afferent density is quite constant. In the cat's superior colliculus, therefore, one might expect the density of W-cell terminations to be fairly uniform because such afferents dominate the retinotectal pathway. We have tested this hypothesis.

Monosynaptic inputs from the main W-cell projection mediate the late negative potential (LNP), a collicular field potential elicitable from the optic pathway. Using a fixed stimulus, we measured the maximum amplitude of the LNP at 90 topographically identified tectal sites in 5 cats. LNP amplitude varied 5-fold over the map and was systematically related to the representation of azimuth. Amplitudes were smallest in the area-centralis and vertical-meridian representations (azimuths 0 - 3°), averaging 26% of the maximum amplitude in that colliculus and never exceeding 38%. Amplitudes were largest in the representations of the contralateral periphery (mean = 57% of max.; azimuths >10°) and of the ipsilateral visual field (mean = 54% of max.) There was little systematic variation as a function of elevation. Controls excluded artifactual sources of variation such as non-uniform afferent activation and drift in electrode properties.

The results imply that W-cell input weakens in the area-centralis representation, matching anatomical evidence for thinning of retinal input in this region. Although topographic gradients in afferent or cellular architecture may also be involved, a plausible explanation for these variations is that collicular magnification is not proportional to retinotectal W-cell density.

Supported by EY06108 and a Sloan Foundation Fellowship to DMB.

315.4

DISTRIBUTION AND ULTRASTRUCTURE OF NEURONS LABELED BY AN ANTIBODY TO GAMMA-AMINOBUTYRIC ACID IN THE SUPERIOR COLLICULUS OF THE RHESUS MONKEY. R.R. Mize, R.F. Spencer, and C.J. Jeon, Dept. of Anatomy and Neurobiology, Univ. of Tennessee Health Science Center, Memphis, TN, 38163 and Dept. of Anatomy, Medical College of Virginia, Richmond, VA, 23298.

Three classes of neuron labeled by antibodies to gamma-aminobutyric acid (GABA) have been identified in the cat superior colliculus (SC). It is not known whether these classes are found in other species. To study this, we examined SC sections from Rhesus monkeys which were labeled by antibodies raised directly against conjugated GABA. Labeled neurons were distributed throughout the monkey SC, but they were most heavily concentrated within the zonal and superficial gray layers. These cells were almost all small neurons and had variable morphologies. Both horizontal and small granule neurons were identified. At the electron microscope level, three types of labeled profile were found. Presynaptic dendrites (PDSs) contained pleomorphic vesicles and received input from other synaptic terminals. Putative axon terminals with flattened synaptic vesicles were also identified. A third type of profile was identified in a series of 26 sections reconstructed with the aid of a computer. This profile was a vesicle-containing dendritic spine. Both PSDs and the dendritic spine received input from the same retinal terminal. In addition, synaptic terminals with flattened vesicles contacted PSDs, revealing a synaptic circuitry which could support disinhibition. These results show that three basic GABAergic cell types can be found in the SC of both cats and monkeys and may represent an organization common to all mammals. Supported by USPHS grants EY-02973 (R.R.M.) and EY-02191 (R.F.S.).

315.5

TWO CLASSES OF AXON TERMINAL ARE LABELED BY AN ANTIBODY TO CHOLINE ACETYLTRANSFERASE IN THE SUPERFICIAL LAYERS OF THE CAT SUPERIOR COLLICULUS. C.J. Jeon*, R.F. Spencer, and R.R. Mize (SPON: S. Afsharpour) Dept. of Anatomy and Neurobiology, Univ. of Tennessee Health Science Center, Memphis, TN 38163 and Dept. of Anatomy, Medical College of Virginia, Richmond, VA, 23298.

The cat superior colliculus receives a dense cholinergic (ACh) input which is thought to arise from two brainstem sources, the pedunculopontine and lateral dorsal tegmental nuclei and the parabigeminal nucleus. The tegmental input projects densely to the intermediate gray layer and sparsely to the superficial layers. The parabigeminal input probably projects only to the superficial layers. We have examined fibers labeled by an antibody to choline acetyltransferase, the synthetic enzyme of ACh, to determine if they differ in morphology and synaptic ultrastructure. Two classes of fiber were found within the zonal and upper superficial gray layers - thin fibers with few varicosities and thicker fibers with numerous varicosities. When fiber diameter was measured with an image analyzer, a bimodal distribution was found. At the electron microscope level, we also found two classes of immunoreactive profile. The first was a small axon terminal containing round synaptic vesicles and forming asymmetric synaptic contacts. These terminals frequently synapsed onto other dendrites. The second class was a larger varicose profile which also contained round synaptic vesicles but rarely formed conventional synaptic contacts. When present, these contacts were symmetric. These results suggest that the two cholinergic inputs which innervate the superficial layers of the cat superior colliculus may differ in fiber diameter and synaptic ultrastructure. Supported by USPHS grants EY-02973 (R.R.M.) and EY-02191 (R.F.S.).

315.6

QUANTITATIVE DISTRIBUTIONS OF AMINO ACIDS AND RELATED ENZYME ACTIVITIES IN LAYERS OF RAT SUPERIOR COLLICULUS. C.D. Ross[1], J.A. Parli*[1], R.J. Ryder*[1], W.B. Farms*[2] and D.A. Godfrey[2]. [1]Dept. of Physiology, Oral Roberts University School of Medicine, Tulsa, OK 74171 and [2]Dept. of Otolaryngology, Medical College of Ohio, Toledo, OH 43699.

Samples microdissected from layers of the superior colliculus from 5 rats, albino and pigmented, were analyzed for concentrations of amino acids by HPLC (fluorescence of OPA derivatives) and for activities of glutaminase (GLNase), aspartate aminotransferase (total: t-AAT; cytosolic isoenzyme: c-AAT), and malate dehydrogenase (MDH) by fluorometric methods. (amino acids: mmol/kg dry wt; enzyme activities: mol/kg dry wt/hr @ 37°C, except MDH, @ 25°C)

Layer	Asp	Glu	Gln	Gly	Tau	GABA	GLNase	t-AAT	c-AAT	MDH
SGS(IIa)	12	29	37	9	22	38	8	50	25	20
(IIb)	12	27	31	6	14	37	8	47	23	16
SO (III)	10	25	23	5	9	24	6	40	20	13
SGI(IVa)	9	25	20	5	8	22	6	42	20	14
(IVb)	9	25	20	6	8	21	5	39	19	14
SAI (V)	9	25	21	7	8	21	5	38	20	12
SGP-SAP	9	25	20	7	8	20	5	36	17	12
rat brain	12	32	14	7	22	17	9	46	22	12

The greatest gradient was superficial to deep, with highest amino acid concentrations and enzyme activities being in SGS. A small caudal to rostral gradient was seen in some cases. No marked differences were noted between medial and lateral locations or strains of rats. (EY-03838)

315.7

ANATOMICAL ORGANIZATION AND FUNCTIONAL EFFECTS OF THE NORADRENERGIC INPUT TO THE HAMSTER'S SUPERIOR COLLICULUS. R.D. Mooney, C.A. Bennett-Clarke, N.L. Chiaia, N. Sahibzada and R.W. Rhoades. Dept. of Anatomy, Medical College of Ohio, Toledo, OH 43699.

Antiserum directed against dopamine-ß-hydroxylase (DBH) was used to demonstrate the noradrenergic (NE) input to the hamster's superior colliculus (SC). DBH immunoreactive fibers were present in all SC laminae, but they were most dense in the ventral one-half of the stratum griseum superficiale, the stratum griseum intermediale, and the stratum album intermedium. Combination of retrograde tracing with fluorogold and immunocytochemistry demonstrated that the source of the NE projection to SC was the locus ceruleus and that this projection was almost completely crossed. Iontophoretic application of NE generally reduced both the spontaneous and stimulus evoked discharges of SC cells. This effect was observed for both visual neurons in the superficial laminae and somatosensory cells in the deep layers. Application of NE generally did not enhance signal to noise ratios for SC neurons. The effects of NE upon the activity of SC cells were usually reduced or completely blocked by co-administration of the ß-antagonists propranolol and sotalol. In the few cells tested, the α-antagonist corynanthine enhanced the suppressive effects of NE upon the activity of SC neurons. With one exception, application of NE or ß-antagonists did not change the receptive field properties of SC neurons. Complex somatosensory cells in which noxious stimulation reduced spontaneous activity and/or the discharges evoked by low threshold stimuli did have their receptive field properties altered by application of ß-antagonists. During application of sotalol or propranol, application of noxious stimuli no longer suppressed the activity of these neurons.

Supported by EY 04170, EY 08015, BNS 85 00142, and funds from the State of Ohio Research Challenge.

315.8

THE SUPERFICIAL GRAY LAYER OF THE HAMSTER'S SUPERIOR COLLICULUS CONTAINS SEROTONIN IMMUNOREACTIVE NEURONS. L.L. Parsons, C.A. Bennett-Clarke, R.D. Mooney, N.L. Chiaia and R.W. Rhoades (SPON: M. Rayport). Dept. of Anatomy, Medical College of Ohio, Toledo, OH 43699.

Two different polyclonal antisera (Inc Star and Accurate) were used to assay for serotonin immunoreactive (SI) cells in the superior colliculus (SC) of hamster and rat. No SI cells were observed in any portion of the rat's SC, although a dense meshwork of SI fibers were visible in all layers of the colliculus in this species. In addition to SI fibers, numerous SI neurons were observed throughout the depth of the stratum griseum superficiale (SGS) of the hamster's SC. Most SI cells in the hamster's SC had horizontally oriented dendrites, but a number of neurons with vertically oriented dendrites and marginal cells were also seen. Serotonin immunoreactive neurons were not observed in the stratum opticum or any of the deep SC laminae. Pretreatment of hamsters with colchicine and pargyline greatly increased the number, but did not alter the laminar distribution, of SI cells. This treatment did not result in the appearance of SI neurons in the rat's SC. Pretreatment with reserpine resulted in a complete loss of SI cells in the hamster's SC. Injection of fluorogold (FG) into the dorsal and ventral lateral geniculate nuclei (two targets of the SGS of the hamster's SC that contain SI fibers) and subsequent immunocytochemical processing for SI revealed numerous FG labelled cells, but no double labelled neurons, in the SGS. Double labelled cells were visible in nucleus raphe dorsalis. Ablation of the superficial SC laminae did not reduce the density of SI in any known targets of the colliculus. Both of these results suggest that SI cells in the hamster's SC may be interneurons.

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315.9

TACHYKININ IMMUNOREACTIVE CELLS IN THE SUPERIOR COLLICULUS OF RAT AND HAMSTER PROJECT TO THE PARABIGEMINAL NUCLEUS. C.A. Bennett-Clarke, R.D. Mooney, N.L. Chiaia and R.W. Rhoades (SPON: H.Waller). Dept. of Anatomy, Medical College of Ohio, Toledo, OH 43699.

A combination of immunocytochemistry for tachykinins using a polyclonal antiserum directed against substance P (Inc Star), retrograde tracing with fluorogold (FG), and ablation techniques were used to demonstrate that tachykinin immunoreactive (TI) neurons in the stratum griseum superficiale (SGS) of the superior colliculus (SC) in both hamster and rat project to the ipsilateral parabigeminal nucleus and that axons arising from these cells appear to be the sole source of TI in that structure. Immunocytochemical experiments demonstrated a dense meshwork of TI fibers (probably of retinal origin) and numerous TI cells in the SC of both hamster and rat. Tachykinin-immunoreactive neurons were almost completely restricted to the SGS. Many had dendrites that were horizontally oriented, although some TI cells with vertically oriented dendrites and a small number of immunopositive marginal cells were also noted. Injections of FG into the parabigeminal nucleus labelled numerous superficial layer cells in the ipsilateral SC of both rat and hamster. A number of these neurons also contained TI. In both species, retrogradely labelled and double labelled cells could be seen in all portions of the SGS, but they were most numerous in the rostromedial part of the colliculus. Ablation of the superficial SC laminae in either hamster or rat resulted in a nearly complete loss of TI in the ipsilateral parabigeminal nucleus. Such lesions did not reduce TI in other targets of the superficial SC laminae such as the ventral lateral geniculate nucleus.

Supported by EY 04170, EY 08015, BNS 85 00142, and funds from the State of Ohio Research Challenge.

315.10

BILATERAL PROJECTIONS OF THE PARABIGEMINAL NUCLEUS IN MACAQUE. J.F. Whitney, J.S. Baizer, and D.B. Bender*. Dept. Physiology, School of Medicine, SUNY at Buffalo, Buffalo, NY 14226.

In many non-primates, the superior colliculus contains a representation of both visual hemifields. Each colliculus projects to the parabigeminal nucleus (Pbg) ipsilaterally, while the Pbg projects to the colliculus bilaterally. In the primate, each colliculus has a map of only the contralateral hemifield. Nonetheless, we have found that the parabigeminal nucleus projects to both ipsilateral and contralateral colliculi. We made injections of fluorescent retrograde tracers (fast blue and diamidino yellow) into the representation of the upper or lower quadrants in the colliculus of macaque monkeys. In the Pbg, labelled cells were found both ipsilateral and contralateral to the injection site. The ipsilateral projection was topographically ordered. Injections in the upper quadrant representation in the colliculus labelled cells in the posterodorsal half of Pbg, while injections in the lower quadrant representation labelled the anteroventral half of the Pbg. However, the contralateral projection arose mainly from the anterior part of the Pbg. Supported by EY02254 and MH42130.

315.11

Fine Structure of HRP Labelled Terminals Projecting to the Tectum from Two Sensory Pathways. R.M. Meszler, P.H. Hartline and R.V. Stirling. Anatomy Dept., Dental School, Univ. of Maryland, Baltimore, MD 21201 and Eye Research Institute, Boston, MA 02114.

The morphological substrate for dual sensory input to the tectum of the rattlesnake was studied using anterograde labelling with HRP. Iontophoretic injections of HRP were placed in the optic chiasma to label retinal projections and nucleus RC for the infrared receptor pathway. After 7-10 days survival, vibratome slices of the aldehyde fixed tecta were incubated with DAB and prepared for light and electron microscopy. In tecta in which both retinal and RC projections were labelled there was a clear separation between the two terminal fields with the boundary being in the middle of the stratum griseum centrale (SGC). The retinal projections consisted of fine axonal branches with numerous varicosities extending throughout the superficial layers and into the outer SGC. The synaptic boutons contain clear spherical vesicles and form asymmetrical contacts with dendritic processes. In contrast, the RC terminals come from large diameter axons which enter the stratum fibrosum centrale (SFC) and divide into progressively smaller branches which extend into the deeper portion of SGC. The terminals branches of these projections form asymmetrical contacts with small dendritic processes and contain clear spherical vesicles with an occasional granular vesicle. Supported by NIH grant #1R01 NS21989 and NSF grant #BNS8411566.

315.12

MULTIMODAL INTERACTIONS IN RAT SUPERIOR COLLICULAR CELLS. Kimberly M. Preucil* and Douglas A. Weldon. Department of Psychology, Hamilton College, Clinton, NY 13323.

In rats, as in other species, anatomical and physiological evidence indicates a convergence of inputs from several modalities in the deep layers of the superior colliculus (SC). Multimodal interaction effects in superficial layer cells have been demonstrated in some species but not in others. In the present study, we examined the influence of combined auditory and visual stimulation on 126 cells in the SC of Long-Evans rats anesthetized with urethane. Visual (light flash, 300 msec) and auditory (65 dB white noise, 100 msec) stimuli were presented either alone or in combinations, with the stimulus onsets either simultaneous or displaced in time by 100, 200, 300, or 400 msec.

The presentation of the auditory stimulus inhibited the response to visual stimuli in 12% of the neurons in the superficial layers of the SC. Of the 49 neurons in the deep layers, an interaction effect occurred in 33% of the bimodal cells responsive to both visual and auditory stimuli, and these effects were either facilitatory or inhibitory. In both the superficial and deep layers, the presentation of the auditory stimulus sometimes produced a sharpening of the response to the visual stimulus.

315.13

LAMINAR ANALYSIS OF A 3D RECONSTRUCTION OF THE SUPERIOR COLLICULUS

A.W. TOGA, B.A. PAYNE* & E.M. SANTORI,
Laboratory of Neuro Imaging, Department of Neurology, UCLA School of Medicine. Los Angeles, CA. 90024

Recent evidence points to the existance of lattices in the superior colliculus (SC) of acetylcholinesterase (AChE) and cytochrome oxidase. These lattices may reflect an organizational principle in the various layers of the SC. Wallace (1988) notes that the two lattices are not in spatial register, but occur at the same depth. We have developed a set of algorithms to examine and display spatial relationships within different brain laminae and have applied these techniques to the study of lattices within the rat SC.

Serial (coronal) sections of tissue alternately stained for cytochrome oxidase or AChE were digitally scanned, and aligned. Sections were reconstructed into three dimensional models. Two models were reconstructed, one for each stain. Lamina profiles were computed using one of three techniques. In the first, a volume showing distance from the surface of the SC was computed and used to "erode" the surface at various depths. This subsurface is equidistant from the surface of the stratum griseum superficial (SGS). Densitometric data representing cytochrome oxidase or AChE is then mapped upon this surface. In the second approach to identifying lamina, a ratio method was employed. A top and bottom surface are identified. These two levels are used to form a ratio for computing a surface below the SGS. In this way the subsurface is no longer equidistant from the surface of the SGS. The third method requires the investigator to manually identify the specific lamina upon which the densitometric data are mapped. All methods illustrate the spatial relationship between the two lattices for cytochrome oxidase and AChE.

315.14

IMMUNOHISTOCHEMICAL CHARACTERISTICS OF NEURONS IN THE SUPERIOR COLLICULUS OF ADULT HAMSTERS A. Jourdain, G.M. Bray and A.J. Aguayo. Center for Research in Neuroscience, The Montreal General Hospital and McGill University, Montréal, Québec, Canada, H3G 1A4.

Immuno-reactivity (i.r.) for GABA, substance P (SP), parvalbumin (PV) and calbindin (CB) in the superior colliculus (SC) of adult hamsters was analyzed for laminar distribution and for correlation with neuronal type. Neurons with PV or GABA i.r. were evenly distributed throughout the superficial SC and were also observed in the deeper strata. By contrast, CB-i.r. neurons tended to be concentrated in the stratum zonale, in the lower stratum griseum superficiale (SGS), and in the deeper strata where large, multipolar neurons were heavily labelled. Fewer neurons showed SP i.r. and most were located in the lower SGS and upper SO.

Within the superficial SC, each of the antibodies labeled more than one type of neuron. Many of the GABA and PV-i.r. cells appeared to be horizontal or pyriform neurons. CB and SP i.r. labeled marginal and vertical neurons as well as horizontal cells.

These different patterns of neuronal i.r. presumably reflect the complexity of neural circuitry in the SC. The extent to which the neurons immunohistochemically identified in this study represent post-synaptic targets of retino-collicular terminals remains to be determined.

315.15

STEADY DIFFUSE LIGHT DECREASES, FLASHING DIFFUSE LIGHT INCREASES, COLLICULUS METABOLIC ACTIVITY: CHANGE DETECTOR? R.M. COOPER, G.A. THURLOW, and A. GAFKA*. Behavioral Neuroscience Group, Psychology Dept., University of Calgary, Calgary, Alberta, Canada, T2N 1N4.

Past work demonstrated that exposure to steady diffuse light (eye covered with translucent mask) results in a strong depression in 2-deoxyglucose (2-DG) uptake in the hooded rat superficial superior colliculus relative to 2-DG uptake in darkness; metabolic activity of visual cortex remains similar under the 2 conditions. In the present study, rats were also exposed to diffuse light but it was flashed at 2, 5 or 8 hz. Again, effects were minimal in cortex but flashing diffuse light strikingly increased SC 2-DG uptake. The increase and decrease, respectively, in metabolic activity under the two spatially unstructured light conditions suggest that the SC is involved in more than receptor orientation and that it is peculiarly sensitive to visual change, perhaps functioning in some alerting capacity.

315.16

N-ACETYLASPARTYLGLUTAMATE IS RELEASED FROM CHICK RETINAL NEURONS AND OPTIC TECTUM UPON STIMULATION OF ACTION POTENTIALS IN THE OPTIC TRACT. D.A. Eagles, L.C. Williamson, and J.H. Neale. Dept. Biology, Georgetown Univ., Washington, D.C. 20057

N-acetylaspartylglutamate (NAAG) is found in high concentration in neurons throughout the nervous system including retinal ganglion cells in avian and mammalian species. We have developed a specific, highly sensitive (1-2 pg) radioimmunoassay (RIA) for NAAG to quantify peptide release in the central nervous system. The RIA was validated with HPLC and IC50 values were: NAAG, 2.5nM; NAA, 100uM; asp-glu, 1mM; and glu, >1mM. NAAG release was quantified in chick retina following depolarization.

To determine if this peptide is released under more physiological conditions electrophysiological stimulation was employed in the optic tract. Chick optic tecta and associated optic nerves were removed and placed in Tyrode's buffer A suction stimulating electrode was placed on the cut optic nerve ending and a recording electrode was inserted into the optic tectum to determine the optimal stimulation for eliciting action potentials in the optic tract. NAAG was released upon depolarization by high potassium in chick retina and by electrophysiological stimulation of the optic tract in the optic tectum. Supported by NIDA grant DA 02297.

315.17

GAD IMMUNOREACTIVITY IN THE CENTRAL VISUAL SYSTEM OF RANA PIPIENS. C. Tyler, K.V. Fite, and G.J. DeVries. Neuroscience and Behavior Program, University of Massachusetts, Amherst, MA 01003.

Immunocytochemistry was used to assess the presence of glutamic acid decarboxylase (GAD), the synthesizing enzyme for gamma aminobutyric acid (GABA), in the primary visual nuclei and terminal visual fields of the frog central visual system. GAD localization was mainly confined to primary terminal zones of optic nuclei. GAD-like immunoreactivity appeared in all primary visual nuclei, with the most dense immunoreactivity occuring in the pretectal terminal fields (posterior thalamic neuropil, nucleus mesencephali, and uncinate). Less intense immunoreactivity was also detected in the basal optic root nucleus. In the optic tectum the 6th and 8th laminae of the superficial optic tectum showed heavy and less dense GAD-like immunoreactivity, respectively. Results from comparing GAD-like immunoreactivity in normal and unilateral enucleated frogs will be presented.

315.18

MORPHOLOGY AND ELECTROPHYSIOLOGY OF IDENTIFIABLE SUBSETS OF VISUAL NEURONS IN THE OPTIC TECTUM OF GOLDFISH. D.M.Chen* and S.C.Sharma. Dept. of Ophthalmology, New York Med. Col., Valhalla, New York 10595.

Goldfish tectal cells were intracellularly recorded with HRP-filled glass micropipettes (70-90MΩ,5%HRP in 0.5M Kcl-Tris buffer). Iontophoretic injections (6-10nA pluses for 40sec) in 19 identifiable cells and their subsequent histological localization revealed that when one tectal neuron was injected with HRP, one or sometimes more adjacent neurons (which were not penetrated by recording electrode) also showed HRP labeling. Sixteen of these twin or multiple labeled neurons have their somata localized in or near the Stratum periventricular . These cells extended their slender dendritic process towards the surface with lateral branches in Central grey zone and terminal dendritic ramifications in the Stratum opticum. Three somata of remaindering cells were localized in the Stratum Fibrosum et Griseum Superficiale with their apical dendrites ramifying in the Stratum marginale and Stratum opticum. Intracellular recording revealed that the impaled tectal neuron showed a changing response patterns. These patterns alternated in the neuron. Present data provides credence to the existance of synaptic contacts between somata and processes of desmosome-like junctions, as well as tight junctions amongst processes of neurons in the Stratum periventricular (Supported by NEI grant 01426).

VISUAL SYSTEM: DEVELOPMENT AND PLASTICITY IV

316.1

EFFECTS OF LONG-TERM VISUAL CORTEX DAMAGE ON SPATIAL AND TEMPORAL FREQUENCY PROCESSING BY NEURONS IN THE CAT'S POSTEROMEDIAL LATERAL SUPRASLYVIAN (PMLS) CORTEX. W. Guido, L. Tong, and P.D. Spear. Dept. of Psychology and Center for Neuroscience, University of Wisconsin, Madison, WI 53706.

Previous studies indicate that neurons in the cat's PMLS cortex show functional compensation (normal direction selectivity and ocular dominance) following neonatal but not adult damage to visual cortical areas 17, 18, and 19. The purpose of the present study was to examine spatial and temporal frequency processing by PMLS neurons after long-term neonatal or adult visual cortex lesions. Recordings were made from PMLS neurons in adult cats that had ipsilateral areas 17, 18, and 19 removed on the day of birth (54 cells), 8 weeks of age (40 cells) or as an adult (39 cells). Neurons were stimulated with drifting sine-wave gratings of various spatial frequencies, contrasts, and temporal frequencies, and the responses were Fourier-analyzed. There were no significant differences in the spatial or temporal properties of PMLS neurons among any of the three groups. Most neurons had optimal spatial frequencies from 0.05 to 0.4 c/deg, spatial resolutions from 0.1 to 1.6 c/deg, and contrast sensitivity from 3 to 100 (33% to 1.0% contrast). When tested at the optimal spatial frequency, most PMLS neurons had optimal temporal frequencies from 3 to 10 Hz and high temporal frequency cut-offs from 10 to 20 Hz. The results in cats with a long-term lesion are similar to those in normal cats and cats with acute (24 hr) visual cortex damage. Together, these findings suggest that: 1) Normal spatial and temporal processing by PMLS neurons is independent of inputs from ipsilateral areas 17, 18, and 19. 2) Long-term visual cortex damage in adult cats does not lead to progressive secondary changes in PMLS cortex. 3) Cats with a neonatal lesion do not show compensation for the spatial and temporal properties of area 17, 18, or 19 neurons that were removed.

316.2

LACK OF BINOCULARITY CHANGES THE SPATIO-TEMPORAL CHARACTERISTICS OF NEURONS IN VISUAL AREA 18 OF THE CAT. S. Bisti*, G.P. Biral* and C. Trimarchi*. (SPON: H. Hollander). Inst. of Neurophysiology CNR, Pisa, I-56100.

Area 18 neurons shift their spatial frequency tuning curves along the spatial frequency axis on changing the temporal frequency of drifting stimuli. The higher the velocity of stimuli the lower the preferred spatial frequency (Bisti et al. J. Physiol., 359: 259, 1985). This spatio-temporal coupling could be instrumental in mechanisms involved in the perception of movement in depth (normally condition associated with convergent eye movements). We tested whether a disruption of binocularity during development may affect the spatio-temporal properties of area 18 neurons. We recorded single unit activity of neurons from three types of preparation, all with disturbed binocular vision: monocularly deprived, strabismic and young split-chiasm cats. In all three models, the spatio-temporal selectivity of area 18 neurons were uncoupled: spatial selectivity was invariant with velocity. These results reinforce the suggestion that spatio-temporal coupling of area 18 neurones is associated with the perception of motion in depth.

316.3

MECHANISM OF ANOMALOUS RETINAL CORRESPONDENCE: ALTERATIONS OF RECEPTIVE FIELD POSITION IN THE LATERAL SUPRASYLVIAN (LS) AREA OF STRABISMIC CATS. N.E.J. Berman, S. Grant*, M. Wilkes*, S.D. Shipp*, and R.I. Wilson*. Dept. of Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, KS 66103; Dept. of Anatomy, Medical College of Pennsylvania, Phila., PA 19129; Dept. of Anatomy, Univ. College London, London, UK WC1E 6BT.

Binocularity and binocular correspondence of receptive fields were determined in area 17 and LS of normal cats and of cats reared with convergent strabismus induced surgically at 3 weeks. Strabismus always caused a breakdown of binocularity in area 17, and the few remaining binocular neurons had receptive field positions arising from positions of normal retinal correspondence in the two eyes. A similar effect was seen in area LS in cats with pronounced strabismus (11° to 30° crossed). The group of cats with mild strabismus (<10°), however, showed nearly normal proportions of binocular neurons in area LS, and their receptive field locations arose from anomalous retinal positions which established visual correspondence of receptive fields in the two eyes. These receptive fields, which were of normal size, appeared to have shifted horizontally on the retina in an uncrossed direction, compensating for the degree of strabismus present. Similar processes could provide the basis of visual compensations that are common in humans with mild congenital strabismus. Supported by the MRC, Wellcome Trust and MH38399.

316.4

ORIENTATION-SELECTIVECELLS CAN EMERGE FROM A HEBBIAN MECHANISM THROUGH INTERACTIONS BETWEEN ON- AND OFF-CENTER INPUTS. K.D. Miller, Dept. of Physiology, UCSF, San Francisco, CA 94143.

A model of populations of ON-center and OFF-center geniculocortical inputs interacting via a Hebbian mechanism can yield orientation-selective cells. The receptive fields of the resulting cells consist of alternate bands of ON- and OFF- inputs. Oriented cells emerge when like-center inputs are correlated at near distances, and anti-correlated at further distances, while opposite-center inputs are anti-correlated at near distances, and correlated at further distances. Such correlations and anti-correlations at near distances have been observed in the retina (D. Mastronarde, 1983, *J. Neurophys.* **49**, 303). Those at further distances have not been observed, but could be present in the LGN or in non-oriented cortical input layers.

This model for the development of orientation selectivity is a variation of that of Linsker (1986, *PNAS* **83**, 7508). His model was biologically problematic in using both positive and negative synapses. While these could be implemented through two populations of synapses, one inhibitory and one excitatory, the "two populations" must be a single population in terms of the statistics of their activation. The current model instead uses two populations (ON- and OFF-center inputs) each of which makes only excitatory synapses but which are mutually anticorrelated.

Linsker's model suggests a form for the cortical organization of orientation selectivity. The current model leads to different constraints on the total synaptic strength supported by an individual cell than Linsker's, and therefore to differing cortical organization of orientation.

Supported by grants to M.P. Stryker from the System Development Foundation and the NSF.

316.5

Mechanisms underlying direction selective adaptation in neurons of the cat striate cortex. Stuart G. Marlin*, R. M. Douglas, and M.S. Cynader (SPON: A. Billy), Depts. of Psychology and Ophthalmology, University of British Columbia, Vancouver, British Columbia, Canada, V5Z 3N9.

We have previously shown that prolonged unidirectional motion induces changes in the direction selectivity of neurons in the cat striate cortex. To understand the mechanisms by which these changes occur we examined the effects of prolonged unidirectional motion on responses to sequentially flashed light bar stimuli (apparent motion) a procedure which is widely used to elucidate mechanisms of direction selectivity. We obtained quantitative measurements of the optimal spatial (Dopt) and temporal (Topt) displacements for sequentially flashed light bar stimulation. We then presented the two stimuli at Dopt using either preferred (Topt) or nonpreferred (-Topt) direction sequences and compared these responses with those to single flashes at each position. Responses to simultaneous presentation, and smooth motion (both preferred and nonpreferred direction) were also obtained. We compared the preadaptation responses in each stimulus condition to those obtained after 1 min of prolonged smooth motion in either the preferred or the nonpreferred direction. Prolonged stimulation in the preferred direction decreased the direction selectivity of cortical neurons and, in addition to small decreases in the responses to single flashes, resulted in proportionately larger decreases in the direction selective interactions associated with sequentially flashed stimuli. Conversely, prolonged nonpreferred direction stimulation resulted in increases in direction selectivity, small increases in the responses to single flashes and a proportionately larger increase in the interactions associated with direction selectivity. These results suggest that adaptation induced alterations in direction selectivity result from alterations of the sequence specific interactions within the receptive fields of cortical neurons and do not result from adaptation of responsivity at a precortical locus. (supported by NSERC of Canada, Grant # a9939 to M.S. Cynader)

316.6

FORCED CO-ACTIVITY IN VISUAL CORTEX REVEALS COMPLEXITY IN SIMPLE RECEPTIVE FIELDS. D. Shulz *, D. Debanne * and Y. Frégnac *, (SPON: M.J. Friedlander). Lab. de Neurobiologie et Neuropharmacologie du Développement. Université Paris XI, Orsay, 91405, FRANCE.

Two types of of receptive fields ("Simple" and "Complex" RF) are classically distinguished in visual cortex of adult mammals, on the basis of the spatial overlap of their responses to the static presentation (ON) and extinction (OFF) of a visual stimulus. These exclusive classes are thought to result from separate stable connections. Previous studies in the anesthetized and paralyzed cat, using protocols of cellular conditioning, allowed us to show that forced co-activity can modify the functional selectivity of a cortical cell in response to moving stimuli (Frégnac et al. *Nature*, 333:367, 1988).

A similar differential pairing procedure was applied with static stimuli to study the role of temporal correlation between pre- and post-synaptic activity in the plasticity of the spatial organization of the RF. The ON and OFF responses to a slit of light, shown repeatedly in a f ixed position, were respectively increased and blocked by brief opposite iontophoretic actions. Spatial generalization of possible effects were quantitatively measured across the RF.

Seventy-seven cells were recorded in area 17 of kittens and adult cats, reared normally. 53 were used for control of temporal stability of the RF organization, and 24 cells were submitted to 51 pairings. Long-lasting modifications of the ON/OFF ratio were observed in 11 out of the 24 paired cells (46%). In 7 of the modified cells the change was selectively restricted to the "paired" zone of the RF. The relative ON/OFF preference shifted in most cases (10/11) towards the response (ON or OFF) associated with the imposed increase in responsiveness, which supports the prediction of our working hypothesis. Largest effects were observed during the critical period.

This work is supported by grants from CEE (ST2J-0416-C) and NEDO to Y.F.

316.7

CORTICAL VISUAL EVOKED POTENTIALS IN RHESUS MONKEY INFANTS DEPRIVED OF DIETARY TAURINE. M.Neuringer and J.A.Sturman. Oregon Regional Primate Research Ctr., Beaverton, OR 97006 and Institute for Basic Research in Developmental Disabilities, Staten Island, NY 10213.

We are examining visual system development in rhesus monkeys deprived of dietary taurine from birth. One group of monkeys was fed a taurine-free soy protein human infant formula, while a second group received the same formula supplemented with 70 umole/100 ml taurine. We previously reported impaired visual acuity development in the taurine-deprived infants and degenerative changes in retinal photoreceptors, particularly cones in the foveal region.

Cortical visual evoked potentials (VEPs) to full-field flashes showed no consistent differences between groups. However, differences were seen in the VEP to phase-reversed square-wave gratings recorded at 6 spatial frequencies at 4, 8 and 12 weeks of age. Taurine-supplemented infants showed clear spatial tuning, and a shift in peak amplitude to higher spatial frequencies with age. In the taurine-deprived infants, amplitude vs. spatial frequency functions were shallow and showed no shift in peak spatial frequency from 8 to 12 weeks. These findings were correlated with abnormalities in the morphology of striate cortex at three months of age, including poor laminar organization and poor differentiation of pyramidal neurons and astrocytes.

These results provide new evidence for the importance of dietary taurine in primate nervous system development.

Supported by NIH grants HD-18678 and RR-00163

316.8

CYTOCHROME OXIDASE ACTIVITY IN THE ADULT PRIMATE VISUAL SYSTEM AFTER RECOVERY FROM BILATERAL FOVEAL ABLATIONS A.A. Skavenski and R.W. Sikes (SPON: L.E. White Jr.) Departments of Psychology and Physical Therapy, Northeastern University, Boston, MA 02115.

Following bilateral foveal ablations, neurons in the foveal projection zone of area 17 lose their response to light. Within three months, about half of these cells begin to respond to visual stimuli presented to the intact peripheral retina. What alterations in cytochrome oxidase activity (cytox) accompany this physiological plasticity?

Bilateral lesions of about 3 deg arc diameter were made in the foveae of M. nemestrina monkeys. Following recovery, the brains were processed for cytox. The lateral geniculate nucleus (LGN) showed a clear reduction (45%) in cytox in the parvocellular layers of the foveal region as compared to the non-foveal region while magnocellular layers appeared normal. In the foveal region of area 17, a comparable reduction (42%) in cytox was seen mainly in layers 4A and 4Cb; the chief target layers of the parvocellular LGN. Thus, it appears the parvocellular stream does not underlie this physiological recovery.

316.9

EFFECTS OF APHAKIA, COMBINED WITH OCCLUSION, ON AREA 17 OF INFANT RHESUS MONKEYS. M. Tigges, J. Tigges, J.R. Wilson, and R.G. Boothe. Yerkes Reg. Prim. Res. Center, & Dept. of Anat. & Cell Biol., Emory Univ., Atlanta, GA 30322.

In 5 newborn rhesus monkeys, unilateral lensectomies were performed and the aphakic eyes corrected with contact lenses. In 2 monkeys, the fellow eye was occluded almost continuously with opaque occluder lenses up to 2 years. In 3 monkeys, the fellow eye was occluded partially for short periods per day. When the monkeys were between 19 and 42 months old, 1 eye was enucleated; 4-14 days later, area 17 was reacted for cytochrome oxidase (CO) as a marker for deprivation-induced changes in the ocular dominance system. In all monkeys, layer 4C in the representation of the central visual field of area 17 exhibited darkly and lightly reacted CO stripes. Preliminary measurements showed that in all partially occluded monkeys, stripes connected to the occluded eyes were wider than stripes connected to the aphakic eyes. After continuous occlusion, the dark and light stripes in one monkey appeared to be of approximately equal width. In the second monkey, stripes related to the aphakic eye were slightly wider. These data demonstrate that an occluded eye is capable of maintaining a considerable amount of cortical territory when competing against an aphakic eye. Care and use of the monkeys complied with NIH guidelines. Supported by grants EY 06001, EY 05975 and NIH RR-00165.

316.10

CYTOARCHITECTURE OF OCULAR DOMINANCE COLUMNS IN OWL VISUAL CORTEX. J.D. Pettigrew and I.C. Gynther. Vision, Touch and Hearing Research Centre, Univ. of Queensland, St. Lucia, Qld. 4067, Australia.

The physiology of binocular interaction in the owl's visual cortex is very similar to that found in the striate cortex of cat or monkey. In particular, monocular concentric LGN inputs are used for binocular processing so that feature extraction is delayed until after the pathways from both eyes converge. We have taken advantage of two unusual features of the owl's visual cortex which facilitate investigation of the circuitry underlying binocular interaction, viz;- 1. the owl's LGN is monocular so that anterograde monosynaptic labelling of the whole input array from one eye is possible with a variety of different markers; 2. ocular dominance columns are not normally present in the owl but appear as a result of deprivation. After monocular deprivation, reconstructed ocular dominance columns formed stripes running orthogonally to the vertical meridian; after reverse monocular deprivation, a similar pattern was seen but stripes were narrower and no longer in register in the magnocellular versus the parvocellular regions of the LGN input layers. The fine morphology of identified neurons, and their possible relationships to ocular dominance column boundaries, are being studied with Lucifer Yellow injections in the brain slice preparation after being labelled with retrograde fluorescent tracers from output target regions of the visual cortex such as tectum, LGN and peri-ectostriatum. In this way we hope to learn more about the post-synaptic changes which may accompany the marked presynaptic rearrangements following deprivation.

316.11

ANALYSIS OF THE PATTERN OF OCULAR DOMINANCE IN NORMAL AND VISUALLY DEPRIVED CATS.

K. M. Murphy, D. G. Jones*, & R C. Van Sluyters.*
School of Optometry, University of California, Berkeley CA.

In primary visual cortex, inputs from the two eyes are largely segregated in layer IV, forming the anatomical correlate of physiologically identified ocular dominance columns. We have qualitatively and quantitatively analyzed the 2D spatial array of cortical ocular dominance beads in both normal and monocularly deprived cats. The pattern of ocular dominance for one eye was visualized following intraocular injection of WGA-HRP and subsequent unfolding and flattening of the cortex. In normal cats, labelled regions serving one eye form an irregularly branching and beaded pattern. Inputs from an eye deprived of pattern vision from the time of natural eye opening form many isolated, unconnected ocular dominance beads. Yet, the deprived eye retains a large degree of territory in layer IV. Inputs from the nondeprived eye of monocularly deprived cats are less segregated, however, ocular dominance beads are discernible. Statistical analyses of the 2D arrangement of ocular dominance beads reveal that this pattern is similar for normal cats and both eyes of monocularly deprived cats. The arrangement is nonrandom, indicating regularity in the array of beads. The average spacing of ocular dominance beads is about 675 μm. Analysis using Voronoi polygons and Delaunay triangles demonstrates that the cat pattern has no overall orientation, whereas the pattern of cytochrome oxidase blobs for one eye of a monkey is clearly oriented. These analyses indicate that monocular deprivation in the cat does not perturb the overall spatial arrangement of ocular dominance beads.

316.12

CONCENTRATION-DEPENDENT SUPPRESSION BY LITHIUM OF EFFECTS ON MONOCULAR DEPRIVATION IN KITTENS. T.Ohashi* and T. Kasamatsu (SPON: K. Nakayama). Smith-Kettlewell Institute, San Francisco, CA 94115.

Lithium (Li) reduced effects of monocular deprivation on ocular dominance in kitten visual cortex and the Li's effect was variable depending on individuals (Ohashi et al., 1988). Here we examined whether there are correlations between plasma concentrations of Li and the effects of monocular deprivation. Fifteen kittens (4-7 weeks old) were i.p. injected with either 25mg/kg Li_2CO_3, 81.8mg/kg RbCl, or saline alone, twice a day for 3 weeks. Li in plasma was measured with ion potentiometry. The ocular dominance distribution (Hubel and Wiesel, 1962) was determined in area 17 after brief monocular deprivation.

We found that 1) Li had no discernible effects on receptive-field properties of recorded cells; 2) the expected shift in ocular dominance following monocular deprivation was blocked. There was a positive correlation between the Li content in plasm and the proportion of binocular cells. Three kittens with plasm Li higher than 1.0mEq/L at the end of monocular deprivation showed high binocularity (B=0.54, 180 cells) and 5 kittens with Li lower than 0.5mEq/L showed low binocularity (B=0.34, 300 cells); 3) 7 control kittens injected with either RbCl (N=3) or normal saline (N=4) showed the usual shift with low binocularity (RbCl,B=0.33, 210 cells; saline,B=0.22, 240 cells). We thus concluded that Li reduces ocular dominance plasticity in a concentration-dependent manner.

316.13

ANTISERUM AGAINST THE ASTROGLIAL S-100 PROTEIN INTERFERES WITH OCULAR DOMINANCE PLASTICITY IN KITTEN VISUAL CORTEX. C.M. Müller* (SPON: European Neuroscience Association), Max-Planck-Institute for Brain Research, 6000 Frankfurt 71, F.R.G.

During a restricted postnatal period the circuitry of the kitten visual cortex undergoes an experience-dependent modification. Previous studies show that cortical malleability is limited to periods where cortical astroglial cells are immature, and plasticity can be restored in adult animals by transplantation of young astrocytes cultured from kitten cortex. One possibility for a glial involvement in cortical plasticity is via biochemical signals released from astrocytes. As the S-100 protein can be detected in cortical astrocytes from the beginning of the critical period and has been shown to be involved in plastic changes in the CNS, I tested the influence of an antiserum against this protein on ocular-dominance (OD) shifts resulting from monocular deprivation (MD). Cannulas attached to osmotic minipumps (Alza 2002) containing antiserum against S-100 (1:10 or 1:20; Dakopatts), or non-immune-serum, were implanted into kitten visual cortex. This procedure was followed by one week of MD. Electrophysiological assessment of the OD distribution after this period revealed a concentration-dependent retardation of ocular-dominance shifts in the hemisphere infused with the antiserum. Normal OD-shifts were found in control hemispheres receiving infusion of non-immune-serum. The infused antiserum was immunocytochemically localized exclusively in the extracellular compartment after termination of the electrophysiological experiments. The data suggest that the S-100 protein may be a crucial factor in the mediation of ocular-dominance plasticity. This might be due to the known neuronotrophic action of this protein. Release of S-100 protein by astroglial cells may be one mechanism of a glia-neuron interrelation in cortical plasticity.

316.14

A NEUROCHEMICAL BASIS OF OCULAR DOMINANCE PLASTICITY MAINTAINED IN DARK-REARED CATS. T. Kasamatsu, T. Shirokawa and V.S. Ramachandran. Smith-Kettlewell Eye Res. Inst., San Francisco, CA 94115 and Dept. Psychol., Univ. Calif., San Diego, La Jolla, CA 92093.

Rearing newborn kittens in the dark has retarding effects on the normal maturation of receptive fields in visual cortex, effectively postponing the end of cortical susceptibility to abnormal visual experience beyond the usual age limit (Cynader and Mitchell, 1980).

We studied whether the brain noradrenergic (NA) system plays a role in this matter. One hemisphere of dark-reared cats(6-18 months) was directly infused with either 4mM 6-hydroxydopamine(6-OHDA), or 10mM D,L-metoprolol (β_1 adrenoreceptor antagonist, or 10mM D-metoprolol (biologically inert)dissolved in 0.4% ascorbate saline. The other hemisphere was infused with either the vehicle solution alone or 0.05mM L-NA. The microinfusion, together with monocular lid suture, lasted for the next 4 weeks after the cats were brought to light. We found that 1) an expected shift in ocular dominance was partially suppressed in the 6-OHDA-infused hemisphere, though the shift toward the open eye was obvious in the control hemisphere, 2) the blockade of the ocular dominance shift was seen in the cortex infused with D-metoprolol, and 3) the shift was more strongly expressed in the NA-infused cortex than control. We conclude that the NA-β_1 adrenoreceptor system is necessary for expression of cortical plasticity in visual cortex of dark-reared cats. (Supported by USPHS EY06733.)

316.15

PLASTICITY IN ADULT AND ADOLESCENT CAT VISUAL CORTEX. K. Fox, N. Daw, and H. Sato. Dept. Cell Biol, Washington Univ. Med. Sch., St. Louis, MO 63110

NMDA receptors and LTP are thought to be related to plasticity. Both occur in the cortex of adult cats, though visually activated NMDA receptors are restricted to superficial layers. If both attributes are sufficient for plasticity then layers II and III of adult visual cortex should be plastic. If both attributes are necessary for plasticity only layers II and III should be plastic. Monocular deprivation has become a standard test for experience dependent plasticity, but the literature contains results on just 6 adult cats and 7 adolescents, none of which were assayed for plasticity as a function of layer. We therefore re-examined ocular dominance (OD) plasticity in older animals as a function of layer. Three month monocular deprivations were imposed starting at 8 months (6 animals). Both hemispheres were assayed for ocular dominance over two days. In the 8-11 month group, 532 cells were tested for ocular dominance. A moderate but significant shift toward the open eye occurred in layers V and VI as well as in layers II and III. Thus, OD plasticity in adolescents can occur through mechanisms which do not involve NMDA receptors. The shift in layer IV was small, rather variable between animals and insignificant. We are now studying the effect of 3 month eye sutures in two older age groups; 15 months and adults of at least 7 years.

316.16

EFFECT OF NOREPINEPHRINE AND ACETYLCHOLINE DEPLETION ON PLASTICITY IN KITTEN VISUAL CORTEX. B. Gordon, B. Mitchell*, K. Mohtadi*, E. Roth*, Y. Tseng*, F. Turk* Inst. Neurosci., Univ. of Oregon, Eugene, OR 97403.

In 1985 Bear and Singer reported that depleting both norepinephrine (NE) and acetylcholine (ACh) decreased visual cortex plasticity assayed by the effect of monocular deprivation (MD). We have amplified this result. We depleted cortical NE and ACh in 35-42 day kittens by lesioning the white matter behind the cingulate gyrus. One eye was sutured on the day of the lesion. We recorded from the visual cortex 7 days later. NE content was measured by HPLC. ACh depletion was inferred from depletion of choline acetyltransferase (ChAT) activity. NE depletion averaged 53% in the successfully depleted animals. Depletion of ChAT activity was consistent with NE depletion. In the first group of animals (N=5) the lesion was contralateral to the deprived eye. Plasticity was decreased but only on the lesioned side where 70% of the cells were driven by the deprived eye. On the unlesioned side only 16% of the cells were driven by the deprived eye. In the second group (N=2) the lesion was also contralateral to the deprived eye, but it was unsuccessful; that is, NE was not depleted on the lesioned side. In these animals only 14% of the cells were driven by the deprived eye. In the third group (N=4) the lesion was ipsilateral to the deprived eye. In these animals only about 26% of the cells on the lesioned side were driven by the deprived eye. The fourth group (N=2) was lesioned but not visually deprived. Animals in this group had normal ocular dominance histograms. We conclude that depletion of NE and ACh does decrease plasticity; that is, it protects the deprived eye from losing its ability to drive cortical cells. Surprisingly, depletion protects only the normally dominant contralateral pathway; the ipsilateral visual pathway remains plastic.

316.17

IBOTENATE-STIMULATED PHOSPHOINOSITIDE TURNOVER: A BIOCHEMICAL CORRELATE OF THE CRITICAL PERIOD FOR SYNAPTIC PLASTICITY. S. M. Dudek and M. F. Bear. Center for Neural Science, Brown University, Providence, RI 02912.

One distinguishing characteristic of experience-dependent synaptic modifications in the kitten striate cortex is that they are primarily restricted to a finite period of postnatal development often refered to as the "critical period." Though estimates of the length of the critical period have varied, there is general agreement that sensitivity to lid closure begins in kittens at about 3 weeks of age, peaks during the fifth week, and gradually disappears between 12 and 16 weeks. We have discovered that this is precisely the developmental profile for stimulation of phosphoinositide (PIns) turnover by the excitatory amino acid ibotenate as measured in synaptoneurosomes prepared from kitten striate cortex.

Ibotenate stimulates PIns turnover at a novel site as neither NMDA, KA nor AMPA are effective. The transient rise in ibotenate-stimulated phosphoinositide hydrolysis at 5 weeks of age does not occur in the striate cortex of kittens raised in complete darkness. Unlike ibotenate, the muscarinic agonist carbachol stimulates PIns turnover in striate cortex at all postnatal ages and is unaffected by dark-rearing. Together, these data indicate that a novel type of excitatory amino acid (EAA) receptor mechanism linked to phospholipase C is transiently expressed in striate cortex during the second and third postnatal months, and that its expression is triggered by visual experience.

PIns turnover leads to the formation of two intracellular second messengers, inositol triphosphate and diacyl glycerol. Thus, these data suggest that excitatory synaptic transmission during the critical period is characterized by unique patterns of second messenger activity, and raise the possibility that PIns hydrolysis may play a central role in the experience-dependent modification of visual cortex. Theoretical considerations have led us to suggest that EAA stimulated PIns turnover may promote the weakening or elimination of synapses when input activation fails to coincide with target depolarization. (Supported by ONR contract N00014-81-K0136 and NIH grant NS06929)

316.18

GAP-43 LEVELS IN CAT STRIATE CORTEX PEAK AT THE HEIGHT OF THE CRITICAL PERIOD. L. I. Benowitz, W. R. Rodriguez*, G. T. Prusky and M. S. Cynader (SPON: J. M. Gilbert) McLean Hospital, Harvard Med. Sch., Belmont, MA 02178; Eye Care Center, Univ. British Columbia, Vancouver, B. C. V5Z 3N9

The maturation of feature detectors in the cat striate cortex normally occurs within the first two months of life. The effects of physiological manipulations in altering the underlying synaptic organization are maximal at around postnatal day 30, the peak of the so-called *critical period.* One molecule that may play a role in determining the critical period could be the neuron-specific phosphoprotein GAP-43, since this protein has been implicated in the development and restructuring of synaptic relationships in other systems. A monospecific antibody prepared in sheep against purified rat GAP-43 was used in the ABC-peroxidase method to stain free-floating sections derived from kittens aged 0, 15, 30, 45, and 60 days postnatal and from adult cats. In neonates, the protein was essentially absent from the cortical mantle outside of the marginal zone (i.e., layer I), but was heavily concentrated in the thalamocortical fibers in the submarginal layer. By day 15, the cortical neuropil began showing increasing levels of the protein, and by day 30 GAP-43 levels peaked in the striate area and in much of the rest of the cortex, particularly in layer I and secondarily in the IV-V boundary. Thereafter, levels declined, and in the adult visual cortex, only very low levels of the protein could be detected. Since GAP-43 appears to transduce physiological activity patterns to alter the functions and structure of the nerve terminal membrane, its presence at the peak of the critical period is consistent with the notion of this protein contributing to activity-dependent tuning processes in this system. *Support: NIH EY 05690 and MRC of Canada.*

316.19

PERSISTENT HIGH-LEVEL EXPRESSION OF GAP-43 FOLLOWING DARK-REARING THROUGH THE CRITICAL PERIOD. SM de la Monte*, GW Mower, K Rosen, MC Fishman, Howard Hughes Medical Institute, Massachusetts General Hospital, Children's Hospital Medical Center, Harvard Medical School, Boston, MA 02114.

GAP-43 is a neuron-specific growth-associated protein expressed at high levels during neuronal growth and regeneration. Recently we and others demonstrated GAP-43 expression to be developmentally regulated such that in the immature brain the levels are globally high, whereas in the adult the levels are generally low, save a few regions including the hippocampus and olfactory bulb, where active restructuring of synapses may continue throughout life. In the present study we examined GAP-43 expression in the visual cortex of cats reared in complete darkness throughout the critical period of visual development. Dark-rearing is known to prolong physiological plasticity of this region. Norther blot analysis of Area 17 demonstrated 7-fold higher levels of GAP-43 mRNA and in situ hybridization disclosed twice as many grains per neuronal cell body in the 10 day old kitten as compared with 5 month old cats. GAP-43 mRNA levels in dark-reared cats were 40% to 70% higher than light-reared cats, and neuronal cell bodies contained the same density of in situ hybridization grains as in the kitten. Similarly, higher levels of immunoreactive GAP-43 in both neuronal perikarya and neurites were present in the dark-reared visual cortex as compared with light-reared controls. The findings suggest: 1-GAP-43 expression is developmentally regulated in the cat visual cortex; and 2-the developmental regulation of GAP-43 gene expression can be modulated by visual experience.-

316.20

COEXISTENCE OF SOMATOSTATIN AND MAP-2 IN DEVELOPING RAT VISUAL CORTEX. S.C. Feldman Dept. of Anatomy, New Jersey Medical School, Newark, NJ 07103.

Perinatal visual cortex (VC) is characterized by large numbers of somatostatin (SRIF)-immunoreactive cells and fibers. Prior to the formation of the cortical layers, i.e., D6, two types of SRIF cells are seen: those which resemble typical neurons, and those which are relatively atypical. The former are present in small numbers distributed throughout the cortical plate. The latter are numerous, transient and limited to the ventral one-half of the plate. The identity of these has been questioned. To determine if these cells are neuronal, sections of VC from animals aged E20 and days 0 and 8 were stained for both SRIF and MAP-2. MAP-2 is a microtubule-associated protein limited to neuronal dendrites. Sections were first reacted for MAP-2 or SRIF using the peroxidase-anti-peroxidase technique; the sections were then reacted for the other protein using avidin-biotin and alkaline phosphatase. Preabsorption of the antibody with synthetic SRIF (10μM) gave the same results as MAP-2 alone. In all sections, both types of SRIF-positive cells were labeled with MAP-2, except on Day 8 when the atypical cells were no longer seen. No SRIF cells were labeled with the glial antigen S-100. These results suggest that the atypical cells may be neurons, and lend support to the hypothesis that these cells are immature, possibly migrating, neurons.

316.21

ELECTROPHYSIOLOGICAL AND NEUROCHEMICAL DIFFERENCES IN THE VISUAL CORTEX OF LIGHT/DARK-AND DARK-REARED RATS. B.W. Bakkum, J.D. Port*, R.S. Cohen and L.A. Benevento Dept. of Anatomy and Cell Biology, U. of Illinois at Chicago, Chicago, IL 60612 and Dept. of Anatomy, U. of Maryland Baltimore Coll. of Dental Surgery, Baltimore, MD 21201.

Sprague-Dawley rats were raised to the age of two months in either complete darkness (Dk) or a 14hr on-10hr off lighting schedule (Lt/Dk). Single units were recorded from area 17 during visual stimulation consisting of whole field flashes, light and dark moving slits or bars and moving checkerboards. The animals were recorded from immediately after removal from their Dk or Lt/Dk environment. 321 cells were recorded. 92% of the Lt/Dk cells, but only 66% of the Dk cells, were visually responsive. When compared to Lt/Dk cells, Dk cells had a higher rate of spontaneous activity characterized by either a high tonic discharge or bursts every 1-4 seconds in the absence of stimulation. Dk cells had less finely tuned responses. For example, 21% of Dk cells, but no Lt/Dk cells, responded only to flashes, while 23% of the Lt/Dk cells, but no Dk cells, responded only to a moving bar. Only Lt/Dk cells were found to be direction sensitive and capable of responding to fast rather than slow moving stimuli. Orientation selectivity was found in 30% of Lt/Dk cells but in only 5% of Dk cells. Based on these changes in inhibitory function, we investigated the effects of dark-rearing on the number of GABA and somatostatin immunoreactive cells in area 17 of three month old rats. In all six cortical layers of area 17, there was a decrease in GABA-positive cells in Dk versus Lt/Dk rats ($p<0.001$). No changes were detected in numbers of somatostatin cells. Taken together, these data suggest that dark-rearing affects the development of normal inhibitory connections in area 17 of the rat visual cortex. Supported by NIH grant NS 15889.

WEDNESDAY PM SYMPOSIA

318

SYMPOSIUM **MESOPONTINE CHOLINERGIC NEURONS: THE NEURONAL SUBSTRATE OF THE ASCENDING RETICULAR ACTIVATING SYSTEM?** PB Reiner, UBC (Chair); M Steriade, Laval; K Semba, UBC; SR Vincent, UBC; C Leonard, NYU. DA McCormick, Yale; RW McCarley, Harvard

The concept that there exists in brain one or more systems involved in control of arousal has long intrigued neurobiologists. The landmark studies of Shute & Lewis suggested that mesopontine cholinergic neurons might be the elusive cell group. In recent years, a great deal has been learned about the biology of these neurons, and this symposium serves as an opportunity to review the merits of the hypothesis that these cholinergic neurons constitute the neuronal substrate of the ascending reticular activating system.

Mircea Steriade will review the concept of the ARAS in both its historical and modern usages, and will present data on these neurons' physiological properties *in vivo*. *Kazue Semba* will discuss the afferent and efferent connectivity of mesopontine cholinergic neurons. *Steve Vincent* will present data on the various transmitter-candidates which have been identified in these neurons in mammals and other vertebrates. *Chris Leonard* will discuss the physiology of mesopontine cholinergic neurons *in vitro*, as well as their responses to transmitter candidates such as acetylcholine. *Dave McCormick* will discuss the effects of acetylcholine on membrane properties of thalamic and cortical neurons *in vitro*. *Bob McCarley* will discuss the projections of mesopontine cholinergic neurons to the the medial pontine reticular formation, and the effects of acetylcholine in the MPRF on both neuronal physiology and behavior.

319

SYMPOSIUM: HEBBIAN SYNAPSE: LEARNING RULES AND MECHANISMS. J. Lisman, Brandeis Univ. (Chairperson); T. Brown, Yale Univ.; T. Sejnowski, Salk Inst. & UCSD; R. Nicoll, UCSF

In a now classic treatise, Hebb proposed that active synapses are strengthened if their activity leads to firing of the postsynaptic cell. Tom Brown will give a historical overview of this concept and describe its applicability to long-term potentiation (LTP) in the CA1 region of hippocampus. The key role of the NMDA receptor in LTP will be described. Brown's talk will conclude with a review of both theoretical and experimental work suggesting that synaptic plasticity governed by the Hebb rule underlies aspects of learning and memory. Terry Sejnowski will describe a process complimentary to the Hebb process that decreases synaptic strength when pre-synaptic and postsynaptic activity do not occur together. Roger Nicoll will describe the involvement of Ca in triggering different phases of LTP and the role of non-NMDA receptors in the expression of enhanced synaptic efficacy. John Lisman will describe how the Ca/Calmodulin kinase molecules contained within the postsynaptic density could serve as a memory storage device capable of storing graded synaptic weights and controlling synaptic efficacy. To conclude this symposium, Terry Sejnowski will discuss the relationship between learning and neural development (some of which depends on the NMDA receptor), and identify important cellular and network aspects of these processes that remain to be investigated.

VISUAL CORTEX IV

320.1

SYNCHONOUS, COHERENT BURST-FIRING BY NEURONS IN LAMINA III OF RAT VISUAL CORTEX *IN VITRO*. R. B. Langdon, D. M. Kuhn*, and M. Sur, Dept. of Brain and Cognitive Sciences, Mass. Inst. of Tech., Cambridge, MA 02139, USA.

We have studied field potentials in isolated slices of rat area 17 to observe gross features of aggregate neuronal behavior in this neocortical circuit. The data indicate that the major route by which excitation ascends after white matter stimulation in our (coronal) slices is by direct retrograde activation of layer III pyramidal cells. These neurons often respond to low-amplitude single-shock stimulation of the white matter by firing in unison one short-duration (10 to 15 msec) burst at roughly 300 Hz. Individual spikes during such bursts are synchronous and phase-locked tightly to the stimulation ('coherent'). These bursts depend upon neurotransmission that is blocked by CNQX, but not APV. Foci of coherent bursting spread horizontally for up to 1.6 mm at roughly 0.1 m/sec. During this horizontal spreading, burst activity remains focused in layer III. Preliminary data suggest that burst amplitudes can be potentiated by stimulating with 35 msec trains at the natural burst frequency (12 trains, 5 sec apart).

The bursting somewhat resembles epileptiform activity in media that contain bicuculline or penicillin (e.g., Connors, ***Nature*** **310**:865, 1984), but occurs in normal, drug-free medium and is lamina-specific and stimulus-locked. Preliminary data suggest that similar bursts can follow optic chiasm or LGN stimulation in supragranular cat visual cortex *in vivo*. We conclude that lamina III neurons can burst at high frequencies and are interconnected in a way that renders them prone to fire coherently. Coherent firing of specific ensembles of lamina III neurons may play a role in the representation of features in visual space in area 17, and their transmission to other regions of cortex.

Supported by NIH Grant EY07023.

320.2

OCCURRENCE OF OSCILLATORY NEURONAL RESPONSES IN CAT VISUAL CORTEX DEPENDS ON STIMULUS COHERENCY. A.K.Engel*, P.König*, C.M.Gray* and W.Singer (SPON: European Neuroscience Association). Max-Planck-Institute for Brain Research, 6000 Frankfurt 71, F.R.G.

We have previously shown that a fraction of cells in cat area 17 exhibits an oscillatory firing behaviour in a frequency range of 40-60Hz (Gray and Singer, PNAS 86, 1698-1702). Currently we investigate the dependence of the oscillatory responses on various stimulus parameters. In particular, we attempt to test the influence of stimuli which do not consist of a single continous contour. To detect rhythmic firing patterns, we computed autocorrelograms of single and multiunit responses. The frequency and the amplitude of the modulation of the autocorrelograms were evaluated quantitatively.

Our results show (i) that both the frequency and modulation strength of the oscillatory responses are independent of stimulus orientation. (ii) The response frequency increases with stimulus velocity, but the relative modulation strength of the autocorrelogram is unchanged. Above a cut-off velocity of 5-7deg/sec, the oscillatory behaviour disappears. (iii) The strength of the oscillatory response depends on binocular stimulation. (iv) Stimulation with random-dot patterns eliminates the rhythmicity of the response. (v) Simultaneous presentation of two light bars with different orientations reduces the strength of the oscillation in a significant fraction of cases.

We conclude that if a continuous light-dark contour is applied as a stimulus, the cells of area 17 show a strong oscillatory modulation of their responses for a wide range of stimulus orientations and velocities. However, if the continuity of the stimulus is disturbed, the oscillatory modulation is attenuated. These results suggests that oscillatory responses are particularly susceptible to the coherency of the visual stimulus. In addition, cooperativity between inputs from the two eyes may also be encoded in the oscillatory firing behaviour of the neurons.

320.3

SYNCHRONIZATION OF OSCILLATORY RESPONSES IN CAT CORTICAL AREA 17 REFLECTS GLOBAL COHERENCY OF VISUAL STIMULI. P.König*, C.M.Gray*, A.K.Engel* and W.Singer. Max-Planck-Institute for Brain Research, 6000 Frankfurt 71, F.R.G.

We have previously shown that oscillatory neuronal responses in cat visual cortex can transiently synchronize (Gray et al., Nature 338, 334-337). It is our current working hypothesis that this synchronization serves to link features in different parts of the visual field and, in particular, encodes the coherency of an object. We currently test this hypothesis by recording with multiple electrodes from area 17 and subjecting the data to cross-correlation analysis.

Our data indicate that (i) stimulation of overlapping receptive fields with a continuous contour leads to highly synchronized oscillatory activity in most of the responsive cortical columns. Disruption of the stimulus coherency interferes with this synchronization. (ii) Cortical sites with spatially separate receptive fields and similar orientation preferences can strongly be synchronized if they are concurrently stimulated with a single long light bar. If a gap is inserted into this stimulus, synchronization becomes weaker. The interaction disappears completely, if the two stimulus fragments are moved in opposite directions. (iii) Cells with non-overlapping receptive fields and dissimilar orientation preferences tend to synchronize better if stimuli with identical orientations are presented on both fields. (iv) Binocular stimulation in appropriate retinotopic correspondence facilitates the synchronization of responses in spatially separate columns.

In conclusion, the data available are consistent with the hypothesis that phase-synchronization is sensitive to global stimulus features, such as spatial continuity, similiarity of orientation of contours, coherent motion and disparity. Commonality of these features among various parts of an object may thus be reflected in the synchronization of oscillatory cortical responses.

320.4

STIMULUS-SPECIFIC INTERCOLUMNAR INTERACTIONS OF OSCILLATORY NEURONAL RESPONSES IN THE VISUAL CORTEX OF ALERT CATS. C.M.Gray, A.Raether*, W.Singer (SPON:J.E. Skinner), Max-Planck-Institute for Brain Research,6000 Frankfurt/M,71, F.R.G.

Previously, we have shown that a subpopulation of cells in the cat visual cortex exhibit stimulus-specific oscillatory responses at a frequency near 50 Hz (Gray and Singer,PNAS,86, 1989). These oscillatory responses exhibit inter-columnar synchronization which depends on their spatial separation, similarity of orientation preference and the global properties of visual stimuli (Gray,et.al., Nature,338,1989). These findings suggest that the synchronization of oscillatory responses in different columns might be a way to establish relations between common features in different parts of the visual field. To substantiate this hypothesis we considered it crucial to demonstrate the occurrence of oscillatory responses and their intercolumnar synchronization in alert animals. We recorded neuronal responses from up to 8 floating micro-electrodes in the striate cortex of kittens and adult cats while the animals viewed a slowly drifting square wave grating, the orientation of which changed continuously through 360 degrees over a period of 20 sec. Auto- and cross-correlation analysis of the neuronal responses revealed that 1) a large fraction of neurons exhibit oscillatory responses to stimuli of optimal orientation at a frequency between 40-60 Hz, 2) neurons in spatially separate columns synchronize their respective oscillatory responses when their orientation tuning overlaps, and 3) the magnitude of the oscillatory responses and their synchronization is greater with binocular stimulation than with monocular stimulation. These findings closely resemble those obtained under anesthesia and thus provide support our hypothesis stated above.

320.5

NEURAL CONNECTIVITY AND PHASE COHERENCE IN THE FIRING PATTERNS OF STRIATE CORTICAL NEURONS. C. Koch, D. M. Kammen* and P. Holmes*†. (SPON: M. Nelson). Divisions of Biology and of Eng. and Applied Science, Caltech, Pasadena, CA, 91125. † Permanent address: Dept. of Theor. & Applied Mechanics, Cornell University, Ithaca, NY 14853.

Stimulus specific collective oscillations have been reported in olfactory (Freeman, W. J. Elect. Clin. Neurophys., 44:369, 1979) and more recently in visual cortical (Gray, C. M. *et al.* Nature, 338:334, 1989) areas. The oscillations observed in the striate cortex of anaesthetised cats (Area 17) are remarkable in that cells tuned to similar orientations but spatially widely separated on the cortical surface (up to 7mm) exhibit stimulus dependent frequency locking with no apparent phase delay.

We have developed an analytically tractable model for a population of cells that receive as feedback from a single comparator neuron a signal proportional to the average activity of the entire population. No direct connections between the excitatory neurons are present. This model exhibits the rapid onset of frequency and phase locking and robust noise tolerance that is consistent with the data from Area 17.

In circuits based on direct lateral connections between the excitatory cells (as is the case in the striate cortex) we find that coherent oscillations can be generated, however, frequency and phase coherence are exceptional rather than generic results. Furthermore the *onset latency* of oscillation is a strong function of the neural population size.

We can therefore conclude that while direct lateral connections are certainly crucial in the function of the visual cortex it is probably the feedback circuits that are central to the establishment of long range dynamic coherence in response to visual stimuli.

320.6

WITHDRAWN

320.7

A DETAILED COMPUTER SIMULATION OF RECEPTIVE FIELD PROPERTIES IN PRIMARY VISUAL CORTEX OF CAT. U. J. Wehmeier, F. Wörgötter and C. Koch, Division of Biology, 216-76, Caltech, Pasadena, CA 91125.

Simple cells in mammalian visual cortex exhibit a number of salient receptive field properties such as orientation selectivity, direction selectivity, and contrast gain control. These cells also display a high degree of receptive field structure, including ON and OFF subfields which have been shown to be mutually antagonistic and usually non-overlapping. In our detailed computer simulation of a patch of cat visual system, we examine a number of possible circuits which produce behaviour consistent with electrophysiology.

Our simulation (Wehmeier & Koch, 1988) has been extended to model approximately 30,000 cells with passive integration and spiking behaviour comprising a 5° by 5° patch of visual angle from the retina, the LGN, and the early cortical layers. The retina is modelled by DOG units with both ON- and OFF- center receptive field profiles. The retinal activity is subsequently passed to two populations of geniculate cells (ON and OFF), and relayed to the cortical input layers (comprising excitatory pyramidal cells and inhibitory stellate and basket cells).

A number of classes of simple cell receptive fields have been synthesized by circuits combining inhibitory and excitatory interactions between ON and OFF subfields. Single unit responses to both moving and flashed stimuli have been produced and compared to experimental intracellular records (Ferster, 1988). A detailed analysis of circuits involving inhibitory feedback has been done to demonstrate how orientation and direction tuning may be simultaneously induced in cortical simple cells within the constraints of known tuning properties. Both long and short range asymmetrical inhibition are shown to be effective in creating direction specificity. The simulated cortical response patterns were tested by presenting moving bars and gratings to our retina and comparing tuning curves to extracellular responses of real cells.

320.8

RECIPROCAL CONNECTIONS WITHIN CAT VISUAL CORTEX (AREA 18). J. D. Boyd* and J. A. Matsubara (SPON: D. E. Mitchell). Depts. of Ophthalmology, Anatomy, and Neuroscience Group. Univ. of British Columbia, Canada, V5Z 3N9.

In visual cortex, columns of neurons interlink specifically with some, but not other, nearby columns. This pattern of intrinsic connections can be visualized by small, focal injections of tracers such as WGA-HRP and is exploited in models attempting to explain properties of visual cortical neurons such as receptive field size, center-surround antagonism, ocular dominance and orientation selectivity. We, and others, have assumed that the intrinsic connections in visual cortex are reciprocal, since WGA-HRP labeled patches contain both anterograde, terminal labeling and retrograde neuronal labeling. However, because it is often difficult to quantify or even distinguish the dust-like, anterograde reaction product from collateral transport or artifact, the exact nature of reciprocity among the intracortical networks remains uncertain.

We examined the spatial relationship of cells labeled with WGA-HRP to axonal terminal fields labeled with Phasaeolus vulgaris leucoagglutinin (Pha-L). A cocktail of 4% WGA-HRP and 2.5% Pha-L was iontophoresed through a micropipette placed stereotaxically in area 18 of the cat. Alternate sections were reacted for HRP histochemistry or Pha-L immunocytochemistry.

Most PHA-L labeled terminal fields were punctate, averaging 0.4 mm in diameter. Between 5 and 10 distinct fields, with an interfield spacing of about 1 mm, were present surrounding a single cocktail injection. While all patches of retrogradely labeled cells overlapped with PHA-L labeled terminal fields, we observed more punctate terminal fields than retrogradely labeled patches. The additional punctate fields of terminals interdigitated between patches of retrogradely labeled cells. These results suggest a far more complicated arrangement of local connectivity, comprising separate anterograde and reciprocal networks, than was previously known. (Funded by MRC MA-9150 to J.M.)

320.9

DISTRIBUTION OF GABA-IMMUNOREACTIVE NEURONS WITHIN THE INTRACORTICAL PATCHES IN CAT VISUAL CORTEX (AREA 18): A DOUBLE LABEL STUDY. J.A. Matsubara and J.D. Boyd* Depts. of Ophthal., Anatomy and the Neurosciences Group. Univ of Brit. Columbia. V5Z3N9 Canada.

The local, intracortical connections in visual cortex have been studied by using small, focal injections of the retrograde tracer, WGA-HRP. The pattern of labeling arising from such injections is columnar, patchy and periodic, exhibiting a repeat spacing of about 1 mm. Our earlier studies, combining physiological mapping with injections of WGA-HRP, revealed that the interconnections linked together sites with different preferred orientations, which led us to speculate that an inhibitory connection between such sites might serve to enhance orientation tuning in visual cortex.

We looked for anatomical evidence for a direct, inhibitory connection between interconnected sites by combining retrograde labeling using WGA-HRP and immunohistochemistry using an antiserum against GABA. Sections were first reacted using the TMB method, stabilized (Horn and Hoffman, 1987) and then processed for GABA immunohistochemistry. The resulting WGA-HRP labeled cells were filled with a black, granular precipitate while the GABA cells were light brown and opaque in color. Double labeled (dl) neurons contained both reaction products.

We found three populations of dl neurons. The dl cells in the first set were distributed randomly in all directions in the injection halo zone. These GABA-positive cells were likely filled with WGA-HRP by diffusion and not active transport. The second set of dl cells was found beyond the injection halo zone, usually in association with the WGA-HRP labeled cells comprising the intrinsic patches. There was a strong tendency for the dl cells in this set to be associated with some, but not all, of the patches within 1 mm of the injection site center; dl cells were never associated with the long range, or more distant patches. The third set consisted of dl neurons which were found outside of the patches. These cells were within 1 mm from the injection center in the superficial layers and spanned distances up to 2 mm in the deeper layers.

320.10

BICUCULINE INDUCED DISRUPTION OF OPTICALLY DETERMINED OCULAR DOMINANCE BANDS IN MACAQUE STRIATE CORTEX. G.G. Blasdel and M.M. Haglund*. Dept. of Neurobiology, Harvard Medical School, 220 Longwood Ave., Boston, MA 02115.

In Monkey striate cortex the transfer of visual information from layer 4C to overlying layers 2 and 3 is characterized by several transformations that appear to exchange spatially continuous maps in 4C for orientationally continuous ones above. The requirements of this exchange are at odds with the well known vertical alignment of ocular dominance through layers. And this conflict suggests the possible involvement of additional inhibitory mechanisms.

We tested this possibility in two macaque monkeys by examining how ocular dominance, visualized with optical techniques thought to reflect upper layer activity (Blasdel and Salama, 1986), is affected by the GABA blocker bicuculine. After the animals had been prepared and ocular dominance bands visualized, we superfused their cortices with solutions of bicuculine (10, 20, and 40μM in saline) and repeated the procedure. We found that bicuculine at concentrations of 20μM or more disrupts the expression of ocular dominance bands even though activity patterns associated with orientation are still apparent. The disruption persists for as long as bicuculine is present in the superfusate. And when it is removed, the original ocular dominance patterns return. This reversible disruption of ocular dominance is unlikely to derive from pathological changes associated with bicuculine (e.g., abnormal bursting, seizures, etc.) since single unit recordings (obtained after the optical recordings and in the presence of 20μM bicuculine) revealed normal numbers of visually responsive upper layer cells, many of which were orientation selective. Moreover, obvious bicuculine associated effects were never observed with concentrations below 40μM.

These results suggest that ocular dominance in the upper layers depends strongly on (and may even be established by) inhibitory mechanisms.

(Supported by EY06586 and by the Klingenstein Foundation)

320.11

INTERACTIONS BETWEEN ADJACENT ACTIVE CORTICAL REGIONS IN MACAQUE V1 VISUALIZED BY OPTICAL IMAGING OF INTRINSIC SIGNALS. *R.D. Frostig, C.D. Gilbert, D.Y. Ts'o, A. Grinvald & T.N. Wiesel.* IBM Research Division & Lab. of Neurobiology, The Rockefeller Univ., NY 10021.

The existence of long range horizontal connections within area V1 suggests that the activity in one cortical site can be influenced by activity several millimeters away. Previous studies using real-time optical imaging with voltage sensitive dyes showed activation of cortical areas both within and outside the topographic representation of the visual field stimulated.

In this study we first explored the spread of activity measured by optical imaging of **intrinsic signals**. A comparison of a map of orientation columns resulting from a small visual stimulus (a drifting grating within a 0.4° stationary window) with an electrophysiological map obtained with electrode penetrations at a number of sites indicated a correspondence in the areas of activation measured by these two methods. Thus maps obtained from optical imaging using voltage sensitive dyes and intrinsic signals are different: the intrinsic signal images cortical regions in which many neurons are firing whereas the dye signal spreads beyond that region, possibly because it also detects subthreshold postsynaptic activation. (Lieke et al., Neurosci Abstr. 14:1122, 1988). Because the dye signal is also **fast**, it is useful to combine these two imaging techniques to investigate long range cortical interactions.

A second part of this study was to explore interactions between nearby cortical regions by comparing the pattern and magnitude of activation from a small visual stimulus alone to that resulting from a combination of the small stimulus and surrounding stimuli. We found that the addition of the surround attenuates the signal obtained from the small stimulus alone and are currently studying the dependency of these interactions on stimulus orientation. One may speculate that the horizontal connections may contribute to this type of interaction. (supported by IBM and NIH grants NS-14716, EY05253, the Rita Allen Foundation and NSF grant 8351738).

320.12

SYNAPTIC PHYSIOLOGY OF HORIZONTAL AND INTERLAMINAR CONNECTIONS IN SLICES OF THE CAT'S VISUAL CORTEX. *J.A. Hirsch and C.D. Gilbert.* Laboratory of Neurobiolgy, The Rockefeller University., New York, NY 10021.

Neurons in the cat's visual cortex are linked by fibers which extend for millimeters parallel to the cortical surface as well as by interlaminar connections. In order to examine the origins and influences of intrinsic cortical inputs to single neurons, we have made intracellular recordings from layer 2+3 in brain slices prepared from area 17 and compared synaptic responses to stimuli delivered to 2+3 at various distances from the impaled cell, or below it in layer 4. Synaptic responses were measured when the membrane potential was held near the threshold for spontaneous activity since IPSPs were indistinguishable at rest.

Electrical stimulation of layer 2+3 within about 150 μm of the impaled neuron, or in layer 4, evoked a mixture of EPSPs and IPSPs followed by a long slow IPSP of higher threshold. Weak shocks applied 0.7 to 2.5 mm away from the recording site, in the same lamina, typically evoked an EPSP or an EPSP followed by a brief IPSP; the magnitude of the potentials grew with increasing stimulus strength. At these longer distances, the long slow IPSP was not seen.

To localize further the origins of the observed synaptic potentials, we separated layer 2+3 from 4 by severing the connections between them; the effects of stimulating layer 2+3 in these preparations resembled those seen in intact slices. Currently we are applying small amounts of excitatory agents at varying distances from the impaled neuron to map the lateral distribution of cells which innervate it. This distribution will be compared to the arrangement of orientation columns revealed by retrograde transport of fluorescent label. (Supported by NIH EY06010, NS22789 and NSF BNS351738)

HYPOTHALAMIC-PITUITARY-ADRENAL REGULATION II

321.1

INDUCTION OF C-FOS GENE EXPRESSION IN RAT CORTEX DURING STRESS. J.C. Miller, E.A. Stone, D. Filer*, A.J. Friedhoff. Millhauser Laboratories of the Department of Psychiatry, New York University School of Medicine, 550 First Ave., N.Y., N.Y. 10016.

The proto-oncogene, c-fos, is a regulatory gene which encodes a nuclear protein that can be induced rapidly and transiently by a variety of extracellular factors, including agents affecting neuronal activity. Induction of c-fos is believed to be an initial event in signal transduction involving long term adaptive responses. Earlier, we studied the induction of c-fos mRNA in the cortex during restraint stress, but did not find a significant effect. Recently, two other groups reported that low levels of adventitious disturbance could also cause the effect in controls necessitating unusual precautions to prevent unintended stress. Using these precautions we subjected rats to restraint stress as described previously. Twenty four hours prior to the stress all animals were gently handled for 1 minute to reduce emotional reactivity. On the day of the experiment the control rats were rapidly decapitated (5 sec). The stressed animals were then subjected to restraint for 2 hours and killed 1 hour later. The c-fos mRNA in the cortex was assayed using Northern transfer and hybridization. A marked induction of c-fos mRNA in cortex of the stressed rats was found. Thus, induction of c-fos gene expression represents a sensitive method for investigating stress induced neuronal activation in the CNS.

321.2

ADRENALECTOMY INDUCES PROLONGED C-FOS EXPRESSION IN HYPOTHALAMIC CRF NEURONS. L. Jacobson[1],* F.R. Sharp[1,2] & M.F. Dallman[1]* (SPON: C. Van Dyke). Departments of Physiology[1] and Neurology[2], University of California, San Francisco, CA 94143.

Neuronal c-fos gene expression has been suggested to be a marker of neural activity (Science 237:192, 240:1348). To determine if Fos expression may be used to identify brain regions responding to removal of corticosterone (B) feedback by adrenalectomy (ADX), rat brains were processed for immunocytochemistry at 1,3 & 7 d after ADX or sham-ADX (SHAM) using a polyclonal antibody to Fos residues 132-154. All rats were perfused within 15 min of anesthesia & within 3 h of lights-on. All treatment groups exhibited basal Fos-like immunoreactivity (FLIR) in several areas, including the suprachiasmatic nucleus, cerebral cortex, and amygdala. FLIR did not differ between SHAM rats & rats not subjected to surgery. Only ADX rats exhibited strong FLIR in the parvocellular hypothalamic paraventricular nucleus; this FLIR was observed at 1,3 & 7 d after ADX, but was prevented by implantation at ADX of a sc pellet providing physiological B levels. Virtually all PVN neurons expressing FLIR double stained for CRF; some parvocellular neurons also expressed both FLIR and vasopressin. We conclude that neuronal FLIR correlates with known changes in neuroendocrine activity after ADX.

321.3

CIRCADIAN VARIATION OF NEUROPEPTIDE mRNA IN THE PARAVENTRICULAR NUCLEUS AND PITUITARY.

S.P. Kwak, E.A. Young, H. Akil and S.J. Watson. Mental Health Research Institute, University of Michigan, Ann Arbor, MI. 48109.

Endogenous glucocorticoid levels in plasma of many mammals fluctuate in a cyclic pattern. The nadir and apex of the rhythm correlate with "sleeping" and "waking" hours of the animal, respectively, and is thus thought to be influenced by a central mechanism sensitive to the circadian clock. Corticotropin releasing hormone and proopiomelanocortin gene products in the hypothalamus and the anterior pituitary are well known for their involvement in mediating steroid release from the adrenals, as well as being the sites of negative steroid feedback. Therefore, we were interested in determining the regulation of these genes with respect to the glucocorticoid rhythm.

RNase protection assay was used to analyze mRNAs of CRF, type 1(MR), type 2(GR) glucocorticoid receptors, and dynorphin from the paraventricular nucleus, as well as POMC mRNA from the anterior pituitary. Rats were housed at 12 hour day/night cycle (lights on at 7AM) and were decapitated at various times of the day. Initial analysis yielded no profile change in POMC mRNA and ACTH content, while corticosterone in plasma rose at 4PM and peaked 2 hours after lights off (9PM at 15.5ug/dl). Steroids returned to low levels by 4AM. CRF mRNA increased slightly from 2AM to 6PM, but decreased sharply between 7-9PM and returned to baseline at 12AM. DYN, MR, and GR mRNAs did not show any change which coincide with steroid levels. It therefore appears that CRF mRNA may be influenced by the steroid flux. We are currently investigating critical time periods. (SJW supported by Grant # DA02265).

321.4

EXPRESSION AND REGULATION OF CORTICOTROPIN-RELEASING FACTOR MESSENGER RNA IN A PONTINE MICTURITION CENTER (BARRINGTON'S NUCLEUS).

T. Imaki*, J. L. Nahon*, P.E. Sawchenko, C. Rivier, and W. Vale, Peptide Biology Laboratories, The Salk Institute, La Jolla, CA 92037.

Corticotropin-releasing factor (CRF) is distributed in a number of extrahypothalamic cell groups that are involved in autonomic responses. Barrington's nucleus (pontine micturition center) has been reported previously to contain CRF-like immunoreactivity , suggesting a physiological role for CRF in micturition . To explore the regulation of CRF mRNA expression in this nucleus, we examined the changes of CRF mRNA levels after stress, by *in situ* hybridization histochemistry. Male albino rats were exposed to electric footshock (1.5 mA, 1 sec duration, 60 times/30 min) and sacrificed 2 and 24 h after stress. Another group was exposed to same paradigm twice daily for 3 days and sacrificed 24 h after the last shock session. Sections were hybridized with an ^{35}S-labeled prepro CRF cRNA probe. CRF mRNA was expressed mainly in Barrington's nucleus and parabrachial nucleus at the level of dorsal pons. Both the number of labeled cells and the mean grain density per cell in Barrington's nucleus was significantly increased at 2 and 24 h after acute stress, as well as after chronic stress. These results indicate that, as in the hypothalamus, CRF mRNA in Barrington's nucleus is positively regulated by stress, and suggest a role for CRF in stress-induced urination.

321.5

CIRCADIAN CHANGES IN THE preproCRH mRNA CONTENT WITHIN THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS OF RATS OF BOTH SEXES AS MEASURED BY *IN SITU* HYBRIDIZATION.

Alan G. Watts & Larry W. Swanson†, Neural Systems Lab, The Salk Institute & †Howard Hughes Medical Institute, La Jolla, CA 92037.

Secretion of adrenal corticosterone into plasma of the non-stressed rat is characterized by a regular daily surge found at the beginning of the activity phase. This surge is stimulated by increases in the secretion of ACTH from the anterior pituitary, which in turn is stimulated by the release of CRH from terminals in the median eminence into the hypophysial portal circulation. The origin of the CRH terminals is the medial parvicellular part of the hypothalamic paraventricular nucleus (PVH). In order to investigate the mechanisms involved in controlling circadian hypothalamo-hypophysial-adrenal function, we have measured both the plasma concentration of corticosterone, and (by *in situ* hybridization) the content of the mRNA coding for the precursor of CRH in the PVH, in male and female rats at different times of the day. We used rats of both sexes to investigate the possible effects of the greater fluctuation in plasma corticosterone concentrations found in female rats.

Male or ovariectomized-estrogen treated female rats were perfused at 00.00h, 07.00h, 13.00h or 19.00h with a 4% paraformaldehyde solution in which the pH was changed from 6.5 to 9.5. Sections through the PVH were hybridized for 20h with a cRNA probe derived from a cloned sequence of the preproCRH gene (kindly provided by Dr. K. Mayo). After post-hybridization washes, the slides were exposed to Cronex 4 X-ray film for 4 days, then dipped in autoradiographic emulsion, exposed and developed. For semi-quantitation, the integrated optical density of the PVH on the film sections from experimental animals was compared and expressed relative to that of adrenalectomized controls on the same film. Plasma corticosterone concentrations were measured at the same time points in different groups of similarly treated animals.

There was a decrease in the content of preproCRH mRNA in the PVH of rats of both sexes between midday and midnight. This decline was much more prominent in female rats, where the increase in plasma corticosterone was approximately twice that found in males. Our results suggest that changes in the content of preproCRH mRNA throughout the day contribute to the diurnal rhythm in hypothalamo-hypophysial-adrenal function, and that these changes may be inversely related to circulating corticosterone concentrations.

This work was supported by NIH grant NS16686.

321.6

CORTICOTROPIN-RELEASING FACTOR (CRF) CONCENTRATIONS EXHIBIT A CIRCADIAN RHYTHM IN BOTH HYPOTHALAMIC AND EXTRAHYPOTHALAMIC BRAIN REGIONS: DIFFERENTIAL SENSITIVITY TO CORTICOSTERONE.

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It has recently been reported that CRF concentrations in cerebrospinal fluid exhibit a circadian rhythm. We have measured the regional brain concentrations of CRF in rats killed in the early morning or late afternoon. The effect of chronic corticosterone administration on CRF concentrations was also studied.

Regional brain concentrations of CRF exhibited marked differences depending upon the time of sacrifice. When compared to rats killed at 0900 hrs, CRF concentrations in rats killed at 1530 hrs were elevated in a number of brain regions. The afternoon rise in CRF concentrations was generally insensitive to exogenous corticosterone administration in extrahypothalamic areas. In rats killed at 0900 hrs, corticosterone failed to alter CRF concentrations or CRF receptor, in extrahypothalmic brain areas, but decreased hypothalamic CRF concentrations and eliminated the observed circadian hypothalamic CRF increase in animals killed at 1530 hrs. These findings support a lack of effect of glucocorticoids on CRF neurons in extrahypothalamic brain areas. This is the first report of circadian changes in neuropeptide concentrations in extrahypothalamic brain areas. Supported by NIMH MH-42088.

321.7

SELECTIVE PLASTICITY OF VASOPRESSIN CONTAINING CRH AXONS DURING REPEATED DAILY STRESS.

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CRH neurosecretory axons in the external zone of the rat median eminence consist of two types: vasopressin (VP) containing (VP+) and VP deficient (VP-). Several types of short term stress, including immobilization, cause selective activation of the CRH+/VP+ axons with no effect on the VP- axons. To examine the effects of repeated daily stress on this system, Wistar rats were handled or immobilized for 150 min daily for 9 days. Rats were studied 24 h after the last exposure, or were immobilized for 60 min immediately before fixation. Median eminences were immersed in glutaraldehyde, embedded in LR White, and serial ultrathin sections were immunoperoxidase labeled for CRH and a proVP derived peptide. Values are given as number of swellings per sample area of pericapillary zone ± S.E.M.

Condition	CRH+/VP+ axons	CRH+/VP- axons	N (# animals)
Handling (daily) + 24 h rest	11.1±2.8	34.5±3.9	6
Stress (daily) + 24 h rest	24.1±4.2	25.6±3.4	6
Handling (daily) + 60' immob.	6.8±2.5	31.5±2.5	5
Stress (daily) + 60' immob.	22.3±3.6	28.4±2.9	6

Two-way ANOVA showed that daily stress produced a significant effect, but only on the VP+ axons ($P<0.0005$), possibly due to increased production of secretory vesicles containing CRH and VP in those neurons.

321.8

EFFECTS OF FENFLURAMINE TREATMENT ON CORTICOTROPIN-RELEASING FACTOR (CRF) ACTIVITY IN RAT BRAIN.

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Fenfluramine is used clinically in the treatment of obesity. Its weight-reducing effects, through decreased food intake and altered autonomic metabolic parameters, are postulated to be mediated by brain serotonin neurons. CRF, a peptide with potent anorectic effects, has recently been reported to reduce body weight in obese rats through its well-characterized autonomic actions. Since serotonin potently stimulates hypothalamic CRF secretion, we addressed the hypothesis that the weight-reducing effects of fenfluramine are mediated, in part, through altered CRF release. Specifically, we examined the effects of subacute d,l-fenfluramine HCl treatment (1-24 mg/kg, s.c., b.i.d. 4 days) in rats on CRF content in discrete regions of brain and on corticosterone levels in plasma. Dose-dependent reductions in hypothalamic CRF-like immunoreactivity were observed; maximal effects (~60% reduction) were noted at doses of fenfluramine > 4 mg/kg. The decreases in hypothalamic CRF appeared to be a consequence of increased CRF release since plasma corticosterone levels were reciprocally increased in a dose-dependent manner. In contrast, high-dose fenfluramine treatment (24 mg/kg) produced significant increases in CRF content in hippocampus, midbrain and spinal cord with no significant alterations in peptide levels in cerebral cortex, striatum, thalamus, pons/medulla and cerebellum. These data, demonstrating selective fenfluramine-induced alterations in CRF activity, suggest that CRF may represent an important endogenous neurotransmitter mediating the weight-reducing effects of the drug.

321.9

HAMSTERS WITH ACTIVE CORONARY VASOSPASM ARE AT INCREASED RISK FROM STRESS. B. Natelson, W.N. Tapp, J. Ottenweller*, E. Grover*, T. Pritzel* & S. Drastal*. Neuro-behav. Unit, VAMC & Dept of Neurosci., NJ Med. Sch., E.Orange, NJ 07019

Cardiomyopathic hamsters (CMH) develop cardiac micronecrotic lesions due to small vessel spasm at 2-3 months of age. Then, although incidence of new lesions falls, CMHs' hearts fail. We have shown that CMHs in the lesion producing period of their lives succumb to stress (PB&B 3:331, 1989). Older CMHs only succumbed when their heart failure was severe (FASEB J 2:2268, 1988). So, we posited that the presence of the vasospastic process was a risk factor for CMHs of similar ages confronting stress. Outcome of cold-restraint stress was compared between 2.5 mo old and 5-6 mo old CMHs. When 5 daily 2 hr sessions were used, deaths occurred in both groups. But mortality rates tended to diverge after the 3rd stress session: 58% of the older CMHS survived while only 25% of the younger ones did ($p = .11$). A second experiment used a less intense stressor; hamsters were kept in the cold for only 1 of the 2 hr stress sessions: 54% of the older CMHs survived despite their having enlarged hearts while only 15% of the younger ones did ($p < .05$). This experiment shows that CMHs with coronary vasospasm are at increased risk of dying prematurely due to stress when compared to CMHs in which active vasospasm has been replaced by cardiac compensatory changes. This animal model may help understand the role of stress in human coronary vasospastic disease in the absence of arteriosclerosis.

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321.10

STRESS AND IL-1-RELATED ELEVATIONS OF BRAIN TRYPTOPHAN ARE DUE TO AUTONOMIC ACTIVATION. A. J. Dunn.
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Footshock or restraint stress cause elevations of the concentration of free brain tryptophan, in addition to the activation of cerebral catecholaminergic and serotonergic systems. Immune challenges (Newcastle disease virus) influenza virus infection, and interleukin-1 (IL-1) administration cause similar changes in brain norepinephrine metabolism and tryptophan. The increases of cerebral tryptophan caused by all these treatments were uniform across all brain regions studied. Footshock causes small increases in plasma tryptophan. The neurochemical effects of footshock or restraint are not altered by adrenalectomy, suggesting that adrenal hormones (corticosterone, epinephrine, norepinephrine) are not involved. However, pretreatment with the autonomic ganglionic blocker, chlorisondamine (2.5-mg/kg IP) prevented the increase in brain tryptophan that followed 20 min electric footshock, 30 min restraint, or IP administration of 200 ng human recombinant IL-1α. The chlorisondamine pretreatment did not prevent the changes of cerebral catecholamine catabolism associated with these treatments. This suggests that the increases in cerebral tryptophan are caused by activation of the autonomic nervous system. We presume that the autonomic activation in some way alters cerebral tryptophan uptake, which would explain the rather uniform regional distribution of the increase in cerebral concentrations. If so, then infection and IL-1 apparently cause activation of the autonomic nervous system.

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321.11

SELECTIVE BREEDING OF RATS FOR SUSCEPTIBILITY TO THE EFFECTS OF UNCONTROLLABLE SHOCK. P.A. Scott*, M.A. Cierpial, & J.M. Weiss (SPON: G. Marsh). Dept. of Psychiatry. Duke Univ. Medical Center, Durham, NC 27710.

One effect of exposure to uncontrollable electric shock in rats is to decrease motor activity as measured in a swim test. However, not all animals within a given population show this effect. To investigate whether there is a genetic component to this variation, we attempted to selectively breed Sprague Dawley rats to be either susceptible (ie., affected) or resistant to this effect of uncontrollable shock. Initially, nine litters of animals were exposed to shock and given the swim test. Animals from both the most affected and the most resistant litters were then brother-sister inbred and the offspring tested for susceptibility to uncontrollable shock. Offspring from the same parents were divided into same-sex triplets, with one animal receiving 3 hrs of randomly-spaced uncontrollable shock (3-hr), one animal receiving 1 hr of randomly-spaced uncontrollable shock (1hr), and one control animal being placed in the shock apparatus for 3 hrs without exposure to shock (control). Heritability proved to be a factor in susceptibility to the effects of uncontrollable shock in that 3-hr animals from affected parents showed significantly less activity in the swim test compared to control animals from the same parents while 3-hr animals from resistant parents showed no decrease in activity compared to their control animals.

321.12

RESTRAINT-STRESS SELECTIVELY ENHANCES DOPAMINE AND ACETYLCHOLINE RELEASE IN LIMBIC STRUCTURES. ENDOCRINE-NEURAL INTERACTION. L. Angelucci*, S. Puglisi-Allegra°, P. Casolini*, A. Zocchi*° and A. Imperato* (SPON : M. Karobath). Institute of Medical Pharmacology, University "La Sapienza", I-00185 Rome. ° Institute of Psychobiology and Psychopharmacology (CNR), I-00185 Rome.

We have utilized brain dialysis to directly evaluate in vivo the effects of restraint-stress on dopamine (DA) and acetylcholine (ACh) release in the main dopaminergic and cholinergic areas. Restraint-stress, while not altering the release of DA and the output of DOPAC and HVA in the striatum, induced a significant increase in the accumbens. The enhancement of DA release is even more pronounced in the prefrontal cortex,field of termination of DA-ergic neurones exclusively arising from the VTA. In order to elucidate the role of the pituitary-adrenocortical-axis in the DAergic and cholinergic activation, the effect of restraint-stress in adrenalectomized rats, was investigated. In this case, the stress-induced changes in DA and ACh release were much less. Moreover systemic corticosterone, mimicking the increase of its plasma levels after stress, enhanced DA and ACh release. Our results suggest that stress activate DA and ACh release in limbic areas and the pituitary-adrenocortical-axis plays a role in that.

DRUGS OF ABUSE: COCAINE II

322.1

COCAINE-INDUCED TOXICITY AND BODY TEMPERATURE RESPONSES TO COCAINE IN SPONTANEOUSLY HYPERTENSIVE RATS. R.W. Rockhold, Y. Ishizuka, S. Carver, B. Hoskins and I.K. Ho. Dept. of Pharmacol. and Toxicol., Univ. of Mississippi Med. Ctr., Jackson, MS 39216.

Heterogeneity in rectal temperature (RT) responses to cocaine are expressed by restraint in SHR under ambient temperatures of 22-24°C. The maximal change in RT is positive (hyperthermia) in certain SHR (designated SHR_H) while others show only a lowering in RT (SHR_L). The magnitude and direction of this response in SHR correlated negatively with increases in the time-to-onset of convulsions (Tc) following i.v. cocaine infusion (1.25 mg/kg.min). Wistar-Kyoto (WKY) rats show only hypothermic responses. Freely moving SHR and WKY show hyperthermic responses. The influence of ambient temperature on RT responses to cocaine was examined further in restrained SHR. Hypothermic responses only were observed to i.p. cocaine (10 mg/kg) in SHR maintained for 2 hr at 4°C, while hyperthermic responses were seen in a 37°C environment. Decreases in RT were significantly ($p<0.05$) different between SHR_H and SHR_L, respectively (-1.10 $\pm$ 0.15, n=4 vs -2.16 $\pm$ 0.25, n=8), at 4°C. No difference was obtained between sub-groups in increases in RT observed in a 37°C environment. An involvement of dopaminergic receptors in the divergent RT responses seen to cocaine at 22-24°C was studied in restrained SHR and WKY. Pretreatment with the dopamine D-1 receptor antagonist, SCH23390 (50 ug/kg, s.c.), converted cocaine-induced RT responses to hypothermia in all SHR and WKY, but did not alter Tc. In contrast, a D-2 receptor antagonist, sulpiride (50 mg/kg, i.p.), produced hyperthermia in all SHR and WKY and decreased Tc only in SHR. Sulpiride and SCH23390 blocked all cocaine-induced RT responses in both strains but increased Tc in only WKY. The results indicate that dopamine receptors mediate RT responses in restrained SHR and that thermoregulatory systems interact with cocaine toxicity. (DA 04264).

322.2

COCAINE ANALGESIA IS MEDIATED BY CENTRAL NOREPINEPHRINE AND OPIOID SYSTEMS. L.C. Terry, K. Berger*, J.A. Kiritsy-Roy, J.L. McNulty*, R.D. Janke*. Neuroendocrine Lab., Univ. of Michigan and VA Med. Center, Ann Arbor, MI 48105.

Cocaine has recently been shown to produce central analgesia in rats by an action that is believed to occur at the supraspinal level (Lin et al., Brain Res. 479:306, 1989); analgesia was partially attenuated by dopamine D_1 or D_2 blockade but not naloxone (1 mg/kg ip). Because cocaine inhibits reuptake of catecholamines and serotonin, the purpose of this study was to determine the role of norepinephrine (NE), alpha and beta adrenergic receptors, serotonin (5HT) and opioids in cocaine analgesia. Analgesia was quantitated using the hot plate test at 52°C with a maximal exposure time of 45 sec to prevent foot pad tissue damage. Fasted, naive rats were used, and drugs were injected intraperitoneally (ip). For central administration, drugs were infused acutely or into chronic indwelling lateral cerebral ventricular cannulae (icv). Central administration of cocaine and its metabolite benzoylecgonine (BE) caused dose-related analgesia in the range of 38-250 ug icv. BE was approximately twice as potent and had a longer onset and duration of action than cocaine. The onset of analgesia was the same (5 min) after icv or ip cocaine, indicating it must cross the blood-brain barrier rapidly. Also, the NE reuptake blocker desmethylimipramine caused analgesia similar to cocaine. Cocaine analgesia was blocked by pretreatment with the selective NE neurotoxin DSP-4, but not by $alpha_{1 \text{ or } 2}$, beta or 5HT receptor blockade with corynanthine, yohimbine, propranolol or methysergide, respectively. Finally, a high dose of naloxone (10 mg/kg ip) blocked cocaine analgesia, and submaximal doses of cocaine and morphine acted synergistically to produce analgesia. These results indicate that cocaine and its metabolite benzoylecgonine act on the CNS to produce analgesia in the rat, and this analgesia is mediated by both the NE and opioid systems.

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322.3

AUGMENTATION OF DOPAMINERGIC TRANSMISSION IN THE BASAL GANGLIA FOLLOWING ACUTE COCAINE ADMINISTRATION. S.P.Banerjee, A.M. Cosentino*, C.Manetto* and T.I.Lidsky. CUNY Med. Sch. NYC

Dopaminergic (DA) antagonists block self-administration of cocaine (Coc) by rats. This observation has suggested that increased DA transmission underlies cocaine self-administration. This suggestion, however, appears to be in conflict with the electrophysiological demonstration that Coc supresses the spontaneous activity of DA cells located in the substantia nigra (SN) and ventral tegmental area. In the present experiment, we assessed the functional effects of Coc, delivered intravenously, by analyzing somatosensory field potentials evoked in the striatum. Administration of Coc attenuated striatal responses in a dose-dependent fashion (1 - 6 mg/kg). These data suggest that the functional effects of cocaine are to enhance dopaminergic activity which, in turn, suppresses striatal sensory responses.

(NS 21418)

322.4

COCAINE BINDING IN THE HUMAN BRAIN WITH POSITRON EMISSION TOMOGRAPHY N.D. Volkow, J.S. Fowler, A.P. Wolf, S. Dewey, D. Schlyer, B. Bendriem, R. MacGregor, R. Hitzemann, J. Logan. Brookhaven National Laboratory, Upton, NY 11973

Animal studies investigating the binding of cocaine (C) in the brain have reported a high affinity binding site related to the dopamine transporter which has been implicated in the reinforcing actions of C. In order to investigate the binding characteristics of C in the human brain, we studied the distribution and kinetics of carbon-11 cocaine (C*) in 6 volunteers using PET. These studies showed fast uptake and clearance of C* by the brain, with maximum concentration occurring 4-6 min. postinjection and clearance of 50% of the radioactivity by 20 min. Maximal concentration of C* occurred in the striatum which showed a concentration twice that in cortex and cerebellum. Administration of desipramine, 60 min. prior to injection of C* did not affect the binding of C* suggesting that the binding of C* is mainly associated to the dopamine transporter. The time activity curve for uptake and washout of C* from the striatum corresponded to the reported time response to the subjective effects of intravenous C suggesting that the euphorigenic properties of C may be related to the rapid binding and clearance of C in the striatum. Research supported by USDOE, OHER, and NIH NS-15638.

322.5

COCAINE EFFECTS ON SEXUAL BEHAVIOR IN THE MALE RAT. Scott D. Mendelson and Joan M. Lakoski, Dept. Pharmacol. & Tox., The University of Texas Medical Branch, Galveston, TX 77550

The effects of acute and chronic cocaine treatment on sexual behavior were addressed in sexually experienced male rats (Sprague-Dawley, 300-400 gm). Cocaine HCl (3.75, 7.5, or 15 mg/kg, i.p.) or saline (n=15, repeated measures) were given 10 min prior to evaluation of sexually motivated level searching behavior (LS) and 15 min prior to introduction of a receptive female rat; no significant effects on LS, mount, intromission or ejaculation latencies as well as post-ejaculatory intervals, and frequencies of the above were observed. Cocaine (15 mg/kg, i.p.) or saline (n=5) administered 1 min prior to LS and 6 min prior to introduction of the female revealed no significant effects on LS but a significant increase in the latencies to mount and intromission ($p<0.02$).

Repeated exposure to cocaine (15 mg/kg, 2X daily, 6 days) produced no alteration in LS evaluated at 1 min but increased mount latencies at 6 min ($p<0.005$) as compared to controls. Examination of sexual behavior 24hr following repeated cocaine revealed no significant differences between groups. As stimulation of catecholaminergic activity has been found to enhance and serotonergic function, in general, inhibit male sexual behavior, these data suggest that cocaine's effects may be mediated via serotonergic pathways. Supported by NIDA DA 04296.

322.6

A PROGRESSIVE-RATIO SCHEDULE FOR COCAINE ADMINISTRATION: EFFECTS OF COCAINE DOSE AND DIAZEPAM. S.I. Dworkin, A. D'Costa*, N.E. Goeders and E. Hoffman*. Dept. of Physiol. Pharmacol., Wake Forest University, Bowman Gray Sch. of Med., Winston-Salem, NC 27103 and Depts. of Psychiat. and Pharmacol. & Ther., LSU Sch. of Med., Shreveport, LA 71130

Male Fisher rats were prepared with chronic indwelling jugular catheters and trained on a progressive-ratio schedule of cocaine self-administration. The progressive-ratio factor was increased from 1 to 25 over the course of initial training. Sessions were terminated after 2 hours or whenever a response did not occur for 15 min. Responding maintained by two cocaine doses (0.33 and 0.67 mg/infusion) was evaluated using the progressive-ratio 25 schedule. The total number of infusions received during the session was not different for the two doses. However, the interinjection intervals maintained by the low dose were directly related to the size of the ratio, thus as the ratio was increased the inter-injection intervals also increased. The larger dose result in more consistent intervals between injections that were not dependent on ratio size. Both substitutions of diazepam (0.20 or 0.25 mg/infusion) and the co-infusion of diazepam with the low dose of cocaine markedly attenuated self-administration. However, the rate of responding did not appear to be affected by the drug combination. Thus it appears that diazepam can attenuate the reinforcing efficacy of cocaine as indicated by the progressive-ratio procedure. (Supported by NIDA 03628)

322.7

BLOCKADE OF THE DISCRIMINATIVE STIMULUS (DS) EFFECTS OF COCAINE IN RHESUS MONKEYS WITH THE D1 DOPAMINE ANTAGONIST SCH 39166. K.E. Vanover*, M.S. Kleven, R.E. Chipkin, and W.L. Woolverton. The Drug Abuse Research Center, The University of Chicago, Chicago IL 60637.

An increase in dopamine (DA) neurotransmission in the brain is thought to mediate many of the behavioral effects of cocaine. Recent research suggests that D1 DA receptors play an essential role in the DS effects of cocaine (Kleven, et al., Psychopharmacology, 95:427, 1988). The purpose of the present experiment was to determine whether the DS effects of cocaine could be blocked by the selective D1 DA antagonist SCH 39166 (Chipkin, et al., J. Pharmacol. Exp. Ther., 247:1093, 1988). Rhesus monkeys (N=3) were trained in a 2-lever, food-reinforced, drug discrimination paradigm to discriminate cocaine (0.2 or 0.4 mg/kg, i.m., 10 min pre-session) from saline. Before test sessions, in which responding on either lever was reinforced, the monkeys were injected with various doses of cocaine alone or in combination with SCH 39166. Administration of cocaine alone (0.025 - 0.2 mg/kg, i.m.) resulted in a dose-related increase (0 - 100%) in the percent of responses that occurred on the drug-appropriate lever. Administration of SCH 39166 (0.05 or 0.1 mg/kg, i.m.) 50 minutes before an injection of cocaine (0.05 - 3.2 mg/kg, i.m.) reduced drug-appropriate responding from 100% to 0% at least at one dose combination in all monkeys. In all monkeys, the blockade was overcome by increasing the dose of cocaine, resulting in an 8 - 32 fold parallel shift to the right in the cocaine dose-response function. The results are further evidence that D1 DA receptors are involved in the DS effects of cocaine. (Supported by NIDA Grant DA-00250).

322.8

EFFECTS OF THE D1 ANTAGONIST SCH 23390 ON BEHAVIOR MAINTAINED BY COCAINE OR FOOD. M.S. Kleven and W.L. Woolverton. Department of Pharmacological and Physiological Sciences, Drug Abuse Research Center, The University of Chicago, Chicago, IL 60637.

Recent evidence that the D1 antagonist SCH 23390 blocks the discriminative stimulus effects of cocaine suggests that D1 dopamine receptors play a necessary role in the effects of cocaine (Kleven et al., Psychopharmacology, 95: 427, 1988). The purpose of the present experiment was to determine whether SCH 23390 selectively decreases cocaine self-administration. Rhesus monkeys (N=4) were trained to press a lever in daily experimental sessions under a 3 component multiple schedule of cocaine or food reinforcement. In the first and third components, food was available under a fixed-ratio (FR) 30 schedule. In the second component cocaine (0.025 or 0.05 mg/kg/inj, i.v.) was available under a FR 30 schedule. A time-out was scheduled after the delivery of each reinforcer. When behavior was stable, monkeys received continuous infusions of several doses of SCH 23390 (0.4-6.4 mg/kg/day) for at least the same number of sessions (5-12) that were required for responding to decline to low levels when the monkeys were allowed to self-administer saline. Infusion of low doses of SCH 23390 (0.2-0.8 mg/kg/day) decreased responding maintained by cocaine with minimal effects on responding maintained by food. Higher doses (1.6-6.4 mg/kg/day) decreased both food- and drug-maintained responding. The effects of SCH 23390 on drug-maintained responding progressively diminished over several days of continuous infusion such that at the end of the infusion period responding approximated control rates. In sessions following termination of daily infusions of higher doses of SCH 23390 (3.2-6.4 mg/kg/day), responding for cocaine occurred at low rates (5-10% of control), and recovery to control rates took as long as 2 weeks. These results suggest that a D1 antagonist can decrease the reinforcing effect of cocaine at doses which have minimal effects on responding maintained by food. However, the effects of SCH 23390 diminish over time and exposure to SCH 23390 may result in long-lasting changes in the effects of cocaine. (Supported by NIDA Grant DA-00250).

322.9

CHRONIC COCAINE IN VIVO MODIFIES PROLACTIN RELEASE IN THE PRESENCE OF DOPAMINE IN VITRO. NS Pilotte, RL Johnson*, EM Dax*. Neuroendocrinology Laboratory, Addiction Res. Ctr., Nat'l Inst. Drug Abuse, Baltimore, MD 21224.

Circulatory concentrations of prolactin (PRL) are reduced shortly after the administration of cocaine to rats treated chronically with the drug. In contrast, PRL increases 22 hr after cocaine. To determine if cocaine alters PRL release directly at the anterior pituitary gland (APG), we infused male Lewis rats with cocaine (1 mg/kg IV) or 0.15 M NaCl (0.1 ml/kg IV) every 12 min for 2 hr for 10 days. After the last session, the APGs were removed and prepared for use in a reverse hemolytic plaque assay (RHPA). PRL secretion was observed from single cells during a 1 hr incubation using a RHPA 24 hr later.

There were more plaque-forming cells from cocaine-treated rats but plaques with diameters > 0.10 mm occurred more often in APGs of saline-infused rats. Application of 3.3 μM cocaine to the AP cells did not alter basal or thyrotropin-releasing hormone-stimulated PRL-mediated plaque formation. The application of 10^{-6} to 10^{-11} M dopamine (DA) suppressed plaque development in a dose-related manner in both groups. However, plaques were smaller and occurred less frequently from cells of cocaine-treated rats at each concentration of DA. Chronic cocaine appears to increase the sensitivity of lactotropes to DA. One mechanism by which cocaine affects PRL release may be by an alteration in the density of DA receptors.

322.10

[^{3}H]CFT: A NOVEL HIGH AFFINITY LIGAND FOR COCAINE RECEPTORS. B.K. Madras, M.A. Fahey*, R.D. Spealman*. Harvard Medical School, New England Regional Primate Research Center, Southborough, MA 01772.

The cocaine analog, 2β-carbomethoxy-3β-(4-fluorophenyl)-tropane (CFT also designated WIN 35,428) is 3-10 times more potent than (-)-cocaine both in vivo and in vitro. [^{3}H]CFT was evaluated as a radioligand probe for cocaine receptors in caudate-putamen membranes of monkeys (M. fascicularis). Kinetic, saturation and competition studies indicated that [^{3}H]CFT, in a manner analogous to [^{3}H]cocaine, bound to at least two components but with affinities 5-10 times those of [^{3}H]cocaine and with dissociation rates at least 10 times slower. In competition studies, the IC_{50} values of cocaine congeners and other monoamine uptake inhibitors at [^{3}H]CFT binding sites correlated highly with their IC_{50} values at [^{3}H]cocaine binding sites. All cocaine congeners displaced [^{3}H]CFT fully and recognized both binding components of [^{3}H]CFT. In contrast, several monoamine uptake inhibitors structurally unrelated to cocaine displaced only about 90% of specifically bound [^{3}H]CFT and appeared to recognize only one [^{3}H]CFT binding component. [^{3}H]CFT and [^{3}H]cocaine appear to bind to a similar spectrum of sites. However, because of its higher affinity and slower dissociation rate, [^{3}H]CFT is a superior radioligand probe for cocaine receptors.
Supported by DA05648, DA00499, DA00088, RR00168.

322.11

CHRONIC COCAINE-INDUCED ALTERATIONS IN DOPAMINERGIC PRE- AND POSTSYNAPTIC MARKERS. L. M. Terry, E. Keran *, L. Hearn*, J. Sanchez-Ramos, J. Pablo, M. Basile and D. C. Mash. Departments of Neurology and Pharmacology, University of Miami School of Medicine, the Dade County Medical Examiner's Office and the College of Health, Department of Medical Laboratory Science, Florida International University, Miami, FL., 33101.

Repeated cocaine administration leads to a variety of behavioral and neurochemical effects. We have examined the effect of chronic administration of cocaine on dopaminergic uptake sites and D1 and D2 receptor subtypes in the rat brain using quantitative in vitro autoradiography. Sprague Dawley rats were implanted with Alzet minipumps containing cocaine (30 mg/kg/day) or saline. At the time of sacrifice, heart blood levels of cocaine were determined by Gas/Liquid chromatography. Dopaminergic receptor subtypes (D1 and D2) were labeled in slide-mounted sections of the rat brain with [^{3}H]-SCH23390 and [^{3}H]-raclopride, respectively. Dopaminergic uptake sites were labelled by differential [^{3}H]-mazindol uptake site autoradiography. Both D1 and D2 receptor subtypes were downregulated in the striatum by chronic cocaine administration. Dopaminergic uptake sites appeared to be unchanged in either the mesolimbic or striatal terminal fields. The density of D2 receptors in the substantia nigra and ventral tegmental area was unchanged, while the number of D1 receptors was markedly reduced in the substantia nigra. These results suggest that chronic cocaine administration may differentially regulate pre- and postsynaptic dopaminergic markers in cell body and terminal regions.

322.12

Binding Characteristics of Cocaine and Tropococaine to Muscarinic and Sigma Receptors in the Human Brain. D. C. Mash, A. J. Ruttenber*, C. V. Wetli*, W.J. Weiner, Y. Itzhak, and J. Pablo. Depts. of Neurology, Biochemistry and Pathology, University of Miami School of Medicine and the Dade County Medical Examiner Dept., Miami, FL. 33101 and the Center for Environmental Health, CDC, Atlanta, GA. 30333

Complications from cocaine abuse include acute psychiatric disturbances, such as dysphoria, agitation, assaultiveness, paranoia, psychosis and hallucinations. Although the precise mechanisms underlying these behavioral disturbances are not known, they may be related to the targeted effects of cocaine at specific neurotransmitter systems and receptors. Fatal excited delirium has been reported in recreational cocaine users having significantly lower blood levels of cocaine than those usually associated with fatal cocaine toxicity (Wetli, C.V. and Fishbain, D.A., J. Forensic Sci. 30:873, 1985). The reinforcing property of cocaine is attributed to its ability to block the reuptake of dopamine. We report here that cocaine exhibits high affinity for muscarinic and sigma receptor sites labelled in human brain membranes. Competition of cocaine with radiolabelled muscarinic agonists and antagonists gave Ki values in the low micromolar range (~ 5 μM). Putative sigma receptor sites in the human brain were labelled with [^{3}H]-ditolylguanidine (DTG). Competition of cocaine with [^{3}H]-DTG in human brain cerebellar membranes resulted in complex inhibition curves that were best fit by a two site model (IC50 values of 1 x 10-5 M and 5 x 10-4 M). Tropococaine, a 'street' contaminant of cocaine, had a ten-fold higher potency than cocaine at both muscarinic and sigma receptors. The direct action of cocaine at muscarinic and sigma receptors may contribute to the dysphoria and delirium experienced by some cocaine abusers. These observations further suggest that chronic cocaine abuse may lead to multiple receptor site alterations in the human brain and thereby contribute to the pathogenesis of cocaine-induced psychosis and the phenomenon of sudden death.

ISCHEMIA

323.1

EFFECTS OF THE 21-AMINOSTEROID U74006F ON POST-ISCHEMIC BRAIN LIPID PEROXIDATION AND RECOVERY OF EXTRACELLULAR CALCIUM E. D. Hall, K.E. Pazara* and J.M. Braughler. CNS Diseases Research, The Upjohn Co., Kalamazoo, MI 49001

The effects of U74006F, shown previously to improve neuronal survival in gerbils following a 3 hr. period of unilateral carotid occlusion (UCO), has been examined on post-ischemic lipid peroxidation (depletion of brain vitamin E) and recovery of cortical extracellular calcium (Ca++)e. Male gerbils were treated with vehicle (0.05 N HCl) or U74006F (10 mg/kg) i.p. at 10 min. before UCO and again immediately after UCO. Brain vitamin E was decreased at 2 hrs. after reperfusion in the vehicle treated animals by an average of 60%. In U74006F treated gerbils, the 2 hr. post-ischemic loss was only 39.7% ($p<0.002$ vs. vehicle). UCO also produced a drop in (Ca++)e from 1.05 mM before ischemia to 0.11 mM by the end of 3 hrs. in both vehicle and U74006F treated gerbils. By 2 hrs. after reperfusion, (Ca++)e recovered to only 0.22 mM in the vehicle animals compared to 0.56 mM in the U74006F group ($p<0.03$ vs. vehicle). Cortical blood flow, mean arterial pressure and pCO2 were the same in both groups. The results indicate that U74006F inhibits post-ischemic lipid peroxidation and that perhaps secondary to this membrane protective effect, the processes responsible for the reversal of the ischemia-induced intracellular Ca++ accumulation are preserved.

323.2

^{45}CALCIUM ACCUMULATION AND INTRACELLULAR CALCIUM DURING IN VITRO "ISCHEMIA". M.P. Goldberg, M.C. Kurth*, R.G. Giffard and D.W. Choi. Dept. of Neurology, Stanford Univ. Med. Sch., Stanford, CA 94305.

We explored the relationship between Ca^{++} influx and neuronal injury in an in vitro model of cerebral ischemia. Dissociated murine neocortical cell cultures were exposed to medium lacking both oxygen and glucose, resulting in widespread neuronal death 24 hr later. With 30-60 min oxygen + glucose deprivation, cellular accumulation of media $^{45}Ca^{++}$ quantitatively paralleled resultant neuronal injury. No $^{45}Ca^{++}$ accumulation was observed in glial cultures under similar conditions. Such "ischemic" $^{45}Ca^{++}$ accumulation was increased by removing either Mg^{++} or Na^{+} from the exposure medium, and blocked by addition of the NMDA antagonists 1 mM APV, 100 μM dextrorphan (DX), or 100 μM CGS-19755. In contrast, neither $^{45}Ca^{++}$ accumulation nor neuronal death was significantly altered by addition of 100 μM nifedipine, a concentration sufficient to block $^{45}Ca^{++}$ accumulation evoked by KCl depolarization. In cultures previously loaded with the intracellular free Ca^{++} indicator fluo-3, intense fluorescence was observed in neurons (but not glia) immediately following deprivation of oxygen + glucose; and this high Ca^{++} signal could be blocked by NMDA antagonists. Present observations provide further evidence linking Ca^{++} influx via NMDA channels to ischemic neuronal injury.

323.3

EFFECTS OF CAFFEINE ON ISCHEMIC NEURONAL INJURY STUDIED USING MAGNETIC RESONANCE IMAGING AND HISTOPATHOLOGY. R.M. Brownstone, G.S. Sutherland*, J. Peeling*, F. Dai*, H. Lesiuk*, and J.K. Saunders*. Depts. Physiol. and Surgery, Univ. Manitoba, Winnipeg, R3E OW3; and the National Research Council of Canada.

The phenomena of post-ischemic selective neuronal vulnerability and ischemic maturation have led to the suggestion that intrinsic neuronal properties are in part responsible for eventual cell death (see Siesjö & Bengtsson, J. Cereb. Blood Flow Metab. 9:127, 1989). Adenosine may mediate a common underlying mechanism in ischemic neuronal damage (Hagberg et al, J. Neurochem 49:227, 1987). To test this hypothesis, the effects of caffeine on neuronal damage were assessed in rats subjected to forebrain ischemia. Six rats were given intravenous caffeine (20 mg/kg) 30 minutes prior to the ischemic insult ("Acute"), whereas 8 control rats received equivalent quantities of i.v. normal saline. Eleven rats were fed increasing oral doses of caffeine over a 3 week period (up to 90 mg/kg/d; "Chronic"). Post-ischemic high resolution (100 um) multi-slice, multi-echo magnetic resonance images were obtained daily for 3 days. All animals were then sacrificed via perfusion-fixation, and the brains studied histologically.

The control rats demonstrated MRI changes indicative of neuronal damage primarily confined to the striatum at 24 hours, with hippocampal damage evident at 48 hours. By 72 hours, the changes were beginning to resolve. Three of six of the "acute caffeine" rats showed significantly more pronounced neuronal damage in both the striatum and the hippocampus by 24 hours. The rest were similar to control. In the "chronic caffeine" rats, the neuronal injury was significantly less pronounced than in the control group. The histopathologic findings were consistent with these MRI changes.

These results support the hypothesis that adenosine plays an important role in reducing the neuronal damage following forebrain ischemia. It is suggested that the accelerated neuronal injury seen in the rats treated with an acute dose of caffeine was secondary to the direct antagonism of adenosine by caffeine, whereas in the rats chronically fed caffeine, there was an upregulation of adenosine receptors which resulted in a decrease in the neuronal damage.

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323.4

MK-801 INCREASES CEREBRAL BLOOD FLOW IN A RAT MODEL OF TEMPORARY FOCAL CORTICAL ISCHEMIA. A.M. Buchan, D. Xue*, A. Slivka*, C. Zhang*, J. Hamilton* and A. Gelb*. Laboratory of Cerebral Ischemia, Robarts Research Institute, London, Ontario, Canada.

The NMDA antagonist MK-801 has been reported to have potent cytoprotective properties in the face of both global and focal cerebral ischemia. Pre and post treatment with MK-801 lessens the volume of cortical infarction in a variety of models. These experiments were performed to test the hypothesis that this effect is related to increased regional cerebral blood flow (rCBF) rather than NMDA antagonism.

Male Wistar rats, anaesthetized and monitored physiologically had both common carotids (RCCA,LCCA) and the right middle cerebral artery (RMCA) exposed. Right cortical rCBF was monitored with a laser Doppler flowmeter. Prior to occlusion rCBF was 106 ± 29 ml/100g/min (± SD) (N=28) with a tandem occlusion of the RCCA and RMCA this fell to 27 ± 7% of baseline (N=11) but no infarcts occurred unless the LCCA was also occluded. With both CCA and RMCA occlusion rCBF was 5.3 ± 1.8% (N=18). Prior to baseline measurements a further group of animals were given saline or MK-801, 5mg/kg i.p., baseline rCBF obtained and temporary clips (2-4 hrs) placed on the LCCA and RMCA, RCCA was permanently occluded.

Time	Saline	MK-801
Baseline rCBF(±SD)	92.9 ± 27	106 ± 32
% ±SD (N)	100 ±0% (8)	100 ±0% (10)
RMCA,RCCA,LCCA occluded	5.8 ±0.5% (8)	17.6*** ±2.3% (10)
LCCA/RMCA reperfused	63 ±13% (7)	80 ±10% (7)
At 24 hours	92 ±12% (7)	121 ±30% (4)

*** = p<.001 (Mann-Whitney U) MK-801 vs. Saline

Animals treated with MK-801 received 2 further doses of 2.5mg/kg at 6 hours and 12 hours. Cortical infarcts at 24 hours were smaller for MK-801 than for saline treated animals, but as can be seen in the table rCBF was significantly higher in drug treated animals. These in vivo findings are supplemented by our studies of canine basilar artery strips. MK-801 in clinically relevant dilutions (10-100 μ Mol) produced dose dependant but endothelium independent vasodilation of artery contracted with KCl and 5HT.

We conclude that the protective effects of MK-801 in focal cerebral ischemia are at least in part related to increased rCBF rather than NMDA antagonism.

323.5

EFFECTS OF NORMO- AND HYPERGLYCEMIA ON REVERSIBILITY AND EXACERBATION OF OUTCOME AFTER CLIP RELEASE AFTER MCA OCCLUSION IN CATS. G.M.de Courten-Myers*,R.E.Myers,M. Kleinholz * and K.R.Wagner(SPON:T.Mandybur).Dept. of Path.,UC College of Med.,and Res.Serv.,Cincinnati VAMC, Cincinnati,OH 45220.

Factors influencing the effects of restoring blood flow to areas of focal brain ischemia remain poorly defined but have come of interest because of fibrinolytic stroke treatment. We investigated pathologic outcome after temporary (4 and 8 hours) middle cerebral artery (MCA) occlusion in anesthetized cats rendered hyper- (20 mM for 6 hrs) or normoglycemic (6mM), respectively. Surviving animals were killed 2 weeks survival to assess infarct size (% MCA territory). Acute deaths resulted from total MCA territory edema with infarction.

Duration occlusion	Glycemia (mM)	Edema death	Infarct	Size mean±SE	Intact
4 hrs	20	7/13(54%)	0/13(00%)	0	6/13(46%)
8 hrs	20	4/9 (44%)	4/9 (44%)	9±3.0%	1/9 (11%)
4 hrs	6	1/12(08%)	5/12(42%)	2±0.3%	6/12(50%)
8 hrs	6	2/10(20%)	6/10(60%)	12±0.2%	2/10(20%)

Hyperglycemia at and following occlusion affected outcome negatively, causing more frequent edema deaths ($p<0.02$). In surviving animals of both glycemia groups, longer occlusion increased infarct frequency and size and decreased intact survival.

323.6

CARRIER MEDIATED TRANSPORT OF LACTIC ACID IN CULTURED NEURONS AND ASTROCYTES. M. Nedergaard*, S.A. Goldman, S. Desai*, and W.A. Pulsinelli (SPON: F. Plum) Dept. of Neurology, Cornell Univ. Medical College, N.Y., 10021.

Lactic acid is a key intermediate in the process of ischemic brain infarction, but the mechanism by which lactic acid crosses brain cell membranes is not well defined. As a measure of lactate transport, we examined neuronal and glial rates of intracellular acidification to lactate exposure at constant extracellular pH (pH_e) using microspectrophotometry and the pH sensitive dye BCECF. Cells were obtained from trypsin-dissociated E16 embryonic rat forebrain. Intracellular pH (pH_i) in CO_2-free HEPES buffered solution was 7.12±0.18 (n=42) in neurons, and 7.01±0.13 (n=36) in astrocytes. Exposure to L-lactate (1-80 mM) at constant pH_e (7.3) caused a reversible decrease of pH_i by 0.1-0.2 in both cell types. The absolute magnitude of intracellular acidification of neurons and glial cells with D-lactate was significantly less than with L-lactate. The initial rate of acidification was a saturable function of L-lactate concentration; K_m was below 1 mM, the maximum acidification rate was~0.3 pH units/min. The rate of acidification was not affected by lowering pH_e to 6.5. Thus under physiological concentrations of lactate and H^+ ions this carrier is saturated. During cerebral ischemia the net effect of this carrier may be the rapid equilibration of transmembrane lactic acid concentrations.

323.7

EXTRACELLULAR ACIDOSIS PROTECTS CULTURED NEURONS FROM INJURY INDUCED BY EITHER COMBINED OXYGEN-GLUCOSE DEPRIVATION, OR GLUTAMATE. R.G. Giffard, H. Monyer and D.W. Choi. Depts. of Anesthesia and Neurology, Stanford Univ. Med. Sch., Stanford, CA 94305.

Brain ischemia in vivo is accompanied acutely by extracellular acidosis, a derangement considered to contribute to resultant neuronal injury. We examined the effect of pure acidosis upon the neuronal injury induced by oxygen and glucose deprivation in vitro. Murine cortical cell cultures subjected to combined hypoxia and glucose deprivation ("ischemia") for 45 - 60 min at pH 7.4 developed widespread neuronal degeneration over the next day. If the bathing medium pH was lowered to 6.0-6.4, resultant neuronal injury was markedly reduced. The basis for this protective effect of acidosis may be downmodulation of NMDA receptor activation (Morad et al., Soc. Neurosci. Abstr. 14:791, 1988), reducing glutamate neurotoxicity. We previously reported that the addition of 500 μM glutamate to the cultures for 5 min at pH 7.4 was sufficient to destroy most of the neuronal population; lowering pH to 6.0-6.4 also attenuated this direct glutamate-induced neuronal degeneration. Acidosis in vivo is indisputably associated with increased severity of infarction; however present observations raise the interesting possibility that acidosis per se may actually reduce the intrinsic vulnerability of neuronal cells to ischemic damage.

323.8

TISSUE LACTATE AND pH AS MARKERS OF INFARCTS FROM CEREBROVASCULAR OCCLUSIONS IN CATS. R.E.Myers,K.R.Wagner,M. Kleinholz* and G.M.de Courten-Myers.Res.Serv.,Cincinnati VAMC and Dept.Path., UC College Med., Cincinnati,OH 45220

Lactate accumulation in tissue >17-20 umoles/g correlates with brain injury in anoxia. We presently test whether a similar relation exists in infarction from middle cerebral artery (MCA) occlusion. We compared hyper- (20 mM) and normoglycemic (6 mM) animal groups in parallel biochemical and pathologic studies and determined lactate and pHi topographically after 0.5 and 4 hrs of occlusion. The % of lactate >17 umoles/g within the ischemic territory in 14 sample sites was correlated with infarct sizes also expressed as % of MCA territory derived from groups with the same durations of occlusion with clip release or with permanent occlusion with 2 wks survival for pathologic study. Acute deaths resulted from total territory infarction and edema.

The % high lactate sites after 0.5 and 4 hrs of occlusion correlated well with permanent occlusion infarct probability and with 4 hr occlusion-clip release outcome in normo- and hyperglycemic groups. However, hyperglycemic 0.5 hr occlusion lactate markedly overestimated 0.5 hr release infarction. pHi determinations revealed significantly less acidosis with similar lactate concentrations after 0.5 than 4 hrs occlusion indicating 1) the accompanying marked acidosis and not lactate most consistently correlates with brain damage and 2) intracellular buffering early after occlusion may protect against injury with early restoration of blood flow.

323.9

HYPOXIA ISCHEMIA INDUCES HEAT SHOCK PROTEIN IN NEONATAL RAT BRAIN. Donna M. Ferriero, Hernani Q. Soberano*, Roger P. Simon, Frank R. Sharp, Manual Gonzalez. Department of Neurology, UCSF, San Francisco, CA 94143-0870

The expression of heat shock protein in rat brain was evaluated in a model of neonatal hypoxia-ischemia. One week old rats were subjected to left carotid artery coagulation and exposure to 8% O_2/92% N_2 for 2 h (moderate injury) or 3.5 h (severe injury). Animals were sacrificed at 1, 12 and 24 h after the hypoxic insult. Immunoreactive cells containing a 70 kD heat shock protein (HSP 70) were seen in regions of the ipsilateral cortex as early as 1 h after the complete insult. After 12 h, neurons of the ipsilateral hippocampus and cortex stained intensely for HSP 70 in the moderately injured group. In the severely injured brains, bilateral staining was observed in neurons and vessels of hippocampus and cortex. Therefore, HSP 70 immunocytochemistry may serve as an early marker for neuronal injury from hypoxia-ischemia in the neonatal rat brain and more importantly may identify previously unrecognized areas of central nervous system vulnerability.

323.10

PAF-ACETHER PRODUCTION IN SPINAL CORD AFTER ISCHEMIA-REPERFUSION **Lindsberg P J*, Yue T-L*, Frerichs K U*, Hallenbeck J M*, Feuerstein G. (SPON:M CARPENTER). Dept. of Neurology USUHS, Bethesda, MD 20814.**

PAF-acether is a potent mediator of inflammatory responses and modulates cardiovascular functions in systemic and microvascular circulation. PAF has been shown to decrease blood flow in the central nervous system (CNS), to induce blood-brain barrier (BBB) damage and has therefore been suggested to play a role in ischemic CNS injury. However, no evidence of PAF-production in injured CNS-tissue has been reported. Lumbar spinal cord ischemia (10 min) was produced in six rabbits with an inflatable balloon-tipped Swan-Ganz catheter and verified by on-line laser-Doppler flowmetry through a laminectomy (L5). Spinal cord samples were obtained after 2 hours of reperfusion for PAF-assay and edema measurement. PAF was extracted by the method of Bligh-Dyer, isolated by TLC and assayed by measuring the release of ^{3}H-serotonin from washed rabbit platelets. Serotonin-release was higher in all ischemic samples than in controls ($p<.05$), suggesting PAF-like activity in CNS injured by ischemia-reperfusion. Coincidently, the water content of ischemic samples was increased by 3.1 ±.7 % ($p=.01$) indicating postischemic edema formation. Our data suggest that PAF exists in injured CNS tissue and may have a role in the pathophysiology of postischemic edema and delayed neuroinjury after ischemia-reperfusion.

323.11

POSTISCHEMIC NEURONAL FLOODING WITH IgG: AN EARLY PREDICTOR OF RAT HIPPOCAMPAL CELL DEATH. C. Williams*, L. Jenkins, and J. Povlishock (SPON: J. Astruc), Depts. of Anatomy & Surgery, Med. Coll. of VA, Richmond, VA 23298.

Previous studies of transient cerebral ischemia (TCI) have demonstrated neuronal populations, notably Hippocampal CA1 neurons, that show little or no initial post-ischemic change, yet die within several days. Biphasic local blood-brain barrier dysfunction and late neuronal flooding with exogenous and endogenous protein tracers thought to be associated with cell death, have also been described with TCI. We studied the possibility that neuronal flooding with endogenous circulating immunoglobulins might be an early and definitive marker of impending cell death by subjecting rats to global TCI and evaluating them after postischemic survivals of 4h to 72 h. Brain tissue was reacted for the immunocytochemical visualization of IgG at the electron microscopic level. In all animals, the insult produced early hippocampal interstitial labeling, indicating increased vascular permeability to IgG. Initially, this presence was not associated with neuronal flooding, despite some neurons displaying modest ultrastructural change. By 24h post-ischemia, however, neurons showing progressive changes all flooded with IgG, and by 48h, these innundated neurons demonstrated dramatic changes consistent with the onset of cell death. Thus, it appears that early intra-neuronal flooding with IgG is an indicator of impending cell death. Supported by NS-20193 and T32 NS-07288.

323.12

ANATOMY OF PENUMBRA IS MODIFIABLE WITH NMDA ANTAGONISTS AND PREDICTS REGION OF INFARCTION R Simon, F Sharp, K Shiraishi* Dept Neurol, UCSF, SF, CA 94143-0870

The pattern and time course of metabolic alterations in cortex and basal ganglia of rat have been studied using 2-deoxyglucose (2-DG) administered 5, 30, 60, 120, 140 min and 24 and 72 hrs following permanent MCA occulsion (N=5 per time point); the effect of the potent NMDA antagonist CGS 19755 (10mg/Kg) administered 5 min prior to,5 min or 60 min following MCA occulsion was then studied. In MCA occlusion rats a pattern of increased 2-DG was reproducibly seen: by 5 min 2-DG was decreased in lateral but increased in medial caudate-putamen (CP) and lateral neocortex; over the subsequent 30 min in CP and 120 min in cortex increased 2-DG uptake preceded decreased 2-DG uptake and infarction. The regional anatomy of the altered 2-DG uptake predicted the anatomy of the eventual infarct (defined on H&E sections at 72 hrs s/p MCA occlusion). Although alternate explanations for increased 2-DG preceding infarction are numerous, excitatory amino acid -induced hypermetabolism which preceeds and predicts the region of infarction is possible. This hypothesis was tested by studying the pattern of 2-DG uptake and resultant infarct in the same rat MCA model in the setting of CGS 19755. Total infarct size was decreased 64%, 50% and 0% in 5 min prior, 5 min post and 60 min post MCA occlusion respectively. 2-DG images showed altered metabolism corresponding closely to the regions of ultimate infarction. These data 1) support a role for excitation-induced hypermetabolism in the pathogenesis of ischemia; 2) suggest that attenuation of hypermetabolism may contribute to the neuroprotective effect of NMDA antagonists; and 3) suggest that the time window of effectiveness of NMDA antagonists may be dictated by the time course of the metabolic changes described above.

323.13

POSTISCHEMIC HIPPOCAMPAL GLUCOSE UTILIZATION AFTER LONG-TERM RECOVERY T. Beck*, A. Wree*, A. Schleicher* (SPON:D.J. Ennulat). Dept. Pathology, Columbia University, New York, NY 10032; Anat. Inst. Univ. Würzburg, FRG; Anat. Inst. Univ. Köln, FRG

Previously we showed local cerebral glucose utilization (LCGU) to be increased in CA1 sector 1 week postischemia (Beck et al., JCBFM 9 Suppl, 1989, in press).

In the present report, hippocampal glucose utilization was determined in male Wistar rats 2 and 3 weeks as well as 3 months after a 10 min forebrain ischemia induced by clamping of the carotid arteries and lowering blood pressure to 40 mmHg.

Despite severe neuronal damage as assessed by histological techniques, local cerebral glucose utilization (LCGU) was significantly increased in the pyramidal and radiatum layers of the CA1 sector up to 3 weeks postischemia, while in other hippocampal areas, and dentate gyrus decreases in LCGU prevailed compared to non-ischemic controls. At 3 months postischemia LCGU in CA1 sector was reduced compared to controls, while staining for GFAP was pronouncedly increased.

The increases in LCGU are suggested to either reflect long-lasting postsynaptic hyperexcitation of pyramidal cells or to point to ongoing presynaptic hyperactivity. Metabolism of glial cells is unlikely to contribute to these effects.

324.1

Vision calibrates the map of head movement in the optic tectum of developing barn owls. S. du Lac and E.I. Knudsen. Dept. Neurobiology, Stanford University, Stanford Ca 94305.

The optic tectum contains mutually aligned maps of visual and motor space: the size and direction of orienting movements that result from microstimulation at any given site in the tectum match the position of visual receptive fields recorded at that site. We tested whether visual experience is required for normal visuo-motor registration by comparing tectal maps in normal barn owls with those in owls raised with both eyelids sutured closed. Microstimulation with standard parameters was applied to the optic tectum and the resulting head movements were measured with a skull mounted search coil. As expected from a previous study, visual deprivation resulted in strabismus in 4 of 5 owls; when visual receptive fields were adjusted by the amount that the eye deviated from normal orientation, the visual map of space in the tecta of blind reared owls was essentially normal. In contrast, the topography of the motor map was conspicuously abnormal. Stimulation-induced head movements, although kinetically normal, rarely corresponded with visual receptive field locations measured at the site of stimulation. Movements elicited from different sites in a single tectum could be either hypo- or hypermetric relative to the movement predicted from the receptive field location. In addition, the direction of evoked movements tended to be inaccurate. A motor map, albeit degraded, did develop in the tecta of blind-reared owls: movements changed from small and ipsilateral to large and contralateral as the stimulation site moved from rostral to caudal, and from upward to downward as the site moved from dorsal to ventral. Thus, the tendency to form a systematic representation of movement vector does not depend on vision. However, vision is required for the motor map to attain the precise topography and accurate alignment with the visual map that exists in the tecta of adult owls.

324.2

EFFECTS OF VISUAL-AUDITORY EXPERIENCE ON SACCADIC LOCALIZATION OF AUDITORY STIMULI. D.D. Kurylo, P.H. Hartline and R.L.P. Vimal*. Eye Research Institute, Boston, MA, 02114.

Cats can be trained to look towards auditory targets, as reported previously (Hartline and Northmore, Soc Neurosci Abstr, 12:1277, 1986). It was also reported that when brief (50 msec) visual and auditory stimuli were presented simultaneously, though at different locations, cats often oriented their eyes to a location between the two stimuli (Vimal, Kurylo, and Hartline, Soc Neurosci Abstr, 14:957, 1988). We now describe how pairing of stimuli in this way influenced subsequent localization of auditory test stimuli that were presented alone. For example, when visual stimuli were offset to the left of auditory stimuli during paired visual-auditory presentations, subsequent localization of auditory alone stimuli was shifted to the left, compared to localization of auditory alone stimuli before pairing. Localization of auditory stimuli could be displaced in this way both horizontally and vertically. This effect occurred after as few as 50 pairings. Even when visual-auditory discrepant pairing occurred at only three spatial positions, subsequent changes in auditory localization generalized to regions in space previously unpaired. Similar changes in localization of auditory stimuli also occurred when cats were in the dark, or when a novel visual background was used during post-pairing auditory test trials. This eliminates the possibility that during paired trials cats learned to associate visual landmarks with each speaker and looked at those landmarks after auditory test stimuli. This effect suggests that the localization of auditory stimuli by adult cats may be continuously altered by experience.

324.3

MULTISENSORY INTERACTIONS IN CAT SUPERIOR COLLICULUS DURING ACTIVE FIXATION

C.K. Peck and J.A. Baro. School of Optometry, University of Missouri-St. Louis, St. Louis MO 63121

In the intermediate and deep layers of the superior colliculus (SC), visual and auditory receptive fields are in alignment when the eyes and ears are directed straight ahead. However, during normal orienting behavior, the eyes and ears can move independently and the consequent misalignment of visual and auditory receptive fields might be expected to reduce the probability of appropriate behavioral responses to multisensory targets. Passive displacement of the ears not only shifts the optimal location for auditory targets (Middlebrooks and Knudsen, J. Neurophysiol. 57: 672, 1987) but also depresses the response of SC neurons to multisensory stimulation at a given location in space (Meredith and Stein, Soc. Neurosci. Abstr. 12: 1034, 1986).

We have examined the effects of misalignment of receptor organs on the responses of visual-auditory neurons in SC while cats made active fixating eye movements to targets at different spatial locations. Under conditions of active, voluntary realignment of the eye, most multisensory neurons exhibited strong enhancement of responses to multisensory stimuli at a given spatial location when the eyes were deviated up to 20 deg. The effects of larger deviations could not be assessed due to the limitations of the cat's oculomotor range. Because passive displacements of visual and auditory targets by 10 deg. have previously been shown to suppress multisensory interactions in cat SC (Meredith and Stein, Brain Res. 365: 350, 1986), the present results suggest that active and passive misalignments of receptor organs are processed differently by the nervous system. Thus, misalignment of sensory maps during active fixation does not invariably degrade or suppress interactions among multisensory targets. Supported by NS-21238.

324.4

AUDITORY AND VISUAL STIMULUS INTERACTION IN THE GENERATION OF HUMAN SACCADIC EYE MOVEMENTS. C.J.Lueck*, I.J.Crawford*, C.J.Savage*, C.Kennard. Dept. of Neurology, The London Hospital, London E1 1BB, U.K.

The hypothesis that auditory and visual spatial representations move with respect to each other during eye movements (Sparks, D.L., Brain Beh. Evol. 31:49, 1988) was tested in man. Four normal subjects were studied using the magnetic scleral search coil technique. Auditory targets were arranged horizontally at 0°, -15°, and +15°. Visual interference was provided by simultaneous onset of one of twelve light emitting diodes arranged horizontally between -25° and +25°.

When starting with head and eyes pointing straight ahead, primary saccades showed shorter latency if the lights were on the same side of the midline as the target buzzer than if they were on opposite sides. Saccade amplitude was reduced equally by a light anywhere in the opposite hemifield. A light in the same hemifield affected saccade amplitude in a linear manner depending upon its position. Final eye position was affected in the same way as primary saccades. When the eyes started from an eccentric position, the relation between light position and saccade amplitude suggested that visual and auditory spatial representations do not remain in fixed relationship to each other during eye movements.

324.5

OBLIQUE GAZE SHIFTS IN HEAD FREE CATS. M.Crommelinck*, E.Olivier*, P.Monteyne*(SPON: M.Meulders). Lab. de Neurophysiologie, Louvain Univ. Sch. of Med., B-1200 Brussels, Belgium.

It has been shown, in head fixed cats and monkeys, that the duration of the smaller component of oblique saccades is increased, as compared to pure horizontal or vertical saccades, to match the duration of the largest component. Is this phenomenon also present when the head is free to move?

Eye and head movements were recorded by the search coil technique in alert cats trained to fixate visual targets. Preliminary results obtained from 640 oblique movements clearly show a stretching of the duration of the smaller component of eye movements, related to the amplitude of the other one. For saccades, the angle of which lies within 25 deg from either the horizontal or the vertical, however, one of the components is too small to be adapted. As far as gaze is concerned, there is no significant effect of one component on the duration of the other. The same is observed for the horizontal and vertical components of head movements, which seem to be independent of each other. These observations suggest that the vestibulo-ocular reflex is not operating with a unitary gain during these head free movements. Indeed, if this was the case, the effect of head rotation on gaze would be compensated for and, like the saccade, the gaze components would be adapted. The fact that head trajectories are not rectilinear (both components are not adapted in duration) does not mean that their amplitude is not precisely controlled. Indeed, the head takes a constant part in the gaze displacement (a mean of 77 and 82% for horizontal and vertical components respectively).

324.6

GAZE CONTROL IN THE HEAD-FREE CAT. I. COMPENSATION FOR STIMULATION-INDUCED PERTURBATIONS IN GAZE POSITION. D. Pélisson*, D. Guitton and D.P. Munoz. (SPON: L. Wolfe) Montreal Neurol. Inst. and McGill Univ., Montreal, H3A 2B4, Canada.

It is thought that saccades are controlled by signals representing target and instantaneous eye positions coded with respect to the head. To determine the frame of reference relevant to gaze (= eye + head) control, we extended to the cat whose head is unrestrained the original study of Mays and Sparks (Science, 208: 1163, 1980). We stimulated the superior colliculus (SC) to perturb initial gaze position before the onset of a gaze shift made in the dark to a flashed target. Gaze shifts compensated for this perturbation and reached the target with normal accuracy, despite the absence of visual feedback. This result indicates that gaze shifts were coded in either a body-centered or spatial frame but we could not distinguish between these 2 alternatives because the cat's body was fixed. When we perturbed an ongoing gaze shift by briefly stimulating a SC during the movement, the deviated gaze shift did not stop but compensatory adjustments to the trajectory occurred "on-line" so as to assure gaze accuracy. This experiment suggests that coordinated eye-head orienting movements are controlled by a feedback system which compares desired and actual gaze positions.

324.7

GAZE CONTROL IN THE HEAD-FREE CAT. II. SPATIO-TEMPORAL VARIATIONS IN THE DISCHARGE OF SUPERIOR COLLICULUS OUTPUT NEURONS. D.P. Munoz, D. Guitton and D. Pélisson*. Montreal Neurol. Inst. and McGill Univ., Montreal, H3A 2B4, Canada.

When a cat, whose head is unrestrained (head-free), orients to a target of interest, the following events occur in the crossed tecto-reticular (TR) pathway. 1) An ensemble of TR neurons (TRNs), centered at the site in the superior colliculus (SC) that will generate the gaze (= eye + head) shift, displays first a low frequency preamble discharge. 2) The TRNs composing this active zone then discharge a burst which precedes the gaze displacement by 15-20ms. 3) At the termination of the movement the initial active zone is silenced and fixation-related TRNs, located in the rostral poles of both SCs, begin to discharge. We have reported this information during the past five years. Here, we present the results of experiments in which we recorded simultaneously from 2 or more TRNs located at different sites in the SC between the initial active zone and the rostral fixation area. The results show that these TRNs are sequentially activated during a head-free gaze shift, indicating that the zone of TRN activity moves across the SC map, away from the initial site, towards the fixation zone, such that the instantaneous location of this zone reflects current gaze motor error.

324.8

MODULATION OF GAZE VELOCITY PURKINJE (GVP) CELLS DURING VESTIBULO-OCULAR REFLEX (VOR) WITH NEAR AND FAR VISUAL TARGETS. L.H. Snyder* and W.M. King. Department of Physiology, University of Rochester, NY 14642.

GVP cells, located in the cerebellar flocculus, modulate with the sum of eye and head movements (gaze) and have been implicated in control of the VOR, an oculomotor reflex that counter-rotates the eyes to stabilize images on the retina during head rotation. During rotation about a vertical axis, compensatory eye velocity is increased when near targets are viewed (Blakemore and Donaghy 1980). We investigated the role of horizontal GVP cells in such "near enhancement" of the VOR. Monkeys equipped with binocular scleral search coils were trained to fixate near (10 cm) or far (220 cm) LED targets. Brief pulses of whole-body rotation (25 d/s for 175 ms) were applied immediately after extinguishing all lights, including the LED target. Averaged eye velocity traces showed that the enhanced near target VOR response diverged abruptly from the far target response ~11 ms after the onset of eye movement. Averaged GVP cell discharge also diverged, but no earlier than 2 ms before the eye velocity divergence (28 observations on 11 cells), suggesting that these GVP cells are outside the pathways that drive near enhancement.

Eight of 11 cells did increase their tonic discharge rate with fixation of near compared to far targets (70 versus 40 hz, mean of 11). This change in firing rate may modulate the sensitivity of cells in more direct VOR pathways, and thereby alter the eye movement response to a given vestibular input.

Supported by NIH Grants GM07356 and EY04045.

324.9

THE LATENCY AND GAIN OF THE HORIZONTAL VESTIBULO-OCULAR REFLEX IN GOLDFISHES DETERMINED BY VESTIBULAR POSITION AND VELOCITY STEPS DURING LEARNING AND MEMORY. R. Baker, A. M. Pastor*, M. Weiser* and J. McElligott. Dept. Physiol. & Biophys. NYU Med. Ctr., New York, NY 10016, and Dept. of Pharmacol., Temple Univ. School of Med., Phil. PA 19140.

In goldfishes with symmetrical VOR gains (eye velocity/head velocity) in the dark of 0.9-1.0 for each eye, vestibular position and velocity steps were utilized to evaluate the latency and profile of horizontal eye movements before, during and after adaptive gain modification. Although electrical stimulation of the vestibular nerve initiated eye velocity at 12-14 msec the latency for both position and velocity steps ranged from 14-21 msec. Differences in latency, time to peak velocity and amplitude could sometimes be observed between movement of the two eyes. Utilizing vestibular frequencies from 0.125-0.5 Hz and amplitudes of 8-32°/sec, goldfishes were trained toward gains of 2.5, 0 or -1.0 with sinusoidal vestibular and visual stimuli for 3-6 hours and then re-trained to 1.0. In all experimental paradigms, irrespective of the extent of learning and memory, the VOR gain measured with vestibular velocity and position steps paralleled that found with sinusoidal stimuli. The latency for increases in VOR gain appeared to follow the earliest detectable eye velocity response for both position and velocity steps. In goldfishes trained to either perfect suppression or inversion (eye gain of 0 and -1.0), latency for the gain modification also followed onset of eye velocity. The initial unaltered eye velocity response was always of small amplitude and short time course, reaching the new gain setting within 0.1-0.2 sec. These data are similar to studies showing an early un-modifiable eye velocity response; however, given the magnitude of change in vestibular sensitivity the goldfish data suggests possibly only one, modifiable, vestibulo-ocular pathway underlying learning and memory. Supported by EY 02007 and NS 13742.

324.10

MONOCULAR ADAPTIVE GAIN CONTROL OF THE VESTIBULO-OCULAR REFLEX IN THE GOLDFISH, *Carassius auratus*. M. Weiser*, A. M. Pastor* and R. Baker Dept. Physiol. & Biophys., NYU Med. Ctr., New York, N.Y. 10016.

Sinusoidal vestibular and visual stimuli were used to study normal VOR and OKN performance as well as adaptive VOR gain control. In eight goldfish with VOR gain (eye velocity/head velocity) of 0.9-1.0 in the dark and 1.0 in the light, presentation of full field optokinetic stimuli produced nearly symmetrical (left eye vs. right eye) horizontal eye movement gain (eye velocity/world velocity) equal to 0.6-0.8 at 0.125 Hz and 16°/sec peak velocity. When one eye viewed the moving stimulus and the other eye the stationary stimulus, optokinetic gain was 0.2-0.3 and <0.1, respectively. When each eye was trained simultaneously towards a visual/vestibular gain of 0 and 2.0 initial performance ranged from 0.5-0.8 to 1.2- 1.5. After learning for 3-6 hours the eye movement gains stabilized near 0.2 and 1.8. The VOR memory tested in the dark was always biased more towards the gain of 0 than 2.0 with an average final value of 0.2 and 0.9 for each eye. Thus in all experiments the eye velocities were 25°/sec disparate during learning and about 12°/sec different during the subsequent test of VOR memory. Although the rapid re-setting eye movements were of markedly different amplitude and even direction, they occurred synchronously. Vestibular sensitivity for the eye training towards a higher gain actually decreased by 1-5°/sec; however, the gain was always the same in the eye trained towards 0 for the VOR both in light and dark. Ablation of the cerebellum showed that it was not necessary for establishing and maintaining the direction of the gain changes, but it was important for attaining larger gain amplitudes. Therefore, connections between the vestibular nuclei play a role in determining gain bias and the velocity difference between the two eyes. We conclude that the brainstem-cerebellar circuitry in the goldfish can produce significantly greater differences between individual eye velocities than is the case in mammals. Supported by EY02007 and NS13742.

324.11

ACTIVITY OF MEDIAL RECTUS MOTONEURONS IN GOLDFISH DURING VESTIBULAR AND VISUAL EVOKED EYE MOVEMENTS. A. M. Pastor* and R. Baker. (SPON: J. O'Neill). Dept. Physiol. NYU Med. Ctr., N Y, N.Y. 10016.

Goldfish were implanted with intracranial bipolar electrodes placed bilaterally on both oculomotor and vestibular horizontal canal nerves. Movements of both eyes were recorded with scleral search coil techniques and identified medial rectus motoneurons were studied during spontaneous saccades, fixations, and sinusoidal visual-vestibular stimulation producing VOR gains from 0 to 2.5. Electrical stimulation of the medial rectus nerve in the orbit and the whole IIIrd nerve showed that medial rectus motoneurons activated from the orbit were surrounded by vertical motoneuronal pools . All medial rectus motoneurons exhibited a discharge correlated with both eye position and eye velocity during every oculomotor behavior. The vertical oculomotor motoneurons that were antidromically identified showed no relationship to any horizontal eye movement or spontaneous tonic activity, except during 'blinks'. Single electrical shock stimulation of the horizontal canal nerve at 2xThr produced contralaterally directed twitch-like changes in the position of both eyes. Latency for the adducting (medial rectus) and abducting (lateral rectus) eye averaged 14 and 16 msec, respectively. Since IIIrd and VIth cranial nerve stimulation produced eye movements at the same latency (7 msec) the observed 2 msec difference in the evoked VOR latency indicates a differential central processing time for the two horizontal canal vestibulo-ocular pathways. Peristimulus time histograms of medial rectus motoneuronal activity triggered by threshold stimulation of the vestibular nerve clearly showed ipsilateral increases and contralateral decreases in excitability at a short latency. The data suggests a reciprocal vestibular inhibitory and excitatory regulation of the medial rectus subdivision as has been found for goldfish abducens motoneurons. Given the differences in eye movements induced by electrical stimulation of the vestibular nerve we conclude that the neural circuits underlying horizontal eye movements are structurally and, to a large extent, functionally separated; however, their behavior is well co-ordinated for visual and vestibular stimuli producing symmetrical eye movements. Supported by EY02007 & NS13742.

324.12

CENTRAL VELOCITY STORAGE: CONVERGENT EVOLUTION IN SOME TELEOSTS AND REPTILES? N. Dieringer* and W. Graf (Sponsor: E.E. Brink). Physiol. Inst., Univ. Munich, Munich, FRG and The Rockefeller Univ., New York, NY 10021

OKAN and VOR time constants longer than that of vestibular afferents are behavioral expressions of the function of a velocity storage mechanism. Differences in the ocular reflex responses of goldfish (Carassius auratus) and toadfish (Opsanus tau) suggest the presence and absence of a functioning velocity storage network, respectively. Lack of velocity storage was also found in other bottom living teleosts (e.g. northern sea robin, Prionotus carolinus, catfish, Ictalurus spec.) as well as in amphibians (urodeles and anurans) as described earlier (Dieringer et al., J. Comp. Physiol. 153, 1983). A non-functioning velocity storage mechanism appears to be concomitant with absence of an inhibitory vestibular commissure at the neuronal level (as in frog and toadfish) and, furthermore, with a reduced role of locomotor capabilities for feeding and escape at the behavioral level. Presence of a storage mechanism can thus be considered a physiological mechanism for behavioral strategies requiring stabilizing reflexes at rapid changes of speed of locomotion. In view of the poor locomotor capabilities of fish-like ancestors of modern amphibians and reptiles, a convergent evolution of a velocity storage mechanism in some teleosts and in reptiles appears to be likely. (Supported by NATO Grant 366/88 to N.D. and NIH grant NS20358 to W.G.)

324.13

DISTRIBUTED PARALLEL PROCESSING IN THE VERTICAL VESTIBULOOCULAR REFLEX. T.J. Anastasio* and D.A. Robinson. Dept. of Ophthalmology, Johns Hopkins Univ. Sch. of Med., Baltimore, Maryland, 21205.

Rather than bearing any simple relationship to semicircular canal (SC) or extraocular muscle (EM) geometry, as predicted by vector analysis, the sensitivity-vectors (SVs) of vestibular nuclear neurons (VNNs) in the cat are dispersed in various directions (Baker et al., Brain Res. 294: 133-137, 1984). We constructed neural network models of the vertical VOR that can simulate this diversity.

Vertical VOR networks had three layers of units, input, hidden and output, representing vertical SC afferents, VNNs and cyclovertical EM motoneurons, respectively. Hidden-to-output connections were fixed in a reciprocal pattern; adaptable input-to-hidden connections were randomized prior to learning. Networks were trained to make compensatory responses to rotations about horizontal axes using the back-propagation learning algorithm.

Following training, the SVs of output units matched those desired (to 1 deg). SVs of hidden units in four-hidden-unit networks lined-up near those of the outputs, as expected from vector analysis. In contrast, those in 40-hidden-unit networks were divergent, as for cat VNNs. This similarity suggests that VNNs encode orientation information in a distributed manner, and that learning networks may be more helpful than vector analysis in predicting the behavior of VNNs.

MORPHOGENESIS AND DIFFERENTIATION

325.1

THE SALAMANDER BRAIN AS A PAEDOMORPHIC VERTEBRATE BRAIN. G. Roth[1]*, C. Naujoks-Manteuffel[1]*, K.C. Nishikawa[2], A. Schmidt[1]*. [1]Brain Res. Inst., Univ. of Bremen, F.R. Germany; [2]Sch. of Biol. Sci., Univ. of Kentucky, Lexington, KY 40506.

The salamander brain is usually considered primitive in view of the absence of multiple lamination and distinct nuclei, as compared to anurans and other vertebrates. However, there is evidence that the salamander brain is paedomorphic, i.e., secondarily simplified; (i) essentially the same morphological and functional types (projectivity, response properties) of tectal, pretectal and thalamic neurons are found in salamanders and frogs; (ii) the distribution of AChE and neuropeptides (BOMB, LENK, SP) in the visual system is similar to that found in frogs; (iii) the basic pattern of nerve cell migration is the same as in frogs, but migration is retarded in salamanders; the most derived salamanders (Bolitoglossini) show the least degree of cell migration; (iv) radial glial cells involved in cell migration are present, but seem to fail tc transform into astrocytes, as happens in other vertebrates. In general, late ontogenetic processes are retarded or fail to occur.

325.2

RETINAL REGENERATION IN GOLDFISH: USE OF CELL SPECIFIC MONOCLONAL ANTIBODIES TO ASSESS THE REESTABLISHMENT OF NORMAL CYTOARCHITECTURE. P.A. Raymond and L.K. Barthel*. Dept. Anat.& Cell Biol., Univ. Michigan, Ann Arbor, MI 48109.

We have generated a panel of monoclonal antibodies (Abs) that recognize specific cells or layers in goldfish retina, and we have used these reagents, and other Abs, to follow the degeneration and subsequent regeneration of the retina in adult goldfish following a neurotoxic lesion. Ouabain, injected intravitreally (5 to 20 μM), completely destroys neural retina, which regenerates in several months. Retinal sections (1 day to 10 months after ouabain injection) were processed for immunocytochemistry with:

Antibody	Specificity
ROS-1, ROS-2	rod outer segments
PLEX-1, PLEX-2	certain plexiform/fiber layers
RET-1, RET-2	amacrine & ganglion cell layers
NN-1, NN-2	microglia, pigment cells & other
MUL-2	Müller glia
ENDO-1	endothelial cells

and with rhodopsin Abs (RET-P1, rho4D2) and Müller Abs (CAC, GS). Double labeling with BrDU (or with ^{3}H-Tdr) proved that the neurons had regenerated. Restoration of cytoarchitecture was remarkably accurate. Supported by EY-04318.

325.3

GOLGI STUDY OF LAYER I THREE-DIMENSIONAL ORGANIZATION IN THE HUMAN CEREBRAL CORTEX. Miguel Marin-Padilla. Department Pathology, Dartmouth Medical School, Hanover, NH 03756.

The combined study of rapid Golgi preparations of layer I, cut parallel, perpendicular and tangential to the long axis of the gyrus, has permitted for the first time, the visualization and reconstruction of its three-dimensional organization. The autopsy material used included the cerebral cortex of 29,30,31,32,34, and 36 week old premature infants. Based on the observations made, the morphology and orientation of the Cajal-Retzius (C-R) cell is herein redefined in new terms. C-R cells are flat stellate neurons with several short and long primary dendrites radiating in all directions within a plane parallel to the pial surface. Dendrites may reach lengths up to 300 micrometers. Most C-R cells are located in the upper third of layer I. They have a descending axon that give off several fine collaterals that also radiate in all directions within the middle third of layer I. Axonic collaterals may reach lengths up to 500 micrometers. The main axon descends toward the lower third of layer I where it becomes a long and thick tangential fiber. The main axon can be followed for a long distance without reaching its termination. They also radiate in all directions. Together they constitute a prominent axonic band in the lower third of layer I. These axons are among the first to undergo myelinization in the cortical grey matter. Thus, dendrites, axonic collaterals and the main axon of C-R cells radiate in all directions and occupy distinct planes within layer I.
Supported by NINCDS grant #NS22897.

325.4

LIVE OBSERVATION OF DYNAMIC EVENTS IN THE FORMATION OF RAT CEREBRAL CORTEX BY LASER MICROSCOPY. M.W. Cooper*, A.B. Waxman and S.J. Smith*. (SPON: P. Forscher) Section of Molecular Neurobiology, Howard Hughes Medical Institute, Yale Medical School, New Haven, CT 06510.

We are using laser confocal microscopy to observe cellular proliferation, migration, differentiation and synapse formation events in tissue slices acutely isolated from cerebral cortex of rat embryos at days E14 to E19. Subcellular resolution is obtained by using the fluorescent, cationic membrane probe Di-I $C_{18}(3)$ as a vital stain. Selective staining of discrete subpopulations of neurons and glia results from localized application of the fluorescent stain. In 768 x 480 pixel images created with incident energy densities of less than 3×10^{-2} Joules/cm^2 at 514 nm, we are able to obtain signal-to-noise ratios adequate to resolve individual filopodia and other very fine cellular processes at depths of 50 to 100 microns into cortical slices. At these energy levels, we are able to record time-lapse video sequences at frame intervals of 15 to 30 seconds for periods of hours with little or no evidence of phototoxicity.

Growth cones observed within embryonic cortical slices exhibited cyclic extension and retraction of filopodia similar to those previously observed under cell culture conditions. Putative immature neurons exhibited migration along radial processes, as inferred in previous studies of fixed tissues. Migration velocities were observed to vary as a function of radial position, being slowest in the cell-dense ventricular zone and fastest in the distal imtermediate zone and cortical plate. One surprising observation was the free and rapid (> 1 um/sec) migration of macrophage-like cells within immature cortical volumes. On occasion, these rapidly migrating cells were observed to adhere to and cause rearrangement of neural or glial processes. (Supported by the HHMI and NIH grant NS16671 to SJS).

325.5

SPONTANEOUS INTRACELLULAR CALCIUM FLUCTUATIONS OCCUR IN NEURONS OF THE EMBRYONIC HIPPOCAMPUS. A.B. Waxman, M.W. Cooper*, and S.J. Smith*. Section of Molecular Neurobiology, Howard Hughes Medical Institute, Yale Medical School, New Haven, CT 06510.

We are using a scanning laser microscope and the fluorescent Ca indicator fluo-3 to measure changes in cytosolic Ca in embryonic hippocampal neurons. Parallel studies are being carried out in intact hippocampi explanted from day E17 to E19 rat embryos and on cultured cells dissociated from similar tissues. Use of the laser microscope in a confocal mode allows measurement of indicator fluorescence with excellent spatial resolution and signal-to-noise ratio at depths of 50 to 100 microns into the explanted hippocampus. The same laser microscope is used with a wider detector aperture setting for very efficient fluorescence measurements on the cultured cells in monolayer. In both cases, 768 x 480 pixel images are collected with energy densities below 3×10^{-2} Joules/cm^2, energy levels where little or no cellular photodamage is evident.

Spontaneous elevations of intracellular Ca occur at irregular intervals both in cultured neurons and in neurons within explants. The Ca elevations last between 1 and 10 sec, and occur at intervals on the order of hundreds of seconds. These Ca transients may reflect membrane electrical activity and opening of voltage dependent Ca channels, but we have not yet investigated this possibility directly. Multiple cells within either cultures or explants often exhibit closely synchronized Ca elevations, which may indicate that the Ca transients are synaptically driven, but some form of gap junctional signalling is also a possibility, given the early developmental stages involved. We have also observed that these Ca-increase events occur more frequently as cells make their initial intercellular contacts. (Supported by the HHMI and NIH gram NS16671 to SJS).

325.6

EVIDENCE THAT POSTNATAL DEVELOPMENT OF THE MOUSE OLFACTORY BULB PROCEEDS BY GRADUAL ADDITION OF NEURAL ELEMENTS. S.L. Pomeroy, A.-S. LaMantia and D. Purves, Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110

We have undertaken a quantitative analysis of the mouse olfactory bulb from birth until maturity in order to ask whether the development of the mammalian brain proceeds by gradual construction or by selection from an initial excess of neural elements.

The brains of CF1 male mice of known age (up to 24 weeks postnatal) were removed and prepared for light and electron microscopy. An assessment was made of: (1) olfactory bulb size; (2) glomerular size and number in cresyl violet-stained serial sections; (3) the extent of mitral cell dendrites visualized by confocal microscopy after retrograde labeling with dil; and (4) the number of glomerular synapses in electron microscopical sections. Serial sections of the olfactory mucosa were also examined to evaluate the size of the sensory epithelium and the density of receptor cells.

We found that the size of the olfactory bulb and mucosa, the number of glomeruli, the complexity of mitral cell dendrites, and the number of glomerular synapses all increase from birth to maturity. These results indicate that neuronal assemblies such as glomeruli and their constituent elements are gradually added during development. Whether the increasing number of glomeruli in postnatal life represents simple addition of these neuronal ensembles, or the net effect of concurrent addition and elimination, cannot be determined from these observations in fixed tissues. The work in the following two abstracts addresses this issue directly by following the natural history of identified glomeruli in living animals. (Supported by USPHS Grant 11699, Training grant 07027, and Javits Center Grant).

325.7

OBSERVATION OF THE OLFACTORY BULB OVER TIME IN LIVING MICE. A.-S. LaMantia, S.L. Pomeroy and D. Purves, Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110

We have developed a technique to examine directly the development and adult plasticity of neuronal ensembles in the brain of an individual animal over weeks to months. To obtain images from living animals, CF-1 mice of various ages were anesthetized, and the right olfactory bulb exposed surgically. After briefly superfusing the intact dura with hypertonic NaCl (1.6M), the bulb was stained topically with the styryl dye RH414. Within several minutes an extensive pattern of fluorescently labeled glomeruli appeared. This pattern was then recorded using a laser-scanning confocal microscope with a 10% transmittance neutral density filter (MRC Lasersharp-500, BIO-RAD). Confocal microscopy is superior to standard epifluorescence microscopy for this purpose because of its ability to attenuate out of focus fluorescence from the overlying dura and afferent fiber layer.

Examination of bulbs imaged in vivo and then immediately fixed and stained en bloc with Sudan Black showed that all of the glomeruli seen in the histological preparation are also apparent in the living animal. Light and electron microscopical examination of bulbs fixed acutely or several weeks after imaging showed the architecture and synaptic organization of the bulb to be unaffected by these procedures.

Therefore, a normal pattern of neural elements may be seen at least twice in the brain of a living animal without compromising the integrity of the tissue examined. This method of in vivo brain imaging can be used in mice from birth through maturity with similar effectiveness. In the following abstract we describe the use of this approach to monitor the development and stability of olfactory glomeruli.
(Supported by USPHS Grant 11699, Training grant 07027, and Javits Center Grant.)

325.8

ADDITION OF FUNCTIONAL NEURONAL ASSEMBLIES VISUALIZED IN THE OLFACTORY BULB OF LIVING MICE. D. Purves, A.-S. LaMantia, and S.L. Pomeroy, Dept. of Anatomy & Neurobiology, Washington Univ. School of Med., St. Louis, MO 63110

The arrangement of olfactory glomeruli was visualized in the same developing mouse on two separate occasions to assess the developmental plan in this part of the mammalian central nervous system.

Neonatal mice (4-7 days postnatal) were anesthetized by hypothermia and the olfactory glomeruli visualized by confocal microscopy after vital fluorescent staining, as described in the previous report. The pups were then allowed to recover and returned to the litter. One to three weeks later, the juvenile mice were perfused transcardially with 1% glutaraldehyde and their brains removed. After postfixation overnight, the olfactory bulbs were stained with Sudan Black and the final pattern of glomeruli compared with the earlier in vivo image.

In animals whose olfactory bulbs were re-examined after a week, the pattern was generally similar to that seen initially, although glomerular growth was apparent and some new glomeruli had been added. After 2 weeks, further additions to the initial population were seen with little or no evidence of glomerular deletion. After 3 weeks the additions were so substantial that comparison with the original image was often difficult. In contrast, the pattern of glomeruli was quite stable over intervals of several weeks in a series of adult animals.

Evidently the basis for the overall increase in glomerular number cited in the first of these three reports is the gradual construction and subsequent persistence of new functional units as the animal matures. These observations in living animals, taken together with histological counts of glomeruli in animals of different ages, indicate that this process continues up until about the time of sexual maturity and then ceases.

325.9

CELL REARRANGEMENT AND DIVISION IN SHAPING OF THE AVIAN NEURAL PLATE. G. C. Schoenwolf and I. S. Alvarez.* Univ. of Utah School of Med., Salt Lake City, UT 84132 and Univ. of Extremadura, Badajoz, Spain.

Shaping of the neural plate, one of the most striking events of neurulation, involves rapid craniocaudal extension. In this study, we determined the roles of two processes in neural plate extension: neuroepithelial cell division and cell rearrangement. Quail epiblast plugs of constant size were grafted either just rostral to Hensen's node or paranodally and the resulting chimeras were examined at selected times postgrafting. By comparing the size of the original plug, the number of cells it contained, and the distribution of cells within it to those same features of the grafts in developing chimeras, we were able to ascertain that during transformation of the flat neural plate into the closed neural tube (a period requiring 24h), the graft undergoes a maximum of 3 rounds of craniocaudal extension. Such extension is accompanied by 2 rounds of mediolateral cell rearrangement and 2 rounds of cell division, some of which occurs in the transverse plane of the neural plate and some in its longitudinal plane. Thus, craniocaudal extension during neural plate shaping is mediated by both mediolateral cell rearrangement and cell division, with the former playing a greater role in extension than does the latter. In addition, widening of the cranial part of the future brain occurs during neural plate shaping. Our results suggest that cell division in the transverse plane plays a major role in this process. Supported by NIH grant no. NS18112.

325.10

CELL TRANSPLANTATION IN THE ZEBRAFISH EMBRYO: IS THE *SPT-1* MUTATION CELL AUTONOMOUS? ROBERT K. HO*, DONALD A. KANE*, & CHARLES B. KIMMEL (SPON: W. ROBERTS). INSTITUTE OF NEUROSCIENCE; UNIVERSITY OF OREGON; EUGENE, OR. 97403

In the zebrafish gastrula, laterally positioned involuting cells converge dorsally to form mesodermal structures in the trunk. The mutation *spt-1* (spadetail) changes the direction of this movement: In *spt-1* homozygotes the same cells move caudally to the tailbud and eventually form mesodermal tissues of the tail. The mutation does not appear to affect ectodermal precursors.

We wish to know if *spt-1* acts in a cell autonomous manner in prospective trunk mesodermal cells and if the anomalous cell movements at gastrulation are specific only to mesodermal precursors. We have labeled mutant and wildtype cells so they can be distinguished from each other, co-transplanted the mixture of labeled cells into unlabeled sibling hosts, and followed the migration patterns and fate of these cells. Our results show that, irrespective of host genotype, cells transplanted to the prospective trunk mesoderm position of the gastrula migrate incorrectly (caudally) if they are mutant and correctly (dorsally) if they are wildtype. Cells placed in prospective ectoderm positions migrate correctly irrespective of their genotypes. We suggest that the *spt-1* mutation specifically and cell autonomously perturbs convergence of the mesoderm. Supported by NIH HD22486 and the Helen Hay Whitney Foundation.

325.11

CRANIAL MUSCLES AND MOTONEURONS THAT INNERVATE THEM ORIGINATE FROM THE SAME AXIAL LEVELS IN THE ZEBRAFISH

T.F. Schilling*, R.M. Warga* and C.B. Kimmel. (spon. D. Kimble) Inst. of Neuroscience, U. of Oregon, Eugene, OR 97403.

Do particular mesodermal and ectodermal structures in the vertebrate head both develop from single head segments? In the zebrafish, the positions of muscles of the eye, jaw, and series of gill arches do not correspond in any clear way to those of the motoneurons innervating these muscles, that are present in a series of brain segments. Differences in the two patterns could arise if mesodermal cells that contribute to a muscle originate at the same axial level as the corresponding motoneurons, and later move to new positions. Such a movement occurs in the chick embryo.

We made fate maps for head mesoderm in 12 and 24 hour zebrafish embryos in which muscle progenitors were labeled with lineage tracer, and correlated these positions with those of brain segments at the same stages, derived from antibody staining patterns. At 12 hours, cranial muscles mapped at approximately the same axial levels as the motoneurons that innervate them. In contrast, by 24 hours, the progenitors of all of the cranial muscles had moved rostrally, and certain of them had inverted their relative positions along the axis. These movements distorted the earlier map and brought the mesodermal cells into positions where they differentiated a day later, out of register with the corresponding neural segments.

We conclude that neural segments and their mesodermal targets are aligned early in cranial morphogenesis. Subsequent movements distort this original simple relationship to establish a complex pattern of cranial innervation.

Supported by NSF grant BNS-8708638 and NIH GM-07257.

INTERACTIONS BETWEEN NEUROTRANSMITTERS III

326.1

SYNAPTIC INTERACTIONS BETWEEN SUBSTANCE P TERMINALS AND DOPAMINERGIC NEURONS IN THE RAT SUBSTANTIA NIGRA: AN ULTRASTRUCTURAL DOUBLE LABELLING IMMUNOCYTOCHEMICAL STUDY I. Mendez*, K. Elisevich, and B.A. Flumerfelt. Depts. of Anatomy and Clinical Neurological Sciences, University of Western Ontario, London, Canada, N6A 5C1.

Evidence that substance P (SP) immunoreactive striatal neurons project to the substantia nigra and influence the nigral dopaminergic output has been provided mainly by physiological, biochemical and pharmacological studies. However, the synaptology of SP-dopaminergic interactions in the substantia nigra is less clear. SP immunoreactive terminals and tyrosine hydroxylase (TH) immunoreactive neurons were simultaneously colocalized with the PAP method using two different chromogens with distinct reaction products easily differentiated at the light and electron microscope levels. SP immunoreactive structures were first localized with 3'3-diaminobenzidine, then TH immunoreactivity was localized using benzidine dihydrochloride. SP immunoreactive terminals made symmetrical and asymmetrical synaptic contacts on TH-positive dendrites in the substantia nigra pars compacta and reticulata. The present results give direct morphological evidence that substance P immunoreactive neurons, likely located in the striatum, provide a direct input to dopaminergic neurons in the substantia nigra, in support of the view that substance P acts as a modulatory neurotransmitter in the striato-nigral loop. (Supported by MRC of Canada)

326.2

ULTRASTRUCTURAL BASIS FOR INTERACTIONS BETWEEN OPIOID PEPTIDES AND DOPAMINE IN RAT STRIATUM. V.M. Pickel and J. Chan. Div. Neurobiology, Dept. Neurology and Neuroscience, Cornell Univ. Med. Coll., New York, NY 10021.

We examined the cellular substrate for known functional interactions between neurons containing opioid peptides and dopamine, the principal catecholamine in the dorsolateral striatum of adult rat brain. Single, vibratome sections were dually labeled using a rat monoclonal antibody against leucine-enkephalin and a rabbit polyclonal antiserum against the catecholamine synthesizing enzyme, tyrosine hydroxylase (TH). Ultrastructural analysis of dually labeled sections unequivocally established the direct synaptic input from TH-immunoreactive terminals to neurons containing leucine enkephalin-like immunoreactivity (LE-LI), as suggested by more indirect analysis of adjacent sections (Kubota et al., *Brain Res.* 367:374, 1986). Additionally, we demonstrated that striatal terminals containing LE-LI and TH (1) form symmetric synapses on common spiny dendrites both with and without detectable LE-LI and (2) show appositions with each other and with unlabeled axon terminals. We conclude that in the dorsal striatum opioid and non opioid spiny neurons are directly inhibited by terminals containing dopamine and/or opioid peptides and that additional presynaptic interactions may account for functional relationships between these transmitters. (Supported by NIH grant DA-04600)

326.3

CCK/DOPAMINE /GLUTAMATE INTERACTIONS IN THE HIPPOCAMPUS/ NUCLEUS ACCUMBENS/VENTRAL TEGMENTAL AREAS.
De Witte Ph., Heidbreder C.* & Gewiss M.* Lab. Psychobiology, Univ. of Louvain, Belgium.

Neurons with co-localized cholecyctokinin (CCK-8) and dopamine are present predominantly in the ventral tegmental area and project mainly to the caudal part of the medial nucleus accumbens. The nucleus accumbens also received excitatory glutamatergic neurons coming from the hippocampus via the fimbria. This dopamine system may have different influence depending on the activity of the hippocampal formation and originating particularly from the subiculum. The hippocampal formation also contains excitatory CCK-8 interneurons innervating pyramidal cell bodies. The intracranial self-stimulation (ICSS) with electrodes implanted into the mesolimbic system can be modulated by intracerebral injection of CCK-8.
A decrease in ICSS is observed following injection of CCK-8 into the ventricle (150 picomoles), into the rostral part of the accumbens (150 picomoles) and into the subiculum (300 femtomoles). Glutamate injected in the same dosage gave raise to the same depressive effect of ICSS. Since CCK-8 inhibits ICSS at a dose 500 time lower into the subiculum than in the cerebral ventricles and since CCK-8 interneurons are present in this hippocampal formation it is thus tempting to relate the inhibitory CCK-8 action on ICSS by a stimulatory pathway coming from the subiculum to the accumbens via the fimbria.

326.4

CELLULAR LOCALIZATION AND EXPRESSION OF MAO IN CNS: QUANTITATIVE ENZYME RADIOAUTOGRAPHY AND IN SITU HYBRIDIZATION HISTOCHEMISTRY. J. Saura Marti*, N.C. Lan, J.C. Shih, M. Da Prada* and J.G. Richards. (SPON: W. Haefely). Pharma Research CNS, F. Hoffmann-La Roche AG., CH-4002 Basel, Switzerland.
Monoamine oxidase (MAO), a flavin-containing enzyme located in the outer membranes of neuronal and glial mitochondria, exists in two forms, MAO-A and MAO-B, which are identified by their inhibitor sensitivity and by their substrate selectivity. Both forms are important for neurotransmitter regulation. Fluctuation in functional MAO activity may be associated with neurological disorders such as depression and Parkinson's disease.
Ro 41-1049 [N-(2-aminoethyl)-5-(m-fluorophenyl)-4-thiazole-carboxamide. HCl] and Ro 19-6327 [N-(2-aminoethyl)-5-chloro-2-pyridine-carboxamide. HCl] are novel reversible and selective inhibitors of MAO-A and -B respectively. As radioligands they have been used to map the distribution and density of the enzymes in microscopic regions of rat CNS, peripheral organs and human brain. Extremely good correlations between binding and enzyme activity were obtained for various parameters, e.g. tissue selectivity, IC_{50} values for inhibitors, age-related changes and species-related differences. Furthermore, the distribution, density and selectivity of binding sites *in vitro* and *in vivo* were very similar, e.g. MAO-A: locus coeruleus, paraventricular thalamus, solitary tract nucleus, inferior olive, raphé nucleus; MAO-B: ependyma, circumventricular organs, hypothalamus, hippocampus, cerebellar Bergman glia cells, inferior olive, posterior pituitary. Using [^{35}S]cRNA probes, e.g. for human MAO-B, it was possible to identify the cellular localization of enzyme synthesis in 5-HT raphé neurones.
The possible role of MAO-A and MAO-B in the aetiology of depression and Parkinson's disease might be elucidated by studying their distribution and expression in diseased human brain using these selective, quantitative and high resolution techniques.

326.5

MODULATION OF ^{3}H-NOREPINEPHRINE (^{3}H-NE) RELEASE FROM THE URINARY BLADDER (UB) OF RAT: EFFECT OF IMIPRAMINE. G.T. Somogyi*, J.M. Perel and W.C. de Groat (SPON: A. Mallinger) Clinical Pharmacol. Program, Western Psychiatric Inst. and Clinic and Dept. Pharmacol., Univ. of Pittsburgh, Pittsburgh, PA 15213.

Cholinergic modulation of ^{3}H-NE release was studied in rat UB strips prelabelled with ^{3}H-NE. ^{3}H-NE uptake was very prominent in the UB base where the noradrenergic innervation is most dense. Electrical field stimulation (30V, 2 HZ, 240 shocks) markedly increased ^{3}H-NE outflow from the superfused tissue. The quantity of ^{3}H-NE released was approximately equal during three consecutive periods of stimulation. Activation of presynaptic muscarinic receptors by 0.1 uM oxotremorine (OXO) led to a 46% reduction of the ^{3}H-NE release. Either 1 uM atropine (ATR) or 1 uM imipramine administered with OXO increased the release above the control (147% and 158% respectively). Both 1 uM ATR and 1 uM yohimbine (YOH) increased the release of ^{3}H-NE (167% and 311% of the control respectively). ATR given after YOH had a smaller facilitatory effect than YOH given after ATR (176% and 366% respectively). We conclude that the release of ^{3}H-NE is modulated via endogenous transmitters acting on both muscarinic and alpha$_2$ adrenergic presynaptic receptors and that the latter provide the most prominent control. Imipramine, a drug that is used to treat hyperactive UB conditions exerts a strong antagonistic effect on muscarinic presynaptic receptors and thereby increases the release of NE; an action which very likely contributes to the therapeutic effects of the drug. Supported by USPHS Grant MH 30915, NSF BN-82-08348, NIH AM 316 888.

326.6

SIGMA ANTAGONIST MJ 14802 INCREASES NEUROTENSIN CONCENTRATION IN THE RAT NUCLEUS ACCUMBENS AND CAUDATE NUCLEUS. B. Levant, G. Bissette, and C.B. Nemeroff. Depts. Pharmacol. and Psychiat., Duke Univ. Med. Ctr., Durham, NC 27710.

Treatment with typical antipsychotic drugs, such as haloperidol, selectively increases the concentration of neurotensin (NT) in the nucleus accumbens and caudate nucleus of the rat. These increases in NT concentration may mediate the therapeutic and side effects, respectively, of antipsychotic drugs. Many antipsychotic drugs have been shown to posess high affinity for the sigma receptor. This study evaluated the effects of selective blockade of sigma and D_2 dopamine receptors on regional brain NT concentrations. NT concentrations in discrete brain regions of adult, male, S-D rats were measured by a sensitive and specific radioimmunoassay. Acute treatment with the selective sigma antagonist MJ 14802 (3 - 60 mg/kg, i.p.) increased the concentration of NT in the nucleus accumbens and caudate nucleus in a dose-related manner ($P<0.01$ at 35 mg/kg). Chronic treatment with MJ 14802 (21 days, 35 mg/kg/day) produced similar increases in NT concentration in these brain regions ($P<0.01$). Neither acute nor chronic treatment with the selective D_2 antagonist sulpiride (100 mg/kg, i.p.) produced the pattern of NT alterations observed after administration of haloperidol. Thus, sigma receptors, rather than dopamine receptors, may mediate the effects of typical antipsychotic drugs on regional NT concentration. Supported by NIMH MH-39415.

326.7

THE MELATONIN RECEPTOR IS COUPLED TO A G-PROTEIN. J.T.Laitinen and J.M.Saavedra., Lab. Clin. Sci., NIMH,Bethesda, MD 20892.

We used quantitative autoradiography to characterize 2-iodo-melatonin binding sites in the rat suprachiasmatic nuclei (SCN) and area postrema (AP) and compared them with those in the chick retina (Dubocovich, FASEB J., 2:2765,1988).

In all three areas, saturation studies revealed high affinity binding sites (K_d ~50 pM) with varying binding capacity (B_{max} ~10, ~30 and ~70 fmol/mg protein for SCN, AP and chick retina, respectively). Displacement studies gave similar results. At least in the SCN, melatonin binding exhibited a diurnal rhythm, a likely reflection from the oscillation of the receptor between two affinity states (Laitinen et.al.,Endocrinol.,124:1585, 1989). To clarify this further, we studied the effect of guanyl and adenyl nucleotides on melatonin binding. Only the guanyl nucleotides dose-dependently inhibited melatonin binding in the three areas. Preliminary data also suggests partial shift of the receptor from high to low affinity state in the presence of 50 μM GTP-gamma-S.

Thus, GTP-binding protein(s) likely participates in melatonin signal transduction. Moreover, our data might explain the discrepancy in affinities reported for the receptor in rat brain.

326.8

IMMUNOCYTOCHEMICAL DEMONSTRATION OF MUSCARINIC ACETYLCHOLINE RECEPTOR PROTEINS IN RAT AND HUMAN NEOCORTEX. INTERACTION WITH CORTICAL PROJECTIONS FROM THE MAGNOCELLULAR BASAL NUCLEUS. P.G.M.Luiten, Gy.Gaál*, R.P.A.Gaykema*, A.D.Strosberg* and H.Schroeder*. Dept. of Animal Physiology, University of Groningen, Groningen, The Netherlands.

Earlier we investigated the projection patterns from the magnocellular basal nucleus (MBN) to the cortex in the rat employing anterogradely transported Phaseolus vulgaris lectin (PHA-L). These projections were analyzed with LM and EM methods and match with cholinergic marker enzyme distribution. Presynaptic labeling with the tracer raised our interest in postsynaptic cholinergic receptors which we investigated with a monoclonal antibody (M35) raised against purified muscarinic receptor proteins.

Application of M35 to rat and human cortical tissue revealed basically identical patterns of immunoprecipitate. At LM level staining of predominantly pyramidal cell types occurred in layer V and somewhat less frequent in layers II/III showing immunoreactive perikaryal cytoplasm and basal and apical dendrites. At the EM level the subcellular distribution revealed the presumed pathway of receptor protein synthesis in the soma, dendritic transport system and postsynaptic membrane incorporation. Double-label pre and postsynaptic staining by peroxidase methods combined with the use of electron-dense particles in the EM indicate PHA-L labeled MBN terminals making symmetric and asymmetric synaptic contacts with M35 labeled neurons.

326.9

ANTI-PEPTIDE ANTIBODIES TO m1 MUSCARINIC RECEPTORS SPECIFICALLY LABEL CORTICAL NEURONS. P. F. Strang, D. C. Mash and D. D. Flynn. Depts. of Pharmacology and Neurology, University of Miami School of Medicine, Miami, FL., 33101.

Muscarinic receptor subtypes (M1 and M2) have been postulated on the basis of pharmacological evidence. While recent molecular biological data have demonstrated five distinct primary sequences of the muscarinic receptor, the pharmacological and biochemical properties attributed to each receptor subtype remains unclear. In the present study, monoclonal antibodies (mcabs) were produced against a peptide corresponding to a unique portion in the i3 cytoplasmic loop of the porcine m1 receptor sequence (Kubo et al., *Nature* 323: 411, 1986). Anti-peptide mcabs were screened by ELISA, immunoblot, immunoprecipitation and immunohistochemical procedures. Immunocytochemical localization studies were performed on paraformaldehyde fixed sections of the rat brain. The sections were processed to identify bound primary antibody by either the PAP (double bridge procedure) and/or biotin-avidin methods. Control sections were obtained by eliminating the primary antisera or by adding a nonspecific IgG instead of the mcabs. Sagittal sections reacted with mcabs revealed distinct labelling of pyramidal cells and apical dendrites throughout the cerebral cortex. Scattered neurons were also visualized in the olfactory tubercle and parasubiculum. No cell or fiber labelling was observed in the thalamus, pons or medulla. These results suggest that the m1 mcabs may recognize a subpopulation of the pharmacologically-defined M1 receptor subtype in the rodent brain. Supported by NS19065 and NS 25785.

326.10

SUBSTANCE P IMMUNOREACTIVITY IN THE RAT INTERPEDUNCULAR NUCLEUS AND SYNAPTIC INTERACTIONS WITH CHOLINE ACETYLTRANSFERASE AND GLUTAMIC ACID DECARBOXYLASE. M.D. Kawaja*, B.A. Flumerfelt, S.P. Hunt and A.W. Hrycyshyn. Dept. of Anatomy, University of Western Ontario, London, Canada & MRC Molecular Neurobiology Unit, Cambridge, U.K.

Habenular projections of the fasciculus retroflexus (FR) contain substance P (SP) or choline acetyltransferase (ChAT) activity, and provide two prominent topographically organized afferent inputs to the interpeduncular nucleus (IPN) of the ventral midbrain. The IPN also possesses neurons which stain immunohistochemically for SP or glutamic acid decarboxylase (GAD). In the present study the cellular and synaptic distribution of SP-immunoreactive profiles in the rat IPN were examined to determine their synaptic relations with either ChAT-positive FR axons or GAD-positive neurons. Both single and double antigen immunohistochemistry were employed using the chromogens diaminobenzidine and benzidine dihydrochloride. SP-positive axons and terminals were found throughout the nucleus, and SP-positive dendrites and somata were confined to the rostral portion. SP-positive FR terminals in several subnuclei formed contacts with dendritic processes solely, including those of GAD-positive neurons. Terminals of ChAT-positive FR axons contacted SP-positive dendrites. These results provide evidence for synaptic interactions between putative excitatory SP- and ChAT-positive FR axons and IPN neurons possessing GAD or SP activity. (Supported by the MRC of Canada)

326.11

PARADOXICAL INCREASE IN INTRACELLULAR CALCIUM (Cai) IN RESPONSE TO GABA IN CULTURED CHICK BRAIN CORTICAL CELLS. M.K. McMillian, L.G. Miller, S. Chesley,* F. Lopez,* A. Schatzki,* Div. of Clinical Pharmacology, Tufts-New England Medical Ctr., Boston, MA.

GABA acts as an inhibitory neurotransmitter in many neuronal systems, increasing Cl^- conductance through the $GABA_A$ receptor channel, and inhibiting Ca^{2+} currents and cAMP accumulation through the $GABA_B$ receptor. In dissociated 8 day old chick embryo cortical cells, cultured for 2-10 days on collagen-coated plates, activations of excitatory amino acid receptors (by glutamate, NMDA, quisqualate or kainate) produce large increases in Cai as measured by changes in Fura 2 fluorescence (ratio method). Surprisingly, GABA also increased Cai in these cells, but not in glial cultures. The increase in Cai was smaller than that obtained with glutamate analogs, but was similar to that obtained with acetylcholine, norepinephrine and ATP. Muscimol (1-50 μM) increased Cai and this effect was blocked by picrotoxinin, suggesting that the $GABA_A$ receptor was involved. The $GABA_B$ antagonist phaclofen (10 μM) did not block. While an indirect effect of GABA on Cai is possible, muscimol effects were observed after exposure to all other aforementioned agonists and their antagonists, and after naloxone. Depolarization of these cells with 40 mM KCl produced a larger effect on Cai than GABA and blocked a subsequent GABA effect.

CARDIOVASCULAR REGULATION III

327.1

INTRACELLULAR ANALYSIS *IN VIVO* OF CARDIOVASCULAR NEURONS IN THE ROSTRAL VENTROLATERAL MEDULLA. A.R. Granata and S.T. Kitai, Dept. of Anat. and Neurobiol., College of Medicine, The Univ. of Tenn., Memphis, TN 38163.

Neurons located in the rostral ventrolateral medulla (RVL) at the level of the C1 adrenergic neurons, which project to the intermediolateral nucleus (IML) in the spinal cord, provide an important excitatory drive to sympathetic preganglionic neurons. The purpose of this study was at first to identify these neurons and to characterize the changes in membrane potential occurring during the cardiac cycle. We used a conventional technique to record intracellular potentials. Rats were anesthetized (urethane plus ketamine), paralyzed, and ventilated and arterial blood pressure and EKG monitored. RVL neurons were antidromically activated following IML stimulation (axonal conduction velocity of 2.6-12 m/sec). After penetration, the majority of these neurons discharge at high regular frequencies. Hyperpolarizing currents injected through the recording electrode reduced the frequency of discharge and then action potentials fired at a regular time with respect of pulsatile arterial wave. Further hyperpolarization (current above 1nA) completely suppressed spike potentials but underlying depolarization remained (700-1100 uV; 200 sweeps averaged). The depolarizing potentials always preceeded the pulsatile arterial wave and their amplitude increased with hyperpolarizing current. A second group of neurons were antidromically activated from the IML (axonal conduction velocity of 20-34 m/sec) and monosynaptically activated from the NTS. The recorded neurons were intracellularly injected with biocytin and visualized under avidin-Texas red fluorescent microscopy. After that, the tissue was processed for phenylethanolamine N-methyltransferase (PNMT) immunoreactivity. All the injected neurons were located in C1 area of the RVL. We can conclude that a population of bulbospinal neurons in the RVL discharge spike potentials arising from EPSPs. These EPSPs are synchronized with the cardiac cycle. (Supported by AMER. HEART ASSOC. GRANT #861294 to A.R.G. and NIH GRANT NS20702 to S.T.K.).

327.2

ANESTHETIC-DEPENDENT CONTRIBUTIONS OF ROSTRAL LATERAL TEGMENTAL FIELD (rLTF) NEURONS TO SYMPATHETIC NERVE DISCHARGE (SND). M.J. Kenney, S.M. Barman and G.L. Gebber. Dept. Pharmacol., Mich. St. Univ., E. Lansing, MI 48824.

The LTF of the caudal medulla (0-4 mm above obex) contains many neurons whose basal discharges are correlated to the 2- to 6-Hz rhythm in SND of barbiturate-anesthetized cats (Gebber and Barman, J. Neurophysiol. 54: 1498, 1985). In contrast, few such neurons were found in the rLTF (4-7 mm above obex) in these preparations. The current study shows that the concentration of rLTF neurons with sympathetic nerve-related activity is dramatically increased under α-chloralose anesthesia. Unit spike-triggered averaging of inferior cardiac SND revealed that the discharges of ~50% of rLTF neurons were synchronized to an aperiodic sharp spike-like event in SND. On occasion, LTF unit activity was correlated to both the aperiodic event and the 2- to 6-Hz rhythm in SND. Microstimulation at rLTF unit recording sites elicited an increase in SND with temporal characteristics similar to that following spontaneous unit activity. Importantly, rLTF neurons with sympathetic nerve-related activity could not be found following midcollicular decerebration in chloralose-anesthetized cats. These results support the view that an aperiodic spike-like component of SND in chloralose-anesthetized cats is mediated by rLTF neurons that are driven by inputs from the forebrain. (Supported by NIH Grants HL33266 and HL13187.)

327.3

VISCEROTOPIC ORGANIZATION OF AFFERENTS OF NUCLEUS TRACTUS SOLITARII TO VENTROLATERAL MEDULLA. R.E. Gomez*, D.A. Ruggiero, S.L. Cravo*, E.P. Mtui*, and D.J. Reis. Div. of Neurobiology, Cornell Univ. Med. Coll., NY, NY 10021. (SPON: J.E. LeDoux)

The nuclei reticularis rostroventrolateralis (RVL) and caudoventrolateralis (CVL) integrate cardiorespiratory (C-R) reflexes and receive afferents from nucleus tractus solitarii (NTS). Yet, the precise viscerotopic organization of the projection is unknown. Sprague-Dawley rats (300-350 g; n=20) were anesthetized with chloral hydrate (0.5 g/kg). *Phaseolus vulgaris* leucoagglutinin (PHA-L) was iontophoresed (7 µA anodal, 7 sec on/off, 15 min) into the caudal two-thirds of NTS. PHA-L labeled varicosities outlined the pyramidal and columnar organization of RVL and CVL. In other experiments, retrograde tracers, Fluoro-Gold, rhodamine-microbeads or WGA-HRP were deposited into RVL and CVL and the distribution of backfilled cells were mapped in medulla. RVL deposits labeled cells in n. retroambiguus (nRA), RVL, n. reticularis dorsalis (RD) and NTS. In NTS, cells were concentrated in dorsal (lateral), medial (parvicellular and subpostremal), intermediate and medial commissural subnuclei. Fewer cells were labeled in lateral and interstitial nuclei. CVL deposits labeled RVL, n.RA, RD and NTS. In NTS most neurons were in ventral, ventrolateral, interstitial, intermediate and lateral commissural subnuclei; few cells were labeled in dorsal or medial divisions. Control injections adjacent to RVL or CVL labeled few cells in NTS. Double-labeled NTS cells followed injections into regions transitional between RVL and CVL. In conclusion, cells of origin of NTS afferents to RVL and CVL are distributed viscerotopically, in a dorsomedial to ventrolateral direction. Single cells projecting to both RVL and CVL and 'interreticular' connections suggest anatomical substrates for C-R integration.

327.4

AFFERENTS FROM VENTROLATERAL MEDULLA TO NUCLEUS TRACTUS SOLITARII AND SPINAL CORD. E.P. Mtui*, R.E. Gomez*, D.A. Ruggiero, M. Anwar* and D.J. Reis. Div. Neurobiology, Cornell Univ. Med. Coll., NY, NY 10021. (SPON: D.A. Fischman)

Neurons in the ventrolateral medulla (VLM) synthesize catecholamines (CA) and neuropeptides; maintain vasomotor tone and modulate cardiopulmonary reflexes relayed by the nucleus tractus solitarii (NTS). Sprague-Dawley rats (300-350g) were anesthetized with chloral hydrate (0.5 g/kg). We first sought to determine whether neurons in nucleus reticularis rostroventrolateralis (RVL) project to the NTS and spinal cord. *Phaseolus vulgaris* leucoagglutinin (PHA-L) iontophoresed into RVL (7 µA anodal, 7 sec on/off, 15 min) labeled terminals in NTS (dorsal-lateral, ventral, intermediate and commissural) subnuclei and intermediolateral cell columns (IML). Projection neurons in VLM were defined by injecting Fluoro-Gold (FG) and rhodamine microbeads (Rhod) into the thoracic cord and NTS. Cells throughout VLM contained FG or Rhod and were admixed with double-labeled cells in RVL. In the caudal VLM, prespinal cells in nucleus retroambiguus were dorsomedial to NTS-projection cells. In order to determine the contribution of CA neurons to the projections, single- and double-retrogradely labeled cells were photographed and tissues processed immunocytochemically (PAP) for tyrosine hydroxylase (TH). TH-immunoreactive backfilled cells were restricted to RVL, whereas most double-labeled (FG/Rhod) cells were immunonegative. To confirm the VLM projection to NTS, WGA-HRP was injected into NTS and tissues were processed for both retrograde transport and TH. Cells containing WGA-HRP and TH were skewed medially in the C1 adrenergic area of RVL; most projection neurons were immunonegative. In conclusion, adrenergic and primarily non-adrenergic projection neurons in VLM may modulate both cardiopulmonary reflex excitability and sympathetic vasomotor tone.

327.5

ANATOMICAL SUBSTRATES OF VENTRAL SURFACE CHEMORECEPTOR REFLEX. D.A. Ruggiero, T.A. Milner, M. Anwar and D.J. Reis. Div. Neurobiology, Cornell Univ. Med. Coll., NY, NY 10021.

Structures integrating cardiopulmonary responses to chemoreceptor and pharmacologic stimulation of the carotid body (CB) and ventral medullary surface (VMS) are unknown. We sought to determine by light and electron microscopy (EM) the anatomical substrates integrating chemoreceptor functions of the CB and VMS. Sprague-Dawley rats (300-350 g) were anesthetized with halothane (2% in 100% O_2). (1) Transganglionic transport of wheat germ agglutinin-horseradish peroxidase (WGA-HRP) injected into CB labeled punctate varicosities in caudal divisions of nucleus tractus solitarii (NTS). (2) *Phaseolus vulgaris* leucoagglutinin (PHA-L) iontophoresed into NTS labeled terminals in a) nuclei reticularis rostroventrolateralis (RVL) and caudoventrolateralis (CVL) and b) along a thin sheet lining the glutamate sensitive area of the rostral VMS subjacent to RVL. (3) PHA-L deposits in RVL labeled terminals in CVL and along a similar strip of VMS. (4) Histochemical demonstration of transport of solid WGA-HRP (in tygon rings, 0.4mm i.d.) applied onto VMS combined with immunocytochemical labeling for serotonin or the adrenaline-synthesizing enzyme, phenylethanolamine N-methyltransferase (PNMT) backfilled monoaminergic and non-monoaminergic perikarya in RVL and areas of NTS which received CB afferents. (5) EM revealed that within 0.5μm of the VMS a) PNMT-immunoreactive (ir) terminals formed synapses with unlabeled dendrites and b) PNMT-ir dendrites were ensheathed by astrocytic processes. These data suggest that cardiopulmonary portions of the NTS link chemoreceptors of the CB and VMS via monoaminergic neurons in the nucleus RVL. (Supported by NIH 18974 and an AHA Established Investigator Award (DAR)).

327.6

CONTRIBUTION OF ADRENALINE SYNTHESIZING NEURONS IN THE VENTROLATERAL MEDULLA TO CIRCULATORY CONTROL. J. Ciriello and T.-X. Zhang. (SPON: R. McLachlan) Department of Physiology, University of Western Ontario, London, Canada N6A 5C1.

We have recently described an asymmetric distribution of phenylethanolamine N-methyltransferase (PNMT) containing neurons in the rostral ventrolateral medulla (VLM) of the Wistar Kyoto (WKY) rat. On the basis of this observation we investigated the contribution of PNMT neurons of the C1 region to the cardiovascuar responses elicited during chemical stimulation. The VLM was systematically explored in alpha-chloralose anesthetized and atropine treated WKY rats for pressor and cardioacceleratory sites using microinjections (12-25nl) of L-glutamate. At the end of each experiment the animal was perfused with Zamboni's fixative and transverse brain stem sections were processed immunocytochemically for the demonstration of PNMT. It was found that in 16 of 24 (66%) WKY rats studied, glutamate injections elicited increases in arterial pressure on one side of VLM only, whereas increases in heart rate were elicited from both sides. The pressor sites were found only in those areas containing PNMT neurons. These data support the hypothesis that adrenaline neurons in VLM are involved in vasomotor control, and suggest that an alternate population of neurons in the same VLM region provides an excitatory input to spinal cardioacceleratory neurons. (Supported by MRC of Canada)

327.7

Activation of 5-HT_2 Receptors at the Intermediate Area of the Ventrolateral Medulla Leads to Changes in Cardiorespiratory Activity. A.K. Mandal, K.J. Kellar, and R.A. Gillis. Department of Pharmacology, Georgetown University School of Medicine, Washington, D.C. 20007.

Recently, we proposed that serotonin (5-HT) may have a dual effect on cardiorespiratory activity at the intermediate area (IA) of the ventrolateral medulla (JPET 248(2):851-857). Specifically, we have shown that activation of 5-HT_{1A} receptors at the IA results in hypotension, bradycardia, and respiratory stimulation as caused by an increase in respiratory rate (f). Application of 5-HT to the IA also causes hypotension and bradycardia. These effects of 5-HT are intensified by prior blockade of 5-HT_2 receptors. The greater responses obtained by 5-HT after blockade of 5-HT_2 receptors may have been due to 5-HT's activating 5-HT_2 receptors, leading to increases in blood pressure (BP) and heart rate (HR). To test this hypothesis, a specific agonist for 5-HT_2 receptors, 1-(2,5-dimethoxy-4-iodophenyl)-2-amino-propane or DOI, was applied bilaterally to the IA of chloralose- anesthetized cats in a dose of 30 ug/side. DOI significantly increased BP (39±7 mmHg, $p<0.05$) and decreased f (-5±0 breaths/min, $p<0.05$) (n=8). Pretreatment with the 5-HT_2 antagonist, ketanserin (25 ug/side), prevented the cardiorespiratory effects of DOI (n=7). N-methyl-D-aspartate (NMDA) applied to the IA (11 ug/side) produced a similar increase in BP as observed with DOI; however, ketanserin did not counteract the hypertensive effect of NMDA. Ketanserin by itself caused a significant decrease in BP (-18±5 mmHg, $p<0.05$). These observations indicate that 5-HT_2 receptors and 5-HT_{1A} receptors located at the IA have oppposing effects on cardiorespiratory activity. Furthermore, since ketanserin by itself produced a decrease in blood pressure, we suggest that 5-HT_2 receptors at the ventrolateral medulla are under tonic activation.

327.8

AFFERENT SOURCES OF SUBSTANCE P-LIKE IMMUNO-REACTIVITY IN THE ROSTRAL VENTROLATERAL MEDULLA. R. Giuliano, T.A. Milner and D.J. Reis. Div. of Neurobiology, Cornell Univ. Med. Coll., NY, NY 10021

Functional (*Brain Res.* 245:279, 1982) and anatomical (*Neurosci. Lett.* 55:255, 1985) evidence implicates the undecapeptide substance P (SP) in the cardiovascular functions of the rostral ventrolateral medulla (RVL). Terminals containing SP-like immunoreactivity (SPLI) form synapses with neurons of the C1 adrenergic group (C1 area) in the RVL (*J. Comp. Neurol.* 270:427, 1988). We sought to determine the sources of SP innervation of this major circulatory control region. Immuno-peroxidase labeling of a polyclonal antiserum against SP was combined with histochemical identification of wheat germ agglutinated horseradish peroxidase (WGA-HRP) in colchicine-treated rats. Unilateral pressure injections of WGA-HRP were made into the C1 area. In single sections processed for immunocytochemical localization of SP and retrograde labeling of WGA-HRP several afferent sources of SPLI to the RVL were detected. Dual-labeled neurons were seen primarily in the laterodorsal tegmental nucleus, lateral and medial divisions of the parabrachial nucleus and raphe nuclei of pons and medulla. Other dual-labeled neurons were distributed in the perifornical area, bed nucleus of the stria terminalis, lateral and dorsal hypothalamic areas and periaqueductal central gray. Single dual-labeled cells occurred scattered in the RVL contralateral to the injection site, the nucleus tractus solitarii and A1 area. In several regions (including periaqueductal central gray, central amygdaloid nucleus and various hypothalamic nuclei) cells con-taining SPLI were surrounded by anterograde label from the RVL. We conclude that (1) nerve terminals containing SPLI which innervate the RVL may derive from both local and extrinsic sources and that (2) RVL neurons may influence neurons with SPLI in certain brain areas.

327.9

ELECTROPHYSIOLOGICAL CHARACTERIZATION OF NUCLEUS TRACTUS SOLITARII (NTS) AND ROSTRAL VENTROLATERAL MEDULLA (RVLM) CONNECTIONS. S.K. Agarwal*, A.J. Gelsema and F.R. Calaresu, University of Western Ontario, London, Ontario, Canada N6A 5C1

The hypothesis that the decrease in arterial pressure (AP) elicited by stimulation of the NTS is mediated by NTS neurons providing a monosynaptic inhibitory input to neurons in the RVLM was tested in two sets of experiments by recording activity of spontaneously firing units in the RVLM and NTS in paralyzed and artificially ventilated male Wistar rats under urethan. In the first set, RVLM vasomotor units (inhibited by activation of baroreceptors [1-2 μg phenylephrine i.v.] and displaying a rhythmicity of their spontaneous discharge in synchrony with the cardiac cycle) were inhibited by single pulse stimulation of depressor sites in the NTS (n=13, latency 26.7 ± 2.6 ms, threshold 73 ± 16 μA). In the second set of experiments, to test the possibility that this inhibitory connection was monosynaptic, pressor sites in the RVLM were stimulated electrically (range 30-500 μA) while recording from 21 spontaneously active units in the NTS. No antidromic activation was observed. These results support recent findings suggesting a multisynaptic connection between the NTS and RVLM. (Supported by MRC of Canada).

327.10

CHEMORECEPTOR INPUTS TO NON-RESPIRATORY CELLS IN THE NUCLEUS OF THE TRACTUS SOLITARIUS (NTS). S. W. Mifflin, Department of Pharmacology, University of Texas Health Science Center, San Antonio, TX. 78284-7764

Experiments were undertaken to examine the carotid body chemoreceptor input to non-respiratory cells in the NTS. In pentobarbital anesthetized, mechanically ventilated and paralyzed cats the lingual artery was cannulated for injection of CO_2 saturated .15M HCO_3. Using K-citrate filled electrodes intracellular recordings were obtained from 14 cells which were depolarized by close arterial injection of <100ul of CO_2 saturated HCO_3 and responded to carotid sinus nerve stimulation with an EPSP at latencies of <5ms. Signal averaging revealed no fluctuation of membrane potential in phase with central respiratory activity or lung inflation in any cell. At end-tidal CO_2s of 3.5-4.5% 6 cells spontaneously discharged action potentials at very irregular frequencies. Electrical stimulation of the carotid sinus nerve evoked EPSPs with latencies of 2.05-4.33ms (3.24±.79ms). None of these cells responded to stimulation of the superior laryngeal nerve. After recording, the electrode was cut in place. Reconstructed recording sites for 12 cells were concentrated from obex to 1mm caudal to obex and dorso-medial (n=9) or dorso-lateral (n=3) to the tractus. These non-respiratory cells in the NTS might be the initial links in the reflex chain whereby arterial chemoreceptors regulate the cardiovascular and respiratory systems. (Supported by NIH grant HL-41894)

327.11

CARDIOVASCULAR ACTION OF ANGIOTENSIN II AND SARALASIN MICROINJECTION INTO THE INFERIOR OLIVE NUCLEUS OF THE RAT BRAIN STEM. Frank Wang* and M. Ian Phillips, Dept. of Physiology, University of Florida, Gainesville, FL 32610.

Several brain stem sites are involved in mediating cardiovascular responses to Ang II. In rats, one of the areas with high density of Angiotensin II receptors which has been overlooked is the inferior olive nucleus. We hypothesized that endogenous Ang II may exert neural control on blood pressure through an action on the inferior olive. Microinjections of Ang II into the lateral inferior olive (n=10) results in significant, dose related elevation of mean arterial pressure (MAP). 0.5 nmole, 1.0 nmole, and 2.0 nmole Ang II microinjections were associated with increased of 8.5 ± 1.9 mmHg, 13.1 ± 1.0 mmHg, and 23.3 ± 6.0 mmHg, respectively. Microinjections of Sar^1Ala^8 Ang II (saralasin) (n=8) were associated with significant decreases of MAP in a dose-related manner. 1.0 nmole and 2.0 nmole of saralasin alone were associated with MAP decreases of 14.7 ± 2.2 mmHg and 21.0 ± 5.5 mmHg. Sequential microinjection of 1 nmole saralasin and Ang II into the inferior olive nucleus (n=4) showed Ang II increases in MAP were reversed by the receptor antagonist. In conclusion, the findings indicate that endogenous Ang II exerts neural control on blood pressure through an action on the inferior olive nucleus because saralasin microinjection lowered normal MAP. The results imply that constant stimulation on the inferior olive nucleus by angiotensin contributes to the maintenance of normal blood pressure.

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327.12

Inhibitory amino acid-induced depression of postsynaptic responses to visceral sensory input in the solitary tract nucleus. P.D. Feldman, H.D. Brinegar* and R.B. Felder. Dept. Int. Med. and Cardiovascular Center, Univ. of Iowa, Iowa City, IA 52242.

Microinjection studies suggest a role for the inhibitory amino acids glycine (**GLY**) and gamma-aminobutyric acid (**GABA**) in modulating the transmission of cardiovascular sensory information within the solitary tract nucleus (**nTS**). Action potential responses of nTS neurons to fixed-intensity electrical stimulation of afferent fibers in the solitary tract were recorded extracellularly from superfused rat brain slices. Post-stimulus time histograms of spike responses were accumulated over 50 sequential stimulations both before and during infusion of GLY or GABA. A total of 29 neurons was studied. Infusion of 100 nM GLY induced a decrease of responsiveness in 15 of the 25 neurons tested, with a mean change (±SEM) of -75.9±5.6%. Infusion of 100 nM GABA similarly induced a decrease of responsiveness in 10 of the 25 neurons tested, with a mean change of -71.8±6.4%, while increased responsiveness was seen in 3 neurons (mean±SE = +45.7±11.4%). Six cells unaffected by GLY and 6 unaffected by GABA were tested further at doses up to those inducing non-selective membrane effects (0.5-10 uM). However, responsiveness remained unchanged. Twenty-one of the 29 neurons had been tested with both agonists. Both agonists affected the responsiveness of 7 cells, while the remaining cells were affected by only GLY (N=4), GABA (N=3), or by neither (N=7). These results indicate that both GLY and GABA may depress the transmission of visceral sensory information within the rat nTS.

UPTAKE, STORAGE, SECRETION AND METABOLISM III

328.1

VESAMICOL DECREASES BOTH QUANTAL AND SYNAPTIC VESICLE (SV) SIZE AT THE NEUROMUSCULAR JUNCTION. A. Nagel* and A.G. Engel. Muscle Research Laboratory, Mayo Clinic, Rochester, MN 55905.

Vesamicol (AH5183), an inhibitor of acetylcholine (ACh) uptake into SVs, reduces quantal size after prolonged nerve stimulation. However, at >7.5 µM the drug also acts as a postsynaptic inhibitor. To further assess the presynaptic effects of the drug, mouse phrenic nerve-diaphragms were incubated with 10 µM vesamicol at 21°C; after 22 min they were stimulated at 4 Hz for 30 min, and then allowed to recover for 45 min. Control muscles were similarly treated in the absence of vesamicol. Before incubation and after recovery, portions of vesamicol and control preparations were fixed for electron microscopy and SV diameters were determined by morphometric methods. In 3 control muscles, the mean MEPP amplitude was 100, 70 and 94% before, immediately after, and 45 min after stimulation; the mean SV volume was the same before stimulation and after recovery. In 3 vesamicol muscles the mean MEPP amplitudes before drug exposure, 22 min after drug exposure, immediately after stimulation, and 45 min after stimulation were 100, 81, 46, and 37%, respectively; the mean SV volume was reduced to 58% of that before drug exposure ($p<0.001$). The results reconfirm the known presynaptic effects of vesamicol, indicate that SV size is related to SV ACh content, imply that quantal size is related to SV size, and are in harmony with the SV hypothesis of neuromuscular transmission.

328.2

CHARACTERIZATION OF THE VESAMICOL (AH5183) RECEPTOR IN VP_1 CHOLINERGIC SYNAPTIC VESICLES. B.A. Bahr and S.M. Parsons. Dept. of Chem., Univ. of Calif., Santa Barbara, CA 93106

Purified synaptic vesicles (SVs) from Torpedo exhibit transport of acetylcholine (ACh) which is inhibited by 1-trans-2(4-phenylpiperidino)cyclohexanol (vesamicol). Detergent screening established that solubilization of active vesamicol receptor (VM-R) requires the steroidal bile acid nucleus. We synthesized the nonionic detergent N,N-bis(3-D-gluconamidopropyl)cholamide (BIGCHAP) and found it to preserve [3H]vesamicol (VML) binding better than other steroidal derivatives. In a BIGCHAP-Tween80 mixed system, the purified VM-R appears to be a diffuse glycoprotein on SDS-PAGE at 240 kDa which silver stains poorly. Identification of the VML binding protein will be achieved with a new photo-affinity analogue, (±)-trans-5(4-azidobenzoyloxy)-3-hydroxy-2-(4-phenylpiperidino)tetralin. Azido-vesamicol inhibited VML binding (K_d=20.8±8.9nM) with a K_I of 2-5µM. SVs trapped on filters and incubated with azido-vesamicol irreversibly lost 60-80% of their VML binding sites (12-16/SV) when photolyzed.

A biotinylated vesamicol analogue competitively inhibited VML binding with a K_I of 126±49nM. (±)-N5-d-Biotinyl-trans-5-amino-2-hydroxy-3(4-phenylpiperidino)tetralin (Rogers et al, J.Med.Chem. 32, 1989) inhibited ACh transport with an IC50 of 138±19nM in the absence and ≫100µM in the presence of avidin. Avidin occlusion recovered 87% of VML binding (k_1=0.21 /min) from biotinyl-vesamicol saturated SVs, whereas only 4% of the ACh transport was recovered after 1HR with avidin. Transport inhibition was reversible when using centrifugative pelleting to remove neat or biotinylated vesamicol from SVs, with transport recoveries of 55-95%. This demonstrates a significant lag time the ACh transporter requires to reactivate following removal of a vesamicol-based inhibitor.

328.3

INTRASYNAPTOSOMAL SEQUESTRATION OF ^{3}H-METHYLENEDIOXYAMPHETAMINE (MDA) AND ^{3}H-AMPHETAMINE (AMPH). R. Zaczek, S. Culp* and E.B. De Souza. Neurobiology Laboratory NIDA/ARC, Baltimore, MD 21224.

We exmined the incorporation of ^{3}H-MDA and ^{3}H-AMPH into rat brain synaptosomes. Saturation studies revealed that ^{3}H-MDA interacted with two saturable sites in synaptosomes which were sensitive to tissue boiling: K_D high=295nM, B_{max}=32pmoles/mg protein.; K_D low=45µM, B_{max}=5.2nmole/mg protein. The high capacities of these sites argue against a bimolecular interaction of ^{3}H-MDA with monovalent protein binding sites. Disrupting the integrity of the synaptosomal membranes by sonication, incubation in hypotonic media and in the presence of the detergent digitonin reduced ^{3}H-MDA and ^{3}H-AMPH incorporation. These data indicate that the incorporation represents a sequestration phenomenon. Incorporation of both compounds was reduced by preincubation of the tissue at 37°C or in hypotonic buffer at 4°C, suggesting that the sequestration is maintained by an intrasynaptosomal factor which is lost under these preincubation conditions. ^{3}H-MDA incorporation was pH dependent and temperature sensitive. The synaptosomal sequestration of both ^{3}H-MDA and ^{3}H-AMPH were inhibited by permeant cations suggesting that the factor which maintains sequestration is anionic. Pharmacological profiles of ^{3}H-MDA and ^{3}H-AMPH sequestration were identical. The rank order of inhibition (desipramine>amphetamine>MDA>methylphenidate) did not correspond to the lipophilicity of the test drugs. The intrasynaptosomal internalization of ^{3}H-MDA and ^{3}H-AMPH described in the present report may be important in the molecular mechanism of monoamine release induced by the amphetamines.

328.4

RELEASE OF ENDOGENOUS DOPAMINE FROM RAT RETINA. C.J.Gibson. Dept. of Pathology, University of Western Ontario, London, CANADA N6A 5C1

A simple superfusion apparatus has been developed for the measurement of endogenous release of the rat retinal transmitter dopamine (DA). Perfusion chambers consisted of inverted Swinnex filters (Millipore Corp, Bedford, MA; SX00 013 00) through which a modified Kreb's buffer is pumped at a flow rate of 0.5 ml/min. Fractions are collected every 15 minutes and analyzed for DA by HPLC with coulometric detection. DA release was stimulated by increasing levels of potassium (from 15 to 55 mM). Basal release averaged 12 + 5 pg/retina and rose 7-fold to 95.5 + 23 pg/retina when exposed to 40 mM potassium (K) Kreb's buffer. This represented 8.9 + 2.3% of total retinal DA. In the absence of calcium, no release was evoked by the addition of 40 mM K (11 + 4 pg/retina). In contrast release caused by the introduction of 50 uM tyramine was calcium independent. Exposure of dark-adapted retina to light also increased endogenous DA release, from 9 + 4 pg/retina to 14 + 6 pg/retina. This simple system should prove useful in determining what factors may normally control retinal DA release.

328.5

PREFERENTIAL PRODUCTION OF 3-HYDROXYANTHRANILIC ACID FROM ANTHRANILIC ACID IN RAT BRAIN SLICES. H. Baran and R. Schwarcz. Maryland Psych. Res. Ctr., Baltimore, MD 21228.

As assessed by HPLC with electrochemical detection (Baran & Schwarcz, submitted), 3-hydroxyanthranilic acid (3HANA), the bioprecursor of the excitotoxin quinolinic acid, is present in the rat brain in very low concentrations (approx. 12 fmol/mg tissue).

In mammalian tissues, 3-hydroxykynurenine (3HKYN) is believed to serve as the bioprecursor of 3HANA. However, we were unable to produce substantial quantities of 3HANA from 3HKYN in rat brain tissue. In our search for alternative bioprecursors of 3HANA, we observed that anthranilic acid (AA) can be 3-hydroxylated by cerebral slices in vitro. Incubation of cortical slices was performed at 37°C in Ringer solution containing 10 μM 4-chloro-3HANA, a potent 3-hydroxyanthranilate oxygenase inhibitor (cf. Walsh et al., this meeting). Incubation with 1 mM AA or 3HKYN produced 89.0 ± 9.3 and 9.0 ± 0.3 (5 min) or 187.5 ± 11.2 and 51.6 ± 7.9 (120 min) fmol newly synthesized 3HANA/mg tissue, respectively (N = 3 each). Preferential 3HANA synthesis from AA was also noted at lower precursor concentrations. 3HANA production from AA was linear up to 5 min.

Work in whole cell preparations will aid in the assessment of the enzymatic conversion of AA to 3HANA in the brain under physiological and pathological conditions.

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328.6

ANTHRANILATE HYDROXYLASE IN RAT BRAIN. R. Schwarcz, H. Baran and E. Okuno, Maryland Psychiatric Research Center, Baltimore, MD 21228.

Experiments with slices of rat cerebral cortex suggest the existence of anthranilate hydroxylase (AAOH), the enzyme responsible for the synthesis of 3-hydroxyanthranilic acid (3HANA) from anthranilic acid (AA) (cf. Baran and Schwarcz, this meeting). We therefore decided to examine its presence in cell-free rat brain homogenate. Standard assays were carried out at 37°C in 30 mM MES buffer, pH 6.0, containing 0.5 mM NADPH. 3HANA was determined by HPLC with electrochemical detection (Baran & Schwarcz, submitted). Enzyme activity could be unequivocally identified in several fractions obtained from homogenate after differential centrifugation in 0.32 M sucrose. The 3500 x g, 10 min precipitate contained the highest specific activity and was used in subsequent experiments. Heat treatment for 1 minute increased AAOH activity (maximum at 80°C). In the presence of 1 mM AA and 10 μM 4-Cl-3HANA (cf. Walsh et al., this meeting), 63 pmol 3HANA/mg protein were produced. The synthesis of 3HANA was linear until 1 h. In the presence of 0.1 mM tetrahydrofolic acid, under otherwise standard conditions, enzymatic activity was increased and greatly accelerated. Kinetic analysis showed a V_{max} of 120 pmol/h/mg protein and a K_m of 111 μM.

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328.7

BRAIN BUT NOT LIVER TISSUE SLICES RETAIN QUINOLINIC ACID UPON PRECURSOR LOADING. C. Speciale, D. Clary* and R. Schwarcz. Maryland Psych. Res. Ctr., Baltimore, MD 21228.

The endogenous excitotoxin quinolinic acid (QUIN) may play a role in a variety of N-methyl-D-aspartate (NMDA) receptor-mediated events in the brain. Rat brain slices synthesize QUIN following exposure to its immediate bioprecursor 3-hydroxyanthranilic acid (3HANA; Soc. Neurosci. Abstr., 14: 479.9, 1988). We have now studied the comparative ability of brain and liver slices to retain QUIN following a preloading regimen (64 μM 3HANA, 60 min at 37°C). Slices loaded with newly synthesized QUIN (=100%), were further incubated with fresh media containing 10 μM 4-chloro-3HANA to prevent additional QUIN synthesis (cf. Walsh et al., this meeting). Efflux was estimated by measuring QUIN in tissue and medium after incubations for 30, 60 or 90 min at 37°C.

In contrast to liver slices, which rapidly lost QUIN within the first efflux period (tissue content after 30 min: 3 ± 1% of controls), brain slices retained QUIN throughout the 90 min procedure (tissue content as % of controls: 52 ± 3 (30 min), 45 ± 4 (60 min), 32 ± 4 (90 min); n=4). In all experiments, QUIN concentrations in the incubation media adequately reflected QUIN loss from the tissue. Brain-specific mechanisms regulating QUIN expulsion into the extracellular compartment may be of relevance for NMDA receptor-mediated processes. Supported by USPHS grant NS 16102 and a Fogarty Fellowship to C.S.

328.8

MULTICOMPONENT ANALYSIS OF TRYPTOPHAN AND TYROSINE METABOLISM IN HUNTINGTON'S DISEASE: EVIDENCE FOR REDUCED FORMATION OF KYNURENIC ACID

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Department of Neurology, Mass.General Hosp. & Harvard Medical School, Boston MA. 02114 and ESA Corp., Bedford, MA. 01730.

Metabolism of tryptophan occurs by a branched pathway. One branch produces the excitatory amino acid (EAA) antagonist kynurenic acid and the EAA agonist quinolinic acid while a second branch produces the neurotransmitter serotonin. In the present study multiple components of the tryptophan and tyrosine metabolic pathways were measured in postmortem putamen of 35 controls and 30 Huntington's Disease (HD) brains using reverse phase high performance liquid chromatography (HPLC) with 16 sensor electrochemical detection. Measurements of kynurenic acid were also verified using reverse phase HPLC with fluorescence detection. Both concentrations of individual components of the tryptophan and tyrosine pathways as well as ratios of precursors to their respective metabolites were measured to assess the rate of turnover through various parts of the pathways. These ratios have the advantage that they are not affected by tissue shrinkage and gliosis. In addition, these ratios cancel out the effects of premortem nutritional status; a significant variable due to the fact that metabolism of tryptophan and tyrosine are exquisitely precursor responsive. In HD putamen there were significant increases in dopamine, 5-hydroxyindoleacetic acid, 5-hydroxytryptophan and serotonin concentrations. The ratios of precursors to products within the tyrosine metabolic pathway and the serotonin branch of the tryptophan pathway were unchanged in HD putamen as compared to controls. The absolute concentrations of kynurenine metabolites were not significantly changed in HD putamen, however there was a significant 2-fold ($p < 0.01$) increase in the ratio of kynurenine to kynurenic acid. This suggests that there may be reduced formation of kynurenic acid in HD. Since kynurenic acid is an endogenous inhibitor of EAA mediated neurotransmission and can block against EAA mediated neurotoxicity *in vivo*, a relative deficiency of this compound could directly contribute to the neuronal degeneration in HD.

328.9

α-KETOGLUTARATE AND PYRUVATE AS CO-FACTORS OF KYNURENINE TRANSAMINASE IN RAT BRAIN. E. Okuno, D.A. Parks*, M. Nakamura and R. Schwarcz. Maryland Psychiatric Research Center, Baltimore, MD 21228.

The endogenous excitatory amino acid antagonist kynurenic acid is biosynthesized from kynurenine (KYN) via a single aminotransaminase reaction using 2-oxo acids as amino-acceptors. We have described pyruvate (Pyr)-preferring KYN-transaminases (KPT) in rat kidney and brain (Ishikawa et al., submitted) and proved their identity biochemically and using anti-KPT-antibodies. We now examined the properties of brain KPT at physiological KYN concentrations. Assay of eluates from a DEAE-Sepharose column using ^{3}H-KYN (2 μM) as a substrate revealed only one enzyme which could use both α-ketoglutarate (αKG) or Pyr as a co-factor. With either oxo acid, the enzyme reacted with anti-KPT antibodies, indicating that transamination was catalyzed by a single enzyme protein. In crude brain homogenate, the enzyme showed a pH optimum of 8.5 with αKG and 8.2 with Pyr. Kinetic analysis revealed a low K_m-value (17 μM) for KYN with 1 mM αKG and a much higher K_m (910 μM) with 1 mM Pyr. Using 2 μM KYN, K_m-values for αKG and Pyr were 150 and 160 μM, respectively. Enzyme activity was also detected with other oxo-acids, namely 2-oxoadipate, phenylpyruvate, 2-oxo-isocaproate, glyoxylate and the most effective compound, 2-oxo-4-methylthiobutyrate.

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328.10

SODIUM-DEPENDENT INHIBITION OF KYNURENATE SYNTHESIS BY QUISQUALATE IN RAT BRAIN. J.B.P. Gramsbergen, W.A. Turski and R. Schwarcz. Maryland Psychiatric Research Center, Baltimore, MD 21228.

The excitatory amino acid (EAA) receptor antagonist kynurenic acid (KYNA) can be produced by rat brain slices upon exposure to L-kynurenine (J. Neurochem., 52:1629, 1989). KYNA synthesis takes place predominantly in astrocytes and may be regulated by neuron-glia interactions (Soc. Neurosci. Abstr., 14:479.12, 1988).

We have now investigated the effects of EAAs on KYNA production. In cortical slices of adult rats, at 1 mM, several EAAs (including glutamate and aspartate) blocked KYNA synthesis by ≥45%. At a lower dose (200 μM), however, only quisqualate (QUIS; -80%), L-α-amino-adipate (αAA; -67%), L-cysteate (-35%) and L-cysteine sulfinate (-32%) blocked KYNA synthesis significantly. Other EAAs such as AMPA, kainate, NMDA, ibotenate and quinolinate were inactive. The QUIS effect could not be blocked by EAA antagonists (including CNQX), tetrodotoxin or in high Mg^{2+}/Ca^{2+}-free medium. In contrast, the inhibitory effect of QUIS, as well as that of αAA, were blocked in Na^+-free medium, and QUIS was not effective in the brain of 1 day old rats or in liver slices. In ibotenate-lesioned striatal slices, QUIS was as effective as in control tissue.

These results suggest that the effects of QUIS and αAA may be mediated by sodium-dependent receptors or carriers on astrocytes. Supported by USPHS grant NS 16102.

328.11

4-CHLORO-, 4-FLUORO- AND 4-BROMO-3-HYDROXYANTHRANILIC ACIDS ARE POTENT COMPETITIVE INHIBITORS OF 3-HYDROXY-ANTHRANILIC ACID OXYGENASE. J.L. Walsh*, [1]William P. Todd*, [1]B.K. Carpenter* and R. Schwarcz (SPON: M. Nakamura). Md. Psych. Res. Ctr., Baltimore, MD 21228 and [1]Dept. Chemistry, Cornell University, Ithaca, NY 14853.

4-Chloro-3-hydroxyanthranilic acid (4-Cl-3HANA) is known to inhibit liver 3-hydroxyanthranilic acid oxygenase (3HAO). Since 3HAO is responsible for the neosynthesis of the endogenous excitotoxin quinolinic acid (QUIN), 3HAO inhibitors and their molecular mechanisms of inhibition are of interest in the study of QUIN neurobiology.

4-Cl-3HANA and its fluoro- and bromo-analogs have now been prepared and tested for their ability to block 3HAO activity in human and rat brain homogenates, and to inhibit QUIN production from 3HANA in rat brain slices. All three drugs inhibited 3HAO activity or QUIN production with identical relative potencies, i.e. 4-Br-3HANA > 4-Cl-3HANA > 4-F-3HANA. In homogenates, IC_{50} values ranged from 2-4 nM (4-Br-3HANA) to 17-24 nM (4-F-3HANA).

Enzyme inhibition was assessed by following the very rapid (6 sec) 3HAO-catalyzed production of ß-carboxy-muconic-ω-semialdehyde from 3HANA. Although the drugs bind very tightly to the enzyme, kinetic analysis demonstrated the inhibition to be competitive in nature.

4-Halo-3-hydroxyanthranilates represent model 3HAO inhibitors which may be of therapeutic value in cases of enhanced QUIN production in neuro-psychiatric diseases.

328.12

POTASSIUM DEPENDENT SODIUM FACILITATION OF DOPAMINE (DA) TRANSPORTER BINDING IN FRONTAL CORTEX. A. Hitri, D. Venable* and R.J. Wyatt. Neuropsychiatry Branch, NIMH Neurosciences Center at St. Elizabeths, Washington, D.C. 20032.

The sodium dependency of the DA transporter binding is a relevant biochemical characteristic to uptake. In the brain, 3H-GBR 12935 labeled DA uptake sites have been found to be both Na dependent and independent (Andersen, P.H., J. Neurochem. 48: 1887, 1987); Na dependent sites are either enhanced or inhibited by increasing concentrations of this ion. We attempted to characterize the Na inhibited 3H-GBR 12935 labeled DA uptake sites in the frontal pole (FP). The FP of three postmortem human brains were individually homogenized in 50mM Tris buffer pH 7.9. The binding reaction was carried out in the presence of 30, 60 and 120 mM NaCl, with or without the addition of 5mM KCl. Consistent with a previous report (Janowsky, A. et al., J. Neurochem. 46: 1272, 1986), our data indicate a Na concentration dependent decrease in Bmax, in a potassium free medium (Bmax=202+56; 139+50; 111+36 for 30, 60 and 60mM NaCl). With the addition of potassium ions, the effect of Na was reversed (Bmax=93+29; 261+78; 239+76, for 30, 60 and 120mM NaCl). The affinity constants were not changed in either case. The FP and striatum are different, striatal DA uptake sites do not require potassium for Na enhancement, while in the FP there is a permissive effect of potassium ions on the Na dependent increase in Bmax.

SOMATIC AND VISCERAL AFFERENTS III

329.1

INDUCTION OF JUN AND FOS PROTO-ONCOGENE PROTEINS J. Leah *, T. Herdegen * and M. Zimmermann.
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The production of jun and fos proteins was examined, immunocytochemically, after neuronal stimulation and axonal transport block in pentobarbital anaesthetised rats.

Both jun and fos appeared in nuclei of numerous, somatotopically corresponding spinal dorsal, but not ventral horn or DRG neurones 1 hr after cutaneous, muscle or visceral noxious stimuli; and in the trigeminal, solitary tract and lateral reticular nuclei following noxious stimulation of the nasal mucosa. Both appeared here, and in brain sensory nuclei after electrical stimulation of sciatic nerve C-fibers. Non-noxious, A-fiber activation was much less effective. Both appeared in many neurones in similar regions after general CNS activation by i.p. metrazol.

Jun, but not fos, appeared after 4 d and persisted for 20 d following transection or crush of the axons of DRG or motoneurones. Jun also appeared in spinal projection and cortical neurones after axotomy; and in many CNS neurones after local block of axonal transport by microinjection of colchicine, and in motoneurones and DRG neurones after sciatic nerve axonal transport block by local vinblastine.

Thus, jun and fos can both be induced transynaptically and by direct stimulation. Jun is differentially induced by axonal transport block, probably by absence of a trophic factor, and it may code for those proteins required for axonal regeneration.

Supported by the Deutsche Forschungsgemeinschaft.

329.2

EARLY ECTOPIC DISCHARGES ARE GENERATED AT THE DORSAL ROOT GANGLION IN RATS WITH A PAINFUL PERIPHERAL NEUROPATHY. K.C. Kajander, S. Wakisaka* and G.J. Bennett*. NAB, NIDR, NIH, Bethesda, MD 20892

We have reported pathophysiological changes in primary afferent fibers (Soc. Neurosci. Abstr. 14:912, 1988) in an animal model of painful peripheral neuropathy (Pain 33:87-107, 1988). One of these abnormalities is the appearance of ectopic discharges in myelinated fibers as early as 1 day after the injury. The present experiments were designed to determine the origin of these discharges.

Microfilament recording techniques were used to record sciatic nerve afferents from the L4-5 dorsal rootlets of rats 1-3 days after nerve injury. Microfilaments were severed proximally to eliminate the possibility of activity originating in the spinal cord. Three kinds of experiments were conducted to determine the site of origin of the ectopic spikes. First, the sciatic nerve was transected about 5 mm proximal to the injured region while recording from afferents with ongoing ectopic discharge. Nerve transection never eliminated the activity (16 fibers tested to date). Second, we determined the critical interval for collision between antidromic ectopic spikes and othrodromic shock-evoked spikes. Computations using each fiber's conduction velocity, critical interval and refractrory period indicated that the ectopic spikes originated near the dorsal root ganglion (DRG). Third, potassium channel blockers, gallamine triethiodide or 4-aminopyridine, applied directly to the DRG increased the activity of all fibers exhibiting ongoing ectopic discharge. Application of the blockers to the region of injured nerve had no effect. We conclude that the ectopic discharge originates in the DRG at or near the afferent's cell body.

329.3

REPRESENTATION OF SHAPE IN THE RESPONSES OF CUTANEOUS MECHANORECEPTIVE AFFERENT FIBERS. M.A. Srinivasan* and R.H. LaMotte (SPON: S. E. Kapadia). Research Lab. of Electronics, MIT, Cambridge, MA 02139 and Dept. Anesthesiology, Yale Univ. Sch. Med., New Haven, CT 06510.

The distribution of surface curvature of an object defines its shape. In order to investigate the peripheral neural representation of shape, we recorded responses of slowly and rapidly adapting mechanoreceptive afferent fibers (SAs and RAs) to cylinders differing in curvature, and to a pattern of curvatures forming a smooth wavy surface applied to the monkey fingerpad. The discharge rates of both SAs and RAs to indentations by the cylinders increased with curvature. SA responses discriminated the curvatures very well both during the ramp and steady phases, in comparison to the ramp responses of RAs. RA responses were well modulated by the curvatures when the cylinders were sinusoidally vibrated: Their response thresholds decreased with increases in curvature. When the wavy surface was stroked at constant force over the fingerpad, both SAs and RAs responded with bursts to the convex portions and pauses to the concave. The width and frequency of SA responses were correlated with the wavelength and radius of the skin curvature, respectively. The RAs responded only to the leading half of the convex portions with poorer modulation. These findings are consistent with our earlier hypotheses [J. Neurosci. 7(6) 1655-1695]: SA discharge rates increase with increases in the depth of indentation and curvature change at the receptive field, and SAs do not respond to the unloading of curvature; RA discharge rates increase with increases in the velocity of indentation and rate of curvature change at the receptive field. (PHS grant NS 15888 and ONR contract N00014-88-K-0604)

329.4

A 3-D CONTINUUM-MECHANICS MODEL OF SKIN PREDICTING MECHANORECEPTOR RESPONSES TO SCANNED, EMBOSSED STIMULI. J.R. Phillips. University Laboratory of Physiology, Parks Road, Oxford. OX1 3PT. U.K.

Neural images reconstructed from primary mechanoreceptive afferent responses to embossed letters scanned across the skin, have distorted features (Phillips et al., Proc. Natl. Acad. Sci. USA, 85:1317-1321, 1988). The objective of this study was to extend the static, 2-D model of skin described previously (Phillips & Johnson, J.Neurophysiol. 46:1204-1225, 1981) to three dimensions, and to account for the response features observed using scanned stimuli. Skin is modelled as an ideal medium in which the principle of superposition of forces applies. Moving stimuli are modelled as assemblies of point loads applied normal to the skin surface summed with frictional forces applied tangential to the surface. Time dependent effects are modelled as receptor properties. The 3-D model accounts qualitatively for many of the effects observed in slow adaptor responses to scanned stimuli, assuming that compressive strain is the effective stimulus at the receptor terminal. In particular, the model accounts for the asymmetry in responses observed between the leading and trailing edges of letters and also between horizontal and vertical elements of horizontally scanned letters. Asymmetry results from superimposing stresses derived from the action of skin frictional forces.

329.5

MECHANISMS OF TWO-POINT DISCRIMINATION USED BY RAPIDLY-ADAPTING (RA) MECHANORECEPTORS IN GLABROUS SKIN. E.P. Gardner and C.I. Palmer. Dept. of Physiology, NYU Sch. of Med., NY, NY 10016.

In order to measure spatial acuity of phasic cutaneous mechanoreceptors in macaque monkeys, we displayed horizontal bar patterns spaced 1-13 mm apart on a computer-controlled OPTACON stimulator. Two-point resolution was measured by simultaneously pulsing pairs of rows at rates of 100, 50 and 25 Hz; each pair was shifted in tandem across the hand in order to simulate tangential motion. Receptive field diameter appears to be the critical determinant of spatial resolution of gaps between two bars. RAs fire continuously if bar spacing is less than the field diameter, but do not summate inputs when both active rows are contained within the field. 75% discharge one spike/pulse as bar patterns cross the field, yielding a uniform spike train whose frequency reflects OPTACON pulse rates, but fails to indicate gaps between bars. When bar spacing exceeds the field diameter, the spike train splits into two sets of impulses as first one and then the second bar traverses the field. Burst onset latencies reflect both bar spacing and apparent speed of motion across the hand. 20% of RAs show a pause in firing at 3 row bar spacing (3.6 mm), while nearly all resolve 4 row (5 mm) gaps. Another 20% differentiate gaps narrower than the field diameter. These RAs respond to both probe indentation and retraction at the field center, firing two spikes/pulse; they show double peaked response profiles to bar patterns spaced at least 2.5 mm apart, whose timing corresponds to bar spacing. None resolve bars spaced 1.2 mm apart, as double spike responses occur only to the leading edge.

Although no gap occurs in RA response profiles when closely spaced bar patterns cross their fields, their duration of firing distinguishes bars spaced less than the field diameter. Spike train duration is directly related to bar spacing; at subthreshold separations, RAs show linear increases in total spikes/sweep as spacing between active rows widens. Furthermore, less overlap of receptive fields contacted by each stripe occurs as bar spacing widens, leading to enlargement of the pool of RAs activated. These population mechanisms may explain psychophysical observations that humans can discriminate probes spaced 1 mm apart, and can differentiate single row gaps on the OPTACON. Most RAs do not differentiate 2 narrow bars spaced less than the field diameter from a single filled bar spanning a similar length of skin. Thus, RA afferents appear to use similar temporal coding mechanisms for subthreshold spatial resolution and for distinguishing bars of varying width. *(Supported by USPHS Research Grant NS11862.)*

329.6

TWO-POINT DISCRIMINATION AND TEXTURE CODING BY PACINIAN CORPUSCLES (PCs) IN PRIMATE GLABROUS SKIN. C.I. Palmer and E.P. Gardner. Dept. of Physiology, NYU School of Medicine, NY, NY 10016.

In order to measure spatial acuity of Pacinian corpuscle afferents in the median and ulnar nerves of anesthetized macaque monkeys, we displayed horizontal bar patterns spaced 1-13 mm apart on a computer-controlled OPTACON stimulator placed on the hand. Two-point resolution was measured by simultaneously pulsing pairs of rows at rates of 100, 50 and 25 Hz; each pair was shifted in tandem across the skin in a row by row sequence in order to simulate tangential motion at speeds of 120-30 mm/s. Like RA afferents, receptive field diameters of PCs also appear to be the critical determinant of spatial resolution of gaps between two bars. However, RAs display finer spatial resolution than PCs, due to their smaller fields, and more regular firing patterns. PCs fire continuously to bar patterns spaced less than the field diameter, displaying multiple-spike bursts throughout much of the field. None of the PCs studied resolve bar patterns spaced 1 or 2 rows apart (1.2-2.5 mm). Only half the PC population shows pauses in activity signalling gaps between bars spaced 5 mm apart, whereas nearly all RAs tested resolve this spacing. Resolution of OPTACON bar patterns by the entire PC population is observed only at bar spacings of 1 cm (8 rows) or more. Spatial resolution is also impeded by the tendency of PC afferents to summate closely-spaced inputs near the field center; this results in response profiles with a single, large-amplitude broad peak. Moreover, irregularities in PC firing patterns usually preclude the use of multiple-spike bursts at subthreshold spacings for distinguishing individual stripes.

Like RAs, PC afferents can use their duration of firing to distinguish bars spaced less than the field diameter, even if there is no gap in the response profile. However, total spike output from PCs is a less reliable metric than from RAs due to greater variability in trial to trial responses, and the tendency of PCs to summate adjacent stimuli. High frequency (100 Hz) sweeps evoke weaker responses than low frequencies (25 Hz), due to response attenuation at the far end of receptive fields, and decreased probability of burst responses to individual OPTACON pulses. These characteristics of PC receptive fields, together with receptor adaptation mechanisms to high pulse rates, endow PC afferents with poor spatial sensitivity, and may explain their inability to encode spatial frequencies of periodic gratings scanned across the hand. *(Supported by USPHS Research Grant NS11862.)*

329.7

STUDIES OF THE INTRAFASCICULAR ORGANIZATION OF HUMAN NERVES: R.G. Hallin* and R. Ekedahl* (SPON: K. Tornqvist). Dept. of Clin. Neurophysiol., Huddinge University Hospital, 141 86 Huddinge, Sweden.

Microneurography in man was carried out in median nerve sensory fascicles with 200-250μm diameter concentric needle electrodes with a recording lead diameter of 20-30μm. Such electrodes permit detailed studies of activity within even a part of a fasicle.

Multiunit fields of myelinated fibres innervating palmar skin did not correspond to the innervation zone of one or two adjacent digital nerves but instead often comprised spatially separate parts of such skin territories. Upon repeated intrafascicular electrode adjustments there were successive somatotopically organized transitions from fields composed by separate parts to fields which were coherent or vice versa. The receptive fields of single A and C fibres were all localized within the previously screened multiunit areas.

These findings suggest the presence of a somatotopy in human limb nerves comparable to that previously established in the spinal cord and somatosensory cortex.

329.8

POSTOCCLUSIVE HYPEREMIA IN FELINE CORTICAL GREY MATTER IS MEDIATED BY TRIGEMINAL AXONS. M.A. Moskowitz, D.E. Sakas, E.P. Wei, M.G. Buzzi, C. Ogilvy, H.A.Kontos. Mass. Gen. Hosp, Boston, MA 02114. Med. Coll. VA, Richmond, VA 23298.

Marked hyperemia may develop in brain following temporary cessation of blood flow, and is associated with morbidity following cardiac arrest, stroke and head injury. Blood flow was compared in 10 symmetrical brain regions using radiolabeled microspheres under resting conditions, during ischemia, and with reperfusion in cats 2-3 weeks after chronic trigeminal ganglionectomy, trigeminal rhizotomy or sham operation. Pentobarbital anesthetized animals (n = 16) were subjected to 10 minutes of combined brachiocephalic-left subclavian occlusion and hypotension (BP < 50 mm). Blood flow values were within normal range and symmetrical at rest. During ischemia, blood flow was not detected in brain. Within 30 minutes after reestablishing flow, values increased to nearly 5 fold above resting and were symmetrical in rhizotomy and sham cats. However, values were attenuated by as much as 65% ipsilateral to the ganglionectomy within cortical grey matter corresponding to the territories supplied by anterior, middle and posterior cerebral arteries. These data demonstrate that axon reflex-like mechanisms mediate cortical hyperemia following recirculation and are likely to contribute to the tissue damage (e.g., edema formation) associated with recirculation injury.

329.9

LOW AND HIGH THRESHOLD MECHANOSENSITIVE UNITS IN THE LUMBAR FACET JOINT. JM Cavanaugh[1,*], T Yamashita[1,*], TV Getchell[2], AA El-Bohy[2,*], AI King[1,*] (SPON: ML Getchell[2]). Bioengineering Center[1] and Dept. of Anatomy and Cell Biology[2], Wayne State University, Detroit, MI 48202.

Our aim was to characterize somatosensory units in the lumbar facet joint, proposed to play a key role in low back pain. **METHODS:** Rabbits (3-4 kg) were anesthetized with Nembutal iv, and an L4-L5 laminectomy was performed. L5 dorsal rootlets were cut at their proximal ends, split, and draped over bipolar recording electrodes. The left L5/L6 facet joint was searched with 1-2 mm diameter rods for mechanosensitive units. Identified units were stimulated electrically to obtain conduction velocities (cv's) and with von Frey hairs to determine threshold. **RESULTS:** Seventeen units have been identified at this joint: 6 in joint capsule, 2 in ligamentum flavum and 9 in regions common to capsule and tendon. Of these, 5 had thresholds > 6g and 8 < 6g. Two had cv's < 2.5 m/s (Group IV), 9 had cv's of 2.5-20 m/s (Group III), and 6 > 20 m/s. Eight of these responded to joint movement that caused tissue stretch. Ten other mechanosensitive units were found in muscle tendon inserting into the facet; 6 had cv's of 2.5-20 m/s and 4 > 20 m/s. **CONCLUSIONS:** The facet joint contains low to high threshold mechanoreceptors, some of which respond to stretch. The results indicate that both proprioceptors and nociceptors are present in this joint. Supported by a Whitaker Foundation Grant (JMC), NIH Grant NS-16340 (TVG), and WSU Research Stimulation Fund (TY).

330.1

SYNTHESIS-MODULATING DOPAMINE AUTORECEPTORS ON PC12 CELLS. D.L. Rosin, D. Bagaglio* and R.H. Roth. Department of Pharmacology, Yale School of Medicine, New Haven, CT 06510.

The presence of release-modulating dopamine (DA) autoreceptors in PC12 cells has previously been demonstrated (N.D. Courtney et al., Soc. Neurosci Abstr. 14:926, 1988). We therefore reasoned that these cells may also possess synthesis-modulating DA autoreceptors. PC12 cells were grown in DMEM with 5% heat-inactivated horse serum and 10% fetal bovine serum. At the time of the experiment, cells were preincubated in a HEPES-modified Krebs-Ringer buffer (pH 7.4) at 37° for 30 min prior to incubation with K^+ or drugs. Catecholamine synthesis was determined by measuring the accumulation of DOPA in a 10 min period following inhibition of DOPA decarboxylase by NSD 1015; DOPA was measured by HPLC-EC. The addition of 30 or 60 mM K^+ for 10 min prior to NSD increased DOPA accumulation in a concentration-dependent manner. The selective DA D_2 antagonist, sulpiride (10 μM), added 15 min before NSD, also increased basal synthesis but did not significantly increase K^+-stimulated DOPA accumulation. The increase in DOPA accumulation produced by K^+ or sulpiride could be attenuated by the DA autoreceptor-selective agonist, EMD 23 448 (10 μM, added 10 min before NSD). Basal synthesis was also inhibited by the addition of EMD alone. These results suggest that, in addition to DA release, catecholamine synthesis is also regulated by DA autoreceptors in PC12 cells. (Supported by USPHS Grant MH 14092 and the Scottish Rite Schizophrenia Research Council).

330.2

EPINEPHRINE IS SYNTHESIZED BUT NOT STORED IN RAT SPINAL CORD. A.F. Sved and J.N. Salter*. *Department of Behavioral Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260.*

Epinephrine (EPI) levels in the intermediolateral cell column of the rat spinal cord (SC) are undetectable, although there is a dense projection to this region from neurons in the medulla containing all the enzymes required for EPI synthesis. One explanation for this finding is that EPI in the nerve terminals is degraded as soon as it is synthesized, so that no EPI is actually stored in synaptic vesicles. To test this hypothesis, EPI, norepinephrine (NE), and dopamine (DA) levels were measured in SC of rats pretreated with an inhibitor of monoamine oxidase (MAO), the major enzyme involved in EPI degradation. Pargyline treatment (75 mg/kg ip, 4 hrs prior to sacrifice; n=6) significantly increased EPI, NE, and DA levels. However, the increase in the level of EPI in thoracic SC (<0.2 to 1.3±0.1 pg/mg tissue; >600%) was much larger than that for DA (134%; 20.5±1.1 to 27.5±1.1 pg/mg tissue) and NE (158%; 209±7 to 332±16 pg/mg tissue) in the SC or other regions of the central nervous system (130-170% increase from control). These results suggest that the interaction of MAO with EPI synthesis is such that high MAO activity can prevent the storage of EPI in certain nerve terminals containing the enzymes required to synthesize EPI. Thus, PNMT-containing catecholamine neurons are not necessarily adrenergic.

330.3

REGULATION OF RAT ADRENAL MEDULLARY PHENYLETHANOLAMINE N-METHYLTRANSFERASE. D.L. Wong, Y.S. Yoo and C.L. Bildstein*. Stanford Univ. Sch. Med., Stanford, CA 94305

Rat adrenal medullary phenylethanolamine N-methyltransferase (PNMT) is regulated hormonally and neurally. Neural stimulation induces PNMT synthesis and requires an intact splanchnic nerve. Hormones, in contrast, act permissively. Steroids restore PNMT to basal levels but not beyond. They regulate PNMT degradation via its cosubstrate, S-adenosylmethionine. PNMT transcriptional activity should reflect the above. PNMT mRNA should concommitantly increase with protein synthesis unless mRNA pools are not fully utilized. Hormonal changes should not alter PNMT mRNA. To examine neural effects, male Sprague Dawley rats were administered reserpine (10mg/kg, alternate days, 1 week), and changes in adrenal PNMT activity, protein (immunotitration) and mRNA (Northern analysis) were assessed in individual animals. Respective changes were 1.8, 1.4 and 2.0-fold. These results suggest that PNMT mRNA pools may not be fully utilized for protein synthesis and that neural induction may elicit a more active form of the enzyme. A time course shows that mRNA increases rapidly (≤24 hr) while responses in enzyme activity and protein are protracted. Hormonal studies were similarly consistent with translational events. Hypophysectomy depleted PNMT activity and protein by ~90 percent while mRNA showed only a 13 percent reduction. ACTH administration (4 I.U. daily, 1 week) restored enzyme activity and protein to ~62 percent of normal values with a slight, albeit insignificant further reduction in mRNA.

330.4

EXPRESSION OF TYROSINE HYDROXYLASE AND ITS mRNA IN CULTURED HYPOTHALAMIC-MIDBRAIN NEURONS. W. Kedzierski*, N. Aquila-Mansilla* and J.C. Porter (SPON: R.M. Stewart). Dept. OB/Gyn and Physiology, U.T. Southwestern Med. Ctr., Dallas, TX 75235

Trypsin dissociated neurons from the hypothalamus-midbrain of 20-day old fetal rats were cultured in serum-free, chemically defined medium. Tyrosine hydroxylase (TH) and its mRNA were quantified using an immunoblot assay and an S1 nuclease protection assay, respectively. During culture, DOPA and a lesser amount of dopamine were secreted into the medium. DOPA and dopamine secretion could be blocked in a dose-dependent manner by 3-iodo-L-tyrosine and α-methyl-L-tyrosine, specific inhibitors of TH. In one experiment, neurons from 140 rat fetuses were combined and distributed among 140 24-mm wells, using 1.5 million cells per well. The rate of secretion of DOPA, the quantity of TH mRNA, and the mass of TH were determined weekly for 11 weeks. The results of this study are shown in Table 1.

Table 1. TH and TH mRNA in cultured neurons and DOPA secretion.

Age of culture (weeks)	TH mRNA (amoles/well)	TH mass (fmoles/well)	DOPA secretion (pmoles/18 h/well)
1	1.6	3.2	0.4
2	2.0	5.8	1.3
3	2.3	6.4	1.5
4	2.8	11.5	4.9
5	2.7	15.3	8.6
6	2.8	21.1	9.2
7	2.8	25.2	7.2
8	2.6	17.5	8.8
9	2.9	15.0	7.9
10	2.1	15.9	8.1
11	2.0	10.5	7.5

After 1 week of culture, there was 1.6 amoles TH mRNA per well, increasing to 2.8 amoles at the end of the 4th week. By the end of the 11th week, there was 2 amoles TH mRNA/well. The mass of TH was lowest (3.2 fmoles/well) after the first week, increasing to 25.2 fmoles by the end of the 7th week. The quantity of TH fell to 10.5 fmoles/well by the 11th week. DOPA secretion increased from 0.4 pmoles/18 h/well after the first week to 9.2 pmoles by the 6th week, then fell slowly until the 11th week. These studies demonstrate that TH gene expression occurs in neurons in culture as indicated by TH mRNA, TH synthesis occurs, and these neurons secrete DOPA for several weeks in serum-free medium. (Supported by NIH grant DK-01237.)

330.5

CHARACTERIZATION OF cDNA FOR AROMATIC L-AMINO ACID DECARBOXYLASE (AADC) FROM BOVINE ADRENAL MEDULLA U.J. Kang and T.H. Joh. Lab. Mol. Neurobiol., Cornell Univ. Med. Coll. Burke Rehab. Center, White Plains, NY 10605

AADC (EC 4.1.1.28) catalyzes the syntheses of dopamine and serotonin, two major neurotransmitters in the nervous system. We previously reported isolation of the lambda gt-11 recombinant clone containing the cDNA for AADC from bovine adrenal medulla (Albert et al, J Biol Chem 1262:9404,1987). Nucleotide sequence analysis of this cDNA shows an open reading frame starting from base 18, extending to base 1478 followed by a 554 bp untranslated region and the poly (A) tail. The deduced amino acid sequence shows a 60% homology to the complete coding region of the epidermal form of drosophila AADC mRNA (Morgan et al, EMBO J 5:3335,1986). Primer extension reactions using two different primers from base 2 to 19 and 112 to 130 consistently produce a distinct band extending 68 bases from the 5' end of our cDNA. This fragment probably represents the 5' untranslated region corresponding to the first exon of drosophila AADC gene. The hepta-peptide stretch representing the pyridoxal phosphate binding site is completely conserved between the two species and is also the same as the peptide sequence from pig AADC. Four of the seven amino acids are shared with feline glutamic acid decarboxylase and 417 bases surrounding this site are well conserved between the two enzymes (54%) (Kobayashi et al, J Neurosci 7:2768,1987). The 3' untranslated region has no significant homology to that of drosophila AADC. It contains, however, about a 200 bp stretch of novel Alu-type repeat sequence (Duncan, Nuc Acids Res 15:1340,1987).(supported by AG00406 and MH 24285)

330.6

SEQUENCE ANALYSIS OF BOVINE DOPAMINE B-HYDROXYLASE cDNA. O. Hwang, K.S. Kim*, and T.H. Joh. Cornell Univ. Med. Coll., Burke Rehab. Center, White Plains, N.Y. 10605.

The enzyme dopamine B-hydroxylase (DBH) catalyzes the conversion of L-DOPA to norepinephrine. Bovine adrenal medullary DBH has been extensively studied for elucidation of its apparent structural complexity. Towards an understanding of the mechanism underlying synthesis of multiple forms of DBH, we isolated and characterized a bovine DBH cDNA. We previously reported the isolation of a 2.3 kb DBH cDNA which was missing it's 5' end (Hwang et al, Neurosci. Abs.14:26, 1988). Here we report the complete sequence of the full length cDNA.

For the isolation of the 5' end fragment, we constructed a random primed lambda gt11 cDNA library and obtained several clones by screening with the 240 bp EcoRI-AvaI fragment as a probe. Analysis of the translated region reveals 87% homology with that of human DBH (Lamouroux et al, EMBO J.6:3931, 1987) and indeed hybridizes to a 3.1 kb human adrenal medullary message. The untranslated region, on the other hand, exhibits a lower homology of 51% compared to the human (Kobayashi et al,Nuc. Acid Res.17:1089, 1989). In addition, 304 bases upstream of the poly A tail lies the stretch of AGTAAA, which was identified as a second polyadenylation site in human. If alternative splicing does occur in bovine DBH as demonstrated for human, however, the second site must be utilized at a much lower rate since our cDNA probe could not detect a smaller message by Northern blot analysis.(supported by MH24285-14)

330.7

MODULATION OF THE LOW THRESHOLD CALCIUM CONDUCTANCE IN GUINEA-PIG THALAMIC NEURONS BY AGONISTS AND ANTAGONISTS OF DOPAMINE RECEPTORS *IN VITRO*. E. Geijo-Barrientos and R. Llinás. (SPON: V. DeCrescito). Dept. Physiol. & Biophys. NYU Med. Ctr. NY 10016.

Thalamic neurons are innervated by fibers originating in the periventricular dopaminergic system (Moore and Bloom, Ann. Rev. Neurosci. 1:29:1978). We have shown (Geijo-Barrientos and Llinás, Soc. Neurosci. Abstr. 1988) that dopamine (DA) increases the magnitude of the thalamic low threshold calcium current (LTC), while haloperidol and forskolin decrease it. In order to further elucidate the role of DA receptors in the modulation of this current, we have investigated the effects of quinpirole, a selective D2 receptor agonist and the two isomeric forms of the antipsychotic drug flupentixol. The experiments were performed *in vitro*, in the guinea-pig thalamic slice preparation using current clamp and single electrode voltage-clamp techniques. 5μM quinpirole induced either a decrease of the LTC or no marked change. Those neurons that demonstrated a change showed a decrease of the LTC in the range of 50% of the control value, with no change in the voltage dependence of the steady state inactivation of the current. The two isomeric forms of flupentixol were used to discard the possibility of a non-specific effect of haloperidol on the low threshold calcium conductance. The cis- and trans- forms of flupentixol were tested at concentrations of 1-10 μM, and the cis-flupentixol induced a decrease in the LTC in 90% of the cells tested, while the trans-flupentixol had a very reduced effect. In the latter case only a small decrease of the LTC in 20% of the cells was observed. This result, which agree with the different affinity of this two isomers for the DA receptors (Cros and Owen, Eur. J. Pharmacol. 65:341:1980), suggest that the effect of these DA blockers on the Ca current is not due to the inespecific high membrane solubility of these drugs. The above results suggest that DA could generate the increase of the LTC by acting simultaneously on D1 and D2 receptors, not unlike what has been proposed in other neurons (Walters et al., Science, 236:719:1987). The issue of interest here is that DA, which has been related to the genesis of several psychiatric disorders, may contribute to the modulation of the thalamo-cortical activity through its actions on the thalamic LTC.

330.8

DOPAMINERGIC CELLS AND FIBRES IN THE MEDULLA OBLONGATA OF CAT REVEALED BY ANTI-DOPAMINE IMMUNOHISTOCHEMISTRY. T.F.C.Batten*, P.N.McWilliam* and A.Maqbool* (SPON: European Neuroscience Association). Dept. of Cardiovascular Studies, Leeds University, Leeds LS2 9JT, U.K.

Formaldehyde fluorescence histochemical localization of catecholamines in the brain failed to provide evidence for dopamine neurons in the medulla oblongata (Dahlstrom, A. & Fuxe, K., *Acta Physiol. Scand.* 62(suppl.232):1, 1964), but recent studies utilizing antisera to catecholamine synthetic enzymes, including our own work on the cat (Batten, T.F.C. & McWilliam, P.N., *J.Physiol.* 399:76, 1988) have shown cells in the caudal dorsal motor vagal nucleus (DMVN) which appear to be immunoreactive for tyrosine hydroxylase (TH) alone, suggesting they may be dopaminergic. We have now used antibodies to dopamine-glutaraldehyde conjugates (prepared by Drs. H.W.M. Steinbusch and R.M. Buijs) to re-examine this region on Vibratome sections of cat brainstem after rapid perfusion with phosphate-buffered 5% glutaraldehyde. Dopamine-immunoreactive (DA-IR) cell bodies were indeed found in the DMVN at the level of, and at levels caudal to the obex. More surprisingly significant numbers of "A1 catecholamine" cells of the ventrolateral medulla also exhibited DA-IR at levels between the obex and spinal cord. DA-IR fibres were scattered in the reticular formation, but were particularly numerous in the DMVN, spinal trigeminal nucleus and dorsal column (gracile and cuneate) nuclei. Thus, dopamine immunohistochemistry has allowed us to demonstrate previously unrecognized systems of dopaminergic cells and fibres in the lower brainstem of the cat.

330.9

DOPAMINERGIC NEURON CULTURES FROM THE SUBSTANTIA NIGRA AND THE VENTRAL TEGMENTAL AREA. S. Masuko, S. Nakajima and Y. Nakajima. Dept. of Anat. and Cell Biol. and Dept. of Pharmacol., Univ. of Illinois, College of Med. at Chicago, Chicago, IL, 60612 and Dept. of Biol. Sci. Purdue Univ., W. Lafayette, IN 47907.

We have developed dopaminergic neuron cultures separately obtained from the substantia nigra (SN) and the ventral tegmental area (VTA) of new born rats by using our previously reported method in which brain nuclei dissected out from brain slices are dissociated and cultured (Masuko et al., J. Neurosci., 1987, 6: 3229). Double immunocytochemical treatments to tyrosine-hydroxylase and GABA in 2-3 week-old cultures showed the presence of 41% dopaminergic (DA) and 38% GABAergic neurons in SN cultures, whereas in VTA cultures there were 65% DA and 21% GABAergic neurons. DA neurons were characterized by 2 to 5 thick primary processes originating from the cell body. These processes had sparse arborization. Varicosities were mainly found on the distal segments of processes. In contrast, GABAergic neurons had highly branched thick and thin primary processes with intensive arborization, their processes having numerous varicosities. The coexistence of DA and cholecystokinin was found in 70% of DA neurons in the SN and in 30% of DA neurons in the VTA. Electrophysiological studies on cultured DA neurons revealed that the action potentials elicited by constant current depolarization adapted and stopped firing. This is in contrast to the non-adapting action potentials recorded from the cultured locus coeruleus neurons. Supported by NIDA grant (DA05701) and NIH grants (AG06093 and NS24711).

330.10

PURIFIED CULTURES OF VENTRAL TEGMENTAL AREA (VTA) DOPAMINE NEURONS. Stephen Rayport, David Sulzer* & Deirdre Batson*. Dept. Psychiatry and Ctr. Neurobiol. & Behav., Columbia Univ. and New York State Psychiatric Inst., New York, NY 10032.

Highly purified preparations of midbrain dopamine (DA) neurons would advance both cellular physiological studies *in vitro* as well as offer an improved source of such cells for brain transplantation. Typically, DA neurons constitute only a few per cent of the cells dissociated from embryonic midbrain. A 10-fold enrichment for DA neurons has been achieved using fluorescence activated cell-sorting to NSP-4, a selectively expressed surface antigen (DiPorzio *et al.*, 1987); but, because the initial numbers of DA cells were extremely low in the E13 mice used, the fraction of DA cells in the final cultures did not exceed 1% (~100 out of 20,000 cells plated in 1 cm^2 wells).

To develop VTA cultures composed principally of DA neurons, we took advantage of the high concentration of DA cells in postnatal VTA and of their comparatively large size (*e.g.*, Loughlin & Fallon, 1985). In our routine midbrain cultures, where about 5% of the cells were tyrosine hydroxylase-positive (TH+), the average diameter of TH+ cells was 14 ± 0.5 μm (mean ± s.e.m.), while TH- cells measured 10 ± 0.2 μm. When we dissected VTA (encompassed in 2 mm^3) from postnatal rats and dissociated it utilizing methods that favor survival of large cells (*e.g.*, Kay & Wong, 1986; Huettner & Baughman, 1986 & 88), 25 to 30% of the cells were TH+. To enrich cultures for large DA cells further, we fractionated the cell suspension on a 0-10-25% Percoll step gradient (*e.g.*, Hatten, 1985). Minimally 35% of the cells in the large cell fraction (top of the 25% layer) were TH+.

When purified cultures were incubated with 5-hydroxy-dopamine, which makes catecholaminergic vesicles electron dense, accumulations of synaptic vesicles in the soma perimeter and in numerous *en passant* varicosities were seen. The majority of large cells in purified cultures also showed the broad action potentials characteristic of midbrain DA cells.

330.11

A COMPARISON OF THE RELATIVE POTENCIES OF DOPAMINE UPTAKE INHIBITORS IN INHIBITING DOPAMINE UPTAKE AND [^{3}H]GBR 12935 BINDING. S. Izenwasser, L.L. Werling, and B.M. Cox. Dept. of Pharmacology, Uniformed Services University, Bethesda, MD 20814-4799.

It is thought that inhibition of dopamine uptake may be the mechanism by which cocaine produces its reinforcing effects. There are, however, a number of compounds which also inhibit dopamine uptake but which are not widely abused. It is therefore possible that cocaine may act preferentially in those brain regions which are specifically involved in drug reinforcement. In the present study, the effects of various dopamine uptake inhibitors were examined on both competition against [^{3}H]GBR 12935 binding and on dopamine uptake in the striatum, medial prefrontal cortex, nucleus accumbens and olfactory tubercle of rat. There were no significant differences in either binding affinity or ability of these drugs to inhibit dopamine uptake among the different brain regions. Furthermore, the order of potencies for the compounds tested was similar for both inhibition of dopamine uptake and displacement of [^{3}H]GBR 12935 binding. GBR 12909 and amfonelic acid were each approximately 40-100 times more potent than either cocaine or methylphenidate in both assays. Thus, for these compounds, there do not appear to be significant differences in drug sensitivity in the brain regions tested and there seems to be a correlation between potency for inhibition of dopamine uptake and competition against [^{3}H]GBR 12935 binding. Carbamazepine, however, potently inhibited dopamine uptake but did not inhibit binding of [^{3}H]GBR 12935 at concentrations up to 100 μM. This suggests the possibility that dopamine uptake may be regulated by more than one mechanism.
(Supported by a grant from the National Institute on Drug Abuse).

330.12

DARP, A NOVEL POTENT DOPAMINE-RELEASING PROTEIN: IN VITRO AND IN VIVO STUDIES. G.D.Chang, A.D.Ramirez*, C.Gross*, F.Marcus*, V.D.Ramirez. Department of Physiology and Biophysics, University of Illinois, Urbana, IL 61801; Chiron Corporation, Emeryville, CA 94608.

DARP, a putative protein discovered in the rat adrenal gland (Chang and Ramirez, Brain Res. 463:385, 1988), has been purified from bovine adrenals to a high degree of purity. This protein was used 1) to study its DA-releasing activity from corpus striatum (CS) fragments superfused in vitro and 2) to examine its capacity to evoke behavioral responses in rats bearing a push-pull cannula in the caudate nucleus (CN). The in vitro data indicate that the DA-releasing activity of DARP is a Ca^{2+} dependent phenomenon. In preparations infused with DARP for 1 h, a rapid decline in activity occurs by 40 min. Infusion of DARP for 20 min at 20 μg/ml induced a marked DA release (> 8-fold) that peaks by 20 min whereas an identical pulse 60 min later barely increased DA release. Contrariwise, infusion of DARP into the CN of freely behaving male rats at 10 μg/ml for 15 min but not at 1 μg/ml evoked strong amphetamine-like stereotyped motor behaviors within 10 min which ceased completely 5 min after termination of the infusion when the rat was again calm. An identical infusion of DARP 25 min later re-initiated similar short-lived stereotyped behaviors. Robust increases in DOPAC and DA were observed with higher levels during the second infusion. Lastly, DARP partially purified from bovine brain also stimulated the in vitro DA release.

330.13

DOPAMINE RELEASE AND METABOLISM IN THE FRONTAL CORTEX AND STRIATUM BY TYPICAL AND ATYPICAL NEUROLEPTICS. F. Karoum, M. Egan* and R.J. Wyatt. Neuropsychiatry Branch, NIMH Neurosciences Center at St. Elizabeths, Washington, D.C. 20032.

The effects of haloperidol and clozapine on frontal cortex (Fx) and striatal (ST) dopamine (DA) metabolism, and release were evaluated in rats. DA metabolism was assessed from changes in 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA). DA release was estimated by the accumulation of 3-methoxytyramine (3MT) following microwaving, 10 min after pargyline (65 mg/kg). The neuroleptics were administered i.p. one hour before sacrifice. Biochemical analyses were by mass fragmentography. Up to 0.1 mg/kg haloperidol did not change ST DOPAC or HVA. They were, however, significantly elevated in the Fx (0.05 and 0.1 mg/kg ip). In contrast, clozapine significantly elevated DA metabolism in both the ST and Fx (5, 10, 20 mg/kg). Haloperidol increased 3MT accumulation in both the Fx (min. dose 0.2 mg/kg) and the ST (min. dose 0.05 mg/kg). Thus, in the ST DA release appears more sensitive to haloperidol than in the Fx. 3MT accumulation was increased by clozapine in the Fx, but reduced in the ST (min. dose 5 mg/kg). The data suggest a dissociation between DA release and metabolism. Differential stimulation of DA release and metabolism in different brain regions may underlie certain clinical differences between haloperidol and clozapine. Additional data on other neuroleptics will be presented.

BLOOD-BRAIN BARRIER I

331.1

EXPRESSION OF THE GLUCOSE TRANSPORTER mRNA IN RAT AND HUMAN CEREBRAL CORTEX DEMONSTRATED BY IN SITU HYBRIDIZATION. R. N. Kalaria, M. Usiak*, T. Golde*, L. Younkin, S. I. Harik and S. Younkin.
Departments of Neurology, Pathology and Pharmacology, Case Western Reserve University, Cleveland, Ohio 44106

We have previously described the enrichment of the glucose transporter protein in brain intraparenchymal vessels by D-glucose displaceable Cytochalasin B binding and immunocytochemistry. Here, we used in situ hybridization to study the expression of the glucose transporter (GT) mRNA in rat and human brain. The plasmid prGT-Sac containing a 1.65 kilobase fragment of the rat brain glucose transporter cDNA cloned into a pBluescribe M13+ (Strategene) was used to produce a ^{35}S-labeled 346 nt cRNA probe for GT mRNA. To control for non-specific binding, sections adjacent to those hybridized with the antisense GT probe were hybridized with a non-complementary probe of quivalent length, concentration and specific activity. Hybridized sections were stained with cresyl violet and hematoxylin to visualize parenchymal structures. As expected from studies on the distribution of the protein, the probe for GT RNA labeled pial and intracortical vessels and some neurons in both human and rat brain. In rat brain, this probe also produced many grains over ependymal cells lining the lateral ventricle.

Supported by a grant from the ADRDA and NIA ADCRC.

331.2

DISTRIBUTION OF THE HEXOSE TRANSPORTER IN THE EYE. S.I. Harik, R.N. Kalaria and G. Perry. Depts. of Neurology and Pathology, Case Western Reserve Univ. Sch. of Med., Cleveland, OH 44106.

It is accepted that "tight" intercellular junctions (zonulae occludens) underlie the blood-ocular barriers that protect the eye from injurious substances in the systemic circulation. These barriers also prevent the diffusion of water-soluble nutrients like glucose which are essential for metabolism. We hypothesized that cells that have barrier characteristics must also have a high density of the hexose transporter protein (HTP) to assure steady glucose supply. We tested this by immuno-cytochemical studies using specific antibodies to HTP.

Adult Wistar rats were anesthetized and perfused-fixed in situ. The eyes were embedded in paraffin and sections were stained by the peroxidase-antiperoxidase method, using a monoclonal antibody to the C-terminus of HTP of human erythrocytes. The following cells stained intensely: (1) endothelium of blood vessels in retina and optic nerve, (2) retinal pigmented epithelium, (3) ciliary body nonpigmented epithelium, (4) posterior epithelium of the iris, and (5) vessels of the iris. All other cell types in the eye and retro-orbital tissues, including striated muscles and lacrimal glands, did not stain intensly for HTP. Thus, we find high density of HTP in all locations of the eye where "tight" junctions were discribed by ultrastructural studies.

331.3

DEXAMETHASONE DECREASES HEXOSE UPTAKE BY ISOLATED CEREBRAL MICROVESSELS. S.R.Chipkin* and A.L.McCall.(Spon: D.Rosene) Depts. of Med. & Physiol., B.U. Sch. Med. Boston, MA 02118

Dexamethasone (DEX) decreases protein entry into the brain and depresses hexose transport into adipocytes and aortic endothelial cells. To determine whether DEX affects hexose transport by the brain microvasculature, we studied ^{3}H-2-deoxy-D-glucose (2DG) transport into isolated cerebral microvessels (ICMV) after DEX preincubation. Calf ICMV were prepared by homogenization and filtration as previously described (Microvasc. Res. 35:167, 1988). After isolation, ICMV were preincubated with DEX or phosphate buffered saline for 1-2 h and then assayed for hexose transport. ICMV were exposed to ^{3}H-2DG (100 nM; 6-8 uCi/ml) and ^{14}C-sucrose (to correct for nonspecific uptake) for up to 10 min. Uptake was stopped with cold 1mM phloretin (in 0.5% ethanol). ICMV retention of 2DG was linear and anion exchange chromatography demonstrated that almost all 2DG was phosphorylated, implying that 2DG uptake reflects transport. Inhibition of 2DG uptake (pmol/mg protein/hr) into ICMV after preincubation for 2h in DEX (10^{-9} to 10^{-3}M) at 23 degrees C is shown below.

{DEX}	0	10^{-9}	10^{-8}	10^{-7}	10^{-6}	10^{-5}	10^{-4}	10^{-3}
2DG uptake	9.68	7.83	5.99	6.40	6.60	5.04	4.21	1.96
(x +SE)	1.3	0.95	0.96	1.7	1.7	1.1	0.4	0.4

Rates of 2DG uptake varied with individual ICMV preparations. DEX inhibition of 2DG uptake was greater at 37 than 23 degrees, and was more marked after 2 h than 1 h. Conclusions: 1) Brief DEX exposure in vitro inhibits hexose transport into ICMV. 2) Inhibition is concentration and temperature dependent. 3) Longer preincubations may inhibit uptake to a greater degree.

331.4

L-PHENYLALANINE TRANSPORT ACROSS THE BLOOD-NERVE BARRIER IS STEREOSPECIFIC, SATURABLE AND SODIUM INDEPENDENT. K.C. Wadhwani, Q. R. Smith and S. I. Rapoport. Lab. Neurosciences, NIA, NIH, Bethesda, MD 20879.

L-Phenylalanine (L-phe) transport across the blood-nerve barrier was studied using an in-situ perfusion technique. Hepes-buffered Ringer's solution containing tracer amounts of [14C]-L-phe and [3H]-dextran was perfused at 5 ml/min into the hindlimb of an anesthetized rat. After 2 min of perfusion, a segment of sciatic nerve was removed, frozen in liquid isopentane, desheathed in dry ice and processed for radioactivity counting. A portion of biceps femoris muscle was also removed and processed. The permeability-surface area products at the blood-nerve barrier [PA(bnb)] to [14C]-L phe decreased, from 58+9 to 1.6+0.3 x10(-5) ml/s.g, as the concentration of unlabeled L-phe in the perfusate increased, from 0mM to 10mM. D-Phe had less of an inhibitory effect in [14C]-L-phe uptake than L-Phe. There was no significant difference in PA of the biceps femoris muscle in the above perfusion media. Replacing NaCl by TrisCl in the perfusion medium did not alter PA(BNB). PA(per) of [14C]-L-phe at the epi-perineurial barrier, measured at 2 min of incubation was 1.4+0.2 x10(-5) ml/s.g (n=6), and was uneffected by 10 mM L-phe in the incubation medium. The results demonstrate concentration depend unidirectional transport of Phenylalanine at the BNB, most likely at the endoneurial capillaries.

331.5

BLOOD-NERVE TRANSFER OF ^{22}NA IN RAT SCIATIC NERVE. A. Weerasuriya* (SPON: C.H. Hockman). Peripheral Nerve Center, Dept. Neurology, Mayo Clinic & Fdn., Rochester, MN 55905.

Peripheral nerve fibers and associated glial cell function within a specialized extracellular compartment--the endoneurial microenvironment. Access to this milieu interieur from other extracellular spaces is limited and controlled by the blood-nerve interface (BNI) consisting of the endoneurial vascular endothelium and perineurium. To investigate the effects of changes in plasma ion concentration on axonal excitability, blood-nerve transfer rates of ions need to be quantified. This was done for ^{22}Na with the i.v. bolus injection technique and estimation of radiotracer in desheathed rat sciatic nerves. Employing a multiple time point analysis and tracer circulation periods of 3 to 12 minutes, graphically determined permeability coefficient-surface area product (PS) to ^{22}Na of BNI is 1.02±0.09 (SEM)x10^{-4} ml.s^{-1}.g endoneurium^{-1} and residual endoneurial plasma volume (V_p) is 1.56±0.66 (SEM) µl.g endoneurium^{-1} (n=17 and r^2=0.884). PS to ^{22}Na of the perineurium alone measured in situ is 1.01±0.15 (SEM)x10^{-5} ml.s^{-1}.g endoneurium^{-1} (n=5). Therefore, it is concluded that blood-nerve exchange of Na (and probably other ions as well) occurs across both components of the BNI and the relative contributions of the endoneurial microvasculature and perineurium are approximately 9:1 in rat sciatic nerve. (Borchard Fund and NS14304-P4 to J. F. Poduslo).

331.6

RETROGRADE TRANS-SYNAPTIC TRANSFER OF A BLOOD-BORNE PROTEIN. J. Villegas* and R. Broadwell. Divs. Neurosurg. & Neuropath., Univ. MD Sch. of Med., Balto., MD 21201

A 0.5% solution of the lectin wheatgerm agglutinin (WGA) conjugated to HRP was administered intravenously to adult rats and mice. Post-injection survival times were 6-48hr. The animals were fixed by perfusion and vibratome sections of their brains and posterior pituitary glands were incubated in a TMB or DAB medium. Cerebral vessels, perivascular phagocytes, the supraoptic (SON) and paraventricular (PVN) nuclei, median eminence, and posterior pituitary gland were conspicuously HRP-positive at 6-24hr and noticeably less so at 48hr. Cranial motor nuclei and other neuronal groups remained unlabeled. At 24hr, reaction product was evident in the Golgi saccules of cerebral endothelia and pericytes and in the perivascular clefts but not beyond the basal lamina. Endocytic vesicles and endosomes were HRP-positive in posterior pituitary terminals and underwent axonal retrograde transport to SON and PVN perikarya which harbored labeled dense bodies, inner Golgi saccules, and secretory granules. Reaction product occupied the extracellular and synaptic clefts, and endocytic vesicles in glial cells and presynaptic terminals within the SON and PVN nuclei. The results suggest that the absorptive endocytosis of a blood-borne lectin-HRP conjugate promotes transcytosis of the protein through the blood-brain barrier and neurosecretory neurons. Supported by NINDS grant #NS18030.

331.7

TRANSCYTOSIS OF A ß-ENDORPHIN -CATIONIZED ALBUMIN CHIMERIC PEPTIDE THROUGH THE BLOOD-BRAIN BARRIER (BBB) IN VIVO. W. M. Pardridge, D. Triguero*, and J. Buciak*. (SPON: J. Walsh). Department of Medicine and Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024-1682.

One strategy for drug delivery through the BBB is the production of chimeric peptides, i.e., the covalent coupling of a nontransportable peptide (e.g., ß-endorphin) to a transport vector compound that is normally transcytosed through the BBB (e.g., insulin, transferrin, cationized albumin, or cationized immunoglobulin G). [D-Ala^2]ß-endorphin (DABE) was covalently coupled to cationized albumin with the disulfide-based coupling reagent N-succinimidyl 3-(2-pyridyldithio)-proprionate (SPDP), following iodination of the DABE. Transcytosis into rat brain was studied with an internal carotid artery perfusion technique coupled with a capillary depletion step to differentiate true transcytosis into the postcapillary compartment of brain parenchyma versus simple binding and/or endocytosis at the brain microvasculature. The unconjugated [^{125}I]DABE was not measurably transcytosed into brain parenchyma during a 10 minute perfusion. However, the [^{125}I]DABE-cationized albumin chimeric peptide was rapidly transcytosed into brain and achieved a postcapillary volume of distribution of 32 ± 10 µL/g brain at 10 minutes. This volume of distribution was comparable to that achieved when [^{125}I]cationized albumin was perfused alone. Conclusions: The use of cationized albumin as a transport vector allows for the transcytosis through the BBB and delivery into brain parenchyma of ß-endorphin in vivo.

331.8

TRANSCYTOSIS OF BLOOD-BORNE FERROTRANSFERRIN AND INSULIN THROUGH THE BLOOD-BRAIN BARRIER. R. Broadwell, A. Wolf, and M. Tangoren*. Div. Neurosurg., Univ. MD. Sch.Med., Balto.,MD 21201

Ferrotransferrin, apotransferrin, and insulin conjugated to HRP (1:1; 10-20mg in 0.5-1cc) were confirmed (excluding apotransferrin) by receptor binding essay to sustain their biological activity and were infused into the internal carotid artery of adult rats; post-injection survival times were 10-60mins. Cerebral endothelia incorporated the ligands readily by receptor-mediated endocytosis. At 10mins., HRP reaction product was in endothelial endocytic vesicles, endosomes, and secondary lysosomes; reaction product was not evident in the perivascular clefts or in perivascular phagocytic cells. At 1hr, perivascular phagocytes and clefts in discrete areas of the CNS, such as the striatum, were HRP-positive in animals injected with insulin and ferrotransferrin conjugates. Presumptive exocytic vesicles occasionally were seen in continuity with the abluminal plasmalemma and exhibited "puffs" of HRP-reaction product extruding into the perivascular clefts. The apotransferrin (iron-free) conjugate was not in perivascular clefts and phagocytes. The results suggest that blood-borne ferrotransferrin and insulin undergo transcytosis through the blood-brain barrier. The transendothelial route most likely utilizes the endosomal apparatus and transfer vesicles arising from endosomes as opposed to a direct vesicular transfer of the peptides. Supported by NINDS grant #NS18030.

331.9

ANTISERUM TO BOVINE BRAIN CAPILLARY MEMBRANES BINDS A 45K, 53K, AND 200K TRIPLET OF BRAIN CAPILLARY-SPECIFIC PROTEINS (BSPs). R.J. Boado*, J. Yang*, and W.M. Pardridge (SPON: H. Weiner). Department of Medicine and Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024-1682.

The brain capillary endothelium which makes up the blood-brain barrier (BBB) in vivo, is endowed with a number of proteins that are either highly enriched or specific to that cell. To begin characterization of BSPs, an antibody specific to the brain microvasculature was prepared by immunizing rabbits with bovine brain capillary plasma membrane proteins. Following absorption of the antiserum with the acetone powders of rat kidney and rat liver, the antiserum specifically illuminated the cerebral microvasculature in brain. There was no immunostaining of any structures in bovine myocardium, liver, kidney, or urinary bladder epithelium. The only other structure found to be immunostained by the antiserum was the basolateral membrane of the bovine choroid plexus epithelium. Western blotting on nylon filters showed that the antiserum bound specifically to a 45K, 53K, and 200K triplet of proteins in the bovine brain capillary fraction, whereas there was no staining of proteins in a bovine brain synaptosomal preparation that was 98% depleted of microvasculature. Conclusions: These proteins are specific to the brain capillary and are not found in neurons or glia, or in endothelium of other organs. Their co-localization to the basolateral membrane of the choroid plexus epithelia suggests that these proteins may be integral components of BBB transport mechanisms.

331.10

BRAIN CAPILLARY ENDOTHELIAL 46K PROTEIN IS CYTOPLASMIC ACTIN BASED ON AMINO ACID SEQUENCING AND LIMITED PROTEOLYSIS STUDIES. T.B. Choi*, D.M. Nowlin*, J. Yang*, J. Calaycay*, J.E. Shively*, and W.M. Pardridge (SPON: W. Oldendorf). Department of Medicine and Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024 and Beckman Research Institute of City of Hope, Duarte, CA 91010.

The most abundant protein in the brain capillary which makes up the blood-brain barrier (BBB) in vivo is a protein that migrates at a molecular weight of ~46K on SDS-PAGE. To determine the identity of this protein, it was extracted from gels and desalted over Sephadex G25. The protein was digested with trypsin and two peptides of 11 and 18 amino acids in length, respectively, gave a 100% match with cytoplasmic actin. The SDS-PAGE gel-purified 46K protein was also subjected to limited proteolysis using S. aureus V8 protease, and the peptide fragments were separated by subsequent SDS-PAGE. This resulted in the formation of a prominent 31K doublet and these fragments were of identical molecular weight to those generated from limited proteolysis of bovine actin. Electron microscopic immunoperoxidase studies of primary cultures of bovine brain capillary endothelium showed immunoreactive actin was intimately associated with the plasma membrane. Conclusions: The brain capillary 46K protein, which comprises ~10-15% of total brain capillary protein, is cytoplasmic actin. This protein is believed to, in part, anchor the brain capillary cytoskeleton, and abnormalities in actin function in the brain capillary endothelium may lead to changes in BBB permeability.

331.11

EFFECTS OF INTERNAL CAROTID ADMINISTRATION OF MPTP ON RAT BRAIN AND BLOOD-BRAIN BARRIER. N.J. Riachi and S.I. Harik. Dept. of Neurology, Case Western Reserve Univ. Sch. of Med., Cleveland, OH 44106

Unlike primates, rats are resistant to systemic MPTP, but their substantia nigra dopamine (DA) neurons are destroyed by direct MPTP infusion. This may be due to their unique "biochemical" blood-brain barrier (BBB), which prevents MPTP and MPP+ from reaching DA neurons. To study this further, we gave MPTP to rats via the internal carotid artery, which delivers 16% of MPTP dose to the ipsilateral cerebrum, with less than 0.5% to the contralateral cerebrum or cerebellum. This method yields 40-fold higher MPTP in the ipsilateral cerebrum than in a similar injection into the right atrium of the heart. Carotid injections of 1 to 3.5 mg of MPTP resulted in 10 to 30% depletion in ipsilateral striatal DA, but without detectable behavioral abnormalities. When 5 mg of MPTP were given, all rats died from acute cardiorespiratory failure. Pargyline did not alter the lethality of MPTP but prevented DA reduction. We also studied the possibility that sequestration of MPTP and MPP+ in endothelial cells could cause BBB abnormalities. However, we found that BBB diffusion of [^{14}C]amino-isobutryic acid was not altered. Thus, we conclude that the rat is resistant to MPTP toxicity even when large doses are given into the brain circulation, and that the BBB integrity is not affected.

331.12

NEOVASCULARISATION OF THE CNS IN EAE. B.Watkins*, P.Glynn*, A.Gautam* and R.Small . (SPON: M.Dubois-Dalcq). Institute of Neurology, Queen Square, London WC1N 3BG, UK.

Inflammatory diseases of the CNS such as multiple sclerosis and experimental allergic encephalomyelitis (EAE) involve focal breakdown of the blood-brain barrier (BBB), which can be visualised by magnetic resonance imaging and horseradish peroxidase (HRP) tracing techniques. The factors involved in BBB dysfunction are not understood, but may include changes in the endothelial cell population during the disease process. We have examined the population dynamics of CNS endothelial cells in a Lewis rat adoptive transfer EAE model by injecting 5'-bromo-2-deoxyuridine (BUdR), a thymidine analog, to label dividing cells. At the height of the disease, EAE rat spinal cord contained many BUdR$^+$ endothelial cells in both large and small blood vessels. In addition, a marked increase was seen in the number of blood vessels in EAE spinal cords. In contrast, no BUdR$^+$ endothelial cells were seen in the spinal cord blood vessels of control rats. These results suggest that inflammatory disease of the CNS involves mitotic activation of normally quiescent CNS endothelial cells which may function in (a) hypervascularisation of the CNS and/or (b) the repair of endothelial cells damaged by infiltrating cells. We are currently combining BUdR prelabelling with HRP perfusion to correlate focal leakage with endothelial cell division, and to study the relationship between neovascularisation and induction of BBB function.

CALCIUM CHANNELS IV

332.1

INACTIVATION OF CALCIUM CURRENT IN CULTURED ADULT MOUSE PANCREATIC B-CELLS IS MEDIATED BY CALCIUM AND VOLTAGE. W.F. Hopkins, L.S. Satin, and D.L. Cook*, Depts. of Physiology/Biophysics and Medicine, Univ. of Washington and VA Medical Center, Seattle, WA 98108.

We have previously shown two components to calcium (Ca) current inactivation in clonal insulin-secreting HIT cells (Satin and Cook, Pflügers Arch, in press, 1989). The fast (<200 msec) Ca-dependent and slow, voltage-dependent components are revealed when long depolarizing pulses are used. To determine if similar Ca currents exist in adult mouse B-cells, we voltage-clamped whole B-cells while blocking K and Na currents as done in HIT cells. The inactivation during short (100 msec) depolarizing pulses was proportional to peak Ca current elicited by the pulse (Plant 1988). On the other hand, the slow inactivation during long (10 sec) pulses was prominent for conditioning pulses below the Ca current threshold (-40 mV) and at very positive (>+50 mV) potentials. Using either Ca or barium (Ba) as charge carrier, this component of inactivation was half-maximal at -30 mV and maximal (>90%) at 0 mV. Peak Ca or Ba current significantly decreased as holding potential was made more positive in the range of -100 to -40 mV. These results suggest that, like HIT cells, mouse B-cells possess fast and slow components of Ca current inactivation mediated by calcium influx and voltage, respectively.

Supported by NIH grant DK29816 and the Veterans Administration

332.2

A DOMAIN MODEL FOR CA^{2+} INACTIVATION OF CA^{2+} CHANNELS. A. Sherman*, J. Keizer*, and J. Rinzel* (SPON: W. Rall). Mathematical Research Branch, NIDDK, NIH, Bethesda, MD 20892, and Institute of Theoretical Dynamics, UC-Davis, Davis, CA 95616.

Most models of Ca^{2+} inactivation assume that Ca^{2+} concentration is elevated in a "shell" near the membrane and acts on both closed and open channels, with a time scale determined by the rate of filling of the shell. In cells with low channel densities (a few μm^{-2}), such as pancreatic β-cells, rat clonal pituitary cells, and bovine chromaffin cells it is likely that high Ca^{2+} exists only at the mouths of open channels. Further, in β-cells it is believed that macroscopic Ca^{2+} oscillates in parallel with bursts of action potentials on a time scale of seconds, suggesting that it cannot also be responsible for inactivation on the 10-100 ms time scale. Following a suggestion of Chad and Eckert [*Biophys. J.*, 45:993, 1984], we propose that domains (~500 μM) form and disappear instantaneously as channels open and close, and that Ca^{2+} inactivates open channels on a time scale determined by the kinetics of binding to the channel. If activation is much faster than inactivation, our mathematical model predicts 1) U-shaped inactivation curves, 2) U-shaped dependence of the inactivation time constant on membrane potential, 3) that inactivation is greatest where the magnitude of Ca^{2+} current is greatest, and 4) that inactivation is an apparent function of total calcium entry during the pre-pulse of a two-pulse experiment, even though whole cell Ca^{2+} current plays no direct role. We fit data of Plant [*J. Physiol.*, 404:731, 1988] on β-cells and show that this model is consistent with previous models for bursting based on slow feedback of cytosolic Ca^{2+} on calcium-activated K^+ channels. In contrast to some investigators we conclude that Ca^{2+} inactivation plays no role in generating bursts in these cells.

332.3

DIVALENT CATION PERMEABILITY THROUGH CHROMAFFIN CELL CALCIUM CHANNELS. K.S. Wilcox and R.J. Bookman*. Dept. of Physiology and Howard Hughes Medical Institute, University of Pennsylvania, Philadelphia, PA 19104-6085.

We examined the permeability of various divalent cations through voltage-dependent calcium channels in bovine adrenal chromaffin cells using the whole cell patch clamp technique. Chromaffin cells were isolated and maintained in primary tissue culture at 37°. Internal and external solutions were designed to minimize all ionic conductances except for calcium. A range of concentrations (2.5, 5.0, or 10.0 mM) of either $BaCl_2$, $SrCl_2$, or $CaCl_2$ were added to the bath. According to the relative reversal potentials, the permeability sequence through calcium channels was $Ba^{++}>Ca^{++}>Sr^{++}$. Both Ba^{++} and Sr^{++} shifted the voltage-dependent activation of the Ca^{++} channel current to a more hyperpolarized potential, whereas deactivation kinetics at -80 mV were unaffected. Whole cell current measurements revealed that Ba^{++} and Sr^{++} produced more charge entry than equimolar Ca^{++} for a given level of depolarization. These results enable us to select external divalent conditions to yield equal charge entry at different pulse potentials, thus setting the stage for the determination, through measurement of membrane capacitance changes, of the relative ability of these divalent cations to support exocytotic secretion from chromaffin cells.

The generous support of R.G. Johnson, Jr. is gratefully acknowledged.

332.4

THE EFFECTS OF Pb^{2+} AND Zn^{2+} ON THE VOLTAGE ACTIVATED CALCIUM CHANNEL DEPENDS ON THE Ca^{2+} CONCENTRATION. **D. Büsselberg[1,2], M.L. Evans[1], H. Rahmann[*2] and D.O. Carpenter[1] (SPON: C. Allen). [1]NYS Dept. of Hlth. & Sch. of Pub. Hlth., Albany, NY 12201, USA. [2]Univ. of Stuttgart-Hohenheim, D-7000 Stuttgart 70, FRG.**

We have shown that Pb^{2+} and Zn^{2+} block dose and voltage dependent calcium currents in Aplysia neurons. The blockade by Pb^{2+} but not Zn^{2+} appears to be specific for the calcium current. Using conventional two electrode voltage clamp techniques we have analyzed the effects of varying calcium on these blocking actions. The concentration of Pb^{2+} which reduced the current by 50% was 24.0 μM in 10 mM Ca-ASW and 69.2 μM in 40 mM Ca-ASW. The Hill coefficient was close to 1.0 in both solutions. For the same reduction 2.7 mM Zn^{2+} was needed in 10 mM CA-ASW and 4.0 mM Zn^{2+} with 40 mM calcium. We calculate a Hill number between 1.3 and 1.6 for the dose-response curve of Zn^{2+}. With increasing calcium in the external bathing solution there was a reduction in the degree of blockade for a given concentration of Pb^{2+} or Zn^{2+}. The blockade by Pb^{2+} was highly voltage dependent, increasing with depolarization. In contrast, the blockade by Zn^{2+} showed almost no voltage dependence. The addition of Pb^{2+} shifted the peak of the current voltage-relation to hyperpolarized voltages whereas Zn^{2+} moved it to depolarized voltages. Our results suggest that Pb^{2+} and Ca^{2+} compete for the same binding site in or near the channel. While Zn^{2+} can also cause a near total blockade of Ca^{2+} current it appears to act primarily if not exclusively through a charge screening effect as indicated by the lack of voltage dependence of blockade and the shift of activation, inactivation and the current voltage relation.

332.5

"LVA" Ca^{++} CURRENTS EVIDENT IN CORTICAL NEURONS ONLY AT ELEVATED (≥30°C) TEMPERATURE. K. Giffin & J.M. Nerbonne, Dept. of Pharmacology, Washington Univ., St. Louis, MO. 63110.

Previously, we reported that Ca^{++} currents in rat visual cortical neurons comprise two kinetically-distinct components (Giffin et al., **Neurosci. Absts. 13**: 1010, 1987). Both components displayed voltage- and time-dependent properties consistent with High Voltage-Activated ("HVA") Ca^{++} channels and no Low Voltage-Activated ("LVA") Ca^{++} currents were seen. Here, we present evidence that "LVA" channels are present in these cells.

Callosal-projecting cells were identified in vitro following in vivo retrograde labeling with rhodamine "beads". Whole-cell voltage-clamp recordings were obtained in bath solution containing (in mM): 140 TEACl, 4 KCL, 2 $MgCl_2$, 5 $CaCl_2$, 10 Hepes 5 glucose. Pipets contained (in mM): 140 CsCl, 10 EGTA, 10 Hepes, 5 glucose, 3 Mg*ATP, 0.1 Tris*GTP. During (125 ms) depolarizations from -80 mV at 20-22°C, the "HVA" current activates to a transient peak and subsequently decays to a plateau; the currents begin to activate at -20 mV, peak at +10-+20 mV and reverse positive to +50 mV (n=13). At 32°C, the rates of "HVA" current activation and inactivation are increased and current amplitudes are reduced. In addition, a third current was observed in 3/5 cells examined at 32°C; this current begins to activate at ≈-40 mV, peaks at ≈-30 mV and is only evident during depolarizations from HPs ≤-80 mV, consistent with the presence of "LVA" Ca^{++} channels. The temperature sensitivity of the "LVA" current may explain differences in physiological properties of cortical neurons in slices and dissociated culture. (Support: NSF #BNS8809823 and NIH #T32-HL07275).

332.6

CALCIUM CHANNELS IN RAT NEURONS: HIGH-THRESHOLD CHANNELS THAT ARE RESISTANT TO BOTH w-CONOTOXIN AND DIHYDROPYRIDINE BLOCKERS. D.W.Y. Sah, L.J. Regan, and B.P. Bean. Dept. of Neurobiology, Harvard Medical School, Boston, MA 02115.

We studied w-conotoxin and dihydropyridine block of Ca channel currents in freshly dissociated neurons from young (< 2 wks.) rats. Only cells with no evident T-type current were studied, using 5 mM Ba as charge carrier. The table shows block of peak current by w-conotoxin (10 uM) and nitrendipine (3 uM); similar results were obtained with 3-10 uM nimodipine.

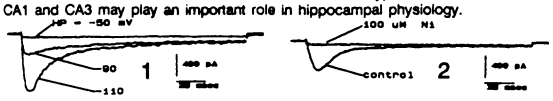
	% block (mean ± s.e.m), h.p. -70 to -90 mV		
Cell type	Nitr.	w-CTX	w-CTX + Nitr.
DRG	18 ± 3	64 ± 2	67 ± 2 (n=8)
SCG	20 ± 3	61 ± 7	78 ± 7 (n=4)
spinal	33 ± 7	36 ± 4	56 ± 4 (n=5)
cortical	24 ± 3	40 ± 5	70 ± 4 (n=5)
CA1	66 ± 7	9 ± 9	99 ± 1 (n=4)
Purkinje			8 ± 4 (n=4)

In contrast to the current picture of N and L channel pharmacology, these results show that most neurons contain a substantial fraction of high-threshold current that is resistant to block by both w-conotoxin and dihydropyridines. (CA1 cells are an exception and Purkinje cells are an extreme case.) Such channels might underlie the many instances of synaptic transmission not blocked by these agents.

332.7

MULTIPLE CALCIUM CHANNEL TYPES EXIST IN ACUTELY ISOLATED GUINEA PIG HIPPOCAMPAL NEURONS FROM THE CA3 REGION. D.J. Mogul and A.P. Fox* (SPON: B. Arnason) Dept. of Pharmacology/Physiology, Univ. of Chicago, Chicago, IL 60637

Previous electrophysiological studies of calcium channels in the CA1 region of mammalian hippocampus have found no evidence of a small conductance, rapidly-inactivating, low-depolarization (T-type) Ca channel (Kay & Wong, 1987; Kay, 1988). The existence of such a channel may provide an important component in spontaneous epileptiform events since it has been suggested that this channel is responsible for pacemaker potentials in various tissues (Llinas & Yarom, 1981). Spontaneous bursting in the hippocampus, which occurs upon application of convulsive agents, always begins in the CA2-CA3 pyramidal region and propagates into the CA1 region. Furthermore, application of the anti-convulsants phenytoin or ethosuximide results in reduction of a low-depolarization rapidly-inactivating Ca channel in neuroblastoma cells (Twombly & Narahashi, 1986) or thalamic neurons (Coulter et. al., 1988). Therefore, we sought to determine what Ca channels exist in the hippocampal CA3 region and, in particular, whether the T-type channel is present in this region. Acutely dissociated adult guinea pig CA3 pyramidal cells (Kay & Wong, 1986) were voltage-clamped using both the whole-cell and single-channel configurations. Fig. 1 shows whole-cell current recordings elicited upon depolarization to -30 mV from a holding potential (HP) of -110, -90, or -50 mV. Barium (5 mM) was the external charge carrier with TEA and TTX outside; Cs, EGTA, Mg-ATP & GTP inside. With HP=-50 mV, a single component inward current showing little to no inactivation was observed during a 230 ms test pulse. With HP=-110 mV, a large rapidly inactivating inward current was observed. This early current was abolished upon application of 100 uM Ni^{2+} (see fig. 2) consistent with T-type channels observed in DRG neurons (Fox et al., 1987). Single-channel recordings suggest the presence of three different types of unitary Ca channel activity, each with a different slope conductance. Our results indicate that differences in the types of Ca channels between CA1 and CA3 may play an important role in hippocampal physiology.



332.8

MULTIPLE CALCIUM CHANNELS IN ADRENAL CHROMAFFIN CELLS. C-R. Jan*, M. Titeler, and A.S. Schneider. Dept. Pharm. & Tox., Albany Med. Coll., Albany, N.Y. 12208.

Radioligand binding of ^{125}I-ω-conotoxin (CTX) and ^{3}H-nitrendipine to membranes from bovine adrenal medulla was investigated in order to test for the presence of multiple Ca^{2+} channels in adrenal chromaffin cells. CTX is a blocker of both N and L-type Ca^{2+} channels in neurons. Nitrendipine is a specific blocker of L-type Ca^{2+} channels. Specific binding sites for both ^{125}I-CTX and ^{3}H-nitrendipine were detected in this study. The K_D was found to be 215 ± 56 pM for ^{125}I CTX binding, and 500 ± 170 pM for ^{3}H-nitrendipine binding; while the B_{max} was 99 ± 18 pmol/g protein for ^{125}I-CTX binding. Dihydropyridines and diltiazem failed to affect ^{125}I-CTX binding, while the binding was blocked by unlabeled CTX and Ca^{2+}. ^{3}H-nitrendipine binding was not affected by CTX, while it was inhibited by dihydropyridines. In fura-2 measurements, BAYK8644 (L-type Ca^{2+} channel agonist) potentiated the K^{+}-evoked rise in cytosolic $[Ca^{2+}]$, and this effect could be blocked by nitrendipine, nifedipine and nimodipine. CTX did not affect the BAYK8644-potentiated Ca^{2+} transients. the results suggest that the binding sites for CTX and nitrendipine are different and indicate the possible presence of both N and L-type Ca^{2+} channels in bovine adrenal chromaffin cells. Supported by NIH grant DK39220 to A.S. Schneider.

332.9

DISSOCIATION OF RECEPTOR OPERATED AND VOLTAGE SENSITIVE CALCIUM CHANNELS IN SYNAPTOSOMES AND CULTURED PRIMARY NEURON PREPARATIONS. M.G. Hamilton*, and T.W. Sawyer* (SPON: James W. Maas). Defence Research Establishment Suffield, Ralston, Alberta, Canada, T0J 2N0.

We have examined the effects of activation of both receptor operated channels (ROC's) and voltage sensitive Ca^{++} channels (VSCC's) on intracellular free Ca^{++} levels ($[Ca^{++}]i$) in chicken synaptosomes and primary neuronal cultures using FURA-2. In chicken synaptosomes, K^{+} caused an immediate elevation in $[Ca^{++}]i$ that was unaltered in Mg^{++} free Krebs'. ω-Conotoxin GVIA (CgTx; 0.1-5μM) caused a significant and dose-dependant reduction of the rise in $[Ca^{++}]i$ in both normal and Mg^{++} free Krebs', but the dihydropyridine antagonist (-)202-791 (up to 5μM) was without effect. In cultured chicken cortical neurones, where CgTx reduced the rise in $[Ca^{++}]i$ after K^{+} depolarization, (-)202-791 also reduced the K^{+} induced rise in $[Ca^{2+}]i$, and when combined with CgTx, the $[Ca^{2+}]i$ increase was abolished. Activation of ROC's using NMDA caused a rise in $[Ca^{++}]i$ only in the chicken neuronal preparation and only in the absence of Mg^{++} ions. CgTx or (-)202-791 had no effect on the NMDA-induced rise in $[Ca^{++}]i$. AP-7 and (±)CPP both inhibited the effect of NMDA. Conversely, neither AP-7 nor (±)CPP affected K^{+} stimulated increases in $[Ca^{++}]i$. The data suggests the presence of ROC and L type VSCC's on nervous elements other than synaptosomes with principally N type channels on nerve endings.

332.10

MEMBRANE POTENTIAL, ION GRADIENTS AND INTRACELLULAR CALCIUM IN SYNAPTONEUROSOMES. M. Benuck, M.E.A. Reith and A. Lajtha. Center for Neurochemistry, NS Kline Inst., Ward's Island, New York NY, 10035.

Previous investigations on the increased turnover of phosphatidylinositol (PI) in neural tissue upon depolarization (KCl) or sodium channel activation (veratridine) have implicated membrane potential and ion gradients as important factors in determining the release of inositol trisphosphate. Indirect evidence from our laboratory suggests participation of the Na+/Ca2+ exchanger in PI turnover. In the present study, Ca2+ levels were measured in mouse cerebrocortical synaptoneurosomes with Fura 2 AM. Addition of KCl or veratridine led to an increase in [Ca2+]i. TTX blocked the effect of veratridine, in contrast to KCl depolarization. Omission of Na+ from the external medium reduced the effect of KCl and veratridine. Verapamil (100 uM), an inhibitor of the Na+/Ca2+ exchanger and a calcium channel blocker, reduced [Ca2+]i in the presence of KCl or veratridine. In contrast, the calcium channel activator, BAY K 8644, or blocker, nimodipine, did not alter the effect of KCl. Other blockers of the Na+/Ca2+ exchanger, Mg2+ (5 mM) and La3+ (10 uM), did not significantly reduce the increase in [Ca2+]i by KCl or veratridine. The results suggest that, in addition to the Na+/Ca2+ exchanger, other factors are involved in regulating [Ca2+]i in synaptoneurosomes.

332.11

CALCIUM-DEPENDENT EXOCYTOSIS AND THE TYPE OF CALCIUM CHANNELS IN A SMALL-CELL LUNG CANCER CELL LINE. Yong I. Kim, J.J. Pancrazio and M.P. Viglione. Depts. of Biomedical Engineering, Neurology and Neuroscience, University of Virginia Health Sciences Center, Charlottesville, VA 22908.

Small-cell carcinoma (SCC) of the lung is associated with a wide variety of paraneoplastic syndromes, including the Ca^{2+} channel disorder Lambert-Eaton syndrome (LES). Using whole-cell patch-clamp techniques, we studied voltage-gated Ca^{2+} currents (I_{Ca}) and the Ca^{2+}-induced changes in cell membrane capacitance (C_m) in human SCC cell line NCI-H146. I_{Ca} was recorded with 10 mM $[Ca^{2+}]_o$ after I_{Na} and I_K were blocked. Depolarization from a holding potential (V_h) of -80 mV to test potentials at -60 to +90 mV evoked I_{Ca} with no or minimal inactivation during 40 or 400 msec excitation. Maximum I_{Ca}, which occurred at about +20 mV, ranged from 20 to 200 pA among different cells tested (n=23 cells). When V_h was changed to -40 mV, both the time course and maximum amplitude of I_{Ca} remained relatively unchanged. The cell's ability for exocytotic secretion and its Ca^{2+} dependency were tested by injecting Ca^{2+} buffer through the recording patch pipette. With 5.8 mM Ca^{2+}/6 mM EGTA buffer, C_m rose from its initial value of 8.85 pF (n=10) (immediately after the establishment of whole-cell configuration) to 16.36 pF within about 4½ min. In contrast, cytoplasmic application of 1.2 mM Ca^{2+}/6 mM EGTA buffer resulted in no change in C_m within 5 min.

These data suggest that i) these SCC cells express L-type Ca^{2+} channels and ii) secretory events in these cells are mediated via Ca^{2+}-dependent exocytosis. Although the presence of T- or N-type channels cannot be excluded from this and other SCC cell lines, L-type channels appear to constitute the majority of the Ca^{2+} channel population in NCI-H146 cells (supported by NIH NS18607 and Muscular Dystrophy Association).

332.12

A VOLTAGE-INDEPENDENT, DIVALENT-SELECTIVE ION CHANNEL IS LOCALIZED ON THE NEURITES OF LYMNAEA NEURONS IN PRIMARY CULTURE. S. A. Scott* and J. A. Strong, Dept. of Biol. Sci., Purdue Univ., W. Lafayette, IN 47907.

Previous reports[1-3] have described an unusual voltage-independent, divalent-selective ion channel in neurons of the molluscs Lymnaea and Aplysia. Although the channel is present in high density, it virtually never opens during cell-attached recording. Channel activity is unmasked by formation of a cell-free patch, as if a cytoplasmic inhibitor keeps the channel closed. Previous studies of this channel in Lymnaea used cell-free patches taken from the soma of acutely isolated neurons[2]. In tissue culture dishes, these cells attach and grow extensive neurites. We now report that following such attachment and growth the channel disappears from the cell soma. We made inside-out cell-free single channel recordings in $BaCl_2$//K-aspartate-10 mM EGTA. Channel activity was observed in 40% of patches taken from unattached neurons, but no channels were seen in any of the patches (N=15) taken from the soma of attached cells maintained in primary culture for 1 or more days. This suggests that the channel is localized to the neurites of attached, growing cells. In support of this idea, we observed channel activity in 75% of patches taken from the neurites, as opposed to the soma, of attached cells.

[1]Chesnoy-Marchais, J. Physiol 312:315 [2]Yazejian & Byerly, J.Membr Biol 107:63 [3]Strong et al., Soc Neu Abst 13:1011.

332.13

CALCIUM CURRENTS IN DEVELOPING MOUSE SPINAL NEURONS. M. Mynlieff, D.P. McCobb and K.G. Beam. Dept. of Physiology, Colorado State Univ., Fort Collins, CO 80523.

Voltage clamp "whole-cell" patch recording was used to measure calcium currents in dissociated spinal neurons from 14 day embryonic (E14) and 7 day postnatal (P7) mice. At E14 the period of motoneuron (MN) cell death is just beginning; at P7 MN cell death is complete and polyneuronal innervation of individual muscle fibers is being eliminated. All recordings were made 24 hr after dissociation; data were taken from the largest neurons, i.e., those cells most likely to be MNs. In the case of the P7 mice, a number of recordings were made from MNs identified by retrograde labeling with the carbocyanine dye dil prior to dissociation. Three calcium currents were distinguished in the cells on the basis of kinetics and voltage dependence. There was a low voltage activated current (Vtest>-50 mV) with characteristics similar to the T current described in sensory neurons. The current reached maximal size at Vtest=-20 to -10 mV with a relatively slow time to peak (20 ms). Half-maximal inactivation of the current was achieved with a 1 s prepulse to -50 or -40 mV. Strong depolarizations (Vtest>-20 mV) activated an inactivating current and a current which was sustained for at least 200 ms. Both currents reached a maximal size with Vtest=+20 to +30 mV. Half-maximal inactivation of the inactivating current was achieved with a 1 s prepulse to -20 or -10 mV. The inactivating and sustained currents resembled the N and L currents described in sensory neurons, respectively. The voltage dependence of the activation and inactivation of all three currents was similar in E14 and P7 neurons. There was a large amount of variability between cells in the magnitude of each current (normalized by linear cell capacitance), some of which may be attributed to a heterogenous cell population. Despite this variability, the average N and L currents were 2.5 fold larger in P7 neurons than in E14 neurons. A developmental increase in N and L currents has been found previously in chick MNs (McCobb et al., Neuron, in press). Study was supported by NIH grant NS-26416 to KGB.

332.14

CALCIUM CURRENTS IN ADULT RAT SENSORY NEURONS. A.Formenti*, A.Pollo*, M.Taglialatela* and E.Carbone* (SPON:European Neuroscience Association). Dept. Anat. Physiol., 10125 Torino, Italy.

Ca currents in freshly dissociated adult rat sensory neurons (DRG) were studied by the whole-cell patch-clamp. Low-threshold (LVA, T) Ca currents turned-on transiently at about -50mV and inactivated fully within 100ms in a voltage dependent manner. This current persisted during bath application of 3.2 μM ω-CgTX or 30 μM Cd and was strongly depressed by 500 μM Amiloride. High-threshold (HVA, L and N) Ca currents turned-on between -10 and 0 mV, inactivated slowlyand incompletely even after prolonged depolarizations. Bath application of 3.2μM ω-CgTX blocked 80 to 90% of this currents. Residual ω-CgTX-resistant currents (10 to 20%) proved to be sensitive to DHP-derivatives. At -60mV holding potential (Vh), 1μM Nitrendipine reduced by 60% the size of this component while BAY K 8644 (1μM) had an agonistic action independent of Vh. BAY K 8644 enhanced the size and speeded-up the inactivation kinetics of CgTX-resistant currents. Inactivation was maximal at potentials positive to -20mV and persisted in the presence of 500μM Amiloride. Our data suggest a multiple action of BAY K on HVA Ca currents and the possible existence of multiple types of Ca channels in adult rat DRG.

332.15

PATCH CLAMP STUDIES OF CALCIUM CHANNELS IN CA1 HIPPOCAMPAL NEURONS. T.J. O'Dell* and B.E. Alger. Dept. Physiol., Univ. Maryland School of Medicine, Baltimore, MD 21201.

Previous whole-cell voltage clamp data suggested that dihydropyridine-sensitive, high-voltage-activated (L-type) calcium channels like those found on other neurons were present on hippocampal CA1 neurons. However, no single channel recordings have tested this hypothesis.

We used the cell-attached patch configuration of the patch-clamp technique to study single calcium channels on pyramidal cells enzymatically dissociated from the CA1 region of guinea pig hippocampus. The cells were bathed in 140 mM KCH_3SO_3 saline to zero the membrane potential and barium ions were used to carry the charge (110 mM $BaCl_2$ in the pipette solution). Single channels with a slope conductance of 23.6 + 3.4 pS (mean ± S.D., 12 patches) were detected during step depolarizations to potentials more positive than -30 mV in about 50% of the patches. Channel openings were also plentiful during maintained (≥1 min) depolarizations to or beyond -20 mV. Channel percent open time was enhanced by bath application of 5.0 μM BAYK 8644 and reduced by application of 1.0 - 5.0 μM nifedipine. These findings are consistent with previous reports and indicate the presence of L-type calcium channels on hippocampal CA1 pyramidal cells. We are investigating the actions of protein kinase C activators on L-channel activity. Supported by NIH NS22010 (B.E.A.) and NS08669 (T.J.O.).

332.16

LOW- AND HIGH-THRESHOLD CA^{2+} CURRENTS IN RAT SPINAL DORSAL HORN NEURONS P. D. Ryu, and M. Randic, Dept. of Vet. Physiol. and Pharmacol., Iowa State University, Ames, IA 50011, USA.

We have used single-electrode voltage-clamp technique and transverse spinal cord slice in an attempt to characterize voltage-dependent Ca^{2+} conductances underlying the low- and high-threshold Ca^{2+} spikes in 2-4 week old rat dorsal horn neurons. Cs^+-filled microelectrodes and solutions containing TTX/TEA/Ba^{2+}/zero-Ca^{2+}/Cs^+ were used to minimize fast voltage-dependent Na^+ and K^+ channel currents. The low-threshold transient inward current and the high-threshold transient and sustained components of the inward current were reversibly depressed by removing external Ca^{2+} ions and enhanced when Ca^{2+} was replaced by Ba^{2+}. The low- and the high-threshold Ca^{2+} conductances were characterized on the basis of their voltage- and time-dependence and sensitivity to the Ca^{2+} channel agonist and antagonist drugs. The low-threshold transient current was selectively blocked by Ni^{2+} and amiloride whereas the high-threshold sustained current was preferentially enhanced by Bay K 8644 and reduced by nifedipine. Cd^{2+} ions blocked both transient and sustained components of the high-threshold current but only slightly reduced the low-threshold transient current. SP reversibly increased the high-threshold transient current in 15 out of 24 neurons tested and in about half of examined cells the low-threshold Ca^{2+} current. The results indicate the existence of multiple types of Ca^{2+} conductances in immature rat spinal dorsal horn neurons that are modulated by SP. Supported by grants from NIH, NSF and USDA.

332.17

EFFECTS OF THYROID HORMONES ON CALCIUM CHANNEL EXPRESSION IN CULTURED RAT SKELETAL MUSCLE. S.R. Sampson and Chaya Brodie*. Dept. of Life Sciences, Bar-Ilan University, Ramat-Gan 52900, Israel.

Thyroid hormones (TH) influence Na-channels in cultured myotubes by 2 opposing mechanisms: (i) up-regulation by stimulation of synthesis, and (ii) down-regulation by what appears to be to an increase in cytosolic Ca^{2+} (Brodie, C. and Sampson, S.R., The Physiologist, 31, 133, 1988). In this study, we examined effects of TH on the number of Ca-channels as measured by specific binding of [^{3}H]PN200-110 to cultured skeletal myotubes derived from 1-day-old neonatal rats. Cells (6-7 DIV) were treated with T_3 (10 nM) for 12-48 hr. TH-treatment caused an increase in Ca-channel density beginning 12 hr after addition of the hormone to the growth medium. Peak effects were obtained after 24 hr. Scatchard analysis of the properties of PN200-110 binding showed that B_{max} increased from control levels of 45.8 to 70.7 fmoles/dish, and Kd increased from 0.22 nM to 0.85 nM (total protein values were unchanged by TH-treatment). Thus, TH causes an increase in number of Ca-channels with decreased affinity for the ligand, similar to TH effects on Na-K pumps and Na-channels in cultured skeletal muscle. We conclude that the effect of TH to up-regulate Ca-channels may be an important component of the mechanism by which these hormones increase cytosolic Ca^{2+} and regulate Na-channel expression in skeletal muscle.

332.18

DIHYDROPYRIDINE (DHP) -SENSITIVE Ca^{++} CHANNELS AND ANKYRIN ARE LOCALIZED IN THE TRIADS OF SKELETAL MUSCLE. B.E. Flucher*, M.E. Morton, S.C. Froehner and M.P. Daniels. Lab. of Neurobiology, NINDS, Lab. of Biochem. Genetics NHLBI, NIH, Bethesda, MD and Dept. of Biochem., Dartmouth Medical School, Hanover, NH.

The DHP receptor of skeletal muscle is thought to function both as a voltage-sensitive Ca^{++} channel and as a voltage sensor in excitation-contraction (E-C) coupling. Cell fractionation studies suggest that the receptor is concentrated in the transverse (T-) tubular system. In order to test this hypothesis a monoclonal antibody (1A) against the α_1 subunit of the Ca^{++} channel was used for immunofluorescence staining, and for immunogold labeling of low temperature resin embedded rat diaphragm muscle. The α_1 polypeptide was localized in the junctional T-tubule membrane but not detected in unspecialized regions of the T-tubules. In contrast, no concentration of the voltage-gated Na^+ channel was observed in the triads (using the monoclonal antibody[1] A/B2; Haimovich et al., 1987). The concentration of the DHP receptor α_1 subunit in the triads is consistent with a role of this protein in E-C coupling. Ankyrin-like molecules are thought to immobilize various integral membrane proteins (erythrocyte anion transporter, nerve Na^+ channel and kidney Na^+/K^+ ATPase) in specialized domains of the plasma membrane. In muscle we have previously demonstrated ankyrin-immunoreactivity in the synaptic folds of the neuromuscular junction, colocalized with Na^+ channels. Here we show the localization of an ankyrin-immunoreactive molecule at the junction between sarcoplasmic reticulum and T-tubules (using an affinity purified antibody[2] against erythrocyte ankyrin; Bennett and Stenbuck, 1979). Thus, ankyrin may be involved in the specialization of two different membranous structures in muscle cells.

1,2 Generously supplied by R. Barchi and V. Bennett, respectively.

332.19

THE EFFECT OF LYOTROPIC ANIONS ON Ca^{2+} CURRENTS, CHARGE MOVEMENT AND Ca^{2+} TRANSIENTS OF FROG SKELETAL MUSCLE. D.E. García-Díaz*, M.J. Delay** and J.A. Sánchez***. *Depto. de Fisiología, Fac. de Medicina, UNAM. **Dept. of Medical Physiology, University of Calgary, Alta., Canada and *** Depto. de Farmacología, CINVESTAV-IPN, México.

Thiocyanate (SCN^-) and other lyotropic anions facilitate the activation of charge movement in skeletal muscle (García-Díaz & Sánchez, Biophys. J. 33, 334a, 1988) the present experiments examine further its action on charge movement, slow Ca^{2+} currents (ICa) and Ca^{2+} transients. Methods: the triple vaseline gap technique (Hille & Campbell, J. Gen. Physiol. 67, 265, 1976). Internal solution (mM) for voltage clamp experiments: TEA_2EGTEA=20, Cs-glutamate=89, Na_2ATP=2, MOPS=4. Ca^{2+} transients were measured under current clamp conditions in Cl^- Ringer's and internal solution: EGTA=1, K-aspartate=121.5, $MgCl_2$=2, MOPS=5, Na_2ATP=2, T=22-24°C. Arsenazo III (1 mM) or Anti-pyrylazo III (2 mM). Results: time to peak of ICa during large depolarization (ms) was 512±80(14) and 330±36(14) after SCN^-. I-V curve shifted -21±2.7 mV(14) after SCN^-. On-charge shifted (mV) from -39.9±9.1(6) to -50±2.0(6). Internal SCN^- produced similar results on ICa and charge movement. Tail currents were fitted with 2 exponentials (ms): τ_1=15.1±2.8(11) and 67.0±20.4(11) after SCN^-. Off-charge step to -10 mV was slowed down ca 2.4 times. Peak amplitude of Ca^{2+} transients increased by 70-100% by SCN^- in both Arsenazo and Antipyrylazo experiments.CONACYT/H.F.

332.20

Ca channel activity in fetal skeletal muscle cells from normal and "Muscular Dysgenesis" mutant mice. R Bournaud*[2], T Shimahara*[1] and I Inoue*[1]. (SPON: Y Bruner) [1]Lab. de Neurobiol. Cell. et Moléc. CNRS Gif-sur-Yvette France, [2]Lab. de Physiol. Gén. Univ. Paris XII 94000 Créteil. France.

Experiments were performed in enzymatically dissociated intercostal muscle cells from 13-19 days old fetuses using the whole cell patch clamp technique. During this period of the myogenesis, all muscle cells studied from normal and Muscular Dysgenesis (*mdg/mdg*) mutant fetuses showed low threshold T type Ca currents; this current was reversibly blocked by amiloride. In normal fetuses the high threshold L type Ca current (sensitive to PN 200-110 and Bay K8644) began to be expressed on the 14th or 15th day of gestation and the number of cells having this current increased until the 19th day. At birth, all normal muscle cells showed the L-type current. Even though the L type current is totally absent in *mdg/mdg* muscle cells from 14 days old fetuses, an "L type like current" appeared in such cells at 16 days and older. This current was also sensitive to dihydropyridines. The number of cells showing the "L-type like" current increased to the end of gestation. The maximum amplitude of this current never exceeded the maximum amplitude of the T type current. These results agree with our previous observations in primary culture of dysgenic muscles. (Supported by a grant from A.F.M)

332.21

DISTRIBUTION OF L-TYPE CALCIUM CHANNELS IN THE ADULT RAT NERVOUS SYSTEM. R.E. Westenbrock*, M.A. Ahlijanian, and W.A. Catterall (SPON: J. DeVito). Department of Pharmacology, School of Medicine, University of Washington, Seattle, WA 98195.

Voltage-sensitive calcium channels are involved in the control of calcium-dependent cellular processes such as action potential generation, muscle contraction, and secretion of hormones and neurotransmitters. L-type channels mediate slowly activated, long-lasting calcium currents that are blocked by dihydropyridine calcium channel antagonists and enhanced by dihydropyridine calcium channel agonists. A monoclonal antibody that recognizes neuronal L-type calcium channels (MANC-1) has been recently developed (Ahlijanian and Catterall, *Neurosci. Abst.*, 1989). In this study, MANC-1 has been used in combination with the indirect peroxidase-anti-peroxidase technique to investigate the distribution of L-type calcium channels in the brain and spinal cord of adult rats. Light microscopic studies revealed that L-type calcium channels are localized in neuronal somata along the entire rostral-caudal extent of the brain and spinal cord. The MANC-1 staining is confined to a narrow zone around the periphery of the soma. Both interneurons and projection neurons exhibited this pattern of staining. For example, in the hippocampus L-type calcium channels in interneurons in the stratum radiatum, stratum oriens, and hilus are labeled with MANC-1 as are pyramidal neurons in Ca1-Ca3 and granule cells in the dentate gyrus. In addition, dense MANC-1 staining is present in proximal regions of major dendrites of projection neurons such as hippocampal pyramidal cells and spinal motor neurons. These results indicate that L-type calcium channels have a discrete distribution on neurons in cellular regions which generate calcium-dependent action potentials.

332.22

IDENTIFICATION OF α_1- AND α_2-LIKE SUBUNITS OF THE NEURONAL L-TYPE CA^{2+} CHANNEL WITH A MONOCLONAL ANTIBODY. M.K. Ahlijanian and W.A. Catterall. Department of Pharmacology, School of Medicine, University of Washington, Seattle, WA 98195.

Voltage-dependent, dihydropyridine-sensitive, L-type Ca^{2+} channels (CaCh) play an important role in controlling the dynamics of intracellular Ca^{2+} concentration in brain cells, but their biochemical properties are unknown. Purified L-type CaCh from rabbit skeletal muscle (SkM) consist of 5 specifically associated subunits: α_1 (175 kDa), α_2 (145 kDa) which is disulfide-linked to δ (24 kDa), β (54 kDa), and γ(30 kDa). Using SkM microsomes as immunogen, a mouse monoclonal antibody (MANC-3) was developed which immunoprecipitates purified CaCh from rabbit SkM radiolabeled by binding of [^{3}H]PN-200-110, by iodination, or by phosphorylation with the catalytic subunit of cAMP-dependent protein kinase. Immunoprecipitation of radiolabeled SkM CaCh is half maximal at 25 nM and is blocked by incubation of the antibody with unlabeled SkM CaCh. Immunoblotting suggests that MANC-3 recognizes the α_1 subunit of the SkM CaCh. Partially purified brain CaCh radiolabeled by binding of [^{3}H]PN-200-110 or by iodination is also immunoprecipitated by MANC-3. This immunoprecipitation is half maximal at 54 nM and is also blocked by unlabeled SkM CaCh. Immunoprecipitation, SDS-PAGE, and autoradiography of the iodinated neuronal CaCh preparation yields a main protein band of 165 kDa. Reduction of disulfide bonds prior to electrophoresis reveals two distinct bands of 170 and 145 kDa. These data suggest that, similar to the SkM CaCh, rabbit brain L-type CaCh contains of at least two noncovalently associated, high molecular weight polypeptides: an α_1-like subunit of 165 kDa and an α_2-like subunit of 145 kDa that is disulfide-linked to a smaller polypeptide of approximately 25 kDa. MANC-3 may be an important tool for purification and characterization of neuronal L-CaCh.

332.23

MONOCLONAL ANTIBODIES TO THE CALCIUM CHANNEL BLOCKER ω-CONOTOXIN. L.P. Fortier, J. Rafrafi, J. Tremblay and R. Hawkes. Lab. Neurobiology, Hôpital Enfant-Jésus, Quebec, Canada, G1J 1Z4.

Murine hybridoma cell lines were constructed that secrete antibodies to the Ca-channel blocker ω-conotoxin (Ctx). Anti-Ctx recognizes Ctx on Western blots, and can be used to localize Ctx binding sites in tissue sections. Fresh rat cerebellar slices were incubated for 1h in 10^{-6}M Ctx, then aldehyde fixed and the Ctx binding sites identified by using peroxidase immunocytochemistry. When Ctx was omitted from the first incubation no staining resulted. Neuronal somata and dendrites of all cerebellar main neuronal types are immunoreactive. On the Purkinje cells the reaction product is not distributed uniformly. Strong staining is seen on the somata and at the branch points of the large dendrites, while the secondary dendrites, spines, and primary dendritic shafts are all more lightly stained. The labelling on cells in deep cerebellar nuclei appear as small discrete dots. These data suggest that the distribution of Ctx binding sites is subtly regulated, and that anti-Ctx may prove a valuable tool to study Ca-channel distributions within neurons.

LIGAND-GATED ION CHANNELS: CHOLINERGIC

331.1

KINETIC CHANGES OF THE NICOTINIC IONIC CHANNEL INDUCED BY (-)METHYLSCOPOLAMINE. J.F.P. Melo[*1] and Y. Aracava[*1,2] (SPON: J.G. Krikorian) [1]Lab. Mol. Pharmacol. II, UFRJ, Brazil; [2]Dept. Pharmacol. Exp. Ther., Univ. Maryland Sch. Med., Baltimore, MD 21201.

The effects of (-)scopolamine on the kinetics of endplate currents of the nicotinic ionic channels activated by acetylcholine were previously studied (Adler *et al., Mol. Pharmacol.* 14:514, 1978). The aim of the present study is to evaluate the action of (-)methylscopolamine on single channel currents recorded from the perijunctional region of isolated interosseal muscle fibers of the frog Lepdodactylus ocellatus by using patch clamp technique. In the presence of (-)methylscopolamine two populations of currents were noticeable: one dominated by single openings and another by long bursting events. Most of the kinetic alterations produced by (-)methylscopolamine could be interpreted on the basis of sequential blockade of the open state of the channels: i) the mean channel open time was shortened with hyperpolarization and blocker concentration; ii) the burst duration and number of openings per burst were increased with drug concentration; iii) the blocked state was prolonged with hyperpolarization but its duration, although longer than that under control conditions, was independent of the concentration of scopolamine analog. The rate of channel blockade was increased with hyperpolarization of the membrane, and with the blocker concentration while the rate of unblocking reaction decreased by negative transmembrane voltage. Thus, (-)methylscopolamine produced an open channel blockade of the nicotinic receptor. (Support: CNPq, FINEP & CAPES, Brazil; US Army Med. Res. & Devel. Comm. Contract DAMD17-88-C-8119 to Dr. E.X. Albuquerque[2])

331.2

ACTIONS OF ENANTIOMERS OF ESEROLINE, A PHYSOSTIGMINE METABOLITE, ON THE NICOTINIC ACETYLCHOLINE RECEPTOR-ION CHANNEL COMPLEX. W.M.Cintra[1,2*], K.-P.Shaw[1*], G.T.Scoble[1*], Q.S.Yu[3], A.Brossi[3] and E.X.Albuquerque[1,2]. (SPON: G.J. Markelonis) [1]Dept. Pharm. Exp. Ther., U. MD Sch. Med., Baltimore, MD 21210; [2]Lab. Mol. Pharm. II, Fed. Univ. RJ, Brazil & [3]Lab.Chemistry, NIDDKD, NIH, Bethesda, MD 20892.

Eseroline is a major metabolite of the reversible anticholinesterase agent physostigmine. End plate currents (EPCs) from frog sartorius muscle reveal that the time constant of the EPC decays (τ) increased at concentrations ranging from 10^{-7}M to 10^{-5}M of (+) eseroline but no change in (τ) was observed in the presence of (-) eseroline at this range of concentration. At 10^{-4}M, an increase in (τ) was observed with both enantiomers and biphasic decays of EPC were observed at hyperpolarized potentials. No increase in the EPC amplitude was observed with either enantiomer in accordance with their weak anticholinesterase activity as compared with (-) physostigmine. Using the frog interosseal muscle fibers for patch clamp recordings in the cell attached configuration, a concentration-dependent reduction in the mean open time of ACh-activated channels (400 nM) was observed with both enantiomers of eseroline. In the presence of eserolines the channels appeared as square-wave current pulses interrupted by many flickers forming long bursts of openings and closings. The mean channel open time decreased with membrane hyperpolarization while both intraburst closed intervals and burst duration increased. Histograms of open times of ACh-activated channels in the presence of eserolines was fitted by a single exponential function, burst times were fitted by double exponential functions. Correlation was made between the biphasic decays in the EPCs and the kinetics of single channel currents. Both enantiomers of eseroline are open channel blockers of ACh-activated channels; they did not unveil stereoselectivity on the ion channel site(s). (Support: U.S. Army Med. Res. & Devel. Comm. Contract DAMD17-88-C-8119).

331.3

NONCOMPETITIVE BLOCKADE OF THE PERIPHERAL AND CENTRAL NICOTINIC ACETYLCHOLINE RECEPTORS (AChR) BY MK-801 AND PCP. A.S. Ramoa[1,2], M. Alkondon[2*], Y. Aracava[1,2*], J. Irons[3*], G.G. Lunt[3], S.S. Deshpande[2*], S. Wonnacott[3] and E.X. Albuquerque[1,2]. [1]Lab. Molec. Pharmacol. II, Fed. Univ. Rio de Janeiro, Brazil, [2]Dept. Pharmacol. & Exp. Ther., Univ. of Maryland Sch. of Med., Baltimore, MD 21201 & [3]Dept. Biochem., Univ. of Bath, BA2 7AY, England.

MK-801and PCP, drugs known to block the NMDA-activated channels, were tested on the nicotinic AChR located in the peripheral and central nervous systems to disclose functional homologies existing between these receptors. Endplate currents (EPC) from frog sartorius muscle, ACh-activated single channel currents from frog interosseal muscle fibers, and anatoxin (AnTX)- or ACh-activated single channel currents from identified retinal ganglion neurons were used in this study. Also, biochemical assays of receptor function were carried out to assess the effect of MK-801. MK-801 (5-80 μM) produced a voltage- and concentration-dependent acceleration of the EPC decay and a decrease in the peak amplitude of the EPCs. The drug (1-10 μM) decreased the frequency of ACh-induced openings in muscle fibers, as well as their mean open time and burst duration. Currents recorded from outside-out patches of retinal ganglion neurons in presence of either AnTX or ACh looked very similar to NMDA-activated currents in terms of conductance. Interestingly, these currents were blocked by MK-801 and PCP, as evidenced by a reduction in the frequency of openings as well as a shortening of open time at concentrations of MK-801 (0.2-2.0 μM) and PCP (1 μM) which block the NMDA receptors. MK-801 (10-25 μM) also inhibited the (-)nicotine-evoked release of [^{3}H]GABA from hippocampal synaptosomes without affecting the binding of (-)nicotine. MK-801 inhibited the binding of [^{3}H]perhydrohistrionicotoxin to the Torpedo membranes, indicating noncompetitive blocking activity. These results disclose a functional homology between the AChR and the NMDA-related ion channels. (Support: CNPq and FINEP, Brazil; NIMH P-50MH44211; US Army Med. Res. & Devel. Comm. Contract DAMD17-88-C-8119; NIH Grant NS25296).

331.4

INTERACTIONS OF ACRIDINE ANALOGS WITH NICOTINIC ION CHANNELS: EVIDENCE FOR HOMOLOGY AMONG FUNCTIONALLY DISTINCT PROTEINS. R.A.M. Reis[1*], Y. Aracava[1,2*], C.M. Himel[2*] and E.X. Albuquerque[1,2]. (Spon: R. Sjodin) [1]Lab. Mol. Pharmacol. II, UFRJ, Rio de Janeiro, Brazil and [2]Dept. Pharmacol. and Exp. Ther., Univ. Maryland Sch. of Med., Baltimore, MD 21201.

1,2,3,4-Tetrahydro-9-amino acridine (THA), a cholinesterase (ChE) inhibitor, has been used in the treatment of Alzheimer's disease (Summers et al., New Engl. J. Med., 315:1241, 1986). More recently, THA has been reported to reduce NMDA-mediated neurotoxicity (Choi et al., Eur. J. Pharmacol. 154:73, 1988) in a manner similar to that described for noncompetitive blockers such as phencyclidine (PCP). In light of the findings that PCP acts on both NMDA and nicotinic macromolecules, thus supporting the notion of conserved ion channels, we studied the actions of THA and 1,2-propane-9-amino acridine araphane (1,2-pAA), a weaker ChE inhibitor than THA, on the muscle nicotinic acetylcholine receptor ion channel (AChR). Single channel currents activated by acetylcholine (ACh) were recorded from the perijunctional region of the frog interosseal muscle fiber. Both THA and 1,2-pAA (0.5-10 μM) blocked the open channels activated by ACh and induced a marked reduction of the opening frequency such that at 10 μM almost no channel activity could be recorded. Both effects were enhanced by membrane hyperpolarization. Also, the analyses of the open-channel currents showed that THA induced bursting-like activity whereas with 1,2-pAA only randomly separated brief events were observed. These results strengthen the notion of homology between nicotinic and NMDA channels (see Costa et al., this meeting). Therefore, although it is clear that these compounds alter the nicotinic AChR, great caution must be taken in relating effects on the AChR to their effectiveness in Alzheimer's disease. (Support: FINEP & CNPq, Brazil; US Army Res. & Devel. Comm. Contract DAMD17-88-C-8119)

333.5

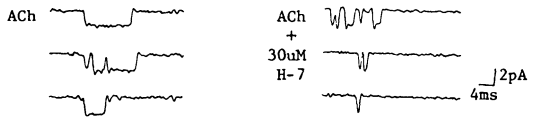
EFFECTS OF FORSKOLIN AND ANALOGS ON NICOTINIC RECEPTOR-MEDIATED $^{22}Na^+$ FLUX AND ON VOLTAGE-DEPENDENT $^{45}Ca^{++}$ FLUX AND VOLTAGE-DEPENDENT $^{86}Rb^+$ EFFLUX IN PHEOCHROMOCYTOMA PC12 CELLS. Y. NISHIZAWA, K.B. SEAMON and J.W. DALY. National Institutes of Health and Food Drug Administration, Bethesda, MD 20892.

Forskolin (FSK), a polar 6-8-piperidinoacetoxy analog (HL706) and an analog, 1,9-dideoxy-FSK that is inactive at adenylate cyclase, were examined for effects on nicotinic receptor-mediated $^{22}Na^+$ flux, high potassium-induced $^{45}Ca^{2+}$ flux through L-type calcium channels and high potassium-induced $^{86}Rb^+$ efflux through calcium-dependent potassium channels in PC12 cells. FSK and analogs completely blocked carbamylcholine-elicited flux at 30 uM. 1,9-Dideoxy-FSK was most potent (IC_{50} 1.6 uM) with FSK and HL706 2 to 3-fold less potent. HL706 and 1,9-dideoxy-FSK had little effect on desensitization of the nicotinic response, while FSK slightly antagonized the desensitization evoked by high concentrations of carbamylcholine. Neither an adenosine analog, NECA, that elevates cyclic AMP, nor 8-bromo-cyclic AMP had any effect on desensitization. The results suggest that FSK and analogs either directly block or are noncompetitive blockers of nicotinic receptor-mediated ion flux in PC12 cells, but that unlike many noncompetitive blockers, they do not markedly enhance desensitization. 1,9-Dideoxy FSK at 30 uM, but not FSK or HL706, markedly inhibited $^{45}Ca^+$ flux and $^{86}Rb^+$ efflux in PC12 cells suggesting generalized effects of this analog on ion channels.

333.6

COMPOUNDS COMMONLY USED TO ALTER PHOSPHORYLATION ACT DIRECTLY ON THE CHANNELS TO DECREASE CURRENTS THROUGH NICOTINIC ACETYLCHOLINE RECEPTORS. T. Reuhl*, J. Pinkham**, J.R. Moorman* and J.A. Dani. Dept. of Molecular Physiology & Biophysics, Baylor College of Medicine, Houston, TX 77030, and *Section of Molecular Neurobiology, Yale Univ., New Haven, CT 06510.

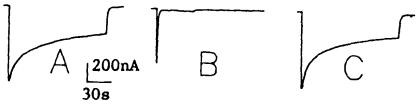
Nicotinic acetylcholine receptor (nAChR) channels were expressed in Xenopus oocytes, and macroscopic currents induced by 50 μM ACh were measured with a two electrode voltage clamp. Consistent with the work of Wagoner and Pallotta (Science, 1988, 240:1655-7), we found that external application of 20 μM forskolin rapidly decreases ACh-induced currents. Bath application of 3-isobutyl-1-methyl xanthine (IBMX, a phosphodiesterase inhibitor) or of 1-(5-isoquinolinylsulfonyl)-2-methylpiperazine (H-7, a protein kinase inhibitor) also decreases ACh-induced currents. The decrease of ACh-induced currents was concentration dependent and was reversed by washing the drug out of the bath. Single-channel measurements were made with excised outside/out patches containing nAChR channels. When o/o patches were rapidly exposed to 0.5 μM ACh in the presence of H-7, the mean open time and the open probability decreased but the conductance was unchanged (Fig.). The rapid effect of IBMX and H-7 on ACh-induced currents and the action of H-7 on excised patches indicate that these compounds directly alter nAChR channels independent of their action on enzymes that participate in protein phosphorylation. Supported by NIH grant NS21229.



333.7

SOLUTIONS EXPOSED TO CERTAIN POLYPROPYLENE TISSUE CULTURE TUBES DECREASE CURRENTS THROUGH NICOTINIC ACETYLCHOLINE RECEPTOR CHANNELS EXPRESSED IN XENOPUS OOCYTES. J.A. Dani and T. Reuhl* (SPON: E. Stefani). Department of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, TX 77030.

Acetylcholine-induced macroscopic currents through nicotinic acetylcholine (ACh) receptor channels expressed in Xenopus oocytes were studied using a two electrode voltage clamp. Exposing our electrolyte solution to Falcon polypropylene tubes decreased the current induced by application of ACh. Our experiments were with the Falcon 2098 50 ml polypropylene tubes from lot numbers 72180188 and 80290188 and with a Falcon 2097 15 ml polypropylene tube from lot number 83560283. Rapid infusion of 50 μM ACh in frog Ringer's solution produced robust currents that decayed in minutes (Fig. A). Simultaneous application of 50 μM ACh in Ringer's solution that had been shaken in the Falcon tubes produced currents with smaller peak currents that decayed in seconds (Fig. B). The currents progressively recovered as the Falcon-exposed solution was washed out of the 0.4 ml chamber for 19 minutes at a flow rate of 2 ml/min (Fig. C). Other tests indicated that a water soluble substance was coming off the Falcon tubes. Spectrophotometry revealed a significant absorbance near 200 nm after distilled water was shaken in a Falcon tube. When distilled water was shaken in 5 Falcon tubes and lyophilized, a white precipitate was formed. We thank Dr. J. Pinkham for providing the mRNA used in this study. Supported by NIH grant NS21229.



333.8

MULTIPLE PHASES OF DESENSITIZATION OF ACETYLCHOLINE RECEPTOR FROM ELECTROPHORUS ELECTRICUS. D. J. Cash, A. M. Rao* & G. G. Mayes*, Biochemistry Dept., Univ. of Missouri-Columbia and Neurochemistry Unit, Missouri Inst. of Psychiatry, School of Med. Columbia, MO 65211

Acetylcholine mediated cation flux and receptor desensitization were measured with quench flow techniques with native membrane vesicles. Three phases of desensitization were observed with half times of approximately 30ms, 250ms and 7s. The fast phase is due to a new receptor (or form of the receptor) distinguishable from the previously studied receptor. This is demonstrated by the constant initial activity of the second phase of desensitization over a wide range of acetylcholine concentration. The second phase is due to the previously studied receptor. The third phase is desensitization of activity remaining in a preequilibrium after the prior desensitization of the same receptor in the second phase. This is demonstrated by the large dependence of the initial activity of the third phase on acetylcholine concentration. The distinguishable receptors are on the same membrane. The different phases of desensitization are characterized by different dependencies on pH. The distinguishable receptors differ in their inhibition by high concentrations of bromoacetylcholine and their inactivation on reduction with dithiothreitol. This characterization of these responses is necessary in order to interpret correctly the effect of pH or bromoacetylcholine on this system. Supported by a grant from NINCDS (DHS 5ROI NS 20377-03).

333.9

FUNCTIONAL PROPERTIES OF SYNAPTIC ACETYLCHOLINE RECEPTORS IN FAST TWITCH FIBERS OF DYSTROPHIC MICE. L. P. Henderson. Department of Physiology, Dartmouth Medical School, Hanover, NH 03756.

Focal extracellular measurements of miniature endplate currents (mepcs) were made from flexor digitorum brevis (fdb) muscle of 2 month old, male C57BL/6J-dy^{2J} and control mice. Mepcs from control mice decayed with an average time constant of 1.2 ms, whereas the time constant of synaptic current decay from dystrophic fibers was significantly faster (0.8 ms). In order to ascertain whether this difference reflected a true difference in the functional properties of acetylcholine receptors (AChR), single channel recordings were made from the synapses of dissociated dystrophic fdb fibers. Two amplitude classes of events (65 and 45 pS) were present in recordings from dystrophic endplates, as has previously been reported for control fdb fibers (Brehm and Kullberg, P.N.A.S. Vol 84: 2550, 1987). However, the open duration of the 65 pS channel was briefer in many fibers (τ<1 ms, Vp=+80mV, cell-attached) than in control fibers (τ~2ms, Vp=+60 mV, cell-attached). In addition, openings of the 45 pS channel, which constituted ~3% of the total events in control fibers, were more prevalent in recordings from dystrophic fiber endplates (~20%). The mean channel open time of the 45 pS channel was also brief (τ<1 ms, Vp=+80 mV, cell-attached). These data suggest that the dystrophic phenotype affects the expression of AChR in innervated fast twitch fibers.

333.10

CHOLINERGIC CHANNELS IN *XENOPUS* MYOCYTES ACTIVATED BY COMBINATIONS OF AGONISTS. Anthony Auerbach, Dept.of Biophysical Sciences, SUNY, Buffalo, NY. 14214

Cholinergic channels usually open only after binding two agonist molecules. The rates of channel opening and closing depend on the nature of the bound agonist. In order to understand how channels behave when two different agonist molecules are bound, embryonic *Xenopus* myocytes were patch-clamped (cell-attached configuration) with pipette solutions of ACh (5 or 10 uM) plus either choline (Ch; 50-500 uM) or carbamylcholine (CCh; 10-200 uM). The applied potential was +40 mV and the temperature was 18 °C. Both large (g_{60}) and small (g_{40}) conductance channels were examined.

Preliminary results are given below (t_c = predominant closed interval time constant, t_o = predominant open interval time constant; times are in ms, concentrations are in uM; 2-4 patches for each experimental condition):

	t_c			g_{40}	
ACh/Ch	g_{40}	g_{60}	ACh/CCh	t_c	t_o
5/0	18	36	10/0	4.7	5.4
5/100	10	20	10/100	0.9	4.7
0/100	-	-	0/100	5.0	1.8

With 100 uM Ch alone the channel opening rate is very low and t_c is too long to be measured.

These results indicate that doubly-liganded channels occupied by ACh and either Ch or CCh open and close at rates similar to channels occupied by two ACh molecules. In this regard, the action of Ch is particularly interesting since endplates are exposed to choline produced by the hydrolysis of ACh during synaptic transmission. Supported by NS235130.

333.11

EARLY DEVELOPMENTAL CHANGES IN ACETYLCHOLINE RECEPTOR CHANNEL KINETICS IN CULTURED XENOPUS MYOCYTES

J. Rohrbough* and Y. Kidokoro. Dept. of Physiology, U.C.L.A. School of Medicine, Los Angeles, 90024.

Developmental changes in single acetylcholine receptor (AChR) channel properties were studied in aneural *Xenopus* myocyte cultures using single-channel recording techniques. Cultures were prepared from stage 15-18 embryos, and recordings taken at developmental stages ranging from several hours to five days after AChR first appear in the membrane.

The burst durations of both low and high conductance channels decreased during development. At 50 mV applied hyperpolarization, the low conductance burst duration decreased from 24 ms at stage 24 to 6 ms at stage 47, a 4-fold decrease; high conductance burst duration also decreased 2.5-fold in the same period, from 10.6 ms to 4 ms. Much of the decrease for both channel types occurred during the first day in culture. Most low conductance burst duration histograms were not well-fit with a single exponential, having an excess of brief openings. The excess could be largely accounted for by singly-liganded openings. The burst durations were determined by the best single exponential fit to the main, slow component. High conductance burst durations were well-fit by single exponentials. The percentage of high conductance channel events, which was initially a few percent, increased gradually beginning around stage 34 to 60% at stage 47. Neither the single channel conductances nor the voltage dependency of burst duration changed with development. To identify the kinetic parameter(s) associated with the burst duration changes, closed time analysis was done at high temporal resolution. The developmental decrease in burst duration could be due to changes in true channel mean open time ($1/\alpha$), or to changes in β or k_{-2}, which would be reflected in the mean closed time of gaps within bursts and the number of gaps per burst. For the low conductance channel, the brief component of the closed time was 25 μs, and mean number of gaps per burst was 1.95. Neither value changed with development. Therefore, we conclude that the channel closing rate constant, α, is the kinetic parameter changing.

333.12

THE POSSIBLE IMPORTANCE OF THE NEURONAL NICOTINIC SUBUNIT β_4 TO THE KINETIC PROPERTIES OF THE ADRENAL CHROMAFFIN CELL AChR.

R. L. Papke, R. Duvoisin*, J. Boulter, and S. Heinemann. Molecular Neurobiology Laboratory, Salk Institute, La Jolla, CA 92037

At least four genes coding for neuronal nicotinic acetylcholine receptor subunits (α_3, α_5, β_2, & β_4) are expressed the PC-12 cell line, which was derived from an adrenal tumor. The α_3 subunit can form functional receptors when expressed with either of the beta subunits. Cell-attached patch clamp recordings were made from *Xenopus* oocytes injected with mRNA coding for the agonist binding subunit, α_3, in combination with message for either the β_2 or β_4 subunits. Multiple conductances are observed with both combinations and may relate to the presence of receptors with different ratios of alpha and beta subunits (Papke *et al.* in preparation). The primary open state of receptors formed with the β_4 subunit was of higher conductance and had open times 4 to 6 fold longer than those formed with β_2. Openings of the $\alpha_3\beta_4$ receptors were grouped in bursts with an average of 8 openings per burst. Bursts of the $\alpha_3\beta_2$ receptors had an average of only 1.5 openings. The open time and burst durations of the $\alpha_3\beta_4$ receptors are similar to what has been described for adrenal chromaffin cell AChR.

In situ hybridization studies have shown that the genes for the α_3 and β_2 subunits are expressed in many parts of the brain (Wada *et al.* J of Comp. Neur., May 1989), while the expression of β_4 in the brain is largely restricted to a portion of the medial habenula (Duvoisin *et al.* in preparation). However, significant amounts of β_4 are expressed in the adrenal gland.

333.13

MAPPING BY SITE-DIRECTED MUTAGENESIS OF NICOTINIC ACETYLCHOLINE RECEPTOR RESIDUES INVOLVED IN LIGAND BINDING.

W. H. M. L. Luyten and S.F. Heinemann. Molecular Neurobiology Lab, Salk Institute, San Diego CA92138.

We have previously demonstrated (Soc. Neurosc. Abstr. 40:3 1986) that a nicotinic acetylcholine receptor fragment, comprising residues 87-208 of its α-subunit, contains a large part of the binding site for α-bungarotoxin (α-btx), as well as for smaller cholinergic ligands. To define more precisely the residues contributing to this binding site, we changed single amino acids in α87-208 by site-directed mutagenesis. Because a positively charged moiety of cholinergic compounds has been postulated to interact with a negative charge in the binding site, all conserved negatively charged residues were mutagenized to His one by one: Asp89, Asp97, Asp99, Asp111, Glu129, Asp138 Asp152, Glu161, Asp163, Asp166, Glu175, Asp180 and Asp200. The mutant proteins were expressed in an *E. coli* lon$^-$ strain; all had to be solubilized from inclusion bodies by denaturation/ renaturation. All mutant proteins accumulated to comparable levels, and all bound α-btx on blots. In solution, α-btx binding to all mutants showed biphasic association as well as dissociation kinetics, consistent with prior evidence (*loc. cit.*) for two non-interconvertible sites with different affinities. Only one mutant (Asp166->His) showed essentially unaltered kinetics compared to wild-type. For the high affinity site, all other mutants had lower association rate constants (k_+) than the wild-type, and fell into three classes based on their dissociation rate constants (k_-). One class showed comparable reductions in k_+ and k_-, suggesting that all binding site residues were intact, but that orientational or conformational impediments in the mutant slowed complex formation. In the two other classes, k_- changed by a larger or smaller factor than k_+, leading to increased or decreased K_Ds, and suggesting that interactions with binding site residues had been altered. Most of the mutants were not stable during the long incubations required to reach equilibrium in competition binding experiments with small cholinergic ligands, so that the effect of the mutations on their affinity could not be evaluated.

333.14

NEURONAL NICOTINIC ACETYLCHOLINE RECEPTORS IN DROSOPHILA MELANOGASTER.

E. Sawruk*, B. Schmitt* and H. Betz. (SPON: W. Oertel), Zentrum für Molekulare Biologie, Im Neuenheimer Feld 282, 6900 Heidelberg, FRG.

Two genes - ard and als - are known to encode subunits of the neuronal nicotinic acetylcholine receptor (nAChR) in Drosophila. Genomic Southern blot analysis using probes derived from these genes suggested the existence of additional nAChR subunits in Drosophila. Screening a Drosophila genomic library with these probes we have isolated several crosshybridizing DNA clones. An alignment of the amino acid sequence predicted from partial nucleotide sequences revealed that two of the clones isolated showed significant homology to putative transmembrane regions of ard and als. Using fragments of these genomic clones as probes additional positive clones were isolated from an embryonic cDNA library of Drosophila. Our data indicate that considerable diversity of acetylcholine proteins exists in insects.

LIGAND-GATED ION CHANNELS: GLUTAMATE

334.1

EFFECT OF BETA ADRENERGIC BLOCKERS ON N-METHYL-D-ASPARTATE (NMDA)-ACTIVATED CHANNELS OF RAT HIPPOCAMPAL NEURONS.

V. Radhakrishnan* and E.X. Albuquerque (Spon: N. Brookes). Dept. of Pharmacol. and Exp. Ther., Univ. Maryland Sch. Med., Baltimore, MD 21201.

NMDA receptors have been implicated in neuronal plasticity, and excessive activation of these receptors may result in seizures and/or injury to the cell (Cotman, C.W. et al., Ann. Rev. Neurosci. 11:61-80, 1988). Antagonists to excitatory amino acids have strong anticonvulsant actions and can protect against epileptic seizures and cell death (Meldrum, B.S. et al., Neurosci. Lett. 39:101-104, 1983). Beta adrenergic blockers have been shown to possess anticonvulsant and mood impairment properties, but it is not known if they affect the NMDA receptors. In the present study, the effects of (±)-propranolol and its stereoisomers were tested on the NMDA-activated single channel currents of cultured hippocampal neurons of fetal rats, using the patch clamp (outside-out configuration) technique. (±)-Propranolol (5-50 μM) reduced the mean open time of NMDA-activated channels in a concentration-dependent manner. A greater reduction in channel lifetime was evident at -80 mV (50-55% reduction with 50 μM propranolol) than at -120 mV (30-35%). At positive potentials, the block was less pronounced. The frequency of activation of NMDA channel currents was also reduced by propranolol in a concentration-dependent manner. A reduction was also observed in the frequency of bursts, though the mean burst time remained unaltered. The long clusters seen after NMDA were not evident after propranolol. The enantiomers of propranolol and the racemic mixture acted in similar manner without showing stereospecificity of responses. It is quite likely that the blockade of NMDA-activated channels may also contribute to the depressive symptoms and alterations in emotional states induced by propranolol. (Support: US Army Med. Res. & Devel. Comm. Contract DAMD17-88-C-8119)

334.2

ZINC REDUCES THE NUMBER OF LARGE-CONDUCTANCE NMDA CHANNEL EVENTS.

G.L. Westbrook & P. Legendre*†. †INSERM, Bordeaux, France and Vollum Institute, Oregon Health Sciences Univ., Portland, OR 97201.

The transition metal cations, Zn and Cd inhibit the NMDA receptor/channel in a non-competitive manner. Whole-cell voltage clamp studies indicate that this inhibition is voltage-independent unlike a number of channel-blocking divalent cations and drugs. However, Zn does cause small reductions in both mean opentime (MOT) and single channel conductance ($\sqrt{}$) of the large conductance level, calculated from spectral analysis of NMDA current noise (Mayer, Westbrook & Vyklicky, J. Neurophysiol., **60**, 645, 1988). To further examine this action of Zn , we have used outside-out patches from cultured neurons of the olfactory bulb and hippocampus of newborn rats.

Extracellular solution contained (mM): Na 165, K 2.5, Ca 1, Mg 0, HEPES 10, glucose 10; glycine .005; picrotoxin, strychnine, and tetrodotoxin were also added. Pipette solution contained (mM): Cs 140, Ca 0.5, EGTA 5, Mg 1, HEPES 10, Cl 126, Fl 14. Cation salts were ultrapure; NMDA (1-10 μM) and Zn (1-30 μM) were applied by flowpipes. Patches from both cell types nearly always contained more than one NMDA-activated channel. As expected, several conductance levels were apparent, consisting primarily of large conductance levels of 45 pS (MOT 5-10 ms) and 35 pS (MOT 1-3 ms); and a much lower frequency of 10 pS events. At -60 mV or +40 mV, the most prominent effect of Zn was a dose-dependent reduction in the frequency of the large conductance events such that at 30 μM Zn, the number of events was 25-30% of control. Amplitude histograms demonstrated a relative increase in the proportion of 10 pS events in the presence of Zn. The MOT of the 45 and 35 pS states were reduced as well. Zn also caused a small, dose-independent reduction in the single channel conductance which was accompanied by an increase in the current variance of the open channel; this appeared to be due to an increase in small (appx. 5 pS) transitions around the 35 and 45 pS levels. This contrasts with the smooth reduction in $\sqrt{}$ in the presence of elevated Ca. Supported by INSERM, NATO Fdn., NIH and the McKnight Fdn.

334.3

INORGANIC LEAD (Pb^{++}) SELECTIVELY BLOCKS N-METHYL-D-ASPARTATE (NMDA)-ACTIVATED CHANNELS. M. Alkondon*[1], V. Radhakrishnan*[1], A.C.S. Costa*[1,2], M. Nakatani[2] and E.X. Albuquerque[1,2]. (Spon: D. Weinreich) [1]Dept. Pharmacol. & Exp. Ther., Univ. of Maryland Sch. of Med., Baltimore, MD 21201 & [2]Lab. Molec. Pharmacol. II, Fed. Univ. Rio de Janeiro, Brazil.

The heavy metal lead has been recogonized as an environmental toxicant which causes a deficit in cognition in children and learning impairment in experimental animals. In view of the recent evidence that implicates NMDA receptors in the processes of learning and memory, we studied the effect of Pb^{++} on glutamate receptors. Using cultured hippocampal neurons (7-21 days old) of fetal rats, we studied the single channel currents (outside-out patch configuration) activated by NMDA and quisqualic acid (Quis). $PbCl_2$ reduced the frequency of openings (1-40 μM), the mean channel open time (5-40 μM) and the mean burst time (10-40 μM) of the single channel currents activated by NMDA in a concentration-dependent manner. These effects were observed at both negative and positive membrane potentials (-100 mV to +80 mV), unlike that observed with another cation, Mg^{++}, which elicited its blocking effect only at hyperpolarized potentials. On the Quis-evoked currents, however, >70 μM $PbCl_2$ was required to produce any significant kinetic alteration. The single channel conductance of channels activated by either NMDA or Quis was unaltered by Pb^{++} at the concentrations used in this study. Selective impairment of NMDA-type of glutamate receptors thus leading to a reduced influx of Ca^{++} and interference with the Ca^{++}-dependent processes may underlie some of the behavioral alterations observed in Pb^{++} neurotoxicity. (Support: US Army Med. Res. & Devel. Comm. Contract DAMD17-88-C-8119 & CAPES Proc. 4987/88-2).

334.4

REDUCTION OF NMDA RECEPTOR CURRENT BY MK-801: SINGLE CHANNEL KINETICS. R.L. Macdonald, N.M. Porter, and R.E. Twyman. Dept. of Neurology, Univ. of Michigan, Ann Arbor, MI. 48104

It has been suggested that the reduction of NMDA receptor current by MK-801 occurs via *fast* open channel block (*Huettner and Bean, PNAS 85:1307, 1988*). In the present study, we further characterized the actions of MK-801 on NMDA receptor currents by studying the kinetic properties of the main conductance state (50 pS) of the NMDA receptor. Outside-out patches from cultured mouse spinal and cortical neurons were held at -75 mV in nominal Mg^{2+} solutions. Data were digitized at 20 kHz (2 kHz Bessel filter). Frequency distributions of open times, closed times, and burst durations were analyzed by curve fitting to sums of exponential functions.

MK-801 (1 and 10 μM) reduced the current evoked by NMDA (50 μM) and glycine (2 μM) primarily by decreasing the frequency of channel opening, with a small decrease in mean open time. Frequency distributions of NMDA evoked openings were best fit by two exponential functions whose time constants were not altered but whose proportion of longer openings was reduced by MK-801. NMDA openings occurred in short bursts, and MK-801 decreased mean burst duration. Burst duration frequency histograms were best fit by two exponential functions whose time constants were not altered. The proportion of longer duration bursts was reduced by MK-801. The number of openings per burst also was decreased by MK-801. Neither intraburst nor interburst closed time constants were affected by MK-801, but the proportion of long duration interburst closures was increased by MK-801.

These results suggest that MK-801 may reduce NMDA receptor current by *slow* open channel block or by binding to an allosteric site on the receptor-channel. *Supported by NIH DA04122 to RLM and NS08216 to NMP.*

334.5

KINETIC PROPERTIES OF NMDA-ACTIVATED CHANNELS IN EMBRYONIC *XENOPUS* SPINAL NEURONS. Yinong Zhang* and Anthony Auerbach, (SPON: C. Bowman). Dept. of Biophysical Sciences, SUNY, Buffalo, NY 14214

NMDA receptors in *Xenopus* play important roles in motor pattern generation and in the plasticity of retino-tectal connections. We are studying the properties of single channel currents elicited by combinations of NMDA and glycine in spinal neurons from 1-day old *Xenopus* embryos. Experiments have been carried out at 24 °C on cell-attached patches of cells grown in culture for 1-7 days.

Xenopus NMDA receptors resemble those of mammalian systems in that the predominant channel conductance is ~50 pS and channel activity is inhibited by Mg^{++} and potentiated by glycine. Open interval durations can be fitted by the sum of 2 exponentials. With channel currents of 4 pA (estimated V_m = -80 mV), >90% of openings are from the slower component which has a time constant of ~1.2 ms. Open interval durations increase with depolarization but do not vary with [NMDA] (1-1000 uM) or added [glycine] (up to 100 uM). Closed interval duration distributions are complex. Under all experimental conditions we have examined, 2 fast (t = 0.1, 1.0 ms) and one very slow (t > 200 ms) are apparent, with an intermediate component(s) in the range 2-30 ms. At very high [NMDA] and [glycine] channel activity is reduced, suggesting that these molecules may compete with each other. With prolonged application of NMDA plus 10 or 100 uM glycine, there is a gradual rundown of activity, but this effect is less marked than the profound desensitization of *Xenopus* cholinergic receptors.

NMDA-activated currents are present on about 60% of the neurons. In some cells, two distinct amplitudes of NMDA currents were apparent. Some of the complexity in channel kinetics may arise from inhomogeneity of channels with regard to both cell type and channel type in a single cell. Supported by NS235130.

334.6

RAPID ACTIVATION AND DESENSITIZATION OF QUISQUALATE-TYPE GLUTAMATE CHANNELS IN EMBRYONIC MOTONEURONS. D.O. Smith, J.L. Rosenheimer, H. Hatt*, and F. Zufall*. Dept. of Physiol., Univ. of Wisconsin, Madison, WI 53706 and Physiol. Inst., Tech. Univ. Munchen, West Germany.

Ionic currents in response to 100- to 300-ms applications of glutamate or quisqualate were measured in motoneurons isolated from embryonic chick spinal cords. A piezo-coupled device was used to produce rapid solution exchanges. In whole-cell recording mode, the inward currents rose in 2 to 8 ms and then decayed to a steady-state level of about 10% of the peak amplitude with a time constant of about 30 ms. In outside-out recording mode, activation could be achieved within <1 ms after glutamate application. The average current desensitized to the lower conductance states with a time constant of about 5 ms. Channel activity was grouped in 6-ms bursts; mean open and close times were 2.8 and 1.5 ms, respectively. Depolarization increased burst length and open time, and increasing agonist concentration prolonged burst length. This rapidly desensitizing quisqualate-type receptor may mediate fast excitatory synaptic transmission in spinal motoneurons. Supported by NIH grant NS13600 and SFB 220.

334.7

NMDA-STIMULATED CALCIUM INFLUX IN RAT CORTICAL SYNAPTOSOMES. M.A. Oleshansky, M.L. Koenig, D.E. Mark*, and M.A. Jackson*. Walter Reed Army Institute of Research, Washington DC 20307-5100.

N-methyl-D-aspartate (NMDA) has been reported to open a non-specific cation channel in a variety of neuronal cell types. As Ca^{2+} influx is a critical component in neurotransmitter release, we have examined the effect of NMDA on Ca^{2+} influx and intracellular Ca^{2+} levels in rat brain synaptosomes.

$^{45}Ca^{2+}$ influx was measured in crude (P2) and purified (P3) synaptosomes prepared from rat cortex. NMDA stimulated $^{45}Ca^{2+}$ influx in P2 which was maximal at 30 sec. In the presence of 5 or 50 mM K^+, NMDA stimulated ^{45}Ca influx in a concentration-dependent manner over a range of 1 to 50 uM with maximal stimulation (150% of control) seen at 50 uM NMDA. The dose-response curve for NMDA was shifted to the left in the presence of 50 mM K^+. NMDA-stimulated ^{45}Ca influx was also apparent but less pronounced in P3. These findings demonstrate that NMDA stimulates Ca^{2+} influx into synaptosomes and suggest that depolarization may be releasing endogenous factors including glycine or glutamate which are supplementing NMDA-stimulated Ca^{2+} influx.

Preliminary experiments with Indo-1 in purified cortical synaptosomes failed to show any significant effects of NMDA on intracellular Ca^{2+} levels. Depolarizing concentrations of K^+ produced only small changes in intracellular Ca^{2+} levels. The effect of an interaction of NMDA and glycine on intracellular Ca^{2+} levels in this system is currently under investigation.

334.8

Excitatory Amino Acid (eaa) Antagonists Decrease the Medium and Slow Afterhyperpolarization (m-ahp/s-ahp) and Hyperpolarize Neurons of the Basolateral Amygdala (bla). P. Shinnick-Gallagher and A.C. Anderson. Dept. of Pharmacology, Univ. of Texas Med. Br., Galveston Tx 77550 (spon: K.D. Phelan)

The effects of endogenous eaa's on intrinsic membrane properties were studied using eaa antagonists, dl-2-amino-5-phosphonovaleric acid (dl-apv, 50uM), 7cl-kynurenic acid (7clkyn, 10uM), and 6-cyano-7-nitroquinoxaline-2,3-dione (cnqx, 5uM). The eaa antagonists induced a voltage independent decrease (10 to 50%) in the m-ahp following 100 msec depolarizing pulses in 77% and a decrease in the s-ahp (20 to 60%) in 66% of the experiments in kindled cells; in control cells the m-ahp/s-ahp decreased 33 to 83% in 60% of the experiments. At peak drug effect the membrane was hyperpolarized 1 to 7 mV in kindled and 2 to 3 mV in control neurons followed by a rebound depolarization from 1 to 14 mV (median: 5mV) above baseline upon washout. Input resistence decreased 5 to 20% in dl-apv, <5% in 7clkyn, and 10 to 15% in cnqx in kindled and control neurons. Previous studies have shown that n-methyl-d-aspartate increases the ahp in CNS neurons and this appears to be true in bla neurons. The current data supports the hypothesis that an tonic release of eaa's modulate voltage gated conductances and resting excitatory tone in both control and kindled bla neurons. (Supported by NS24643)

335.1

PROTEIN KINASE C AND cAMP-DEPENDENT PROTEIN KINASE PHOSPHORYLATE THE PURIFIED $GABA_A$ RECEPTOR. M.D. Browning[1], M. Bureau[2], E. Barnes[1], and R. Olsen[2]. (Spon: J. Sikella) [1]Department of Pharmacology, University of Colorado HSC, Denver, CO 80262; [2]Department of Pharmacology, UCLA Sch Med, Los Angeles, 90024.

A number of recent studies have suggested that phosphorylation of the $GABA_A$ receptor could modulate receptor function. Activators of protein kinase C (PKC) and cAMP-dependent protein kinase (A kinase) have been shown to influence $GABA_A$ receptor function. In addition, Sweetman et al. (J. Neurochem. 51,1274,1988) have reported that a kinase associated with the partially purified preparation of the receptor could phosphorylate the α-subunit of the receptor. This kinase activity was not influenced by cAMP, cGMP, Ca^{2+}, calmodulin, or phosphatidylserine. To explore the issue directly, we have examined the ability of specific kinases to catalyze significant phosphorylation of the $GABA_A$ receptor which has been purified to near homogeneity.

The $GABA_A$ receptor was purified as previously described using benzodiazepine affinity chromatography. The purified receptor possessed no detectable kinase activity. PKC and the A kinase catalyzed the phosphorylation of both the β- and α-subunits of the receptor. However, most of the PO_4 incorporation was associated with the β-subunit. Two muscimol binding polypeptides designated β_h (higher molecular weight; M_r 59,000) and β_l (lower molecular weight; M_r 57,000) were present in the preparation. The higher molecular weight polypeptide, β_h, was phosphorylated specifically by the A kinase. β_l was phosphorylated specifically by PKC. β_h and β_l gave distinct patterns in a one-dimensional phosphopeptide analysis. Taken together these data suggest that there are two forms of the β-subunit of the $GABA_A$ receptor and that these two forms of the β-subunit are phosphorylated by distinct kinases. (supported by PHS grants NS22071 to R.O. and NS26377 and DK 40483 to M.D.B.)

335.2

MONOCLONAL ANTIBODIES RECOGNIZING THE BENZODIAZEPINE RECEPTOR OF CHICK EMBRYO BRAIN. H.S. Yin and S.S. Fan*, Dept. of Anatomy, College of Medicine, National Taiwan University, Taipei, Taiwan, R.O.C.

Monoclonal antibodies(mAbs) have been produced by immunizing BALB/c mice with the benzodiazepine(BZD) receptor proteins from 20 day chick embryo brain. The fluorogram and gel slice extraction of SDS-PAGE gels of the brain membranes photolabelled by ^{3}H-flunitrazepam(FNZ) showed that the binding sites were located at 51KD and 48KD proteins. The proteins from 48KD to 51KD were eluted from gels and then injected into the mice. Screening was performed by ELISA, immunoblotting, and radioligand binding after the splenocytes were fused with NS-1 myeloma cells. Six positive clones were obtained. Western blot of ^{3}H-FNZ-labelled membranes showed that the mAbs recognized a major radioactive 51KD and a minor 50KD bands. Immunoblotting of two-dimensional slab gels revealed same bands with pIs ranging from 5.6 to 6.0. Cultured brain neurons stained with the mAbs showed that the immunoreactivity was localized on the membranes of cell soma and neurites of certain neurons. Immunostaining of paraffin sections of brain tissue labelled neurons located at various areas including hippocampus, optic tectum and cerebellum. These results indicate that the mAbs recognize the BZD receptor of chick embryo brain. Futher characterization of the receptor using these mAbs is in progress.

335.3

THE PRODUCTION AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES DIRECTED AGAINST THE γ-AMINOBUTYRIC ACID ($GABA_a$) RECEPTOR. P. A. Gallombardo and J. F. Tallman. Abraham Ribicoff Research Facilities, Yale University School of Medicine, 34 Park St., New Haven, CT 06508

The molecular complex which mediates the effects of the neurotransmitter GABA and the anxiolytic benzodiazepines has been purified; and the genes encoding the receptor subunits have been sequenced. Molecular genetic techniques, including *in situ* hybridization histochemistry, have revealed that multiple combinations of subunits can form an array of receptor subtypes. Monoclonal antibodies directed against the rat brain $GABA_a$ receptor were produced to be used as biochemical and immunocytochemical probes for assessing the function and localization of specific receptor subunits. The monoclonal antibodies were produced by the immunization of Balb/c mice with purified rat brain $GABA_a$ receptor followed by the formation of antibody secreting hybridomas. The resulting antibodies were characterized by solid phase radioimmunoassay, immunoblot, and epitope protection assay. All of the antibodies reacted with a 50kD protein in preparations of purified receptor and brain membranes. This protein was identified as the benzodiazepine binding subunit by photolabeling the protein with ^{3}H-flunitrazepam and performing two dimensional immunoblots. The specificity of two of the antibodies was further demonstrated by immunoprecipitating the solubilized benzodiazepine binding site. The antibodies could be divided into five epitope groups. Immunoreactive proteins were identified in rat astrocytes and in three species of Nemathelminthes. These cross reactive proteins may be homologues of the $GABA_a$ receptor γ subunit.

335.4

DIFFERENTIAL DISTRIBUTION OF $GABA_A$ α1,α2 AND α4 RECEPTOR mRNAs IN THE RAT NERVOUS SYSTEM. N. Brecha, C. Sternini, K. Anderson*, K. Bhakta*, M. Khrestchatisky*, A.J. MacLennan*, M.Y. Chiang*, R.W. Olsen and A.J. Tobin. Depts. of Med., Anat. & Cell Biol., Pharm. and Biol. and CURE, UCLA Sch. Med. and VAMC-West Los Angeles, LA, CA, 90024.

The $GABA_A$ receptor consists of several structurally distinct components including α, β and γ subunits. Three distinct rat $GABA_A$ α cDNAs (1, 2 and 4) have been isolated. The cellular localization of $GABA_A$ α mRNAs in the rat brain was studied using *in situ* hybridization histochemistry with ^{35}S-labeled rat α1, α2 and α4 RNAs. Cryostat sections were incubated in antisense or sense RNAs, washed at high stringency and processed for autoradiography. The α1 transcripts have a wide distribution along the neuroaxis. Regions with heavy labeling include the olfactory bulb, hippocampal formation, cortex, inferior colliculus, cerebellum and cerebellar nuclei. Hypothalamic, thalamic and midbrain structures are also labeled. Low to undetectable α1 mRNA levels are found in the caudate and in many regions of the pons and medulla. The α2 and α4 transcripts have a more limited distribution, with heavy labeling in the olfactory bulb and hippocampal formation and moderate labeling in the cortex. The α2 mRNA is also expressed in the caudate. Areas with low to negligible levels of α2 and α4 mRNAs include the inferior colliculus, cerebellum, deep cerebellar nuclei and many regions of the pons and medulla. These studies show that the α transcripts are differentially expressed in the brain. Some areas contain all three α mRNAs, while in other areas there are marked differences in their localization patterns and levels of expression.

335.5

FUNCTIONAL EXPRESSION AND REGIONAL DISTRIBUTION OF A AND B SUBUNITS FOR THE GABA A RECEPTOR. M. Khrestchatisky*, A.J. MacLennan*, M.Y Chiang* W. Xu*, M. Jackson, K. Anderson, C. Sternini, N. Brecha, R.W Olsen and A.J. Tobin. Departments of Biology, Medicine, Anatomy, and Pharmacology, UCLA, Los Angeles, CA 90024. (SPON: M. Wexler).

Four cDNAs (α1, α2, α4 and β) from a rat hippocampal cDNA library encode isoforms of the subunits of the GABA receptor. α1, α2 and β polypeptides are very similar to the bovine α1, α2 and β polypeptides. The α4 polypeptide differs in the presumed extracellular and intracellular domains from previously reported subunits and is thus a novel isoform. The cDNA probes detect mRNAs with distinct sizes and regional distributions in the rat brain. When injected into Xenopus oocytes, combinations of in vitro transcribed RNAs for the α and β subunits produce GABA- dependent inward currents. In some cases, picrotoxin alone applied to oocytes elicits an outward current. These responses suggest that α and β polypeptides can produce GABA-gated ion channels that can also open spontaneously. Supported by NS 21908 and 22256.

335.6

ANALYSIS OF GABA-A RECEPTOR SUBUNIT COMPOSITION WITH SUBTYPE-SPECIFIC ANTISERA. S. Endo*, G.B. Smith*, L. Deng*, J.D. Young* and R.W. Olsen. Depts. of Pharmacology and Biological Chemistry, UCLA School of Medicine, Los Angeles, CA 90024.

Antisera were produced against subunit subtype-specific peptide sequences of the $GABA_A$ receptor from bovine brain. Putative extracellular and cytoplasmic domains of α_1, α_2, α_3 and β receptor subunits as published were evaluated and unique sequences identified. From these, sequences predicted to be highly immunogenic were selected. Peptides corresponding to the putative cytoplasmic domain between membrane spanning regions 3 and 4 were synthesized and purified by reverse phase HPLC. The purified peptides were characterized by amino acid analysis. A C-terminal cysteine was added to each to facilitate coupling to carrier protein; the peptides were conjugated to KLH and injected into rabbits. Antisera to peptides from α_1, α_2, and β_1 have been screened by indirect ELISA using synthetic peptides and shown to give half maximal reaction at dilutions of 100,000. Affinity purified receptor shows bands on SDS gels at 52-54 kD which are recognized by α antisera. In addition, the β subunit at 58 kD, and additional bands at 35, 45, and 70 kD, are being investigated for specific labeling on Western blots and immunoprecipitation with antisera.

Supported by NIH grants NS22071 and HD06576.

335.7

ONTOGENY OF THE $GABA_A$/BENZODIAZEPINE RECEPTOR COMPLEX IN THE RAT BRAIN: LOCALIZATION OF α_1-SUBUNIT mRNA AND LIGAND BINDING SITES. C. Gambarana, R. Pittman*, G.A.Ordway*, and R.E. Siegel. Dept. of Pharmacology, Case Western Reserve University, Cleveland, OH 44106.

The $GABA_A$/benzodiazepine receptor complex mediates the actions of the major inhibitory transmitter in the brain, γ-aminobutyric acid (GABA). The action of GABA at the receptor is modulated by two classes of clinically important compounds, the benzodiazepines (BZD) and barbiturates. It appears that the receptor complex is composed of multiple subunits and that its composition may differ in different brain regions.

Much remains to be learned concerning the importance of the different subunits in the formation of a functional $GABA_A$/benzodiazepine receptor complex. To approach this issue, expression of the receptor in the developing rat brain was examined with in situ hybridization and receptor autoradiography. Using a probe complementary to the mRNA encoding the α_1 subunit, only low levels of message were detectable during the first postnatal week. At this time, the mRNA was most prominent in the cortex, hippocampus, and in the inferior colliculus. A dramatic rise in mRNA levels occurred in the second week, particularly in the cerebellum where increased levels of message were paralleled by increases in the binding of [^{3}H]RO 15-1788 and [^{3}H]GABA, ligands for BZD and GABA sites, respectively. In the cerebellum, the α_1 mRNA and binding sites continued to increase until day 28, and then decreased to adult levels. These findings will allow us to assess the relation between receptor expression and GABAergic synapse formation, as much is known about the ontogeny of the cerebellum.

335.8

MODULATION OF $GABA_A$ RECEPTOR CHANNEL EXPRESSED IN XENOPUS OOCYTES, BY FULL AND PARTIAL AGONISTS OF THE BENZODIAZEPINE RECEPTOR. L. Prado de Carvalho*, P. Curutchet*, J. Stinnakre[1]* and J. Rossier. Laboratoire de Physiologie Nerveuse and Laboratoire de Neurobiologie Cellulaire et Moléculaire[1], C.N.R.S., 91198-Gif sur Yvette, CEDEX, France.

Voltage-clamped oocytes injected 2-4 days before recording with chick brain poly(A^+)RNA were used to compare the ability of benzodiazepine (BZ) receptor full and partial agonists to enhance Gaba induced current. Diazepam (DZ) and flunitrazepam enhanced current induced by 10 μM Gaba in a dose dependent manner. Maximal effect (190% of controls) was attained with micromolar concentrations. Long application (30 min) of agonists did not change the initial potentiating effect: thus equilibrium is rapidly attained and BZ receptors do not desensitize. Ro 16-6028 and Ro 17-1812 had only small enhancing effects up to 1 μM, but at 100 μM were as potent as DZ. Clonazepam enhanced Gaba current at sub-micromolar concentrations but the Gaba response did not surpass 150% of controls.

Ro 16-6028 and Ro 17-1812 have a higher affinity than DZ (*Eur. J. Pharmacol.*, 156: 169, 1988), thus their lower effect at 1 μM on the oocyte is in agreement with their partial agonist profile in other systems. Effects observed above 1 μM may deserve further studies.

335.9

EXPRESSION OF mRNA IN XENOPUS OOCYTES DEMONSTRATES GENETIC DIFFERENCES IN ETHANOL SENSITIVITY OF THE $GABA_A$ RECEPTOR COMPLEX K.A. Wafford, R.A. Harris and T.V. Dunwiddie. Dept. of Pharmacology, Univ. of Colo. Hlth. Sci. Cntr., and V.A. Medical Center, Denver, CO 80262

Ethanol has a number of effects on the brain, producing sedation and anesthesia. Genetically selected lines of mice and rats have been generated which display differing degrees of sensitivity to these effects of ethanol. Biochemical studies suggest differences in the $GABA_A$ receptor of these lines, for example in long sleep and short sleep mice. Increasing evidence suggests that ethanol enhances the opening of $GABA_A$ chloride channels and increases GABA-mediated $^{36}Cl^-$ influx into mouse brain microsacs. Messenger RNA was extracted from LS and SS mouse brain using a Fast-Track isolation kit and 50ng injected into defolliculated oocytes. Cells were incubated for 2-3 days before recording. Currents were recorded under voltage-clamp conditions and drugs were applied by perfusion. $GABA_A$ receptors were expressed in oocytes and application of GABA elicited a similar inward current in both lines of mouse, the GABA current was enhanced by pentobarbital (100μM) and diazepam (0.1μM). Ethanol (10-50mM) in the presence of GABA had a large potentiating effect on LS GABA receptors (20mM enhanced the initial current by ~57%) but had no significant effect on SS GABA receptors. Ethanol also had a "long-term" potentiating effect which peaked at ~20 min. GABA responses were enhanced by concentrations of ethanol which had no effect alone. Hence the diffences in ethanol sensitivity of LS and SS mice can be at least partially explained by some difference in one or several of the GABA receptor subunit genes. Supported by the V.A. and grants AA06399.

335.10

CHRONIC ETHANOL EXPOSURE ALTERS BENZODIAZEPINE (BZ) RECEPTOR LIGAND MODULATION OF GABA-ACTIVATED CHLORIDE FLUX. K.J. Buck* and R.A. Harris. Dept.of Pharmacology, UCHSC, Denver, CO 80262; and VA Med. Res. Serv., Denver, CO 80222.

We found that chronic ethanol exposure attenuated the ability of flunitrazepam (FNZ) to modulate muscimol-activated $^{36}Cl^-$ uptake, and enhanced the actions of BZ inverse agonists, Ro15-4513 and DMCM. Cortical membrane vesicles were prepared from ICR mice given a liquid diet containing 6% (v/v) ethanol, or an equicaloric diet with sucrose substituted for ethanol, for 10 days. Muscimol-activated $^{36}Cl^-$ uptake was measured as previously described (Life Sci. 39: 2005, 1986). Attenuatation of FNZ's effect was the result of reduced efficacy in microsacs prepared from nonwithdrawn mice, but not those withdrawn for 6 or 24 hr. Sensitization to the actions of BZ inverse agonists was reflected by increased efficacy. Ro15-4513 efficacy was increased in microsacs prepared from nonwithdrawn mice and those withdrawn for 6 hr, 24 hr or 8 days, and was greatest in those withdrawn for 6 hr.

Our results indicate a role for the chloride channel in the development of cross-tolerance to FNZ following chronic ethanol exposure. Furthermore, they demonstrate that chronic ethanol exposure sensitizes the channel to the actions of BZ inverse agonists. This may be responsible for sensitization to BZ inverse agonists observed behaviorally in ethanol-dependent animals. Moreover, these changes in chloride channel function may play a role in the development of tolerance or physical dependence to, or withdrawal from, ethanol or other intoxicant/sedative drugs. Supported by the VA and AA06399.

335.11

EFFECTS OF CONTINUOUS DIAZEPAM ADMINISTRATION ON GABA-A SUBUNIT mRNA IN RAT BRAIN. C. Heninger, N. Saito*, J.F. Tallman, R.S. Duman, K.M. Garrett*, M.P. Vitek and D.W. Gallager. Dept. of Psychiatry, Yale Univ. School of Med, New Haven, CT 06508.

Chronic treatment with the benzodiazepine agonist diazepam (DZ) results in the development of tolerance and physical dependence. Our laboratory has documented regionally specific differences in GABA sensitivity both electrophysiologically and biochemically after chronic exposure to DZ. In order to investigate a possible molecular basis for these changes, we have measured levels of GABA-A receptor α and β subunit mRNAs after chronic treatment with vehicle or DZ implants. Levels of mRNA were examined by Northern blot using cDNA clones for GABA-A α and β receptor subunits, and resulting autoradiograms were analysed by densitometry. Levels of α subunit mRNA in cerebral cortex were decreased compared to levels resulting from vehicle treatment. Levels of GABA-A β subunit mRNA were similar after chronic vehicle or DZ treatment. Acute DZ (2 hr) did not alter levels of either α or β specific mRNA. These results suggest that changes in GABA receptor subunit mRNAs may be related to the GABA sensitivity changes seen with chronic DZ treatment. Asssociation of such changes with chronic treatment will be assessed by comparing regional differences in GABA-A subunit mRNA with regional changes in binding to ligands specific for the benzodiazepine recognition site and the GABA recognition site.

335.12

MAPPING THE BENZODIAZEPINE PHOTOAFFINITY LABELLING SITE WITH $GABA_A$ RECEPTOR ANTIBODIES. F.A. Stephenson* and M.J. Duggan* (SPON: P.A. Kirkwood) School of Pharmacy, Brunswick Square. London WC1N 1AX, England.

Analysis of the amino acid sequence of the $GABA_A$ receptor polypeptides has predicted a model for the protein with a large extracellular N-terminal hydrophilic domain and four hydrophobic transmembrane regions M1-M4. The location of the drug binding sites within the protein sequence are not known. We have focused on the identification of the agonist benzodiazepine binding site. The $GABA_A$ receptor was purified from adult bovine cerebral cortex, photoaffinity labelled with [^{3}H] flunitrazepam and subjected to complete and limited cyanogen bromide chemical cleavage. The products of these reactions were analysed for radioactivity, for immunoreactivity in Western Blots with anti-α1 1-15, anti-α1 324-341 and anti-α1 413-429 $GABA_A$ receptor antibodies and for reactivity with biotinylated Concanavalin A. Using the deduced amino acid sequence of the 1 subunit and these results, it was shown that the site of benzodiazepine agonist photoaffinity labelling does not lie within the sequence α1 1-58 and α1 149-429.

335.13

A STUDY OF NEUROTRANSMITTER RECEPTORS AND VOLTAGE-ACTIVATED CHANNELS OF THE RAT CEREBELLUM USING mRNA-INJECTED XENOPUS OOCYTES. D. Ragsdale* and R. Miledi. Laboratory of Cellular and Molecular Neurobiology, Department of Psychobiology, University of California, Irvine 92717.

We have investigated the neurotransmitter receptors and voltage-activated channels of cerebellar cells by isolating mRNA from rat cerebellum, injecting it into Xenopus oocytes and then examining the functional receptors and channels expressed in the oocyte membrane using a conventional two-electrode voltage-clamp to record membrane currents. We found that "cerebellum oocytes" had a distinct pattern of expression of receptors and channels. They gave large responses to GABA and glutamate but small responses to acetylcholine, kainate, glycine and serotonin. They also exhibited large voltage-activated Na^+, K^+ and transient outward currents. This pattern of responses was clearly different than that seen in oocytes injected with mRNA from rat cerebral cortex. We presume that the relative sizes of the various drug-and voltage-activated currents in cerebellum and cortex oocytes reflect the levels of mRNA encoding receptors and channels in these two brain regions, and this is supported by northern blot analysis which indicated that the degree of hybridization of a Na^+ channel probe to different cerebellum and cortex mRNA preparations correlated well with the relative sizes of the voltage-activated Na^+ currents in cerebellum and cortex oocytes.

SECOND MESSENGERS: PROTEIN PHOSPHORYLATION

336.1

EFFECT OF LONG-TERM TREATMENT WITH ANTIDEPRESSANT DRUGS ON PHORBOL ESTER-BINDING SITES IN RAT BRAIN. K. Osada*, M. Asakura*, M. Shibata*, H.Kubota*, A.Sato*, J. Adachi*, and K. Hasegawa* (SPON: M.OKAMOTO) Dept. of Neuropsychiat. St. Marianna Univ. Sch. Med., 2-16-1, Sugao, Miyamae-ku, Kawasaki, Kanagawa, 213, Japan

We examined the effects of long-term treatment with antidepressants on the binding characteristics of [^{3}H]-phorbol 12,13-dibutyrate (PDBu) which binds to a regulatory domain of Ca^{2+}-phospholipid dependent protein kinase (PKC) in rat hippocampal membranes. Treatment with antidepressants (desipramine, imipramine, clomipramine, mianserin, fluoxetine) for 14 days caused a significant inhibition of the Ca^{2+}-chelator (EGTA)-induced dissociation of [^{3}H]PDBU binding from membranes following incubation with Ca^{2+} and [^{3}H]PDBu. However there was no significant change in the [^{3}H]PDBu binding of membranes washed with 5 mM EGTA. Therefore, the difference between the binding to membranes by preincubation with [^{3}H] PDBu which does not completely dissociate by EGTA and the remaining binding even after washing with EGTA was significantly increased following treatment with antidepressants. This subtructive binding might represent a newly synthesized membrane-inserted PKC. These data suggest that long-term treatment with antidepressants made PKC stabilized to EGTA following incubation with Ca^{2+} and phorbol esters, suggesting the facility of formation of membrane-inserted PKC.

336.2

CHARACTERIZATION OF PHORBOL ESTER-BINDING SITES IN MEMBRANE FRACTIONS OF RAT BRAIN M.Asakura*, K.Osada*, T.Tsukamoto*, J. Imafuku*, J. Nakanishi* and K. Hasegawa* (SPON:T. HAMA) Dept. of Neuropsychiat. St.Marianna Univ. Sch. Med. 2-16-1 Sugao, Miyamae-ku, Kawasaki, Kanagawa, 213 Japan

Characteristics of [^{3}H]phorbol 12,13-dibutyrate (PDBu) binding to Ca^{2+}-phospholipid-dependent protein kinase C (PKC) were examined in rat brain membrane fractions. The equilibrium binding of [^{3}H]PDBu displayed upward curvilinear Scatchard plots, showing an apparent existence of multiple affinity states of binding sites or negative cooperativity. The high-affinity state of [^{3}H]PDBu binding sites was markedly reduced at 50 μM of Ca^{2+}-chelator (EGTA) without change in maximum binding sites. However, there was a 60 % reduction in total binding sites with a linear plots after washing with 100 μM EGTA. The PKC bound to membranes is controlled by not only Ca^{2+} but also phorbol esters. Therefore, the membrane-bound PKC induced by [^{3}H]PDBu in a dose-dependent manner may contribute to an apparent low-affinity state of curvilinear Scatchard plots. Prior incubation with higher concentrations of [^{3}H]PDBu diminished the PKC release by low concentration of EGTA. Furthermore, incubation of membranes with phosphatidylserine (PS) also inhibited the PKC release by EGTA. Accordingly the chelator-stable PKC induced by Ca^{2+}, phorbol esters or PS might be the membrane-inserted PKC.

336.3

PROTEIN KINASE C ISOZYMES IN PRIMARY CULTURES OF CEREBELLAR GRANULE CELLS: GLUTAMATE-STIMULATED MEMBRANOUS ASSOCIATION OF TYPE II PKC. F.L.Huang[1], D.-M. Chuang[2] and K.-P.Huang[1]*. [1]NICHD, NIH, Bethesda, MD and [2]NIMH, Washington, D.C.

Our previous studies have shown that both type II and III, but not type I, protein kinase C (PKC) isozymes were present in the granule cells of adult rat cerebellum (BBRC 87, J. Neurosci. 88). During postnatal cerebellum development, expression of these isozymes were under separate controls. In the present study, type II and III PKCs, but not type I, were also found in the primary cultures of granule cells prepared from 8-day old rats. Expression patterns of these isozymes were different. Except for a slightly elevated level at 4-5 days, type II PKC was expressed at a relatively stable level throughout 13 days in culture. On the contrary, type III PKC was expressed at a low level initially and the content increased as the culture continued up to 13 days. Treatment of the 8-day cultures, when type II PKC was the predominant species, with PMA resulted in the down-regulation of PKC, whereas PKC in the 13-day culture, when type III PKC was predominant, was less susceptible to down-regulation by PMA. Treatment of the culture with glutamate, the excitatory amino acid, caused a reduction of both type II and III PKCs from the cytosol and an increase of these isozymes in the membrane fraction. Though the time course and extent of translocation varied from culture to culture, an accumulation of type II, but not type III, PKC in an unique membranous fraction which was not extractable by 0.5% NP40 containing buffer, was consistently observed. These results suggested that type II and III PKCs, with their dissimilar expression patterns and responses to glutamate, may also be endowed with dissimilar and specific functions in granule cells of cerebellum.

336.4

PROTEIN KINASE C ISOFORMS IN RAT HYPOTHALAMUS AND MIDBRAIN CENTRAL GRAY. U.E. Olazabal, R.H. Lustig, D.W. Pfaff, and C.V. Mobbs Rockefeller Univ., New York, NY 10021.

We have shown previously that phorbol esters infused into the midbrain central gray (MCG) facilitate the estrogen-dependent behavior, lordosis. Therefore, we examined the distribution of protein kinase C (PKC) isoforms in the MCG and ventromedial hypothalamus (VMH), brain regions which control lordosis. PKC isoforms were detected by 1- and 2-dimensional gel electrophoresis followed by immunoblot analysis using a monoclonal antibody directed against PKC (Amersham). Further immunoblot analysis used three different polyclonal antibodies against synthetic peptides which were reported to recognize respectively alpha, beta, or gamma isoforms (the kind gift of Makowske et al., JBC 263:3402-3410). All three isoforms were present in VMH, MCG, and hippocampus, although the relative amounts of each isoform varied between regions. The gamma PKC isoform migrated slightly more slowly, suggesting a higher molecular weight, than the other isoforms, and was absent in pituitary. At least six different PKC isoforms were detected on 2-D gels using the monoclonal PKC antibody. Further, we have used this antibody to demonstrate PKC by immunocytochemistry in paraformaldehyde-fixed tissue. We conclude that at least 6 PKC isoforms exist in the VMH and at least three PKC isoforms exist in the MCG.

336.5

DIFFERENTIAL ASSOCIATION OF FOUR PKC SUBSPECIES WITH SUBCELLULAR COMPONENTS OF NEURONS. N. Saito, A. Kose*, A. Ito*, T. Tsujino*, M. Hirata*, C. Yoshihara*, and C. Tanaka* Dept. of Pharmacology, Kobe University School of Medicine, Kobe 650, Japan.

Protein kinase C (PKC) is known as a Ca^{2+}/ phospholipid dependent protein kinase involved in various signal transduction in tissues. This enzyme has been shown to be a large family consisting of more than four PKC-subspecies (α,βI, βII and γ) by molecular cloning studies. But the physiological difference between the function of each subspecies is unclear. We demonstrate here the regional and intracellular localization of each subspecies of PKC in the rat to elucidate the specific functional role of each PKC subspecies. Immunochemical and immunocytochemical studies revealed the different localization of these four subspecies; γ-PKC is rich in hippocampus and cerebellum, βI-PKC is seen in pontine nucleus and triangular septal nucleus, βII-PKC is abundant in CA1 region of the hippocampus, caudate-putamen and amygdaloid complex. α-PKC is diffusely present through the brain. Under electronmicroscopy, γ-PKC is seen in perikaryon, dendrite, nucleus, and axon. βI-PKC is mainly present in the periphery of the neuronal perikarya. βII-PKC is seen to be associated with Golgi complex of the neuron and α-PKC is abundant in the nucleus. The different localization of each subspecies of PKC in the neuronal components suggests that each subspecies is involved in the specialized function in the different neurons.

336.6

THE PROTEIN KINASE C INHIBITOR STAUROSPORINE REDUCES EXCITABILITY OF HIPPOCAMPAL CA1 NEURONS. **J. B. Denny*, J. Polan-Curtain*, C. Salvador*, D. L. Armstrong and M. J. Wayner. Division of Life Sciences, University of Texas at San Antonio, San Antonio, TX 78285.**

We have investigated the role of the signal-transducing enzyme protein kinase C in hippocampal neurons using staurosporine. This microbial alkaloid specifically inhibits protein kinase C at nanomolar concentrations. In the present study intracellular recordings were made from CA1 neurons before, during and after a 30 min bath application of 50 nanomolar staurosporine.

Hippocampal slices (400 um thick) were isolated from Sprague-Dawley rats aged 37-41 days and placed in a constant perfusion recording chamber mounted on a microscope stage. Resting membrane potential, input resistance, EPSP and action potential amplitude were measured. Passive membrane properties were not affected; however, EPSP amplitude was reduced by 30% and action potential amplitude was reduced by 19% (n=6). None of these effects were observed when only the staurosporine solvent, dimethylsulfoxide, was applied (n=5). The effects of staurosporine were reversed during a 40 min saline wash. These results indicate that protein kinase C is necessary for normal excitatory responses to synaptic activation within the CA1 region of the hippocampus.

Supported in part by NIH Grant RR08194.

336.7

ESTRADIOL MODULATION OF PROTEIN KINASE C (PKC) ACTIVITY IN THE RAT PITUITARY IN VIVO AND IN VITRO. S.V. Drouva, I. Gorenne*, E. Laplante*, E. Rérat*, A. Enjalbert, C. Kordon U. 159 INSERM, 2ter rue d'Alésia, 75014 Paris (France).

Ovarian steroids are known to affect numerous levels of pituitary regulation. The present study was designed in order to investigate PKC activity in pituitary tissue of ovariectomized rats (OVX) or OVX treated with 17 β-estradiol (E2) ; to evaluate the distribution pattern of the enzyme and possible E2-induced variations among the different cell types of pituitary cells in primary cultures separated by unit gravity gradient sedimentation ; finally to analyze E2/PKC interactions on hormone secretion in pituitary cell cultures treated or not with E2. Partially purified soluble (S) and particular (P) PKC was assayed by 1,2 diolein or TPA induced phosphorylation in the presence of Ca^{2+}, PS, histone III S and γ32P(ATP). E2 treatment significantly increased pituitary PKC levels of both S and P fractions on in vivo or in vitro models in a time dependent manner (30 min 96 h). The steroid effect was specific since stereoisomers were inactive. PKC activity was found in all pituitary cell types. E2 treatment (10^{-9} M for 72 h) significantly increased the enzyme activity in all cell types with a preferential effect on a subpopulation of lactotrophs. In addition, administration of E2 to cell cultures strongly increased the TPA induced LH and PRL release ; it had no effect on TPA evoked GH secretion.

336.8

DECREASE IN CALCIUM CALMODULIN-DEPENDENT PROTEIN KINASE II ACTIVITY IN RAT HIPPOCAMPAL SLICES IN MAGNESIUM-FREE MEDIUM. W.W. Anderson, S.B. Churn*, and R.J. DeLorenzo. Dept. of Neurology and Dept. of Pharm., Med. Col. of Virginia, Richmond, VA 23298.

Induction of seizures by kindling leads to a long-lasting decrease in calcium-calmodulin dependent protein kinase II (CKII) activity (Wasterlain and Farber, PNAS 81:1253, 1984; Goldenring et al., Brain Res. 377:47, 1986). Removal of extracellular Mg induces electrographic seizures and epileptiform bursting in rat hippocampal slices (Anderson et al., Brain Res. 319:215, 1986). We therefore investigated whether exposure of hippocampal slices to Mg-free medium would also decrease CKII activity.

Experiments were performed on hippocampal slices from female Sprague-Dawley rats (115-260 gm). Extracellular recording and stimulation were performed in CA3. Experimental slices (3 to 8 slices pooled from one animal per experiment) were incubated in 2 mM Mg, then exposed to Mg-free medium for 1 hr, and frozen. The same number of control slices were run concurrently in 2 mM Mg. Calcium-calmodulin stimulated CKII autophosphorylation in homogenates was measured by standard techniques. Biochemistry was performed on those experiments where control slices showed no bursting.

Phosphorylation of the CKII 50 kDa subunit in homogenates isolated from slices exposed to Mg-free medium was decreased by 37.5 $\pm$ 5.5 % (mean $\pm$ sem) compared to controls. The decrease was observed in all experiments (n=7) and was significant ($p<.005$, paired Students t-test).

The results indicate that exposure of slices to Mg-free medium causes a decrease in CKII activity, as does kindling. They are also consistent with the hypothesis that the induction and/or generation of seizure activity involves a decrease in the ability of calcium and calmodulin to stimulate CKII activity.

336.9

DEVELOPMENTAL EXPRESSION OF THE GRANULE CELL-ENRICHED CALMODULIN-DEPENDENT PROTEIN KINASE (CaM KINASE-GR). C-A. Ohmstede,* K.F. Jensen and N. Sahyoun. (SPON: B. Cooper), Wellcome Research Laboratories and NTD, HERL, USEPA, Research Triangle Park, NC 27709.

In the adult rat cerebellum, CaM kinase-Gr is found primarily in granule cells. Antibodies to CaM kinase-Gr stain a heterogeneous population of these neurons. In fixed tissue, this staining is primarily localized to the nucleus; however, staining of unfixed tissue and ultrastructural localization have demonstrated a more extensive distribution within the granule cell including cytoplasm, dendrites and axons. No staining was observed in the Purkinje cells of adult animals. To further characterize this enzyme, we investigated its distribution with the developing cerebellum. Western blots from postnatal cerebellum had suggested a specific developmental profile which may reflect important events in the maturation of neuronal circuitry. On postnatal day 7, CaM kinase-Gr was present in Purkinje cells but was not detected in the proliferating cells of the external germinal layer. By postnatal day 14, the enzyme distribution reflected that of the adult cerebellum. These data suggest that CaM kinase-Gr may play an important role in the maturation of Purkinje cells as well as in the mature function of granule cells.

336.10

DNA-PROTEIN INTERACTIONS WITHIN THE 5'-FLANKING REGION OF THE CaM KINASE II α-SUBUNIT GENE. T. Sunyer* and N. Sahyoun. (SPON: J. Reinhard), Wellcome Research Laboratories, Research Triangle Park, NC 27709

Ca^{2+}/calmodulin-dependent protein kinase II (CaM Kinase II) is an abundant neuronal enzyme whose level and polypeptide composition are dependent on the neuronal subtype and stage of development. CaM Kinase II isoenzymes are also present in non-neuronal tissues. These observations led to the study of DNA-protein interactions which may regulate the expression of the α-subunit of the enzyme. Three restriction fragments were isolated from the 5'-noncoding region of the α-subunit gene. Fragment A (170 bp) contained the transcription initiation site; fragment B (120 bp) included GGGCG and TATATAA sequences 76 and 160 bp upstream from the mRNA start site; and fragment C extended 106 bp further upstream. Only "B" appeared to bind specifically to nuclear protein when assayed by the gel mobility shift method. Protein extracts from purified nuclei obtained from rat brains at different ages (E16, newborn, 2-week old and adult) resulted in similar DNA-protein complexes. Likewise, nuclear extracts from the cerebellum and forebrain yielded similar mobility shifts. Nuclear extracts from rat liver, kidney and spleen produced tissue-specific electrophoretic mobility shifts of fragment B. These results indicate that fragment B may bind to different nuclear proteins from different tissues.

336.11

EXPRESSION OF THE CALCIUM CALMODULIN PROTEIN KINASE II GENE AND THE YEAST ADENYLATE CYCLASE GENE IN NEURONS FROM HSV-1 VECTORS. A. I. Geller and R. L. Neve. Dana Farber Cancer Institute and Childrens Hospital, Boston, MA. 02115.

To study second messenger physiology, in vivo, we are using defective HSV-1 vectors (Science 241, 1667, 1988) to express, in neurons, altered second messenger enzymes which should not be regulated, instead they should always be active. The increased activity resulting from a second messenger enzyme which is always active may allow us to determine its role in such processes as neurotransmitter release, regulation of voltage gated ion channel activity, and long term potentiation. Specifically, expression of a segment of the calcium calmodulin protein kinase II gene or the yeast adenylate cyclase gene encoding the catalytic domain of each enzyme should result in enzymes which are always active. Previous work supports our approach: Calcium calmodulin protein kinase II protein is composed of a domain structure in which the calcium-calmodulin binding domain and the catalytic domain are located on distinct segments of the polypepetide; following limited proteolysis the catalytic domain of calcium calmodulin protein kinase II is equally active in the absence or presence of calcium. Similarly, the yeast adenylate cyclase protein is composed of a domain structure in which the regulatory and catalytic domains are located on distinct segments of the polypeptide; expression of the catalytic domain in yeast results in a ten fold increase in the concentration of cAMP. We are now studying virus stocks of HSV-1 vectors which may express the catalytic domain of the calcium calmodulin protein kinase II gene or the yeast adenylate cyclase gene. Expression of a calcium calmodulin protein kinase II which is always active may permit us to determine its role in neurotransmitter release. Elevation of cAMP levels might activate the cAMP dependent protein kinase, perhaps revealing its role in regulating the activity of the voltage gated sodium channel. HSV-1 vectors expressing catalytic fragments of second messenger enzymes may prove to be a useful tool to modify neuronal physiology in the adult brain.

336.12

CYCLIC AMP AND CA/CALMODULIN STIMULATED PROTEIN PHOSPHORYLATION IN RAT CEREBELLUM, STRIATUM AND HIPPOCAMPUS. J.W. Kasckow, S.T. Cain and C.B. Nemeroff. Dept. of Psychiatry, Duke Univ. Med. Ctr., Durham, NC 27710.

We analyzed cAMP and Ca/calmodulin-dependent protein phosphorylation in various brain regions. Male, Sprague-Dawley rats were decapitated and the caudate, hippocampus and cerebellum rapidly dissected and homogenized. S_2 and P_2 fractions were obtained by centrifugation. Samples were phosphorylated for 60 seconds with 10 μM ATP in the presence of $CaCl_2$/calmodulin, cAMP or vehicle. Phosphorylated proteins were separated on 11% polyacrylamide gels. Autoradiograms of the dried gels were scanned and phosphate incorporation quantified using microdensitometry. In cerebellum, only the S_2 fraction showed marked stimulation of cAMP-stimulated protein phosphorylation. Furthermore, in the cerebellar P_2, but not S_2, we observed the cAMP-independent phosphorylation of 45 kDa, 43 kDa and 30 kDa proteins. In cerebellum, in contrast to hippocampus and caudate, the phosphorylated form of the β subunit (64 kDa) was enriched relative to the α subunit (53 kDa). In caudate, but not in hippocampus or cerebellum, two proteins of molecular weights 38 kDa and 25 kDa showed cAMP enhanced phosphorylation. Supported by NARSAD and MH-39415.

336.13

G_s ACTIVATION OF SYNAPSIN PHOSPHORYLATION IN NEOSTRIATAL AXON TERMINALS. N. Aronin and K. Chase* Dept. of Medicine, Univ. of Mass. Med. Sch., Worcester, MA 01655

Previous studies in this laboratory showed in neostriatal axon terminals that adenylate cyclase activity was coupled to the signal transducing protein, G_s. Here we examined whether G_s is located in axon terminals of intrinsic neurons in the neostriatum and whether G_s activation of adenylate cyclase is sufficient for synapsin phosphorylation. First, quinolinic acid (40 μg in 1 μl), which destroys intrinsic neurons but not afferent fibers, or vehicle (1 μl) was injected into neostriatum (n=24 rats) and G_s was measured in neostriatal synaptosomes by cholera toxin-induced ^{32}P-NAD ribosylation after protein separation on SDS-PAGE. Quinolinic acid produced a 50% decrease in G_s in neostriatal synaptosomes compared to vehicle treated tissues. Substance P, a peptide found only in intrinsic neurons of the neostriatum, was markedly reduced in the synaptosomes of quinolinic acid treated animals. Second, synaptosomes were treated with an activator of G_s, 10mM NaF. After back-phosphorylation of acid-extracted synapsin, the 10k cAMP-dependent fragment of synapsin was identified by limited proteolysis on SDS-PAGE. A two-fold increase in cAMP-dependent synapsin phosphorylation was found.

Adenylate cyclase in the neostriatum and cortex can be regulated by several signaling mechanisms (i.e., voltage-dependent activation, Ca^{++}/calmodulin) in addition to G_s. These studies suggest that in axon terminals of neostriatal neurons, G_s activation is sufficient to produce phosphorylation of synapsin. Since there is no evidence for synaptic inputs onto axon terminals of neostriatal neurons, these findings provide additional support for non-synaptic, receptor mediated, presynaptic regulation of neostriatal neurons. Supported by the NSF and the Joseph Healy Foundation.

336.14

OPIOID-INHIBITED PROTEIN PHOSPHORYLATION IN BRAIN MEMBRANES FROM NORMAL AND MORPHINE-DEPENDENT RATS. L. M. Fleming and S. R. Childers. Dept. Pharmacology, Univ. Florida Coll. Med., Gainesville, FL 32610.

Previous studies have shown that opioid-inhibited adenylate cyclase (AC) decreased membrane protein phosphorylation by cAMP-dependent protein kinase when membranes were incubated with opioid agonists in the presence of the AC substrate App(NH)p before addition of 32P-ATP. In rat striatal membranes, we detected two such phosphoproteins (MW 63 kDa and 85 kDa) whose forskolin-stimulated phosphorylation (in the presence of App(NH)p) was inhibited by 20-25% by opioid agonists. This inhibition was naloxone-reversible, and required both sodium and GTP. Inhibition by D-ala enk was maximal in striatum; inhibition was also seen in frontal cortex but not in cerebellum. In thalamus, where mu receptors are highest, inhibition of forskolin-stimulated protein phosphorylation by DAGO was observed. Two dimensional electrophoresis (NEPHGE & SDS-PAGE) confirmed that opioid agonists inhibited forskolin-stimulated phosphorylation of at least two bands; the 63 kDa band had a relative pI of 6.0 while the 85 kDa doublet had a highly basic pI at approx. 9.0. While the identity of the 63 kDa band remains unknown, the 85 kDa doublet is thought to be synapsin, since its molecular weight and pI correspond to reported values of synapsin. To determine the effect of chronic morphine treatment on opioid-inhibited protein phosphorylation, rats were implanted s.c. with morphine pellets (75 mg; 1/day) for 1-7 days. In membranes from thalamus, phosphorylation of both 63 kDa and 85 kDa bands was increased by chronic morphine treatment. Chronic morphine treatment increased App(NH)p-stimulated phosphorylation by 160%, while increasing forskolin+App(NH)p-stimulated phosphorylation by 180%. Morphine treatment did not change phosphorylation of bands not stimulated by forskolin. The increase in App(NH)p and forskolin stimulation was observed relatively early in the chronic morphine treatment (after 2-3 days), but was not observed in membranes from rats injected acutely with morphine (10 mg/kg). These results suggest that a molecular mechanism of tolerance may involve two principal proteins (63 kDa and 85 kDa) of opioid-inhibited AC in brain membranes.

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336.15

PHOSPHORYLATION OF DARPP-32 AND PROTEIN PHOSPHATASE INHIBITOR-1 IN RAT CHOROID PLEXUS. G.L. Snyder, J.-A. Girault*+, J. Chen*, J. Kebabian, and P. Greengard+. Abbott Labs, Abbott Park, IL 60064 and +The Rockefeller University, New York, NY 10021.

DARPP-32 and inhibitor-1 are two related proteins which become potent inhibitors of protein phosphatase-1 upon phosphorylation by cAMP-dependent protein kinase. DARPP-32, but not inhibitor-1, is enriched in dopamine-innervated cells possessing the D1 dopamine receptor. We have examined the regulation of the phosphorylation of DARPP-32 and inhibitor-1 by agents which raise cAMP levels in choroid plexus, a tissue containing high levels of both proteins. Rat choroid plexus were incubated in warm, oxygenated RPMI medium with or without drugs for 15 min. Proteins were acid-extracted, separated by SDS-PAGE and transferred to nitrocellulose. The levels of the phosphorylated forms of DARPP-32 and inhibitor-1 were determined by immunoblotting with a monoclonal antibody specific for the phosphorylated forms of DARPP-32 and inhibitor-1. This antibody was prepared against a phospho-peptide encompassing a sequence surrounding the cAMP-dependent phosphorylation site shared by DARPP-32 and inhibitor-1. Forskolin (10uM) and isoproterenol (10uM) increased the levels of two immunoreactive bands of Mr 29,000 and 36,000, which were identified as inhibitor-1 and DARPP-32, respectively, using antibodies specific for each of these proteins. The phosphorylation state-specific monoclonal antibody should provide a useful tool for the study of the regulation of the phosphorylation of DARPP-32 and Inhibitor-1.

336.16

PHOSPHORYLATION OF DARPP-32 BY CASEIN KINASE II Jean-Antoine Girault, Hugh C. Hemmings Jr., Kenneth R. Williams*+, Angus C. Nairn, and Paul Greengard. Lab. of Molecular & Cellular Neuroscience, The Rockefeller University, New York NY and *Dept. of Mol. Biophysics & Biochemistry, Yale University, New Haven CT.

DARPP-32 is a dopamine- and cAMP-regulated phosphoprotein (Mr=32,000) enriched in dopaminoceptive neurons that possess the D1 dopamine receptor. DARPP-32, when it is phosphorylated by cAMP-dependent protein kinase on a threonine residue, is a potent inhibitor of protein phosphatase-1. In rat striatal slices labeled with [^{32}P]-phosphate, DARPP-32 was also phosphorylated on serine under basal conditions. The protein kinase responsible for this phosphorylation has been identified as casein kinase II. Purified DARPP-32 was a good substrate for purified casein kinase II (stoichiometry>2mol phosphate/mol protein, apparent K_m=3.4 μM, k_{cat}=0.32 sec^{-1}). The main residues phosphorylated in vitro were Ser_{45} and Ser_{102}. The k_{cat} of phosphorylation of Ser_{102} was 5-fold higher than that of Ser_{45}. The residue phosphorylated in striatal slices was identified as Ser_{102}. Phosphorylation of DARPP-32 by casein kinase II did not affect its ability to inhibit protein phosphatase-1 which depended only on phosphorylation by cAMP-dependent protein kinase. Phosphorylation of DARPP-32 by casein kinase II facilitated its phosphorylation by cAMP-dependent protein kinase with a 2.2-fold increase in the V_{max}. DARPP-32 phosphorylated by casein kinase II was dephosphorylated by protein phosphatase-1 and -2A. This dephosphorylation was inhibited by the concomitant phosphorylation of the cAMP site. In conclusion phosphorylation of DARPP-32 by casein kinase II and cAMP-dependent protein kinase exhibit positive interactions in vitro. Phosphorylation of DARPP-32 by casein kinase II in intact cells may regulate the responsiveness of DARPP-32 to cAMP-dependent protein kinase and therefore to dopamine. Supported by USPHS MH40899.

336.17

REGULATION OF THE PHOSPHORYLATION OF DARPP-32 BY CASEIN KINASE II. Stevin H. Zorn, Jean-Antoine Girault, and Paul Greengard, Lab. of Molecular and Cellular Neuroscience, The Rockefeller University, 1230 York Ave., New York, NY 10021.

DARPP-32 is a dopamine- and cAMP- regulated phosphoprotein (Mr 32,000) enriched in dopaminoceptive neurons possessing D_1 receptors. It is phosphorylated on threonine by cAMP-dependent protein kinase and on serine by casein kinase II (CKII) (Girault et al., accompanying abstract). We assessed the activity of CKII in brain during development and found it to be high as early as embryonic day 16. This activity persisted throughout development. We also studied the regulation of CKII by hormones and neurotransmitter agents in rat brain striatal slices. Kinase activity was measured directly in extracts from slices by employing a specific peptide substrate. The state of serine phosphorylation of DARPP-32 immunoprecipitated from striatal slices prelabelled with [^{32}P]-orthophosphate was also evaluated. A small but significant increase in the activity of CKII was observed in extracts of striatal slices exposed to insulin (100nM) compared to vehicle-treated slices. Insulin also enhanced the phosphorylation of DARPP-32 on seryl residues in prelabelled striatal slices. These data suggest that the phosphorylation of DARPP-32 by CKII in intact striatal cells may be regulated by insulin. (Supported by USPHS MH40899)

336.18

REGULATION OF PROTEIN TYROSINE PHOSPHORYLATION IN RAT LOCUS COERULEUS BY CHRONIC ANTIDEPRESSANTS. J. Mattessich* and E.J. Nestler (SPON: J.F. Tallman). Lab. of Mol. Psych., Depts. of Psych. and Pharmacol., Yale Univ. Sch. of Med., New Haven, CT 06508.

We have shown previously that chronic glucocorticoids increase protein tyrosine kinase activity and levels of immunoreactivity of c-src, a well-characterized protein tyrosine kinase, in specific regions of rat brain, including locus coeruleus (LC) (Mol. Pharmacol. 35: 265). In an attempt to gain further understanding of the regulation and functional role of protein tyrosine phosphorylation in LC, other psychotropic drugs with known physiological effects on LC neurons were studied.

Immunoblot analysis revealed that chronic administration of imipramine (IMI) or electroconvulsant treatments (ECT) to rats increased c-src levels in LC by 30-50% compared to controls. In contrast, chronic valium and haldol treatments produced no effect on c-src in this brain region. IMI regulation of c-src displayed regional specificity: no change was detected in hippocampus or dorsal raphe. Consistent with the rise in c-src in LC with chronic IMI, levels of some tyrosine-phosphorylated proteins were also increased in this area as studied by immunoblot experiments with an anti-phosphotyrosine antibody.

Future experiments will determine whether regulation of protein tyrosine phosphorylation is shared by other classes of antidepressant drugs or by other types of psychotherapeutic agents known to alter LC function. These investigations will help elucidate the role that protein tyrosine phosphorylation plays in mediating chronic psychotropic drug action in brain.

336.19

TYROSINE PHOSPHORYLATION OF A 40-KD PROTEIN IN HIPPOCAMPUS: RAPID AND TRANSIENT INCREASE INDUCED BY SEIZURES. K.R. Stratton, P.F. Worley, R.L. Huganir, J.M. Baraban. Dept. of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Although tyrosine kinase activity is particularly abundant in brain, little is known about regulation of this intracellular signalling pathway. In recent studies using the hippocampal slice preparation, we have identified a soluble 40-kD protein that exhibits increased tyrosine phosphorylation after exposure of intact slices to muscarinic agonists or phorbol esters that activate protein kinase C (Proc. Natl. Acad. Sci. USA 86:2498, 1989).

To determine whether increased tyrosine phosphorylation of this protein also occurs in vivo, we have used anti-phosphotyrosine antibodies to assay levels of tyrosine phosphorylation in brain homogenates after seizure activity. After maximal electroconvulsive shock (MES) treatment, there is a selective increase in tyrosine phosphorylation of the 40-kD protein. The increase is maximal by 1 min after MES and returns to basal levels by 8 min. This phosphotyrosine containing protein is present in higher levels in hippocampus than cortex and not detected in cerebellum or brain stem. These studies demonstrate that tyrosine phosphorylation of this 40-kD protein is also rapidly regulated in vivo.

PEPTIDES: ANATOMICAL LOCALIZATION IV

337.1

DISTRIBUTION OF INHIBIN SUBUNIT mRNA and IMMUNOREACTIVITY IN THE RAT BRAIN. V.J. Roberts, H. Meunier,* W. Vale and P.E. Sawchenko, The Salk Institute, La Jolla, CA 92037.

Inhibin (α/β_A or α/β_B subunit heterodimers) and activin (β/β dimers) are best known as gonadal glycoprotein hormones, but are also expressed in brain. Regional S1-nuclease analysis and immunohistochemical methods were used to further clarify the central distribution of inhibin/activin subunits. The results may be summarized as follows: 1. mRNA encoding each of the α-, β_A- and β_B-subunits is expressed in all major brain regions examined. The relative abundance of the three mRNA species varies markedly from region to region, with α generally predominating. 2. Antisera that preferentially read the β_A- or β_B-subunit stain cytoplasmically a small population of cells centered in the caudal part of the nucleus of the solitary tract and their axonal projections to the neurosecretory hypothalamus. 3. β_A antisera also stain the nuclei of neurons in several discrete brain regions; β_B antisera stain perikarya in many of the same regions. Cerebellar Purkinje cells, for example display β_A-immunoreactivity (ir) in their nuclei and β_B-ir in their cytoplasm. 4. We have as yet failed to succeed in localizing α-subunit-ir in the CNS. These results suggest that inhibin/activin-related molecules may in some CNS systems play a conventional role in intercellular communication, and in others function intracellularly, the latter consistent with their structural similarity to a larger family of growth and differentiation factors.

337.2

LOCALIZATION OF GTP BINDING PROTEINS $G_{o\alpha}$ AND G_β IN RAT CEREBELLUM. C.T.Lin, W.H.Tsai, L.S.Kao*, K.J.Chang. Institute of Biomedical Sciences, Academia Sinica, Taipei 11529, Taiwan, R.O.C.

The physiological roles of GTP binding regulatory proteins (G_o) are not well established in neurons yet. The purpose of this study was to prepare and characterize polyclonal antibodies against α and β subunits of G_o for the localization study of these G proteins in rat cerebellum in order to provide further information regarding G protein function. For preparation of $G_{o\alpha}$ and G_β antiserum , we used two synthesized peptides Goα (Thr^{262} - Gln - Phe - Glu - Ser - Lys - Asn - Arg - Ser - Pro - Asn - Lys - Glu^{274}) and G_β (Met^1 - Ser - Glu - Leu - Glu - Gln - Leu - Arg - Gln - Glu - Ala - Glu - Gln- Leu^{14}) as antigens. These peptides were attached to a core heptalysine oligomer, each carrying eight copies of these peptide fragments (octopus immunogen) as previously described (K.J.Chang et al. Proc, Nat'l. acad. Sci. USA, 85:4929,1988). Each octopus immunogen was then used to immunize rabbits by conventional method. The antisera were checked at first by dot blot method. After purification of monospecific antibodies by peptide-gel affinity chromatography, the antibodies were used to stain the crude brain membranes by Western blot analysis which showed a single band of 35 kD for G_β and 39 kD for $G_{o\alpha}$. Finally, the antibodies were used to localize the rat cerebellum which had been fixed by perfusion fixation method using 3% formaldehyde. In the cerebellar cortex, anti-G_β reaction product was seen mainly on the cell membrances of some Purkinje neurons and granule cells, with occasionally in the cytoplasm of a few Purkinje neurons. On the other hand, the anti-$G_{o\alpha}$ reaction product was mainly found in the cytoplasm of some Purkinje cells and their dendritic processes. These findings suggest that only certain Purkinje cells and granule cells contain G protein, and some of them may present on the cell membrane while others in the cytoplasm. Supported by NSC, Taipei, Taiwan R.O.C, grants NSC 78-0412-B001-03 and 78-0412-B002-123.

337.3

INTERLEUKIN-2 (IL-2) IN THE RAT BRAIN: RECEPTORS AND IMMUNOREACTIVITY (IR). P.A. Lapchak, D.M. Araujo, J.-G. Chabot, A. Beaudet and R. Quirion. Neuroanat. Lab., Mtl. Neurol. Inst. and McGill Univ., Montreal, Quebec, Canada.

Recent evidence suggests that IL-2 plays a physiological role in the mammalian CNS. In the present studies, the distribution of both IL-2 and specific IL-2 binding sites was examined in rat brain using biochemical, immunohistochemical and autoradiographic techniques. The presence of IL-2 was first demonstrated in homogenates from hippocampus, neostriatum and cerebral cortex by RIA (0.7, 0.65, 0.15 ng/mg tissue, respectively). Immunoautoradiography using a primary polyclonal antibody against IL-2 confirmed the presence of IL-2 like material in the hippocampus and dentate gyrus. ^{125}I-IL-2 was then shown to bind specifically with limited capacity and with high affinity to hippocampal (Bmax=0.48 ± 0.08 fmol/mg protein, Kd=25 ± 6 pM), but not neostriatal or cortical homogenates. Quantitative autoradiographic analysis of ^{125}I-IL-2 binding revealed specific labeling in hippocampus, external plexiform layer of the olfactory bulb, cerebellum, corpus callosum, stria terminalis, commissura anterior pars posterior; very low, but diffuse binding was also detected in cerebral cortex and neostriatum. In summary, our results demonstrate that IL-2 and IL-2 receptors are present in specific regions of the rat brain, where they may be involved in the regulation of neuronal function. (Supported by MRC and FRSQ, Canada).

337.4

IMMUNOHISTOCHEMICAL LOCALIZATION OF DIABETES-ASSOCIATED PEPTIDE IN PORCINE PANCREATIC ISLETS. Y.N. Wang, D. Chang* and J. K. Chang*. Peninsula Laboratories, Inc., Belmont, CA 94002.

Amyloid deposits in the islets of Langerhans of the pancreas are a common finding in noninsulin-dependent diabetes. Recently, a novel 37 amino acid peptide, diabetes-associated peptide (DAP), has been identified in amyloid-containing pancreatic extracts from diabetic patients (A. Clark et al., *The Lancet ii*:231, 1987). It may be possible that DAP is a factor leading to the pathologic abnormalities in noninsulin-dependent diabetes. In this study, we demonstrate that DAP is abundantly located in B-cells of the porcine pancreatic islets by immunohistochemistry. The porcine pancreases were fixed in 4% paraformaldehyde solution and sectioned at 15 μm on a cryostat. Tissue sections were processed for immunohistochemistry by the indirect immunofluorescence method. The DAP antiserum was used at a dilution of 1:200. The secondary antiserum was goat anti-rabbit-FITC at a dilution of 1:60. Specificity was assessed by incubating tissue sections with the antiserum preabsorbed with 10 μM synthetic DAP. Immunoreactive DAP-containing cells were found only in the islets, not in other areas of the porcine pancreas. In the islets, immunoreactive DAP-containing cells were large, polyglonal and heavily stained. Absorption control showed that no crossreaction of DAP antiserum with human CGRP or rat CGRP was observed. The results suggest that DAP is located in islet B-cells of the porcine pancreas and may co-exist with insulin and CGRP in the B-cells. DAP may play an important role in pancreatic endocrine functions.

337.5

INTRACEREBROVENTRICULAR BOMBESIN ADMINISTRATION LEADS TO INCREASED GLUCOSE UTILIZATION IN THE RAT ANTEROVENTRAL THALAMIC NUCLEUS. B.W. Bates*, Z. Merali and P. Ramm. Dept. of Psychology, Brock University, St. Catherines, Ont. L2S 3A1.

The tetradecapeptide bombesin elicits stereotyped grooming behavior in the rat. Mechanisms which have been proposed for this effect include activation of cholinergic and/or dopaminergic receptor systems, particularly those originating in the lower brainstem (Johnston & Merali, *Peptides* 9:245, 1988). We have used ^{14}C2-deoxyglucose (^{14}C2-DG) autoradiography to map local cerebral glucose utilization (LCGU) in an attempt to localize sites at which bombesin exerts its effects.

Rats were prepared with chronic cannulae in the femoral artery and vein. Three days later, the freely moving animals received 0.4 ug bombesin in 5 μl saline into the third ventricle, or vehicle alone. The brains were then processed in the normal fashion for the fully quantitative ^{14}C2-DG method. Rates of glucose utilization were read by computerized densitometry.

The most striking effect was an obvious, consistent, and localized elevation of metabolic activity in the anteroventral thalamic nucleus, ventrolateral aspect. This region stains heavily for AChE and may contain bombesin receptors (Wolf & Moody, *Peptides*, 6:111, 1985). It receives input from the pallidum and projects to frontal premotor cortex. It is included in thalamic regions involved in the integration of motor initiation and control, and may be a component of a functional circuit mediating the behavioral effects of bombesin.

337.6

BRAINSTEM PROJECTIONS TO MEDIAL THALAMIC NUCLEI SUBSERVING ANALGESIA. L.J. Sim and S.A. Joseph. Neuroendocrine Unit, University of Rochester, Rochester, N.Y. 14642

The involvement of medial thalamic nuclei in nociceptive modulation has been demonstrated in numerous lesion and stimulation studies. Medial thalamic stimulation relieves a variety of pain syndromes in the human. The integration of the medial thalamus and brainstem nociceptive regions were investigated in this study. We neuroanatomically identified the origin and neurochemical nature of brainstem inputs to parafasicular (PF) and centromedial (CM) nuclei in the rat. Wheat germ agglutinin-horseradish peroxidase (WGA/HRP) was stereotaxically injected into either CM or PF. After 48 hours, rats were perfused or administered intraventricular colchicine. Colchicine treated rats were sacrificed 24 hours later. WGA was visualized immunocytochemically with nickel enhancement. Appropriate sections were then routinely stained for appropriate neuropeptides or transmitters. Both CM and PF injections resulted in labeled cells throughout the raphe complex, with the densest labeling in dorsal raphe nucleus (DRN), median (MnR) and paramedian raphe nuclei and nucleus raphe magnus (NRM). Retrogradely labeled neurons were also identified in locus coeruleus (LC), ventrolateral periaqueductal gray (PAG) and lateral and posterior dorsal tegmental nuclei after both injections. CM injections also produced labeled neurons in ventral tegmental area and interpeduncular nucleus and PF injections in pontine reticular nuclei, nucleus reticularis paragigantocellularis and spinal nucleus V. Our double label studies demonstrated that the majority of cells projecting to CM and PF from DRN, MnR, PAG and LC are peptidergic and a small percentage contain either serotonin or norepinephrine. These results demonstrate anatomical connectivity between medial thalamic nuclei and brainstem nuclei of the descending pain pathway and are consistent with a role for CM and PF in pain and analgesia. (Supported by Heart Association grant #87 1011 and NRSA grant DA07232-03)

337.7

LOCALIZATION OF NEUROPEPTIDES TO TARGET-SPECIFIC SUPERIOR CERVICAL GANGLION NEURONS. J.I. Luebke and L.L. Wright. Dept. Anatomy, Boston Univ. Sch. Med., Boston, MA 02118.

There is evidence for an organizational system in the peripheral sympathetic nervous system in which populations of neurons projecting to different targets differ in their neuropeptide content. The present studies were initiated to determine whether such a system of chemical coding exists in superior cervical ganglion (SCG) neurons which project to three functionally diverse targets: the pineal gland, the submandibular gland (SMG) and the anterior chamber of the eye (eye). Adult male rats (220-300g) were anesthetized and injected with a 2% solution of fluorogold (FG) in: 1) the pineal gland (0.5 ul), 2) bilaterally in the SMG (5ul), or 3) bilaterally in the eye (5ul). Following the appropriate survival periods, the animals were perfused with paraformaldehyde and SCGs were removed. Frozen sections were serially mounted onto slides and subsequently processed for immunoreactivity (IR) to either neuropeptide Y (NPY), somatostatin (SS), or vasoactive intestinal peptide (VIP) using Vector's ABC-G.O. system. Sections incubated in the absence of either primary or secondary antisera showed no IR. Sections were examined for cells labelled with FG and displaying IR to one of the three peptides. Preliminary data show differences in the expression of NPY and VIP but not SS in populations of neurons projecting to the pineal, the SMG and the eye. The percentages of target-specific neurons IR for each peptide are given below:

	NPY (s.e.)	VIP (s.e.)	SS (s.e.)
Pineal gland (n=4):	23 (2.5)	7 (1.5)	23 (2.8)
SMG (n=2):	23 (4.1)	24 (1.3)	20 (2.3)
Anterior Eye (n=2):	36 (3.1)	14 (3.7)	26 (5.3)

Supported in part by NIH grant NS21577.

337.8

MESSENGER RNAs FOR NEUROPEPTIDES IN PETROSAL GANGLION. *M.F. Czyzyk-Krzeska*, K.B. Seroogy, D.A. Bayliss, N. Mohopatra* and D.E. Millhorn. Department of Physiology, University of North Carolina, Chapel Hill, N.C. 27599.*

We examined petrosal ganglia of the rat using *in situ* hybridization histochemistry to determine the existence of messenger RNAs for neuropeptides [preprotachykinin A (PPT-A), calcitonin gene-related peptide (CGRP), somatostatin, neuropeptide Y (NPY) and cholecystokinin (CCK)] in individual cells. A population of neurons which innervate the carotid body and carotid sinus was retrogradely labeled by perfusing the carotid bifurcation with Fast Blue dye. In another series of experiments the carotid sinus nerve was cut 7 days prior to study. Hybridization was performed with [^{35}P] labeled synthetic oligonucleotides applied to 10 μm thick tissue sections. After hybridization the tissue was processed for film and emulsion autoradiography. We determined that neurons in petrosal ganglia contain mRNAs for PPT-A, CGRP, somatostatin and NPY. There is no evidence for CCK mRNA. The mRNAs for all neuropeptides except somatostatin were present in somata retrogradely labeled with dye from carotid sinus. Somatostatin was found only in the non-labeled cells, which presumably do not project to carotid sinus structures. The number of cells expressing PPT-A and CGRP was decreased following transection of the carotid sinus nerve.

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337.9

PEPTIDERGIC INNERVATION OF THE KIDNEY IN THE MONKEYS, *MACACA FASCICULARIS*, *MACACA MULATTA*, AND IN MAN. J. E. Norvell, Department of Anatomy, Cell Biology and Neuroscience, Oral Roberts University, Tulsa, OK 74171.

Nerve fibers immunoreactive for neuropeptide Y (NPY), vasoactive intestinal polypeptide (VIP), and substance P (SP) are demonstrated for the first time by the indirect immunofluorescence technique in monkey and human kidneys. NPY-like immunoactivity (NPY-LI) was seen in nerve fibers in the connective tissue of arteries and to a lesser extent, veins. Varicose fibers were seen leaving blood vessels and passing between tubules in the inner cortex, or between blood vessels and tubules. These fibers are also seen surrounding afferent and occasionally efferent arterioles associated with glomeruli. VIP-LI nerve fibers, although fewer in numbers, have a similar distribution to NPY-LI fibers. SP-LI was observed as varicose nerve terminals in the adventitia of blood vessels as well as running between tubules of the cortex. This study shows that NPY-LI, VIP-LI and SP-LI nerve fibers have similar distributions in the primate kidney as those reported in other species.

337.10

LAMINAR DISTRIBUTION OF NEUROTRANSMITTERS IN CONVEXITY NEOCORTEX OF DOLPHINS. I.I. Glezer, P.J. Morgane and C. Leranth. Dept. of Cell Biol. and Anatomy of CUNY Med. SCH., New York, NY 10031, Worcester Found. for Exp. Biol., Shrewsbury, MA 01545, Yale Univ. Sch. of Medicine, New Haven, CT 06510.

Immunocytochemical methods were used for light and electron microscopic analysis of transmitter localization in neuronal perikarya and synapses in different layers of visual neocortex of several species of toothed whales. Neuropeptide Y (NPY) is localized in non-pyramidal neurons of variable shapes which are concentrated mostly in layers II, IIIa and VI. Almost all NPY synapses are of en passage type and diffusely dispersed in all cortical layers with a significant concentration occuring in varicose axons running tangentially in the upper third of layer I. Neurons with cholecystokinin (CCK) are localized mainly in layers I,II and VI and belong to the bipolar/fusiform types. Tyrosine-hydroxylase (TH) is found only in tangential axons running for long distances in lower part of layer I. These findings support our views and concepts on the importance of layers I and II as the major input laminae of the whale convexity cortex. (Supported by NSF grant BNS 87-042032 and NIH grants HD 06364, NS 26068.

337.11

SPECIFIC LOCATION OF NEURONS EXHIBITING MOLLUSCAN, SMALL CARDIOACTIVE PEPTIDE (SCP)-LIKE IMMUNOREACTIVITY IN LARVAE OF TRITONIA DIOMEDEA. S.C.KEMPF. Dept. of Zoology and Wildlife Science, Auburn University, AL 36849

Previous investigation (Kempf, Masinovsky, Willows. 1987. J. Neurobiol. 18:217-236) established the presence of neurons in larval T. diomedea labeled by a monoclonal antibody binding molluscan SCPs. Labeling occurred in a single axon in the cerebral commissure and small cells (presumably neurons) in each cerebral ganglion (CG) in the late embryo. During the larval stage 2 pairs of neurons with axons extending to their respective CG became antigenic. Examination of labeled wholemounts, suggested that the pair of neurons with axons extending to the left CG were located in or near the visceral ganglion and the other pair, on the right, outside the central nervous system, near the mantle edge and adjacent to the anus. This investigation establishes these as the actual locations of the neurons using serial sections of whole larvae labeled with a peroxidase marker prior to embedding. In addition, fluorescent markers elicited a third neuron, not observed in the previous study, that is present on both the left and right sides of the larva and has an axon extending to its respective CG.

337.12

IMMUNOCYTOCHEMICAL STUDY OF THE RAT SUPRATENTORIAL DURA MATER. J.T. Keller, C.F. Marfurt, and M.J. Fritts*. Dept. of Neurosurgery and Anatomy/Cell Biology, Univ. of Cincinnati, James N. Gamble Inst. of Med. Res., and The Christ Hospital, Cincinnati, OH 45219; Dept. of Anatomy, Indiana Univ. Sch. of Med., Northwest Ctr. for Med. Ed., Gary, IN 46408.

Because of the recent association of trigeminal dural fibers, neurogenic inflammation, and vascular headache we chose to immunocytochemically examine the neural elements of the rat supratentorial dura mater for the presence and distribution of substance P (SP), calcitonin gene related peptide (CGRP), dopamine-B-hydroxylase (DBH), neuropeptide Y (NPY) and vasoactive intestinal polypeptide (VIP). The supratentorial dura of 30 male Sprague Dawley rats was examined for SP (1:500), VIP (1:700), DBH (1:600), NPY (1:400), CGRP (1:2000). Trigeminovascular (SP, CGRP), sympathetic (DBH, NPY) and parasympathetic (VIP) fiber patterns were identified in association with the middle meningeal artery (MMA) and its branches, and on the superior sagittal sinus (SSS). In addition, a third pattern of fibers coursed obliquely over the dural convexity from the transverse sinus, caudal and inferior, to the SSS, rostral and superior. CGRP, SP, and VIP were found to be more loosely associated with the vasculature than the DBH and NPY positive fibers. In all cases, the SSS was more densely innervated than the convexities. The density of VIP fibers was less than that of other fibers examined. The functional interaction of these elements and the prominent mast cell population previously identified (Keller et al., Neuroscience Abst. 14:972, 1988) warrants further consideration regarding their role in the pathogenesis of headache. (Supported by NIH grant NS 22969)

337.13

GABAERGIC INNERVATION IN CEREBRAL ARTERIES. T. OKUNO and T. J-F. LEE. Dept. of Pharmacology Southern IL Univ. School of Medicine, Springfield, IL 62794-9230. (SPON: K. Sherman)

Our recent studies have demonstrated that cerebral blood vessels receive dense GABAergic innervation (Lee et al., 1989). It has been suggested that neuronal GABA may act as a modulator altering the release of transmitters from the neighboring nerve terminals. The origin and distribution of GABAergic innervation, and its morphological relationship with other autonomic innervations in cerebral arteries therefore were examined using sequential double-labelling immunocytochemical techniques. Results indicate that the distribution pattern of glutamic acid decarboxylase-immunoreactive (GAD-I) fibers is similar to that of GABA-transaminase immunoreactive (GABA-T-I) fibers but is different from those of tyrosine hydroxylase (TH)-I and choline acetyltransferase (ChAT)-I nerve fibers. Some GAD-I fibers, however, were found to overlap with ChAT-I and TH-I fibers. These results demonstrate that GAD-I fibers are distinctive fibers. The close relationships among GAD-I, ChAT-I and TH-I fibers, however, suggest that a functional interaction may occur between GABAergic and other autonomic innervations. Furthermore, GAD- and GABA-T-immunoreactivities were found in the ciliary ganglion cells suggesting that some GABAergic fibers to cerebral blood vessels may originate from the ciliary ganglion. (Supported by NIH HL27763, AHA/IHA and Southern IL Univ. School of Medicine)

337.14

VIP- AND ChAT-IMMUNOREACTIVE FIBERS ARE DISTINCTIVE FIBERS IN CEREBRAL ARTERIES OF THE CAT. F. J-P. Miao*and T. J-F.Lee. Dept. of Pharmacology, Southern Illinois University School of Medicine, P.O. Box 19230, Springfield, Il 62794.

The question as to whether or not vasoactive intestinal polypeptide (VIP) is localized in cholinergic nerves in cerebral arteries has not been clarified. A sequential double-labelling immunohistochemical technique using avidin-biotin complex (ABC) method was therefore employed to examine the possible co-localization of choline acetyltransferase (ChAT) and VIP in nerve fibers innervating the cat cerebral arteries. Isolated arteries were fixed by immersion in buffered periodate-paraformaldehyde-picric acid-lysine-formaldehyde (PPPLF) solution. Diaminobenzidine (DAB) and tetramethylbenzidine (TMB) were used as chromogens to distinguish ChAT and VIP immunoreactivities. Both ChAT-I and VIP-I fibers in cerebral arteries appeared as bundle and fine fibers. Most CHAT-I and VIP-I fibers were independent fibers. ChAT-I and VIP-I fibers, however, were often found to overlap. Overlaying of VIP-I fibers on ChAT-I fibers and relay connections between them were also observed. Only in less than 5% of the fibers examined, did ChAT- and VIP-immunoreactivities appear to be co-localized. These results suggest that acetylcholine and VIP are not co-localized in most neurons innervating cerebral arteries. The intimate relationships between VIP-I and ChAT-I fibers, however, suggests a functional interaction between these two distinctive innervations. (Supported by NIH HL 27763, 24683, and SIU School of Medicine)

338.1

COMPENSATORY ACTIVATION OF SUBSTANCE P BIOSYNTHESIS BY L-DOPA IN STRIATONIGRAL NEURONS OF DOPAMINERGIC DENERVATED RATS. S. P. Sivam and J. E. Krause. Dept. Pharmacol. & Toxicol., Northwest Ctr. Med. Ed., Indiana Univ. Sch. Med., Gary, IN 46408 and Dept. Anat. & Neurobiol., Washington Univ. Sch. Med., St. Louis, MO 63110.

Previous studies showed that L-DOPA treatment to neonatal 6-hydroxydopamine (6-OHDA) lesioned rats led to a marked decrease in the levels of striatonigral substance P (SP), which was linked to a D1 dopamine receptor mediated mechanism. The present study examined the hypothesis that there is a compensatory activation of SP biosynthesis in order to replenish the L-DOPA induced SP depletion. Three-day old Sprague-Dawley rat pups were lesioned with 6-OHDA and were challenged with L-DOPA at about 60 days of age. Animals were killed 80 min, 6 h or 24 h following L-DOPA treatment. L-DOPA treatment (80 min group) produced a greater decrease in SP levels in the striatum and substantia nigra than that observed with lesion alone. The SP levels recovered to lesioned-control values by 6 h. This recovery was accompanied by a marked increase in striatal preprotachykinin-mRNA at 6 h, and mRNA levels were below lesioned-control levels by 24 h. The results indicate that the L-DOPA induced depletion of SP levels in lesioned rats is replenished by an enhanced biosynthesis of SP. Thus, the integrity of dopamine system is essential for the development and maintenance of striatonigral SP neurons.

338.2

CORRELATION OF SUBSTANCE P (SP) AMINO TERMINAL (SP-N) METABOLITES IN VIVO WITH SP-INDUCED DESENSITIZATION IN THE MOUSE SPINAL CORD. O.J. Igwe*, X. Sun*, C. Schamber* and A.A. Larson. Dept. of Vet. Biology, U of Minnesota, St. Paul, MN 55108.

We have previously reported that intrathecal (i.t.) injection of mice with SP or C-terminal fragments of SP results in a behavioral syndrome characterized by reciprocal caudally-directed biting and scratching (CBS). Repeated injection of SP, but not SP C-terminal fragments, results in desensitization (DES) to SP-induced CBS. The major products of SP catabolism are SP N-terminal fragments, which we has shown to be capable of inhibiting SP-induced CBS. We hypothesize that the generation of SP N-terminal metabolites play a role in the development of DES. In this study, peptidase inhibitors- phosphoramidon (PH), bacitracin (BAC) and angiotensin converting enzyme inhibitor (ACEI or SQ20881) - each having distinct effects on the production of SP-N fragments, were used to investigate the accumulation of tritiated SP-N metabolites in vivo during the development of DES to SP using ^{3}H-SP. SP-N metabolites in the spinal cord were quantified by reversed-phase HPLC. The magnitude of SP-induced DES correlates well (r=0.99) with the total SP-N metabolites recovered from the spinal cord in each group. The magnitude of SP-induced DES was also found to be negatively correlated (r=1.00) with total recovered intact ^{3}H-SP. These results, together with our previous studies, indicate that SP-induced DES depends, at least in part, on the concentration of SP N-terminal metabolites in the spinal cord. To determine which enzymes participate in the metaboilism of *endogenous* SP, we examined the effect of these peptidase inhibitors on SP degradation using a synaptic membrane preparation. The order of efficacy for in vivo inhibition of ^{3}H-SP metabolism was PH=BAC>ACEI while that for in vitro synaptic membrane-derived peptidases was BAC>PH>>ACEI. These results suggest that BAC-sensitive endopeptidases may be primarily responsible for SP metabolism in SP synapses of the spinal cord while PH- and ACEI-sensitive enzymes play supporting roles. The rank order of the peptidase inhibitors in attenuating the magnitude of SP-induced DES was BAC~PH<<ACEI. Supported by USPHS grants DA04090, DA04190 and DA00124.

338.3

SEGREGATION OF NEURONS EXPRESSING DIFFERENT SPECIES OF PREPROTACHYKININ mRNAs IN THE RAT TRIGEMINAL GANGLION. **Jian-Hua Zhang*, Yasuhiro Morita, Takashi Hironaka*, Shiro Nakagawa*, and Masaya Tohyama*. Dept. of Anatomy, Osaka Univ. Med. Sch., and Kagoshima Univ. Med. Sch., Japan**

The preprotachykinin-A (PPTA) gene encodes substance P (SP) and substance K (SK) and generates 3 different species of mRNAs (α, β and γ) following alternative splicing. α-mRNA contains a sequence coding for SP, while β- and γ-mRNAs contain sequences for SP and SK. Accordingly, the expression of PPTA-gene was studied by in situ hybridization using several synthetic oligonucleotide probes specific to the PPTA-mRNAs.

A concurrence on the basis of the number, distribution, and hybridization density of labeled neurons indicates one population of trigeminal ganglion cells expresses a large number of γ-mRNA and another population expresses a small number of α-mRNA. Density of hybridization signals in the former was ten times higher than that of the latter. Neurons labeled with different probes showed a similar distribution, but the number of γ-mRNA expressing neurons was over 1.5 times more than that of α-mRNA expressing neurons.(SPON: T. Nagai)

338.4

WITHDRAWN

338.5

Reserpine increases neuropeptide Y precursor gene expression in rat adrenal via transsynaptic control. Hiroshi Higuchi, Atsushi Iwasa* and Hiroshi Yoshida*. Department of Pharmacology I, Osaka University School of Medicine, 4-3-57 Nakanoshima, Kita-ku, Osaka 530, Japan.

Neuropeptide Y (NPY) is an important cotransmitter of catecholaminergic neuron, which regulates blood pressure; central administration of NPY causes hypotension, whereas peripheral administration produces a long-acting pressor response. In order to study whether some antihypertensive drugs may modify the biosynthesis and/or turnover of NPY, the effect of reserpine on NPY and NPY mRNA contents in rat adrenal and brain was studied by NPY radioimmunoassay and quantitative Northern blot analysis. Reserpine (0.5 mg/kg, i.p., 5 days) reduced by 50 % the NPY content in rat adrenal 24 h after the first injection, and then the NPY level recovered gradually so that NPY amount increased conversely 1 week after cessation of reserpine administration. The NPY mRNA level in rat adrenal also increased gradually (obvious in 2 days-treatment), and the increase continued until 2 weeks after cessation of reserpine treatment. In contrast, β-actin mRNA abundance did not change by reserpine treatment. The changes in NPY and NPY mRNA were not observed in rat brain areas. Reserpine-induced increase of NPY mRNA was completely abolished by Splanchnic nerve transection. These findings indicated that reserpine increases NPY precursor gene expression and NPY biosynthesis via trans synaptic control.

338.6

INCREASED LEVELS OF NEUROPEPTIDE-Y mRNA IN LOCUS COERULEUS FOLLOWING RESERPINE TREATMENT. B.J. Wilcox and J.R. Unnerstall. Dept. Neurology and Alzheimer Research Labs, Case Western Reserve Unv. Sch./Med., Cleveland, OH 44106.

Acute treatment of animals with reserpine depletes brain norepinephrine and increases the levels of tyrosine hydroxylase (TH) in locus coeruleus (LC) neurons. Recently, it has also been shown that this treatment increases the relative levels of TH mRNA in LC neurons (Berod *et al.*, *PNAS* 84:1699, 1987). In these experiments, we address the question whether reserpine treatment can also modulate the relative mRNA levels of neuropeptide-Y (NPY) in the LC. Animals were treated with a single subcutaneous injection of reserpine (10 mg/kg) or an equivalent volume of vehicle. Two days following treatment, the animals were sacrificed. A 45 mer anti-sense DNA oligonucleotide directed toward the N-terminal of the coding region of rat NPY mRNA was 3' end-labeled with [^{35}S]dATP and used for *in situ* hybridization histochemistry. In general, the distribution of positively labeled LC neurons agrees well with that seen by immunohistochemistry. A greater grain density was consistently seen over neurons in the dorsal medial portion of the LC when compared to other labeled neurons in both control and reserpine-treated animals. An approximate 50% increase in the average relative grain density per neuron were seen in treated animals when compared to controls. A broadening of the distribution of grains per cell in the treated group was also seen. Greater increases in relative grain densities were seen in sets of neurons in areas where the baseline grain densities were low (e.g. ventral vs. dorsal aspects of the medial LC). These increases in relative NPY mRNA levels in specific subsets of LC neurons may reflect a ceiling effect where neurons with tonic lower levels of NPY activity may be affected to a greater extent by the drug treatment. Conversely, the possibility of specific neural mechanisms affecting individual subsets of peptide-containing neurons cannot be excluded.

338.7

EVOLUTIONARY CONSERVATION OF CCK AND NEUROPEPTIDE Y: cDNA CLONES FOR CHICK CCK AND GOLD-FISH NPY. A. Blomqvist* and D. Larhammar. Dept of Medical Genetics, Uppsala University, Box 589, S-751 23 Uppsala, Sweden.

CCK (cholecystokinin) and NPY (neuropeptide Y) are widely distributed and abundant neuropeptides in the central nervous system of all mammals investigated. Both peptides have highly conserved sequences among mammals. CCK-8 is identical in all mammals known. However, its larger forms, CCK-33, -39, and -58, all display several amino acid differences.

We wished to investigate the evolutionary conservation of CCK-8 and its larger forms by comparing the mammalian prepro-CCK sequences with a non-mammalian sequence. If the various forms of CCK turn out to display different degrees of conservation, it will imply that they have functional roles that differ in importance in an evolutionary perspective. We have used a rat CCK cDNA probe to screen a chick brain cDNA library. Several clones were isolated and are in the process of being characterized.

NPY is a 36-amino acid peptide which is virtually identical in all mammals known. We have recently deduced the chick NPY sequence from a cDNA clone and found that it has only one difference as compared to the mammalian sequences. Also the carboxyterminal part of the NPY precursor, CPON (for C-terminal peptide of NPY), is highly conserved between chicken and mammals which suggests that this peptide too may serve as a neurotransmitter.

Our previous findings of strong sequence conservation of NPY between chicken and mammals was complemented by using a rat NPY probe in so-called zoo blots, i.e. Southern hybridizations to genomic DNA isolated from various animal species. The rat NPY probe detected cross-hybridizing bands in a reptile (crocodile), an amphibian (Xenopus laevis), and a bony fish (salmon). We have used a chick cDNA probe to screen a gold-fish retina cDNA library. Several clones were identified and will be sequenced.

338.8

THE EFFECTS OF AGING AND NUCLEUS BASALIS LESIONS ON NEUROPEPTIDE Y (NPY) LEVELS AND ITS ENCODING mRNA IN THE RAT BRAIN. J.J.Poulakos[1], W.J.Millard[2], G.W.Arendash[3], & E.M. Meyer[1]. Depts. of Pharmacology[1] & Pharmacodynamics[2], Univ. of FL, Gainesville, FL, & Dept. of Biology[3], Univ. of So. FL, Tampa, FL.

NPY is a 36 amino acid peptide found in the brain. Two conditions known to alter brain NPY levels are aging and prolonged cholinergic hypofunction to the cerebral cortex. The present study characterized brain NPY and prepro NPY mRNA levels in Sprague Dawley (SD) and Fischer 344 rats ranging from 6 to 26 months of age. Additionally, cortical cholinergic hypofunction was induced in rats with bilateral lesions to the nucleus basalis magnocellularis (nBM) with ibotenic acid. NPY levels were measured by RIA and prepro NPY mRNA levels were ascertained by dot blot analyses with a rat cDNA probe provided by Dr.J. Allen. Cortical NPY levels progressively decreased in SD rats of 4, 12, 16 and 24 months of age (250 pg/mg tissue - 4 months of age to 100 pg/mg tissue - 24 months of age). Cortical prepro NPY mRNA levels increased approximately 40% from 6 to 16 months of age in Fischer 344 rats and then progressively decreased at 20 and 24 months of age. In nBM lesioned SD rats NPY levels were unchanged while its encoding mRNA levels decreased slightly 2 months post lesioning. These results suggest that aging and cholinergic transmission may be involved in the regulation of cortical NPY levels.

338.9

NEUROPHYSIN EXPRESSION ON CELL MEMBRANE OF A HUMAN LUNG CARCINOMA CELL LINE SUGGESTS ABSENCE OF PROCESSING ENZYME. L.C. Rosenbaum*, G. Nilaver, H.H.M. Van Tol* & E.A. Neuwelt* (SPON: W.R. Woodward) Dept. Neurol., Biochem., Neurosurg., & VIABR, Oregon Hlth Sci. Univ., Portland, OR 97201.

Carcinomas of the lung are sometimes associated with neurophysin (NP) secretion and inappropriate antidiuresis (SIADH) (North et al. Cancer 62:1343, 1988). We have detected NP-reactivity on the surface of human lung carcinoma (LX-1) cells intracerebrally xenografted in nude rats by immunohistochemistry (IHC). Northern analysis and in situ hybridization with a 50 base-pair oligonucleotide probe to the C-terminal region of human pro-pressophysin (PPYsin) demonstrated the expression of PPYsin mRNA in LX-1 tumor xenografts. IHC with NP and vasopressin antibodies, however, revealed no detectable staining in the cytoplasm of LX-1 tumor cells. Similar results were obtained by Western blot analysis of LX-1 cells, suggesting lack of post-translational processing of PPYsin in the cytosol of this cell line. Inability to document SIADH in LX-1 tumor bearing-nude rats implied the tumor to be non-secretory. The accumulation of NP immunoreactivity in the cytoplasm of colchicine-treated LX-1 cultures with reduction of cell membrane staining further suggested the preferential targeting of unprocessed PPYsin to cell membranes in LX-1 tumor cells. All of these findings may be relevant to the lack of SIADH noted with this cell line (supported by PHS grants NIDDKD-37205 & NCI-31770).

338.10

COMPARISON OF BOMBESIN, NEUROMEDIN-C AND NEUROMEDIN-B ON PHOSPHATIDYLINOSITOL TURNOVER IN RAT HYPOTHALAMIC SLICES. M.S. Saporito, R.O. Warwick, Jr., T.J. Shickley and S.K. Murphy*. Philadelphia College of Pharmacy and Science, Department of Pharmacology and Toxicology, and *Department of Biology, Philadelphia, PA. 19104.

Bombesin-like (BN) peptides are biologically active in mammalian CNS. Direct injection into the hypothalamus (HYP) leads to a disruption in thermoregulation and satiety. Although the neuronal mechanisms which mediate these responses remain unclear, BN stimulates phosphatidylinositol (PI) turnover in brain slices. Further, BN, neuromedin-C (NM-C, GRP 10), but not neuromedin-B (NM-B) inhibit K$^+$ evoked release of ^{3}H-serotonin (^{3}H-5-HT) from HYP slices and alter ^{45}Ca influx into HYP synaptosomes. This study compares the effects of BN, NM-C and NM-B on PI turnover with effects of these peptides on ^{3}H-5-HT release and ^{45}Ca uptake.

^{3}H-inositol phosphate (IP_1) accumulation was used as an index of PI turnover. BN and NM-C (1 uM) significantly increased IP, accumulation by 65 and 83%, respectively ($p < 0.05$). NM-B (1 uM) was inactive. These data agree, qualitatively, with reports that BN and NM-C are more active then NM-B in evoking HYP mediated disruptions in thermoregulation and inhibiting ^{3}H-5-HT release from HYP slices. As observed in ^{3}H-5-HT release and ^{45}Ca uptake studies, BN, but not NM-C, activity was abolished by the endopeptidase 24.11 inhibitor phosphoramidon (10 uM). Further, BN and NM-C activities were enhanced (70%) by the peptidyl dipeptidase inhibitor enalaprilat (10 uM). These data indicate that in this HYP slice preparation a) endopeptidase 24.11 generates an active peptide fragment from BN that is similar to NM-C, and; b) peptidyl dipeptidase inactivates BN and NM-C.

This study suggests that IP, accumulation may represent a useful in vitro model for the study of BN-like peptides and strengthens the hypothesis that BN-like peptides function as modulators of neurotransmission in the CNS.

This study was supported by USPHS Grant NS 26007 to ROW.

338.11

SUBCELLULAR DISTRIBUTION OF CORTICOTROPIN-RELEASING FACTOR IN THE RODENT CENTRAL NERVOUS SYSTEM. S.T. Cain, M. Owens, M. Johnson* and C.B. Nemeroff. Depts. Psychiat. and Pharmacol., Duke Univ. Med. Ctr., Durham, NC 27710.

Corticotropin-releasing factor (CRF) is a 41 amino acid peptide with a heterogenous anatomical distribution within the central nervous system (CNS). When administered into the CNS, CRF exhibits a variety of electrophysiological, pharmacological and behavioral effects. A further criterion which must be satisfied for a substance to be considered a neurotransmitter is preferential enrichment in presynaptic terminals and vesicles. We have now analyzed the subcellular distribution of CRF within the CNS by combining density gradient fractionation procedures with a highly specific and sensitive radioimmunoassay for CRF.

Adult, male, Sprague-Dawley rats were decapitated and the brain regions of interest rapidly dissected. A crude synaptosomal fraction (P_2) was prepared by centrifugation. The P_2 fraction was then subfractionated into synaptosomal and mitochondrial components. The concentration of CRF-like immunoreactivity (CRF-LI) in the subcellular fractions was determined by radioimmunoassay. CRF-LI was enriched 50% in the crude synaptosome preparation relative to the homogenate. Within the P_2 fraction, synaptosomal CRF-LI was enriched almost 2-fold relative to the mitochondrial fraction. The synaptosomal enrichment of CRF-LI is consistent with a role for this peptide as a neurotransmitter in the CNS. Supported by NIMH MH-42088.

338.12

CHARACTERIZATION OF SEVERAL ENDOPROTEOLYTIC ACTIVITIES FROM RAT TISSUES WHICH CLEAVE PROSOMATOSTATIN AND MODEL SUBSTRATES AFTER SINGLE ARGININE RESIDUES. Margery C. Beinfeld, Jason C. Viereck*, Julie Bourdais*, Paul Kuks*, Alain Morel*, and Paul Cohen*. Dept. of Pharmacology, St. Louis Univ. Med. Sch., St. Louis, MO 63104 and Universite Pierre et Marie Curie, Paris, France.

During the post-translational processing of neuropeptides most cleavages occur at dibasic residues such as Arg-Lys. However, the prohormones of a number of neuropeptides (such as somatostatin, CCK, dynorphin, PHM, and ANF) are also cleaved at one or more single arginine residues. We found that rat intestinal secretory granules, brain cytosol, and brain synaptosomes all contained enzymatic activity which cleaved a model substrate containing the site which is cleaved when somatostatin 28 is generated in vivo. Both preparations contained both soluble and membrane associated activities.

These enzymes were all active at neutral or slightly basic pH, displayed different molecular weights, Km values, substrate specificity, and inhibitor sensitivity. Based on their inhibitor sensitivity they were either serine, metallo- or thiol proteases. Most of these enzymes could correctly cleave anglerfish prosomatostatin to produce somatostatin 28. One of the enzymes could also cleave some dibasic substrates. Supported by NIH NS18667 and The Fogarty International Center.

338.13

***IN VIVO* RELEASE OF N-ACETYL-ASPARTYL-GLUTAMATE FOLLOWING OPTIC NERVE STIMULATION.** G. Tsai, B.L. Stauch, J.J. Vornov*, J.K. Deshpande and J.T. Coyle. Depts. of Neuroscience, Pharmacology, and Psychiatry, The Johns Hopkins School of Med., Balto., MD 21205.

N-Acetylaspartylglutamate (NAAG), a peptide found in remarkably high concentrations in the vertebrate CNS, may serve as a neurotransmitter or modulator in several putative glutamatergic pathways. To date, the release of endogenous NAAG upon stimulation of an appropriate pathway *in vivo* has not been documented. NAAG is a candidate transmitter of the retinotectal pathway of the rat based on immunocytochemical, lesion and radiolabeling release studies. To determine whether the peptide is released *in vivo*, the optic nerve stump was stimulated for 10 minutes (10Hz, 10 microamp, 0.1 msec duration) immediately after enucleation, while the ipsilateral and contralateral superficial layers of superior colliculi were perfused with Krebs-bicarbonate buffer utilizing the technique of *in vivo* microdialysis. The evoked release of endogenous NAAG was quantified by a liquid phase radioimmunoassay (RIA), according to which the fractions were subject to an anion exchange HPLC chromatography followed by RIA of the eluant. NAAG release was increased by 270±25 % over baseline in superior colliculus contralateral to the stimulation (n=6). This evoked release was markedly inhibited (>95%) by perfusion of the colliculus with calcium-free buffer containing 1mM EGTA (n=6). Reperfusion with normal buffer in the same animal resulted in restoration of NAAG release. These results demonstrate that endogenous NAAG is subject to calcium-dependent release *in vivo* upon electrical stimulation of a defined NAAG-containing pathway and suggest that NAAG may serve as a neurotransmitter/modulator in the optic pathway.

338.14

VISUALIZATION OF NAALADase ACTIVITY WITHIN AN ACRYLAMIDE GEL FOLLOWING ITS PURIFICATION TO HOMOGENEITY. B.L. Stauch, M.B. Robinson, G. Tsai, M.L. Simmons*, and J.T. Coyle, Departments of Pharmacology and Neuroscience, The Johns Hopkins School of Medicine, Baltimore, MD. 21205.

The acidic neurodipeptide, N-acetyl-aspartyl glutamate (NAAG), is thought to function as a neurotransmitter/neuromodulator in the mammalian nervous system. Our laboratory has identified a membrane-bound metallopeptidase, N-acetylated alpha-linked acidic dipeptidase (NAALADase), capable of degrading NAAG to N-acetyl-aspartate (NAA) and glutamate *in vitro* (Robinson et al., J. Biol. Chem. 262, 14498-14506, 1987); and has recently demonstrated that NAAG is degraded by a pharmacologically similar enzyme *in vivo* (Stauch et. al., Neurosci. Let., *in press*). The salient features of this metallopeptidase include its chloride dependency, potent sensitivity to quisqualate (IC50=500nM) and phosphate, and selective stimulation by cobalt. In addition, we have found that NAALADase is an unusually basic protein with a pI of 9.0, as assessed by chromatofocusing chromatography. We have solubilized this peptidase from rat brain and purified nearly to homogeneity by sequential column chromatography including DEAE-Sepharose, CM-Sepharose, Concanavalin A Sepharose, and size exclusion chromatography. SDS gel electrophoresis of the purified protein developed with silver stain revealed one band migrating at 94 kD. To confirm that this band represents NAALADase, we have developed a specific enzymatic activity stain, enabling us to visualize NAALADase activity within the polyacrylamide matrix. This procedure couples the hydrolysis of glutamate from NAAG to the reduction of iodonitrotetrazolium to formazan. Using this methodology, we have demonstrated unequivocally that the band migrating at 94 kD on an SDS gel represents NAALADase. Furthermore, consistent with its exclusive localization to kidney and neural tissue, we have demonstrated NAALADase activity in an acrylamide gel loaded with crude brain and kidney homogenates, but not with liver homogenate.

338.15

EXCITATORY AMINO ACIDS IN AMYOTROPHIC LATERAL SCLEROSIS. JD Rothstein, G Tsai, RW Kuncl, L Clawson*, DR Cornblath*, DB Drachman, A Pestronk, BL Stauch, JT Coyle. Departments of Neurology and Psychiatry, Johns Hopkins Univ School of Medicine, Baltimore, MD 21205.

Excitatory amino acid neurotransmitters have been proposed as potential toxic agents in a number of chronic neurological disorders including ALS. N-Acetyl-aspartyl-glutamate (NAAG) is an endogenous excitatory neuropeptide present in the CNS, with high concentrations in the motor cortex and motor neurons of the spinal cord. To define the changes in excitatory amino acids and NAAG in ALS, we measured cervical spinal cord and CSF glutamate, aspartate, NAAG, and its metabolite N-acetyl-aspartate (NAA) concentrations in patients with well characterized classic ALS. All assays were blinded for diagnosis. Mean age and age ranges were similar for all groups. Cervical spinal cord was studied in 2 groups of patients: ALS (n=8) and non-motor neuron disease controls (n=9). Spinal cord NAAG and NAA levels were similar in ALS and control tissue specimens. Tissue choline acetyl transferase and glutamate amino decarboxylase levels were also unchanged between groups. CSF studies utilized three study groups: ALS (n=18), other neurological diseases (OND; n=13), and hepatic encephalopathy (HE; n=10). Patients with HE have high CSF glutamine and thus served to control for artifactual hydrolysis to glutamate. CSF excitatory amino acids and NAAG were significantly altered in patients with ALS. CSF NAAG and NAA levels were increased 2-3 fold compared to OND specimens. There was a significant 3-fold elevation of CSF glutamate concentration and a doubling of CSF aspartate concentration. There were no significant changes in CSF branched chain, aromatic, or basic amino acids. CSF glutamate concentration was not artifactually elevated by hydrolysis to glutamine, as evidenced by the normal glutamate levels in HE patients. Our data suggest altered excitatory amino acid metabolism in patients with ALS. Increased release of NAAG with its concomitant metabolism to aspartate and glutamate may account for our results. The role of NAAG in the pathogenesis of ALS remains to be explored.

338.16

BIOCHEMICAL AND BIOLOGICAL CHARACTERIZATION OF LONG CHAIN-BIOTINYLATED GNRH ANALOGUES. B.T. Miller, T.J. Collins*, G.T. Nagle and A. Kurosky*. Depts. of Anatomy & Neurosciences and Human Biological Chemistry & Genetics, Univ. Texas Medical Branch, Galveston, TX 77550.

The incorporation of biotin moieties into bioactive peptides and proteins often yields derivatives which retain biological activity. Such biotinylated probes, which form stable complexes with either free or immobilized avidin, can be used for both the localization and the isolation of specific peptide and protein receptors. Proteins and peptides are often biotinylated with commercially available N-hydroxysuccinimide (NHS) esters of biotin. These reagents are chemically reactive toward the epsilon amino groups of lysine residues. Using such a biotinylating reagent which contains an extended spacer arm, we linked biotin moieties to [D-lys^6]-GnRH. We examined the structure of the resultant peptides by HPLC, amino acid compositional analysis and automated peptide sequencing. Biological activity was tested by assessing the ability of the derivatized analogues to stimulate LH release from cultures of dispersed anterior pituitary cells. Our results indicate that different bioactive, biotinylated GnRH derivatives can be synthesized by varying the reaction conditions during biotinylation. Preliminary structural analysis of biotinylated [D-lys^6]-GnRH molecules and other GnRH analogues suggests that residues other than lysine are capable of being biotinylated with NHS esters.

338.17

THE EFFECTS OF PRENATAL METHADONE EXPOSURE ON POSTNATAL mRNA EXPRESSION OF NEUROPEPTIDES IN THE RAT HYPOTHALAMUS. J.W. Nemitz, A.H. Hassen, and J.A. Schriefer. Depts. of Anat., Physiol. and Pharm., WV School of Osteopathic Med., Lewisburg, WV 24901

Adult, female Sprague-Dawley rats made methadone dependent using a subcut. pump (9 mg/kg/day), were mated to produce offspring. The prenatally methadone exposed rat pups were sacrificed at different postnatal days by decapitation. Frozen serial coronal sections (20um) at the level of the hypothalamus were cut and processed for *in situ* hybridization using ^{35}S-oligonucleotide probes (DuPont). Results indicate that significant differences exist between the amount of oxytocin probe observed in the Day 1 controls (animals that have not been prenatally exposed to methadone) as compared to Day 1 methadone animals for the paraventricular, supraoptic and retrochiasmatic supraoptic nuclei of the hypothalamus. The levels of mRNA oxytocin labelling was decreased in the methadone animals. A comparison of the Day 10 control and methadone animals showed no significant differences in the level of oxytocin mRNA for the same hypothalamic nuclei. These data suggest that chronic prenatal exposure to methadone alters the mRNA expression of a pituitary peptide and that this change is rapidly reversed postnatally. Supported by WVSOM funds.

338.18

A POSTERIOR LOBE PROLACTIN RELEASING FACTOR (PRF) DISTINCT FROM OXYTOCIN. W.K.Samson*, R.Mogg*, L.Martin* and R.J.Fulton* (SPON: M.J.Duncan) Anatomy, Univ. MO Sch. of Med., Columbia MO 65212 and Inland Laboratories, Dallas TX 75207.

In addition to oxytocin (OT), bovine and rat neurointermediate lobes (NILs) contain a novel peptidergic PRF. This factor is not OT because removal of endogenous OT by immunoaffinity reduces, but does not abolish, the PRL response to NIL extracts seen *in vitro*. The PRF activity can be resolved from OT on sizing gels and by HPLC. Unlike OT, the bioactivity of this PRF remains after oxidative extraction procedures and is expressed *in vivo* and *in vitro* even in the presence of dopamine. This PRF does not act via OT receptors on the lactotroph, since cell preparations in which the OT responsive subpopulations of lactotrophs have been selected out by preincubation with OT-ricin A chain conjugates still respond to the NIL PRF. These cytotoxin studies have also revealed that the VIP responsive lactotrophs are the same subpopulation as those responsive to OT, but not TRH. In agreement with Ben-Jonathan's pioneering work (ENDO 122:2533), our data provide further evidence for the existence of a nonoxytocinergic PRF in the NIL.

338.19

EXOGENOUS AND ENDOGENOUS ANGIOTENSIN III ARE NOT EQUIVALENT. R.H. Abhold. VCAPP, Wash. St. Univ., Pullman, WA 99164-6520.

This study examined the difference between the ANG III endogenously generated from ANG II (ANG(2-8)g) and exogenously applied ANG III (ANG(2-8)a) In the metabolism of ANG(2-8)g, only ANG(2-7) and ANG(2-4) were directly produced from the parent peptide, ANG(2-7) being the principal product. This contrasts with the metabolism of ANG(2-8)a in which ANG(3-8) is also generated and is the primary product. In vitro, ANG(2-7) and ANG(2-5) inhibited the formation of ANG(3-8), ANG(1-7) and ANG(2-7) inhibited the formation of ANG(2-4), and ANG(1-5) had no discernable effects on fragment formation. In vivo, ANG(1-7) and ANG(2-7) increased the dipsogenic effect of ANG(1-8) whereas ANG(1-7), ANG(2-7), and ANG(2-5) increased that of ANG(2-8)a. None of the ANG fragments themselves possessed intrinsic dipsogenic activity. These data indicate that ANG(2-8)g and ANG(2-8)a are metabolized differently and suggest this difference may be important when pharmacologic and physiological comparisons are made between ANG(1-8) and ANG(2-8).

mRNA REGULATION III

339.1

N-METHYL-D-ASPARTATE CHANNEL BLOCKERS INHIBIT SEIZURE INDUCED RISE IN C-FOS mRNA IN HIPPOCAMPUS OF KINDLED RATS. D.M. Labiner, D.A. Hosford, C. Shin, J.O. McNamara. VA and Duke University Medical Centers, Durham, NC 27705.

c-fos is an early gene which encodes a transcription factor. An afterdischarge (AD) triggers a dramatic rise in c-fos mRNA in the hippocampus of both naive and kindled animals. Understanding the mechanism by which AD effects this may provide a clue to the functional significance of the c-fos induction. We hypothesized that NMDA receptors participate in AD-induced c-fos expression since anatomic distribution of c-fos induction parallels that of NMDA receptors. We therefore examined the effects of NMDA channel blockers on c-fos expression activated by AD.

Hippocampal kindled rats were treated with vehicle, MK-801 (1.5 mg/kg), or phencyclidine (PCP, 5 mg/kg) 60 min prior to a seizure and sacrificed 30 min later. Frozen sections were used for in situ hybridization with a ^{32}P-oligonucleotide probe. Control animals exhibited a dramatic rise in c-fos mRNA bilaterally in the dentate granule cell layer. MK-801 attenuated the rise by 50% ($p<.01$). A similar suppression was observed with PCP. There was no change in AD duration in either group compared to vehicle.

These data indicate that NMDA channel activation is one mechanism by which AD induces c-fos. Interestingly, NMDA channel activation appears to play a critical role in kindling development. Linking c-fos expression to NMDA channel activation thus strengthens the likelihood that c-fos expression serves a causal role in kindling development.

339.2

EARLY GENE EXPRESSION IN THE HIPPOCAMPUS IN THE KINDLING MODEL. M.G.Simonato*, D.A.Hosford, D.M.Labiner, C.Shin, J. Morgan*, T.Curran* & J.O.McNamara (SPON: M.Bowman). Epilepsy Res. Lab, Duke & V.A. Med. Ctrs., Durham, NC, 27705.

Changes in gene expression may explain the longlasting hyperexcitability of the kindling model. Early genes encode transcriptional regulators that may mediate longlasting changes in target gene expression. We therefore examined the effects of a single kindling afterdischarge (AD) on the level of mRNA of 3 early genes that are expressed in other seizure models: c-fos, c-jun, and NGF1a (homologous to zif/268; Saffen et al., PNAS, 1988). Hippocampal AD was produced by stimulating the angular bundle of rats that were sacrificed at multiple timepoints up to 3 hrs later. Sham-stimulated animals served as controls. In situ hybridization of hippocampal sections was performed with radiolabelled oligonucleotide probes (50-mers). Autoradiograms were analyzed by computer-assistance (Loats) to determine the relative levels of mRNA.

In dentate granule cell layer, c-fos and c-jun mRNA were markedly increased ($p < .01$) at 30 min and returned to near-control levels by 60 min; by contrast, NGF1a mRNA exhibited a similar abrupt rise but remained significantly elevated at 60 min before returning to control levels at 120 min. In CA1 pyramidal cell layer, NGF1a mRNA was elevated (40%) by 30 min whereas little (10%) or no increase was detected in c-fos and c-jun, respectively. The apparent differential expression of early genes following the same stimulus may imply that target genes are also differentially controlled in distinct neuronal populations.

339.3

PHORBOL MYRISTATE ACETATE (TPA) AND SERUM ENHANCE NERVE GROWTH FACTOR SYNTHESIS IN FIBROBLASTS.
D. Wion*, R. Houlgatte*, P. Clochon*, D. Mac Grogan*, E. Dicou* and P. Brachet. INSERM U 298, CHR, 49033 Angers, France.

Previous studies performed with mouse L fibroblasts have shown that serum causes in this system a specific elevation of the cellular content of NGF mRNA and increases coincidently the amounts of NGF protein secreted in the medium (Houlgatte et al, Biochem. Biophys. Res. Comm. (1988)150:723-730). Further experiments provided evidences that serum, or a macromolecular fraction of serum, stimulates also the synthesis of NGF in rat primary fibroblasts or in iris transplants. This suggests that serum elements may be involved in the control of NGF synthesis in vivo, for instance in wound mechanisms. The effect of serum in L cells may be mimicked by the protein-kinase C activator, TPA. Conversely, the promoting effect of serum and TPA was abolished by the protein-kinase C inhibitor, polymyxin as well as by the protein synthesis inhibitor, cycloheximid. These results suggest that induction of NGF-gene expression in L cells requires both protein-kinase C activation and protein synthesis.

339.4

FOS PROTEIN EXPRESSION IN CULTURED CENTRAL NEURONS AND ASTROCYTES. Kinya Hisanaga*, Stephen M. Sagar, and Frank R. Sharp (SPON: M. Floeter) Depts. of Neurology and Physiology, UCSF, and VA Medical Center, SF, CA 94121 USA.

Expression of the proto-oncogene c-fos in rat neocortical neurons and astrocytes was examined using in vitro immunocytochemistry for the gene product, Fos. Nearly pure neurons and nearly pure astrocytes were cultured in serum-free medium for 3-5 days, and then stimulated as follows. Remarkable Fos expression in astrocytes was observed 2 hours after mechanical stimulation by changing media (even returning the same medium in which the cells had been cultured), and after treatments with serum (10%), epidermal growth factor (EGF, 100ng/ml), and dibutyryl-cyclic AMP (db-cAMP, 1mM), but not potassium chloride (KCl, 46mM) and nerve growth factor (NGF,100ng/ml). Fos was induced in neurons after treatments with serum, KCl and db-cAMP, but not carbachol (acetylcholine agonist, 1mM), EGF and NGF. Fos immunocytochemistry in cultured cells might be useful for determining the specific factors (e.g. neurotransmitters and trophic factors) responsible for "third messenger" c-fos expression in the nervous system.

339.5

A RAPID AND TRANSIENT INCREASE IN c-fos AND zif/268 mRNA INDUCED BY ELECTROCONVULSIVE SHOCK. S.R. Abu-Shakra*, A.J. Cole, D.W. Saffen*, J.M. Baraban and P.F. Worley (SPON: D. Drachman). Depts. of Neuroscience, Johns Hopkins University, Baltimore, MD 21205.

Recent studies have identified a set of genes referred to as immediate early genes (IEG) that respond rapidly to growth factor stimulation. Several of these IEGs, thought to encode transcription factors, are also activated by neuronal stimulation in vivo. For example, metrazole induced seizures lead to a rapid increase in c-fos and zif/268 mRNAs. To analyze the seizure induced activation of IEGs in brain, we used the maximal electroshock model (MES). mRNA levels were assayed in rat brain sections by in situ hybridization with ^{35}S-labelled anti-sense riboprobes. MES increased both mRNAs in the dentate gyrus and cortex. mRNA levels are maximal by 30 min, return to basal levels by 2 hr, appear below basal levels at 4 hrs and normalize at 24 hrs. Diazepam, carbamazepine, phenobarbital and phenytoin blocked hindlimb extension due to MES, but not the rise of c-fos and zif/268 mRNAs. Additionally, NMDA antagonists MK-801 (1 or 10mg/kg) and CGS-19755 (15mg/kg) blocked hindlimb extension but failed to block zif/268 mRNA increases and only partially reduced the c-fos mRNA response to MES. Our findings suggest that MES elicits a rapid genomic response that is not blocked by anticonvulsants.

339.6

ADENOSINE RECEPTOR ACTIVATION INDUCES A RAPID INCREASE IN C-FOS, BUT NOT C-JUN, EXPRESSION IN NEURON-GLIA HYBRIDS AND FIBROBLASTS R.M.Gubits, J.B.Wollack, H.Yu*,and W.K.Liu*, Dept. of Neurology, Columbia College of Physicians & Surgeons,New York, N.Y.10032

The neuromodulator adenosine, is thought to act through its cell surface receptors as an endogenous anti-convulsant. One step in the signal transduction pathways of several cell surface receptors is the rapid, transient induction of c-fos and c-jun gene expression. Both of these events,i.e.1.)enhanced adenosine release and 2.)the induction of c-fos and c-jun mRNAs, occur with similar kinetics in the brains of animals following metrazol-induced seizures. In order to determine whether these 2 phenomena are related, i.e. whether enhanced expression of c-fos and c-jun mRNAs could occur, in part,as a result of the interaction of adenosine with its receptor, we treated 3T3 fibroblasts and NG108-15 neuroblastoma/ glioma hybrid cells with adenosine agonists and analyzed them for c-fos and c-jun mRNAs and Fos protein.

Both lines responded to treatment with the adenosine agonists, CHA (A1-selective) and NECA (non-selective adenosine agonist) with a concentration-dependent transient increase in c-fos mRNA of 3.5- to 8-fold. c-jun mRNA was present in both treated and untreated cells at approximately the same concentration. The kinetics of the c-fos response were much more rapid for 3T3 cells (peak at 15 min) than for NG cells (peak at 60-90 min). This slower, more prolonged response in the NG cells was quite similar to that of c-fos induction in brains of animals following seizures. Specificity of the response was further investigated by the addition of the non-selective adenosine receptor antagonist, theophylline, just before the agonist drugs. Fos protein induction was demonstrated by immunocytochemical staining of Fos in NECA-treated 3T3 cells. These results suggest that Fos induction is a component of the adenosine receptor signal transduction pathway. Fos and Jun are nuclear proteins, which may interact to regulate the transcription of other genes. Our results are therefore consistent with the hypothesis that part of adenosine's anticonvulsant activity could involve changes in gene expression mediated by Fos and Jun. Supported by Colleen Giblin and St. Gile's Funds for Child Neurology.

339.7

DIFFERENTIAL INCREASE IN LEVELS OF C-FOS AND CALCYCLIN, BUT NOT HEAT-SHOCK PROTEIN, mRNAs IN RESPONSE TO PLATELET-ACTIVATING FACTOR IN A HUMAN NEUROBLASTOMA CELL LINE. **G. Allan* and N.G. Bazan. (SPON: H.E.P. Bazan) LSU Eye Center and Neuroscience Center, New Orleans, LA 70112.**

Platelet-activating factor (PAF) is a potent lipid mediator implicated in a wide range of pathophysiological conditions including cerebral ischemia (BBRC 149:580, 1987) and epilepsy (J. Neurochem. 51:1900, 1988). Post-ischemic recovery of neuronal function may include activation of gene expression by such mediators in either those cells that survive after damage, those that take over functions of lost cells, or both. To investigate PAF effects on neuronal gene expression the neuroblastoma cell line SH-SY-5Y was treated with 1 μM PAF, RNA was isolated after various times of PAF exposure and steady-state levels of the mRNAs coding for c-Fos, calcyclin, heat-shock proteins 24 and 70 (HSP 24 and 70), and ß-actin analyzed by slot- and Northern blotting. Levels of c-Fos mRNA were elevated at between 30 and 90 min PAF exposure. Levels of calcyclin mRNA, preferentially expressed in cycling cells, increased after 6-7 h. Levels of HSP 24, HSP 70, and ß-actin mRNA did not alter during this time. These results suggest that PAF can initiate cascades of neuronal gene expression. (Supported by NIH NS 23003).

339.8

ONCOGENES IN CAT CORTICAL STRUCTURES. G.D. Mower, K.M. Rosen*, M. McCormack* and L. Villa-Komaroff. (SPON:F.H. Duffy). Neurology Research, The Children's Hospital, Boston, MA 02115.

Proto-oncogenes could be genetic elements involved in short and long term changes in neural function. The present study compared expression of a series of oncogenes, by Northern blots, in visual, frontal, and cerebellar cortices of cats. Analyses were done on kittens (1 week), mature (5 month) cats, and in mature cats deprived of all visual experience by dark rearing from birth, to test whether there were environmental effects on gene expression.

Expression of oncogenes showed regional and age related differences. Fos was nearly absent in all cortical regions of kittens and expressed about equally in all regions of mature cats. Myc was highly expressed in all regions of kittens, but persisted only in the cerebellum of mature cats. PDGF-B (sis) was very low in all regions of kittens but expressed in all regions of mature cats, being lowest in cerebellum. H-ras was expressed in all cortical regions and was higher in kittens. Other oncogenes (eg. int, neu) are currently under study.

No marked differences were found between normal and dark reared animals in visual cortex for any oncogene tested to date. This result is surprising since expression of many oncogenes is activity dependent. Moreover, since dark rearing extends the "critical period" of visual cortex, these negative results put into question a simple role of oncogenes in visual cortical plasticity.

339.9

RAPID REGULATION OF GENE EXPRESSION BY NERVE GROWTH FACTOR IN PC12 CELLS. Stephen R.J. Salton* (SPON: Harvey Reisine). Fishberg Research Center in Neurobiology, Mt. Sinai Sch. of Med., NYC, NY 10029.

PC12 cells undergo a transition from an adrenal chromaffin-like cell to a sympathetic neuron-like cell in the presence of nerve growth factor (NGF) but not epidermal growth factor (EGF). NGF and EGF have been shown by a number of investigators to rapidly turn on the transcription of several mRNA's in PC12 cells; the proteins encoded by some of these mRNA's are structurally related to and/or appear to interact with several known transcription factors. I and others (Twiss, J.L. and Landreth G.E., Soc. Neurosci. Abst. Vol. 14, Part 2, p1324, 1988) have used subtractive hybridization to increase the possibility of detecting low abundance mRNA's that are regulated uniquely by NGF. RNA from PC12 cells treated with NGF and cyclohexamide (Milbrandt, J., Science 238, 797) was used to construct a lambda GT-10 library of 2 million recombinants. 100,000 pfu's of the primary library were screened with subtracted cDNA and 75 NGF-induced clones identified. The positives were rescreened with cDNA's from NGF-treated and EGF-treated PC12 cells, v-fos, v-myc, v-fgr, c-fos and actin ß cDNA's, and with oligonucleotide probes to NGF-1A, NGF-1B, and to consensus sequences in Zn^{++}-finger containing transcription factors and steroid hormone receptors. Approximately 55 of the 75 clones were found to hybridize with NGF-1A and 15 with actin ß. Four clones encode mRNA's that are rapidly induced to relatively high levels by NGF; two additional clones encode mRNA's of lower abundance, one of which hybridizes at high stringency with the transcription factor consensus oligonucleotide. Anti-sense oligonucleotides are being used to rapidly assess the functional importance of the encoded proteins during NGF-induced neural differentiation of PC12 cells.

339.10

INDUCTION OF NEUROFILAMENT (NF-H) FOLLOWING DIFFERENTIATION OF NEUROBLASTOMA CELLS IS DUE TO AN INCREASE IN mRNA K.C. Breen* & B.H. Anderton, Department of Immunology, Cranmer Terrace, London SW17 ORE, England. (SPON: BRA)

Dibutyryl-cyclic AMP (dbcAMP) treatment of neuro-2A neuroblastoma cells induces cell differentiation and neurite outgrowth. Undifferentiated cells express heavy neurofilament polypeptide (NF-H) at a low level. Following differentiation, there is a significant increase in NF-H immunoreactivity, the induced filaments being in the phosphorylated form as determined using a specific monoclonal antibody RT97. Immunocytochemical studies showed that the phosphorylated form of hte polypeptide is specifically localised in the neurites and is not present in the cell bodies which only contain the dephosphorylated form. Furthermore, the induction of the NF-H polypeptide was shown to be controlled at a genetic level, as differentiation of the cells results in an increase in mRNA corresponding to NF-H. This is in contrast to NGF-induction of NF-H in PC12 cells which occurs at a post-transcriptional level.

339.11

THE ANDROGEN-BINDING PROTEIN GENE IS EXPRESSED THROUGHOUT THE RAT BRAIN OF BOTH SEXES. Y-M Wang, D. A. Bayliss*, D. E. Millhorn, and D. R. Joseph*. Curriculum in Neurobiology, Departments of Pediatrics and Physiology, The Laboratories for Reproductive Biology, University of North Carolina, Chapel Hill, NC 27599.

The presence of brain identifier sequences in the rat androgen-binding protein (ABP) gene (Molec. Endocrinol. 2:3, 1988) prompted us to look for ABP expression in the brain. Northern blot analyses of poly(A)$^+$ RNA isolated from various brain regions of both sexes with cDNA as probe revealed major mRNA species of 1.7 and 2.3 kb. The highest concentrations were found in the hippocampus, olfactory bulb, striatum and cerebral cortex with no gender differences. A postnatal study emcompassing newborn to mature rats shows the 1.7-kb transcript is expressed at increasing levels with age, whereas expression of the 2.3-kb transcript was maximal between days 30-50. Sequence analysis of brain cDNA clones indicates ABP mRNAs are processed differently in the brain. Expression of ABP in the brain may provide a mechanism to fine-tune androgen effects or ABP may act as an independent neuropeptide. (supported by NIH grants HD-21744, 5-P30-HD18968 and HL 33831).

339.12

COMPLEX DISTRIBUTION OF mRNAs OF ISOFORMS OF THE NA,K-ATPASE IN RAT BRAIN. P.E. Filuk*, W.L. Stahl, D.M. Dorsa and M.A. Miller*. V.A. Medical Center and Univ. Washington Sch. Med., Seattle, WA 98108.

Although three isoforms of the catalytic subunit of the Na,K-ATPase have been identified in nervous tissue, their distribution, regulation and specific function are as yet unknown. We have examined the distribution of mRNAs encoding the α1, α2, and α3 isoforms by in situ hybridization. Brain sections were treated with [^{35}S]-oligonucleotide probes (30 mer) complementary to both coding and non-coding regions of each isoform. Specificity was assessed by competition of labelled probe with excess unlabelled probe and by Northern analysis. Even though all three isoforms were localized in cerebral cortex and hippocampus, their distributions were often distinct. α1 mRNA was associated with laminae 2, 3 and 4 of cerebral cortex. Strong labelling was present in CA1, CA2 and CA3 pyramidal cells with significantly lower labelling associated with the granule cells of hippocampus. α2 mRNA was diffusely distributed in cerebral cortex and in all layers of the hippocampus. Cortical laminae 4 and 5 and all regions of the hippocampus (e.g. CA1, CA2, CA3, dentate gyrus) exhibited high levels of α3 mRNA. In addition, selected nuclei of the thalamus and hypothalamus were labelled by probes to α2 and α3, isoforms of the Na,K-ATPase which may be sensitive to hormonal regulation.

339.13

ACTIVITY-DEPENDENT GENE EXPRESSION IN VISUAL CORTEX NEURONS OF ADULT MONKEYS D.L.Benson,P.J.Isackson,E.G.Jones Dept. of Anatomy and Neurobiology, Univ. of California,Irvine,CA 92717.

Following monocular deprivation in adult monkeys, there is an increase in RNA encoding calcium calmodulin dependent protein kinase type II(CAMK-II) in primary visual cortex. This increase is detected as early as four hours after deprivation by intraocular injection of tetrodotoxin. Parallel immunocytochemical experiments confirm previous studies indicating that the increase occurs in ocular dominance columns corresponding to the deprived eye (SHC Hendry and MB Kennedy, PNAS 83:1536(1986)).

Total RNA, isolated from normal animals and from animals that had been monocularly deprived 4 hours to 7 days, was reverse transcribed. The resultant cDNA was selectively amplified using the polymerase chain reaction (PCR, Saiki, et al,Science 230:1350(1985)) Amplified reaction products were examined on agarose gels and demonstrated an increase in CAMK-II mRNA with deprivation. The transcripts were isolated, cloned, and sequenced. These clones were used to generate antisense RNA for S1 nuclease protection experiments which confirmed the results obtained from the PCR reactions.

Using the same methodology, a monkey specific glutamic acid decarboxylase (GAD) clone has been isolated. S1 nuclease protection experiments indicate a small decrease in GAD mRNA following monocular deprivation. In situ hybridization experiments will more definitively determine the cellular response of GAD and CAMK-II to a decrease in afferent activity. Supported by NIH Grants EY 07193 and NS 24747-03.

339.14

HIGH-LEVEL EXPRESSION AND SPECIFIC REGULATION OF APOLIPOPROTEIN D mRNA IN ENDONEURIAL FIBROBLASTS OF REGENERATING RAT PERIPHERAL NERVE. P.Spreyer*, H.Schaal*,G.Kuhn* and H.W.Müller. Mol. Neurobiol. Lab., Neurology Dept., Univ. of Düsseldorf, FRG.

A cDNA clone containing the entire coding region of rat apolipoprotein D (apo D) was isolated from a cDNA library of regenerating rat sciatic nerve (SN) by a differential hybridization procedure selecting for sequences enriched in the distal stump following crush lesion. Northern blots revealed that apo D mRNA was present in small amounts in noninjured mature SN and brain, but not in neonatal rat brain. In spatio-temporal analyses moderately increased levels of apo D mRNA were detected in the proximal segment of crushed SN as well as in the proximal and distal stumps of nonregenerating transected and ligated SN at 1 and 4 weeks after injury. In contrast, in the distal stump of crushed SN the steady state level of apo D mRNA transiently increased at least 40-fold above control levels at the time when axons regenerate into this nerve segment. Cellular localization of apo D transcripts could be detected by in situ hybridization in endoneurial fibroblasts. These results suggest that stimulation of high-level expression of apo D mRNA is a specific molecular event of endoneurial fibroblasts in nerve regeneration.

339.15

THE HORMONAL REGULATION OF GLUTAMINE SYNTHETASE IN CULTURED ASTROCYTES. W.A. Schreier*, A.C. Passaquin*, K. Huff, and J. de Vellis. (SPON: Z.H. Zhang) Dept. of Anatomy & Cell Biology, U.C.L.A. School of Medicine, Los Angeles, CA 90024.

The hormonal regulation of glutamine synthetase was examined in cultures of astrocytes from neonatal rat cerebrum. The effects of three hormones: hydrocortisone (HC), insulin, and thyroid hormone (T3) were investigated. Both the enzymatic activity of glutamine synthetase (GS) and the level of the corresponding mRNA were followed during hormonal treatments. All three hormones enhanced the specific activity of GS, however the effects of insulin and T3 were quite variable. Whereas the HC-induction of GS activity was correlated with a corresponding increase in GS mRNA, no significant change in the level of GS mRNA was observed after insulin or T3 induction. This suggests that insulin and T3 regulation of GS activity in astrocytes is a post-transcriptional event in contrast to the transcriptional regulation of GS by HC. Supported by USPHS grant HD-07032, NIH grant HD-06576 and DOE contract DE-FC03-87-ER60615.

339.16

EXPRESSION OF MYOSIN LIGHT CHAIN 2 mRNAs IN THE DEVELOPING RAT BRAIN. D. L. Feinstein and R. J. Milner, Research Institute of Scripps Clinic, La Jolla, CA, 92037.

In order to gain a better understanding of the molecular basis for the diversity of astrocyte function, we have isolated cDNA clones for molecules whose expression is specifically regulated in these cells. cDNA libraries were constructed from primary cultures of neonatal rat brain astrocytes. Clone pAT11 hybridized to a mRNA species that was expressed at highest levels in the embryonic brain, absent from liver or kidney RNA samples, and which was greatly enriched in astrocyte versus whole brain RNA. The DNA sequence of clone pAT11 indicated that it encoded smooth muscle myosin light chain 2 (smMLC2), a regulator of myosin contraction. A second mRNA species, detected by cross-hybridization with the open reading frame of clone pAT11, was expressed at low levels in astrocytes and in the embryonic brain, but at high levels in the postnatal day 23 brain. This mRNA was also the predominant MLC2 species present in RNA from neuronal cultures. Screening of a rat cortex cDNA library resulted in the isolation of clone RLC3-8, which encodes a novel MLC2 and is derived from the larger mRNA species. The tissue distribution of this mRNA was that expected of a nonmuscle isotype. We conclude that at least two forms of MLC2 are expressed in rat brain and that their expression is regulated developmentally and in a cell-specific manner. Supported by a fellowship from the National Multiple Sclerosis Society and grants from NIH (NS21815, NS22347).

339.17

INSULIN-LIKE GROWTH FACTOR II GENE EXPRESSION IN CEREBELLAR GRANULE CELLS. K.M. Rosen*, R. O'Brien*, B. Yankner, and L. Villa-Komaroff.
Dept. of Neurology, The Children's Hospital, Harvard Medical School, Boston, MA 02115

We have examined the expression of the insulin-like growth factor II gene in mitotically-active murine cerebellar granule cells. The cerebellum was dissected free of meninges and choroid. Percoll gradient fractionation of dissociated tissue yielded a homogeneous population of granule cells. Immunohistochemical staining of these granule cell preparations for GFAP indicated < 1% of the cells were glial cells. RNA was isolated from granule cells immediately after purification and from glial cells maintained in culture for 7 days. IGF-II gene expression was characterized using a highly sensitive S1 nuclease protection assay. A high specific activity single stranded DNA probe was generated by subcloning a human IGF-II cDNA restriction fragment into bacteriophage M13. No expression of IGF-II was detected in RNA from glial cells. A low but reproducible level of IGF-II mRNA expression was detected in RNA from granule cells isolated at postnatal days 2, 3 and 4. These results indicate that the IGF-II gene is expressed in neuronal cells during the period of granule cell differentiation.

339.18

INTERLEUKIN 1-ß MESSAGE LEVELS INCREASE IN ALZHEIMER'S DISEASE WHILE GAP-43 MESSAGE LEVELS ARE DIMINISHED. K. E. Rogers*, A.B. Wadhams* and P.D. Coleman.
Department of Neurobiology and Anatomy, University of Rochester, Rochester, NY, 14642.

Interleukin-1(IL-1) is an immunomodulator which has been shown to be produced by microglia and astrocytes. In addition IL-1 is an astroglial mitogen which can also induce the expression of ß-amyloid precursor gene. Thus, we have compared the message levels of IL-1ß in AD to their normal aged matched controls in the superior frontal gyrus. Poly-A+ mRNA was isolated from 500 mg samples using the method of Badley, et al. Typically, 12 mg or more of mRNA was obtained with an A260/280 ratio of 2.0. A 2.0 ug sample of mRNA was electrophoresed on a denaturing agarose gel to assess the quality of the mRNA. A series of dilutions was then slot blotted and probed with a radioactive IL-1ß cDNA probe obtained from D. Cosman (Immunex Corp.). The resulting autoradiogram was densitometrically scanned. Since virtually all mRNA is contaminated by rRNA, all samples were normalized for the amount of polyA+ mRNA present in each sample. Results showed an average of 65% increase in IL-1ß mRNA in young AD SFG. Conversely, in old AD SFG an average decrease in IL-1ß mRNA of 14% was seen. These data are consistent with data of Mountjoy et al., 1983, indicating a smaller loss of neurons in very old AD as compared to younger AD cases. Identical studies were done using a probe for the growth associated protein, GAP-43. These studies indicated that GAP-43 message was decreased in AD SFG as compared to the normal controls. Currently, mRNA blots from AD and normal control SFG are being probed with IL-α

339.19

REGULATION OF THE GROWTH-ASSOCIATED ALPHA-TUBULIN GENE, T ALPHA 1, IN SYMPATHETIC NEURONS *IN VIVO* AND *IN VITRO*. F. Miller, T.C. Mathew*, H.C. Wilson*, and R.B. Campenot.
Department of Anatomy and Cell Biology, University of Alberta, Edmonton, Alberta, Canada T6G 2H7.

Previous reports have shown that expression of T alpha 1 alpha-tubulin mRNA is specifically correlated with neuronal growth in developing and mature mammalian neurons. To investigate how T alpha 1 mRNA expression is regulated by extraneuronal influences, we have experimented with sympathetic neurons of the rat superior cervical ganglion (SCG). Unilateral transection of the carotid nerve close to the SCG (short cut) leads to induction of T alpha 1 mRNA in injured neurons as well as in uninjured, contralateral neurons. In contrast, transection of sympathetic axons projecting to the iris by unilateral enucleation (long cut) results in increased T alpha 1 mRNA only in ipsilateral ganglia. The contralateral increase in T alpha 1 mRNA following a short cut may be due to increased available nerve growth factor (NGF) in bilaterally-innervated target organs, since, in cultured sympathetic neurons, NGF regulates T alpha 1 mRNA in a dose-dependent fashion. T alpha 1 mRNA may also be regulated transneuronally: unilateral decentralization of the SCG by transection of the afferent preganglionic input leads to increased T alpha 1 mRNA in both the deafferented and in contralateral sympathetic neurons.

339.20

EXPRESSION AND LOCALIZATION OF CALMODULIN DEPENDENT PROTEIN PHOSPHATASE (CALCINEURIN) mRNA AND PROTEIN IN RAT BRAIN. M.L. Billingsley, J.W. Polli* J. K. Krady* and R. L. Kincaid*.
(SPON: R. Pelligrino) Dept. Pharmacology, Penn State Univ. College of Medicine , Hershey, PA 17033 and Section on Immunology, NIAAA, Rockville, MD 20892.

Calmodulin-dependent protein phosphatase (CN) is present at high levels in rat brain. In order to identify neurons and brain regions expressing CN, we used immunocytochemistry, immunoblotting and *in situ* hybridization to explore this question. Immunocytochemistry revealed that the highest levels of CN were found in hippocampus, substantia nigra, striatum, amygdala, neocortex and cerebellar Purkinje cells; however, neurons throughout the brain had detectable amounts of CN. Immunoblotting confirmed this pattern of localization, and subcellular fractionation showed CN to be associated with both cytosolic and membrane compartments. Analysis of developing rat brain indicated that CN expression increased postnatally, with near-adult levels observed at postnatal day 20. A series of specific 200-300 bp fragments corresponding to the antisense strand of a cDNA clone for CN were radiolabelled and used for *in situ* hybridization. CN mRNA was highest in the hippocampus, followed by striatum, cerebellum, amygdala and neocortex. The patterns of CN expression using both immunochemical and hybridization methods were in concordance, suggesting that regional differences in CN may reflect differences in transcription. Supported by grants from NIH (MLB) and the PMA Foundation (JWP).

OPIATES, ENDORPHINS AND ENKEPHALINS: BEHAVIORAL EFFECTS

340.1

NALOXONE REDUCES SOCIAL LOCOMOTOR ACTIVITY IN RATS. C.P.J. Dokla and J.K. DeFilippis*. Dept. of Psychology, Fairfield University, Fairfield, CT 06430.

Social affiliation (Panksepp et al., Biological Psychiatry, 13:607, 1978), exploration, and locomotor activity (Arnsten et al., Life Sciences, 25:1035, 1979) are thought to be modulated by opioid mechanisms. File (Psychopharmacology, 71:41, 1980) reported that naloxone (NAL), an opioid antagonist, significantly reduced activity in rats tested in pairs but not individually during a 10-min session. In the present study, groups of male Long-Evans hooded rats received NAL (1 or 4 mg/kg,ip) or vehicle only (isotonic saline) 30 min prior to testing sessions. Individual locomotor activity was measured in two photobeam-type activity boxes (41-cm^3) using daily 30-min sessions for 5 days. Following a 1-week washout period, activity was measured in pairs of rats from each group using daily 15-min sessions for 4 days. Locomotor activity was not significantly affected by NAL in individually tested rats, although retention (recovery) of activity habituation was improved by NAL (1 mg/kg group compared to the 4 mg/kg group only, $p < .05$). In the social (paired) activity tests, both doses of NAL reduced activity over the 4 days of testing ($ps < .01$). The present results suggest a role for opioids in social affiliation.

340.2

BIOCHEMICAL AND BEHAVIORAL EFFECTS OF INTRA-MR INJECTIONS OF OPIATE AGONISTS. M.A. Klitenick and D. Wirtshafter,
Dept. of Psychology, Univ. of Illinois, Chicago, IL 60680.

Recent studies have reported pronounced behavioral and biochemical effects following various manipulations of the midbrain raphe nuclei. In previous studies we have shown that injections of morphine, DAGO or DPDPE into the median raphe (MR) elicit increases in locomotor activity (LA). In an attempt to further clarify the role of opiates within the MR we decided to investigate the biochemical effects of intra-MR injections of DAGO or DPDPE following activity measures within a photocell cage.

LA was increased ($p<.05$) after intra-MR DAGO (437.5ng) compared to vehicle controls, confirming previous results. Levels of DA, DOPAC and HVA in the striatum (CAU) as well as 5-HT and 5HIAA in the hippocampus (HIPP) were not altered ($p>.5$). Intra-MR DAGO injections resulted in decreased levels of DA ($p<.05$) and increased levels of DOPAC and HVA ($p<.05$). Intra-MR injections of DPDPE (10µg) elicited an increase ($p<.05$) in LA compared to vehicle controls. In HIPP, only 5HIAA/5-HT was lower ($p<.05$) in DAGO-injected animals. Within the NA, DA levels were lower then controls ($p<.01$), DOPAC and HVA levels were higher ($p<.001$), and both 5-HT and 5HIAA were lower than control values ($p<.01$). No significant effects were found within the CAU for levels of DA, 5-HT or their metabolites.

(Supported by NIH NS21350)

340.3

NALOXONE DIFFERENTIALLY DISRUPTS THE ACQUISITION AND THE EXPRESSION BY MALE RATS OF A CONDITIONED PLACE PREFERENCE FOR AN ESTROUS FEMALE. B.J. Mehrara* and M.J. Baum, Dept. of Biology, Boston University, Boston, MA 02215.

Experiments were conducted to explore the possible contribution of endogenous opioid peptides to sexual reward in adult male rats. Sexually experienced males were allowed to mate with an estrous female in an initially 'non-preferred' chamber; on alternate days they were placed alone in an initially 'preferred' compartment. Males injected s.c. with saline prior to 8 such conditioning sessions acquired a conditioned place preference (CPP) for the initially non-preferred compartment whereas control subjects which were simply placed alone in each chamber on alternate days acquired no CPP. Administration of naloxone (1 or 5 mg/kg s.c.) 5 min. prior to each conditioning session had no effect on the acquisition of a CPP for an estrous female; however, these treatments attenuated the expression of a previously established CPP in gonadally intact males. Two weeks after castration males given saline prior to each conditioning session readily acquired a CPP despite their reduced mating performance. However, in contrast to gonadally intact males, acquisition of a CPP was blocked in castrates treated with a high dose of naloxone (5 mg/kg s.c.). Naloxone (1 or 5 mg/kg s.c.) also blocked the expression of a CPP in castrates. In male rats opioid inputs to neural reward circuits may normally be activated by incentive stimuli, but not by the primary rewarding stimuli associated with an estrous female. (supported by HD21094 and MH00392 to M.J. Baum)

340.4

ROLES OF OPIOID AND BENZODIAZEPINE SYSTEMS IN MEDIATING EFFECTS OF SOCIAL COMPANIONS ON ULTRASONIC DISTRESS CALLS IN INFANT RATS. **S.E. Carden & M.A. Hofer. Columbia Univ. & N.Y.S. Psychiatric Inst., New York, NY 10032.**

Low doses of morphine (0.125mg/kg) and chlordiazepoxide (2.0 mg/kg) are equally effective in lowering the rate of ultrasonic distress calls in 10-day-old rat pups. The presence of a single anesthetized littermate in the test chamber reduces vocalization to a comparable degree.

We sought to evaluate the impact of opioid and benzodiazepine antagonists on the comforting effects of littermate or dam during testing in a novel environment.

Naltrexone, but not RO 15-1788, blocked the reduction in vocalization associated with the presence of a social companion. The opioid antagonist disrupted quiet contact between test pup and companion while the benzodiazepine blocker facilitated the behavior.

The presence of a companion may provide comfort by inducing a release of endogenous opioids and, in addition, may inhibit the release of an endogenous anxiogenic ligand to the benzodiazepine receptor.

340.5

PLACENTAL OPIOID-ENHANCING FACTOR DOES NOT MODIFY MORPHINE-INDUCED HYPERTHERMIA. M.B. Kristal, E.F. Ferguson*, J.J. Bruschetti* & A.C. Thompson*. Dept. of Psychology, SUNY at Buffalo, Buffalo, NY 14260.

We have reported that Placental Opioid-Enhancing Factor, ingested in amniotic fluid (AF), enhances opioid-mediated analgesia in rats. The present study assessed the effect of AF ingestion on opiate-mediated hyperthermia. This problem was of interest for two reasons. (1) The tail-flick test, used to assess pain threshold, is dependent on tail-skin temperature. If AF enhances morphine-induced hyperthermia and decreases tail-skin temperature, the use of the tail-flick test to assess analgesia would be confounded. (2) Is the opioid-enhancing effect of AF ingestion limited to analgesia?

Body temperature was monitored before and after rats were treated with 3mg/kg ip morphine sulfate (or .9% saline) and an orogastric infusion of AF (or a control). Three doses of fluid were assessed (.125 ml, .25 ml, and .75 ml) in 3 separate groups. Each rat served as its own drug x infusate control.

No significant interaction between AF (at any dose) and morphine-induced hyperthermia was found. As expected, this dose of morphine significantly increased body temperature at all post-injection test times (30,90, & 135 min).

The results suggest that (a) the assessment of analgesia after AF/morphine treatment is not confounded by concurrent changes in body temperature, and (b) that the effect of AF on opioid-mediated analgesia is not generalizable to all other opioid-mediated processes.

Supported by NSF grant BNS 86-01818 awarded to MBK.

340.6

OPIOID RECEPTOR REGULATION OF MATERNAL BEHAVIOR IN RATS. P.E. Mann, C.H. Kinsley, P.M. Ronsheim, and R.S. Bridges. LHRRB, Harvard Medical School, Boston, MA 02115.

Opiates inhibit maternal behavior (MB) in rats. Central or systemic administration of morphine (MOR) disrupts MB in steroid-primed, pup-induced virgin and lactating rats. MOR, a putative mu agonist, interacts with different opioid receptor subtypes. The aim of the present study was to characterize those opioid receptor subtypes mediating opiate disruption of maternal behavior. Virgin, Sprague-Dawley rats were mated and then implanted with lateral ventricle cannulae on day 13-15 of gestation. On postpartum day 5 mothers were tested for MB 30 min after vehicle (VEH) injections (5 ul) into the lateral ventricle. On day 6 the rats received one of the following opioid receptor agonists (5 ul) 30 min before testing: B-endorphin (BE; mu/epsilon; 0.29, 0.72, 1.45, or 2.9 nm), [D-Pen^2,D-Pen^5]-Enkephalin (DPDPE; delta; 2.9, 29 nm), U-50,488H (kappa; 2.9, 29, 145 nm), SKF-10047 (SKF; sigma; 2.9, 29, 145 nm), or DAGO (mu; 0.29, 0.72, 1.45, 2.9 nm). Both BE (mu/epsilon) and DAGO (mu) significantly disrupted MB at the 2.9 and 1.45 nmolar doses compared to vehicle. Retrieval was affected by the 2.9 dose of BE and the 1.45 and 2.9 doses of DAGO. DPDPE (delta), U-50,488 (kappa) and SKF (sigma) failed to affect full MB or retrieval at any of the doses tested. These results suggest that disruption of maternal behavior by opiates and opioid peptides is regulated by the mu opioid receptor.

340.7

DELTA AND KAPPA OPIATE RECEPTOR CONTROL OF SEPARATION DISTRESS: J. Panksepp, P. Lensing* and G. Bernatzky*, Dept. of Psychol., Bowling Green State Univ, OHIO and Univ. of Salzburg, AUSTRIA.

The mu-opioid receptor system exerts powerful inhibition over isolation-induced distress vocalizations (DVs). We have now evaluated delta and kappa receptor agonists and antagonists (ant.) effects on DVs of 1-8 day old domestic chicks. For delta opioid receptors, ED-50s for DADLE and DPDPE (icv into 4th ventricle) were approx. 1.0 nmoles and for DSLET 0.5 nmoles. The delta receptor ant. ICI-154129 increased DVs following ip (10 mg/kg) and icv (10 ug) injections, but ICI did not antagonize the inhibitory effects of icv DPDPE (1 nmole) or morphine (1 ug). For kappa receptors, U-50,488 reduced DVs by about 50% at doses above 5 mg/kg but also produced sedation. These effects were reversed with the kappa specific ant. Mr-2266. U-50,488 icv up to 15 ug had little effect on DVs, while Mr-2266 at similar doses (ip and icv) reliably elevated the frequency of DVs. Delta receptor control of DVs is weaker than mu receptor control. Kappa-specific involvement in the inhibition of DVs remains ambiguous, but the Mr-2266 effects suggest a reduction of kappa tone may also promote this type of emotional arousal.

340.8

CNS BETA-ENDORPHIN: ITS ROLE IN GROWTH AND DEVELOPMENT AND IN BIOLOGICAL RESPONSES TO MATERNAL SEPARATION IN RAT PUPS. J. Bartolome, M. Bartolome*, B. Lorber*, A. Chao* and S. Schanberg*. Dept. Pharmacol. Duke Univ. Med. Ctr., Durham, N.C. 27710.

Accumulated evidence indicates that endogenous opioid peptides may have an important role in the regulation of perinatal development. The question remains as to which neuropeptides are involved and through which mechanisms do they regulate growth. This study examines the effects of central administration of beta-endorphin (BE) on important growth determinants. The responses to BE were then compared to those seen after a short-term separation of pups from their dams to find out whether the growth deficits observed in the deprived pups could be explained by changes in brain BE levels.

Intracisternal injection of BE to rat pups reduced ornithine decarboxylase (a key growth-regulatory enzyme) activity, and inhibited DNA synthesis in both brain and liver. Inhibition of DNA synthesis was still evident 12 hr after BE exposure. In addition, BE treatment prolonged the apparent half-life of plasma insulin, a classical trophic factor. Essentially the same pattern of biochemical and endocrine responses was seen in maternally deprived rat pups.

The results obtained strongly suggest a regulatory role for CNS BE in growth and development. The data also suggests that BE may be a prime mediator of the biological and physiological responses seen following interruption of mother-pup interactions. (Sup. by NIH Grants RO1-NS25738 and RO1-MH13688)

340.9

EVIDENCE THAT MU AND KAPPA OPIOID RECEPTOR SITES MEDIATE SUCKLING-INDUCED PROLACTIN SECRETION IN LACTATING RATS. M. Baumann and J. Rabii. Department of Biological Sciences, Rutgers University, Piscataway, NJ 08855.

Several investigators have reported that the opioid antagonist naloxone can significantly inhibit suckling-induced prolactin (PRL) release in the rat. However, the specific opioid receptor subtype(s) involved are not known. The relatively high doses of naloxone required to completely block the suckling-induced PRL surge [Selmanoff and Gregerson, 1986] suggest the importance of a non-mu receptor mechanism. We have employed selective opioid antagonists to determine the role of mu and kappa receptors in mediating PRL release during suckling *in vivo*.

Lactating mothers were implanted with intracerebroventricular (ICV) cannulae and chronic indwelling jugular cannulae. On day 10 of lactation, litters were removed for 4 hours and mothers were treated with opioid antagonists or vehicle. Pups were returned and allowed to suckle for 30 minutes. The irreversible mu opioid antagonist ß-funaltrexamine (ß-FNA) was injected ICV at doses of 2.0 and 10.0 nmol, 4 hours before pup return. Nor-binaltorphimine (nor-BNI), a putative kappa antagonist, was injected ICV at doses of 5.0 and 20.0 nmol, 45 minutes before pup return. Serial blood samples were withdrawn prior to and at 10, 20, 30 and 60 minutes after the onset of suckling.

Suckling stimulated a rapid and dramatic rise in plasma prolactin in all vehicle treated females. ß-FNA, on the other hand, suppressed suckling-induced PRL secretion in a dose-related manner. The 10.0 nmol dose of ß-FNA caused almost total abolition of the response. Likewise, the higher dose of nor-BNI completely blocked the surge of PRL during suckling. These data imply that both mu and kappa opioid receptors are physiologically relevant mediators of PRL release during lactation in the rat. (Supported by the Busch Memorial Fund and the Anne B. and James H. Leathem Scholarship Fund.)

340.10

ATTENUATION OF THE MORPHINE INDUCED ANALGESIC RESPONSE DURING LACTATION IN THE RAT. J. Janik, M. Baumann, P. Callahan and J. Rabii. Dept of Biological Sciences, Rutgers University, Piscataway, NJ 08855.

We have previously reported that lactating females are not sensitive to morphine or methadone stimulation of prolactin release. However, this model does show a prolactin secretory response to both β-endorphin and d-ala-d-leu-enkephalin (DADLE). The purpose of this study was to further characterize the sensitivity of the lactating model to morphine administration by measuring the morphine induced analgesic response using a hot water tail immersion test.

Lactating female rats between days 6 and 10 postpartum received saline or morphine at 2.5, 5.0 or 10.0 mg/kg (iv). Cycling females in the diestrous stage of the estrous cycle were similarly treated and served as controls. Some of the animals in each of the groups were pretreated with the μ_1 specific antagonist naloxonazine (NAZ; 20 mg/kg, iv) 24 hours prior to the morphine. A latency of 15 seconds was considered complete analgesia and the animal's tail was removed from the hot water bath.

Although the lactating model did exhibit analgesia following morphine administration, the analgesic response was attenuated compared to the non-lactating rats. In addition, NAZ pretreatment did not alter the analgesic response in the lactating model suggesting that the analgesia was produced by a receptor subtype other than the μ_1 site. However, NAZ pretreatment did attenuate the analgesia observed in the diestrous control rats. These results are consistent with the results obtained regarding the sensitivity of the prolactin secretory response to morphine during lactation. It seems likely that the μ_1 opiate receptor subtype is not active during lactation. (Supported by the Busch Memorial Fund and the Anne B. and James H. Leathem Scholarship Fund).

340.11

THE EFFECT OF ACUTE STRESS ON LEVELS OF IMMUNOREACTIVE BETA-ENDORPHIN IN DISCRETE BRAIN REGIONS IN RATS PRENATALLY EXPOSED TO ETHANOL. R.A. Baker and W.J. Shoemaker. Neuroscience Program and Dept. of Psychiatry, Univ. of Connecticut Health Center, Farmington, CT 06032.

Among many functions proposed for beta-endorphin is a role in modulation of stress. Several studies have reported depletion of immunoreactive beta-endorphin from certain brain regions after footshock (Izquierdo et al.1984, Netto et al.1985, Netto et al.1988). We have attempted to localize more specifically the regions affected by footshock stress in both normal rats and rats prenatally exposed to ethanol. Two baseline control groups were used, a) rats handled daily for 14 days prior to sacrifice and b) rats not handled prior to sacrifice. Rats were sacrificed by decapitation at 3, 10 and 30 minutes after a five second continuous 0.8 milliamp shock. Levels of beta-endorphin were measured by radioimmunoassay in the mediobasal hypothalmus, remainining hypothalamus, amygdala, mediodorsal thalamus, midbrain, medulla/pons and septal area including the nucleus accumbens. Levels in all brain regions, except the amygdala showed decreased beta-endorphin levels at 3 minutes post-shock, with the most dramatic effect seen in the septal region, in agreement with prior reports. Beta-endorphin levels after foot-shock in rats prenatally exposed to ethanol is particularly relevant since these rats have abnormal peptide levels (Shoemaker et al. 1983) and respond abnormally to behavioral measures of stress (Kehoe et al. 1988). Supported by NIAAA #06279.

340.12

UNILATERAL MICROINJECTIONS OF |D-ALA^2,N-ME-PHE^4,GLY^5-OL|-ENKEPHALIN (DAGO) INTO THE VENTRAL PALLIDUM (VP) PRODUCES CONTRALATERAL TURNING IN RATS. D.C. Hoffman, T.E.G. West* and R.A. Wise. Dept. Psych., Concordia Univ., Montreal, Canada, H3G 1M8.

Turning behavior following unilateral microinjection of the selective mu agonist, DAGO, into the VP of rats was investigated. Male Long-Evan rats were chronically implanted with bilateral cannulae aimed at the VP. Testing involved placing each rat in a cylindrical container and measuring the number and direction of 360° turns over a 120-min test period. Following tests with no injection and vehicle (Ringers solution), the effects of three doses of DAGO (0.02, 0.2 and 2.0 nmoles; administered in a counterbalanced order) were determined in each rat. Pretreatment with the opiate antagonist, naltrexone (1.0 mg/kg IP), on DAGO-induced circling was subsequently tested, followed again by control tests with vehicle and no injection. DAGO resulted in dose-dependent contralateral circling with both the frequency and duration increasing with larger doses. Circling produced by 2.0 nmoles was blocked in rats pretreated with naltrexone. These results suggest that mu receptors in the VP are involved in motor behavior and because this enkephalinergic projection likely originates in the nucleus accumbens, these neurons may represent an important output pathway in mediating locomotor activity associated with dopaminergic release in the accumbens.

340.13

LETHALITY OF MORPHINE IN MICE INFECTED WITH TOXOPLASMA GONDII. C. Chao*, B.M. Sharp*, C.Pomeroy*, G. Filice* and P.K. Peterson* (SPON: S.E. Nicol). Hennepin County Medical Center, VA Medical Center, and University of Minnesota Medical School, Minneapolis, MN 55404.

Opiates modulate a variety of immune functions. The present study was designed to investigate the effect of morphine on the pathogenesis of T. gondii infection. Repeated sc injections with morphine sulfate (6 mg/mouse) every 36 h addicted mice and markedly increased the mortality of mice infected with an avirulent strain of T. gondii (86% vs 0% mortality in addicted vs control mice, P<.001). However, addiction to morphine did not seem to be necessary for the lethal effect, since a single challenge with morphine also markedly (P<.001) increased mortality of infected mice when the morphine was administered at day 13 post-infection. Lethality after a single injection of morphine (6 mg) was not observed until day 9 post-infection, a time when significant immune reactivity was evident (i.e., 3-4 fold splenic hypertrophy). Death occurred rapidly - within 2 h of morphine administration. This lethal effect was dose-dependent (LD_{50}=1.75 mg) and was reduced (P < .05) by treatment with naltrexone. Sulfadiazine treatment for 5 days abrogated the lethality of morphine indicating an absolute prerequisite of an active infectious state for this phenomenon. These findings suggest that immune activation by T. gondii infection plays a critical role in morphine-induced mortality in this murine model. (Supported by grants DA-04381 and DA-04196).

340.14

ATTENUATION OF RESPONSE OF POMC PEPTIDES IN AUTISTIC AND SIB CHILDREN TO CHRONIC VERSUS ACUTE NALTREXONE. P.H. Herman, A. Arthur-Smith, K. Verebey* and J. Alrazi*. Brain Res Cen & Dept Psychiatry of Children's Hosp Natl Med Cen & George Washington Univ Sch Med, Washington, DC and PDLA, South Plainfield, NJ.

Naltrexone (NTRX) may be a useful tool in evaluating the proopiomelanocortin (POMC) HPA axis in autistic and self-injurious (SIB) children (Herman et al., Soc Neurosci 14:465, 1988), and this may relate to the therapeutic usefulness of this drug in these children. Here we compared NTRX(0.5,1.0,1.5 and 2.0mg/kg)and placebo administered acutely (once) versus chronically (three times) a week in 8 autistics without SIB (4-12 yrs) and in 5 severe SIBs(10-17 yrs). Blood was drawn 1 hr after oral drug. Plasma irß-endorphin (ß-E), cortisol (COR) and irACTH were measured by RIA, and plasma NTRX and 6ßnaltrexol (6ßNLT) by GC/MS. Acute NTRX resulted in two groups - one showing at least a 50% increase in irß-E, COR and irACTH in response to NTRX (POMC Stim) and a second showing no change (POMC NonStim). There was a highly significant difference in the irß-E, COR and irACTH response of these two groups to every acute NTRX dose (all p's < .0005), and these differences were not due to differences in plasma NTRX and 6ßNLT. These effects were greatly attenuated when the same doses of drug were chronically administered. Plasma NTRX and 6ßNLT concentrations were about twofold higher when drug was chronically versus acutely administered.

340.15

NALTREXONE BLOCKS THE EFFECTS OF PRENATAL EXERCISE ON REFLEX DEVELOPMENT IN THE MALE RAT. S.K. Elliott* and J.L. Voogt (SPON: M.K. Shellenberger). Dept. of Physical Therapy Ed. and Dept. of Physiology, Univ. of Kansas Med. Center, Kansas City, KS 66103.

We have previously reported that high intensity treadmill running during the third trimester of pregnancy in the rat induces delays in neonatal reflex development and permanently impairs exploratory behavior. (Neurosc. Abst. 41.14, 1988). Copulatory potentials in adult male offspring were also impaired, with reduction in SDN-POA volume. The opioid system has been implicated as a mediator of sexual behavior differentiation as well as somatic and brain development. It is also known that ß-endorphins are released during exercise in the rat. The present study investigated the effects of blocking opiate receptors during prenatal exercise on neuromotor development. Third trimester pregnant rats were injected daily with 2 mg/kg naltrexone (N-EX) at 0900, 1300 and 1700. The running protocol began at 1330 daily. Exercised controls received saline injections (S-EX). Non-exercised naltrexone injected pregnant females (N-C) and saline controls (S-C) were also included. S-EX offspring showed impaired reflex and exploratory behaviors. N-EX offspring showed reflex development consistent with the S-C group. However, N-EX exploratory behaviors were as impaired as in the S-EX group. N-C offspring showed accelerated growth and reflex development, but did not display advanced exploratory skills. These results suggest that exercise-induced reflex acquisition delays, but not complex exploratory behaviors, may be mediated by the opioid system. Investigation of copulatory behavior potentials and morphometric analysis of the somatosensory cortex is underway. (NIH-HD22340)

340.16

POTENTIATION OF MORPHINE AND STRESS INDUCED ANALGESIA IN MICE BY ANTAGONISM OF MAMMALIAN FMRF-AMIDE RELATED PEPTIDES. M. Kavaliers and H.-Y. T. Yang, University of Western Ontario, London, Canada and NIMH, St. Elizabeths Hosp. Washington D.C.

Two mammalian FMRFamide-like peptides have been isolated from bovine brain; an octapeptide, FLFQPQRFamide (F-8-F-amide) and an octadecapeptide, AGEGLSSPFWSLAAPQRF-amide (A-18-F-amide). Results of previous studies with rodents have suggested that FMRFamide and endogenous mammalian FMRFamide-related peptides may function as opiate antagonists. In the present study determinations were made of the effects of intracerebroventricular administrations of antibody (IgG) prepared from antisera raised against these peptides on nociception and morphine- and immobilization-induced opioid analgesia in male mice. Both F-8-F-amide-IgG and A-18-F-amide-IgG antisera augmented nociception (thermal response latency) and increased the durations and levels of morphine- and immobilization-induced analgesia in a naloxone reversible manner, with F-8-F-amide-IgG antiserum having a greater effect than A-18-F-amide-IgG antiserum. These observations provide additional support for the proposal that mammalian FMRFamide-like peptides function as endogenous opiate antagonists and have a role in the mediation of antinociception.

PAIN MODULATION: AFFERENT MECHANISMS

341.1

CONDITIONED ANALGESIA IN THE SPINALIZED RAT. J.A.Salinas*, P.A.Illich, M.W.Meagher & J.W.Grau (SPON:R.A.King). Dept. of Psychology, Texas A&M Univ., College Station, TX 77843.

Pairing a neutral stimulus with an aversive event can endow the stimulus with the ability to elicit analgesia. It has been generally assumed that forebrain systems play a critical role in producing this conditioned analgesia. However, there is some evidence that classically conditioned responses can be established in spinalized subjects. The present study assesses whether conditioned changes in pain reactivity can be obtained in spinalized subjects. Six rats received a spinal transection at T2. Eight hrs after the surgery, subjects received differential conditioning (which controls for both pseudoconditioning and sensitization). One stimulus (CS+) was paired with intense tail shock (2-sec, 3-mA), while the other (CS-) was presented alone. Mild shock (10-sec, 1.0-mA) delivered to the left or right paw served as the CS+ and CS-. The subjects received 30 CS+ and 30 CS- trials spaced 1 min apart. Which stimulus served as the CS+ was counter-balanced across subjects. Pain reactivity was then tested one hour later with the tail-flick test. Subjects appeared analgesic during the CS+ relative to the CS-. This difference extinguished over the course of testing. A second experiment was then performed to determine whether the conditioned analgesia is naltrexone reversible. We found that a large dose of naltrexone (14 mg/kg) did not attenuate the conditioned analgesia. Supported by NSF grant BNS 881981 to JWG.

341.2

PREVIOUSLY FOOD DEPRIVED RATS EXHIBIT AN EXAGERATED MORPHINE ANALGESIA. M.K. Biles*, P.A. Illich, and J.W. Grau. Dept. of Psychology, Texas A&M University, College Station, TX 77843.

Previous research has shown that an extended exposure to inescapable shock elicits a strong hormonally mediated opioid analgesia. In addition, it sensitizes the opioid system to being reactivated, 24 hr later, upon exposure to a low dose of morphine (1 mg/kg) (Grau et al., Science, 203: 1409, 1981). Here we determined whether another manipulation which elicits a hormonally mediated opioid analgesia, food deprivation (Hamm et al., Physiol. & Beh., 35: 879, 1985), has a similar sensitizing effect.

Subjects had their food removed and after 24 hrs were given 9 grams of food. After an additional 24 hrs, food was returned. Twentyfour hours after food was returned, subjects were injected with 1 mg/kg morphine or saline. Pain reactivity was assessed 30 min later with the tail-flick test. We found that morphine elicited a much stronger analgesia in previously food deprived subjects. In a second experiment, we assessed pain reactivity over a 2.5 hr period following the injection. We found that previously food deprived subjects exhibited an exagerated analgesia for the entire 2.5 hrs of testing. In both experiments, food deprivation had no impact on the levels of pain reactivity observed in the saline controls.

341.3

SEROTONERGIC AGONISTS PRODUCE ANTINOCICEPTIVE EFFECTS IN RESPONSE TO COLORECTAL DISTENSION. R.M. Danzebrink and G.F. Gebhart. Department of Pharmacology, The University of Iowa, Iowa City, Iowa, 52242.

The antinociceptive effects of intrathecally administered 5-HT and other serotonin receptor agonists were examined in a model of visceral pain (colorectal distension, CRD) in conscious rats. CRD reliably elicits a vigorous pressor response and contraction of the abdominal and hindlimb musculature (a visceromotor response). Antinociception is characterized by inhibition of both pseudaffective responses.

5-HT dose-dependently elevated the visceromotor threshold and produced a slight facilitation prior to dose-dependent inhibition of the pressor response to CRD. The 5-HT_{1A} agonist 8-OH-DPAT, the 5-HT_{1B} agonist RU-24969, the 5-HT_2 agonists DOI, MK-212, and -methyl-5-HT and the 5-HT_3 agonist 2-methyl-5-HT all dose-dependently inhibited the pressor and visceromotor responses to noxious CRD. The antinociceptive effects of 5-HT, RU-24969 and DOI were antagonized by methysergide. Ketanserin antagonized the antinociceptive effects of MK-212 and MDL-72222 antagonized the effects produced by 2-methyl-5-HT in response to CRD. The antinociception produced by 8-OH-DPAT was not antagonized by methysergide and the effects produced by -methy-5-HT were not antagonized by either methysergide or ketanserin. These results demonstrate that 5-HT_1, 5-HT_2 and 5-HT_3 receptors in the spinal cord mediate antinociception in response to noxious CRD in conscious rats.

341.4

INFLAMMATION POTENTIATES RESPONSES TO COLORECTAL DISTENSION IN THE RAT. T.J. Ness and G.F. Gebhart. Departments of Anesthesia and Pharmacology, The University of Iowa, Iowa City, Iowa, 52242

Turpentine (25% in peanut oil) produces inflammation when applied to the gut mucosa of rats as judged by plasma extravasation (Evan's Blue) and neutrophil infiltration. Colorectal distension (CRD) produces behavioral (conditioned avoidance), cardiovascular, visceromotor (abdominal/hindlimb muscle contractions) and neuronal (T13-L2 spinal dorsal horn) responses and counter-inhibitory effects.

When compared with responses in untreated rats, responses in turpentine-treated (colorectal mucosa) rats are potentiated 1,2,6,24 or 48 hours following treatment:

(1) 30 mm Hg, 20 s CRD produces conditioned avoidance in turpentine-treated, but not untreated rats;
(2) resting heart rate is higher in treated rats;
(3) pressor responses to CRD are greater in treated rats;
(4) pressure thresholds for activation of visceromotor responses to CRD are lower in treated rats;
(5) graded neuronal responses are increased 50-300% two hours following treatment with turpentine;
(6) graded inhibition of the tail-flick reflex by conditioning CRD was greater in treated rats.

In the aggregate, these studies suggest that turpentine-induced inflammation increases visceral nociception.

341.5

HYPOGASTRIC AND PELVIC NEURECTOMY CONTRIBUTE DIFFERENTIALLY TO VAGINOCERVICAL STIMULATION-PRODUCED ANALGESIA IN RATS. ST Cunningham, JL Steinman & BR Komisaruk, Institute of Animal Behavior, Rutgers Univ., Newark, NJ 07102.

Vaginocervical stimulation (VS) has been shown to elevate both tail flick (TF) latency to radiant heat and vocalization (VOC) threshold to electric shock. The present study examined the relative contributions of hypogastric neurectomy (HX) and pelvic neurectomy (PX) to VS-produced analgesia (VS-PA) as measured by these tests. Bilateral HX, PX or HXPX were performed 5-10 days prior to behavioral testing. After baseline (BL) was established, VS (400g force) was applied for 5 sec. Values are expressed as % change from BL; * = $p<0.03$; t-tests comparing each treatment group to unoperated controls (n's = 7-10).

	CONTROL	HX	PX	HXPX
TF	347%	169%*	148%*	42%*
VOC	27%	-17%*	52%	-9%*

In summary, HX or PX reduced VS-produced elevation in TF, and HXPX produced the greatest reduction. By contrast, HX and HXPX, but not PX alone, significantly reduced the VOC elevating effect of VS. These findings suggest that the hypogastric and pelvic nerves subserve different components of VS-PA. (Supported by NIH NLS-2-1R01-NS22948 to BRK).

341.6

VAGINOCERVICAL STIMULATION-PRODUCED ANALGESIA AND CORRELATED SPINAL CORD AMINO ACID RELEASE ARE DISRUPTED BY NEONATAL CAPSAICIN TREATMENT. D. B. Masters, C. Beyer*, F. Jordan*^, J. L. Steinman and B. R. Komisaruk. Institute of Animal Behavior and ^Dept. of Chemistry, Rutgers University, Newark, N. J. 07102 USA.

We hypothesized that inhibitory amino acids (Gly, Tau) are released within the spinal cord in response to vaginocervical mechanostimulation **(VS)** and thereby produce analgesia. This hypothesis was tested by HPLC analysis of the amino acid content of microdialysates of the sacral dorsal horn before, during, and after VS (400g force) in the urethane-anesthetized rat. Furthermore, the neurotoxic effect of neonatal capsaicin treatment **(NCT)** was utilized in order to increase pain thresholds to a wide variety of noxious stimuli. Our strategy was to diminish vaginal afferent activity by NCT and observe any corresponding change in amino acid release.

Results from adult female rats show a significant elevation in the NCT group (n=37) compared to controls (n=24) in response thresholds on the following tests: vocalization to tail shock (55%), paw lick to hot plate (76%), and tail flick (20%). By contrast, leg-flexor reflex to hindpaw-pinch was unaffected. VS in control rats significantly increased pain thresholds on all these tests 90-300%. The effect of VS in NCT rats was significantly less than in controls on all four tests. Moreover, NCT rats show a significant decrease in vocalization threshold (10%) during VS compared to pre-VS levels.

The results from *in vivo* dorsal horn microdialysates show that Gly and Tau are significantly increased by VS in control (n=4) but not NCT rats (n=8). However, Asp and Glu increase in response to VS in both groups. All statistics used ANOVA ($p\leq0.05$).

In conclusion, the evidence that VS lowers vocalization threshold in NCT rats indicates that VS releases nociception-producing transmitters and analgesia-triggering transmitters, the former being preferentially spared in vaginal afferents after NCT. The idea that excitatory and inhibitory amino acids mediate these events is consistent with their spinal release demonstrated in this study. Support NIH :1RO1NS22948-02 (BRK)

341.7

VAGAL AFFERENT MODULATION OF NOCICEPTION: CENTRAL PATHWAY. K. Ren, A. Randich and G.F. Gebhart. Departments of Pharmacology and Psychology, The University of Iowa, Iowa City, Iowa, 52242

Vagal afferent stimulation (VAS) at low and high intensities produce facilitation and inhibition, respectively, of spinal nociceptive transmission. Central substrates involved in these VAS-produced effects were further examined in this study. Cold block of the thoracic spinal cord abolished VAS-produced effects on spinal units. VAS-produced inhibition of responses of lumbar dorsal horn units to 50°C thermal stimuli was significantly attenuated by either transection of thoracic dorsal lateral funiculi (DLF) or microinjection of lidocaine into the thoracic ventrolateral funiculi (VLF). VAS-produced facilitation of unit responses was unaffected by DLF transection. Decerebration at the collicular level had little effect on VAS-produced inhibition, but eliminated VAS-produced facilitation. Bilateral microinjection of lidocaine into the region of the locus coeruleus (LC) attenuated VAS-produced inhibition and abolished VAS-produced facilitation. These results indicate that descending pathways engaged by peripheral VAS are located in the VLF and DLF of the spinal cord and at least one relay for the descending modulation produced by VAS is the LC. A rostral forebrain loop appears to be required for VAS-produced facilitation. Supported by NS 24958.

341.8

RELAY BY NUCLEUS TRACTUS SOLITARII (NTS) OF NOCICEPTIVE MODULATION BY VAGAL AFFERENT STIMULATION (VAS). A. Randich, K. Ren and G.F. Gebhart. Departments of Psychology and Pharmacology, The University of Iowa, Iowa City, IA 52242

Both VAS and NTS stimulation (NTSS) modulate spinal nociceptive transmission. Since vagal afferent fibers terminate primarily in the NTS, the functional role of the NTS in VAS-produced nociceptive modulation was examined. Spinal dorsal horn units were recorded extracellularly in anesthetized, paralyzed rats. VAS was applied to the central end of the cut cervical vagus nerve and modulated neuronal responses to 50°C heating of the skin as reported before. NTSS either ipsi- or contralateral to the spinal unit inhibited neuronal responses with equal efficacy. The apparent latency to NTSS-produced inhibition was 50 ± 10ms. NTSS decreased the slope of the stimulus-response functions of spinal units to graded heating without altering the response threshold. Quantitative comparisons revealed similarity between the effects of NTSS and VAS on spinal nociceptive transmission. Microinjection of lidocaine into the NTS significantly attenuated VAS-produced inhibition. VAS-produced facilitation was eliminated by microinjection of lidocaine into the contralateral, but not ipsilateral NTS. The data suggest that the NTS is a relay for VAS-produced modulatory effects. A multisynaptic relay of VAS-produced effects is suggested by the apparent latency to NTSS-produced inhibition. Supported by NS 24958.

341.9

ANTINOCICEPTIVE EFFECTS OF INTRAVENOUS SEROTONIN IN RATS. III. ROLE OF NON-VAGAL AFFERENTS. G.F. Gebhart, S.J. Lewis*, S.T. Meller* and M.J. Brody. Dept. Pharmacology, The University of Iowa, Iowa City, Iowa, 52242.

We have reported that intravenous (IV) serotonin (5-HT) produces a dose-dependent inhibition of the nociceptive tail-flick (TF) reflex in Sprague-Dawley rats through activation of vagal afferent fibers capable of engaging descending inhibitory systems. The aim of this study was to determine whether non-vagal afferents participate in the antinociceptive effects of acute doses of IV 5-HT (6-192 µg/kg) in lightly-anesthetized rats with either bilateral removal of the superior cervical ganglion, or transection of either the carotid sinus (CSN) or glossopharyngeal nerve (glosso). There were no differences in baseline TF latencies between groups and all groups showed a dose-dependent inhibition of the TF reflex to IV 5-HT. Control rats had an ED_{50} of 40 µg/kg. Bilateral removal of the SCG, or transection of the glosso or CSN resulted in varying degrees of increased sensitivity to IV 5-HT (ED_{50} of 25, 10 and 12 µg/kg, respectively). These results suggest that these non-vagal afferents modulate the antinociceptive effect of IV 5-HT mediated by vagal cardiopulmonary afferents.

341.10

CHARACTERIZATION OF EFFECTS OF SEROTONIN ON CORTICAL SENSORY EVOKED POTENTIALS (SEP). K.A. Follett, S.T. Meller*, S.J. Lewis*, M.J. Brody and G.F. Gebhart. Departments of Neurosurgery and Pharmacology, The University of Iowa, Iowa City, Iowa, 52242.

Stimulation in nucleus raphe magnus in rats inhibits the tail-flick (TF) reflex and SEP. Intravenous (IV) serotonin (5-HT) produces profound antinociception as measured by changes in TF latency (Neurosci. Abstr., this volume). The purpose of this study was to determine whether 5-HT also attenuates SEP, thereby providing insight into whether IV 5-HT activates brainstem antinociceptive systems.

Sprague-Dawley rats were anesthetized, paralyzed and artificially respired. Ten cortical SEP produced by electrical stimulation of the hindpaw (10 mA, 0.5 msec, 1/10 sec) were recorded epidurally and computer-averaged. Graded doses (6-288 µg/kg) of 5-HT were administered IV, after which 10 consecutive SEP were again recorded. IV 5-HT produced a dose-dependent reduction of SEP amplitudes. The average dose required for 50% reduction was 32 µg/kg (>90% reduction at 64 µg/kg). The latency to onset of the effect (30-60 sec) and the duration of action (< 1 min to 7-8 min) were directly related to the magnitude of SEP reduction. The doses required and the duration of effect are comparable to those associated with TF inhibition by IV 5-HT, indicating that this effect may be due to activation of cardiopulmonary afferents, which in turn may activate brainstem antinociceptive centers.

341.11

ANTINOCICEPTIVE EFFECTS OF INTRAVENOUS SEROTONIN IN RATS. I. CHARACTERIZATION. S.T. Meller*, S.J. Lewis*, T.J. Ness, M.J. Brody and G.F. Gebhart (SPON: R. Schelper). Dept. Pharmacology, The University of Iowa, Iowa City, Iowa, 52242.

The aim of this study was to systematically examine the effects of intravenous (IV) serotonin (5-HT) on nociception and blood pressure (BP) as 5-HT has been shown to be algesic, and there are 5-HT receptors on cardiopulmonary afferents. IV 5-HT produced a dose-dependent (6-192 µg/kg) inhibition of the tail-flick (TF) reflex (ED_{50}=40 µg/kg). A variety of anatomical and pharmacological manipulations were performed to characterize the antinociceptive effect of IV 5-HT and associated changes in BP. Removal of reflex apnea by artificial respiration did not alter inhibition of the TF reflex by IV 5-HT. Chlorisondamine pretreatment abolished depressor responses to IV 5-HT, but did not significantly alter inhibition of the TF reflex. Acute BP changes of similar magnitude and duration observed with 5-HT were produced by IV nitroprusside, phenylephrine or trimethaphan but did not change TF latency from baseline. Bilateral vagotomy abolished up to 70% of the antinociceptive effect of IV 5-HT. Finally, low thoracic spinal cord transection abolished inhibition of the TF reflex produced by IV 5-HT. These results indicate that the antinociceptive effect is not secondary to apnea, or BP changes and support the notion that IV 5-HT is a potent noxious stimulus capable of activating vagal afferents and eliciting antinociception by engaging descending inhibitory systems.

341.12

ANTINOCICEPTIVE EFFECTS OF INTRAVENOUS SEROTONIN IN RATS. II. ROLE OF VAGAL AFFERENTS. S.J. Lewis*, S.T. Meller*, M.J. Brody and G.F. Gebhart (SPON: J. Godersky). Department of Pharmacology, The University of Iowa, Iowa City, Iowa, 52242.

We have reported that intravenous (IV) serotonin (5-HT) produces a dose-dependent inhibition of the nociceptive tail-flick (TF) reflex in Sprague-Dawley rats through activation of vagal afferent fibers capable of engaging descending inhibitory systems. In that study, bilateral vagotomy abolished about 70% of the antinociceptive effect of 72 µg/kg 5-HT. The aim of this study was to examine which specific vagal afferent nerves might mediate this effect. The antinociceptive effects of acute doses of IV 5-HT (6-192 µg/kg) was examined in lightly-anesthetized rats with either bilateral vagotomy, aortic depressor (ADN), or superior laryngeal nerve (SLN) transections. There were no differences in baseline TF latencies between groups and all groups showed a dose-dependent inhibition of the TF reflex. Control rats had an ED_{50} of 40 µg/kg. Following bilateral vagotomy there was a dramatic shift to the right (ED_{50}=90 µg/kg) of the 5-HT dose-response curve. In contrast, bilateral transection of either the ADN or SLN resulted in a greatly enhanced sensitivity to 5-HT (ED_{50}=15 and 17 µg/kg, respectively). These results indicate that there is a complex interaction between these vagal cardiopulmonary afferents in response to IV 5-HT.

341.13

EFFECTS OF HYPERBARIC STRESS ON RESPONSE PATTERN AND MORPHINE-INDUCED ANALGESIA IN THE FORMALIN TEST. O.-G. Berge, K. Furset* and I. Garcia-Cabrera*, Dept. of Physiol., Univ. of Bergen, N-5009 Bergen, Norway.

Rats administered formalin (0.1 ml, 5 %) in a hind paw, and saline or morphine subcutaneously, were exposed to ambient pressure of either 1 or 48 bar in a helium-oxygen atmosphere. The behavior of the animals was monitored for 35 min at stable pressure, starting 25 min after the injections. The 48-bar groups showed an increase in total motor activity and a dramatic reduction in pain-related behavior, due to reduced licking, biting, sustained lifting and protection of the injected paw. Brief paw-lifting was not altered and there was no increase in freezing at 48 bar. Significant effects of morphine on accumulated time of total pain-related activity were only present at 1 bar. The total number of pain-related responses was much less affected by pressure and was dose dependently reduced by morphine at both pressures. The results demonstrate the advantage of evaluating several criteria in the formalin test, show that hyperbaric exposure alters the response pattern in this test and provide evidence against pressure reversal of morphine analgesia.

341.14

OPIOID MEDIATED DEPRESSION OF NOCICEPTION BY HYPERCAPNIA IN THE RAT.

G.D. Gamble* & R.J. Milne, Dept. of Physiology, University of Auckland, New Zealand. (SPON: S.Pockett)

Moderate hypercapnia elevates pain thesholds in human subjects. We have administered CO_2 in the inspired gas mixture to conscious rats. CO_2 in the range 5 to 10% produced moderate hypercapnia (P_ACO_2 : 40±8 to 90±9 mmHg) and elevated tail flick and leg flexion latencies 2 to 3 fold in both intact and spinalised animals. Tail skin temperatures were unchanged. The effects on withdrawal reflex latencies but not on P_ACO_2 or $P_AO_2$were blocked by naloxone (2mg/kg), and were not present in morphine tolerant animals. The effects were reduced by dexamethasone but were not changed by adrenalectomy or by guanethidine, propanolol or phentolamine. Hypercapnia delayed the onset of the late phase of behavioural responses to formalin injected into the plantar surface of the hindpaw. We conclude that moderate hypercapnia powerfully depresses flexor withdrawal responses to noxious stimuli, by a mechanism involving release of endogenous opioids but not systemic catecholamines. This effect may account in part for the elevation in pain threshold during impaired respiration.

Supported by the Auckland Medical Research Foundation.

341.15

EFFECTS OF DORSAL COLUMN (DC) STIMULATION ON C_8-T_5 SPINOTHALAMIC TRACT (STT) CELLS. **M.J. Chandler, T.J. Brennan, D.W. Garrison, K.S. Kim and R.D. Foreman. Depts. Physiol. and Physical Ther., Univ. Okla. HSC, Okla. City, OK 73190.**

We hypothesized that inhibition of spinothalamic tract (STT) neurons may provide a neural mechanism for attenuation of pain observed clinically with epidural spinal electrical stimulation of dorsal columns (DC). In 11 α-chloralose anesthetized monkeys, extracellular potentials were recorded for neurons (C_8-T_5) antidromically activated from the ventral posterior lateral thalamus. Effects of stimulating ipsilateral DC at C_3-C_5 (30-300 µA, 50-200 Hz, 250 µs) were determined on spontaneous cell activity and on activity evoked by cardiac and by innocuous or noxious somatic stimuli. DC stimulation reduced spontaneous or somatic-evoked activity in 2 of 2 cells excited only by noxious pinch (HT), in 4 of 11 cells excited by both pinch and brushing hair (WDR) and in 6 of 7 cells excited by pinch but inhibited by brushing hair (HTi). Conditioning stimuli from DC at intervals of 20-160 ms reduced spikes evoked by electrical stimulation (1-35 V, 1 Hz, 100 µs) of cardiopulmonary A-δ spinal afferents in 11 cells. Intracardiac bradykinin (BK) increased activity in 5 of 9 STT cells. DC stimulation reduced BK-evoked activity in 3 cells, excited and then inhibited 1 cell, and did not affect 1 cell. Thus, DC stimulation may attenuate pain by reducing activity of STT neurons which receive nociceptive somatic or visceral input. (Medtronics; HL22732)

341.16

TREATMENT WITH B-VITAMINS ENHANCES DIFFUSE NOXIOUS INHIBITORY CONTROLS OF NOCICEPTIVE NEURONS IN THE RAT SPINAL CORD. Q.-G. Fu *, J. Sandkühler and M. Zimmermann (SPON: A.F. HAASE). II. Physiol. Inst. Univ., 6900 Heidelberg, FRG

Diffuse noxious inhibitory controls (DNIC) produce spinal inhibition via a supraspinal loop involving serotonergic mechanisms. We studied the possibility that the analgesic effect of B-vitamins may be related to DNIC.

Rats were treated daily for 7 days with 1 ml/kg s.c. of vitamin B compound (B_1/B_6/B_{12}, 100 mg/100 mg/ 1 mg in 3 ml) or isotonic saline. On day 8, discharges were recorded with microelectrodes from lumbar spinal dorsal horn neurons under pentobarbital anaesthesia. Neurons were activated by noxious radiant heat (50°C, 10s) applied to the hindpaw. DNIC was induced by 35 s of repetitive electrical stimulation (1 ms, 0.8-8.0 mA, 500 ms trains at 50 Hz) starting 5 s before heating, via pairs of needles inserted at tail, ipsilateral forepaw or contralateral hindpaw.

In both groups of treatment the heat-evoked responses were inhibited by electrical stimulation of ipsilateral forepaw or contralateral hindpaw. The degree of inhibition increased at increasing intensity of transcutaneous stimulation. However, in the animals treated with B-vitamins the inhibitory effect was greater than in control animals, and the increment of inhibition with increasing current of stimulation was much steeper than in the controls. Thus B-vitamins enhance DNIC of spinal nociceptive neurons.

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341.17

EFFECTS OF HIGH FREQUENCY TENS ON HEAT PAIN DISCRIMINATION. S. Marchand, M.C. Bushnell and G.H. Duncan. Cent. rech. sci. neurol. and Fac. méd. dent., Univ. Montréal, Canada H3C 3J7.

Numerous clinical reports have demonstrated that high frequency TENS can reduce patients' complaints of pain. We have recently verified, in a controlled laboratory setting, that TENS reduces subjects' evaluations of experimental heat pain (Marchand et al., 1988). In order to further evaluate the role of TENS in modulating sensory-discriminative aspects of nociception, this study assessed subjects' ability to detect small changes in noxious heat stimuli before, during and after high frequency TENS.

Ten subjects detected small differences in the intensity of painful heat stimuli applied to the cheek. Subjects participated in one TENS session and one placebo session, in which they discriminated small changes in painful heat (47°C to 47.1°, 47.25°, 47.4°, 47.55° and 47.7°C) and nonpainful visual stimuli, before, during and after the application of 100 Hz nonpainful TENS or placebo TENS.

When compared with the placebo sessions, nonpainful TENS significantly decreased the subjects' mean percent success for detecting the temperature changes during treatment (21% reduction during TENS, 2.9% *increase* during placebo TENS, $P<0.001$); in addition, this effect persisted after the treatment was terminated (12.2% reduction after TENS, 6.7% *increase* after placebo TENS, $P<0.05$). Correspondingly, the latency to detect the temperature changes was significantly increased during TENS compared to the placebo condition (11.5% increase versus 3.0% decrease, respectively, $P<0.01$), as well as after TENS (13.1% increase versus 4.5% decrease, respectively, $P<0.01$). Neither the percent success nor the latency to detect changes in the visual stimuli was altered by TENS.

These data show that the TENS treatment selectively altered the heat pain discrimination, thus indicating that activity in sensory-discriminative pain pathways is modulated by high frequency nonpainful TENS.

Supported by the Canadian MRC and the Quebec FCAR.

341.18

DOES A COULOMETRIC RELATION DETERMINE WHETHER ANALGESIA IN THE DECEREBRATE RAT IS OPIOID OR NONOPIOID IN FORM? M.W. Meagher, J.W. Grau, & R.A. King, Depts. of Psychology, Texas A&M University, College Station, TX 77843, and UNC, Chapel Hill, NC 27514.

Terman et al. (Science, 226: 1270, 1984) have suggested that the coulometric relation (mA*sec) determines whether shock directly activates an opioid or nonopioid analgesic system at the level of the brainstem: low coulometric products elicit an opioid analgesia, while high coulometric products elicit a nonopioid analgesia. Here we test this hypothesis by evaluating whether a coulometric relation determines the form of the analgesia (i.e. whether its opioid or nonopioid) observed in the decerebrate rat. We first identified the minimum coulometric products required to elicit analgesia in the decerebrate rat on the tail-flick test. We found that either 3, 25-sec, 0.5 mA or 3, 12.5-sec, 1.0 mA shocks were required. We then compared the form of the analgesia elicited by these shock schedules to that elicited by 3, 25-sec, 1.0 mA shocks. (Elsewhere we have shown that the analgesia observed after these shock schedules depends upon supraspinal systems.) All 3 shock schedules induced significant antinociception. However, naltrexone (14 mg/kg) had no effect on the analgesia observed in any of the groups. This suggests that only the nonopioid brainstem analgesic systems may be directly activated by shock. Supported by NIDA grant DA04259-01 to JWG.

341.19

INDEPENDENCE OF MOOD ELEVATION AND ANTINOCICEPTION INDUCED BY RUNNING IN MAN. M. Glusman, W.C. Clark*, M.N. Janal*, and J. Kuhl*. Lab. of Behavioral Physiology, New York State Psychiatric Institute, New York, N.Y. 10032.

Running produces stress induced analgesia and mood elevation in man (Janal, M.N., et al, Pain, 19:13-25, 1984). The present study shows that these effects are independent of each other. Antinocicept-ion requires prolonged hard running for its production, whereas mood elevation is elicited sooner and at lower levels of stress. Furthermore, under particularly stressful circumstances, e.g., a long hard run at an uncomfortably hot temperature, antinociception occurs without mood elevation.

In an initial experiment 37 Ss (13 trained runners and 24 sedentary Ss) ran the Bruce Protocol to 90% of maximal heart rate on a treadmill. Mean duration of exercise was 11.7 min. Mood, measured by visual analog scales, was significantly elevated postrun, whereas pain perception, measured by the ischemic arm pain test, was not significantly altered. In another experiment a 50 min outdoor run at near racing speed produced both antinociception and mood elevation in 12 trained runners. Lastly, a similar run by 5 trained runners in uncomfortably hot weather (mean temp. 30.9 C) produced antinociception and a decrease in mood ratings.

341.20

NOVELTY-INDUCED ANALGESIA IN THE NAPLES HIGH- AND NAPLES LOW-EXCITABLE RAT LINES. A.G. Sadile, H. Welzl*[2] and B. Siegfried*[3]. Inst. Human Physiol. & Med. Biophys., Univ. Naples, Italy; Insts. [2]Behav. Biol. & [3]Pharmacol., ETH & Univ. ZUrich, Switzerland.

Naples High-Excitable (NHE) and Naples Low-Excitable (NLE) rat lines are genetically selected for arousal in response to a novel environment (activity in a Lät-maze). Exposure to a spatial novelty has been reported to induce analgesia (Siegfried, B. et al., Behav.Neurosci., 101:436, 1987). If a differential reactivity to novelty underlies the behavioral differences found in the NHE and NLE rats, differences in novelty-induced analgesia (NIA) between the 2 lines should also occur. Therefore, we measured NIA by the tail-flick latency (TFL) method in NHE/NLE rats and in their controls (random bred and F_2 hybrids) before and at different times after exposure to a complex tunnel maze. All groups did not differ in their baseline TFL. After the exposure to novelty NLE rats increased their TFL to a larger extent than controls whereas the TFL of NHE rats remained close to baseline levels. These differences disappeared with ageing. Since, however, adult (6mo.) as well as ageing (15mo.) NHE/NLE rats were different in activity upon exposure to a novel environment, different mechanisms might underlie novelty-induced analgesia and novelty-induced hyperactivity.

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PSYCHOTHERAPEUTIC DRUGS: ANTIDEPRESSANTS

342.1

LITHIUM FACILITATION OF SYNAPTIC TRANSMISSION DEPENDS ON PROTEIN KINASE C (PKC). M.S. EVANS*, D.B. CLIFFORD and C.F. ZORUMSKI. Depts. of Neurology and Psychiatry, Washington University Sch. of Med., St. Louis, MO 63110.

Lithium is a commonly-used psychotherapeutic agent, but how it influences nervous system function is unclear. Lithium facilitates excitatory synaptic transmission in hippocampus. This is likely to be important for seizure syndromes caused by lithium, but it may also be important for lithium's therapeutic effects. Because lithium affects G proteins and PKC in other systems, we asked if this effect of lithium on synaptic transmission might involve one of these second messengers. We studied lithium's effect on extracellular EPSPs at the Schaffer collateral/CA1 synapse in rat hippocampal slices. Pretreatment of rats with intraventricular pertussis toxin did not alter synaptic facilitation by lithium. Activation of PKC by phorbol esters enhanced synaptic transmission, and occluded the facilitatory effect of lithium. Inhibition of PKC by H7 or spingosine did not markedly affect synaptic tran smission, but these treatments also eliminated lithium's facilitation of synaptic transmission. Lithium facilitates ecitatory synaptic transmission by enhancing transmitter release (Evans, et. al., Soc. Neuro sci. Abstr. 14:1094, 1988); In cultured pituitary cells lithium facilitates corticotropin release by a mechanism involving PKC (Zatz, M. and T.D. Reisine Proc. Nal. Acad. Sci. U.S.A. 82:1286-1290, 1985). suggesting that lithium may have a widespread effect on secretory processes involving PKC. This may account for some of lithium's psychotherapeutic and toxic effects.

342.2

EFFECTS OF CHRONIC LITHIUM TREATMENT ON PROTEIN KINASE C (PKC) AND PKC-MEDIATED PH CHANGES IN HL60 CELLS. J. A. Bitran,* F. Gusovsky, H. K. Manji,* and W. Z. Potter* (SPON: A. J. Nazarali). Lab. of Clin. Sci., NIMH, and Lab. of Bioorg. Chem., NIDDK, NIH, Bethesda, MD 20892.

Lithium effects on signal transduction are of great interest. We studied the effects of chronic LiCl treatment on dibutyryl cyclic AMP differentiated HL60 cells. Chronic treatment (7 days) with 1 or 10 mM LiCl markedly attenuated both the agonist (fMLP) and phorbol ester induced pH changes (thought to be mediated by PKC regulation of the Na/H antiporter activity). In contrast, chronic LiCl did not affect phosphoinositide turnover or agonist induced intracellular [Ca^{2+}] increases. These results suggested a selective LiCl effect on PKC, which we further investigated.

Chronic LiCl treatment resulted in decreases in both cytostolic and membrane associated PKC activity. In addition, immunoblot analysis demonstrated decreased PKC amounts in both fractions following chronic LiCl treatment. Thus, alterations in PKC function may represent a mechanism for the therapeutic actions of lithium. The possibility that modification of PKC function represents a primary (rather than a compensatory) effect of chronic LiCl treatment is currently under investigation.

342.3

THERAPEUTIC DOSES OF LITHIUM ENHANCE HYPOTHALAMIC SEROTONIN TURNOVER IN RATS: A MICRODIALYSIS STUDY. T. Baptista*[2], L. Hernandez*[2], J. L. Burguera*[3], M. Burguera*[3] & B.G. Hoebel[1] (SPON: D. Chou). [1]Dept. Psychol., Princeton Univ., Princeton, NJ 08544-1010, [2]Laboratorio de Fisiologia de la Conducta, Escuela de Medicina, Merida, Venezuela, [3]Departmento de Quimica, Facultad de Ciencias, Merida, Venezuela

Chronic administration of lithium salts improves the symptoms of bipolar disease; however, the mechanism of action of lithium is not well known. Some *in vitro* and *postmortem* studies strongly suggest that central serotonergic neurons might be involved in lithium therapeutic effects. In the experiments reported here microdialysis with removable probes and high pressure liquid chromatography with electrochemical detection were used to assess amphetamine-induced release of serotonin (5-HT) and its metabolite 5-HIAA in the perifornical hypothalamus (PFH) and hippocampus (HP) of freely moving rats before and after chronic lithium chloride administration (2 meq/kg, by intragastric daily injections for 14 days). With this treatment, the serum levels of lithium, as measured by atomic absorption spectrometry were 0.65 $\pm$ 0.2 meq/l. These levels are within the therapeutic window for humans. After lithium treatment, amphetamine-induced 5-HT release was significantly enhanced in the PFH but not in the HP. The basal levels of 5-HIAA in the PFH but not in the HP were also enhanced. We speculate that this effect of lithium on the hypothalamus could be related to the improvement in autonomic and cyclic symptoms of manic-depressive patients undergoing lithium therapy.

342.4

THE TRICYCLIC ANTIDEPRESSANT DESIPRAMINE HAS BIPHASIC EFFECTS ON THE GROWTH OF RAT PHEOCHROMOCYTOMA (PC12) CELLS IN VITRO. D.A. Tanen*, P. Montpied* and S.M. Paul*, (SPON:H. Khachaturian). Section on Molecular Pharmacology, Clinical Neuroscience Branch, NIMH, Bethesda, MD 20892 and Howard Hughes Medical Institute.

Rat pheochromocytoma (PC12) cells have proven useful as in vitro models of adrenal chromaffin cells and can differentiate in response to growth factors to assume the phenotypic properties of sympathetic (noradrenergic) neurons. Tricyclic antidepressants, such as desipramine (DMI), are believed to produce their therapeutic effects by altering the activity of central noradrenergic neurons, although their precise mechanism(s) of action remain obscure. Consequently, we have studied the effects of desipramine on a number of cellular properties of PC12 cells that are related to catecholamine release, biosynthesis, uptake, and metabolism. In the course of our experiments, we observed that incubation of PC12 cells with varying concentrations of desipramine (0.1-20 μM) results in a biphasic effect on cell growth.

PC12 cells were grown (1-14 days) in DMEM supplemented with 7% horse and fetal calf serum. Cells were incubated for various times in the presence and absence of desipramine, harvested, and counted using a Coulter Counter. Cell growth was also monitored by pulse labelling with [^{3}H] thymidine.

Desipramine (2-20 μM) resulted in an increase in cell number following long-term (5-14 days) incubation; whereas lower concentrations of desipramine tended to inhibit cell growth. Desipramine did not appear to alter the differentiation of PC12 cells. Experiments measuring [^{3}H] thymidine incorporation revealed an early (24 hour) effect of desipramine (1-20 μM) on DNA synthesis. These data suggest that desipramine may have an heretofore undescribed effect on PC12 cell growth. Studies on the effects of other chemically-related (and unrelated) antidepressants on PC12 growth will be presented.

342.5

THE EFFECT OF CHRONIC ADMINISTRATION OF ANTIDEPRESSANTS ON α_2 ADRENOCEPTORS IN THE LOCUS COERULEUS (LC). CE Aronson*[1], and GB Kovachich[2,3] (Spon: C O'Brien). Univ. of Penn. Sch. of Vet. Med.[1], Univ. of Penn. Sch. of Med.[2], Vet. Admin. Med. Ctr.[3], Phila., PA 19104.

Many antidepressants acutely suppress the firing rate of the LC, but the suppressed activity returns towards control values with repeated administration of these drugs. This "escape" of the suppressed firing rate may be accompanied by subsensitive responsiveness of the LC to α_2 adrenoceptor agonists. To determine if such reduced responsiveness could be accounted for by changes in α_2 adrenoceptors in the LC, we used the technique of quantitative autoradiography to measure these receptors (ligand: ^{3}H-idazoxan, 3nM, $\pm$ phentolamine, 5μm.) Antidepressants, administered to rats for 14-21 days, were protriptyline, phenelzine, mianserin, citalopram and sertraline. Protriptyline, phenelzine and mianserin produced statistically significant (about 15%) reductions in the binding of ^{3}H-idazoxan whereas neither citalopram nor sertraline caused this effect. Thus, antidepressants that acutely affect the functioning of noradrenergic nerves may reduce α_2 adrenoceptors in the LC. **(Supported by Research Funds from the Vet. Admin.)**

342.6

EFFECTS OF ANTIDEPRESSANTS ON LOCUS CERULEUS (LC) DISCHARGE: COMPARISON BETWEEN A NORADRENERGIC REUPTAKE INHIBITOR AND A SEROTONERGIC REUPTAKE INHIBITOR. A. L. Curtis, D. Parris* and R. J. Valentino. Department of Pharmacology, George Washington University Medical Center, Washington D.C. 20037.

Corticotropin-releasing factor (CRF) which has been hypothesized to be hypersecreted in depression, activates LC neurons and disrupts LC responses to phasic sensory stimuli. The hypothesis tested was that antidepressants have effects on LC discharge characteristics that are opposite to those of CRF. Acute administration of the norepinephrine reuptake inhibitor, desmethylimipramine (DMI, 0.1 and 0.3 mg/kg, i.v.), decreased LC spontaneous discharge rates of halothane-anesthetized rats and altered the response of LC cells to phasic sensory stimuli such that the ratio of evoked-to-tonic discharge was increased. These effects are opposite those produced by CRF. However, tolerance occurred with chronic DMI administration. In contrast, acute administration of the serotonin reuptake inhibitor, sertraline (SER, 1.0 and 3.0 mg/kg, i.v.), had no effect on LC spontaneous discharge, and decreased the ratio of evoked-to-tonic discharge during repeated phasic sensory stimulation. Chronic administration of SER resulted in an enhanced response of LC cells to phasic sensory stimuli and thus an increased ratio of evoked-to-tonic activity. The present results suggest that chronic administration of antidepressants that selectively inhibit serotonin reuptake may oppose the effects of CRF on LC responses to phasic sensory stimuli. The net effect of these drugs may be to enhance LC responsiveness to phasic sensory stimuli and this may be one mechanism of their antidepressant action. Supported by NIH grants MH 40008 and MH42796.

342.7

CHRONIC ANTIDEPRESSANT ADMINISTRATION ATTENUATES STRESS-INDUCED ACTIVATION OF NORADRENERGIC LOCUS CERULEUS (LC) NEURONS. Rita J. Valentino and Andre L. Curtis. Department of Pharmacology, George Washington Univ. Med. Ctr., Washington D.C., 20037.

Corticotropin-releasing factor (CRF) increases LC spontaneous discharge. Likewise, i.v. infusion of nitroprusside increases LC spontaneous discharge by releasing CRF. Because CRF is thought to be hypersecreted in depression, it was hypothesized that chronic antidepressant administration would either antagonize CRF or block stress-elicited CRF release. To test this hypothesis, LC spontaneous discharge was recorded in halothane-anesthetized rats that were chronically administered the norepinephrine reuptake inhibitor, desmethylimipramine (DMI, 10 mg/kg/day, i.p. for 21 days), or the serotonergic reuptake inhibitor, sertraline (SER, 10 mg/kg/day, i.p. for 21 days). I.C.V. administration of CRF (3.0 μg, i.c.v.) increased LC spontaneous discharge rate of chronic DMI and SER rats by a similar magnitude as reported for untreated rats. However, LC activation by nitroprusside infusion was greatly attenuated in rats chronically administered DMI indicating that stress-induced CRF release was decreased in these rats. This effect was not apparent in rats acutely administered DMI (0.3 mg/kg, i.v.), or in rats chronically administered SER. Chronic DMI did not alter the magnitude or time course of nitroprusside-elicited hypotension. The present results suggest that chronic administration of antidepressants which are selective in blocking norepinephrine reuptake may attenuate the hypersecretion of CRF in depression, and this may be one mechanism for their antidepressant effects. Supported by NIH Grants MH 40008 and MH 42796.

342.8

ELECTROPHYSIOLOGICAL EFFECTS OF THE ANTIDEPRESSANT CANDIDATE, NEFAZODONE, ON MONOAMINERGIC NEURONS IN RATS. C.P. VanderMaelen and J.P. Braselton. Bristol-Myers Company, Preclinical CNS Research, 5 Research Parkway, Wallingford, CT 06492.

Nefazodone, an antidepressant candidate chemically related to trazodone, is currently undergoing clinical evaluation. In the present series of experiments, the effects of systemically administered nefazodone on the spontaneous firing rates of noradrenergic neurons in the locus coeruleus (LC), serotonergic neurons in the dorsal raphe (DR) nucleus, and A9 dopamine neurons in the substantia nigra were assessed in male Sprague-Dawley rats anesthetized with chloral hydrate. Standard techniques for extracellular single-unit recording using glass microelectrodes were employed. Nefazodone (0.1-10.0 mg/kg, i.v.) produced a mild excitation of LC neurons (ED_{25} = 2.4 mg/kg, i.v.), while desipramine (1.0 mg/kg, i.v.) produced the expected suppression of firing. In the DR, nefazodone (0.1-3.2 mg/kg, i.v.) produced variable effects, with the predominant effect being a weak inhibition (ED_{25} = 1.3 mg/kg, i.v. for cases where inhibition occurred). By comparison, clomipramine (0.8 mg/kg, i.v. or greater) effectively inhibited DR neuronal firing. No consistent effect of nefazodone was seen for A9 dopamine neurons. These results when compared to results reported in the literature suggest that nefazodone has a unique electrophysiological profile compared to antidepressants of the MAOI, TCA, and 5-HT uptake inhibitor classes.

342.9

ANTIDEPRESSANT-LIKE EFFECTS OF TRAZODONE ON DIFFERENTIAL-REINFORCEMENT-OF-LOW RATE 72-S (DRL 72-S) BEHAVIOR IS MEDIATED BY THE PARENT COMPOUND, NOT THE METABOLITE M-CHLOROPHENYLPIPERAZINE. Gerard J. Marek, Timothy H. Hand, Abby A. Li* and Lewis S. Seiden. Dept. Pharmacological & Physiological Sciences, Univ. of Chicago, Chicago, IL 60637.

The atypical antidepressant drug trazodone tests similar to other antidepressant drugs (increased reinforcement rate and decreased response rate) on a behavioral screen for antidepressant drugs (the DRL 72-s schedule of reinforcement; Seiden et al.,1985). One of the most potent pharmacological actions of trazodone is antagonism of 5-HT_2 receptors. The main metabolite of trazodone in both humans and rodents is the 5-HT_{1B} agonist m-chlorophenylpiperazine (m-CPP). The present experiments were designed to determine if the antidepressant-like effects of trazodone on the DRL 72-s schedule are due to the parent compound or the major metabolite m-CPP. Administration of m-CPP (1-10 mg/kg,ip) to Sprague-Dawley rats 60, 30 or 10 min. before the behavioral session did not mimic the reinforcement rate increasing effects of trazodone (10-20 mg/kg). Next, the enzyme inhibitor proadifen (SKF 525 A; which blocks conversion of trazodone into m-CPP) was administered 3 hrs prior to trazodone. Proadifen (50 mg/kg,ip) by itself was without effect on DRL 72-s behavior. Proadifen administration prior to trazodone caused a > 30-fold leftward shift in the dose response curve for both the reinforcement rate and the response rate. These results suggest that the parent compound, and not the trazodone metabolite mCPP, mediates the antidepressant-like effects of trazodone on DRL 72-s behavior. This research is suppo ted by PHS MH-11191; RSA MH-10562 (L. Seiden) and MSTP Grant GM-07281 (Marek).

342.10

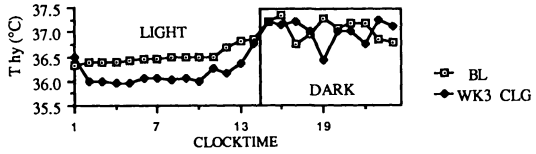
EFFECTS OF ANTIDEPRESSANTS ON BASAL & NE-STIMULATED ^{3}H-INOSITOL PHOSPHATE FORMATION IN RAT CORTICAL SLICES. L.E. Dyck and A.A. Boulton, Neuropsychiatric Research Unit, Dept. of Psychiatry, Univ. of Sask., Saskatoon, Sask., Canada S7N 0W0.

Some antidepressants (ADs) possess considerable affinity for α_1-antagonist receptors, and thus might be expected to interfere with NE-stimulated inositol trisphosphate formation, which is an α_1-receptor-mediated response. We investigated this possibility by incubating rat cortical slices (50 µl, ~1 mg protein) with ^{3}H-inositol (1 µCi) for 1 h to label phosphoinositides, and then measured the formation of ^{3}H-inositol phosphates (IPs) during a 1 h incubation in the presence of 10 mM LiCl. IPs were isolated by adsorption onto an anion exchange column. NE-stimulated IP formation was inhibited dose-dependently by tricyclic ADs, mianserin, citalopram and tranylcypromine (IC_{50}'s, ~ 3-200 µM). By contrast, basal rates of IP formation were stimulated by higher concentrations (≥ 0.1 mM) of the tricyclic ADs, but not by the other ADs. Prazosin did not block tricyclic AD-stimulated IP formation; therefore, the tricyclics did not seem to be acting either as inhibitors of the reuptake of endogenous NE or as partial α_1-agonists. An examination of the types of IPs formed by ADs showed increased amounts of inositol monophosphate and decreased amounts of inositol bisphosphate compared to control. The mechanism(s) of the latter effects is unknown; however, the efficacy of the ADs at inhibiting NE-stimulated IP formation correlated to their reported affinities for of α_1-antagonist-receptors. Supported by Rhone Poulenc Pharma Inc. and Sask. Health.

342.11

ANTIDEPRESSANT DRUG TREATMENT WITH AN MAOI DECREASES HYPOTHALAMIC TEMPERATURE IN SYRIAN HAMSTERS. B. Gao* and W. Duncan* (SPON: J. Passonneau). Clinical Psychobiology Branch, NIMH, Bethesda, MD 20892

We have previously observed that chronic treatment of Syrian hamsters with the antidepressant drug clorgyline (CLG), a type A monoamine oxidase inhibitor, results in REM sleep suppression and a decrease in peritoneal temperature (T_p). Both decreased T_p and REM sleep suppression may be related to altered hypothalamic temperature regulation. The current experiment measured hamster hypothalamic temperature (T_h) and motor activity during baseline (BL) and 3 weeks of CLG or saline (SAL) treament. Using Alzet mini-osmotic pumps, CLG (2mg/kg/day, n=4) or SAL (n=2) was administered s.c. to hamsters housed in LD 14:10 at 22°C. T_h and motor activity (A_m) were monitored continuously during the hamster activity-rest cycle using the Mini-Mitter telemetry system. Compared to the light phase BL, CLG decreased T_h by 0.4°C, 0.3°C and 0.3°C during weeks 1, 2 and 3. Light phase A_m was decreased by 27% during weeks 1, 2 and 3. Compared to the dark phase BL, CLG decreased T_h by 0.2°C and A_m by 17% during week 1. T_h normalized and A_m tended to normalize during weeks 2 and 3. These effects were not observed in SAL-treated hamsters. These results suggest that CLG decreases the hypothalamic temperature set-point during the light (or rest) phase of the hamster circadian cycle.



342.12

NEONATAL DESIPRAMINE ADMINISTRATION TO RATS AFFECTS IMMOBILITY IN THE PORSOLT SWIM TEST AND DIURNAL RHYTHM OF RUNNING WHEEL ACTIVITY. K.D. Dwyer*, K.S. Albrecht* and E.J. Roy. Neural & Behavioral Biology Program, University of Illinois, Champaign, IL 61820.

Neonatal administration of antidepressants to rats has been proposed as an animal model of depression. In the present study we measured behavioral and biochemical indices to examine the validity of this proposed animal model of depression.

Neonatal rats were treated with saline or 5mg/kg desipramine (DMI) each day for 11 days. At 1 mo of age, open field behavior of the rats was examined. At 2 mos of age the animals were tested for immobility in a 15 min Porsolt swim test. 24 hrs later immobility was measured again in a 5 min swim test. Between the 2 swim tests the animals were administered antidepressants or saline. At 4 mos of age the female rats were placed in running wheels to assess diurnal rhythms. Several biochemical indices were measured post-mortem.

Consistent with previous studies, rats treated neonatally with DMI showed increased immobility in the first swim test. However in the second swim test animals failed to increase immobility. The rats treated neonatally with DMI showed a phase-advance in running wheel activity on proestrus, similar to the changes in diurnal rhythms associated with depression. The results of this study indicate that neonatal DMI administration produces changes in the Porsolt swim test and changes in diurnal running activity suggestive of a useful animal model of depression, but further validation is necessary.

342.13

BEHAVIORAL CHARACTERIZATION OF THE NOVEL ANXIOLYTIC/ANTIDEPRESSANT AGENT WY-50,324 H. Morris*, S.M. White, A.T. Shropshire*, M. Abou-Gharbia, C.A. Boast and J.A. Moyer. Wyeth-Ayerst Research, CN 8000, Princeton, NJ 08543-8000

Wy-50,324 [N-(2-(4-(2-pyrimidinyl)-1-piperazinyl)ethyl)tricyclo(3.3.1.1(3,7) decane-1-carboxamide] is a novel adamantyl piperazine derivative that has been shown to have both 5-HT1A partial agonist and 5-HT2 antagonist activity (Moyer et al., Soc. Neurosci. Abstr. 1989). Wy-50,324 reduced mouse isolation-induced aggression (MED = 10 mg/kg i.p.) and in rats weakly reduced avoidance responding (AB50 = 25, 30 mg/kg i.p.), and produced hypothermia. This profile is similar to that of some nonbenzodiazepine anxiolytics. Unlike these agents, Wy-50,324 produced significant effects in Geller-Seifter conflict in rats by increasing punished responding at a dose which did not reduce VI responses (10 mg/kg i.p.). In ancillary studies, Wy-50,324 did not produce catalepsy or antagonize apomorphine-induced effects; it produced limited sedation/ataxia, and showed no ethanol interaction effects in mice. These results indicate that Wy-50,324 has a unique anxiolytic behavioral profile with limited CNS side effects.

342.14

PSYCHOPHARMACOLOGICAL PROFILE OF WY-50,324 - A NOVEL ANXIOLYTIC/ANTIDEPRESSANT AGENT. J.A. Moyer, R.F. Kucharik*, T.H. Andree, S.M. White, D. Grimes*, R.A. Scerni*, R.L. Fenichel* and M. Abou-Gharbia. Wyeth-Ayerst Research, CN 8000, Princeton, NJ 08543-8000

Wy-50,324 [N-(2-(4-(2-pyrimidinyl)-1-piperazinyl)ethyl)tricyclo(3.3.1.1(3,7)) decane-1-carboxamide] is a novel adamantyl piperazine derivative. Like non-benzodiazepine (BZ) anxiolytics, Wy-50,324 has very high affinity at 5-HT1A receptors *in vitro* (Ki = 1nM) and demonstrates partial 5-HT1A agonist activity *in vivo* in rat serotonin syndrome tests (agonist ED50 = 13 mg/kg i.p., antagonist ED50 = 8 mg/kg i.p.). Unlike most non-BZ anxiolytics, Wy-50,324 also has high affinity at 5-HT2 receptors (Ki = 66nM) and shows 5-HT2 antagonist activity in functional tests (quipazine-induced head shakes in rats ED50 = 0.6 mg/kg i.p.; platelet aggregation IC50 = 4.4 µM; isolated rabbit aorta pKa = 6.5). These results indicate that Wy-50,324 has both 5-HT1A partial agonist and 5-HT2 antagonist activity. This combined activity suggests Wy-50,324 is a unique anxiolytic/antidepressant agent. Wy-50,324 has low activity at D2, Alpha 1, Alpha 2, and BZ receptors suggesting a favorable side effect profile.

342.15

IMIPRAMINE AS A DISCRIMINATIVE STIMULUS IN PIGEONS. L. Zhang and J.E. Barrett, Dept. of Psychiatry, Uniformed Services University of the Health Sciences, Bethesda, MD 20814-4799.

Although the major mode of action of imipramine (IMI) is to block the uptake of norepinephrine (NE) and serotonin (5-HT), IMI also affects other neurotransmitter systems. The role of these different systems in determining the behavioral effects of IMI was investigated using a drug discrimination procedure with IMI as the training drug. Key pecking by pigeons was reinforced with food following the completion of 30 pecks on one key following IMI (3.0 or 5.6 mg/kg, i.m.); following saline, pecks on a second key produced food. The tricyclic antidepressants, amitriptyline, doxepin and desipramine all produced drug-appropriate responding. Substitution for IMI also occurred with cocaine and amphetamine, as well as with the NE uptake inhibitors tomoxetine and nomifensine. The 5-HT_{1A} receptor ligand 8-OH DPAT also produced responding on the IMI key. Generalization did not occur with the following drugs: fluoxetine, clonidine, ritanserin and GBR 12909; substitution for IMI was obtained with some pigeons with gepirone, buspirone, and scopolamine. These results confirm that the IMI discriminative stimulus in pigeons is complexly mediated and is based in large part on drugs interacting with NE and 5-HT systems. Effects with 8-OH-DPAT suggest that compounds with activity at the 5-HT_{1A} site may be clinically effective antidepressants and that the activity of IMI may be mediated in part through this receptor site.

342.16

THE ANTIDEPRESSANT PHENELZINE AND METABOLISM OF γ-AMINOBUTYRIC ACID AND ALANINE IN RAT BRAIN. G.B. Baker and I.L. Martin*. Neurochemical Res. Unit, Dept. of Psychiatry, Univ. of Alberta, Edmonton, Canada T6G 2B7 and *MRC Molecular Neurobiology Unit, Univ. of Cambridge Medical Sch., Cambridge CB2 2QH, UK.

Phenelzine (phenylethylhydrazine) is a monoamine oxidase-inhibiting antidepressant effective in treating panic disorder. In addition to elevating brain biogenic amines, this drug has been reported to cause an increase in concentrations of GABA and alanine in rat brain. In our laboratories, the effects of phenelzine on rat whole brain activities of GABA transaminase and alanine transaminase were investigated. Male Sprague-Dawley rats were injected with phenelzine (15mg/kg ip) and killed 1, 2, 4, 8 or 16h after injection. Significant inhibition of both enzymes was still evident at 16h after injection. A study at 4h of doses of phenelzine ranging from 2.5 to 30mg/kg indicated dose-dependent inhibition of both enzymes. Such inhibition may contribute to the overall clinical profile of this drug and to its behavioural and physiological effects in laboratory animals. Funds provided by the British and Canadian Medical Research Councils.

342.17

CHRONIC ANTIDEPRESSANT DRUG (AD) TREATMENT ATTENUATES HYPOTHERMIA CAUSED BY ELECTROCONVULSIVE SHOCK (ECS). C. H.Gleiter[1,2] K.M. Wozniak[1] and J.DeJong[1]*. [1]NIAAA/LCS, Bethesda, MD 20892; [2]HPI Ciba-Geigy, D-7400 Tübingen, FRG.

ECS-induced body temperature decrease in mice has been shown to be markedly attenuated following chronic ECS[1]. Therefore we investigated the effect of acute and chronic AD treatment on ECS-induced hypothermia in NIH Swiss mice. Animals received desipramine (DMI), fluoxetine (FLUOX), amitryptiline (AMI), pargyline (PARG) (all 10 mg/kg i.p.) or saline. Rectal temperature was recorded at 0, 15, 30, 45, 60 ,90 min following a single ECS. ECS (8 mA, 1 s, no anesthesia) was applied 24 h after a single drug injection, and 24 h, 1 week and 2 weeks after the last of 14 once-daily injections. Hypothermic response to a single ECS at the various time points of AD treatment (expressed as area under the temperature-time curve):

	SALINE	DMI	FLUOX	AMI	PARG
single	188±16	235±53	188±63	223±32	203±44
chronic	200±39	82±11**	52±28**	94±28**	87±27**
1 week	174±44	201±32	143±45	163±51	132±37
2 weeks	154±41	137±22	162±40	125±11	165±25

(mean ± SD; n = 9/group; °C x min; ** $p < 0.001$)
Conclusions: 1. Chronic AD treatment attenuates the hypothermic response to a single ECS in a similar way as repeated ECS[1]. 2. This resembles the attenuation by chronic ECS or AD treatment of 8-OH-DPAT or clonidine-induced hypothermia (see [1]). 3. The (common ?) mechanism remains unclear. [1]Gleiter et al. Convul. Ther. 5: in press (1989).

342.18

USE OF S-ADENOSYL-METHIONINE, A NATURALLY OCCURRING METHYL DONOR, IN THE TREATMENT OF DEPRESSION. N.Z. Rosenlicht*,B.L.Kagan*,D.L.Sultzer* and R.H.Gerner. West Los Angeles V.A. Medical Center, Dept. of Psychiatry, UCLA Sch. of Med. and UCLA Neuropsychiatric Inst., Los Angeles, CA 90024.

18 inpatients meeting DSM-III criteria for major depression were randomly assigned to receive 21 days of oral S-adenosyl-methionine (SAM) or placebo. All patients underwent an initial 7 day drug-free washout period. Clinical status was monitored using Hamilton, Caroll, and global rating scales at 0, 3, 7, 14, and 21 days of treatment. Of the 15 patients who completed the study, the SAM treated group showed significant improvement in their depressive symptoms, as measured by the above scales. Other than 1 patient who became manic, despite no prior history of bipolar affective disorder, virtually no side effects were noted.

This study indicates that oral SAM is a safe and effective antidepressant, and that the role of methylation in the pathophysiology of affective disorders is an an area deserving of further study.

342.19

CHRONIC ANTIDEPRESSANT TREATMENT AUGMENTS COUPLING OF THE GTP BINDING PROTEIN, Gs, TO THE CATALYTIC MOIETY OF ADENYLATE CYCLASE. H. Ozawa and M.M. Rasenick, Physiology and Biophysics, University of Illinois College of Medicine, Chicago, IL 60680.

Despite nearly two decades of research, a clear molecular locus for antidepressant action has not been defined. This laboratory and others have postulated that G proteins formed a part of that locus, but additional data were required to make a more clear hypothesis. This study demonstrates that chronic treatment of rats with antidepressant drugs (iprindol or amitryptilline) or electroconvulsive shock, increases activation of adenylate cyclase in membranes prepared from various regions of rat brains. Inhibition of adenylate cyclase is not affected by these treatments and the chemical elimination of Gs abolishes all differences between treated and control groups. Non-neuronal tissues from treated animals showed no differences in adenylate cyclase than those tissues from control animals. The dissociation constant (K_d) and maximal binding (B_{max}) for [^{32}P] AAGTP (an hydrolysis-resistant, photoaffinity GTP analog) to synaptic membrane G proteins was unchanged after antidepressant treatment. Thus, it is unlikely that there was any change in the quantity or GTP-binding capacity of Gs. However, the transfer of [^{32}P] AAGTP from Gi/o to Gs was accelerated by chronic antidepressant treatment. Our findings are consistent with chronic administration of ECS inducing enhanced coupling between Gs and the adenylate cyclase enzyme as well as increasing the interaction between Gs and Gi/o. Supported by MH 39595.

342.20

A DOUBLE-BLIND PLACEBO CONTROLLED STUDY OF CAPTOPRIL IN THE TREATMENT OF MAJOR DEPRESSION. L. Germain*, G. Chouinard*, L. Annable*, (Sponsor: Y. Lamarre), Clinical Psychopharmacology Unit, Department of Psychiatry, McGill University, Montreal, Quebec.

Captopril is a potent inhibitor of the brain converting enzyme (CE). The CE is located in the human hippocampus, septum, amygdala, hypothalamus, frontal cortex, caudate nucleus and mid-temporal cortex, and it degrades several neuropeptides. In order to assess the effect of captopril on mood we studied 14 patients with major depression (DSM-III). Following diagnostic screening, all patients were voluntarily admitted to a research ward for 7 weeks. They were randomly assigned to treatment with captopril (C) or placebo (P) in a double-blind parallel group clinical trial. The dosage of their medication was doubled weekly from 37.5 to 150 mg/day. Their mental status was assessed weekly with the Hamilton Rating Scale for depression (HAM-D) and dexamethasone suppression tests were performed (DST). Analysis of covariance with baseline scores as covariate revealed that the C group (n=8) had significantly ($p=0.06$) lower mean HAM-D scores than the P group (n=6) after two weeks. A significant ($p<0.05$) change of the DST was observed in the C group but not in the P group. These results suggest that captopril may have an antidepressant effect with an early onset of action. Further study is necessary to clarify its long-term effect.

342.21

LONG-TERM TRICYCLIC AND ELECTROCONVULSIVE TREATMENT INCREASES RESPONSIVENESS OF DORSAL HIPPOCAMPUS 5-HT$_{1A}$ RECEPTORS: AN ELECTROPHYSIOLOGICAL STUDY IN THE RAT.

C. de Montigny, Y. Chaput and P. Blier. Dept. of Psychiatry, McGill University, Montréal, Canada H3A 1A1.

Suranyi-Cadotte et al. (Can. Coll. Neuropsychopharm., 10:O-22,1988) have reported an increased number of [^{3}H]8-OH-DPAT binding sites in the rat dorsal hippocampus following long-term tricyclic antidepressant treatment but no change following 5-HT reuptake blocker administration. In the present study, the responsiveness of CA$_3$ dorsal hippocampus pyramidal neurons to microiontophoretically-applied 5-HT and 8-OH-DPAT was compared in control rats and in rats which were administered one the following treatments: the tricyclic imipramine (10 mg/kg, i.p.), the selective 5-HT reuptake inhibitor paroxetine (5 mg/kg, i.p.), the monoamine oxidase inhibitor clorgyline (1 mg/kg, s.c.) daily for 21 days or 7 electroconvulsive shocks (ECS). For the latter group, controls were administered subconvulsive shocks. The efficacy of the stimulation of the ascending 5-HT pathway in suppressing the firing activity of the same postsynaptic neurons was also studied in the same rats. The responsiveness to both 5-HT and 8-OH-DPAT was increased in the ECS and in the imipramine groups whereas it was unchanged in the paroxetine group and decreased in the clorgyline group. In all four treatment groups, however, the efficacy of the stimulations of the 5-HT pathway was increased, thus indicating an enhanced 5-HT transmisssion by all these antidepressant treatments.

Consistent with the demonstration that 5-HT$_{1A}$ receptors mediate the hyperpolarizing effect of 5-HT on dorsal hippocampus pyramidal neurons (Andrade and Nicoll, J. Physiol., 394:99,1987), and the radioligand binding data of Suranyi-Cadotte, the present results provide further evidence that antidepressant-induced modification of the postsynaptic responsiveness of dorsal hippocampus pyramidal neurons to 5-HT is attributable to an alteration of 5-HT$_{1A}$ receptors.

342.22

BEHAVIORAL AND BIOCHEMICAL EVIDENCE FOR DOWNREGULATION OF 5HT1A RECEPTORS BY SERTRALINE.

L.S. Reynolds, M.H. Connolly*, S. McLean and J. Heym. Central Res. Div., Pfizer Inc., Groton, CT 06340.

Clinical trials have shown sertraline, a potent and selective inhibitor of 5HT reuptake, to be an effective antidepressant (AD). As with all ADs, however, there is approximately a 2 week latency before a therapeutic response is achieved. This observation has prompted examination of the effects of chronic AD treatment on monoaminergic function in brain. Our most recent experiments have studied the effect of chronic sertraline treatment on postsynaptic 5HT1A receptors.

Sertraline (10 mg/kg/day) or vehicle was continuously infused into adult male rats via subcutaneously implanted minipumps. After 14 days of treatment, the sensitivity of postsynaptic 5HT1A receptors was assessed by examining the potency of 8-OH-DPAT, a 5HT1A agonist, to elicit reciprocal forepaw treading (RFT). Animals were sacrificed 24 hours later for *in vitro* analysis of 5HT1A receptors in forebrain using radioligand binding with [^{3}H]-8-OH-DPAT. Chronic but not acute administration of sertraline shifted the dose-response curve for 8-OH-DPAT-elicited RFT to the right with nearly a 2 fold decrease in potency. This attenuated behavioral response was accompanied by an 18% decrease in the number of 5HT1A receptors in hippocampus without a change in receptor affinity.

These data further indicate that chronic treatment with sertraline produces changes in monoaminergic function that are not apparent after administration of a single dose and these adaptations may be fundamental for alleviation of clinical depression.

342.23

CHRONIC IDAZOXAN (IDZ) ATTENUATES ISOPROTERENOL - INDUCED CYCLIC AMP PRODUCTION IN C6 GLIOMA CELLS.

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Chronic treatment with a number of antidepressants produces a subsensitivity of rat adrenoceptor linked adenylate cyclase. This effect has generally been attributed to increased synaptic norepinephrine; however, a direct postsynaptic effect has also been implicated by recent in vitro studies (Fishman and Finberg, J. Neurochem. 49:282-9, 1987).

We undertook the present study to investigate the effects of chronic IDZ (a selective alpha-2 antagonist, and a putative antidepressant) on signal transduction in C6 glioma cells. Incubation of C6 cells with 1 or 10 uM IDZ for 5 days resulted in a significant decrease in isoproterenol-stimulated cAMP production. In contrast, forskolin-stimulated cAMP production was unaffected. The effects of chronic IDZ at the receptor and G protein level were also examined, and the results suggest an uncoupling of the beta adrenoceptor from the adenylate cyclase.

BRAIN METABOLISM AND BLOOD FLOW II

343.1

LOCAL CEREBRAL PROTEIN SYNTHESIS IN THE CAT DURING NORMAL DEVELOPMENT.

J. F. Kerrigan*, H. T. Chugani*, D. A. Hovda, J. R. Villablanca, S-C. Huang*, J. Barrio*, and M. E. Phelps. UCLA School of Medicine, Los Angeles, CA 90024, USA

We have measured local cerebral protein synthesis rates (lCPSR) in normal cats using in vivo incorporation of L-(1-^{14}C)-leucine and quantitative autoradiography. Animals were studied at 15 (n=1), 30 (n=1), 45 (n=3), 60 (n=5), 90 (n=3), 120 (n=2), and 180 days(n=1). lCPSR was calculated for 50 brain regions representing visual, auditory, motor, extrapyramidal, cerebellar, thalamic, limbic and brainstem subsets. lCPSR changed over the course of development with most brain regions increasing during early time points, reaching a metabolic peak by 45 days of age, when leucine incorporation rates were up to 2.7x those seen at 15 days. lCPSR for most brain regions then declined, approaching 15 day values by 180 days of age. Some structures, including those involved in motor and visual function, showed a second metabolic peak at 90 days. Although lCPSR for virtually all structures peaked at 45 days, changes in protein synthesis rates with time showed many individual characteristics. Absolute values of lCPSR varied widely from structure to structure, ranging from 2.0 nmole/min/gm in septum to 6.8 nmole/min/gm in medial habenula (45 days). In many structures the maturation of lCPSR correlated well with previosly established developmental neurobehavioral milestones. We plan to use these normative data to evaluate metabolic response during recovery in brain-lesioned animals.

343.2

VASCULAR EFFECT OF ACETAZOLAMIDE ON THE CHOROID PLEXUS.

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Acetazolamide inhibits carbonic anhydrase and produces a marked reduction in cerebrospinal fluid (CSF) production. Studies in vitro suggest that acetazolamide also constricts blood vessels of the choroid plexus. The goal of the present study was to examine effects of acetazolamide on blood flow to the brain and choroid plexus. We measured blood flow (microspheres) to the brain and choroid plexus and production of CSF (ventriculo-cisternal perfusion) in anesthetized rabbits. Under control conditions, blood flow to brain and choroid plexus was 49±5 (mean±SE) and 402±48 ml/min × 100 g, respectively, and CSF production was 9.4±0.9 μl/min. Acetazolamide (25 mg/kg, i.v.) decreased production of CSF by 55±5% but increased cerebral blood flow and blood flow to the choroid plexus more than two-fold. Acetazolamide also produces hypercapnia. In animals in which the hypercapnia was prevented by increases in ventilation, blood flow to choroid plexus tended to increase even further. Thus, acetazolamide decreased production of CSF but, in contrast to predictions based on studies in vitro, acetazolamide produced a marked increase in blood flow to the choroid plexus. These findings indicate that blood flow to the choroid plexus and production of CSF are uncoupled under some conditions.

343.3

IN-VIVO CREATINE KINASE REACTION RATES IN HYPOXIC BRAIN. D. Holtzman, M. Offutt*, L.J. Neuringer*. Neurology, Children's Hospital, Boston, MA 02115 and Nat'l Magnet Lab, MIT, Cambridge, MA 02139.

Brain creatine kinase (CK) forward reaction rates [phosphocreatine (PC) to ATP] were studied in young adult mice made hypoxic with cyanide (KCN) using high field (8.45T) NMR spectroscopy and the saturation transfer technique. pH was measured from chemical shift of the inorganic phosphate (P_i) peak. Sublethal KCN produced one of two responses: 1) no change in PC, 60% increase in P_i with two P_i peaks present, and a transient doubling of the CK rate constant; or 2) a transient 35% decrease in PC, increased P_i with two peaks (pH 7.16 and 6.70), and a 3-fold decrease in CK rate constant. Lethal KCN doses produced a 40-60% decrease in PC followed by a fall in both PC and ATP. There were two P_i peaks (pH 7.15 and 6.4) and a 6-fold increase in CK rate constant. The two P_i peaks suggest two intracellular pools including one with a stable concentration and pH of 7.15 under severe hypoxic conditions. Phosphate flux (PC to ATP) doubles with mild hypoxia but is markedly decreased with moderate hypoxia. [NRSA (NS 08039) and Milton Fund grants to DH, NIH Resource Grant (RR 00995) to LN, and a Mental Retardation Center Grant from NICHD (P 30 - HD 18655)].

343.4

INCREASED CEREBRAL CORTICAL TISSUE WATER CONTENT AFTER 3-4 WEEKS OF HYPOBARIC HYPOXIA (0.5 ATM). J.C. LaManna, M. Cullen*, L.M. Vendel*, W. Austin*, and K.P. Strohl. Depts. Neurology, Medicine and Physiol./Biophys., Case Western Reserve University, Cleveland, OH 44106, U.S.A.

We have previously reported that regional cerebral blood flow is not suppressed by the hypocapnia that accompanies hyperventilation in response to hypobaric hypoxia, demonstrating cerebrovascular adaptation to long term mild oxygen deficit. In this study we report the long term effects of hypoxia on brain water and ion content. Adult male Wistar rats (n=5) were kept in a hypobaric chamber for 3-4 weeks at 0.5 ATM, littermate rats were used as normoxic (n=10) and acute hypoxic (n=12) contols. Rats were anesthetized with chloral hydrate (400 mg/kg, i.p.) for placement of arterial and venous cannulae, and allowed to recover in an hypoxic environment, except for the normoxic controls which were kept in room air at normal pressure. After 3 hrs rats were sacrificed and the mid-brain and bilateral samples of the frontal and occipital cortex were removed and frozen. Acute hypoxic (PaO_2: 47±2 vs 93±2 mmHg) rats were hypocapnic ($PaCO_2$: 23±1 vs 34±1 mmHg) and alkalotic (pH: 7.50±.01 vs 7.38±.01), but otherwise were not different from normoxic controls. Chronic hypoxic rats (PaO_2: 50±3 mmHg) were also hypocapnic ($PaCO_2$: 19±2 mmHg), but had normal arterial pH (7.38±.03) and elevated hematocrit (71±2) compared to normoxic (46±1) or acute hypoxic (49±1) rats. The water content of the cerebral cortical structures was increased to 82% compared to 79% in the control groups. Na^+ and K^+ were also elevated in these cerebral cortical samples. There was no increase in water or ion content of the mid-brain samples. Thus, rats exposed to chronic hypoxia of up to 1 month demonstrated a tendency toward development of cerebral edema in spite of compensatory cerebrovascular responses.

343.5

FACTORS AFFECTING LACTATE RELEASE FROM HIPPOCAMPAL SLICES. H.M.Assaf*,A.J.Ricci*,T.S.Whittingham, J.C. LaManna,R.A.Ratcheson,and W.D.Lust. Lab. of Exper. Neurological Surgery, Case Western Reserve Univ. School of Medicine, Cleveland, OH 44106

Intracellular lactic acid (L) accumulation has been implicated in the evolution of brain damage after ischemia. Since compartmentation of L may play a role in acid-base balance during a number of metabolic stresses, L release was examined in hippocampal (H) slices under a variety of conditions. Gerbil H slices were preincubated for 1 h in artificial cerebrospinal fluid (ACSF) equilibrated with 95%O_2/5%CO_2 (pH 7.4 at 37°C) and then transferred to tubes containing 300 ul of test media. 15 ul aliquots were taken at various times of incubation and assayed for L. The initial rate of L release in cont slices was 6.5 nmol/min/mg prot and increased 2-and 4-fold under conditions mimicking ischemia and anoxia, respectively. Elevated K^+ (60 mM) increased L release by nearly 3-fold. Release was temperature-dependent. Removing Ca^{++} or adding D-Lactate (5mM) to the ACSF had only a minimal effect. Pyruvate (10 mM) decreased L release by more than 50% in cont and anoxic ACSF. If L is released in the uncharged form, loss of cellular protons would tend to minimize large pH fluctuations among the various CNS compartments.

343.6

LACTATE TRANSPORT PROBABLY DOES NOT MEDIATE THE HIGHLY TEMPERATURE-SENSITIVE FALL IN pH_0 EVOKED BY ANOXIA IN HIPPOCAMPAL SLICES. W.Walz[1] and K.Krnjević. Anaesthesia Research Department, McGill University, Montréal, H3G 1Y6. P.Q., Canada.

At 21-22°, brief anoxia (95% N_2 + 5% CO_2 for 2 min) causes inconsistent changes in pH_0 (+0.042 ± 0.034), recorded in CA1 pyramidal layer with pH-selective microelectrodes; but at higher temperatures, it produces an increasing acidosis, reaching 0.092 ± 0.037 pH units at 34-35°. This acidosis depends on anaerobic glycolysis, being abolished by superfusion with glucose-free medium. The possibility that it is directly caused by anaerobic lactate production and its efflux via specific lactate transport was tested by applications of DL-p-hydroxyphenyl-lactic acid (HPL), a selective antagonist of lactate efflux in cultures of hippocampal cells (Walz and Mukerji, 1988, Glia 1, 366). In slices at 33-35°, 8 mM HPL failed to reduce the Δ pH_0's evoked by 2 min anoxic tests. In conclusion, the transient fall in pH_0 produced by 2 min of anoxia is probably not mediated by specific lactate transport, but rather by some other mechanism, such as Na^+-H^+ exchange (Supported by Canadian Medical Research Council).

[1]on leave from Physiol. Dept, Univ. of Saskatchewan, Saskatoon.

343.7

WITHDRAWN

343.8

ENERGY METABOLITES IN THE ISCHEMIC PENUMBRA. B. A. Kaplan* and W.A.Pulsinelli. Dept.of Neurology, Cornell Univ. Sch. of Med.,New York, NY 10021

The penumbra of evolving brain infarction has been defined as a region with reduced cerebral blood flow (CBF), impaired synaptic transmission but preserved ionic gradients and cell morphology. The quantitative relationship between penumbral CBF and energy metabolism is not well defined. We measured CBF with laser doppler probes and energy metabolites fluorometrically at sites in the core and periphery of ischemic cortex in Spontaneously Hypertensive rats subjected to middle cerebral/common carotid artery occlusion for 15, 60 or 240 min. Sites with CBF values between 15-35% of preocclusion values were sampled from both the periphery and core zones of ischemia.

Metabolites (μmol/g) at CBF 15-35% of Preocclusion Values

	CBF(%)	Lactate	Glucose	ATP	PCr
Sham	100	1.67	2.06	2.81	4.89
(n=15)		±0.17	±0.10	±0.11	±0.13
Core	21.4a	8.81a,b	0.27a,b	0.24a,b	0.57a,b
(n=11)	±1.49	±1.14	±0.09	±0.05	±0.09
Periph.	25.7a	5.21a	1.98	2.23	3.86a
(n=10)	±1.81	±0.92	±0.34	±0.28	±0.62

a $p \leq 0.05$ vs.sham, b $p \leq 0.05$ vs. periph. (ANOVA, Bonferroni correction)

Despite the similar degree of ischemia in the core and periphery, high energy metabolites and glucose levels were severely reduced in the core while in the periphery they were maintained near normal. These data are consistent with the supression of energy demands in the periphery of evolving infarcts.

343.9

COMBINED qEEG AND NMR SPECTROSCOPIC ANALYSIS OF BRAIN IN A RABBIT MODEL OF HYPOGLYCEMIC COMA.
K. H. Taber, M. J. Herold* and J. D. Frost*.
Dept.s of Radiology, Neurology and Magnetic Resonance Center, Baylor College of Medicine, Houston, TX 77030.

Combined 31 phosphorus NMR spectroscopic and qEEG measures were used to delineate the metabolic and functional changes that occur in brain during and following induction of hypoglycemic coma. All NMR aquisitions were performed on a Bruker 2.35T MSL Spectrometer utilizing a 1.5 cm surface coil. Physiologic parameters were monitored with a Lifescope II telemetry system (Nihon Kohden). Blood glucose was tested every 5-10 min utilizing a Glucometer II (Ames) glucose meter. EEG was recorded with a modified Neuropak II recording system (Nihon Kohden). Hypoglycemic coma was induced by injection of insulin and reversed following a set duration of coma by infusion of glucose. Blood glucose and EEG changes preceeded 31P spectral alterations in all rabbits. Correlation analysis was used to identify related measures and provide insight into how metabolic alterations are reflected in electrical activity as well as indicate parameters associated with permanent tissue damage.

343.10

THE ROLE OF HEMATOCRIT IN THE REDUCTION OF CEREBRAL BLOOD FLOW MEASURED DURING HYPERGLYCEMIA.
R. B. Duckrow, Department of Medicine, Division of Neurology, The Pennsylvania State University, Hershey, PA 17033.

Cerebral blood flow (CBF) is reduced during hyperglycemia in awake-restrained rats. However, the hematocrit may increase in rats made acutely or chronically hyperglycemic, and this may reduce CBF by increasing blood viscosity. This hypothesis was tested by measuring regional CBF in normoglycemic and hyperglyemic rats after normalizing the hematocrit by isovolemic hemodilution. Chronic hyperglycemia (33 mM) was induced by treatment with streptozotocin. Vascular catheters were placed using halothane/nitrous oxide anesthesia. Wounds were injected with procaine and swabbed with xylocaine ointment. The hip girdle was immobilized with a plaster cast and anesthesia was withdrawn. After a one hour recovery period blood was withdrawn and centrifuged. The red cell fraction or the plasma was resuspended in a glucose solution and reinjected to cause no change or to reduce the large vessel hematocrit by 5. Thirty minutes later CBF was measured using [^{14}C]iodoantipyrine and dissection of brain regions. Normalization of hematocrit by hemodilution increased CBF in all brain regions by 15%. Despite equal hematocrit, hyperglycemia reduced CBF by 20%. This reduction was more prominent in nontelencephalic regions. CBF is reduced during hyperglycemia independent from the effects of large vessel hematocrit. (Supported by PHS NS24109 and an AHA EI Award)

343.11

EFFECTS OF SEVERE HYPOGLYCEMIA ON SYNAPTIC TRANSMISSION AND CARBOHYDRATE METABOLISM IN THE GUINEA-PIG HIPPOCAMPAL SLICE.
J. Waalen* & P. Lipton (Spon. K. Raley), Dept. of Physiol., Univ. of Wisconsin, Madison, WI 53706.

Hypoglycemia profoundly affects cerebral activity but the mechanism is not yet understood. There are significant EEG changes prior to measured changes in ATP but the localizations of the two measurements are not the same. Here we assess the role and control of glucose metabolism in the dentate gyrus of the guinea-pig hippocampal slice. Population spikes were evoked by stimulation of the perforant path at 1/8 Hz. After removing glucose, synaptic transmission is maintained for 10-15' then decreases to zero with a t1/2 of about 8'. ATP and PCr in the molecular layer are still at control levels at the time of complete transmission failure as is Ca^{2+}-dependent K^+ stimulated release of endogenous glutamate from dentate gyrus. Thus, transmission loss due to severe hypoglycemia is not due to energy failure or functional neurotransmitter depletion.

Studies with lactate provide further evidence that glycolysis per se is necessary for synaptic transmission. 20 mM lactate rapidly re-activates transmission abolished by hypoglycemia without changing high energy phosphate levels. This effect is blocked by 2-DOG (20 mM) and by IAA (.2 mM). Thus, return of transmission is a result of re-activating one or more steps in glycolysis. The result suggests there is at least partial gluconeogenesis in the glucose-deprived slice.

343.12

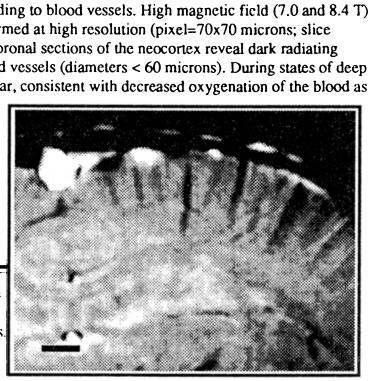
MULTIPLE-QUANTUM COHERENCE IN VIVO NMR SPECTROSCOPY OF 9L GLIOSARCOMA CELLS IMPLANTED INTO ATHYMIC MICE.
T. Nakada and I. L. Kwee. Neurochem Res Lab, VA Med Ctr, Martinez, CA 94553 and Dept of Neurology, Univ of California, Davis, CA 95616.

Multiple-quantum coherence experiments involve precessional and relaxation properties of phenomena arising from transitions between levels with $\Delta m_1 > 1$. Additional spectral editing capability arising from multiple-quantum coherence experiments compared to the single-quantum coherence experiments have been subject of various in vivo spectroscopic studies (Sotok CH, Magn Reson Med 7:364, 1988, Ueshima Y. et al., Magn Reson Imaging 7 (suppl): 190, 1989). Among the various proposed applications of multiple-quantum coherence experiments in vivo, zero-quantum (ZQ) experiments of lactate editing and double-quantum editing (DQ) of adenosine diphosphate (ADP) appears most promising. In this study, we have applied ZQ lactate editing and DQ ADP editing to study the in vivo metabolism of 9L rat gliosarcoma cell lines using subcutaneous implantation of cultured 9L cells into athymic mice. These techniques appear to be useful for monitoring tumor metabolism under various treatment regimens.

343.13

MAGNETIC RESONANCE MICRO-IMAGING OF BLOOD OXYGENATION IN BRAIN.
S. Ogawa*, T-M. Lee*, D.W. Tank and A.R. Kay*. (SPON: C.A. Taylor) AT&T Bell Laboratories, Murray Hill, NJ 07974.

Regional differences in O_2 utilization are currently used in PET imaging to localize brain activity during cognitive tasks. ^{1}H MRI has primarily been used as an anatomical tool. We now demonstrate that gradient echo images at high spatial resolution exhibit contrast dependent on blood oxygentation. The contrast results from the effect of paramagnetic deoxyhemoglobin and manifests as dark lines in the image (SeeFig.), corresponding to blood vessels. High magnetic field (7.0 and 8.4 T) images of rat brain were formed at high resolution (pixel=70x70 microns; slice thickness=700 microns). Coronal sections of the neocortex reveal dark radiating lines corresponding to blood vessels (diameters < 60 microns). During states of deep anesthesia the lines disappear, consistent with decreased oxygenation of the blood as a result of depressed neuronal activity. Results suggest that gradient echo images at high field may serve as a non-invasive method of measuring brain activity at the resolution of cortical columns.



In vivo gradient echo image (coronal section) including neocortex and hippocampus. Scale Bar=500 microns.

343.14

IN VIVO 13C{1H} NMR SPECTROSCOPIC MONITORING REVEALS FORMATION OF BRAIN [1-13C]2DG GLYCOGEN IN ANESTHETIZED RATS.
J.J. Kotyk*ƒ R.K. Deuel†, J.E. Mathisen*†, T.A. Woolsey†, J.S. McCaslandt, and J.J.H. Ackerman*† (SPON R. Sohn†) Washington University School of Medicine†, College of Arts and Sciences*, and Monsanto Co.ƒ, St. Louis, MO 63110.

Recently it has been proposed that brain glycogen is utilized during normal brain activity. 1-[^{13}C] 2DG glycogen can be identified and observed simultaneously with ^{13}C2DG/DG6P by ^{13}C{^{1}H} NMR spectroscopy. We monitored inclusion of [^{13}C]2DG into brain glycogen in Na pentothal anesthetized (35 mg/kg iv) adult rats after an iv bolus of [1-^{13}C]2DG (500mg/kg). Experiments were conducted in a Bruker WH-360 spectrometer (8.5 Tesla) using a two coil coaxial surface coil antenna. Body temperature, respirations, and brain intracellular pH were monitored and control spectra obtained. Ten min. spectral averages were analyzed. [1-^{13}C]2DG/DG6P was detected at 0-10 min., and [1-^{13}C]2DG glycogen first observed in one rat 20-30 min. after iv 2DG; it disappeared and was next detected at 50-60 min. In another rat it appeared at 100-110 min., again at 120-130, and 160-170 min. after iv 2DG. The highest glycogen concentration at any time was 8% of total maximum DG analog pool. No brain intracellular pH or other indications of physiological distress were noted. The data demonstrate formation of brain ^{13}C2DG glycogen in the living rat. Supported in part by NS 17663, NIH RO1GM30331, and the McDonnell Center for Studies of Higher Brain Function.

343.15

pH$_i$ COMPARTMEMTATION IN HIPPOCAMPAL SLICES INDICATED BY SNARF-1 AND NEUTRAL RED. T.J. Sick and J.C. LaManna, Depts. of Neurology, Univ. of Miami, Miami, FL 33101 and Case Western Reserve Univ., Cleveland, OH 44106.

Intracellular pH (pH$_i$) was measured in rat hippocampal slices by absorption spectrophotometry of neutral red (NR) and by spectrofluorometry of SNARF-1. Slices were incubated in either 100 μM neutral red or 20 μM of the acetoxymethylester form of SNARF-1. pH$_i$ was estimated from standard curves determined with either neutral red or the free acid form of SNARF-1 in brain homogenate, titrated to known pH with a standard glass pH electrode. Resting (95% O_2 - 5% CO_2) pH$_i$ by SNARF-1 was more acidic than pH$_i$ measured by NR. Changes in pH$_i$ during anoxia (95% N_2 - 5% CO_2) also differed when measured by SNARF-1 and NR. Following 10 min of anoxia pH$_i$ by SNARF-1 acidified to 0.14 pH units while pH$_i$ by NR did not change significantly from control values. Both pH$_i$ indicators showed large acid shifts upon administration of high CO_2. The data suggest that at least two proton compartments exist in brain and that these compartments differ in their responses to anoxia. It is not yet clear whether the pH$_i$ measurements indicate two different cellular compartments (e.g. neurons and glia) or subcellular compartments (cytosol or organelles).

343.16

KINETIC MODEL OF ^{14}C-2-DEOXYGLUCOSE METABOLISM IN BRAIN SLICES. C.S.Patlak, G.C.Newman, F.E.Hospod, Depts. of Neurology and Neurological Surgery, SUNY at Stony Brook, Stony Brook, N.Y. 11794.

The metabolism of 2DG in brain slices has been analyzed using a kinetic model with five compartments: 1) perifusate, 2) tissue 2DG, 3) 2DG6P, 4) 2DG1P + UDP-2DG, and 5) 2DG-glycogen + 2DG-glycoproteins.

Hippocampal or hypothalamic brain slices, 540μ or 1000μ thick, were incubated in 0.28μCi/ml of ^{14}C-2DG for varying times for uptake and washout experiments. After rapid freezing, the slices were extracted with perchloric acid and the supernatant separated by anion exchange chromatography. Tissue 2DG and acid-labile 2DG1P and UDP-2DG thus are determined as a single fraction. Each data set is derived from 104 to 149 slices from 12 to 18 Sprague-Dawley rats. The results have been analyzed using the solutions of the differential equations of our kinetic model and least squares analysis and will be presented in graphical and tabular form.

Our results reveal the following: 1) Influx and efflux of 2DG from 540μ brain slices proceed with K_1^* and k_2^* similar to those *in vivo*; 2) the 2DG6P-phosphatase reaction rate constant, k_4^*, is about 0.011 min^{-1}, similar to *in vivo*; 3) the rate constant of 2DG6P-2DG1P phosphoglucoisomerase, k_5^*, is similar to k_4^*, but radioactivity is trapped in the tissue; 4) significant amounts of glycogen and glycoproteins accumulate during long incubations consistent with studies *in vivo*. In 1000μ brain slices: 1) K_1^* and k_2^* are reduced; 2) k_3^* increases; 3) there is little or no changes in the other rate constants; 4) but 2DG1P, UDP-2DG and 2DG-glycogen accumulate at a greater rate because of increased tissue 2DG6P.

Analysis of total radioactivity, C_i^*, of uptake experiments 45 min or less with a three parameter model reveals excellent agreement with the calculated rates based upon the analysis of all data with the eight parameter model.

343.17

THE EFFECTS OF PROPOFOL ON LOCAL CEREBRAL GLUCOSE UTILIZATION (LCGU) IN THE RAT. C. Ori*, M. Dam*, G. Richieri*, G. Pizzolato*, G.P. Giron* and L. Battistin* (SPON. N. L. Shinowara). Ist. Anestesiologia e Rianimazione e Clinica Neurologica, Universita di Padova, 35100 Padova, Italy.

Propofol (ICI 35268; 2,6-di-isopropylphenol), an intravenous anesthetic, decreases intracranial pressure, cerebral blood flow, and $CMRO_2$. Therefore, propofol may be a suitable anesthetic to use in patients who suffer from intracranial disorders. In the present study the autoradiographic 2-deoxy-D-[^{14}C]glucose (2-DG) method was used to investigate the LCGU responses to propofol anaesthesia.

Male Fischer-344 rats (3 months old) were anesthetized with propofol (20 mg/kg) 30 min before 2-DG injection. Anaesthesia was maintained with a propofol infusion rate of 12.5, 25, or 50 mg/kg/h and LCGU evaluated as previously described (J. Neurochem. 28: 897, 1977). Propofol decreased LCGU in the auditory, visual, sensory-motor, precentral, and prefrontal cortices, thalamic nuclei, extrapiramidal system (caudate-putamen, globus pallidus, red n. and substantia nigra) and limbic system (hippocampus, presubiculum, medial habenula, and interpeduncularis n.). LCGU decreases were dose-dependent, except for the LCGU reduction in the reticular formation, which was maximum when the lowest propofol dose was used. This effect may be related to the anesthetic state which also occurs with low propofol doses

343.18

BIOCHEMICAL DISTRIBUTION OF PLASMA DERIVED 18F-DL-ERYTHRO-9,10-DIFLUOROPALMITATE IN RAT BRAIN. J.G. Noronha, J.J. DeGeorge*, B. Schmall*, T. Nariai*, J. Bell*, R.D. Finn*, and S.I. Rapoport. Lab. of Neurosci, NIA and Nuclear Medicine, NIH, Bethesda, MD 20892

We previously examined the incorporation of [9,10-3H] palmitate from plasma into brain and now compare its brain distribution with that of [9,10-18F]palmitate, a positron emitter, to determine whether such a probe has potential for imaging brain lipids using positron emission tomography (PET). 18F-palmitate was synthesized with a sp. ac. of 500-1000 mCi/mmol and was infused over 5 min into rats implanted with arterial and venous femoral catheters. After 20 min, rats were killed by a pentobarbital overdose and brains were removed and extracted. Neutral or acidic chloroform:methanol (2:1) extracts were prepared and partitioned with KCl. In the absence of acid, 43% of brain label is in the lipid phase, 25% in the aqueous phase, and 32% in the pellet. Extraction with acid yielded 57% of label in lipid, 43% in aqueous and < 1% in pellet. These results indicate that polar metabolites of 18F-palmitate, both lipid and water-soluble, contribute substantially to brain label. Work is in progress to determine whether 18F-palmitoylCoA or -acetylCoA are aqueous metabolites. [9,10-18F]Palmitate may prove to be a sensitive probe of membrane components using PET.

343.19

HPLC-EC DETERMINATION OF FREE PRIMARY AMINO ACID CONTENT OF CAT CISTERNAL CEREBROSPINAL FLUID. V.R. Roettger, F. Costello*, T.R. Bumbalough*, and M.D. Goldfinger, Department of Physiology & Biophysics, Wright State University, Dayton, OH 45401-0927.

The objective of this work was to assess the primary amino acid content of cat CSF. Adult cats were anesthetized with Nembutal. The dura beneath the atlanto-occipital membrane was exposed. A 27ga needle was inserted through the dura overlying the obex; approx. 2 ml of cisternal CSF was gently withdrawn and stored at -70°C for subsequent analysis. Osmolality of thawed CSF was 310 mOsm/Kg(N=5, range:303-325).

Isocratic reverse-phase HPLC-EC was as in (1) (using MPI) with these modifications: flow-rate - 1.8 ml/min; column temp. = 40°C. Elution times were under 19 min (GABA). Derivatization was as in (1) (25µL sample: 25µL DS; 1.5 min) with these modifications: thiol content was X110; excess thiol was eliminated by post-derivatization/pre-column reaction with Iodoacetamide (5.0µl of 140 mg/ml water). The high thiol content optimized derivatization; Iodoacetamide removed the thiol peak and allowed detection of rapidly-eluting derivatives (eg, Aspartate). Detection limits were between 0.3 (Glutamine) and 2.3 (GABA) picomoles derivatized. CSF was studied as a 50% water dilution. Detector sensitivity was switched during elution with a schedule designed to accommodate the wide ranges of CSF amino acid content.

A water mixture of amino acids (31 mOsm/Kg) simulated CSF amino acid levels. With serial dilution testing, detection with switching was linear (slopes: mixture, 0.99; range .95-1.02; CSF, 1.00, range .93-1.05). CSF components were identified by co-elution and 'spiking' of 50% CSF with standards (up to 2X load). Several peaks were not identified. In spiking tests, observed peak heights varied linearly with expected heights; for each of 3 experiments, all component values were used to generate 1 observed vs expected regression line: avg. slope = 0.97, range:.95-.99.

In 5 cats, CSF concentrations (µmole/L) of identified components had these averages (ranges): Glutamate, 4.1(2.9-5.5); Asparagine, 12.2 (10.0-13.4); Glutamine, 681.5(620.0-827.3); Glycine, 15.1(13.5-16.5); Taurine, 7.1(4.8-12.9); Alanine, 37.5(24.0-52.8); Tyrosine, 10.5 (6.9-17.2); GABA, <0.1.

(1):V.R. Roettger & M.D. Goldfinger. Soc. Neurosci. Abst. 13:1565, 1987.

Supported by: American Heart Assoc. (Ohio Affil.) and Wright State Univ.

343.20

CEREBRAL METABOLIC EFFECTS OF *SIGMA* LIGANDS IN THE RAT. A.Della Puppa and E.D. London. Neuropharmacology Laboratory, Addiction Res. Ctr., NIDA, Baltimore, MD 21224.

The 2-deoxy-D-[1-^{14}C]glucose method (Sokoloff, L., et al., J. Neurochem. 28:897, 1977) was used to map and quantitate the cerebral metabolic effects of *sigma* (σ) ligands. Drugs tested included *d*-pentazocine, (±)BMY 14802 and (±) BW 234U.

Male Fischer-344 rats, 4-6 months of age, were used as previously described (London, E.D., et al., J. Neurochem. 37:217, 1981). Drugs were administered i.p. at a dose of 30 mg/kg. In general, BMY 14802 significantly increased LCGU in the substantia nigra compacta and reticulata, paraventricular hypothalamic nucleus, lateral habenula, facial and cochlear nuclei, locus ceruleus, pontine nuclei, supramammillary nucleus, medial mammillary nucleus, VTA, lateral vestibular and interpositus cerebellar nuclei and cerebellar vermis. *d*-Pentazocine increased LCGU in the substantia nigra compacta and reticulata, paraventricular hypothalamic nucleus, layer 5 of the somatosensory cortex, medial habenula, anterior cingulate cortex, posterior piriform cortex, CA1 and CA3, and subthalamic nucleus. In contrast, BW 234U reduced LCGU in the superficial gray layer of the superior colliculus, inferior colliculus, substantia nigra compacta, lateral habenula, medial geniculate body, abducens and lateral vestibular nuclei and in the auditory cortex. Most of the areas that showed changes in LCGU are known to contain σ receptor sites (Largent, B.L, et al., J. Pharmacol. Exp. Ther. 238:739, 1986). Differences in pharmacological properties, including effects on neuronal excitability, may have resulted in different distributions and effects of the drugs on LCGU.

343.21

MEASUREMENT OF FREE AND TRANSFER RNA AMINO ACYL SPECIFIC ACTIVITIES AND PROTEIN SYNTHESIS IN RAT BRAIN IN VIVO. K.M. Hargreaves*, J.B. Buciak*, and W.M. Pardridge. Department of Medicine and Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024-1682.

Measurement of cerebral protein synthesis requires the determination of amino acid specific activities in both the intracellular-free pool and the amino acyl transfer RNA pool. Following development of methods for measuring these two parameters, brain protein synthesis was measured in ketamine-anesthetized, male Sprague-Dawley rats by an internal carotid artery infusion technique. The brain was perfused for 0.5-30 minutes with buffer containing 10 mM glucose, 3% bovine albumin, 1X concentration of plasma amino acids, 30% rat erythrocytes, and 100 μCi/ml [^{3}H]-leucine. The free-intracellular pool and the tRNA pool of leucine completely equilibrated within 2 minutes, but these two pools did not reach equilibrium with the plasma pool until 10 minutes of perfusion. Moreover, after this point of equilibration, the specific activity of the plasma amino acid pool remained three-fold higher than the specific activity of the free-intracellular and tRNA pool. Protein synthesis rates were constant between 10 and 30 minutes of perfusion and averaged 0.62 ± 0.06 nmol/min/g (mean ± S.E., n = 11) for the whole cerebral hemisphere. Conclusions: The specific activity of the amino acyl tRNA pool is in rapid equilibrium with the free-intracellular pool, but is markedly overestimated by the specific activity of the plasma amino acid pool.

343.22

REGIONAL CEREBRAL GLUCOSE UTILIZATION IN FREELY-RANGING AND RESTRAINED RATS. R.M. Bryan, Jr. Departments of Surgery (Neurosurgery) and Physiology, M.S. Hershey Medical Center, Hershey, PA, 17033.

Regional cerebral glucose utilization (rCMRgl) was measured in freely-ranging and restrained rats to determine the effects of restraint on rCMRgl. Three groups of rats were used in this study: (1) freely-ranging controls; (2) rats restrained by fitting a plaster cast around the pelvis (hindlimb restraint); and (3) rats restrained by immobi- lizing all four limbs with tape (four-limb restraint).

Hindlimb restraint significantly elevated heart rate (35%), blood pressure (19%), plasma glucose (75%), plasma epinephrine (1400%), plasma norepinephrine (458%), and significantly decreased $PaCO_2$ (40.0 mm Hg to 37.4 mm Hg) compared to the freely-ranging controls. These changes are indicative of a stress response. Regional CMRgl decreased significantly in the sensory cortex (9%), motor cortex (9%), and the inferior colliculus (7%). However, CMRgl measured in fourteen other brain regions was not statistically different from the freely-ranging controls. Four-limb restraint did not significantly alter rCMRgl measured in five brain regions. I conclude that restraint stress has very little effect on rCMRgl. Cerebral energy metabolism appears to be controlled differently during restraint stress than during other forms of stress where rCMRgl increases throughout the brain.

343.23

EFFECTS OF HIGH-DOSE *d*-SKF 10047 ON CEREBRAL METABOLISM IN RATS. G.R. Hill*, A. Della Puppa and E.D. London (SPON: D. Jasinski) Neuropharmacology Lab., Addiction Research Center, NIDA, Baltimore, MD 21224.

The effects of high-dose *d*-SKF 10047 (5 mg/kg i.v.) and its potential PCP-like activities on local cerebral glucose utilization (LCGU) were studied by using the 2-deoxy-D-[1-^{14}C]glucose ([^{14}C]DG) method (Sokoloff, L., et al., J. Neurochem. 28:897,1977)

Male Fischer-344 rats were prepared with indwelling femoral and venous catheters (London, E.D., et al., J Neurochem. 37:21, 1981). *d*-SKF 10047 was given i.v. at 2, 5, 10 and 15 min before [^{14}C]DG and LCGU calculated as described elsewhere. *d*-SKF 10047 increased LCGU in the anterior cingulate cortex, retrosplenial granular and agranular cortex, visual cortex, molecular layer of the dentate gyrus, presubiculum, nucleus accumbens, globus pallidus, anterior dorsal, reuniens and reticular thalamic nuclei, zona incerta, subthalamic nucleus, medial and lateral mammillary bodies, substantia nigra reticulata, oculomotor, abducens and pontine nuclei. However, LCGU was decreased in the auditory cortex, inferior colliculus, medial geniculate body, lateral habenula and layer 1-3 of the frontal cortex. The effects of a 5 mg/kg i.v. *d*-SKF 10047 dose widely reproduced cerebral metabolic patterns observed after PCP administration (Weissman, A.D., Brain Res. 435:29, 1987). The similarities between *d*-SKF 10047 and PCP effects on LCGU may be a function of the *d*-SKF 10047 dose. Indeed, such a dose may have stimulated the PCP receptors for which *d*-SKF 10047 has low affinity. Therefore, it is suggested that some of the cerebral activities of PCP and *d*-SKF 10047 high-dose are mediated through common mechanisms.

343.24

REGIONAL ANALYSIS OF [9,10-^{3}H] PALMITATE INCORPORATION IN A METASTATIC BRAIN TUMOR MODEL. T. Nariai*, J.J. DeGeorge*, N. Greig*, S. Genka* and S. I. Rapoport. Laboratory of Neuroscience, NIA, NIH, Bethesda, MD 20892.

We have developed a procedure to examine brain phospholipid synthesis and turnover in vivo, using radiolabelled palmitate (Noronha et al. Trans. Am. Soc. Neurochem. 20:142, 1989). In the present study, we used this technique to investigate lipid metabolism of neoplastic tissue and normal brain following intracerebral tumor cell implantation in Fischer-344 rats. Walker 256 carcinosarcoma, 250 cells in 5 ul EMEM containing 1% low melting point agar, were implanted into the caudate nucleus. Control animals were injected with the same amount of agar to evaluate implantation damage. Seven days after tumor cells were implanted, awake rats were infused intravenously with [9,10-^{3}H] palmitate(^{3}H PA)(6.4 mCi/Kg) for 5 min. Twenty minutes after the start of infusion, rats were killed and brains were removed and prepared for autoradiography and histology. The tumor cell mass (2-10 ul in volume) was well demarcated histologically from surrounding gliotic brain tissue. Incorporation of ^{3}H PA into tumor was heterogeneous, 3.1-6.8 fold greater than in corresponding contralateral brain regions. The area of reactive gliosis surrounding the tumor and the control lesion sites showed less than 1.3 fold increase of ^{3}H PA incorporation. The results indicate that radiolabelled palmitate can be utilized for imaging brain tumors in vivo.

ALZHEIMER'S DISEASE: TRANSMITTERS AND BEHAVIOR

344.1

EVIDENCE FOR NEUROFIBRILLARY TANGLES (NFT'S) IN PONTINE 5-HT NEURONS IN ALZHEIMER'S DISEASE (AD) J.M. Lee* & N.W. Kowall (SPON: E.T. Hedley-Whyte). Department of Pathology & Neurology, Mass. General Hospital, Boston MA 02114.

Midbrain and pontine raphae nuclei were investigated in patients with AD and age-matched controls using the mouse monoclonal antibody PH8. This antibody recognizes similar epitopes of the 3 aromatic amino acid hydroxylase enzymes; tyrosine, phenylalanine and tryptophan hydroxylase (Haan et al., Brain Res.(1987) 426:19-27). Tissue was taken within 24 hours of death and placed in a periodate-lysine-paraformaldehyde fixative. Fifty micron sections were incubated with a 1:1000 dilution of PH8 followed by incubation with a goat anti-mouse peroxidase conjugated secondary (1:300) and developed using DAB as a substrate. Under the conditions described above, controls as well as AD brainstems showed intense reproducible PH8 positive cell body staining in the dorsal raphae (DR,B7), median raphae (B8) and B9 nuclei. Staining of the neuromelanin neurons of the locus ceruleus and substantia nigra was variable and less intense. Counterstaining with 1% thioflavine S revealed that a number of PH8 positive neurons showed a green-yellow fluorescent material under UV light consistent with NFT's in both the dorsal as well as median raphae nuclei of the AD group. The distribution was similar to Tau positive immunoreactive neurons in separately stained sections. In addition, preliminary cell counts and analysis of cell areas revealed trends toward a decreased PH8 positive cell number in the caudal DR and B9 nuclei and a decreased cell area of caudal DR neurons in AD patients.(PH8 antibody available courtesy of Dr. R.G.H. Cotton)

344.2

PRESYNAPTIC PROTEINS IN THE PERFORANT PATHWAY TERMINAL ZONE ARE DEPLETED IN ALZHEIMER'S DISEASE. L.M. Cabalka, B.T. Hyman, T.C. Ritchie, C.R. Goodlett, J.D. Coulter and G.W. Van Hoesen. Depts. of Anatomy and Neurology, Univ. of Iowa, Iowa City, IA, and Dept. of Neurology, Harvard Medical School, Boston, MA.

The perforant pathway arises from layers II and III of the entorhinal cortex and is the source of cortical afferents to the hippocampal formation, terminating in the outer 2/3's of the molecular layer of the dentate gyrus and along distal dendrites of hippocampal pyramidal cells. In experimental animals, entorhinal lesions cause loss of synaptic markers in this terminal zone. A consistent alteration in Alzheimer's disease (AD) is the presence of neurofibrillary tangles in layer II of entorhinal cortex. We have examined the distribution of nerve terminal proteins in the hippocampal formation of five AD patients, three controls, monkeys, and rats, to determine the effect of this pathology on synaptic markers. Tissue was stained using antibodies against synaptophysin (p38), a synaptic vesicle membrane protein present in virtually all nerve terminals, and against NT75, a selectively distributed nerve terminal protein. Control patients, monkeys and rats showed similar patterns of p38 immunoreactivity, with dense staining throughout the molecular layer of the dentate gyrus. All AD patients had neurofibrillary tangles in layer II of entorhinal cortex, and in these patients p38 staining was depleted in the perforant path terminal zone. This suggests that the loss of p38 immunoreactivity in AD is related to entorhinal pathology. Moreover, both p38 and NT75 immunoreactivity were associated with some neuritic plaques in the dentate molecular layer. This result suggests that neuritic plaques may be a focus of aberrant sprouting of nerve terminals, and that normally present nerve terminal proteins may play a role in this process. Supported by NS23783, NS14944, PO NS19632, and IT 32N507294.

344.3

FOUR CLASSES OF ALZHEIMER'S DISEASE: NEOCORTICAL NEUROPATHOLOGY AND ALTERATIONS IN NEUROTRANSMITTER RECEPTOR BINDING. B.A. Vogt, G.W. Van Hoesen, L.J. Vogt* & P.B. Crino. Depts. Anatomy & Behavioral Neurology, Boston Univ. Sch. Med., Boston, MA 02118, Depts. Anatomy and Neurology, Univ. Iowa, Iowa City, IA 52242 & V.A. Hospital, Bedford, MA 01754.

Cases of dementia of the Alzheimer type (DAT) were classifiéd according to which layers had the greatest proportionate loss of neurons in posterior cingulate cortex.These four classes did not differ by age at disease onset, length of disease nor density of thioflavin S-stained neuritic plaques or neurofibrillary tangles.

Pirenzepine binding to cryostat sections was reduced in layers V and VI of all cases but was most pronounced in cases with greatest loss of neurons in these layers. Oxotremorine-M binding was normal in all DAT cases except in the class where neuronal loss was most pronounced in layer IV. This latter class also had greatest up regulation of beta adrenoceptors. Muscimol binding was normal in all layers but layer I, where reduced binding did not appear to be class related. Finally, ketanserin binding was unaltered in any layers in all cases.

In conclusion, neocortical neuron loss is not the same in all DAT cases and alterations in receptor binding are not a generalized response to such changes. Future receptor binding studies will require more detailed assessments of underlying neuropathological alterations.

Supported by NINDS grants NS 18745 and NS 14944.

344.4

RELATIONSHIP OF NGF-RECEPTOR AND NADPH-DIAPHORASE CONTAINING BASAL FOREBRAIN NEURONS IN MONKEYS, AGED NORMAL ELDERLY AND ALZHEIMER'S DISEASE PATIENTS. E.J. Mufson and J.H. Kordower. Inst. for Biogerontol. Res., Sun City, AZ and Univ. of Illinois, Sch. Med., Chicago, IL

Interspersed within the basal forebrain continuum of large magnocellular cholinergic neurons which express the receptor for nerve growth factor are smaller NADPH-diaphorase positive neurons. We determined the relationship between these neuronal populations and whether NADPH-d positive perikarya are involved in the pathologic process of AD. Sections from basal forebrain of normal aged patients obtained postmortem and adult Cebus monkeys were processed for NADPH-d, nerve growth factor receptor (NGFR) and concurrently for both proteins. In both species, NADPH-d positive neurons were seen scattered throughout the basal forebrain with many more located within the region of the nucleus basalis as compared to the septal and diagonal band nuclei. Although a few NADPH-d neurons intermingled within the aggregates of NGFR expressing neurons, most were located either adjacent to or surrounding NGFR neuronal subgroups. In AD patients, many of the small ovoid, fusiform or bipolar NADPH-d neurons appeared shrunken or dystrophic. Sections concurrently stained for NADPH-d, NGFR immunoreactivity and Thioflavin-S revealed numerous tangle bearing NGFR expressing neurons whereas virtually all NADPH-d neurons were Thioflavin-S negative. NADPH-d positive neurons in close proximity to basal forebrain NGFR expressing neurons degenerate in AD, but do not exhibit NFTs as do NGFR containing neurons. Support: Amer. Hlth. Ass. Fdn., ADCRC, NS26146, NS25655.

344.5

BRAIN CHOLINERGIC RECEPTORS IN HUMAN NEURODEGENERATIVE DISEASES. I. Aubert, D. M. Araujo, S. Gauthier, & R. Quirion. Douglas Hospital Res. Ctr. & McGill Univ., Montreal, Quebec, Canada H4H 1R3.

In the present work, we determined whether cholinergic receptor sites are altered in various brain regions of certain neurodegenerative diseases such as Alzheimer's(AD), Parkinson's(PD), and Parkinson's with AD-type dementia(PD/AD). Thus, we characterized the binding of muscarinic and nicotinic ligands to homogenates of human brain. Our results show that in all three disease categories, the maximal density of nicotinic sites, assessed using ^{3}H-methylcarbamylcholine (MCC) as ligand, is markedly reduced in cortical regions and in hippocampus. In PD and PD/AD brains there is also a decrease in the Bmax for ^{3}H-MCC binding to homogenates of caudate-putamen. In contrast, the total density of muscarinic receptor sites,characterized using ^{3}H-QNB, was increased in the caudate-putamen of all brains and in the hippocampus of AD brains (81%). This increase in ^{3}H-QNB binding may be due to an up-regulation of the muscarinic-M1 subtype, since the Bmax for ^{3}H-pirenzepine binding was enhanced (by 20-85%) in these brain regions. The density of muscarinic-M2 sites, assessed with either ^{3}H-AF-DX 116 or ^{3}H-ACh was significantly reduced in all brain regions except caudate-putamen of PD/AD and AD brains, but was not affected in any region of the PD brains. In summary, cholinergic receptors are differentially altered in different brain regions in diseases.(MRC, Canada)

344.6

HETEROGENEITY OF CHOLINERGIC INNERVATION IN SUBFIELDS OF HUMAN HIPPOCAMPUS AS REVEALED BY CHOLINE ACETYLTRANSFERASE ACTIVITY. K. L. Tenebruso*, W. Hoss and R. W. Hamill. Monroe Comm. Hosp./Univ. of Roch., Rochester, N. Y. 14603, Univ. of Toledo, Col. Pharmacy, Toledo, OH 43606.

The distribution of cholinergic innervation of human hippocampus was examined utilizing a radiochemical assay for choline acetyltransferase (ChAT) activity, a marker of cholinergic synapses. Subregional and micropunch dissection of transverse cryostat sections (240-300 microns) were performed in order to isolate CA1, CA2, CA3, CA4/DG, subicular regions, and adjacent cortex (parahippocampal or entorhinal). Micropunch analyses permitted characterization of hippocampal laminae in some cases. Adjacent cresyl violet or acetylcholinesterase (AChE) stained sections provided correlative morphological observations. ChAT activity was highest in the CA4/DG region, relatively midrange and uniform levels existed throughout CA1, CA2 and CA3, and lower levels were found in adjacent cortical regions. Micropunch studies of laminae confirmed the subregional studies: ChAT specific activity appeared highest in the granular cell layer of the dentate gyrus and the polymorphic layer within the hilus of the dentate gyrus (CA4). Thus, the CA4/DG region received the richest cholinergic innervation. Preliminary studies in Alzheimer's disease patients indicate severe ChAT depletion in all hippocampal subregions and laminae. (Rochester Alzheimer Disease Project, PHS Grant, AG 03644).

344.7

BRAIN GANGLIOSIDES IN ALZHEIMER'S DISEASE AND IN RATS WITH FOREBRAIN LESIONS. N.M.K. Ng Ying Kin*, L.H. Pan*, J.H. Louvaris*, Y. Robitaille* and N.P.V. Nair. Douglas Hospital Research Centre, McGill University, Montreal, Canada.

Rats with quinolinic acid-induced lesions of the nucleus basalis magnocellularis (nbm) exhibit central cholinergic dysfunctions and are putative animal models for Alzheimer's disease (AD). Since ganglioside deficiencies have been reported in AD brains, it would be of interest to determine whether that was also the case in the lesioned rats. Unilateral lesions of the rat's nbm were performed as described by El-Defrawy et al. (Neurobiol. Aging, 6, 325, 1985). Cortical tissues from the ipsilateral (affected) and contralateral (controls) sides of the lesions were used in our studies. Gangliosides, assayed for their sialic acid contents, were found to be significantly reduced in affected tissues when compared to controls (N=5). Decreases range from 8 to 26 percent. Similarly, in human post-mortem cortical tissues, ganglioside contents were decreased in different anatomical regions of AD brains (N=11) when compared to controls (N=6). Regional reductions ranging from 6 to 22 percent were observed and were statistically significant . Deficits in younger patients were more pronounced. In summary, deficiencies in gangliosides, which have been reported to possess trophic properties, suggest a role for these compounds in the pathophysiology of AD and the animal model. Studies on the metabolism of gangliosides in the lesioned rats should be informative.

344.8

HUMAN CORTEX HAS THREE SUBTYPES OF NICOTINIC RECEPTORS. E. Giacobini[1], K. Sugaya*[1], V.A. Chiappinelli[4], B. Clark[2] and R. Struble[3]. Depts. Pharmacology[1], Pathology[2] and Psychiatry[3], Southern Ill. Univ. Sch. Med., Springfield, IL and [4]Dept. Pharmacology, St. Louis Univ. Sch. Med., St. Louis, MO

Kappa-bungarotoxin (K-BTX) is a selective antagonist that facilitated characterization and localization of central nicotinic receptors. We have examined brain samples of human frontal and parietal cortex obtained from biopsies and autopsies, using three different ligands of nicotinic receptors, ^{125}I-alpha-BTX, ^{125}I-K-BTX and L-^{3}H-nicotine. In human frontal cortex of neurological controls (21-57 yrs), elderly controls (64-94 yrs) and Alzheimer disease (AD) patients (67-78 yrs), we demonstrated two binding sites for both bungarotoxins, with high and low affinity. With alpha-BTX, there was no statistical difference between young controls, old controls and AD patients. With K-BTX there was a significant decrease in low affinity binding sites between young and old controls and AD patients (42% and 47%, respectively). Computer assisted image analysis and autoradiography mapped out specific localizations of these three binding sites in brain areas. The functional significance of K-BTX sites was investigated by studying ^{3}H-acetylcholine release in human and rat cortex slices. Our results suggest the presence in human brain of three nicotinic receptor subtypes with different localizations which are recognized by bungarotoxins or nicotine. (Supported by NIA AG05416; NINDS NS17574; and R.J. Reynolds Tobacco Co.)

344.9

VISUO-SPATIAL AND MEMORY FUNCTION FOLLOWING ARECOLINE INFUSION IN PATIENTS WITH DEMENTIA OF THE ALZHEIMER TYPE. K.C. Raffaele, J.V. Haxby*, P. Morris*, T.T. Soncrant, and S.I. Rapoport. Laboratory of Neurosciences, National Institute on Aging, NIH, Bethesda, MD 20892.

Decreases in cholinergic markers have been demonstrated in brains of patients with dementia of the Alzheimer type (DAT). DAT patients treated with arecoline, a muscarinic receptor agonist, have shown some improvement in picture recognition, but not on other memory measures. In order to examine further possible performance improvements following arecoline treatment, patients with DAT (with mild to moderate dementia, mean Mini-mental Exam score = 13.8+4.8) were treated with a single infusion of arecoline (5 mg over 30 min.), one hour following methylscopolamine (0.5 mg p.o.). Psychometric testing was performed at 5 time points: the day before the infusion, during methyl-scopolamine pretreatment and at 0, 90, and 330 min. following the arecoline infusion. Patients were tested on 3 memory tasks (Digit Supraspan, Block Supraspan, and Buschke Selective Reminding) and a test of visuo-spatial ability (accuracy in copying figures). Blood samples from several time points during and after the infusion were analyzed to determine plasma levels and half-life of arecoline. Preliminary results from four patients show no significant improvement in memory measures. However, drawing accuracy was improved in two patients.

344.10

COMPARISON OF TETRAHYDROAMINOACRIDINE (THA) AND PHYSOSTIGMINE (PHY) AT INCREASING ACETYLCHOLINE (ACh) IN RODENT BRAIN. D. R. Liston, D. S. Chapin*, S. B. Jones* and J. A. Nielsen. Pfizer Central Research, Groton, CT.

Senile dementia of the Alzheimer type (SDAT) is a neurodegenerative brain disorder characterized by a decrease in neurochemical markers associated with cholinergic neurons. Of the acetylcholinesterase (AChE) inhibitors that have been used to ameliorate the cholinergic deficits present in SDAT, THA is proving to be the most effective clinically. Previously we demonstrated that THA is readily distributed to brain (Liston et al., Soc. Neurosci. 14:1224, 1988). The present studies have examined the effects of THA and PHY on ACh levels in brain. THA and PHY increased ACh concentration in the striatum and frontoparietal cortex of rats. THA induced statistically significant elevation of ACh at 5.6-17.8 mg/kg i.p.(LD50=31 mg/kg), while PHY was effective only at 1 mg/kg i.p., one-half of its LD50. THA increased ACh for at least 120 min, returning to baseline at 240 min, whereas PHY increased ACh at 30 min, but was without effect at 60 min. after dosing. THA increased ACh and had an LD50 in mice similar to that in rats. In both mice and rats, THA reversed the decrease in ACh levels induced by scopolamine. These data in rodents indicate that THA has a broader effective dose range and longer duration of action than PHY. These properties, in conjunction with THA's ability to achieve high drug concentrations in brain, may explain the favorable clinical results obtained with THA in the treatment of SDAT.

344.11

NOVEL PHYSOSTIGMINE ANALOGS FOR ALZHEIMER'S DISEASE THERAPY: PHARMACOKINETIC AND BIOCHEMICAL STUDIES. **D.B. Ellis*, G.M. Bores*, R.S.Hsu*, R.R.L.Hamer* and F.P. Huger.** Depts. Biological and Chemical Research, Hoechst-Roussel Pharmaceuticals, Somerville, NJ 08876.

The recognition of a central cholinergic deficit in Alzheimer's disease (AD) has prompted interest in acetylcholinesterase (AChE) inhibitors as a means of therapy. While cognitive and behavioral effects have been seen with physostigmine, a short duration of action, marked peripheral side effects, narrow therapeutic window and modest cognitive improvement after oral administration limit its use. We present several new physostigmine derivatives with better stability, improved bioavailability, longer duration of action and greater AChE inhibitory selectivity. Substitution of the carbamate nitrogen with a phenyl, cyclooctyl, isoquinolinyl or (R*) α-methylbenzyl yielded selective inhibitors of AChE while a phenethyl group was selective for butyrylcholinesterase. The (S*) α-methylbenzyl and phenylpropyl analogs were non-selective. Stability of these derivatives in acid and plasma was excellent. When given to rats, all were well absorbed and rapidly penetrated the blood-brain barrier. Duration of AChE inhibition in rat brain varied markedly, (R*) α-methylbenzyl >>> cyclooctyl, (S*) α-methylbenzyl, phenyl, isoquinolinyl >> physostigmine. The 3-chlorophenyl and α-methylbenzyl analogs were also active inhibitors of NE re-uptake. The increased duration of action, better stability, decreased acute toxicity and additional noradrenergic activity provide encouraging lead compounds with potential clinical implication for the symptomatic treatment of AD patients.

344.12

EVIDENCE THAT AF-DX 116, A MEMORY FACILITATING MUSCARINIC-M2 ANTAGONIST, CROSSES THE BLOOD BRAIN BARRIER. W. Regenold M.G. Packard and R. Quirion. Depts. of Psychiatry and Psychology, McGill University, Douglas Hospital Research Centre, 6875 La Salle Blvd., Verdun, P.Q., Canada, H4H 1R3.

The cholinergic hypothesis of geriatric memory loss predicts that a muscarinic-M2 autoreceptor antagonist may improve cognitive function in Alzheimer's disease (AD) by increasing acetylcholine (Ach) release. Previous work in our labs has shown that AF-DX 116 binds an M2-like receptor site and enhances in vitro Ach release. Furthermore, on both a win-shift radial maze task (sensitive to hippocampal lesions) and a win-stay task (sensitive to caudate lesions) systemic post-training injections of AF-DX 116 improved memory. In view of these findings and the importance of peripheral drug delivery in AD pharmacotherapy, we investigated the ability of AF-DX 116 to cross the blood brain barrier (BBB). Anaesthetized rats received 1 mCi/kg injections of [^{3}H]AF-DX 116 in 0.4 ml EtOH(45%)-saline soln. Half of the rats were perfused prior to decapitation, and brains were dissected and homogenized. Mean CPM from perfused (P) brain homogenate aliquots were, e.g., 801(cortex) to 382(brain stem) above background and consistently lower than nonperfused(NP) brain, e.g., 801(P) vs. 2022(NP) in cortex and 382(P) vs. 936(NP) in brain stem. These results suggest that AF-DX 116 crosses the BBB, supporting a central action of AF-DX 116 on memory. (Supported by the Medical Research Council of Canada)

344.13

LOCUS COERULEUS CELL LOSS IN NEURODEGENERATIVE DISEASES: COMPUTER IMAGING. K.F. Manaye, W.K. Smith, D.J. Woodward, D.M.A. Mann* and D.C. German. Depts. of Psychiat. & Cell Biol., U. of Texas Southwestern Med. Cntr., Dallas, TX 75235 and Dept. of Pathol., U. of Manchester, UK.

The locus coeruleus (LC) cells are markedly reduced in number in patients with Alzheimer's disease (AD), Parkinson's disease (PD) and Down's syndrome (DS). The purpose of the present study was to examine the topographical pattern of LC cell loss in the above diseased brains. Cell locations were mapped in horizontal sections (10-14) from rostral to caudal using the CARP system from Biographics Inc. 10 AD, 6 PD and 2 DS and 8 age-matched normal brains were examined. Data analysis consisted of calculating the number of cells per section, and the area of the nucleus in the XZ (mediolateral X rostrocaudal) and XY planes (mediolateral X dorsoventral). The cell loss in AD and DS was primarily confined to the rostral portion of the nucleus whereas the loss in PD was comparable from rostral to caudal and markedly destroyed the peripheral cells. These data suggest that cell loss in AD and DS is greatest among the forebrain-projecting cells whereas in PD the loss is more uniform. Research supported by the National ADRDA, AG-08013, NS-25321 and DA-2338.

344.14

CSF LEVELS OF VASOPRESSIN, OXYTOCIN, AND NEUROPHYSINS ARE REDUCED IN ALZHEIMER'S DISEASE. William G. North*, Robert Harbaugh*, and Teddie Reeder*(SPON: H. L. Borison). Departments of Physiology and Neurosurgery, Dartmouth Medical School, Hanover, NH 03756.

The well-documented observation that vasopressin, oxytocin, and related neuropeptides affect behavior, and the finding that vasopressin protects against age-related changes in cholinergic function, prompted us to examine the levels of these peptides, and of neurophysins, in the CSF of patients suffering from Alzheimer's Disease. Neurophysins are gene-related products secreted by vasopressin and oxytocin neurons. For nine (9) such patients (6 men, 3 women) CSF samples wee obtained from a Selker reservoir placed into the right ventricle and attached to a continuous infusion pump for delivering bethanacol chloride. Protein concentrations of the CSF samples were evaluated from differential absorbance at 215 nm and 225 nm. The levels of vasopressin, vasopressin-associated neurophysin (VP-HNP), oxytocin, and oxytocin-associated neurophysin (OT-HNP) were determined for unextracted CSF using our specific RIAs for these substances. Values obtained were compared with those for CSF from 60 mentally healthy patients who were being treated for back pain.

All four substances measured by RIA were reduced in the patients with Alzheimer's Disease. For these patients vasopressin levels (3.6±0.7 fmol/ml) and VP-HNP levels (21.7±1.6 fmol/ml) were less than half of those for control subjects (7.8±0.4 fmol/ml; 54.0+2.0 fmol/ml). Oxytocin levels (2.7±0.6 fmol/ml) and OT-HNP (104.1±3.4 fmol/ml) were 75% and 58% of those for control subjects (3.6±0.3 fmol/ml; 180.1±1.9 fmol/ml). We conclude from these data that Alzheimer's Disease is associated with decreased activity of both vasopressin neurons and oxytocin neurons with axons terminating in the central nervous system.

344.15

IMMUNOHISTOCHEMICAL STUDY OF SUBSTANCE P (SP) AFTER UNILATERAL FRONTAL LOBECTOMY IN THE HUMAN BRAIN. C. Bouras*, P.R. Hof[1], P.G. Vallet*, F. Tagliavini*, Y. Charnay*, J. Constantinidis*. (SPON. P.L. Magistretti). Div. of Morphological Psychopathology, Dept. of Psychiatry, Univ. of Geneva, CH-1225 Geneva, Switzerland, and [1]Research Institute of Scripps Clinic, BCR-1, La Jolla, CA 92037, USA

In a previous study of cases of Alzheimer's disease presenting an asymmetric cortical atrophy, striking differences in SP immunoreactivity between the two hemispheres were described (Bouras et al., in press). In the present study we report on the asymmetric changes in SP distribution in the pallidum (P) and the substantia nigra (SN) of five brains with unilateral frontal surgical ablation for tumoral pathology. The time between surgery and death varied from one week to two years. We performed a quantitative analysis of the staining intensity of the SP-immunoreactive profiles using computer-assisted micro-densitometry. In all five investigated cases the staining intensity was asymmetric and significantly higher in the P (up to 31 %) and the SN (up to 24 %) ipsilaterally to the lobectomy. There were no changes in the SP-immunoreactive cell bodies visualized in the striatum. These data, as compared to previous results obtained in Alzheimer's disease cases, suggest that a loss of cortico-striatal afferents may influence the SP content in the terminal areas of the striato-pallidal and striato-nigral pathways.

344.16

THE VASOACTIVE INTESTINAL POLYPEPTIDERGIC DORSAL AMYGDALOFUGAL PATHWAY IS NOT ALTERED IN ALZHEIMER'S DISEASE. P.B. Cipolloni B.J. Quigley Jr.*, and N.W. Kowall. Dept. Anatomy, Boston Univ., ENR Memorial VA Hosp., Bedford MA 01730 and Neurology Service, Mass.General Hospital, Boston MA 02114.

We have previously shown that several classes of peptidergic neurons in the amygdala including those containing neuropeptide Y, cholecystokinin, substance P and somatostatin, develop dysmorphic features and contribute to senile plaque formation in Alzheimer's disease (AD). In the present study we used immunoperoxidase methods and a commercial antiserum (INCstar) to determine the distribution of vasoactive intestinal polypeptide (VIP) immunoreactivity in the amygdala of normal aged humans and patients with AD. VIP immunoreactive fibers, terminals and cells define the lateral division of the central nucleus more clearly than do other peptide stains. The stria terminalis is also intensely VIP immunoreactive. In Alzheimer's disease there is no change in the intensity or distribution of staining in the central nucleus of the amygdala or the stria terminalis. Occasional dystrophic VIP positive neurites are seen in the central nucleus. Despite the severity of changes affecting the amygdala in AD, the dorsal amygdalofugal pathway as defined by VIP immunocytochemistry is largely unchanged. This finding underscores the selectivity of neuronal degeneration in AD and supports the notion that projection systems to cerebral cortex are preferentially affected in AD.

344.17

GALANIN IMMUNOREACTIVE NEURONS OF THE TUBEROMAMMILLARY NUCLEUS IN NORMALS AND PATIENTS WITH SENILE DEMENTIA OF THE ALZHEIMER TYPE. V.Chan-Palay and B.Jentsch*. Neurology Clinic, University Hospital, CH-8091 Zürich, Switzerland.

The tuberomammillary nucleus in man contains a significant number of variably galanin immunoreactive neurons in a dense field of terminals. The location, quantity and morphological features of these peptide neurons are distinctive and have been studied using interactive computer systems. By these means the galanin containing neurons in this nucleus in six persons, neurologically and psychiatrically unaffected, were studied, quantified and three dimensional reconstructions were made of the entire region. These results were compared to the same nucleus in brains of clinically, psychometrically tested and neuropathologically confirmed cases of senile dementia of the Alzheimer type taken from the Zürich longitudinal study of dementia. There are significant alterations in the morphology of the remaining galanin immunoreactive neurons, and a loss of galanin containing cells. The immunoreactivity of the galanin neurons range from deeply intense to a very light reaction within the same groups of neurons suggesting that there may be co-existence of galanin with other neuroactive substances. We are investigating the probability that they are a subset of the histamine containing neurons of the tuberomammillary nucleus.

344.18

CORTICAL GLUTAMINASE, β-GLUCURONIDASE AND GLUCOSE UTILIZATION IN ALZHEIMER'S DISEASE. E.G. McGeer, H. Akiyama* and P.L. McGeer. Kinsmen Lab. of Neurol. Res., Univ. British Columbia, 2255 Wesbrook Mall, Vancouver, B.C., V6T 1W5, Canada.

Large cortical pyramidal neurons, known to be affected in DAT, stain positively with an antibody to phosphate-activated glutaminase (PAG). Cortical PAG, measured biochemically, is reduced in DAT more than is choline acetyltransferase (ChAT) in the same samples. β-Glucuronidase, a possible marker for reactive astrocytes, is elevated. PET studies show significant deficiencies in cortical glucose metabolism in DAT and serial scans show an exascerbation of the defect over time. In 4 cases, no correlation was found within each brain between the levels of ChAT determined postmortem in various cortical regions and the premortem local cerebral metabolic rates (LCMRs). In the 3 cases with <2 years between scan and death, the LCMRs correlated positively with PAG and negatively with β-glucuronidase activities. Such results are consistent with the hypothesis, advanced initially on the basis of morphological studies on a PET-scanned DAT that the progressive deficiencies in glucose metabolism reflect degeneration of cortical neurons with replacement by glial scar tissue.

344.19

ALTERATIONS IN THE DENSITY OF [^{125}I]PEPTIDE YY BINDING SITES IN THE HIPPOCAMPUS IN ALZHEIMER'S DISEASE. J.R. Unnerstall, B.J. Wilcox and P.J. Whitehouse. Dept. Neurology and Alzheimer Research Labs, Case Western Reserve Univ. Sch./Med., Cleveland, OH 44106.

Alterations in neuronal systems that contain neuropeptide-Y (NPY) have been described in the cortex and hippocampus of Alzheimer's Disease patients. Here, we have utilized the potent NPY analog [^{125}I]Peptide YY ([^{125}I]PYY) to label NPY binding sites in frozen sections of hippocampus taken from 9 AD cases and 7 age-matched controls at autopsy. Quantitative receptor autoradiography was used to assess the distribution and density of binding sites in these tissues. In tissue section, [^{125}I]PYY binds to two sites with K_D's of approximately 50 pM and 6 nM. Sections were labeled with 150 pM [^{125}I]PYY in the absence or presence of 500 nM human NPY to assess the high-affinity binding in autoradiographic assays. The distribution of [^{125}I]PYY binding sites parallels the distribution of NPY cell bodies and terminals described in the literature, with the highest binding seen in CA4 and the molecular layer of CA1-3. High levels of binding were also seen in the stratum radiatum and oriens. Moderate levels of binding are seen in the subiculum. Low levels of binding are seen in the molecular layer of the dentate gyrus. 50% decreases in [^{125}I]PYY binding in the AD cases were seen in the subiculum and the molecular layer of the dentate gyrus. Modest changes in other subregions can be related to the disruption of the laminar organization of the hippocampus. Thus, the greatest changes in [^{125}I]PYY binding can be related to subregions receiving afferents from intrinsic NPY neurons.

344.20

CIRCADIAN RHYTHMS OF MELATONIN AND CORTISOL IN NORMAL AGEING AND ALZHEIMER'S DISEASE. M. Thakur, M. Sharma*, G. Schwartz,*R. Quirion and N.P.V. Nair. Douglas Hosp. Res. Centre & McGill Univ. Montreal, Quebec, Canada H4H 1R3.

We investigated circadian rhythms of cortisol and melatonin in normal ageing and Alzheimer's disease (AD). Control subjects (19-89 yrs) were physically and mentally normal. AD patients (57-76 yrs) were diagnosed as "probable" or "definite" AD cases according to Reisberg Scale. All subjects were admitted to an investigation unit, where hourly blood samples were drawn from 8 a.m. until 8 a.m. the next day. The indoor illumination was restricted to 300 lux during the day and 50 lux during the night. Melatonin and cortisol were estimated by radioimmunoassay. The data was analyzed both for quantitative (24 hrs secretion and peak level) and time related functions (cosinor analysis for acrophase, mesor and amplitude). Preliminary results show significantly higher secretion of cortisol (264.1±55.9µg/dl vs 155.5±65.0µg/dl for controls) and melatonin (488.7±142.1pg/ml vs 295.5±137.6pg/ml for controls) in AD patients. On the contrary mean secretion of cortisol and melatonin decreased with age. However the acrophases of the two hormonal rhythms showed different relationships to age (positive correlation, $r=0.38$, $p<0.001$ for melatonin; and negative $r=-0.56$, $p<0.001$ for cortisol). This may suggest a weakened responsiveness of the circadian system to the day-night cycle and an altered relationship between the two pacemakers in the elderly.

344.21

COMPUTER QUANTITATED ALL NIGHT SLEEP/WAKE EEG IN EARLY ALZHEIMER'S DISEASE. P.N. Prinz, L.H. Larsen*, and M.V. Vitiello*. Dept. of Psychiatry, Univ. of Washington, Seattle, WA 98195.

Waking (clinical) EEG frequencies, while highly variable, average lower in Alzheimer's dementia (AD) than controls. Here we replicate this finding in the all night sleep EEG using computer techniques that minimize unwanted variance (muscle, eye and state influences) by selecting ideal tonic REM or slow wave sleep (SWS) epochs for analysis. Nine hour EEGs from 36 early AD (Mini Mental = 23 ± 3.) and 36 controls were digitized (256 Hz) and processed (AR filter smoother and fourier transform) using a Compaq 386. Results showed that 1) ADs had more slow (0.5 to 6 Hz) and less fast (8-30 Hz) energy during REM than controls; e.g., 16-20 Hz. REM energy (as % of total 2-20 Hz REM energy) averaged 1.82 ± .18 (SD) and 3.49 ± .32 for AD and controls ($F(1,70)=58$; $p<.0000$). 2) ADs have less energy in all frequency bins during SWS than controls; e.g., 0.5-2 Hz SWS energy averaged 7.01 ± 1.37 and 10.85 ± 1.82 for AD and controls ($F=22.6$; $p<.0000$). 3) Alpha frequency shifts toward lower values in AD; e.g., the ratio, [6 to 8 Hz ÷ 8 to 9 Hz] averaged 1.675 ± .39 and 1.447 ± .52 for AD and controls ($F=24.0$; $p<.0000$). These 3 measures together correctly classify >90% of control vs. early AD subjects in discriminate analyses. Supported by PHS Grants MH33688, RR37 and the VA.

344.22

FORGETTING IN ALZHEIMER'S DISEASE (AD): DMS VS DNMS RECOGNITION. D.M. Freed and A.J. Saraj*. Dept. of Psychology, Univ. of Southern Calif., Los Angeles, CA 90089.

Huppert and Piercy (1978) devised a procedure to evaluate rate of forgetting in memory-impaired individuals. Using this technique, control subjects (N=19) and patients with AD (N=19) were shown a set of 90 color slides taken from foreign language magazines. AD patients were provided with additional study time in order to equate their initial recognition performance with that of control subjects. Delayed-match-to-sample (DMS) and delayed-non-match-to-sample (DNMS) recognition was assessed 10 min and 24 hrs after learning.

Overall, AD patients displayed normal rates of forgetting in both DMS and DNMS recognition. A subgroup of AD patients (N=6) displayed an anomalous improvement in DNMS recognition 24 hrs after learning, replicating the findings of an earlier study (Freed et al., 1989). These results suggest that DMS and DNMS recognition are uncoupled in a subgroup of patients with AD. The existence of subgroups of AD patients has important implications for research. Supported in part by a grant from USC's Faculty Research Innovation Fund.

344.23

CHARACTERISTICS OF ODOR MEMORY IN ALZHEIMER'S DISEASE C.S. Seery* and C. Murphy. San Diego State Univ., San Diego, CA, 92182 & UCSD Medical Center, San Diego, CA 92103.

Recent neuroanatomical evidence for damage to the olfactory areas in Alzheimer's Disease suggests that patients with the disease may show selective olfactory dysfunction. Prior research has suggested that Alzheimer's patients show impairment in both olfactory threshold and memory. The present study was designed to investigate olfactory memory in Alzheimer's patients, and to compare types of responses made by patients to those made by controls. Ten patients who met the NINCDS ARDA criteria for probable Alzheimer's Disease were matched according to sex, age, and odor threshold to ten normal controls. Subjects performed a recognition memory task for odors and visual stimuli. ANOVA was conducted to compare odor and symbol hits and false-alarms in the two groups. A significant interaction was found between group and the within subject measure of hits and false alarms. A Newman-Keuls test revealed that patients had significantly more odor false alarms than did the controls, whereas the number of odor hits did not significantly differ between the two groups. Furthermore, patients had significantly more symbol false-alarms than did controls, whereas, once again, the number of symbol hits did not differ between the two groups. Interestingly, although patients had more symbol false-alarms than did the controls, within the patient group, there were significantly more odor false-alarms than symbol false-alarms. These results show that not only are the Alzheimer's patients impaired on odor memory tasks relative to controls, but they also make qualitatively different types of responses--more false-alarms, especially in the olfactory domain. Analysis of odor memory false-alarms may prove to be of diagnostic value, and may be consonant with the idea of olfactory intrusion errors. Supported by NIH grant #AG04085 and #AG08203 to C.M. We acknowledge the assistance of the Alzheimer's Disease Research Center at UCSD.

344.24

GEOGRAPHICAL KNOWLEDGE IN PATIENTS WITH ALZHEIMER'S DISEASE. W.W. Beatty and N. Bernstein*. Alzheimer Disease Research Center, UCSD.

Geographical knowledge, a measure of remote memory for visuospatial information, was studied in 24 mildly or moderately demented patients who met NINCDS-ADRDA criteria for Alzheimer's disease (AD). Patients and age, education, and gender-matched controls completed the US and California-Nevada (CA-NV) maps from the Fargo Map Test (FMT, Beatty, J Clin Psychol, 1988, 44, 61). AD patients were moderately impaired in locating the absolute positions of the gross geographical features emphasized on the US map and more profoundly impaired in locating the specific features (e.g., cities) emphasized on the CA-NV test. When the CA-NV map was scored for relative accuracy, performance by patients and controls improved to the same degree. Regression analyses indicated that patients' performance on the FMT was best predicted by neuropsychological measures that reflect remote memory; measures of anterograde memory and visuospatial function were not as strongly predictive. Our results suggest that AD patients lose the ability to access spatial information stored as a cognitive map at a stage in their disease when capacities for visuospatial analysis and wayfinding by taxon strategies may be relatively well preserved.

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344.25

CLASSICAL CONDITIONING OF THE EYELID RESPONSE IN NORMAL ELDERLY AND IN PATIENTS DIAGNOSED WITH ALZHEIMER'S DISEASE. D. S. Woodruff-Pak, R. G. Finkbiner*, and I. Katz*. Dept. of Psychology, Temple University, Philadelphia, PA 19122 and Philadelphia Geriatric Center, Philadelphia, PA 19141.

The model system of classical conditioning of the eyelid response in rabbits has led to significant progress in the neurobiology of learning and memory. There are striking parallels in learning and retention of this response between rabbits and humans. Age differences in learning this response are large in both species. Two brain structures of demonstrated involvement in eyelid classical conditioning are the cerebellum and the hippocampus. The ipsilateral cerebellum is essential for eyelid conditioning, and the hippocampus plays a modulatory role. Loss of Purkinje cells with age correlates with poorer learning in rabbits and might also be involved with poorer acquisition in older humans. Since the hippocampus is severely damaged in senile dementia of the Alzheimer's type (SDAT), we suspected that patients with SDAT might condition more poorly than normal elderly adults. Medical records of Philadelphia Geriatric Center residents were screened, and patients diagnosed with standard criteria for SDAT (e.g., McKhann et al., Neurology, 34, 1984) were identified and contacted to participate. Age-matched control subjects from PGC were also run. An 80 dB, 500 msec tone conditioned stimulus (CS) was followed 400 msec after its onset with a 5 psi, 100 msec corneal airpuff unconditioned stimulus (US). All subjects demonstrated that they could hear the tone clearly. They also demonstrated that they had a blink reflex to the airpuff. Eyeblinks were measured with an infrared detector, mounted on a headpiece with the air tube. Ninety trials were presented to the subject in a session lasting about 45 minutes. By the end of the session, normal older adults were producing an average of 57% conditioned responses, while patients with SDAT were producing an average of 10% responses. These differences were statistically significant. This simple form of learning is seriously impaired in SDAT. In this regard, classical conditioning of the eyelid response may be useful as a behavioral assessment technique in SDAT.

344.26

LONGITUDINAL MENTAL STATUS CHANGES IN ALZHEIMER'S DISEASE. S.T. DeKosky , F.R. Schmitt,* and C.Knox.* Dept. of Neurology, Lexington VA and Univ. Kentucky Med. Ctrs. and Sanders Brown Center on Aging, Lexington, KY 40511.

Studies of the natural history of Alzheimer's disease (AD) are needed as baselines for assessing effective treatment. Such research also provides information on reliability and validity of brief rating scales used to follow disease progression and response to therapy. Neurotransmitter enhancement might produce improvement in cognition but long term effectiveness of such therapy, or treatments to delay structural changes (e.g., gangliosides or trophic factors) require data on long-term cognitive stability or decline. Longitudinal data from our NIA-Alzheimer's Disease Research Center has allowed for the assessment of cognitive rating scales in a large cohort (N=125) of AD patients meeting NINCDS-ADRDA criteria for diagnosis. Six month followups for up to 18 months show progressive impairment in the Mini-Mental State (MMS; -3.2 points/yr), Memory Information Test (MIT; 3.2 points/yr), and cognitive portion of the AD Assessment Scale (ADAS; 6.2 points /yr). Non-cognitive ADAS scores did not change significantly; use of medications for "non-cognitive symptoms" (e.g., restlessness, anxiety, depression) appeared to stabilize some behavioral ratings. We found slight correlations between level of education with cognitive scores but stronger associations with duration. The three mental status exams were highly correlated with each other. No differences in mental status scores based on family history of AD were seen.

344.27

MEMORY IMPAIRMENTS OF ALZHEIMER'S DISEASE CHARACTERIZED BY THE CERAD PSYCHOMETRIC BATTERY. K.A. Welsh, N. Butters, R.C. Mohs, J.P. Hughes, A. Heyman & CERAD neuropsychology investigators. Alzheimer's Disease Research Center, Duke University, Durham, North Carolina, 27705. CERAD has developed a reliable neuropsychological assessment battery for the evaluation of patients with the clinical diagnosis of Alzheimer's disease (AD). In this study we examined the memory measures of the battery in order to establish the utility of this instrument in characterizing the memory impairments of Alzheimer's disease. Enrolled cases (N=358) were stratified by stage of dementia (mild vs moderate) and were compared to normal controls (N=327) on the list learning task and the delayed recall and recognition procedures. The results of the analyses indicate (1) an attenuated learning rate (slope from linear regression), (2) severely impaired retention, defined as percent savings between the delay recall trial and the last learning trial, and (3) deficient recognition memory in the dementia groups. In addition, retention correlated well with other neuropsychological measures of the CERAD battery, particularly the tests involving verbal retrieval. These data using CERAD measures conform closely to the well documented changes reported in AD using other neuropsychological assessment instruments. The results provide some of the validation information necessary before theoretically important studies applying the CERAD battery to other populations can be addressed.

344.28

CORRELATES OF DEMENTIA: AN EPIDEMIOLOGICAL STUDY IN THE AREA OF BORDEAUX (FRANCE). J.F. DARTIGUES*, C. MESSIER, M. GAGNON*, R. SALAMON*, P. BARBERGER-GATEAU*, M. ORGOGOZO*, and the PAQUID GROUP. Département d'Informatique Médicale, Université de Bordeaux II, 146 rue Léo Saignat, 33076 Bordeaux, FRANCE.
PAQUID is an epidemiological study designed to gather and follow-up a cohort of 4,000 elderly (65 years and older) subjects living at home. These subjects were randomly chosen in the general population of 80 well-defined communities of Gironde and Dordogne (two large districts in the area of Bordeaux, FRANCE). The major goal of PAQUID is to study normal and pathological brain aging. We present the results of the data collected on 2792 subjects who accepted to participate in Gironde. The DSM-III criteria for dementia was met by 99 subjects, corresponding to a 3.55% prevalence. The prevalence increased with age, but more interestingly, the prevalence of dementia also decreased dramatically as educational level increased. Prevalence is 11.6% for subjects with no education, 4.3% for subjects with grade school level of education, 1.3% for subjects with high school level, and 0% for subjects with university degrees. Within the same educational level, the prevalence of dementia decreased as the level of intellectual demands of the subjects' principal occupation increased.

344.29

NEUROLOGICAL FINDINGS IN ALZHEIMER'S DISEASE AND NORMAL AGING. L.J. Thal and D. Galasko (SPON: K. Madden). Dept. Neurosciences, UCSD, La Jolla, CA 92093; Neurology Ser., VA Med. Ctr. San Diego, CA 92161

Abnormal neurological findings including primitive reflexes, parietal sensory deficits, extrapyramidal signs and myoclonus occur in Alzheimer's disease (AD). In order to determine their potential value as markers of AD and their relationship to the stage of AD, we compared standardized neurological examinations in 135 community-dwelling patients with AD and 91 nondemented elderly controls. After correcting for differences in age and education between the two groups, we found that rigidity, stooped posture, graphesthesia, neglect of simultaneous tactile stimuli (face-hand test) and snout, grasp and glabella reflexes were present significantly more often in AD than controls. These findings increased in prevalence in AD patients according to the severity of their cognitive impairment. However, in a multivariate logistic regression model we found that only the grasp reflex, graphesthesia and face-hand test were statistically significantly associated with the degree of cognitive impairment. Although abnormal neurological findings occur regularly in AD, their frequency of occurrence overlaps with normal aging, resulting in a low sensitivity for the early diagnosis of AD. Prospective studies are necessary to identify whether patients with the early onset of neurological abnormalities, particularly extrapyramidal findings, form a distinct subgroup of AD such as those with diffuse Lewy body disease.

344.30

COVERT ATTENTIONAL SHIFTS AND CEREBRAL METABOLISM IN ALZHEIMER'S DISEASE AND NORMAL AGING. P.M.Greenwood*,R.Parasuraman* and J.V.Haxby*(SPON:J.Moran). Cognitive Science Lab.,Catholic Univ.,Washington, DC 20064 and Lab. of Neurosciences, N.I.A.,Bethesda, MD 20892.

Right-left asymmetries in cerebral metabolism of parietal association areas are seen in early dementia of the Alzheimer type (DAT) (Haxby et al.,1985). Since the posterior parietal plays a role in visual attention (Mesulam, 1981), we hypothesized that DAT-induced parietal dysfunction might effect the costs and benefits of cue validity on covert orienting (Posner, 1980). A directed attention task was administered to normal young and old Ss and to DAT patients, in whom resting regional cerebral metabolic rates for glucose were measured by PET. A target letter appeared after a valid, invalid or neutral location cue. In the Detect condition, Ss made a speeded response when the "X" was detected. In the Encode condition Ss made a speeded categorization of the target as a consonant or vowel. The difference in RT between invalid and valid cues was largest for the DAT group ($p<.001$). The normal young and old performed similarly (Nissen & Corkin 1985). Furthermore, RT differences were larger in the DAT group in the Encode condition ($p<.05$) suggesting costs and benefits increase with increased target processing. Results are discussed in relation to asymmetric parietal metabolism in DAT and impaired disengaging of attention after right parietal damage (Posner et al.,1984). (Supported by grants from ADRDA and NIA).

344.31

NEUROPSYCHOLOGICAL CHARACTERISTICS OF A LEWY BODY VARIANT OF ALZHEIMER'S DISEASE. D. Salmon, L. Hansen*, E. Masliah*, D. Galasko*, N. Butters and R. Katzman. Alzheimer's Disease Research Center, UCSD Sch. of Med., San Diego, CA 92103.

A variant of Alzheimer's disease (AD) has recently been described in which neuropathological changes typical of AD occur concomitantly with diffusely distributed neocortical and brainstem Lewy bodies and temporal lobe spongiform changes (Lewy body variant: LBV). The neuropsychological test performance of 9 patients with autopsy verified LBV was compared to that of 9 patients with autopsy verified AD.

Although the two patient groups were matched for age, education and overall severity of dementia (Blessed scores LBV=16.56 errors; AD=17.56 errors), the LBV patients were more impaired than the AD patients on measures of attention, visuospatial/constructional ability, and verbal fluency. The groups did not differ significantly on tests of confrontation naming or memory. The present results suggest that LBV produces a pattern of neuropsychological deficits typical of cortical dementia such as AD (e.g., memory, language impairment), with additional, uncharacteristically severe deficits in cognitive processes usually affected in subcortical dementia (e.g., attention, verbal fluency). This pattern of deficits may be useful for clinically differentiating LBV from AD.

345.1

Non-radioactive detection of NGF-receptor mRNA with digoxigenin-UTP labeled RNA probes. F. Baldino, Jr., E. Robbins, D. Grega[1], S. L. Meyers, J.E. Springer[2], and M. E. Lewis. Cephalon, Inc., West Chester PA. [1]Boehringer Mannheim, Indianapolis, IN., and [2]Hahnemann Univ., Phila., PA.

Non-radioactive detection of mRNA has become an important new histochemical strategy to study the regulation of gene expression. Autoradiographic detection of specific mRNAs with radiolabeled probes has been limited by several factors including exposure time and the degree of cellular resolution. Here we report a method to detect mRNA coding for the NGF receptor (NGFR) with an RNA probe labeled with digoxigenin-UTP.

A NGFR cDNA (3400 bases) was blunt end inserted into the polylinker site of a pGEM vector containing an Sp6 promotor and linearized at an EcoRI restriction site. In situ hybridization was performed on 25 um tissue sections according to previously published methods. Hybrids were detected with anti-digoxigenin-alkaline-phosphatase conjugate reacted with NBT.

Hybridization positive cells were identified along the base of the forebrain extending dorsally along the vertical diagonal band into the medial septum. The distribution of labeled perikarya is consistent with in situ hybridization studies using radiolabled probes complementary to the NGFR message (see J.E. Springer Neurosci. Abs.), and was convergent with the distribution of cholinergic neurons in these regions. Background labeling was barely visible in these sections.

The ability to detect NGFR mRNA with RNA probes labeled with digoxigenin-UTP (dig-UTP) will greatly enhance our ability to better understand the factors which regulate the expression of this gene. Furthermore, RNA probes labeled with dig-UTP permits the high level of resolution required to study the regulation of relatively rare transcripts at the single cell level. We thank Boehringer Mannheim GmbH for their continued support and collaboration.

345.2

LOCALIZATION OF NGF RECEPTOR mRNA IN EARLY CHICKEN EMBRYOS USING IN SITU HYBRIDIZATION J.G. Heuer* and M.A.Bothwell (SPON: O. Smith) Dept. of Physiology & Biophysics, University of Washington, Seattle, Wa. 98195.

We have previously described the cloning of a chicken NGF receptor cDNA (Bothwell et al., Neurosci. Abstr. 14:684, 1988) and its use in detecting NGF receptor mRNA in embryonic day 6 chick embryos. We demonstrate here the localization of NGF receptor mRNA in 40, 55 and 72 hour chick embryos using in situ hybridization. Chick embryos were removed from their eggs, fixed in 4% paraformaldehyde, dehydrated and embedded in paraffin. 8um sections were cut and collected onto polylysine coated slides and in situ hybridization was carried out according to Wilkinson et al., (Development 99:493, 1987) with the omission of the high stringency wash steps. NGF receptor sense and antisense cRNA probes were made using SP6 and T7 RNA polymerases from a cDNA fragment subcloned into a pGEM vector (Promega). We find NGF receptor mRNA expression on a wide variety of non-neuronal cell types including head mesenchymal cells, segmental mesoderm cells, mesenchymal cells entering developing wing and leg buds as well as sclerotome and dermatome cells of developing somites. In addition, we find NGF receptor mRNA expression on several neuronal populations including cells of cranial ganglia 5,7,8,9 and 10, presumptive motoneurons in the spinal cord, cells of the ventral myelencephalon and a subpopulation of cells in the diencephalon. Our findings indicate the presence of NGF receptor mRNA expression on a wide variety of cell types at stages earlier than those previously examined, suggesting that NGF may play other roles in development aside from the classical neurotrophic role.

345.3

KAINIC ACID - INDUCED SEIZURES STIMULATE INCREASED EXPRESSION OF ß-NGF mRNA IN ADULT RAT FOREBRAIN. C.M. Gall, K.D. Murray*, and P.J. Isackson, Department of Anatomy and Neurobiology, University of California, Irvine, CA 92717.

Recent work in our laboratories has demonstrated the recurrent limbic seizures, induced by an electrolytic lesion of the dentate gyrus hilus, result in a rapid bilateral increase in mRNA for nerve growth factor (NGF) in hippocampal neurons and a more delayed increase in neurons of olfactory cortex, neocortex, and amygdala. In the present study, the influence of seizures induced by intraventricular injection of 0.5µg kainic acid (KA) on mRNA for NGF was examined by *in situ* hybridization using riboprobes complementary to ß-NGF and the full preproNGF sequence (Whittemore et al., 1988). Hybridization was localized with both film and emulsion autoradiographic techniques.

The greatest KA-induced increase in hybridization was observed in the dentate gyrus granule cells. As early as 2 hrs post-KA there was a 35-fold increase in the density of hybridization within this cell layer (as evaluated by densitometric analysis of film autoradiograms). By 5 hrs post-KA, this had declined to 10-fold control values. At both of these time points virtually all of the dentate gyrus granule cells appeared to be densely labeled with the ^{35}S-cRNA probe. As in control rats, the majority of neurons within stratum pyramidale of hippocampal regions CA3 and CA1 were not labeled in KA-treated animals. By 12 hrs post-KA, hybridization density within the granule cell layer had declined further and there was a clear increase in the density of hybridization in superficial layers (II, III) of the piriform and entorhinal cortices and more modest increases in superficial neocortex. These data provide further support for the conclusion that seizure activity serves as a powerful stimulant for increased expression of NGF in adult rat forebrain neurons.

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345.4

CELLULAR LOCALIZATION OF ß-NGF mRNA IN RAT FOREBRAIN. J.C. Lauterborn, P.J. Isackson, and C.M. Gall, Dept. of Anatomy and Neurobiology, University of California , Irvine CA 92717.

Nerve growth factor (NGF) is thought to be a neuronally derived trophic factor which has been implicated to be important for the survival of basal forebrain cholinergic neurons. In the present study, the distribution of ß-NGF mRNA was analyzed in rat forebrain by *in situ* hybridization techniques using a ^{35}S-labeled RNA probe complementary to a 771 base sequence encoding the complete preproNGF peptide (Whittemore et al., 1988). Hybridization was visualized by emulsion autoradiographic (AR) methods.

The highest density of labeled cells was seen in the hippocampus with individual well-labeled cells observed in all principal fields as well as the subiculum. In the dentate gyrus, well-labeled cells were scattered within the hilus and a much lower density of AR grains was observed overlying the granule cell layer. Interestingly, a small number of labeled cells were also seen in the tenia tecta. In regio inferior, numerous labeled cells were observed uniformly scattered within both stratum pyramidale and stratum radiatum. By comparision, in regio superior the vast majority of labeled cells were seen within stratum pyramidale. It should be noted that the labeled neurons comprised a small portion of total neurons in each of these areas. Beyond hippocampus, scattered labeled cells were observed within the amygdaloid complex with greatest densities in the cortical amygdaloid nucleus and the anterior amygdaloid area. In addition, a small number of labeled cells were seen in the ventral pallidum and the magnocellular preoptic nucleus; two basal forebrain areas which contain cholinergic neurons. Finally, a moderate density of AR grains was also observed in layer 2 of the entorhinal and cingulate cortices and layers 2 & 3 of piriform cortex. These data provide further information on the distribution of ß-NGF synthesizing cells in limbic brain regions. Moreover, the presence of ß-NGF synthesizing cells in cholinergic-containing ventral forebrain areas is intriguing in light of NGF's probable trophic effect on CNS cholinergic neurons. Supported by NS26748 to C.M.G. and NS24747 to P.J.I.

345.5

CHARACTERIZATION OF TRUNCATED, SOLUBLE FORMS OF THE HUMAN NERVE GROWTH FACTOR RECEPTOR. A.A. Zupan, P.A. Osborne*, and E.M. Johnson, Jr. Department of Pharmacology, Washington University, St. Louis, MO 63110.

We report the purification, by sequential ammonium sulfate precipitation and immunoaffinity chromatography, of at least three truncated forms of the human nerve growth factor receptor ($NGFR_t$) from amniotic fluid with yields of 80 µg/L of starting material. Using ^{125}I-$NGFR_t$ as a tracer in the purification, a recovery of 40% was calculated. The three glycoproteins, with molecular masses of 45, 40, and 35 kDa on SDS/PAGE-12%, were confirmed to be $NGFR_t$'s by amino-terminal sequencing of individual species isolated by electroelution and were designated $NGFR_t$-1, -2, -3, respectively. The apparent binding affinities of these species for NGF were comparable to that of the low-affinity, cell-surface receptor. Isoelectric points ranged from 3.3 - 3.95 with intraspecies microheterogeneity. Each of these proteins retained a complex-type carbohydrate modification on Asn-32 and demonstrated varying degrees of sialation. We propose these molecules to be portions of the receptor's extracellular domain generated by differential proteolytic cleavage proximal to the membrane-spanning sequence. The potential to isolate milligram quantities of human $NGFR_t$ permits structural studies of the intact receptor and the generation of polyclonal antibodies to study its biological function(s). Supported by a Monsanto grant.

345.6

CIRCUMVENTRICULAR ORGANS AND CHOROID PLEXUS OF ADULT RAT CONTAIN HIGH LEVELS OF NERVE GROWTH FACTOR (NGR) RECEPTOR. [1]Qiao Yan,[1]Akio Wanaka,* [2]H. Brent Clark, and [1]Eugene M. Johnson, Jr., [1]Department of Pharmacology, Washington University Medical School, St. Louis, MO 63110 and [2]Department of Laboratory Medicine, Memorial Medical Center, Springfield, IL 62781

Immunohistochemical examination of adult rat brain demonstrated the presence of high levels of NGF receptor in circumventricular organs: Subfornical organs, median eminence, area postrema, and posterior pituitary. Dense staining was also seen in choroid plexus. Biochemical study of the NGF receptor in these tissues by crosslink/immunoprecipitation showed a band at a molecular weight of 90 kDa, consistent with intact receptor. No truncated species of receptor was observed. These tissues also contained significant levels of the 3.9 Kb NGF receptor mRNA, indicating that the receptor is synthesized locally. Immunoelectronmicroscopy indicates that the receptor resides on un-identified fusiform cells which lie both inside and outside the basal lamina in proximity to blood vessels. The NGF receptor in the circumventrical organs were further studied by in vivo labeling of ^{125}I-ligands. Intravenously injected ^{125}I-NGF was specifically accumulated in the area postrema and the posterior pituitary (only areas examined), but ^{125}I-cytochrome C was not. Labeling of ^{125}I was blocked by injection of unlabeled NGF. Experiments are currently underway to identify the cell type(s) bearing the NGF receptor in these tissues.

345.7

DEVELOPMENTAL EXPRESSION OF NERVE GROWTH FACTOR RECEPTOR (NGFR) IN THE RAT VISUAL SYSTEM. M.C. Comelli*, L. Cavicchioli*, P. Candeo*, G. Carmignoto* and L. Maffei*°. (SPON.: G. Calderini). Fidia Research Laboratories, 35031 Abano Terme (PD) and °Istituto di Neurofisiologia, CNR, 56100 Pisa, ITALY.

Retinal ganglion cells of rat retina have been recently proven to be rescued from degeneration by NGF intraocular administrations (Carmignoto, G. et al., J. Neurosci., in press). Since the action of NGF involves a receptor-coupling event, we analyzed the possible expression of the NGFR and its mRNA in the retina and optic nerve of Long Evans hooded rats. The presence of NGFR was detected immunocytochemically in cross sections of the retina by using the monoclonal antibody 192-IgG, which specifically recognizes rat NGFR. NGFR immunoreactivity was present in the retina as well as optic nerve of neonatal rats, whereas it was present only in the retina of adult rats. The pattern of staining is consistent with Müller cells, although it can not be completely ruled out that retinal ganglion cells (RGCs) are also positive. The mRNA for NGFR was also found to be present in the retina both at postnatal day 2 and in adult rats, thereby indicating that the immunoreactivity represents authentic NGFR. These results suggest that the effect of NGF on RGC survival is mediated indirectly through Müller cells and raises the possibility of a specific role of NGF in the development of the visual system.

345.8

REVERSE PHASE-HPLC CHARACTERIZATION OF NGF AND NGF RECEPTOR. C.E. Beck*, D.E. Shan*, J.I. Teng*, K. Werrbach-Perez* & J.R. Perez-Polo, (Spon: L.W. Thorpe) HBC&G Dept., Univ. of Texas Medical Branch, Galveston, TX, 77550.

The first obligatory step to the action of the neuronotrophic factor NGF is binding to a cell surface receptor, NGFR. Murine beta-NGF, here called NGF, is a glycoprotein. NGF purified by reverse phase HPLC was attached to nitrocellulose membranes, reacted with biotinylated lectins and reactions visualized with an avidin-biotin complex coupled to horseradish peroxidase that yielded several monosaccharides. Hydrolysis of NGF followed by analysis by high pressure anion exchange chromatography with pulsed amperometric detection (AE-PAD) gave d-glucose, fucose, mannose, d-galactose and glucosamine. NGFR present on PC12 cells was partially purified by lectin chromatography and RP-HPLC followed by immunoprecipitation with a monoclonal antibody to rodent NGFR and SDS-PAGE analysis. Our results suggest that there is a receptor associated protein of 16 KDa. Supported in part by grants from NINDS and ONR.

345.9

NERVE GROWTH FACTOR (NGF) REGULATES EXPRESSION OF THE NGF-RECEPTOR GENE IN ADULT RAT SENSORY NEURONS R.M.Lindsay, E.M.Shooter, G.Dechant*, H.Thoenen and D. Lindholm. Regeneron Inc., 777 Old Sawmill River Road, Tarrytown, NY 10591-6707

Adult rat dorsal root ganglion (DRG) sensory neurons can be maintained in culture in the absence of NGF. We have therefore used these neurons to study the effect of NGF on the regulation of expression of mRNA encoding the NGF-receptor (NGF-R). In the absence of NGF, NGF-R levels remained constant for 7 days in cultures of adult rat DRG neurons. In the presence of NGF, NGF-R mRNA levels rose 2-3-fold after 2 days, reaching plateau levels (5-6-fold increase) after 5 days. NGF-induced up-regulation of NGF-R mRNA could be shown even after prior NGF-deprivation for 5 days. NGF had no effect on NGF-R mRNA levels in DRG non-neuronal cells. Other polypeptide growth factors (EGF, CNTF, FGF) had no effect on adult rat DRG neuron NGF-R mRNA levels, but 8-bromo-cAMP treatment decreased NGF-R mRNA levels by 65% (cf. controls) after 2 days. NGF also induced a rapid rise in c-fos in DRG neurons but not in non-neuronal cells. These results suggest that levels of expression of the NGF-receptor in vivo may be regulated by endogenous levels of NGF.

345.10

IDENTIFICATION AND CLONING OF ANTISENSE mRNA FOR NERVE GROWTH FACTOR RECEPTOR. A.G. Yakovlev,* E. Costa and I. Mocchetti+. Fidia-Georgetown Institute for the Neurosciences and +Department of Anatomy & Cell Biology, Georgetown University, Washington D.C. 20007.

Northern blot hybridization analysis of rat brain poly(A)$^+$ RNA with pNGFR.1 cDNA (Radeke et al. 1986) clone revealed the presence of two mRNA species related to nerve growth factor receptor (NGFR) one of 3.8 kb and the other of about 1.7 kb. The 1.7 kb hybridization pattern fails to occur in poly(A)$^+$ RNA prepared from PC12 cells or dorsal root ganglia which highly express the 3.8 kb NGFR mRNA. Furthermore, the presence of 1.7 kb band could be detected by hybridizing with a single strand probe containing the coding sequence of NGFR cDNA and not by its antisense strand. These observations prompted us to investigate whether an endogenous antisense NGFR RNA could be present in rat brain. cDNA library of rat brain was constructed and screened with pNGFR.1 probe. Several positive clones were isolated and further analyzed by Northern blot hybridization analysis. One of the positive clone, pBS3 which hybridized to 1.7 kb brain RNA, was partially sequenced. The 3' region of this clone revealed high homology with a segment of NGFR mRNA. pBS3 cDNA was used to hybridize poly(A)$^+$ RNA from rat brain at different stage of postnatal development. We found that the zenit of pBS3 mRNA expression is associated to the nadir of NGFR mRNA expression. We are currently investigating whether the BS3 mRNA is involved in the modulation of NGFR mRNA expression at the translational level.

345.11

TRANSSYNAPTIC REGULATION OF NERVE GROWTH FACTOR GENE EXPRESSION. M. Fabrazzo*, E. Costa and I. Mocchetti+. Fidia-Georgetown Institute for the Neurosciences and +Department of Anatomy & Cell Biology, Georgetown University, Washington D.C. 20007.

The regulation of nerve growth factor (NGF) gene expression in rat brain has been studied to assess the hypothesis deriving from our *in vitro* studies that beta-adrenergic receptor (BAR) stimulation increases NGF gene expression. The hypothesis was verified *in vivo* by investigating whether the acute or prolonged stimulation of BAR by isoproterenol (20ug i.c.v.) increases NGF mRNA content. While BAR activation failed to change NGF mRNA content of various brain structures, the depletion of catecholamine stores by reserpine induced a specific increase of NGF mRNA in cerebral cortex. The time course of this effect shows that a single dose of reserpine (2 mg/kg s.c.) elicits an increase on NGF mRNA content which peaks at 9 hr after the injection. However at 24 hours the content of NGF mRNA return to the control value . Injecting the same dose for two days, the NGF mRNA increase caused by reserpine last longer. In fact, 3 days after the last injection NGF mRNA and protein content are still significantly increased. Therefore, reserpine either might remove a transsynaptic tonic inhibition exerted by a depleted monoamine or increase the content of corticosteroids which, in turn, can positively affect NGF gene expression.

345.12

DETECTION OF NERVE GROWTH FACTOR (NGF) mRNA IN HUMAN BRAIN USING THE POLYMERASE CHAIN REACTION (PCR). M. Fahnestock and L. B. Taylor*. Molecular Biology Department, SRI International, Menlo Park, CA 94025.

We have previously shown that NGF mRNA can be detected in human frontal cortex using sensitive Northern blotting techniques, and that the relative amounts of NGF mRNA in various cortex samples can be estimated using dot blotting. However, the amount of NGF mRNA in human brain is extremely low, and in order to load sufficient mRNA on Northern or dot blots to generate a signal, we have been forced to start with many grams of tissue. This has made the study of NGF in smaller structures of the brain impossible.

We now show that, starting with small amounts of tissue from human cortex or hippocampus, polyA+ mRNA can be purified and tested for the presence of NGF mRNA. One microgram of polyA+ mRNA is sufficient template for the synthesis of cDNA; then NGF-specific primers and Taq polymerase are used to amplify the NGF cDNA by the polymerase chain reaction (PCR). A standard, amplified simultaneously in each sample, allows relative amounts of NGF mRNA to be compared between samples. We show that NGF mRNA can be detected by PCR in human cortex.

345.13

SITE SPECIFIC MUTAGENESIS OF TRP-21 TO LEU-21 IN β-NERVE GROWTH FACTOR (NGF) RETAINS BIOLOGICAL ACTIVITY. Y. Luo* and K.E. Neet. Department of Biochemistry, Case Western Reserve University, Cleveland, Ohio 44106.

Oxidation of Trp-21 with N-bromosuccinimide produces a significant decrease in both biological and binding activity of NGF (Cohen et al. J. Biol. Chem. 255, 2949, 1980; Frazier et al. Biochemistry 12, 3281, 1973). In order to test further the involvement of Trp-21 in the biological and binding activity of NGF, we have replaced the tryptophan with leucine by oligonucleotide-directed mutagenesis. The mutated NGF cDNA was confirmed by dideoxy DNA sequencing and was highly expressed in *E. coli* with a gene fusion vector pRIT2T (Luo & Neet, 18th Ann. Soc. Neurosci. Mtg, 14, 276.7, 1988). The fusion protein was present in inclusion bodies of *E. coli* and was purified through a DE52 anion exchange column in the presence of 6 M urea. Intact mutant NGF protein, NGF(W21L), was cleaved from the fusion protein by trypsin treatment at 0^0 C and purified to homogeneity by reverse phase FPLC. NGF(W21L) was found to promote neurite outgrowth of PC12 cells in defined medium and this biological activity was inhibitable by polyclonal anti-NGF antibodies. Our result indicates that alteration of Trp-21 to a smaller hydrophobic residue, leucine, does not eliminate the biological response of PC12 cells to NGF. Further biochemical characterization and quantitation of this mutant protein are in progress. (Supported by an NIH grant, NS24380)

345.14

SRC AND RAS RELATED ANTIGENS ARE REQUIRED FOR NEURONAL DIFFERENTIATION OF PC12 BY NGF AND b-FGF. N. E. Kremer, J. S. Brugge,* and S. Halegoua*, Dept. of Neurobiology & Behavior, S.U.N.Y. at Stony Brook, Stony Brook, NY 11794, and *Howard Hughes Medical Institute, Dept. of Microbiology, Univ. of Penn., Philadelphia, PA, 19104.

We have used antibody microinjection into multinucleated fused PC12 cells to study signal transduction of NGF and basic FGF (see Hagag et al., 1986, Nature, 319:680). We report that microinjection of a monoclonal antibody against the protooncogene c-src product pp60, or of monoclonal antibody Y13-259 against protooncogene c-Ha-ras product p21, blocks neurite outgrowth induced either by NGF or b-FGF.

Cells were pretreated with NGF for 24 hr, or with b-FGF for 4 days, then microinjected, and scored for neurites 24 hr later. In a typical experiment with NGF, 75% of uninjected control cells or cells injected with a non-specific control antibody exhibited neurites. Only 17% of cells had neurites after microinjection of the anti-src antibody. Similarly, treatment with b-FGF resulted in a neurite frequency of 71%. Injection of the anti-src antibody resulted in a frequency of 26%. Injection of anti-ras antibody similarly inhibited both NGF and b-FGF induction of neurites. Both anti-src and anti-ras antibodies prevented and reversed neurite outgrowth. All antibody injected cells were capable of producing neurites, since they all produced neurites in response to dibutyryl cAMP. Furthermore, antibody injected cells regrew neurites at times beyond 40 hr post-injection.

Our results suggest that a src-related tyrosine kinase and a ras-related G-protein are essential components of both the NGF and b-FGF signal transduction pathways. The relationship between these components and others involved in NGF action, such as C-kinase and A-kinase, are being investigated.

345.15

PROCESSING OF RECOMBINANT NERVE GROWTH FACTOR P.J. Isackson, M. Blaber*, K. Cavanaugh* and R.A. Bradshaw. Dept. of Biological Chemistry and Dept. of Anatomy and Neurobiology, Univ. of California, Irvine, CA 92717.

Beta-NGF and the associated glandular kallikreins, alpha-NGF and gamma-NGF, of the mouse submandibular gland have each been expressed as fusion proteins in E. coli using a plasmid vector containing the araB and araC genes. Induction with arabinose results in the expression of each of these fusion proteins at a level of greater than 1 mg per liter of media. The kallikrein fusion proteins can be solubilized with guanidine-HCl followed by refolding with a glutathione-based redox buffer. Limited digestion of the gamma-NGF fusion protein with trypsin produces a two-chain form of the molecule which has been purified to homogeneity, is enzymatically active against synthetic ester substrates, and recombines with alpha- and beta-NGF to form the 7S NGF complex. In contrast to the kallikreins, the beta-NGF fusion protein is not soluble in neutral pH buffer after the refolding procedure. Treatment of the refolded fusion protein with trypsin produces a form of beta-NGF which is soluble and promotes neurite extension of PC12 cells. Supported by NS24747 to P.J. Isackson and NS19964 to R.A. Bradshaw.

345.16

RAPID AND SPECIFIC INDUCTION OF IMMEDIATE EARLY GENES BY NERVE GROWTH FACTOR IN PC12 CELLS. Jeffery L. Twiss* and Gary E. Landreth. Department of Neurology, Medical University of South Carolina, Charleston, SC 29425

Nerve growth factor rapidly stimulates the expression of a number of "immediate early genes" which encode putative transcriptional regulators. We have screened a cDNA library from NGF-treated PC12 cells and have detected four cDNAs representing mRNA species whose abundance is dramatically increased on NGF treatment of PC12 cells. Two of these clones have been characterized. NGF treatment resulted in the dramatic elevation of mRNA levels of 1N19 within 1 hr, which was sustained for at least 24 hrs. The mRNA levels of a second clone, 7N56, are induced within 10 hrs of NGF treatment and subsequently declined within 24 hrs. EGF and TPA treatment of the cells had no effect on mRNA levels of these genes. Cross hybridization studies demonstrated that these clones are unrelated to other NGF induced genes including c-fos, c-myc, PC2, PC3, PC4. Partial sequence analysis revealed no sequence homology with other gene sequences in Genbank. We have detected a novel gene, 1/5N3, which was maximally induced within 15 min of NGF addition, mRNA levels then fell rapidly in the following 30-60 min. The accumulation of 1/5N3 mRNA was blocked by ā-amanitin. The expression of this gene was also stimulated by EGF and TPA to similar levels. No significant homology within the Genbank database was detected using partial sequence data.

345.17

Induction of 1,2-diacylglycerol by nerve growth factor and basic fibroblast growth factor in PC12 cells is primarily from phospholipids other than the phosphoinositides. J. G. Altin* and R. A. Bradshaw, Dept. of Biological Chemistry, California Coll of Med., Univ. of Calif., Irvine, CA 92717.

Nerve growth factor (NGF) and basic fibroblast growth factor (bFGF) induce differentiation and neurite outgrowth in PC12 cells. In these cells, NGF stimulates an increase in the production of inositol phosphates (IPs) from the cleavage of phosphoinositides with the concomittent release of 1,2-diacylglycerol (DAG). However, compared to carbachol, the amount of IPs produced by both NGF and bFGF are inadequate to account for the DAG, suggesting that other phospholipid pools may also be hydrolyzed. The addition of NGF and bFGF to cells pre-labelled with ^{3}H-inositol and incubated in the presence of 10 mM LiCl, each increased the production of IPs by 120-200% within 30 min. Approximately 85% was recovered as IP, the remainder as inositol bisphosphate and inositol trisphosphate. Under similar conditions carbachol induced a 10-fold increase in IPs. NGF and bFGF also induced a Ca^{2+}-dependent increase in the mass level of DAG; the increase was about 2-3 fold after 15 min and was sustained for at least 30 min. Stimulation of DAG production by 0.5 mM carbachol was similar to that induced by 200 ng/ml NGF and 50 ng/ml bFGF. As the production of IPs by carbachol was 5-10-fold greater than that induced by NGF and bFGF, this suggests that both factors stimulate the production of DAG primarily from a source(s) other than the phosphoinositides. Moreover, since carbachol stimulates the production of DAG but does not induce neurite outgrowth in PC12 cells, it raises the question as to the role of protein kinase C in the signal transduction cascade leading to neurite outgrowth in PC12 cells, and whether the species of DAG produced by the action of NGF, bFGF and carbachol might activate different forms of protein kinase C. Supported by USPHS research grant NS19964 and ACS research grant BC273.

345.18

DIFFERENTIAL INHIBITION OF NGF RESPONSES BY PURINE ANALOGS. C. Volonté, A Rukenstein*, D.M. Loeb* and L.A. Greene*. Department of Pathology,College of Physicians and Surgeons, Columbia University, New York - N.Y., 10032.

Purine analogs are used in this study to dissect specific steps in the mechanism of action of nerve growth factor (NGF). We have previously shown that protein kinase N (PKN), an NGF-activated serine protein kinase that is active in the presence of Mn^{++}, is inhibited in vitro by purine analogs, the most effective of which was 6-thioguanine (apparent K_i = 6 μM). Several different criteria indicated that 6-thioguanine was not a general inhibitor of protein kinases and that it was relatively specific for PKN. Since purine analogs rapidly and effectively enter cells, they were assessed for their actions on both transcription-dependent and -independent responses to NGF. NGF-promoted neurite regeneration is reversibly suppressed, both in PC12 cells and in cultured sympathetic neurons, by the analogs, at concentrations very similar to those that inhibit PKN. Comparable concentrations of the purine analogs also block NGF- and cyclic AMP analog-stimulated induction of ornithine decarboxylase activity. This effect is prevented by pre-exposing the cells to NGF for a very short time (1-3 min). In contrast to its inhibition of neurite regeneration and ornithine decarboxylase induction, 6-thioguanine does not suppress NGF-dependent induction of c-*fos* mRNA expression and in fact, appears to enhance this effect. c-*fos* mRNA induction is transient in the presence of 6-thioguanine and NGF, peaking at about 1 hr and being undetectable by 4 hrs of treatment. Thus, purine analogs such as 6-thioguanine appear capable of differentially suppressing some, but not other actions of NGF. These findings suggest the presence of multiple pathways in the NGF mechanism of action and the possibility of dissecting them with the use of purine analogs. Moreover, these data are compatible with a causal role for protein kinase N in certain of these pathways.

345.19

EFFECTS OF NERVE GROWTH FACTOR (NGF) AND MONOSIALOGANGLIOSIDE GM1 ON NEUROTRANSMITTER RELEASE IN VIVO. D. Maysinger, M. Herrera-Marschitz*, U. Ungerstedt and A.C. Cuello Dept. of Pharmacology & Therapeutics, McGill University, Montreal and Dept. of Pharmacology, Karolinska Institutet, Stockholm, Sweden.

The release of acetylcholine, (ACh), choline, dopamine (DA), DOPAC, 5-HIAA, HVA and adenosine was studied in the rat cortex and striatum by using microdialysis combined with highly sensitive HPLC systems. The release of neurotransmitters and their metabolites was investigated in animals with unilateral cortical devascularizing lesions causing partial retrograde degeneration of cholinergic neurons in the nucleus basalis magnocellularis. Cortically lesioned rats were chronically treated with either NGF or the monosialoganglioside GM1. The drugs were delivered into the lateral ventricle via permanent installed canulae. Thirty days following the treatment, animals were anaesthesized with halothane and microdialysis probes were implanted into the intact or remaining cortex and the left caudate putamen (ipsilateral to the lesion). Unstimulated ACh release was only measurable in the presence of neostigmine. This AChE inhibitor produced transient but significant increase of DA release in the corpus striatum. High KCl concentration caused significant increase in release of all neurotransmitters. Both NGF and GM1 partially or totally (GM1) reestablished the recovery of stimulated ACh release in lesioned rats.

345.20

EFFECTS OF NGF AND/OR GM1 ON HIGH AFFINITY CHOLINE UPTAKE IN THE RAT BRAIN FOLLOWING UNILATERAL DECORTICATION. L. Garofalo* and A.C.Cuello(SPON: E.Pioro). Dept. of Pharmacology, McGill University, Montreal,Canada H3G-1Y6.

Previous studies in our laboratory have shown that treatment with NGF and/or GM1 increases ChAT activity above control levels in the remaining ipsilateral cortex of adult rats following unilateral devascularizing cortical lesions. In order to examine further the effects of such a lesion and factor administration on the functional state of cholinergic terminals we assessed high affinity choline uptake(HACU) in the remaining ipsilateral cortex of rats 1,5, 15 and 30 days post-lesion. Adult male Wistar rats were cortically lesioned unilaterally and treated immediately intracerebroventricularly with either: vehicle, NGF(12ug/day), GM1(5mg/kg/day) or both these factors simultaneously for 7 days using osmotic minipumps. Cortical HACU in the remaining ipsilateral cortex of vehicle treated rats did not differ, at any time examined post-lesion, from control values. Significant increases over control values in HACU were observed 15 days post-lesion only in rats which received both NGF and GM1. At 30 days post-lesion, a 22% and 30% increase over control cortical HACU was noted in synaptosomes from NGF or GM1 treated rats respectively and a 66% increase was obtained in rats which received NGF and GM1 simultaneously. Immunocytochemical studies are currently underway to determine whether these increases may be due to sprouting of cortical cholinergic terminals.

TROPHIC AGENTS VI

346.1

AN APPARENT ABSENCE OF NERVE GROWTH FACTOR RECEPTORS ON MIGRATING NEURAL CREST CELLS *IN VIVO*. J. Speight* and P. Bernd (SPON: J. Jakway). Dept. of Anatomy and Cell Biology, SUNY Health Sci. Ctr., Brooklyn, NY 11203.

Binding studies on relatively undifferentiated short-term (0 & 24 hr) neural crest (NC) cultures have demonstrated that premigratory and early migratory NC cells do not bind ^{125}I-NGF indicating that they do not possess NGF receptors; differentiating, long-term (>5 day) cultures do bind ^{125}I-NGF via specific receptors and are potentially responsive to NGF. 72-hr (stage 19-20) quail embryos were used to examine the presence or absence of NGF receptors on early migratory NC cells *in vivo*. Serial cryostat (10 µm) sections were exposed to ^{125}I-NGF (50 ng/ml in PBS, 90 min, 37°C); controls included an excess of nonradioactive NGF (5 µg/ml). Radioautography was used to localize NGF receptors, while migrating neural crest cells were identified using HNK-1. Preliminary results revealed low levels of specific binding uniformly throughout the embryo; however, the density of silver grain accumulations over the HNK-1+ cells was not greater than that over surrounding tissues, indicating that early differentiating NC cells *in vivo*, as *in vitro*, do not possess receptors for NGF. 8 day (stage 35) embryos were used as a positive control; as expected, there was specific ^{125}I-NGF labelling of dorsal root ganglia. These results further confirm *in vitro* studies and imply that NGF does not play a role in the migration and early differentiation of NC cells. Supported by grants from the March of Dimes (#1-1090), NSF (BNS-8896101), and the Dysautonomia Foundation.

346.2

SELECTIVE EXPRESSION OF NERVE GROWTH FACTOR RECEPTORS ON SENSORY NEUROBLASTS IN NEURAL CREST CULTURES. P. Bernd. Dept. of Anatomy and Cell Biology, SUNY Health Sci. Ctr., Brooklyn, NY 11203.

Previous studies have shown that long-term neural crest cultures (7 days past explantation) contain a subpopulation of cells exhibiting NGF receptors. We have found, however, that the phenotype of cells expressing NGF receptors is dependent upon the culture conditions. In cultures maintained in the absence of exogenous NGF, the primary cell phenotype binding ^{125}I-NGF appears to be Schwann cell precursors, while neuron-like cells do not bind ^{125}I-NGF. In contrast, if cultures are maintained in the continuous presence of exogenous NGF (50 ng/ml), catecholaminergic neuron-like cells with high affinity NGF receptors can be identified. The current study investigated whether sensory neuroblasts, identified by the presence of vasoactive intestinal polypeptide (VIP) or substance P (SP), are likewise affected by culture conditions. Combined radioautographic and immunocytochemical results indicate that VIP-like immunoreactive cells do not have NGF receptors if maintained in the absence of NGF. Preliminary co-localization studies with cultures maintained in the continuous presence of exogenous NGF revealed NGF receptors on cells immunoreactive for SP. Studies are under way to determine whether these receptors are of the high affinity subtype. These findings suggest that sensory neuroblasts may be responsive to NGF, depending upon the microenvironment. Supported by grants from the March of Dimes (#1-1090), NSF (BNS-8896101), and the Dysautonomia Foundation.

346.3

PATTERNS OF IMMUNOREACTIVITY IN CNS AND GENETICALLY ENGINEERED CELL LINES VISUALIZED WITH ANTIBODIES AGAINST NATIVE NGF AND SYNTHETIC PEPTIDE SEQUENCES WITHIN NGF AND PRO-NGF. C. Wetmore[1], T. Ebendal*[2], M. Bygdeman*[3], M. Eriksdotter-Nilsson*[1], L. Strömberg[1], H. Persson*[4], L. Olson[1], Depts. of [1]Histology & Neurobiology and [4]Molecular Neurobiology, Karolika Institute, S-104 01 Stockholm; [3]Dept. of Obst-Gyn, Karolinska Hospital, S-104 01 Stockholm; [2]Biomedicum, Uppsala Univ., S-751 23 Uppsala, Sweden.

We have raised antibodies against purified mouse salivary NGF and against synthetic peptide sequences within NGF and pro-NGF. Two peptides (P5, P6) represent sequences in rat and mouse pro-NGF, one peptide (P3), a highly conserved region of mature NGF (identical in man, mouse, rat and chicken) and one peptide (P4) the presumed loop region of mature NGF. Monoclonal peptides against P3 were also generated. Polyclonal antibodies against native NGF were raised in four different species (mouse, rabbit, sheep, chicken). Antibodies were affinity-purified using the appropriate antigens and were tested in adult male mouse submandibular glands. In adult rat brain P3-IR cells were found in several other regions including cortex and hippocampus. P3-IR was distributed in peripheral cytoplasm. Antibodies against pro-NGF peptides P5 and P6 bound to a similar set of cells in cortex, hippocampus and elsewhere. P5-IR had a perinuclear distribution. P6-IR was prominent both in brain and in spinal cord neurons and in oligodendroglial cells. Antibodies against native NGF raised in rabbit and sheep bound mainly to myelinated fibers. After colchicine treatment, one such antibody also revealed many nerve cells in cortex. Additionally, cells in human fetal spinal cord were strongly immunoreactive with P3 antibodies. 3T3 fibroblasts transfected with the rat NGF gene also contained high numbers of P3-IR cells. Interestingly, antibodies raised against native NGF in chicken bound to the membranes of a rich network of varicose nerve terminals in cortex and hippocampus and to cholinergic nerve cell bodies. This observation suggests the possibility of obtaining anti-idiotypic antibodies recognizing the NGF receptor following immunization with NGF. Further studies including preabsorption tests and experimental perturbations will define the possibilities and limitations of using immunohistochemistry to study NGF in CNS.

346.4

CORRELATIONS BETWEEN GAP43 EXPRESSION AND THE NGF RECEPTOR IN PRIMARY SENSORY NEURONS. V.M.K. Verge*, W. Tetzlaff, M. Bisby and P.M. Richardson. McGill University and University of Calgary, Canada.

Expression of the gene for GAP43 in normal and injured rat sensory neurons was studied by in situ hybridization with a cDNA probe in correlation with NGF-receptor radioautography or immunocytochemistry on adjacent sections. In normal lumbar DRG, some neurons were heavily labelled by the GAP43 probe while others had labelling near background levels. The highest cytoplasmic concentrations of GAP43 mRNA were found in neurons with high-affinity NGF receptors: much lower concentrations were found in neurons without such receptors. Another NGF-responsive population, sympathetic neurons in the superior cervical ganglion, also displayed high basal levels of GAP43 mRNA. One week following sciatic nerve transection, GAP43 mRNA was increased in all L_4 DRG neurons except for approximately 10%, presumed to lack projections into the sciatic nerve. It is suggested that i) NGF may regulate the synthesis of GAP43 in normal adult sensory neurons. ii) one consequence of the relatively strong expression of the GAP43 gene in NGF-responsive sensory neurons might be superior capacity for collateral sprouting in response to partial denervation. iii) following nerve injury, GAP43 is induced in both NGF-responsive and unresponsive neurons by some unknown signal.

Supported by MRC (Canada) and the Rick Hansen Fund.

346.5

NERVE GROWTH FACTOR RECEPTOR IMMUNOREACTIVITY IN THE SKIN AND ITS RELATIONSHIP TO SENSORY AND SYMPATHETIC FIBERS. A. Ribeiro-da-Silva*, R.L. Kenigsberg*, I. Mazzoni* and A. Claudio Cuello. Dept. of Pharmacology & Therapeutics, McGill University, Montreal, Quebec, Canada H3G 1Y6.

In the rat skin, nerve growth factor receptor (NGFR) immunoreactivity (IR) has been shown to occur in nerves, sensory receptors and patchy areas of the epidermis associated with intraepithelial nerve fibers (Ribeiro-da-Silva, et al., Neurosci. Abstr. 14:903, 1988). In the present study, double labelings for NGFR-IR and substance P (SP)-IR, and NGFR-IR and dopamine beta-hydroxylase (DBH)-IR were carried out using exclusively monoclonal antibodies. Nerves with NGFR-IR often contained fibers with SP-IR. These fibers were observed crossing the NGFR-IR patchy areas of the epidermis, ending closer to the surface of the epithelium. Nerves with both DBH-IR and NGFR-IR occurred mainly in nerve fibers associated with blood vessels. Sensory denervation caused a complete disappearance of SP-IR, and sympathetic denervation a complete depletion of DBH-IR. In the denervated skin, increased NGFR-IR was observed in peripheral nerves containing degenerated axons. This study reveals that most of the NGFR-IR in the skin is associated with sensory fibers.

346.6

EXPRESSION OF NERVE GROWTH FACTOR RECEPTOR IN FOLLICULAR DENDRITIC CELLS OF LYMPHOIDAL TISSUES. S J Thompson*, A M Gown*, M A Bothwell, (SPON: D. Nochlin Department of Physiology & Biophysics, University of Washington School of Medicine, Seattle, WA. 98195.

Nerve growth factor receptor (NGFR) has been localized to follicular dendritic cells (FDC), which are antigen- presenting cells within the germinal centers of lymphoidal tissues. Monoclonal antibodies against human NGFR developed in this laboratory were used to characterize, by immunocytochemical method, the induction of NGFR expression by FDC in response to antigen challenge. FDC immunoreactivity in lymph node, tonsil, spleen, and lymphoid nodules within gut and lung has been noted. In spleen, trabeculae and sinusoids are also immunoreactive. Cross-linking experiments with radiolabelled NGF in one available dendritic cell line have been negative. Preliminary evidence suggests that unactivated lymphoidal tissues in humans and primates do not express NGFR.

NGFR expression in FDC is shared in common with several other cell types which are dendritic in morphology and are thought to be growth-regulatory to associated proliferating cells, including sustentacular cells of the adrenal medulla and myoepithelial cells of mammary tissue.

346.7

Spatial and temporal expression of NGF Receptor on the mesenchymal cells of embryonic rat lung in vivo and in organotypic lung culture. E. F. Wheeler*, S. L. Patterson*, and M. Bothwell. Dept. of Phys. and Biophys., U. of Washington, Seattle, WA. 98195.

The NGFR has been shown to express on a wide variety of neuronal and nonneuronal tissues as a function of development. In a number of developing epithelial organs, NGFR has been observed to express in a temporal manner on mesenchymal cells associated with developing epithelial tissue. Using immunocytochemical analysis and in situ hybridization on whole rat embryos, we have observed precisely timed expression (day 15- 17) of NGFR on the mesenchyme associated with developing bronchio-epithelium of the lung. Organotypic lung culture of day 16 embryonic lung prolongs the period of receptor expression. When whole 14 day rat lungs are subjected to organotypic culture, the expression of NGFR is delayed. However, once expressed on the mesenchyme in 14 day organotypic lung cultures, NGFR is maintained on the cell surface long after it would have disappeared in vivo. Preparation of monolayer lung cell cultures derived from different embryonic lung stages contain a population of mesenchyme cells that maintain receptor expression. The role epithelial cells play in the timed expression of NGFR on these mesenchymal cells will be discussed.

346.8

ELECTRON MICROSCOPY SHOWS NGF RECEPTOR LABELING OF GLIAL-LIKE CELLS IN OLFACTORY BULB. H. Vickland*,[1] J. N. Kott,[2] L.E. Westrum,[1,2,3] S.L. Patterson*,[4] and M. Bothwell[4] (SPON: K. Chan). Depts. Biol. Struct.,[1] Neuro. Surg.,[2] Restor. Dent.,[3] and Physiol. and Biophys.,[4] Univ. of Wash., Seattle, WA 98195.

We have used MAB 192 IgG to localize nerve growth factor receptor (NGFR) in the rat olfactory bulb (OB) in early postnatal (PN) stages and in the adult, and have observed age-related differences in NGFR expression with light (LM) and electron microscopy (EM). In PN ages 2 to 12, intense NGFR immunoreactivity (IR) was observed surrounding the olfactory fila, though the axons themselves appear negative. In adults no comparable NGFR staining was seen in this location, but the OB glomeruli show intense staining and the label appears strongest in a sheath surrounding the glomerulus. By EM, NGFR-IR appeared strongest on the exposed membrane surfaces of glial-like cells that resemble astrocytes. These have long processes surrounding axon fascicles of the fila, and some of the processes appear to contact blood vessels. A similar pattern of glial cells and processes exhibiting NGFR-IR surround the OB glomeruli. This supports the speculation that astrocyte-like cells are possibly involved in the distribution of NGF to other cells of the olfactory system. We are in the process of investigating the pattern of distribution of NGF. (Supported by Grant No. NS09678 to LEW and NS23343 to MB. LEW is an affiliate of CDMRC.)

346.9

NGF IN CHOROID PLEXUS AND CSF. S.L. Patterson* and M. Bothwell (SPON: C.A. Livingston). Dept. of Physiology and Biophysics, Univ. of Washington, Seattle, WA 98195.

In addition to its well characterized role in the development and maintenance of sympathetic and sensory neurons, nerve growth factor (NGF) may also be important in some central neural systems. NGF responsiveness is well documented in the magnocellular cholinergic neurons of the basal forebrain. These neurons project to the hippocampus and the neocortex, which provide NGF trophic support. These two brain regions are generally believed to be the primary source of NGF to these neurons. However, the efficacy of intraventricular infusions of NGF in alleviating age or injury related deficits in cholinergic function suggests the possibility that there might be an endogenous source of NGF associated with the ventricles. We report here the presence of NGF associated with the choroid plexus, and with the CSF, of rat brain. The NGF was localized immunocytochemically, assayed for the ability to promote neurite outgrowth from PC12 cells, and quantitated using antibodies against murine *B* NGF in a two-site enzyme-linked immunoabsorbant assay (ELIZA). The results suggest that the epithelial cells of the choroid plexus are producing biologically active NGF and releasing it into the the CSF in physiologically significant quantities.

346.10

1,1,3, TRICYANO-2-AMINO-1-PROPENE POTENTIATES NERVE GROWTH FACTOR-INDUCED PROTEIN PHOSPHORYLATION IN THE PC-12 CELL LINE. J. West Paul and John P. DaVanzo. Dept. of Pharmacology, School of Medicine, East Carolina University Greenville, NC 27834.

Our laboratory has recently shown that 1,1,3, tricyano-2-amino-1-propene (Triap) potentiates the actions of NGF on the PC-12 cell line. Nanomolar concentrations of Triap potentiate the induction of neurite outgrowth by NGF as well as the induction of tyrosine hydroxylase and choline acetyltransferase. To further investigate the mechanism for this potentiation we examined Triap's effect on protein phosphorylation in NGF treated and untreated PC-12 cells. NGF alone caused a detectable increase in the phosphorylation of proteins with molecular weights of 101, 62, 38, and 21 kilodaltons (Kd) and a decrease in phosphorylation of a protein at 65 Kd. Triap alone increased the phosphorylation of only one protein at 38 Kd. Cells treated with both NGF and Triap increased the phosphorylation of proteins at 101, 62, and 21 Kd with no change in the phosphorylation of the 38 Kd protein. The combination also decreased the phosphorylation of the 65 Kd protein as compared to NGF alone. Triap and NGF together also induced the phosphorylation of proteins at 55 and 17 Kd. Experiments with immunopurified tyrosine hydroxylase showed that Triap alone had no effect on tyrosine hydroxylase phosphorylation but Triap potentiated NGF's induction of protein phosphorylation. These results suggest that Triap's interaction with NGF is more complex than a simple potentiation of phosphorylation. (Our thanks to Hoechst-Roussel Pharmaceuticals Inc. for financial support.)

346.11

DETECTION AND PARTIAL CHARACTERIZATION OF AN NGF-ACTIVATED MICROTUBULE ASSOCIATED PROTEIN 1.2 (MAP 1.2) KINASE ACTIVITY IN PC12 CELLS. H. Tsao*, J.M. Aletta, L.A. Greene (SPON: D. Burmeister). Dept. of Pathology, College of Physician and Surgeons, Columbia Univ. New York,NY 10032

The addition of NGF to intact PC12 cells leads to a rapid increase in the phosphorylation of high molecular weight (HMW) MAP 1.2, also designated MAP 1B and MAP 5. The phosphorylation of this protein in intact cells has been well described (Aletta et al, JCB 106: 1573-1581) and may play a role in neurite outgrowth. In order to further elucidate the mechanism underlying the in vivo observations, an in vitro system was developed. Here we describe the cell free detection of an NGF-stimulated kinase activity capable of phosphorylating both endogenous PC12 cell and exogenous brain MAP 1.2. Nontreated cultures of PC12 cells or replicate cultures exposed to NGF for 1 hr are homogenized at 4 deg in MES/EGTA/$MgCl_2$ pH 6.8 and spun at 100,000g. The supernatants are then incubated either with or without exogenous MAPs for 15 min at 37 deg. Both ATP and GTP can serve as phosphate donors and results indicate that the NGF-stimulated MAP 1.2 kinase activity can be inhibited by either μM levels of heparin or mM concentrations of 2-aminopurine. When bovine brain MAPs are isolated for the assay, MAP 1A, MAP 1.2 and MAP 2 are all present in the preparation. Each of these MAPs appears to be phosphorylated by an NGF-sensitive mechanism in our assay system. However, MAP 1 and MAP 2 appear to have differential sensitivities to heparin and 2-aminopurine. These differences therefore suggest that more than one NGF-regulated kinase may be involved in the phosphorylation of different HMW MAPs.

346.12

NERVE GROWTH FACTOR (NGF) REGULATION OF ARACHIDONIC ACID METABOLISM IN PC12 CELLS. J.G. Webb* (SPON: H. Martin). Dept. of Pharmacology, Medical University of South Carolina, Charleston, SC 29425

The effect of NGF on basal and hormone-stimulated prostaglandin (PG) formation was examined in cultured PC12 cells. PGE_2 and PGI_2 (measured as 6-keto $PGF_{1\alpha}$) released into the incubation buffer were quantitated by RIA. In control PC12 cells, basal production of PGE_2 (630 ± 83 pg/min/mg prot.) was 5-fold greater than PGI_2 (134 ± 26 pg/min/mg prot.) synthesis. Synthesis of both PGs was eliminated by the cyclooxygenase inhibitor indomethacin. Neither norepinephrine (NE) nor bradykinin (BK) stimulated PG synthesis in undifferentiated control cells. Addition of NGF (50 ng/ml) to the culture media for up to 7 days produced time-dependent changes in arachidonic acid metabolism. Basal rates of production of PGE_2 and PGI_2 were increased to 2122±400 and 355±56 pg/min/mg prot., respectively. PG formation from exogenous arachidonic acid (10μM) was also enhanced 4-fold in NGF treated cells. Further, after 7 days of treatment with NGF addition of BK or NE now stimulated PG synthesis. BK (1μM) produced a 2-fold increase in PGE_2 and PGI_2 production and NE (1μM) produced a 50-60% increase in PG synthesis. The results indicate that NGF induced differentiation in PC12 cells promotes the expression of cyclooxygenase activity and imparts responsiveness to hormones which regulate arachidonic acid metabolism.

346.13

SUPRESSION OF NGF ACTIONS BY THE 5-LIPOXYGENASE INHIBITOR CGS 8515. D.J. Steel, J.W.F. Wasley*, and L.A. Greene. Dept. of Pathology, Columbia Univ. Coll. of P & S, New York, NY 10032 and *CIBA-Geigy Corp., Pharmaceutical Div., Summit, NJ 07901.

Recent work suggests that arachidonic acid metabolites may mediate NGF-induced responses in PC12 cells (DeGeorge et al, J. Neurosci Res. 21:323, 1988). Our investigations with selective cyclooxygenase inhibitors indicate no role for this pathway in the NGF-induced differentiation of PC12 cells, using induction and regeneration of neurite outgrowth, and induction of ornithine decarboxylase (ODC) as criteria. However, we find that a selective inhibitor of the 5-Lipoxygenase (5-LO) pathway, CGS 8515 (Ku et al, Biochim. Biophys. Acta 959:332,1988) reversibly antagonizes several NGF-mediated responses in PC12 cells. CGS 8515 blocked both NGF-induced neurite initiation (transcription-dependent) and regeneration (transcription-independent) with an IC_{50} of 900 nM after 2 days of treatment. Similiar doses of CGS 8515 also inhibited the NGF induction of ODC, with a maximal effect between 2 and 6 hours of pretreatment. Examination of protein phosphorylation indicates that an 18 kD protein that is less phosphorylated in response to NGF is more heavily phosphorylated in the presence of CGS 8515, (+/-NGF). A structural analog of this compound that does not inhibit 5-LO had no effect on any NGF-mediated responses examined; other analogs with 5-LO inhibitory activity also blocked NGF effects. However, parallel experiments with other reportedly selective 5-LO inhibitors that are structurally distinct from CGS 8515 did not block responses to NGF. Thus, the role of eicosanoids in the NGF mechanism and the mechanism by which CGS 8515 blocks NGF actions remain to be defined. To this end, PC12 cell eicosanoid composition is being determined with and without exposure to this compound.

346.14

INHIBITION OF NERVE GROWTH FACTOR-INDUCED NEURITE OUTGROWTH OF PC12 CELLS BY A PROTEIN KINASE INHIBITOR WHICH DOES NOT PERMEATE THE CELL MEMBRANE. Y. Matsuda, S. Nakanishi*, H. Kase*, and G. Guroff. Kyowa Hakko Kogyo Co., 3-6-6 Asahimachi, Machida-shi, Tokyo 194, Japan and Section on Growth Factors, National Institute of Child Health and Human Development, Bethesda, MD20892.

Previous studies have shown that K-252a, an alkaloid-like kinase inhibitor isolated from the culture broth of Nocardiopsis sp., selectively inhibits the actions of nerve growth factor on PC12 cells (Koizumi, S., J. Neurosci., 8 : 715, 1988). The present experiments were designed to inspect the action of K-252b, the 9-carboxylic acid derivative of K-252a, on nerve growth factor-iduced neurite outgrowth. K-252b is water-soluble and does not permeate the cell membrane of PC12 cells, whereas K-252a clearly does. On the other hand, K-252b is as potent as K-252a itself in inhibiting nerve growth factor-induced neurite outgrowth. In the same concentration range as K-252a, K-252b also can severely block protein kinase activity in vitro assay system. (Kase. H., Biochem. Biophys. Res. Commun., 142 : 436, 1987). These data can be interpreted to suggest that the site of action of K-252 compounds to block the neurite elongation is located on the cell surface of PC12 cells. Furthermore, K-252b provides a new tool for the dissection and study of ecto-protein kinase-requring process.

346.15

REGULATION OF NERVE GROWTH FACTOR (NGF) AND NGF-RECEPTORS BY CHOLINERGIC DRUGS. J. Alberch*, M. Carman-Krzan* and B.C. Wise. Fidia-Georgetown Institute for the Neurosciences, Georgetown Univ., Washington, D.C. 20007

It is well documented that NGF and its receptor are present in brain and that NGF may be a neurotrophic factor for brain cholinergic neurons. In the present studies, we investigated whether the levels of the NGF protein and NGF receptor are pharmacologically regulated by drugs that affect cholinergic neurotransmission. Membranes prepared from the cortex of 28 day old rats have both high and low affinity binding for ^{125}I-NGF. Scatchard analysis indicated a high affinity site with a K_D of 26 ± 4 pM and B_{max} of 0.65 ± 0.03 fmol/mg protein. A lower affinity site with an estimated K_D of 37 ± 10 nM and B_{max} of 125 ± 33 fmol/mg protein was also detected. Treatment of young rats with scopolamine (5 mg/kg, twice daily for 7 days) resulted in the loss of detectable high affinity binding sites and a decrease in the number of low affinity sites in cortical membranes. The K_D of the low affinity site did not change. The hippocampus (2.2 pg NGF/mg tissue) and cortex (1.2 pg/mg) have the highest levels of NGF in the rat brain as measured with the two-site NGF enzyme immunoassay. After 7 days of scopolamine treatment, there was little change in the levels of NGF in the cortex and hippocampus. In contrast, treatment with physostigmine (1 mg/kg/day for 7 days) resulted in about a 45% decrease in NGF levels in both the cortex and hippocampus. These results suggest that the dynamic state of the NGF system in brain can be modulated by treatment with cholinergic drugs.

346.16

HUMAN B-LYMPHOCYTES EXPRESS FUNCTIONAL NERVE GROWTH FACTOR RECEPTORS.
P. Ehrhard*[1], R. Peck*[2] and U. Otten[1] (SPON: W. Fischli).
[1] Dept. of Pharmacology, Biocenter, University of Basel, CH-4056 Basel, and [2] Central Research Unit, Hoffmann-La Roche & Co., Ltd., Basel, CH-4002 Basel, Switzerland

In addition to its characteristic effects on peripheral sympathetic and sensory neurons and subsets of centrally located neurons, there is increasing evidence that nerve growth factor (NGF) may play an essential role in the modulation of the immune system and the inflammatory response. By using a monoclonal antibody directed against the human NGF receptor a sensitive immunoprecipitation assay for the quantification of human NGF receptors has been established. Our results show that in addition to neural cells, non-neuronal tissues also express significant amounts of NGF receptors: e.g. human spleen and lymph nodes contain 8, respectively 6, fmole receptor/mg protein. Moreover, we found that human B- and (to a smaller extent) T-cells isolated from the blood of healthy donors express specific NGF receptors. These receptors are functional as demonstrated by the findings that NGF induces a dose-dependent proliferation of both B- and T-lymphocytes and immunoglobulin production by the B-cells. Thus, in addition to its specific neurotrophic effects, NGF plays a role in immunoregulation.

346.17

SPECIFIC LECTIN-BINDING RADIOIMMUNO- AND BIOASSAY METHODS FOR DETECTION OF 53 kDa NGF. J. Lakshmanan*, R. Lam*, A. Reviczky*, M. Grodin*, A. Abo-Bakr*, S. Soinila, D. North* and D.A. Fisher* (SPON: M. Weichsel, Jr). Department of Pediatrics, UCLA School of Medicine, Harbor-UCLA Medical Center, Torrance, CA. 90509.

In 1988 using an immunoblot analysis, we reported the presence of a 53 kDa NGF, in addition to 13 kDa NGF, in submaxillary glands of adult male and female mice (BBRC 152:1008-1013). In subsequent investigations we characterized the 53 kDa NGF as a glycoprotein (Clin Res 37:181A, 1989), secretory in nature (FASEB J 3:1572A, 1989) and biologically active (Neurosci Lett, in press, 1989). More recently, we documented that 53 kDa NGF, but not 13 kDa NGF, is present in the salivary glands of immature and young adult male and female mice; 13 kDa NGF appears only late in development (Pediatr Res 25:313A, 1989). We now describe specific lectin- binding radioimmunoassay and bioassay methods for quantifying 53 kDa NGF. These methods in conjunction with immunoblotting should prove useful in determining the tissue distribution and purification of 53 kDa NGF.

346.18

EFFECTS OF TWO PROTEIN KINASE INHIBITORS (STAUROSPORINE AND K252a) ON NGF-MEDIATED PC12 CELL SURVIVAL. D.L. Needels, L.A. DiNatale*, and T.L. Martin. CNS Biology, Bristol-Myers, Wallingford, CT 06492.

In addition to its well known effects on cell morphology, nerve growth factor (NGF) also supports the survival of PC12 cells under serum-free conditions. We have established a colorimetric bioassay for PC12 cell survival based on the metabolism of MTT by viable cells, and used it to quantify the effects of two related protein kinase inhibitors (staurosporine and K252a). These molecules have opposing, but not antagonistic, effects on PC12 cell morphology [*Martin et al., this meeting*].

Although it is thought that at least some of the morphological responses of PC12 cells to NGF are mediated by protein kinases, much less is known about the role of kinases in the NGF survival response. Neither staurosporine (which promotes neurite formation) nor K252a (which inhibits NGF-induced neurite formation) was active at supporting PC12 cell survival at concentrations of up to 200 nM, suggesting separate pathways for neurite-promotion and survival. K252a half-maximally inhibits NGF-induced neurite formation at a concentration of ~100 nM, apparently by blocking a kinase located within an NGF-specific pathway. K252a also inhibited NGF-induced survival, but with the half-maximal response occuring at ~1 nM. The observed cell death was not due to cytotoxicity, since cells supported under other growth conditions died only at much higher concentrations of K252a (≥400 nM). In contrast, staurosporine was incapable of inhibiting NGF-mediated survival of PC12 cells at less than cytotoxic concentrations.

These data suggest a multiplicity of protein kinases involved in the responses of PC12 cells to growth factors, with some specificity in terms of both the stimulating growth factor and the biological endpoint. Interestingly, K252a completely blocked NGF's survival effects at concentrations that have little effect on the morphological response, suggesting distinct pathways. Moreover, K252a is active at concentrations well below its reported K_i for inhibition of protein kinase C (25 nM). Identification of the enzymatic systems affected by these molecules should provide insight into the mechanism of NGF's effects on PC12 cell survival and morphological differentiation.

346.19

THE PARADOXICAL EFFECTS OF TWO PROTEIN KINASE INHIBITORS (STAUROSPORINE AND K252a) ON PC12 CELL MORPHOLOGY. T.L. Martin, J.K. Dickey*, M.P. Rosser*, and D.L. Needels. (Spon: L.A.Riblet). Depts. of CNS Biology, and Screening & Biochemistry, Bristol-Myers Co., Wallingford, CT 06492.

The capacity of PC12 cells to transform from a chromaffin-like cell to one resembling a fully differentiated sympathetic neuron on exposure to nerve growth factor (NGF) or basic fibroblast growth factor (bFGF) is well documented. It has been postulated, based on studies with modulators of kinase activity, that protein kinases play a role in this process of cellular differentiation. For example, the protein kinase inhibitor K252a inhibits the neurite outgrowth response of PC12 cells to NGF [*Koizumi et al. (1988) J. Neuroscience 8, 715-721*]. We report here that a closely related protein kinase inhibitor (staurosporine) has the opposite effect, inducing the morphological differentiation of PC12 cells in the absence of NGF.

PC12 cells were cultured in the presence or absence of growth factors and/or protein kinase inhibitors and visually inspected 48 hr later for the presence of neurites. As previously reported, 50-200 nM K252a blocked the ability of NGF to induce neurite formation, but was inactive in bFGF-treated cultures. Staurosporine, in contrast, was unable to block the NGF response. In fact, staurosporine alone had a robust neurite-promoting effect on PC12 cells at concentrations ranging from 20-200 nM. In an attempt to block this morphological response, staurosporine-exposed PC12 cells were challenged with K252a. In contrast to what would be expected for agents acting through a common effector pathway, K252a did not inhibit staurosporine-induced neurite formation.

These data indicate that more than one kinase plays an important role in the process of neurite elaboration and suggests that there are multiple pathways leading to the common endpoint of neurite outgrowth. Although staurosporine is an inhibitor of protein kinase C, the concentrations of staurosporine required to induce neurites are considerably higher than the K_i for enzyme inhibition (0.7 nM). Identification of the putative protein kinases (or other enzymes) inhibited by these molecules should advance our knowlege of growth factor-mediated neurite outgrowth in PC12 cells.

346.20

MECHANISMS OF NERVE GROWTH FACTOR ACTION IN H_2O_2 RESISTANCE G.R. Jackson*, K. Werrbach-Perez*, and J.R. Perez-Polo. Dept. Human Biol. Chem. and Genet., Univ. of Texas Medical Branch, Galveston, TX 77550.

Nerve growth factor (NGF) regulates neuronal cell death in developing sympathetic and embryonic sensory ganglia, as well as injured magnocellular cholinergic neurons of the basal forebrain. PC12 cells treated with NGF doses ranging from 1 ug to 1 ng/ml display resistance to peroxidative injury generated by H_2O_2. Acquisition of resistance occurs within 24 hr of NGF treatment and requires de novo protein synthesis. Treatment with retinoic acid, which upregulates the low affinity NGF receptor, or the protein kinase inhibitor K252a mimics the resistance conferred by NGF. Pretreatment with a sublethal dose of H_2O_2 (0.5 mM) provides resistance to further insult; NGF treatment of survivors enhances this resistance. NGF increases the specific activity of catalase by 30% in control PC12 cells and by 500% in the survivors of 0.5 mM H_2O_2. Thus, injury and NGF enhance the capability of NGF responsive populations to withstand peroxidative stress. Supported in part by NINDS grant NS-18708.

REGENERATION: CNS II

347.1

AGE-RELATED RECOVERY AFTER NERVE REPAIR IS PROBABLY NOT DUE TO REGENERATION OF NORMAL PERIPHERAL ORGANIZATION. J.T. Wall, P.E. Garraghty, S.L. Florence, and J.H. Kaas. Vanderbilt University, Nashville, TN 37240.

The best examples of sensory recovery after nerve repair are seen in children. Is this recovery due to mechanisms involving peripheral regeneration and/or mechanisms relating to adaptation in the brain? To test if recovery is due to a peripheral capacity of immature nerves to regenerate normal connections with the skin, this study assessed patterns of somatotopic organization of primary afferent terminations in the cuneate nucleus and spinal cord following early repair of a hand nerve. Cutaneous injections of HRP conjugates were made into reinnervated thumb (D1) and normally innervated little finger (D5) locations in a 3.3 year old macaque monkey which had undergone anesthesia and wrist-level median nerve section and repair at 10 days of age. The resulting patterns of transported label were compared to patterns in normal monkeys. The termination patterns of regenerated D1 inputs are highly abnormal in both the cuneate nucleus and dorsal horn. Tightly focussed, rostro-caudally oriented columns of label typical of normal D1 terminals are replaced by more broadly distributed patterns of label. This suggests that immature nerves do not have a general capacity to regenerate normal connections, and that central mechanisms of adapting to changed peripheral organization contribute to sensory recovery after early nerve repair. [Grants NS21105 + NS16446]

347.2

FURTHER EVIDENCE THAT DEVELOPMENTAL PLASTICITY OF THE RUBROSPINAL TRACT RESULTS PRIMARILY FROM THE GROWTH OF NEW AXONS. X.M. Xu, R.R. Pindzola and G.F. Martin. Department of Anatomy and Neuroscience Program, The Ohio State University College of Medicine, Columbus, OH 43210.

Rubral axons can grow around a lesion of their spinal pathway in developing opossums and a critical period exists for that plasticity (Martin and Xu, Dev. Brain Res., 39:303-308). In the present experiments we sought to determine if the axons which grow around the lesion are those originally cut or later growing ones. Pouch young opossums were injected with Fast Blue (FB) into the caudal thoracic cord during the critical period to label rubrospinal neurons. 3-4 days later, the rubrospinal tract was transected 4-5 segments rostral to the injection. The animals were maintained for about 1 month before a second marker, Diamidino Yellow (DY) was injected into the cord between the FB injection and the lesion. Four days later, the animals were sacrificed and the tissues prepared for microscopic examination. Only a few neurons were labeled by FB in the red nucleus contralateral to the lesion, indicating that most axotomized rubrospinal neurons degenerated. In contrast, neurons were labeled by DY alone, indicating that axons which were not present at the time of the FB injection had grown around the lesion to incorporate DY. A few double labeled neurons were also found, however, suggesting that axons of some FB labeled neurons that survived the lesion grew around it to incorporate DY. We conclude that developmental plasticity of the rubrospinal tract results primarily from the growth of new axons around the lesion but that true regeneration may also occur. (Supported by NS-25095).

347.3

THE EFFECTS OF THE FETAL SUBSTANTİA NİGRA GRAFTS ON THE SYMPATHETIC GANGLİA. S.Uysal*, E.Korfalı*, T.Özmen*, İ.H.Ulus, W.D. Knowles. Dept. of Neurosurgery and Pharmacology, Uludağ Univ. Bursa, Turkey and CCF, Cleveland Ohio, USA

In the present study, after the destruction of the nigrostriatal dopaminergic pathways by intraventricular injection of 6-OHDA, fetal substantia nigra graft tissues which were obtained from 15-17 days old fetusus were transplantated into cavities overlying caudate nucleus were investigated for demonstration of the effect of graft tissues on the sympathetic ganglions.

Three months after grafting tyrosine hydroxylase activity of the adrenal, celiac and lomber sympathetic ganglions of grafted rat groups showed an increase of 121% and this increase has been found significant. These results indicate that fetal nigrostriatal dopaminergic grafts has also positive effects upon the distant CNS tissues.

347.4

SCHWANN CELLS AND SCHWANN CELL CONDITIONED MEDIUM SUPPORT SURVIVAL AND NEURITE OUTGROWTH OF LONG-PROJECTION NEURONS OF THE NUCLEUS LOCUS COERULEUS. B.Baharloo* and M.B.Clark. Dept. of Anatomy, Univ. Md. Sch. of Med., Baltimore, MD 21201

Using dissociated cell cultures we have compared the survival and neurite outgrowth of locus coeruleus neurons from postnatal rats in environments of 1) Schwann cells (SCs), 2) Schwann cell conditioned medium (SCM), 3) SCM prebound to the culture dish and on 4) laminin, 5) collagen and 6) polylysine. After 3d in culture neurons were identified by double immunostaining for neurofilament protein and tyrosine hydroxylase. Neurite length and branch points were determined from micrographs of individual neurons. Since initial experiments indicated that neuronal survival and neurite outgrowth were similar on SCs whether or not they assembled a basal lamina, conditioned medium was always prepared from SC cultures which had not assembled basal lamina. Neuronal survival was 3-5x greater in the presence of SCs than on laminin, collagen or polylysine. SCM, either prebound to the culture dish or included in the culture medium as a soluble component, supported the survival of 70-75% as many neurons as Schwann cell beds. Neurite promotion (neurite bearing neurons/total neurons) was greater in the presence of SCs (62%) or prebound SCM (50%) than in soluble SCM (30%) or defined medium (6-20%). Mean neurite length on prebound SCM was approximately 2x that on SCM-soluble or laminin, and 5x that on polylysine; the number of branch points associated with neurons on prebound SCM or on laminin were similar, with an average of 6/neuron as compared to 3/neuron from soluble SCM and 0-1 for neurons on polylysine in defined medium. Finally, 50-75% of neurons in cultures with SCM were TH positive, indicating the survival of specific noradrenergic neurons. Taken together these results suggest that SCs support survival and outgrowth of long projection locus coeruleus neurons and that the products released by SCs may be responsible for these effects. (Supported by NIH NS24252 to MBC.)

347.5

INVOLVEMENT OF SEPTOHIPPOCAMPAL PATHWAY IN COLCHICINE-INDUCED COMPENSATORY RESPONSES IN HIPPOCAMPUS. P. Tandon, M. Bonner, S. Barone Jr., and H.A. Tilson. LMIN, NIEHS/NIH, Res. Tri. Pk, NC 27709.

Our lab has shown that intradentate administration of colchicine produces a hyperstimulation of agonist-induced PI turnover. This response has been related to the process of compensation in the hippocampus occurring in response to neurodegeneration and can be observed one year after the lesion. As the main cholinergic input to the granule cells and pyramidal cells of the CA3 region in the hippocampus is via the septohippocampal pathway, it has been suggested that regeneration of this pathway occurs after the lesion. To further study this hypothesis we stereotaxically injected 2.5 ug of colchicine in the hippocampus and/or septum in various groups of male Fischer-344 rats. Twelve wks after the injection, the animals were sacrificed and hippocampus dissected out. Agonist-induced turnover of PI showed that septal lesions alone did not have any effect on PI turnover in the hippocampus while hyperstimulation of IP release was observed after hippocampal lesion as reported earlier. However, when both the hippocampus and the septum were lesioned, the hyperstimulation of agonist-induced release of IP was reduced. These data demonstrate the importance of the septohippocampal pathway for the persistent hyperstimulation of IP release after intradentate colchicine.

347.6

BINDING PROPERTIES OF A NEWT RETINAL PIGMENT EPITHELIUM ANTIBODY DURING RETINAL DEVELOPMENT AND REGENERATION. *Lisa R. Klein*, P.R. MacLeish and Torsten N. Wiesel* Laboratory of Neurobiology, The Rockefeller University, New York, NY, 10021.

The binding of RPE-1, a mouse monoclonal antibody selective for newt retinal pigment epithelium, was followed in eyes undergoing embryonic development and retinal regeneration. Using the indirect immunofluorescence technique on frozen sections, bright and continuous staining was observed exclusively in the retinal pigment epithelium of normal adult newts but staining became diminished near the ora serrata region and stopped abruptly at the ciliary margin. During development, staining was not detected in the retinal pigment epithelium until the formation of photoreceptor outer segments and was not observed in any other ocular tissue. There was no correlation between the formation of pigment in retinal pigment epithelial cells and their labelling with the RPE-1 antibody. Furthermore, albino salamander embryos showed the same pattern of labelling with RPE-1 as that seen in age-matched pigmented animals. During retinal regeneration, retinal pigment epithelial cells were stained less intensely by the antibody. The newly formed retinal cells were brightly stained and retained varying degrees of pigmentation during the early phase of regeneration. With time, staining in regenerated retina receded so that by the end of regeneration, staining by RPE-1 was once more restricted to the RPE cells. The identification of RPE-1 as a marker for post-mitotic retinal neurons about to undergo differentiation provides a promising approach for further studies of regeneration using molecular tools. (Supported by NIH grants NS22789, and EY05201)

347.7

TECTAL REGULATION OF NEURITIC OUTGROWTH AND EXPRESSION OF NEUROFILAMENT PROTEINS IN GOLDFISH RETINAL EXPLANTS. C.M. Hall*, C.K. Else*, and N. Schechter. Departments of Biochemistry and Psychiatry, SUNY at Stony Brook, NY 11794

Goldfish retinal explants were used to study the regulatory influence of the optic tectum on neurofilament expression during optic nerve regeneration. Neuritic outgrowth was quantitated and the expression of the neurofilament proteins (ON_1 and ON_2) was characterized by immunohistochemistry and by labeling with ^{35}S-methionine, followed by 2D-gel electrophoresis, autoradiography and densitometry. Retinas were explanted at various times after optic nerve crush and were characterized during a two week time period. We demonstrate that the relative expression of ON_1 and ON_2 varied with time after crush and with the time that the explants were kept in culture. Removal of the contralateral optic tectum prior to optic nerve crush enhanced neuritic outgrowth when compared to control explants after sham operation. In these experiments, the expression of ON_1 and ON_2 in the explants was significantly diminished at a time when tectal innervation normally takes place. One interpretation of these results is that tectal contact sustains the expression of the neurofilament proteins. (NIH grant EY-05212 to N.S.)

347.8

GROWTH OF INJURED RABBIT OPTIC AXONS WITHIN THEIR OWN DEGENERATING ENVIRONMENT: POSSIBLE SEQUENCE OF EVENTS.

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Spontaneous growth of axons after injury is limited in the mammalian central nervous system (CNS). We have previously shown apparent regenerative growth of injured adult rabbit optic axons into their own degenerating environment. The newly growing axons, including growth cones, unmyelinated and thinly myelinated axons, were compartmentalized. The present study examines the possible sequence of events leading to this compartmentalized growth using electron microscopy and immunocytochemical analysis. Unmyelinated axons, as a single profile or as bundles, appear preferentially at the margins of the compartment in collagenous tissue under the dura and are channeled by glial processes. The processes protrude from unidentified cells and from astrocytes stained with antibodies to glial fibrillary acidic protein. Myelinated axons are preferentially located at the center of the compartment. Their myelin lamellar periodicity measured by diffractometry is characteristic of CNS myelin. We suggest that the initial protrusion of the axonal tip is into extracellular matrix channeled by glial processes, which then group together into bundles to form a compartment. This growth is reminiscent of developmental growth through intercellular channels. At a site, 2mm distal to the lesion, 8 weeks postoperatively, 14068 axons were counted, (20387, corrected for the grid bar), representing 7% of that in an intact nerve. This offers the possibility for further growth leading to reconnection with the target.

347.9

CHARACTERIZATION OF THE ENVIRONMENT OF REGENERATING AXONS IN THE GOLDFISH OPTIC NERVE: DIFFERENTIAL EXPRESSION OF HNK-1, EMBRYONIC N-CAM (EN-CAM) AND CHONDROITIN SULPHATE PROTEOGLYCAN (CSPG). Yael Shinar*, W. Battisti, M. Schwartz, P. Levitt, and M. Murray. Dept. Anatomy, Med. College of PA, Philadelphia, PA.

The goldfish optic nerve can be used to characterize molecular interactions leading to succesful regeneration within a CNS environment. Here we demonstrate expression of membrane and extracellular epitopes in the goldfish optic nerve that may play a role axonal outgrowth. Using ELISA on nerve homogenates and immunocytochemistry on longitudinal sections reactivity of laminin, CSPG, N-CAM and HNK-1 was observed in both intact and regenerating nerve 22 days post crush. In intact nerves, CSPG and laminin staining showed a linear pattern of staining reminiscent of the fascicular boundaries and suggesting a non-neuronal location. In contrast eN-CAM and HNK-1 appeared to stain the entire fascicle, and thus stain areas containing both axons and non-neuronal cells. In injured nerves, 22 days post crush the normal pattern was conserved proximally to the crushed area. The crush site was intensely and rather uniformly stained by all antibodies. Distally, CSPG and laminin expression is increased and appear to be associated with non-neuronal cells while HNK-1 and en-CAM are also elevated and may be associated with regenerating axons as well. The increased expression of these molecules in the regenerating nerve suggests that they contribute to successful axonal growth. The expression of these epitopes in the intact nerve may contribute to the regenerative potential of the goldfish optic nerve.

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347.10

STUDIES OF MISROUTED REGENERATING IPSILATERAL RETINOTECTAL AXONS IN RANA PIPIENS. D.J. Stelzner and J.A. Strauss, Dept. of Anatomy and Cell Biology, SUNY Health Science Center, Syracuse, New York 13210.

After optic nerve crush injury in Rana pipiens, misrouted regenerating optic axons project to the ipsilateral (ipsi) optic tectum and overlap with the normal intact projection from the other eye. Many collaterals are formed in the optic nerve early in regeneration which retract by 5 mo. p.o. To test whether misrouted optic axons form synaptic connections in the ipsi tectum, these axons were labeled anterogradely with WGA-HRP at 8 wks (N=5), 3 mo (N=5) and 6 mo p.o. (N=3) and processed for LM and EM (Joosten et.al., '87). To test whether misrouted ipsi optic axons are collaterals of normally projecting axons, the retrogradely transported fluorescent labels fast blue (FB) and rhodamine (Rh) were applied to the centrolateral dorsal tectum of each tectal hemisphere at 3 mo (N=4) and 6 mo (N=8) p.o. Labeled synaptic profiles were observed in ipsi tectum at all survival times tested after nerve crush injury but were in greatest number at 6 mo. p.o. A large percentage of retinal ganglion cells were double labeled with FB and Rh 3 mo p.o. but few remain double labeled 6 mo. p.o. Thus, misrouted ipsi axons form and maintain contract in the already innervated tectum but collaterals of axons also projecting to normal targets are lost as reinnervation is completed (Grant NS14096).

347.11

FROG TECTAL AXONS FAILING TO REGENERATE WITHIN THE CNS DO SO WITHIN PERIPHERAL NERVE IMPLANTS. Y.-H. Hung and D.J. Stelzner(SPON: J.A. Horel). Dept. of Anatomy and Cell Biology, SUNY Health Science Center, Syracuse, New York 13210.

Tectal efferent axons, located adjacent to the optic tract, fail to regenerate past diencephalic lesions in Rana pipiens even though optic axons regenerate after the same injury (J. Comp. Neurol. 255: 511-525). We tested whether tectal efferents can regenerate within peripheral nerve implants. Segments of frog sciatic nerve were implanted into the anterolateral (N=13) or centrolateral (N=13) dorsal surface of the optic tectum using an angled micropipette. After 12 p.o. wks, the distal end of the nerve was cut and HRP applied. All animals survived for 3-7 d.p.o., were perfused with mixed aldehydes, brains frozen sectioned in the transverse or sagittal plane, and reacted with DAB or TMB histochemistry. Labeled cells were not seen in control cases (N=6) where the nerve was crushed before HRP applied, but were found in all experimental operates. The vast majority of labeled cells were in close proximity to the graft insertion site. Most were located in laminae 5 or 8, the origin of the tectal efferent projections, but a smaller number were labeled in lamina 2. In several cases labeled neurons could also be identified in the posterior thalamus and rostral midbrain tegmentum. Like the mammalian CNS, tectal neurons unable to regenerate within the CNS, can grow within peripheral nerve implants. (Supported by grant NS 14096).

347.12

SURVIVAL OF DISPLACED RETINAL GANGLION CELLS AFTER OPTIC NERVE REGENERATION IN THE FROG HYLA MOOREI. S.A. Dunlop, M.F. Humphrey and L.D. Beazley. Psychology Dept., University of Western Australia, Australia.

Approximately 50% of orthotopic retinal ganglion cells (RGCs) die following extracranial optic nerve crush in Hyla moorei. The remaining RGCs form functional contacts in the optic tectum. Some RGCs are located in the inner cell layer of the inner nuclear layer. Here we investigated whether these "displaced" RGCs were similarly affected by optic nerve crush. Displaced RGCs were identified in retinal wholemounts and sections after retrograde transport of HRP applied to the optic nerve.

In normal retinae (N=5) the displaced RGCs comprised 0.55% of the orthotopic population. Densities of displaced RGCs were highest in the area centralis and horizontal visual streak similarly to the orthotopic population. In addition the displaced cell density was higher in the naso-ventral and tempero-dorsal peripheries.

In retinae at least 70 days after nerve crush (N=4) the orthotopic RGCs were reduced by a mean of 47% compared to the unoperated partner retinae. The mean proportion of displaced cells was similar to that in normals at 0.62%. Therefore a similar degree of cell death had occurred in the displaced RGCs. In both normal and regenerate retinae the displaced cells were made up of several distinct types. This provides further evidence that all RGCs undergo a similar proportion of death, rather than the death being confined to specific classes.

347.13

FUNCTIONAL SYNAPSES BETWEEN REGENERATED RETINAL GANGLION CELL AXONS AND SUPERIOR COLLICULUS NEURONS IN ADULT HAMSTERS. S.A. Keirstead, M. Rasminsky, Y. Fukuda, D.A. Carter*, A.J. Aguayo and M. Vidal-Sanz*. Montreal General Hospital and McGill University, Montreal, Quebec, H3G 1A4.

An autologous peripheral nerve graft was anastomosed to the ocular stump of the transected right optic nerve in adult hamsters. The other end of the nerve graft was inserted into the ispsilateral superior colliculus (SC) at week 7-8 and the left optic nerve transected at week 9-11.

Extracellular recordings were made near the site of graft insertion in the SC at week 21-26, several weeks after retinal ganglion cell (RGC) axons that regenerate through such grafts form synapses in the SC (Carter et al, Soc. Neurosci. Abstr. 14:654,1988). Units responding to light flashed at the right eye were found in the superficial 500 um of the SC in all 8 animals tested. 25 units responding to light were identified as SC neurons and not RGC axons since they responded more consistently to paired electrical stimulation (ES) of the graft than to single pulses at the same intensity, indicating postsynaptic summation of afferent inputs. Light flash and ES of the graft decreased ongoing activity in another 7 SC neurons.

Regenerated RGC axons were the only possible sources of visual input to SC neurons responding to light. This study demonstrates that axons regenerated over long distances from adult mammalian CNS neurons can form functional synapses with neurons in other regions of the CNS.

347.14

EXTENSION AND PERSISTENCE OF REGENERATED RETINAL GANGLION CELL AXONS IN THE SUPERIOR COLLICULUS OF ADULT HAMSTERS. D.A. Carter*, G.M. Bray, and A.J. Aguayo. Montreal General Hospital and McGill University, Montréal, Québec, Canada, H3G 1A4.

Two months after placement of a peripheral nerve (PN) graft connecting one eye and the superior colliculus (SC) in adult hamsters, regenerated retinal ganglion cell (RCG) axons, which have regrown through the PN graft, extend up to 0.5 mm into the SC and make synaptic contacts with neuronal targets (Soc. Neurosci. Abstr. 14: 654, 1988). Four months after placement of such grafts, SC neurons can be activated transynaptically by stimulation of the retina by light (Keirstead et al., Soc. Neurosci Abstr., 1989). To investigate the morphology of the regenerated projection at 4 months and its later persistence, RGC axons of similarly prepared hamsters were labelled with HRP orthogradely transported from the eye and examined 4-10 months after PN graft insertion into the SC. By light microscopy, labelled axons remained confined to the superficial SC but extended further (up to 1 mm) into the SC. The extension of some axons towards the surface of the SC resembled that of normal retino-collicular arbors. By electron microscopy, the regenerated retino-collicular terminals had a normal appearance. Thus, over intervals of several months, these regenerating CNS axons extend into the neuropile of adult animals and appear to persist.

347.15

RECROSSING OF RETINAL AXONS AFTER EARLY TECTAL LESIONS IN HAMSTERS OCCURS ONLY WHERE VIMENTIN- AND GFAP-POSITIVE MIDLINE CELLS ARE DAMAGED.

D.-Y. Wu*, S. Jhaveri* and G. E. Schneider (SPON: N. Y. Kiang). Dept. of Brain and Cognitive Sciences, M.I.T., Cambridge, MA 02139.

The midline in the developing tectum contains vertically-oriented cells stretching between the ventricle and the pial surface. The cells express vimentin and GFAP and may form a ***barrier*** to keep retinal axons from crossing the midline (Wu, D.-Y. *et al.*, *Soc. Neurosci. Abstr.*, 1988, *14*:1110).

As an initial test of the "barrier" hypothesis, we studied the fate of the midline cells in regions where retinal axons recross abnormally at the dorsal midline following ablation of the right SC and removal of the right eye. Lesions were made in P1 hamster pups and animals were sacrificed on P2, P8, P13, and P15 — 18 hours after HRP was injected into the remaining eye. One series of sections was reacted for visualizing the HRP; adjacent series were immunostained with antibodies to vimentin or to GFAP.

A tectal lesion was always accompanied by withdrawal of midline processes from the pial surface and a deviation of processes away from the lesion site. By P2, retinal axons had crossed the dorsal midline either along a newly-formed bridge above the necrotic tissue, or more ventrally, over the surface of the lesioned SC. The ventral route was present ***only where midline processes were abnormal***, but did not span the entire rostro-caudal extent of the midline damage. Thus, disruption of midline cells is permissive but may not be instructive for retinal axons to spread from one SC to the other.

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PROCESS OUTGROWTH, GROWTH CONES AND GUIDANCE MECHANISMS V

348.1

LOCALIZATION OF TUBULIN DURING AXON OUTGROWTH FROM CHICK TRIGEMINAL NEURONS. M. Sisk, M. Lee, P. Gass, S. Moody and A. Frankfurter. (SPON: S. Klein) Depts. of Anatomy, Biology and Neuroscience, University of Virginia, Charlottesville, VA 22908.

During development tubulin gene expression is differentially regulated. The modulation in abundance of different gene products raises the question of whether specific classes of cells construct biochemically distinct microtubules to perform specific functions. To answer this question we compared the staining patterns in the developing chick trigeminal system produced by two anti-β-tubulin monoclonal antibodies. TuJ1 recognizes exclusively the neuron-specific β-tubulin isotype (Class III) encoded by the gene cβ4 (Sullivan et al. '86, Molec. Cell. Biol. 6:4409). This antibody stains only postmitotic neurons and their processes as early as st13. The only extra-CNS structures that are immunoreactive in the head are epibranchial placodes. In contrast, the other antibody (5H1), which recognizes an epitope common to Class III and at least one other isotype,does not stain cells in the V area until st15. At this time, only axons in the hindbrain marginal zone and in the V ganglion are immunoreactive. In addition, apparently all ventricular processes of hindbrain neuroepithelial cells are 5H1-positive. Furthermore, 5H1 is not brain specific; the entire epidermis is brightly immunoreactive, as are a few mesenchyme cells in the mandibular arch. All neuroepithelial and epidermal staining is lost by st 20, whereas axonal staining persists at least until D10. As development procedes, more and more axons, both in the brain and V ganglion, stain with TuJ1. Within any particular fascicle only a subset of these also stain with 5H1. The fact that 5H1 localizes only to cell processes in select cells suggests that it recognizes a posttranslational modification, whose site we are investigating. Supported by NS21142, NS23158, AFCD344.

348.2

NEURONAL MATURATION AND AXON GUIDANCE IN THE MOUSE TRIGEMINAL SYSTEM: AN IMMUNOHISTOCHEMICAL STUDY. D.Y.R. Stainier* and W. Gilbert. Bio. Labs, Harvard Univ., Cambridge, MA 02138.

We are investigating the outgrowth and pathfinding characteristics of the trigeminal sensory neurons in the mouse, using several specific mAb's. MAb B30 (Stainier and Gilbert, J. Neurosci., 1989) stains the surface of mesencep. trig. neurons (mesV) as well as some sensory neurons in the PNS. MAb E1.9 labels growing axons of various sensory neurons.

By early E9, sparsely distributed E1.9 positive neurons in the trig. ganglion send out short axons. The first outgrowth defines the ophthalmic (oph.) projection and axons grow out as individual fibers, each growth cone growing independently in a common direction. By late E9, B30 immunoreactivity (IR) defines mesV neurons as they send axons from their midbrain location to the brainstem where they exit the CNS at the pontine region. These central axons mix with B30 positive neurons and axons of the trig. ganglion and join one of the three (oph., max. and mand.) projections. By early E10, the oph. projection has already reached past the eye cup where axons are sending short collaterals. While leading axons still behave as pioneers, later ones seem to join nerve bundles.

By late E10, early E11, the leading axons seem to have reached the vicinity of their targets and more axons can be seen joining the nerve bundles as they leave the ganglion. Indeed, B30 IR clearly shows at this stage three distinct axon bundles leaving the ganglion and also reveals the segregation of the trig. neurons according to their general target area. Shortly after that, E1.9 IR disappears while the B30 Ag is detectable throughout synaptogenesis and until P15.

348.3

THE PRIMARY AFFERENT INNERVATION OF THE TRIGEMINAL BRAINSTEM COMPLEX OF FETAL RATS AS DEMONSTRATED BY ANTEROGRADE TRANSPORT OF DI-I. R.S. Crissman, L.L. Parsons, N.L. Chiaia and R.W. Rhoades (SPON: A.V. McGrady). Dept. of Anatomy, Medical college of Ohio, Toledo, OH 43699.

Deposits of Di-I into the trigeminal (V) ganglion were used to trace the primary afferent projection to the brainstem, primarily V nucleus principalis (PrV), in rats killed on embryonic (E-) days 16, 18, 20, the day of birth (P-0) and P-7. On E-16, Di-I labelled fibers were visible throughout PrV. Most of these axons were very simple and followed radially oriented paths from the V spinal tract into the nucleus. Some fibers ended in growth cones and many also gave off side branches at several points along their course. In addition to the radially oriented fibers, some axons left the tract in the rostral part of PrV and coursed through the length of the nucleus, parallel to its long axis. A few of these could be followed as far as the rostral portion of V subnucleus interpolaris and many of them also gave off branches along much of their length. Organization of the primary afferent projection to PrV on E-18 was generally similar to that on E-16. However, at this age, many of the primary afferents that followed radial trajectories into PrV were organized into fascicles that exited the tract at periodic intervals. All of the fibers within a given fascicle appeared to terminate in the same portion of PrV. By E-20, most primary afferent axon arbors were still fairly simple, but some fibers had arborizations that appeared considerably more widespread than those observed in older animals. Organization of radially oriented collaterals into fascicles was also apparent at this age. By P-0, most primary afferents terminating in PrV had fairly restricted arbors, but a few widespread axons were still visible. By P-7, most well-labelledprimary afferents in PrV had formed dense and highly circumscribed arbors.

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348.4

FACIAL NERVE DEVIATION AFTER OTOCYST REMOVAL IN CHICK EMBRYOS. W. Yang*, C.S. Von Bartheld, and E.W Rubel (SPON: R.L. Hyson). Hearing Development Laboratories RL-30, Univ. of Washington, Seattle, WA 98195.

The otocyst was extirpated unilaterally in E3 chick embryos. At E9-12, the embryos were perfusion-fixed and the fluorescent tracer DiI was injected into ipsilateral middle ear structures to label the facial nerve. The tissue was cryosectioned 2-3 months later. After otocyst removal the facial nerve takes an unusual route to the brain. It courses rostrally outside the cranium, penetrates the skull adjacent to the trigeminal ganglion and then turns caudally inside the cranium to enter the brain in the usual location, i.e. at the level of the vestibular nuclei. Motor fibers of the facial nerve appear to make the same detour as the sensory fibers. Auditory brainstem nuclei do not receive projections from the facial or paratympanic nerve when the vestibular or cochlear nerves are absent.

The abnormal course of the facial nerve was also found in some cases with partial otocyst removal, when only a rudimentary cochlea and cochlear nerve remained. However, the facial nerve follows its normal route along the vestibular nerve when a portion of the vestibular endorgan and nerve persisted. In all cases, the facial nerve projections in the brainstem appear similar to normal controls.

We conclude that the vestibular nerve, but not the cochlear nerve normally provides guidance cues for ingrowing facial sensory nerve processes. In the absence of this pathway the trigeminal nerve appears to attract facial nerve axons. The fact that once inside the cranium the nerves are able to find their normal site of entrance into the brain and their normal termination sites suggests highly specific guidance cues. Such cues, apparently, are effective beyond the normal distance from the ingrowing sensory axons. Supported by NIH grants NS 24522 and NS 08578.

348.5

DEVELOPMENTAL EVOLUTION IN THE DISTRIBUTION OF RADIAL GLIAL FIBER GROWTH CONES IN THE MURINE CEREBRAL WALL. T.Takahashi*,J-P. Misson* VS. Caviness Jr.,(Spon.: P.Bhide).Dept. Neurology, Mass. Gen. Hospital, Boston, MA 02114
Bipolar radial glial cells of the developing murine cerebral wall are proliferating population which continually adds fibers during the course of cerebral histogenesis. A quantitative study during the 3rd week of gestation, the principal period of neuronal migration, suggests, however, that a substantial proportion of the fibers extends only as far as the outer IZ-deep subplate zone of the cerebral wall (Gadisseux et al., in prep.). The present analysis, based upon glial immunostaining with RC2 antibody (Misson et al.,Dev.Br.Res,1988,44,95), is a re-examination of this possibility. Specifically this analysis is a quantitation of the density of glial fiber growth cones in relation to depth in the cerebral wall through the interval E17-P2. The glial cell growth cones,as visualized by RC2 staining,are polymorphic. For the purpose of the present analysis two growth cone forms are recognized: a simple rounded or club shaped form, on the one hand, a heterogeneous set of forms made complex because of multiple filopodial extensions and branches, on the other. Only the simple form is considered in the analysis on the assumption that it is related to fiber extension while the complex form is probably a reflection of glial process arborization and astrocytic transformation (Caviness et al., Neurosci. Abstr. 1989). Through P0, growth cones of the simple form are abundant in the cerebral wall but subsequently decline sharply in density. By E17 their distribution is disproportionate with respect to the strata of the cerebral wall. Specifically, the concentration in the ESS is 2X that of the adjacent subplate and 4X that of the overlying CP. By P0, the disparity has lessened such that the concentrations in the ESS through the lower half of the cortex are approximately equalized though still somewhat greater than that in the outer zone of the CP. These observations, consistent with findings of the quantitative study of RGF cited above, suggest that fiber tips amassed within the ESS at E17, surge into the CP during the final days of gestation. The interval of the surge corresponds to the time of assembly of supra- granular cortical layers by neuronal migration.

348.6

SEROTONIN IMMUNOREACTIVE FIBERS YIELD A COMPLETE MAP OF THE BODY SURFACE IN SOMATOSENSORY CORTEX OF PERINATAL RATS. L.M. Yarris, N.L. Chiaia, C.A. Bennett-Clarke, M.F. Jacquin, J.H. Haring, G.J. Macdonald and R.W. Rhoades (SPON: E.C. Johnson). Depts. of Anatomy, Medical College of Ohio, Toledo, OH 43699 and Robert Wood Johnson Medical School, Piscataway, NJ 08854, and Dept. of Anatomy and Neurobiology, St. Louis University School of Medicine, St. Louis, MO 63104.
Serotonergic fibers in developing rats pass through a period during which they are very dense in primary sensory cortical areas (D'Amato, R.J. et. al. PNAS, 84:4322, 1987). We have extended this observation by determining the age at which differential localization of serotonin immunoreactive (SI) fibers can first be detected in the primary somatosensory cortex and whether neonatal peripheral nerve damage alters the organization of the serotonergic innervation of this cortical region. Pups from timed pregnant rats were sacrificed one hr after birth (P-0) and at the beginning of each of the next nine postnatal days and their cortices were processed for the demonstration of SI using conventional techniques. Some of these animals sustained transection of the left ION within 2 hrs of birth. Dense SI was present in the presumptive primary somatosensory cortex by the beginning of P-1. At this age, the region in which these fibers were most dense provided a crude representation of the body surface. By the start of P-2, the map of the body surface defined by areas of high density SI was much more refined. In the region representing the vibrissa pad, an organization related to the rows of whiskers could be seen clearly. By, the beginning of P-3, all of the patches representing the individual vibrissae could be readily seen as well as the representation of the buccal pad, lower jaw, forelimb and hindlimb. Transection of the ION on the day of birth and examination of the cortex on P-9 demonstrated a marked disruption of the vibrissa-related pattern in the somatosensory cortex.
Supported by BNS 85 17537, DE 07734, and funds from the State of Ohio Research Challenge.

348.7

MODEL OF OCULAR DOMINANCE COLUMNS AND PATTERN FORMATION: ANALOGY TO FERROMAGNETIC DOMAINS. H. Potter, (SPON: M. Livingstone). Dept. Neurobiology, Harvard Med. Sch., Boston, MA 02115.
The formation and behavior of the patterns of functional domains of two biological tissues—the ocular dominance columns in the vertebrate visual cortex and the dermal condensations that will give rise to feathers in the developing chick skin—bear a striking resemblance to the behavior of the pattern of alternating magnetic domains in ferromagnetic crystals. In all three cases, striped patterns can be interchanged with patches or blobs by altering the competitive advantage of one type of domain over the other—for instance, by suturing one eye closed at birth, by disrupting cell-cell interactions with antibodies, or by exposing the crystals to an external magnetic field. These similarities suggest a general model for pattern formation during development, that is based solely on cell or synapse adhesion and motility as governed by the same simple energy relationships that underlie the formation and behavior of ferromagnetic domains.

348.8

EARLY INGROWTH OF INDIVIDUAL CALLOSAL AXONS INTO RAT NEOCORTEX. S. Catalano * and H.P. Killackey, Depts. of Anatomy and Neurobiology and of Psychobiology, University of California, Irvine, CA 92717 (SPON: R. Josephson)
The morphology of single callosal afferents in rat neocortex at various timepoints in development were examined using the fluorescent tracer DiI. Rat pups were sacrificed and then perfused transcardially with 4% buffered paraformaldehyde. Crystals of DiI were then implanted in neocortex, and the brains stored in fixative for up to six months. During this time the DiI diffuses through the lipid membranes of cells in the aldehyde fixed tissue (P. Godement et al, Development 101:697, 1987), labeling callosal axons anterogradely and callosally projecting cells retrogradely in the contralateral cortex. Brains were then sectioned with a vibratome, and sections were counterstained with bisbenzamide to visualize the cortical laminae, allowing direct identification of fiber position within the laminae.
By the nineteenth day of gestation, cortex can be subdivided into a marginal zone, cortical plate, subplate, and intermediate zone. At this stage, presumptive callosal afferents can be observed which have penetrated the subplate and reached the lower border of the cortical plate. These fibers exhibit a simple morphology; most are unbranched along their entire length, and are tipped by a distinctively small growth cone. Given that the subplate contains cells destined to become layers V and VI (layers in which callosal axons will terminate), it appears as though some callosal axons reach the vicinity of their target much earlier than has been previously demonstrated. (Supported by NSF grant BNS 87-19311).

348.9

DEVELOPMENT OF CALLOSAL ARBORS IN THE HAMSTER SM CORTEX. C.R. Norris* and K. Kalil. SPON: J. Lilien. Neuroscience Program & Dept of Anatomy, University of Wisconsin, Madison, WI 53706.
We have followed the extension of callosal axons into the cortex and their transformation into axonal arbors. First, at the LM level, we are interested in the role of lateral branching in the development of topographic connections. Second, at the EM level, we are examining the time course of synapse formation by callosal axons.
We used DiI to label callosal growth cones arising from the sensorimotor cortex in infant hamsters. Callosal growth cones begin to extend across the callosum at birth and first enter the contralateral cortex at about 48 hours postnatal. Axons then grow perpendicular to the cortical layers with little lateral branching until they reach layer 1, at about 9 days postnatal. Thus, in the initial stages of growth into the cortex, growth cones appear to establish their cortical territory with straight unbranched axons before the axons give rise to the lateral branches. Furthermore, the site at which growth cones enter the cortical target corresponds topographically to their neurons of origin in the contralateral cortex. Therefore, initial callosal connections are point to point connecting reciprocal regions of the cortex, as in the adult. The present results are not inconsistent with previous studies using retrograde tracers to study the development of callosal connections, which suggested that early callosal arbors spread over a wider cortical territory than those in the adult; rather it is likely that pruning of widespread arbors is a later developmental event.
To examine the time course of transformation of callosal growth cones into synapses, combined HRP-EM methods were used. Preliminary data suggest that at 3-4 days postnatal callosal axon tips in the deep layers of cortex do not establish synaptic contacts. Moreover, even at 10 days postnatal, callosal axons still exhibit small but identifiable growth cones. Taken together, these results suggest that axons first establish their cortical topography and then branch laterally to form synaptic connections with cortical targets. (Supported by NIH grant NS14428.)

348.10

AXON TRAJECTORY AND GROWTH CONE MORPHOLOGY IN THE DEVELOPING CORPUS CALLOSUM. Dale Hogan* and N.E.J. Berman (SPON: S.R. Nelson). Dept. of Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, KS 66103.
Visualizing the pattern of growth of CNS tracts is an important step in gathering evidence for putative axonal guidance mechanisms. The developing corpus callosum was studied in neonatal rats by inserting the carbocyanine tracer DiI into the visual cortex of fixed brains. This technique allows visualization of pioneer axons, individual axons in the growing front and complex growth cone morphologies. On the day of birth, the growing fascicle is just crossing the midline with the pioneers .5-1 mm. ahead of the fascicle. The pioneers are seen in both the dorsal and ventral aspects of the callosum. The axons in the bundle appear to remain parallel to each other implying that they are arranged topographically as they enter the callosum. In the most rostral regions of the visual callosum, more axons have crossed the midline and the pioneers extend further into the contralateral hemisphere than in the more caudal region. Many of the growth cones in the main fascicle show complex filopodial and lamellepodial morphologies suggesting that they are growing slowly and actively sampling their environment. Pioneer axon growth cones were observed to be smaller and rounder, implying that they are growing faster and following a preexisting substrate. (Supported by MH38399 and BNS8819971)

348.11

HEREDITY OF COLLISIONS BETWEEN ANTERIOR COMMISSURE AND COLUMNS OF THE FORNIX IN MOUSE BRAIN. B. Cassells and D. Wahlsten. Dept. of Psychology, University of Waterloo, Waterloo Ontario, CANADA N2L 3G1. Axons of the anterior commissure (AC) normally pass anterior to the columns of the fornix (CF) in a compact cylindrical bundle. However, BALB/c inbred mice show a high incidence of CF-AC collisions (Wahlsten, Brain Res. 68: 1-18, 1974) in which part or all of the anterior part of the AC passes through the CF. To more fully describe the inheritance of the collision, C57BL/6 CRBL mice, which do not show this anomaly, and BALB/cCRBL mice were bred to produce reciprocal F1 hybrids and backcrosses. These mice were perfused as adults and every other 25 µm sagittal section throughout the extent of the septum was stained with Sudan Black B for myelin and examined at 40 X for CF-AC collisions and related morphology. The defect showed nearly complete (94%) penetrance in inbred BALBs. Collision risk was increased additively by incompletely penetrant recessive BALB/c autosomal (64%) and X-linked (30%) factors. The inheritance of corpus callosum defects, also seen in BALBs, was entirely independent of the inheritance of CF-AC collisions. Study of morphology related to the collisions led to the hypothesis that hereditary factors produced premature differentiation of neurons sending axons via the AC; this produced an increased rate of growth in the anterior but not posterior limbs of the AC due to differences in primary axonal outgrowth/guidance mechanisms and the resulting dyscoordination produced the CF-AC collisions.

348.12

GROWTH AND BRANCHING OF CORTICAL AXONS: IMPLICATIONS FOR TARGET SELECTION BY DEVELOPING AXONS. D.D.M. O'Leary and T.Terashima, Dept of Neurosurg, Washington Univ, St. Louis, MO. 63110

Layer 5 of the mammalian neocortex is the source of projections to a number of subcortical targets. We have studied the elaboration of these projections from two distinct regions of cortex, motor and visual, and find that subcortically projecting layer 5 neurons have similar axon extension and branching programs, although their mature projections are quite distinct. Cortical axons and their collateral branches were labeled by injections of the axon tracer, DiI, made in vivo into motor or visual cortex of E20 to adult rats. The primary axons arising from layer 5 neurons extend out of cortex through the internal capsule, cerebral peduncle, pyramidal tract and into the spinal cord. The subcortical targets of layer 5 neurons are later innervated by collaterals which branch from the primary axons. We have focused on 5 major branch points: tectal (which innervates several targets, with the superior colliculus as its distal target), mesencephalic (which innervates the midbrain nuclei such as the red nucleus, etc.), pontine (innervates basilar pons), inferior olive, and dorsal column nuclei. Each collateral forms well after the parent axon grows past the future branch point, with the more rostrally located branches forming first. Both motor and visual cortical axons form and extend all 5 branches, but those from visual cortex are delayed. We have yet to observe any indication that the growth cones of the primary axons recognize the future branch points, nor do we see any filopodial remnants left behind that may mark them. At a later stage, certain components of the initially similar axon trees are eliminated: Motor cortical axons lose most of their tectal collateral, while visual cortical axons lose the primary axon and all collaterals caudal to the pontine branch point. Thus, the development of layer 5 subcortical projections has 3 phases: extension of primary axons, delayed formation of collateral branches, and selective loss of axon segments. These findings indicate that in this system target selection is not the responsibility of the growth cone of the primary axon.

348.13

SEROTONIN AGONISTS DECREASE BRANCHING OF FRONTAL CORTEX NEURONS. L. Sikich*, J. Hickok*, and R.D. Todd. (SPON: K. Isenberg). Department of Psychiatry, Washington University School of Medicine, St. Louis, MO 63110.

Serotonin (5HT) has long been known to influence neurite elongation in identified invertebrate neurons. However, the morphological effects of 5HT upon individual neurons in mammals has not been previously examined.

Various serotonergic drugs were added to dissociated cultures of fetal rat cortex after one day in vitro. Following 90 hours of drug exposure, the number of neurites and branch points along each neurite, the somal area and the total neuritic extent were measured in 100 cells from each treatment group. Treatment with 5HT or the $5HT_{1A}$ agonist 8-OH-2-(Di-*n*-propylamino)-1,2,3,4-tetrahydronaphthalene (8-OH DPAT) decreased the mean number of branches and mean neuritic extent by ~50% and ~25% respectively. These effects were blocked by coculture with the $5HT_{1A}$ antagonist spiperone. Results were similar in cultures from both E17 and E19 animals.

These results demonstrate that 5HT acts via $5HT_{1A}$ receptors to modify specific facets of neuronal morphology. Whether these changes represent decreases in all cortical neurons or in specific subgroups of neurons is currently unknown. Such morphological modifications could influence the pattern of connections and synaptic contacts and the survival of neurons in the mammalian frontal cortex.

348.14

DIBUTYRYL cAMP REDUCES THE INHIBITORY EFFECT OF AMITRIPTYLINE ON NEURITE OUTGROWTH OF CHICK EMBRYONIC CEREBRAL EXPLANTS. K. L. Wong*, E. Gonzales*, and A. I. Farbman. Department of Neurobiology and Physiology, Northwestern University, Evanston, IL 60208.

Recent data from this laboratory have shown that: 1) dibutyryl cAMP (dbc AMP), or phosphodiesterase inhibitors such as isobutylmethylxanthine and theophylline, enhance neurite outgrowth of chick embryonic olfactory explants [J. Neurobiol. (1988) 19: 681], and 2) amitriptyline (AMI), a typical tricyclic antidepressant, inhibits neurite outgrowth of both chick embryonic olfactory and cerebral explants in a dose-dependent manner [Brain Res. (1988) 457: 281]. These data lead to the possibilities that cAMP could be involved in neurite outgrowth, and that AMI may inhibit neurite outgrowth via the alteration of the intracellular level of cAMP.

In chick embryonic cerebral explants, 1 or 10 µM of dbc AMP results in a significant 19 and 27% increase, respectively, in the percentages of explants that expressed neurite outgrowth relative to the controls (1 µM: $t=2.93$, $df=8$, $p<0.025$; 10 µM: $t=4.42$, $df=8$, $p<0.005$). More importantly, combined treatment of AMI and dbc AMP reduces the inhibitory effect of AMI treatment alone. For example, 1 or 10 µM of dbc AMP (in the presence of 8 µM of AMI) results in a significant 26 and 37% increase in neurite outgrowth over that of 8 µM of AMI treatment alone. (1 µM: $t=3.57$, $df=6$, $p<0.015$; 10 µM: $t=5.06$, $df=6$, $p<0.0025$). Likewise, both 1 and 10 µM of dbc AMP (in the presence of 4 µM of AMI) noticeably reduce the inhibitory effect of of 4 µM of AMI (1 µM: $t=2.96$, $df=6$, $p<0.05$; 10 µM: $t=3.43$, $df=6$, $p<0.015$).

PROCESS OUTGROWTH, GROWTH CONES AND GUIDANCE MECHANISMS VI

349.1

ANALYSIS OF CELLULAR INTERACTIONS THAT MEDIATE THE DEVELOPMENT OF MOTOR AXON SEGMENTATION. R. A. Oakely and K. W. Tosney. Neurosci. Program & Biol. Dept., Univ. of Michigan, Ann Arbor, MI 48109

Motoneurons extend axons only through the anterior half of each somite and the development of this segmental pattern is dependent on sclerotome. We have developed an *in vitro* assay to investigate the cellular mechanisms responsible for motor axon segmentation. We remove the viscera, notocord, and spinal cord and cut strips of several consecutive somites that are cultured ventral side up on a laminin substratum. These conditions expose the sclerotome and yet retain the physical relationships among anterior and posterior populations. Motoneurons are labeled with DiI, dissociated and deposited on the explants.

We find a clear hierarchy of substratum preference as detected by the extent of labeled neurite outgrowth after 20 hours of culture. Neurite outgrowth is minimal on the posterior half sclerotome and is more extensive on the anterior half sclerotome. In addition, the behavior of neurites at tissue borders indicates that the posterior sclerotome is impervious to axon invasion from somata on the spinal cord basal lamina or on the anterior sclerotome, consistent with the role of the posterior sclerotome as a barrier to axon advance. Our results suggest that a contact mediated mechanism may be responsible for motor axon segmentation. A preliminary analysis of the trajectory of neurites suggests that a long distance cue may also contribute.

Despite a clear preference for anterior over posterior as a substratum, axons grow much more robustly on the laminin substratum than on any portion of the explant. In addition, outgrowth is extensive on the remaining spinal cord basal lamina and on the myotome. These results suggest that the sclerotome is a relatively hostile environment for motor axon outgrowth and that the posterior sclerotome is more hostile than the anterior.

Supported by NIH grant #NS-21308.

349.2

TWO GROWTH ASSOCIATED PROTEINS: CHANGES IN MEMBRANE LOCALIZATION DURING DEVELOPMENT. M. Merline*, E. Floor, and K. Kalil. (SPON: E. Schweitzer). Dept of Anatomy and Neurosciences Training Program, University of Wisconsin, Madison, WI 53706.

We previously described a 33kDa brain-specific membrane protein whose expression during development is well correlated with axon outgrowth in the hamster CNS (Kalil and Perdew, *J. Neurosci.*8:4797-4808, 1988). To gain insight into the functional role of this protein, we examined how its subcellular localization changes during development and compared this localization with that of another growth associated protein, GAP-43, which is thought to play a role in axon outgrowth.

Subcellular fractionation was carried out on hamster brains ranging in age from 3 days postnatal to adult. Brains were homogenized and a low-speed supernatant run on Sephacryl S-1000 columns to separate subcellular particles according to size. Membrane particles from each column fraction were pelleted and their proteins separated by SDS-PAGE. The proteins were analyzed by Western blotting with a monoclonal antibody to the 33kDa protein and a polyclonal antibody to GAP-43 (kindly provided by L. Benowitz). In young animals the 33kDa protein appeared to be distributed over membrane particles of a wide range of sizes. By contrast, in adult brains the protein was enriched in a fraction containing small particles 30 nm in diameter, a size somewhat smaller than synaptic vesicles. Results with GAP-43 suggest that in young brains this protein is associated primarily with large membrane particles.

The results suggest that these proteins may play a role in intracellular membrane processing during axon outgrowth. The residual stores of the 33kDa protein on a population of small particles in the adult brain could perhaps be involved in further synaptic changes once axon outgrowth is completed. (Supported by NIH Grants NS 24890 to E. Floor and NS 14428 to K. Kalil.)

349.3

NEW MACRO-EXPLANT (ME) TISSUE-CULTURE MODEL TO STUDY SPINAL-CORD (SC) NEURONS. C.Mariotti*, V.Askanas, W.K.Engel, C.S.Lee*. Neuromuscular Ctr, U. of So. Cal., L.A., 90017.

We have developed a system for culturing separately the entire longitudinally-cut ventral and dorsal parts of the whole SC of 12-14 day rat embryos, on polylysine-collagen mixture, in F14 medium + 10% FBS. At the time of explantation and throughout the period of culturing (up to 21 days) CAT activity in dorsal MEs (DMEs) was negligible (2% of that in the ventral MEs (VMEs). In 5 experiments, neurite outgrowth, measured by computerized video image analysis (CVIA) (RAS, Amersham), at 12 days was 3-fold in VMEs and 2-fold in DMEs ($p<.02$) that of 5-day-old explants. Optical-density (OD) analysis of neuron-specific enolase (NSE) immunostain showed that at 5 days 37% of neurites in VMEs and 31% in DMEs belonged to the highest OD bin (indicating strongest staining) vs. 19% and 21% of 12-day VMEs and DMEs ($p<0.05$). To the contrary, 44% of 12-day VME-neurites and 43% of DME-neurites belonged to the lowest OD vs. 30% and 35% in 5-day VMEs and DMEs. About 35% of all neurites belonged to the middle OD level. CAT activity was the lowest in 5-day VMEs. It increased 113% ($p<0.05$) at 12 days, 50% at 16 (p=NS) days, and 106% ($p<0.05$) at 21 days. This new system allows study of motor neurons and the influence on them of putative growth, survival and maturation factors in an SC milieu more closely resembling the in vivo condition. Our established parameters can now serve as a basis for such studies.

349.4

CARBON FILAMENTS PROMTE AXONAL GROWTH ACROSS THE SITE OF SPINAL CORD TRANSECTION IN ADULT RATS. M. Dauzvardis* and T. Khan, (SPON: G. Gaik) Neuro-Regeneration Lab., RR&D Center, VA Hospital, Hines, IL 60141.

Small diameter (4-8μ) carbon filaments have been used in the orthopedic repair of ligaments in the knee (Weiss, A.B. et al., Clin. Orthop. & Related Res., 196:77, 1985). In these studies, fibroblasts apparently used the filaments as framework for the laying down of new collagen. Carbon filaments have also been shown to support the growth of embryonic spinal cord in tissue culture (Khan, T. et al. Abtrc. Soc. Neurosci. 11:586, 1985). In the present study, we analyzed the ability of carbon filaments to function as a "scaffolding" for the growth of regenerating axons in spinalized rats.

Seven 200g Wistar rats sustained total spinal cord transection at level T8-T9 with the use of a #11 scapel blade followed by withdrawal of fine wire hook through the transection site. In five of these animals the resulting transection gap was then filled with a 5mm long bundle of approximately 10,000 carbon filaments of 4.0μ diameter. Two rats served as surgical controls. After a six-week survival period, all animals received bilateral cortical injection of 1% WGA-HRP. No fibers were observed below the lesion area in the two surgical control animals, while the spinal cords of 2 out of 5 animals with carbon filament implants were found to contain labeled axons as far as two spinal segments below the transection site.

Carbon filaments, by providing a favorable adhesion surface and also a possible guiding function, may prove useful in the treatment of spinal cord injury. Supported by funds from Veterans Affairs, Rehabilitation R&D Service, Rehab. R&D Grant #B423R.

349.5

CELLULAR ELEMENTS ARE REQUIRED FOR AXONAL REGENERATION IN THE LAMPREY SPINAL CORD. **D.I. LURIE* AND M.E. SELZER. DAVID MAHONEY INST. OF NEUROL. SCIENCES AND DEPT. OF NEUROLOGY, U. OF PENN., SCH. OF MED., PHILA., PA 19104.**

Larval sea lampreys recover from complete spinal transection. This involves axonal regeneration across the zone of injury. We wish to determine whether cellular elements in the injury zone are required for this regeneration. A 3mm freeze lesion was performed which severed axons and destroyed neurons and glia but left the connective tissue components of the meninges intact. The lesion was devoid of cellular elements after 4 weeks. The remaining connective tissue formed a continuous bridge between the proximal and distal stumps of the cord. Nine Petromyzon marinus larvae received a freeze lesion at the level of the 7th gill and were allowed to recover for at least 10 weeks. Muller and Mauthner axons were injected intracellularly with HRP. Whole-mounts of brain and spinal cord were prepared. None of the animals recovered coordinated swimming even after 19 weeks. Fifty-five branches of 29 axons were labelled with HRP. Although these neurites regenerated in a directionally specific manner in the proximal stump, none of them grew into the lesion site. We conclude that regenerating spinal neurites will not grow into a connective tissue matrix devoid of both neurons and glia. (NIH grants NS 14837 and NS 25581).

349.6

ADHESION AND NEURITE OUTGROWTH ON THROMBOSPONDIN AND A 140 kDa FRAGMENT. K.S. O'Shea, L-H.J. Liu*, & V.M. Dixit* Anatomy & Cell Biology, Univ Mich, Ann Arbor, MI 48109.

To assay the effects of thrombospondin (TSP), a trimeric glycoprotein constituent of extracellular matrices, on adhesion, neuroepithelial cells isolated from spinal cords of day 13 mouse embryos were cultured on TSP (1-50 ug/ml), its NH_2-terminal heparin-binding domain (25 ug/ml), a 140 kDa fragment containing the COOH domain but lacking the heparin-binding amino-terminal region (25 ug/ml), laminin (25 ug/ml), or BSA (25 ug/ml), in serum-free N2 medium. Anti-TSP (50 ug/ml) or GRGDS (500 ug/ml) was added to additional wells coated with TSP. After 2h, coverslips were washed, the Hoescht dye was added and adherent cells counted. Neurite outgrowth from anti-alpha-MSH stained cells was determined after eight h in vitro. TSP, like laminin, supported both attachment and neurite outgrowth as did the 140 kDa fragment, but not the heparin-binding domain. Anti-TSP antibodies inhibited attachment and neurite outgrowth on TSP; GRGDS had no effect. Interestingly, neurite outgrowth on the 140 kDa fragment was nearly as efficient as on laminin, while on the intact molecule, outgrowth was slightly less.
Supported by NIH grant HD-23867.

349.7

AXONIN-1, AN AXONALLY SECRETED GLYCOPROTEIN: I. IMMUNOHISTOCHEMICAL LOCALIZATION IN DEVELOPING NERVE FIBER TRACTS. E.T. Stoeckli*, P. Streit#. M.A. Rüegg* and P. Sonderegger. Biochemisches Institut & #Hirnforschungsinstitut, Universität Zürich, CH-8057 Zürich.

Axonin-1 is a glycoprotein that is released from axons of cultured neurons, as revealed by metabolic labeling in a compartmental cell culture system (Stoeckli, E.T. et al., Eur. J.Biochem., 180:249-258, 1989). It has recently been purified from the ocular vitreous fluid of the chicken embryo (Rüegg, M.A. et al., EMBO J., 8:55-63, 1989). In the present study, the expression of axonin-1 in the developing nervous system of the chick was investigated immunohistochemically at the light and electron microscopic level. In both the developing retina and spinal cord, axonin-1 was found mainly associated with nerve fiber tracts. In the spinal cord, the staining disappeared after hatching. Immunoelectron microscopy of the optic fiber layer of the retina located the axonin-1 immunoreactivity of axon bundles to the surface of the axonal membrane. The specific location in bundled axons and the early disappearance after birth suggests implication in the formation of nerve fiber tracts.

349.8

AXONIN-1, AN AXONALLY SECRETED GLYCOPROTEIN: II. IDENTIFICATION OF A MEMBRANE-ASSOCIATED HOMOLOGUE INVOLVED IN NEURITE FASCICULATION. M.A. Rüegg*, E.T. Stoeckli*, R.B. Lanz*, P.Streit# and P. Sonderegger (SPON: H.-C.Bauer). Biochemisches Institut und #Hirnforschungsinstitut der Universität Zürich, CH-8057 Zürich.

The location of axonin-1 immunoreactivity at the surface of developing nerve fiber tracts (Stoeckli, E.T. et al., accompanying abstract) closely parallels the tissue distribution of cell adhesion molecules involved in neurite fasciculation. The presence of anti-axonin-1 Fab fragments during axon growth in vitro resulted in antibody binding to the axonal surfaces and in a marked perturbation of the fasciculation pattern. Hence, a fraction of axonin-1 is associated with axonal membranes and, by operational criteria, qualifies as a cell adhesion molecule. The major proportion of membrane-associated axonin-1 co-solubilized with the integral membrane proteins. Structurally, the integral membrane form was found to be highly similar to soluble axonin-1, hence, adhesive properties are postulated for secreted axonin-1. As a soluble adhesive protein, it may function as a regulator of cell adhesion around its most likely site of secretion, the growth cone.

349.9

A SMALL SUBPOPULATION OF MYOCYTES MAY BE REQUIRED FOR PROPER MORPHOGENESIS OF IDENTIFIED MOTONEURONS IN EMBRYONIC ZEBRAFISH. B. Debu, S.H. Pike*, R. Bremiller*, C.B. Kimmel and J.S. Eisen. Institute of Neuroscience, University of Oregon, Eugene, OR, 97403

Identified primary motoneurons (PMNs) innervate cell-specific regions of each muscle segment in the zebrafish. En route to their targets, PMN growth cones extend along a common pathway until they reach a choice point where each growth cone selects a cell-specific pathway.

Labeling with the anti-engrailed antibody, 4D9 reveals a set of 3-6 muscle cells, termed primary medial myocytes (pmms) at the choice point; these cells differentiate hours before adjacent muscle cells. Pmms are absent from trunk segments and PMNs project abnormally in embryos bearing the spt-1 mutation in which somitogenesis is disrupted. Mosaic analysis involving transplantation of single PMNs showed that PMN phenotype depends on the genotype of the host, not on the genotype of the PMNs. These observations suggest that pmms may be required for proper pathfinding by the PMNs.

To test this hypothesis, we ablated pmms in wild type embryos using laser-irradiation. PMN pathfinding was abnormal in segments in which pmms were ablated, but was normal in segments in which muscle fibers adjacent to the pmms were ablated. These results support the hypothesis that pmms are required for proper pathway navigation by the PMN growth cones. Supported by HD22486 and NS23915.

349.10

FORMATION OF THE MAUTHNER CELL LATERAL DENDRITE IN ZEBRAFISH IS INDEPENDENT OF ITS EARLIEST SENSORY CONTACTS. K. Hatta*, W.K. Metcalfe, M.S. Curry * and C.B. Kimmel. Inst. of Neuroscience, U. of Oregon, Eugene, OR 97403.

Are specific axo-dendritic contacts required for correct dendritic development of a postsynaptic cell? The lateral dendrite of the Mauthner cell (M-cell) receives synaptic inputs from sensory neurons located in the trigeminal (Vg), vestibular (VIIIg), and lateral line (LLg) cranial ganglia. Axons from the Vg are the first of the inputs to contact the M-cell, and they eventually synapse most proximally on the lateral dendrite. They are followed by the VIIIg and then LLg inputs, which make contacts progressively more laterally on the dendrite. Thus the spatial positions of the inputs precisely reflects their time of arrival at the dendrite.

At the time the first Vg axons arrive at the M-cell, its lateral dendrite has not yet formed, and Vg growth cones contact the M-cell body. This relation suggested the hypothesis that the Vg contact is necessary for the M-cell to initiate proper differentiation or to develop its lateral dendrite.

To test this hypothesis, we used a laser to ablate Vg cells 1-2 hr before they contact the M-cell. The lateral dendrite and other morphological features of M-cells deprived of their early Vg inputs and examined 5 days later appeared normal. Thus, the outgrowth of the lateral dendrite may either be programmed intrinsically in the M-cell, or dependent upon some environmental signal other than its first input. Supported by NS17963 and Naitoh Foundation.

349.11

GROWTH CONE MORPHOLOGY OF COMMISSURAL INTERNEURONS IN THE CHICK SPINAL CORD. H. Yaginuma*, S. Homma*, R. Künzi* and R. Oppenheim (SPON: N. Okado). Dept. of Anatomy, Wake Forest Univ. Medical School, Winston-Salem, NC 27103.

Previous studies have shown that spinal commissural interneurons (CI) located in dorsolateral and intermediolateral regions are one of the earliest developing populations in the chick spinal cord. To study the mechanisms involved in axonal guidance in this pathway, we have examined the morphology, trajectory, and interaction with the substrate, of commissural growth cones. Growth cones were labelled in vitro with HRP or DiI and observed by light and electron microscopy.

Developing growth cones showed different shapes depending on their position in the pathway. Both before and after passing through the floor plate, growth cones were lamellepodial or filopodial in shape. By contrast, within the floor plate, the majority exhibited blunt or varicose shapes, with only one or a few filopodia at the leading edge and small protrusions on the sides. Within the longitudinal fiber tracts of the marginal zone (MZ) growth cones were lamellepodial or spindle shaped with filopodia. When observed with the electron microscope, most growth cones in the floor plate were found to contact the basal lamina. Close contacts between growth cones and longitudinal axons in the MZ were also observed. The present results indicate that different guidance mechanisms are used along the CI pathway.

349.12

FLOOR PLATE ANTIGEN P84 SUPPORTS ADHESION AND OUTGROWTH OF CULTURED CEREBELLAR NEURONS. W. Chuang and C. Lagenaur. Dept. of Neurobiology, Anatomy, and Cell Science. Ctr. for Neuroscience, Univ. of Pittsburgh, Sch. of Med., Pittsburgh, PA 15261.

We have previously described a novel adhesion molecule, P84, which is restricted to the floor plate of mouse embryonic spinal cord, but is widely expressed in postnatal CNS. Previous studies showed that nitrocellulose immobilized P84 supports adhesion and outgrowth from postnatal cerebellar neurons. Both monoclonal and polyclonal anti-P84 antibodies stained the surfaces of cultured postnatal central neurons. Addition of polyclonal Fab fragments directed against P84 to the growth medium inhibited cell adhesion and outgrowth on P84. Intact monoclonal antibodies to P84 also inhibited adhesion and outgrowth on P84 antigen. Taken together, these findings suggest that the adhesive activity of isolated P84 antigen observed in vitro resides in a site exposed on the surface of living cells. Immunoaffinity purified P84 is composed of proteins with molecular weights of 167, 85, and 66 kD. In Western blot, polyclonal antibodies to P84 stain these three proteins. Affinity purified antibodies eluted from each P84 band also stained all P84 proteins indicating that these bands all share common antigenic determinants. When these same affinity purified antibodies were used to stain sections of embryonic spinal cord, they produced a staining pattern identical to that seen with monoclonal anti-P84 stainings. Cells taken from embryonic day 15 tectum (which does not yet express P84) were not able to attach and extend neurites on P84, but were able to grow on laminin, poly-L-lysine, and 8D9 antigens. Postnatal cortex shows weak staining for P84, and indeed, this tissue will grow on a P84 substrate. These findings indicate that the ability of neurons to grow on P84 is temporally and regionally regulated.

349.13

FLOOR PLATE CELL MORPHOLOGY REVEALED BY TRANSGENE EXPRESSION. R. Campbell*, R. McGowan*, C. Sapienza*, and A. C. Peterson. Ludwig Institute for Cancer Research, Montreal, Canada.

The neural tube floor plate is thought to participate in the guidance of a subset of axons (commissural axons) within the developing neural tube. We are studying a transgenic mouse line in which an *hsp68-lac* Z construct is ectopically expressed within the floor plate from E10 to E14. Staining for beta-galactosidase activity using chromogenic substrates reveals a subset of cells with a morphology suggesting an intimate association with decussating neurites.

In this same line, insertion of the transgene has caused a mutation of the *dystonia musculorum* (*dt*) locus, leading, in homozygous mice, to a profound degeneration of peripheral sensory axons soon after birth. The transgene's ectopic expression in this line appears to result from its site of insertion within the genome, as other lines made with the same contruct show no such expression (Kothary, R., Clapoff, S., Brown, A., Campbell, R., Peterson, A., and Rossant, J., *Nature*, **335**: 435, 1988.). Since this insertion site appears to be the *dt* locus, this raises the possibility that regulatory elements belonging to the *dt* gene itself are directing transgene expression to the floor plate.

349.14

ACTIVE MOVEMENTS OF MEMBRANE ANTIGENS TO THE EDGES OF GROWTH CONES OF CORTICAL NEURONS. **D.B. Wayne, N.L. Baumrind , A.L. Pearlman and M.P. Sheetz. Depts. of Cell Biol. & Neurol., Wash. Univ. Med. School, St. Louis, MO 63110.** **The neuron-specific antibody, 2A1, immunolabels the perimeter of murine cortical neurons in vitro (Baumrind et al., this volume). This distribution could result from either active concentration of the antigen at the cell's leading edge or passive trapping at the substrate surface. To distinguish between these we used antibody coated gold particles to follow the movements of antigen by video-enhanced DIC microscopy. 80% of 2A1-conjugated gold particles are found along the growth cone perimeter and many are located on filipodia. They exhibit diffusive movements indicating that their position is not fixed by substrate attachment. An antibody to N-CAM (abNCAM) is similarly distributed, whereas transferrin or rat IgG bind primarily to the central body of the growth cone. Gold particles coated with 2A1 or abNCAM move predominantly in a random manner, but occasionally move outward to the leading edge of lamellipodia or to the end of filopodia at 1-2μm/sec. Detailed position analyses indicate that the latter movements are active, not diffusive. The active forward movement of 2A1 and N-CAM and their preferential distribution at the growth cone edge suggest that membrane molecules involved in cell-cell or cell-substrate interactions may be actively concentrated at the leading edges of growth cones and filopodia (see Sheetz et al., this volume for a possible mechanism).**

349.15

ASTROCYTES MATURED IN VITRO SUPPORT RETINAL NEURITE OUTGROWTH. March D. Ard and Mary B. Bunge. Dept. Anatomy, U. Mississippi Med. Ctr., Jackson, MS, and Dept. Anatomy & Neurobiology, Washington U. Sch. Med., St. Louis, MO.

Short-term (5-10 d) astrocyte cultures consist of flat cells, but in long-term (28-40 d) cultures, astrocytes develop a stellate shape with thin processes and are packed with more glial filaments. Embryonic retinal neurite outgrowth appears to be supported equally well in both types of cultures.

Primary astrocytes from neonatal rat cerebral cortex were subcultured once onto collagen-coated coverslips in medium with 20% serum. Embryonic d 15-16 rat retinal explants were placed on 1 d subcultures. After 4 d, vigorous neuritic outgrowth reached a length of 2.1 mm (average length of longest bundle of neurites from 8 cultures). EM showed the neurites in fascicles within intercellular channels between cell layers or on the upper surface of the astrocytes. Neurites contacted astrocyte surfaces but neither the collagen substratum nor the abundant extracellular matrix (ECM). ECM formed in short-term cultures consisted of an occasional segment of basal lamina-like material, collagen fibrils near the substratum, and accumulations of a filamentous meshwork material.

When explants were added to long-term astrocyte subcultures, neuritic outgrowth was again vigorous, reaching 2.0 mm in 4 d (average of 10 cultures) As in younger cultures, neurites grew within intercellular channels or on top of the astrocyte layers, in contact with astrocyte surfaces but not with the collagen substratum or ECM. ECM in long-term cultures differed from that of short-term cultures in that the intercellular meshwork material had been replaced by more dispersed and widespread wispy material yet to be identified. Basal lamina-like material had formed into occasional ring structures enclosed by cellular processes, reminiscent of astrocytic end feet in CNS. The stellate shape of the astrocytes and their increased production of glial filaments together with the basal lamina-like structures may indicate polarization of the cells with maturity. Supported by NIH grant NS15020 and the National Multiple Sclerosis Society.

349.16

A ROLE FOR TENASCIN IN NEURITIC OUTGROWTH ON ASTROCYTES. **H. M. Geller, J. P. Grierson, R. E. Petroski. Department of Pharmacology, UMDNJ-Robert Wood Johnson Medical School, and The Graduate School, Rutgers University, 675 Hoes Lane, Piscataway, NJ 08854.**

In vivo, astrocytes are thought to play a key role in axonal guidance. *In vitro*, a monolayer of purified protoplasmic (type 1) astrocytes seems to model this function by providing an optimal substrate for the adhesion and process outgrowth of dissociated embryonic rat hypothalamic neurons at low density. We have found that the astrocyte monolayer is often inhomogeneous. Most of the culture consists of regions of very "flat" large astrocytes which support extensive neuritic processes as determined by immunoreactivity with anti-neurofilament antibody. Other regions contain smaller astrocytes which reach confluence at a higher density and have a variegated or "rocky" appearance under differential interference contrast microscopy; these regions display a virtual absence of neurites. We have conducted a detailed immunocytochemical investigation of the potential sources of heterogeneity in the GFAP$^+$ astrocyte monolayer by immunocytochemical techniques using antibodies directed against extracellular matrix and putative cell adhesion molecules. No correlation was found with the expression of NCAM, thrombospondin, laminin, or fibronectin and the presence or absence of neurites. A high correlation exists between the expression of the myotendinous antigen M1 (tenascin, cytotactin, J1) and the flat and rocky astrocytes type: flat astrocytes with many neurites express moderate levels of punctate M1 immunoreactivity whereas regions with a high level of fibrillar M1 immunoreactivity displayed low neuritic invasion and a rocky appearance. These results suggest a that there is coordinate expression of the "rocky" trait and tenascin and that tenascin expression may play a role in directing neural outgrowth. Supported by NIH NS 25168.

349.17

ANTIBODY AGAINST MYELIN-ASSOCIATED INHIBITOR OF NEURITE GROWTH DECREASES FREQUENCY OF NEURITE EXCLUSION AREAS R.D. TODD (SPON: E.H. Rubin). Department of Psychiatry, Washington U. School of Medicine, St. Louis, MO 63110.

Primary cultures of rat frontal cortex contain transiently present, large A2B5+, GFAP-, galC- cells (called CINEAS: cells in neurite exclusion areas) which do not support the movement of neurons or the growth of neurites across their surfaces (R.D. Todd, Soc. Neurosci Abst. 13:257). Caroni and Schwab (Neuron 1:85-96, 1988) have described monoclonal antibodies, IN-1 and In-2, which neutralize the effects of the CNS myelin-associated inhibitor of neurite growth. The effects of these two antibodies on CINEA number are reported here.

High density (8 x 10^5 cells per 35 mm poly-lysine coated plate) cultures of E17.5 rat frontal cortex cells were grown in the presence of serial dilutions of IN-1 or IN-2 hybridoma supernatants. Cultures were scored for CINEA number three days after plating. IN-1 had no effect on CINEA number with dilutions of 1:200 to 1:2. In contrast, IN-2 decreased CINEA number in a dose related manner for dilutions of 1:200 to 1:50 (from 240 CINEAs per 10^6 cells to 100 per 10^6 cells). Increasing IN-2 concentrations 25-fold (1:2 dilution) resulted in no further decrease in CINEA number. These results are compatible with factors related to myelin-associated inhibitor having a developmental role in neurite growth, neuron migration, or both.

REGENERATION: CELL BIOLOGY

350.1

[COLD] FUSION IN HYPOTONIC SALINES OF SEVERED MYELINATED AXONS. T.L. Krause* and G.D. Bittner. Dept. of Zool., Coll. of Pharm., and Inst. for Neurosci., U. Texas, Austin, TX 78712.

Severed nerve axons, myelinated or unmyelinated, typically take weeks to months to regenerate in invertebrates or vertebrates. Several years ago we (Bittner et al., Brain Res. 367: 351-355, 1986) reported that two severed segments of an unmyelinated invertebrate axon, the crayfish medial giant axon (MGA), dissected free of the animal could be morphologically and physiologically reconnected in vitro within minutes using polyethylene glycol (PEG). Morphological fusion was apparently successful in only 3-7% of all trials; physiological reconnection occurred even less frequently.

We now report a much more frequent ability to fuse severed segments of an invertebrate myelinated giant axon, the MGA of the earthworm (*Lumbricus terrestris*). Apparent morphological fusion has been achieved in 80-90% of all in vitro attempts by applying a 50% solution of 4000 MW PEG at 20°C. The PEG solution is introduced through a micropipette for one minute directly upon severed, apposed, MGA segments in a 30% hypotonic saline solution. Axoplasmic and axolemnal continuity have been used as two measures of morphological fusion success. In addition, we have often observed diffusion of Lucifer yellow dye between two axonal segments fused by this technique. However, Lucifer dye does not diffuse across the lesion site if severed segments are apposed, but PEG is not applied.

Unlike certain others, this [cold] fusion [bio] technology does not require palladium electrodes. Like other putative cold fusion technologies, unambiguous proof of complete fusion may be hard to demonstrate. We are presently examining the ability of PEG-fused axons to conduct action potentials across the lesion site and to move HRP and other markers across the lesion site by slow or fast axonal transport. Supported by Texas Advanced Technology Grant #14-2202 to GDB.

350.2

A CONDITIONING LESION ENHANCES THE RATE OF DEVELOPMENT OF MECHANOSENSITIVITY IN AN ACUTELY CUT NERVE. G.M. Koschorke*, R.A. Meyer, and J.N. Campbell. The Johns Hopkins Univ. School of Medicine, Baltimore, MD 21205

Myelinated afferents develop mechanosensitivity at the site of a peripheral nerve ligation within hours of the ligation. We postulate that a mechanical-to-electrical transducer is transported to the nerve ligation site. Since a nerve injury is known to amplify axonal transport, a conditioning lesion should enhance the rate of development of mechanosensitivity. In anesthetized monkeys, the sural nerve was crushed, ligated, and 1 cm of nerve was removed distal to the ligature. Seven days later, the nerve was again crushed, ligated and cut 1 cm proximal to the conditioning lesion. Small strands were dissected from the parent nerve 6 cm proximal to the ligature, and action potential activity from myelinated afferents was recorded. Control experiments were done in acutely ligated nerves without a conditioning lesion. The percentage of myelinated afferents that responded to mechanical stimulation at the site of the acute injury was significantly greater following a conditioning lesion (45% at 10 hours) than in the control experiments (23%, $p \leq 0.001$). These results support the hypothesis that a mechanical-to-electrical transducer is conveyed via axonal transport to the periphery and becomes functional in regenerating membranes. This transducer may account for the mechanosensitivity of myelinated, low-threshold mechanoreceptors.

350.3

LENGTH OF LESION HAS NO EFFECT ON SPEED OF REGENERATION IN THE RAT SCIATIC NERVE. J.J. Campbell* and B. Pomeranz. Dept. of Zoology, University of Toronto, Toronto, Canada, M5S 1A1.

This study was conducted to determine the effect of length of lesion and the corresponding loss of Schwann cells on the rate of regeneration.

Twelve adult male Wistar rats received lesions of the right sciatic nerve near the sciatic notch. Three lesions of different lengths were randomized amongst these animals: the crush lesion was 3 mm long; the short freeze lesion was 9 mm long; and the long freeze lesion was 18 mm long.

On post-operative days 25, 30 and 35, EMG's were recorded using one of two methods. Five animals (2 crush and 3 short freeze) underwent one EMG recording session with the sciatic nerve placed on stimulating hook electrodes, and pin recording electrodes inserted in 12 identified sites in the foot. Seven animals (4 crush and 3 long freeze) underwent a technique of repeated EMG recording sessions, modified from Kerns et al. (*J. Neurosci. Meth.*, 19:259-266, 1987). The sciatic nerve was stimulated transcutaneously through micro-alligator clips, firmly pinching the shaved skin of the rat in the upper thigh over the sciatic nerve, and EMG's were recorded through fine pin electrodes as before. This method permitted repeated EMG's to be recorded over several days, producing a time-course for regeneration.

There was no difference in the rate of regeneration between the three lesion groups, indicating that the destruction of Schwann cells does not affect the rate of regeneration of a lesioned sciatic nerve.

350.4

THE MODULATION OF FAST AXONAL TRANSPORT SPEED DURING NERVE REGENERATION. M.B. Atkinson* and A.C. Breuer. (SPON: A. Tramposch) Neurology and Brain and Vascular Research, Cleveland Clinic Foundation. Cleveland, OH 44195.

Axonal transport plays a critical role in neuronal regeneration after injury. Retrograde (RETRO) transport has been hypothesized as an injury signal mechanism to the cell soma. Synthesized molecules for regeneration are transported from the cell soma by either anterograde (ANTERO) fast axonal transport (FAT) (Eg. plasma membrane components, growth associated proteins) or slow axonal transport (cytoskeletal elements). The rate at which the components are delivered to the growth cone is likely the rate limiting step of regeneration. We report the rate for the FAT system at various time points following transection and anastomosis of male, Sprague-Dawley rat sciatic nerve as determined by video microscopy and computer image enhancement techniques. Time points include 24, 48, 72 hours and 1 week post anastomosis which resulted in ANTERO FAT speed *decreases* of 44.8% ($p<0.005$), 12.1% ($p<0.05$), 25.3% ($p<0.01$), and 20.7% ($p<0.01$) respectively and RETRO FAT speed *decreases* of 9.2% ($p<0.01$), 15.7% ($p<0.001$), 18.4% ($p<0.001$), and 22.1% ($p<0.001$) respectively. We hypothesize that the decrease of FAT speeds in both the ANTERO and RETRO directions may be related to a shift in molecular synthesis towards the production of cellular components needed for regeneration at the expense of other molecular species (including both ANTERO and RETRO translocator motor proteins). The decrease in FAT speeds reflects a reduced delivery rate. Augmenting FAT speeds using Ca^{++} channel agonists (Cell Calcium 9: 293-301, 1988) increases the organelle delivery rate and may enhance axonal regeneration.

350.5

ALTERED MICROTUBULE ASSOCIATED PROTEIN (MAP) PHOSPHORYLATION DURING OPTIC NERVE REGENERATION. Denis Larrivee, Dept. Physiology, Cornell Univ. Med. Coll., New York, NY 10021.

Many axonal phosphoproteins in the goldfish optic nerve show changes in phosphorylation related to the progress of regeneration (Larrivee & Grafstein, J. Neurosci. 9:574, 1989). One of these phosphoproteins, designated P9, undergoes a prominent pI shift that is first apparent 3 weeks after the nerve is injured and persists to at least 7 weeks. Because P9 is similar in molecular weight and pI to several low molecular weight MAPs, its association with microtubules was explored during regeneration. The protein a)could be precipitated with taxol, b) was immunoreactive with a chartin antibody (courtesy of Dr. F. Solomon), and c) copurified with tubulin on gel permeation columns. Thus, P9 is likely to be associated with microtubules and to contain epitopes shared with chartins. In regenerating nerves, ^{32}P labeling of P9 was highest in a tubulin fraction that was soluble in low-salt buffer: by contrast, its labeling in normal nerves was highest in the non-soluble tubulin compartment. Even extraction with high-salt buffer (0.3M NaCl) did not separate P9 from tubulin in normal and regenerating nerves. Intraocular injection of ^{3}H-proline during regeneration preferentially labeled P9 associated with non-soluble tubulin. Thus, changes in phosphorylation of P9 during regeneration may be involved in releasing tubulin into a soluble axonal pool.

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350.6

TRANSPORT OF SCb TUBULIN INTO AXON SPROUTS IS INCREASED BY A CONDITIONING LESION. I.G. McQuarrie and J.M. Jacob. Neural Regeneration Ctr., VA Med. Ctr., Cleve., OH 44106.

When axons are injured, the nerve cell body response includes an increase in tubulin synthesis associated with an increase in the amount of tubulin being carried by SCb, the faster subcomponent of slow axonal transport. When a conditioning lesion initiates these events, a second lesion can test the effect of supplemental SCb tubulin on the growth of newly-formed axons (Jacob & McQuarrie, J. Cell Biol. 107:454a, 1988). Rats received either a conditioning crush or sham-crush of distal sciatic nerve branches, followed after one week by injection of 35-S methionine into the lumbar spinal cord. One week later, a second crush was made immediately ahead of the labeled SCb wave. Nerves were harvested 4-12 days later for SDS-PAGE of consecutive 3 mm segments, fluorography, gel punching, and liquid scintillation counting. The SCb rate was normal (3.3 mm/d) in both conditioned and sham-conditioned axons, but tubulin labeling was 50-100% greater in conditioned axons. Actin, calmodulin, and MAPs participated in this increase. The outgrowth rate was 4.8 mm/d in conditioned axons vs. 4.3 mm/d in sham-conditioned axons. These findings support the idea that axonal outgrowth depends on the availability of tubulin, actin, and other cytomatrix proteins (McQuarrie & Lasek, J. Neurosci. 9:436, 1989).

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350.7

AXOTOMY INCREASES AXONAL TUBULIN TRANSPORT AT THE FRONT OF SCb. J.M. Jacob and I.G. McQuarrie. Neural Regeneration Center, VA Medical Center, Cleveland, OH 44106.

When peripheral nerve motor axons are interrupted in mammals, the nerve cell body response includes an increase in tubulin synthesis. This produces an increase in the amounts of 35-S methionine-labeled tubulin being carried by SCb, the faster subcomponent of slow axonal transport (Hoffman and Lasek, Brain Res. 202:317-333, 1980). We measured the rate and amount of transport of selected SCb proteins: tubulins, microtubule-associated proteins (MAPs) at 58-67 kD, actin, and calmodulin. Rats received either a crush or sham-operation of distal sciatic nerve branches, followed one week later by injection of 35-S methionine into the lumbar spinal cord. Nerves were harvested 1, 2, or 3 weeks later for SDS-PAGE of consecutive 3 mm segments, fluorography, gel punching to remove proteins, and liquid scintillation counting. There was no change in the rate of the SCb peak: 3.5 mm/d after crush vs. 3.4 mm/d after sham-operation. A 150% increase in SCb tubulin labeling was confined to faster-moving elements of the crushed axons, and formed a new peak which advanced at 5-6 mm/d. Actin, calmodulin, and MAPs participated in this localized increase. These findings define the increase in SCb transport that has been shown to accelerate axonal outgrowth at the site of a second nerve injury (Jacob and McQuarrie, J. Cell Biol. 107:454a, 1988).

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350.8

CYTOSKELETAL CHANGES DURING AXONAL GROWTH AND REGENERATION IN CULTURED SENSORY NEURONS. M.R. Gilbert*, B.L. Harding*, P.N. Hoffman, J.W. Griffin. Johns Hopkins Univ. Sch of Med., Baltimore, MD 21205

Previous studies have shown that axonal growth during development and regeneration is associated with changes in the levels of cytoskeletal mRNAs (Hoffman, PN, J Neurosci 9:893, 1989). In neonatal animals, levels of class II β tubulin and GAP-43 mRNAs are elevated, whereas levels of NF-L are low. Later in development, tubulin and GAP-43 mRNA levels decrease while NF-L mRNA levels increase. Axotomy of peripheral sensory fibers in mature rats results in a recapitulation of the developmental pattern of gene expression. We compared these in vivo properties with cultured neurons, utilizing DRG explant cultures grown in N2 defined media with NGF. For the study of growth-associated changes, mRNA levels for class II β tubulin, GAP-43, and NF-L were measured in ganglia at the time of dissection and at 2, 4, 6, and 8 weeks in vitro. There was a progressive decrease in tubulin and GAP-43 mRNA levels over the 8 weeks. In contrast to the in vivo system, NF-L mRNA levels in cultures did not increase with age. Axotomy of cultured DRG neurites, performed at 4 weeks in vitro, caused a prominent induction of tubulin mRNA, but little change in NF-L mRNA levels. These results indicate that many of the changes in cytoskeletal mRNA levels during growth and regeneration are similar in both the in vivo and in vitro models.

350.9

ASSOCIATION OF AXOTOMY - INDUCED CHANGES IN RAT DORSAL ROOT GANGLION NEURONS WITH THE PROGRESS OF AXONAL REGENERATION. M. R. Wells and U. Vaidya. Dept. of Neuroscience, New York College of Osteopathic Medicine, Old Westbury, N. Y. 11568 and Dept. of Neurology SUNY Stony Brook, N.Y. 11794-8121

A unilateral crush lesion of the sciatic nerve in rats produces a biphasic response in some metabolic and morphological parameters in the L5 and L6 dorsal root ganglia (DRG). The present study examined the possible association of the progress of axonal regeneration with these alterations. After unilateral crush lesions of the sciatic nerve at the level of the sciatic notch, the progress of the regeneration of axons in the nerve from the L5 ganglion was examined by axoplasmic transport of [^{3}H] labelled peptides 1-7 days after injury. The data demonstrate that a significant proportion of axons from the L5 ganglia enter the distal stump between 1-2 days after injury. The progress of regenerating axons was linear at approximately 2 mm/day thereafter. The timing of the regeneration of axons suggests that some components of the first of the two major phases of the L5 DRG neuronal reaction to axotomy may be associated with the entry of axons into the distal stump. The second phase may be associated with early contact of axons with hip muscles. Supported in part by the Veterans Administration and NYCOM.

350.10

TARGET AND GROWTH REGULATION OF PROTEINS SENSITIVE TO AXOTOMY. A. Buriani, M.J. Savage, D.W. Burmeister and D.J. Goldberg. Dept. of Pharmacology and Ctr. for Neurobiology & Behavior, Columbia U. Coll. of P&S, 630 W. 168th St, New York, NY 10032.

Among the changes a neuron undergoes after axotomy, variations in the synthesis or metabolism of specific proteins may play an important role in the regeneration of the axonal tree. Using SDS-PAGE, we identified 10 proteins whose incorporation of ^{35}S-methionine consistently changed 1 week after axotomy of the giant cerebral neuron of *Aplysia in vivo*. Axotomized GCNs were placed in culture, where they display vigorous axonal growth, to investigate the regulation of these changes. The labeling of one of the proteins that up-regulate (112 kDa; pI 5.6) was greatly reduced if all growth was blocked by either of 2 methods. The incorporation of label into two other proteins that up-regulate (116 kDa; pI 5.4 and 46 kDa; pI 6.3) was greatly decreased when cells with which GCN makes chemical synapses were included in the cultures, even though growth continues unabated. For the 116 kDa protein, target membranes mimicked the effect of the intact target, but medium conditioned by exposure to targets did not. These results indicate that membrane-membrane contact with target cells (or its loss) and growth (or its cessation) are involved in the initiation or termination of the increased output of certain proteins from the cell body after axotomy.

350.11

CHARACTERIZATION AND IMMUNOLOCALIZATION OF SOLUBLE EXTRACELLULAR GLYCOPROTEINS IN GOLDFISH BRAIN. Finnbogi Thormodsson, Stephen F. Lakos and Bernice Grafstein. Physiology Dept., Cornell U. Med. Coll., New York, NY 10021.

The goldfish brain contains a prominent group of soluble acidic glycoproteins consisting of 2 main subgroups, with MWs about 33K and 38K respectively. The proteins, which can bind to heparin, are largely extra- cellular. We have designated them "eXoGlycoProteins" (XGPs). Expression of XGPs is increased during optic nerve regeneration. Edman degradation sequencing of spots from 2-dimensional gels (performed by Dr. J. Labdon) showed that the first 15 N-terminal amino acids in the most abundant isoform of the 33K sub-group were identical with the sequence deduced from a cDNA clone of "ependymins" (Königstorfer et al., J. Neurochem 52: 310, 1989). The most abundant isoform of the 38K sub-group showed less homology. Antiserum to XGPs produced prominent immunostaining of the pial cells and the outer walls of capillaries, as well as a population of large granular cells in the thick reticular zone underlying the optic tectum, in the vicinity of the third ventricle, and occasionally under the pia. Immunopositive cells in the blood included lymphocytes, monocytes and macrophages. The XGPs therefore appear to be associated with a diverse population of cells of mesodermal origin in the brain and blood, at least some of which belong to the reticuloendothelial system. **[Supported by NIH Research Grant NS-09015 and Training Grant NS-07138.]**

350.12

CHANGES IN β-PREPROTACHYKININ (β-PPT) mRNA AND TACHYKININS IN RAT DORSAL ROOT GANGLIA (DRG) FOLLOWING PERIPHERAL OR CENTRAL AXOTOMY. D.B. Henken[1], A. Tessler[1,2], M.-F. Chesselet[1], and M. Murray[1]. [1]Anatomy Dept., Medical College of PA, and [2]VA Medical Center, Phila, PA.

In normal rat DRG, 15-20% of the total neuronal population contains tachykinins and mRNA for the tachykinins precursor, β-PPT. In order to determine whether peptide and message content change differentially after axotomy, we examined the numbers of cells labelled for tachykinins immunocytochemically or for β-PPT mRNA using in situ hybridization histochemistry following either sciatic nerve or dorsal root section. Neurons labelled for β-PPT mRNA or for tachykinins and unlabelled neurons were mapped, measured and compared in sections from operated and intact sides. One day following sciatic nerve section, no changes were detectable for either the message or the peptide. At 3 days the numbers of neurons labelled for the message had decreased to 4%, but the proportion of cells labelled for the peptide did not change. Two weeks following sciatic nerve axotomy 3.5% of the total neuronal population labelled for β-PPT mRNA while 8% stained for tachykinins. By 6 months following peripheral axotomy, the proportion of cells labelled for both the message and the peptide returned to control levels of 15%. After dorsal root section, at all post-operative intervals, both message and peptide remained at control levels of 15-20%. These results suggest that the DRG soma reacts differently to axotomy of its peripheral or central process at the level of gene expression. Supported by grants from NSF-BNS8616841, NIH-NS24707, USAMRDC-51930002 & VA Med Res Ser.

350.13

IN SITU HYBRIDIZATION DETECTION OF EARLY ALTERATIONS IN RIBOSOMAL RNA (rRNA) LEVELS FOLLOWING AXOTOMY OF HAMSTER FACIAL MOTONEURONS. N. B. Kinderman* and K. J. Jones. (SPON: M. Oblinger) The Chicago Medical School, North Chicago, IL 60064.

Adult hamster facial motoneurons (HFMN) represent a population of motoneurons that, when axotomized, undergo a reactive sequence geared toward survival and regeneration. One of the most robust changes demonstrated both morphologically and autoradiographically to occur after axotomy of HFMN involves alterations in nucleolar functioning at 2 days postoperatively (PO). In this study, we extended our investigation of the nucleolar response through the use of quantitative *in situ* hybridization and rDNA probes to detect how early after axotomy alterations in nucleolar functioning occur. Adult hamsters (100 days) were anesthetized by intraperitoneal injection of Nembutal and subjected to axotomy of the right facial nerve, with the left side serving as control. PO survival times were 6, 18, and 24 h, and 2 and 4 d, with each experimental group containing 3 animals. Fresh, frozen brainstem sections containing the facial nuclear groups were collected and postfixed. A nick-translated rDNA probe complementary to 28S rRNA was prepared and, following prehybridization, hybridized to the sections overnight at Rm T. The sections were subsequently washed, dried and processed for autoradiography. Quantitative analysis was accomplished using a computerized image analysis system to measure number of grains per cell, somal size, and grain density. At 6h, there was an axotomy-induced 10% increase in rRNA levels, relative to the controls. The peak increase (48%) in rRNA levels occured at 24 h, with a decline in the increase present at 2 and 4 d PO (35% and 31%, respectively), relative to the controls. Somal area was found to be initially increased (19%) at 18 h PO, with a peak at 24 h PO (28%), and a return to control values by 4 d PO. No changes in the concentration of rRNA were found at any time PO. Thus, the axotomy-induced changes in rRNA levels, as detected with quantitative *in situ* hybridization, 1) occur significantly earlier than previously thought using different methods and 2) indicate that initiation of the neuronal injury response occurs within hours PO. Supported by a SCRF grant (KJJ).

351.1

METABOLIC CHANGES IN MUSCLE FIBERS REGENERATING IN THE PRESENCE AND ABSENCE OF INNERVATION. S. Sesodia*, R. Choksi* and P.M. Nemeth. Dept. of Neurology, and of Anatomy & Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

We examined the influence of innervation on the energy-acquiring metabolism of muscle fibers in regenerating soleus muscles. In one group of Wistar rats, necrosis of the left soleus muscle was induced by a subdermal injection of Australian tiger snake venom (10μg in 100μl 0.9% NaCl) into the dorsolateral aspect of the lower hindlimb. In a second group, muscles were denervated immediately prior to venom injection by removing a 1 cm segment of sciatic nerve near the sciatic notch. Soleus muscles from both hindlimbs were removed at selected times after the venom injection and processed for histochemical and quantitative microchemical analyses. Fibers identified by myosin ATPase as type 1 in one section were located in, and dissected from, an adjacent lyophilized section. Individual dissected fibers were analysed by microanalytical biochemical techniques for enzymes representative of anaerobic (lactate dehydrogenase, LDH), aerobic (malate dehydrogenase, MDH) and high energy phosphate (adenylokinase, AK) metabolism. In innervated regenerating muscles, LDH was comparable to control levels by 3 days while the other enzymes returned to control levels more slowly. In denervated regenerating muscles, however, LDH returned to control levels with a similar time course as in the innervated regenerating muscles but MDH attained values only 30% of control while AK was 84% of control.

We conclude from these results that innervation is necessary for the restoration of aerobic metabolism in regenerating muscles while anaerobic metabolism seems to be independent of innervation. The results also imply that in the absence of contractile demands, the metabolic needs of denervated muscle fibers can be satisfied by anaerobic metabolism.

351.2

THE HYPER-REINNERVATION OF RAT SKELETAL MUSCLE. T.A. Kuiken*, W.Z. Rymer and D.S. Childress*. Depts. of Biomedical Engineering and Physiology, Northwestern University, Chicago, Illinois, 60611.

Rat MG muscles were hyper-reinnervated by: 1) cutting the MG nerve and implanting it onto the MG muscle together with additional hind limb nerves, thereby increasing the total number of regenerating motoneurons up to 11 fold (26 animals) or; 2) crushing the MG nerve and excising the medial portion (50-70%) of the muscle, thereby decreasing the number of muscle fibers (6 animals).

Muscles hyper-reinnervated with multiple nerves recovered more fully than did the self-reinnervated control muscles. Up to three times more motor units formed in the hyper-reinnervated muscles than were found in normal MG. The mean motor unit size was significantly smaller than in normal or control muscles. Moreover, the range of motor unit size was greatly increased; both normal sized and very small units were found in hyper-reinnervated muscles. Some of these motor units were extremely small, producing less than 200 mg. of tetanic force, which was a full order of magnitude smaller than units in normal muscle. Both fast and slow motor units were present in this population of small motor units. About 25% of the muscle fibers became polyneuronally innervated.

In the muscle reduction experiments, the number of motor units formed was not significantly larger than expected considering the mass of the muscles. However, the distribution of motor unit sizes in some of the experiments appeared to be bimodal, possibly implying that some motoneurons had an advantage in the reinnervation of these muscles. Cross-sections of the muscles were stained for ATPase activity after acid pre-incubation to determine myofiber type. The percentage of slow fibers was reduced by over 50% in these experimental muscles suggesting that fast motoneurons may have an advantage in competitive reinnervation.

351.3

HIGHLY SELECTIVE REINNERVATION OF XENOPUS PECTORALIS MUSCLE FOLLOWING NERVE CRUSH. Y. HARADA* and A.D. GRINNELL. Jerry Lewis Res. Ctr., UCLA Sch. of Med., Los Angeles, CA 90024.

In the pectoralis muscle of Xenopus, 95% of muscle fibers have two distinct endplates, and 80% of these are innervated by different branches of the same axon (mono-neuronal double innervation:MDI). This MDI can be thought to be the result of selective elimination and retention of synapses. To study this hypothesis, reinnervation during several months following nerve crush was investigated in adult Xenopus. Using formamide (up to 4M) to block contraction and reduced K^+ Ringer solution to block action potentials, even small EPP components could be recorded. MDI was judged from simultaneous occurance or failure of EPPs at both junctions at threshold stimulus strength. Reinnervation was mostly complete within 20 days after crush. Initially, one of the two endplates was occupied. Subsequently, the other became occupied selectively by the same axon, without polyneuronal innervation of one junction, or innervation of the two endplates by different axons, at more than control levels. But a foreign motor nerve appeared to innervate endplates randomly. These results indicate highly selective reinnervation from the beginning following nerve crush. Supported by a grant from the NSF.

351.4

ALL PERONEAL MOTONEURONS OF THE RAT SURVIVE CRUSH INJURY BUT SOME FAIL TO REINNERVATE THEIR ORIGINAL TARGETS. John E. Swett, Chang-Zern Hong*, Peter G. Miller*. Anat. & Neurobiol. & Phys. Med. & Rehab., Univ. Calif., Irvine, CA 92717

This is a quantitative study of the motoneurons of the rat's common peroneal nerve following severe unilateral, 2 mm-long crush injury of the sciatic nerve. Recovery from injury was allowed for 14 to 188 days and was measured behaviorally and electrophysiologically. The motoneurons were retrogradely labeled on both sides, the uninjured side serving as a control. On the injured side the nerves were labeled either distally or proximally to the crush site with HRP. Spinal segments L2 to L6 were cut in serial, frozen cross-sections. HRP was reacted using TMB as the chromogen. The normal peroneal nerve contains 634 ±26 motoneurons (22 cases). The number labeled distally from the crush site (22 cases) was 535 ±69 or 84.4% of normal. In cases where the nerve was labeled 5 mm proximally to the injury normal population numbers (648 ±30) were found. The unlabeled 15.6% had not vanished as a result of cell death. It was composed largely of small motoneurons whose axons may not have regenerated distally beyond the crushed zone. Mean soma size of injured neurons increased to maximum 3-6 weeks after injury and then gradually decreased in size over the following weeks to nearly normal values. This transient increase in size was due to two factors: 1) soma swelling in response to axonal injury, and 2) absence of many small motoneurons, presumably γ-motoneurons, that probably failed to regenerate beyond the injury zone long after larger motoneuron axons had reinnervated their targets. As all motoneurons survived, it is conceivable that they could all theoretically reinnervate their original targets under certain conditions providing that proper pathguides are available. (Supported by NIH Grant NS23707-03).

351.5

EXTENT AND PRECISION OF REINNERVATION OF MUSCLE SPINDLES IN THE CAT'S TENUISSIMUS MUSCLE. M.DeSantis. Dept. of Biol. Sci. & WAMI Prog., Univ. of Idaho, Moscow, ID 83843.

Cross-sections (X-sect.) through muscle spindles (ms.sp.) were studied microscopically. The area enclosed by the outer capsule of the spindle,the intracapsular area (ICA), served as the measure of the position at which a nerve terminal occurred along the length of the spindle.

In anesthetized cats the nerve to the muscle was destroyed unilaterally by freezing. A total of 75 X-sect. through ms.sp. were examined for regenerated terminals between 22 and 93 days after denervation. As controls the side contralateral to the denervation was sham-operated, and spindles from unoperated cats were also studied. A total of 118 X-sect. through control spindles were observed; 42% of them had nerve terminals present. The 29 X-sect. with sensory endings were located in regions of the spindle that had ICAs which generally exceeded those where the 18 with motor endings were present (Table 1). Fewer, 35%, of the X-sect. through ms.sp. that were allowed to be reinnervated had nerve endings. This difference between control and reinnervated spindles was due chiefly to fewer motor endings being present after axonal regeneration; the percentages with sensory endings were similar (Table 1). Most of the 21 X-sect. through ms.sp. with sensory endings had larger ICAs than the 8 profiles in which motor endings occurred (Table 1).

The data are consistent with the following two conclusions. Terminals of regenerated afferent and efferent axons redeploy in a way which resembles the innervation of intact ms.sp., and complete repopulation of the spindle by motor endings lags that of sensory endings. (Supported by USPHS grants NS-11026 & RR-05360).

Table 1

Type of	Control		Reinnervated	
Ending	%	ICA range (um^2)	%	ICA range(um^2)
sensory	24.6	3800 to 23000	24.0	2000 to 24000
motor	17.8	1100 to 4400	10.7	580 to 2200

351.6

DISTRIBUTION OF FIBER TYPES IN LONG-TERM SELF-REINNERVATED CAT MUSCLE. P.A. Nemeth*, T.C. Cope and P.M. Nemeth Dept. Physiology, Hahnemann Univ., Philadelphia PA 19102 and Dept. Neurology, Washington Univ., St. Louis, MO 63110 (PMN).

Histochemical studies of reinnervated muscle show clustering of like-type fibers distinct from the usual mosaic pattern. Our aim was to characterize the extent of type-grouping quantitatively.

Myosin ATPase-stained cross-sections from medial gastrocnemius muscles self-reinnervated for >2 years after complete nerve section were compared with those from the contralateral control muscle in 4 adult cats. The spatial relations between 3 muscle fiber types (I, IIa, IIb) were tested for randomness using the method of Venema (*Muscle and Nerve*, 11:301,1988). For 14 regions from reinnervated muscles (≥3 per muscle), 10 were similar to normal, i.e. differences between expected and observed numbers of adjacent fibers of like type fell within the normal range.

The fact that type-grouping was not prevalent in the majority of samples measured so far suggests that both recovery time and the source of reinnervation may be important for re-establishing the spatial distribution of fiber types. Restoration of a mosaic pattern raises the possibility of reorganization of synaptic connections made by regenerating motoneurons. (Supported by NS21023 (TCC) and DK38375 (PMN)).

351.7

ANTENNAPEDIA REGENERATES IN THE STICK INSECT, *Carausius morosus*: SENSORY PROJECTIONS II. W. P. Chan* and J. S. Edwards (SPON: R. D. Longley). Department of Zoology, University of Washington, Seattle, WA 98195.

Receptors on the antennapedia regenerate (AntpR) in the stick insect, *Carausius morosus* project to the deutocerebrum as with the normal antenna (Edwards et al., *Soc. Neurosci. Abstr.*, 12:1574, 1986). Nearly 40 % of the receptors on the flagellum of a normal adult antenna are chemosensory. On the more distal segments, ca. 50 % of these receptors are multiporous (olfactory). No such sensilla are found on the more proximal segments.

On the AntpR, ca. 20 % of the receptors are chemosensory but multiporous sensilla are absent. The anterior portion of the deutocerebrum in the AntpR is reduced (Edwards et al., *J. Neurobiol.*, In press, 1989). Single-sensillum cobalt-fills showed that chemoreceptors on the normal antenna always project to the anterior portion of the deutocerebrum. This condition is similar to the Lozenge^3 mutant in *Drosophila melanogaster* (Stocker, R. F. and Gendre, N., *Dev. Biol.*, 127:12, 1988). An inductive interaction of sensory neurons on deutocerebral target cells also operates during regeneration. Supported by NIH grant NS-07778 to JSE.

351.8

EFFECT OF MIDFIELD CUTS ON INTACT AND REGENERATING SYNAPSES IN THE CRAYFISH. J. S. Rhee* and S. J. Velez. Department of Biological Sciences, Dartmouth College, Hanover, New Hampshire 03755.

The superficial flexor muscle system of the crayfish Procambarus clarkii has been used to study how neurons reconnect with their appropriate target cells under a variety of experimental conditions (J.Neurobiol. 19: 127-152). We had reported that when the nerve was cut in the middle of the muscle field, and the lateral muscle fibers were removed, leaving the medial fibers with the normal complement of synapses, the medial synapses were repressed (no jp's could be recorded at physiological temperatures) as the axon without a target area (axon 4) grew over the medial muscle fibers (Soc. Neurosci. Abstr. 12: 1575). In the present work, similar surgery was done but this time the lateral fibers were left in place, providing a target area for the growth of axon 4. Now we do not observe the physiological repression observed earlier as jp's are detected for all axons at physiological temperatures over the medial fibers. Axon 4 spreads some contacts over the medial field but maximum growth occurs over the lateral fibers. By eight weeks all axons regenerate their normal connectivity patterns. These results suggest that intact synaptic contacts are not disturbed by partial axotomy if the nerve is able to grow over a denervated target area; when this is not possible, hyperinnervation of the remaining target field results in the repression of established synaptic contacts.

351.9

SUBSTRATE BOUND NERVE GROWTH FACTOR (NGF) ENHANCES AND DIRECTS THE REGENERATION OF ADULT RAT DORSAL ROOT AXONS. J.D. Houle and J.E. Johnson. Dept. of Anatomy, Univ. of Arkansas Med. Ctr., Little Rock, AR 72205

Severed adult rat dorsal roots were apposed to an intraspinal transplant of fetal spinal cord (FSC) tissue co-grafted with nerve growth factor (NGF) treated or untreated nitrocellulose strips to determine if substrate bound NGF could stimulate and guide the regrowth of centrally directed sensory axons. Axonal regrowth from the injured roots was assessed 1-3 months post transplantation by calcitonin gene-related peptide immunoreactivity (CGRP-IR).

In animals with untreated nitrocellulose implants individual CGRP-IR axons were observed in dorsal regions of the FSC tissue, primarily away from the nitrocellulose. In contrast, dense fascicles of regenerated CGRP-IR axons lined the entire length and depth of NGF treated nitrocellulose, with many axons extending beyond the graft-host interface ventrally into the adjacent host neuropil. Regeneration of CGRP-IR axons from severed roots was suggested by the retrograde labeling of dorsal root ganglion neurons with fluorescent dye injected into FSC tissue transplants. These results indicate that NGF can have a specific neurotrophic effect on the regeneration of sensory axons within the CNS, i.e. by promoting the extent of axonal regrowth and by directing regrowing fibers towards undamaged regions of the spinal cord. Supported by SCRF/PVA and NIH grants NS26380 and EY07584.

351.10

TROPHIC REGULATION OF GABA RECEPTOR EXPRESSION IN AXOTOMIZED DRG CELLS. R.B. Bhisitkul*, J.D. Kocsis and S.G. Waxman. Dept. of Neurology, Yale Sch. Med. and VA Med. Ctr., West Haven, CT. 06516. The effect of active regeneration on GABA receptor expression of axotomized rat sensory neurons was studied using current clamp and single electrode voltage clamp recording. Axotomy was produced without allowing normal axonal regeneration by transection and tight ligation of the sciatic nerve. 12-19 days post-transection, the GABA-mediated responses of excised L4 and L5 DRG cell bodies were attenuated; the mean depolarization was reduced to 7 mV from 16 mV in controls, and the mean GABA-induced current was 0.4 nA compared to 1.0 nA in controls. Decrements in GABA sensitivity produced by axotomy could be partially prevented by active regeneration of injured axons through the denervated distal nerve segment. Sciatic nerves were transected and ligated with the addition of a discrete nerve crush 3.0 cm proximal to the ligation site, allowing a delimited segment of regeneration without peripheral target reconnection. Under these conditions, the GABA-induced depolarization was considerably improved compared to transection alone. These results suggest that there is a trophic influence within the distal nerve segment which plays a role in maintaining somatic GABA receptors during axonal injury, consistent with the findings that Schwann cell-derived nerve growth factor exerts neurotrophic regulation in sensory neurons during regeneration.

351.11

DEVELOPMENT AND LESION INDUCED CHANGES IN FLUORIDE RESISTANT ACID PHOSPHATASE AND BINDING OF THE LECTIN BANDIRAEA SIMPLICIFOLIA-I IN THE RAT'S TRIGEMINAL BRAINSTEM COMPLEX. F.A. White, N.L. Chiaia and R.W. Rhoades. (SPON: J.G. Foy). Dept. of Anatomy, Medical College of Ohio, Toledo, OH 43699.

Binding of the lectin Bandiraea simplicifolia-I (BS-I) and expression of the enzyme fluoride resistant acid phosphatase (FRAP) identify the same population of dorsal root and trigeminal (V) ganglion cells (Silverman, J.D. and Kruger, L., Somatosensory Res., 5:259, 1988) and these primary afferents appear to be mutually exclusive from those that contain tachykinin immunoreactivity. We have previously shown that adult transection of the infraorbital nerve (ION) leads to a transient decrease in tachykinin immunoreactivity in the medullary dorsal horn (MDH) while neonatal transection of this V branch results in an expansion of the band of tachykinin immunoreactivity in this portion of the brainstem. In the present study, FRAP histochemistry and BS-I binding were used to assess the effects of neonatal and adult ION transection upon the innervation of the MDH by this population of sensory neurons. Transection of the ION in adulthood resulted in transient reduction in BS-I binding that was apparent by 7 days post-lesion, maximal at 30 days post-lesion, and nearly recovered by 60 days after the nerve transection. Neonatal ION transection resulted in a permanent loss of BS-I binding in the ipsilateral MDH. Because we were unable to consistently demonstrate BS-I binding in the brains of very young rats, we used FRAP histochemistry to evaluate the short-term effects of neonatal ION transection. These experiments showed that ION transection had no significant effect upon the density of FRAP staining in the MDH at 6 hrs after neonatal ION transection and that a clear reduction in staining density occurred within 16 hrs of the nerve transection.

Supported by BNS 85 17537, DE 07734, and funds from the State of Ohio Research Challenge.

351.12

ACCURACY OF REGENERATION OF VIBRISSA-RELATED PRIMARY AFFERENT NEURONS AFTER NEONATAL TRANSECTION OF THE RAT'S INFRAORBITAL NERVE. P.S. McCann*, N.L. Chiaia and R.W. Rhoades (SPON: R. Waziri). Dept. of Anatomy, Medical College of Ohio, Toledo OH 43699.

A sequential double labelling paradigm was used to determine the accuracy with which vibrissa-related trigeminal primary afferent neurons reinnervated the correct follicle after infraorbital nerve (ION) transection on the day of birth. Pups were anesthetized by hypothermia and true blue (TB) was injected into the A1 vibrissa follicle bilaterally within 6 hrs of birth. After an additional 6-8 hrs, the ION on the left side was transected. When these rats reached at least 6 wks of age, the A1 vibrissa follicle on both sides was injected with diamidino yellow (DY). Three days later, animals were anesthetized with ether, perfused with formalin, and tissue was processed for fluorescence microscopy. In normal ganglia (N=10), 80% (sd.=8.1) of the TB labelled cells also contained DY. In ganglia ipsilateral to damaged nerves (N=13), 17% (sd.=7.7) of the TB labelled cells also contained DY. This value was significantly lower than that for the normal ganglia, but significantly greater than that which would be expected by chance. Analysis of the areal distributions of labelled cells also indicated that reinnervation of the A1 vibrissa follicle was not random. In mid-horizontal sections through normal ganglia 92% (sd.=6.9) of cells labelled with DY only were located within the mediolateral distribution of the TB labelled neurons. In ganglia ipsilateral to damaged nerves, this value was 68% (sd.=7.8). Thus, the majority of the "foreign" innervation of the A1 follicle after neonatal ION transection came from neurons in the portion of the ganglion that would normally supply it. All of these data indicate that reinnervation of vibrissa follicles after neonatal ION transection, while not completely accurate, is also not random.

Supported by BNS 85 17537, DE 07734, and funds from the State of Ohio Research Challenge.

351.13

NGF PROMOTES NEURITE REGENERATION FROM NERVE CUT ENDINGS OF ADULT DORSAL ROOT GANGLIA CULTURES IN A COLLAGEN GEL.
HORIE,H.,BANDO,Y.*,TSU,H.*, ITO,S.*, FUKUDA,N.* AND TAKENAKA,T. Dept. of Physiol., Yokohama City University, School of Medicine, Yokohama 236, Japan

Dorsal root ganglia with nerve fibers dissected from 3-month-old mice were embedded in a collagen gel.The ganglia were cultured in serum free defined media. Regenerating neurites appeared from nerve cut endings 12 h after in culture.This result shows that neurons could start to regenerate neurites from the ending more quickly than dissociated ones. When ganglia were cultured in a NGF-free medium, average lengths of regenerating neurites were 133±55 um from the short distance nerve cut endings ($d \leq 1$ mm) apart from a ganglion and 106±24 um from the long distance endings ($d > 1$ mm) 24 h after in culture.When the ganglia were cultured in the NGF containig medium (100 ng/ml), the lengths were 174±58 um in the short and 162±98 um in the long 24 h after in culture. The effect of NGF to promote the regeneration were clealy shown from these results.At 96 h after cultured in a NGF containing medium, the average length of neurites from the long distance endings was 575±300 um and 364±176 um from the short.It is said from these results that NGF promotes regeneration more strongly from longer distance nerve endings than short ones.

351.14

PREVENTION OF PRIMARY SENSORY NEURON LOSS FOLLOWING PERIPHERAL NERVE TRANSECTION DOES NOT DEPEND ON AXONAL REGENERATION.
S. Melville, T. E. Sherburn, and R. E. Coggeshall. The Marine Biomedical Institute, Dept. of Anatomy and Neurosciences and Dept. of Phys. and Biophysics, The University of Texas Medical Branch, Galveston, Texas, 77550.

Transection of a major peripheral nerve usually causes the loss of a significant number of sensory cells. The present study shows that placing stumps of a transected nerve in an impermeable tube prevents this loss. We now show that this preservation does not depend on axonal regeneration. In one group of rats, the sciatic nerve was sectioned and left in situ. In another group the stumps, were placed 8 mm apart in an impermeable tube. In a second group the stumps were separated by a 15 mm gap. Thirty days after surgery DRG cells were counted bilaterally. Results show a 27-28% loss of DRG cells after simple sciatic nerve transection, with no loss of cells in the tubed animals. There was a difference in the tubed animals, however, in that regeneration took place with the 8 mm gap but not with the 15 mm gap. We conclude that placing transected rat sciatic nerve stumps in an impermeable tube protects those cells that normally would be lost and this is not dependent upon regeneration of axons. These findings may have clinical significance. Supported by grants NS11255, NS 10161, and NS 07185.

351.15

CYTOLOGY OF REGENERATED SENSORY TERMINALS IN ALLOGRAFTED AND AUTOGRAFTED PRIMATE SKIN.
D. D. Samulack, B. L. Munger*, and R. W. Dykes. Dept. of Anatomy, Penn. State Univ., Hershey PA 17033, Dept. of Physiology, McGill Univ., Montreal, Canada H3G 1Y6

The present study was designed to evaluate the regeneration of cutaneous sensory axons into allografted skin in immunosuppressed (cyclosporin and methylprednisolone) baboons. Autografted skin served as a surgical control. Single axons serving grafted skin were identified electrophysiologically at the forearm level and characterized on the basis of receptive fields and adaptive properties at 5-8 mo after grafting. At necropsy skin was prepared for light and electron microscopy. Regenerated axons were smaller in diameter and had a slower conduction velocity. Both rapidly and slowly adapting responses were identified. Meissner corpuscles only infrequently regenerated in all grafted skin and were often abnormally innervated. Normal sensory innervation of hairs was also never observed in all grafted skin. In the case of severe allograft rejection, no papillary ridges or hair follicles were present and few cutaneous nerves could be identified. To date, Merkel cells have not been identified in any allo-grafted specimen. The apparent lack of cytological specialization of sensory terminals in the face of identifiable adaptive properties implicates the axon is the site of mechanoelectric transduction in cutaneous mechanoreceptive afferents.

351.16

A COMPARISON OF CRYOPROBE AND CRUSH LESIONS IN THE RAT SCIATIC NERVE.
J.M. Kerns, B. Braverman*, A.D. Ivankovich*, A. Mathew* and C. Lucchinetti*, Depts. of Anatomy and Anesth., Rush Med. Coll., Chicago, IL 60612.

Cryoanalgesia has been used for pain treatment because the neural damage is limited and reversible. This experimental study examines the sensory, motor, behavioral and structural recovery following two types of nerve lesions. The sciatic nerve in anesthetized rats was frozen (CP) with a Westco Neurostat 1H3E cryoprobe or crushed (C) with forceps for 60 sec. Sensory tests indicated an earlier and improved mean withdrawal time from a heat source during the first month in the CP group. The CP index of motor recovery (digital twitch tension) returned to normal (96%) by 35dpo, but remained subnormal (81%) in the C group. The recovery of locomotion (SFI) was complete and nearly equal in the two groups by 42dpo. There were no significant group differences in the morphometric results at 63dpo; the fiber number increased 10-15% and the fiber size decreased 24-36% compared to the control side. While the functional recovery following a CP lesion may be earlier than a C lesion, the recoveries are nearly identical after two months. Support by NIH N519769 and BRSG 507RR05477 grants

351.17

ELECTRICAL FIELD EFFECTS ON CRUSHED NERVE REGENERATION.
C. Lucchinetti* and J.M. Kerns (SPON: C. Robinson). Dept. Anat., Rush Med. Coll., Chicago, IL 60612.

Previous studies have demonstrated enhanced recovery following the delivery of an electrical field to a transected nerve. This study examines the effects of such fields on the regeneration of crushed rat sciatic nerve during the first month. The treated (T) nerve group received a battery implant delivering 10uA with the cathode distal. The recovery was compared to an untreated (UT) group and unoperated controls (C). The loss of locomotion behavior and partial recovery (SFI) is identical for the T and UT groups. The index of motor recovery (twitch tension) is also similar (T/C=48% and UT/C=53%) by 28dpo. However, a "window" of enhancement occurs 2-4 days earlier in the T group. Qualitative histology at 28dpo suggests some improvement in the T group, but there are no significant group differences for fiber number. Similarily, the mean number of HRP labeled motoneurons is not significantly different (T=1393, UT=1658, C=1746). We conclude that despite an earlier motor recovery in the T group, no group differences in functional or structural recovery are observed at 28dpo. Support by BRSG 507 RR05477 and the Enelow Fund.

351.18

EARLY STAGES OF REGENERATIVE GROWTH OF ADULT RAT CNS AXONS INTO PERIPHERAL NERVE GRAFTS.
G.Campbell, P.N.Anderson*, M.Turnaine* and A.R.Lieberman*. Dept. of Anatomy & Dev. Biol. University College London.

Grafts of peripheral nerve into adult rat thalamus are invaded by large numbers of regenerating axons from thalamic neurons, as shown by retrograde HRP labelling (Bentley et al., J. Neurocytol. 1985; Campbell et al., unpublished). We have investigated early stages of this regenerative growth. Autografts of sciatic nerve were implanted into caudolateral thalamus of anaesthetised rats. The distal, free end was laid beneath the scalp. Between 5d and 4wk later the brains were processed for EM.

A regenerative response in the CNS, characterised by the production of large numbers of axonal sprouts, is well established around the proximal end of the graft by 5d. The sprouts accumulate below the forming glia limitans often in association with glia. Sprouts cross the limitans, many in contact with glial processes, to enter the border zone between host brain and graft tissue. By 8d, regenerating axons are associated with Schwann cells and astrocytes within the graft and at 2-4 wk the proximal portion of the graft contains large numbers of regenerating axons in Schwann cell or Schwann cell-astrocyte bundles. By 4 wk many axons have grown at least 6 mm along the graft. Most regenerating axons, at both proximal and distal levels of the graft, lay in simple contact with Schwann cells: very few were deeply invaginated into Schwann cell processes. Thus the relationship between them resembled that seen in very early stages of PNS regeneration, which may be due to either slow growth of regenerating CNS axons or abnormal axon-Schwann cell interactions.

351.19

AN IMPROVED MODEL TO STUDY RAT SCIATIC NERVE REGENERATION. K. Harman, J.C. de la Torre. Physiotherapy Program, Univ. of Ottawa Health Sci., Ottawa, Ont. K1H 8M5.

Quantifying sciatic nerve axons in the regenerated distal stump after transection may be used as an indicator of the nerve's response to a treatment. We have improved our rat nerve guide model and developed a simpler, more quantifiable and efficient axon counting technique. To make the nerve firmer, it is rapidly cooled to 0°C, transected with one stroke of a razor blade, creating an interstump gap of 2.5 mm. A marker is butted against each nerve stump and the gap is filled with collagen matrix (CM). The markers provide an accurate reference to the cut nerve end. Cross-sections 1 um thick are taken 5 mm distal to the transection site. The image of the slide is projected directly onto the digitizing tablet using a camera lucida attachment. An image analyzer computes quantity, density and size of regenerated axons at this site. This approach is being applied to test putative growth promoting factors. This model will also be used in studies with longer observation periods to correlate findings in the distal stump to target muscle reinnervation.
Supported by The Easter Seal Research Inst.(Ont.)

351.20

A QUANTITATIVE BIOCHEMICAL ASSAY FOR CATECHOLAMINERGIC NEURITE OUTGROWTH. D.S. Bell,* I.J. Kopin, M.A. Palmatier. HHMI-NIH Research Scholars and CNB, NINDS, Bethesda, MD, 20892.

Catecholaminergic neurons have an uptake and storage mechanism for catecholamines, which is present in neurites. Catecholamine uptake may thereby provide a rapid, quantitative assessment of in vitro neurite outgrowth.

Timed-pregnant Sprague-Dawley rats are euthanized at E-20, according to NIH Animal Care guidelines. Superior cervical ganglia (SCG) are dissected from the fetuses, dissociated, and cultured in serum-free medium with various amounts of NGF. After 24 h in vitro, uptake of ^{3}H-dopamine (DA) is measured. Parallel cultures can be processed for tyrosine hydroxylase (TH) immunocytochemistry.

Low concentrations of NGF are sufficient to support full cell survival for 24 hours with minimal neurite outgrowth. At higher NGF concentrations, DA uptake correlates linearly with increased neurite length. Therefore, dopamine uptake in SCG neurons cultured in serum free medium and low NGF concentrations can provide a quantitative measure of neurite outgrowth. This bioassay is presently being used to characterize and isolate neurite promoting factors from several sources.

CELL LINEAGE AND DETERMINATION III

352.1

DIFFERENTIAL CYTOCHROME OXIDASE STAINING OF DEVELOPING QUAIL NEURAL CREST CELLS IN CULTURE. S. Liu*, D.A. Wilcox*, M. Sieber-Blum and M. Wong-Riley (SPON: T. Trusk). Dept. Anatomy & Cellular Biology, Med. College of Wis., Milwaukee, WI 53226.

The neural crest, a transient tissue of the vertebrate embryo, gives rise to sensory neurons, autonomic ganglia, pigment cells, and other cell types. In quail neural crest cell cultures, there are two populations of sensory cells: non-migrating/early-differentiating and early-migrating/late-differentiating (Sieber-Blum, *Science* 243:1608,'88). In order to determine whether these different types of cells undergo changes in energy demands during cell development, cytochrome oxidase (C.O.) histochemistry was performed on day 1(E3) to day 19 cell cultures. At all stages, pigment cells were negative for C.O. staining. However, sensory neuroblasts increased their C.O. activity significantly ($p<0.0001$) with development. By days 12 and 19, the centrally located sensory neuroblasts were well differentiated and were much larger and more C.O. reactive than the priepheral ones ($p<0.0001$). A heterogeneous pattern of C.O. activity also existed among centrally located neuroblasts. We further distinguished sensory and autonomic neuroblasts by immunostaining C.O.-reacted cultures for the stage specific embryonic antigen-1 (SSEA-1) and dopamine-ß-hydroxylase (DBH), respectively. Sensory neuroblasts were dramatically more C.O. reactive than autonomic cells. We conclude that neuronal energy demands increase during development, indicating that functional activity increases with neuronal maturation. Different C.O. activity between the two populations of sensory cells may be due to their different time course of development and/or different level of functional activity. The autonomic neuroblasts have much lower C.O. staining than sensory neurons, suggesting that at least under these culture conditions and time period, developing sensory neurons may have higher spontaneous or synaptic activity than autonomic ones. Supported by USPHS grants NS18122 (MWR) and HD21423 (MSB).

352.2

A VITAL DYE ANALYSIS OF TRUNK NEURAL CREST CELL MIGRATION IN MICE. George N. Serbedzija, Scott E. Fraser, and M. Bronner-Fraser. Developmental Biology Center, U.C. Irvine, Ca. 92717

Analysis of neural crest cell migration in the mouse has been difficult due to the lack of reliable cell markers. Recently, we found that injection of DiI into the chick neural tube marks premigratory neural crest cells whose endfeet are in contact with the neural tube (Serbedzija, etal., 1988, Soc. Neurosci. 18:427). Here, we use this appraoch to describe neural crest migratory patterns in the trunk of the mouse. Embryos were removed from the mother between E8 and E10, labelled with DiI, cultured for 12 to 24 hours, and then analyzed at the level of the forelimb. 12 hours after DiI injection into E8 embryos, Labelled cells were observed exclusively in the neural tube. 12 hours after DiI injection into E8.5 embryos, labelled cells were found in the neural tube, in large clusters between the dorsal neural tube and the epidermis, and sparsely within the sclerotome. Embryos injected at E9 and incubated for 12 hours contained numerous DiI-labelled cells between the neural tube and the dermamyotome and in the region of the sympathetic ganglia. When E9 embryos were incubated for 24 hours, DiI-labelled cells were found in both the spinal and sympathetic ganglia. In contrast, embryos labelled at E10 contained DiI-labelled cells only in the spinal ganglia. These findings indicate that trunk neural crest cells in the region of the forelimb: 1) initiate their migration at approximately the 15 somite stage (E8.5), 2) populate their derivatives in a ventral to dorsal order, and 3) appear to be restricted to the rostral portion of each sclerotome. This pattern of neural crest migration closely resembles that previously observed in the avian embryos. (Supported by USPHS HD-25138 and BNS 8608356).

352.3

MONOCLONAL ANTIBODY, B-1A11, TO A CELL SURFACE EPITOPE RECOGNIZES A SUBPOPULATION OF EARLY NEURAL CREST CELLS. D.A. Wilcox*, and M. Sieber-Blum (SPON: F.D. Anderson). Department of Anatomy and Cellular Biology, Medical College of Wisconsin, Milwaukee, WI, 53226.

In order to develop a tool for isolating and/or manipulating subpopulations of precursor cells, monoclonal antibodies (mab) were raised against quail neural crest cells. One of them, B-1A11, belongs to the IgG1 subclass and recognizes a trypsin-resistant cell surface epitope on a subset of early neural crest cells in vitro. Positive cells were present in 2 day old cultures. By day 3, about 10% of the neural crest cells within the explant were B-1A11$^+$, whereas in selected areas at the periphery 40-60% were immunoreactive. In older cultures, a subset of sensory neurons, occasional immature pigment cells, and cells resembling nerve supporting cells were immunoreactive, while adrenergic and mature pigment cells did not bind detectable levels of the mab. Using mab against bromodeoxyuridine (BrdU) in day 3 cultures, we determined that during a 2 hr incubation period with BrdU >30% of the B-1A11$^+$ cells were in S-phase. When live, stained cells in primary explants were resuspended and seeded out at clonal density, they reattached and continued to proliferate. The data suggest that B-1A11 recognizes a differentiation antigen and may be useful for analyzing subpopulations of neural crest cells. Supported by USPHS grant HD21423.

352.4

Evidence for death of committed neuronal precursors in the developing adrenal gland. P.D. Henion and S.C. Landis. (SPON: V. Lemmon) Center for Neurosciences, Case Western Reserve Univ. Sch. Med.; Cleveland, OH 44106

The sympathoadrenal lineage of the neural crest gives rise to sympathetic neurons and adrenal chromaffin cells. Cell culture studies have raised the possibility that sympathetic neurons and chromaffin cells derive from a bipotential precursor cell population whose fate is determined by the local environment; consistent with this hypothesis, a subpopulation of clustered cells are present in embryonic adrenal which express the neuronal markers SCG-10 and B2 (Anderson and Axel, 1986).

To define further the properties and fate of the neuronal precursor cells, we stained embryonic and postnatal adrenals with antibodies against other neuronal markers and determined the time course of their expression. SCG-10/B2-IR cells express the neural adhesion molecules L1 and NCAM on E15. B2-IR was no longer observed on E18-19 while SCG-10/L1/NCAM-IR clusters of cells were evident until P6-7. To determine whether the disappearance of staining with SCG-10, L1 and NCAM in the first postnatal week was due to the death of neuronal precursors or the loss of expression of these molecules, we examined plastic sections of P0-P7 adrenals. During this period, clusters of cells in the medulla appeared to degenerate. Analysis of P5 adrenals revealed ultrastructural evidence of degeneration including dilated mitochondria, lack of rough endoplasmic reticulum and granular, electron-dense cytoplasm in numerous granule-containing cells. Thus, our observations confirm the existence of a neuronal precursor cell population in the developing adrenal and provide evidence that at least some of these cells die.

352.5

AN ISOLATED MESENCEPHALIC NEURAL CREST SUBPOPULATION DEVELOPS NEURONAL CHARACTERISTICS IN CULTURE. K. F. Barald Dept. Anatomy and Cell Biology, U.Michigan Med. Sch. Ann Arbor, MI 48109.

Although neural crest cells are known to be very responsive to environmental cues during their development, recent evidence indicates that at least some subpopulations may be committed to a specific differentiation program prior to migration. To address this problem, we have isolated a pure subpopulation of chick mesencephalic neural crest cells by no-flow cytometry after labelling them with monoclonal antibodies (Mabs) to a 75kD cell surface antigen associated with high affinity choline uptake. The Mabs also label all of the neurons of the embryonic chick and quail ciliary ganglion (CG) in vivo and in vitro, suggesting that the neural crest subpopulation that labels with these Mabs may be precursors of CG neurons. Pure populations of antigen-positive (A^+) cells were grown in a variety of media, each of which differently affected its characteristics and development. A^+ cells proliferated in 15% fetal bovine serum (FBS) and high concentrations (10-15%) of chick embryo extract, but did not differentiate, although they retained basal levels of choline acetyltransferase (ChAT) activity. However, in chick serum and high (25 mM as opposed to 7mM) K^+, and heart-, iris- or lung-conditioned media, all of which are known to promote survival &/or cholinergic development of CG neurons, the cells ceased to proliferate and "neuron like" cells indistinguishable from cultured CG neurons appeared in the cultures. Neuron-like cells were not found in liver-, notocord- or neural tube-conditioned media if FBS was used. These results are consistent with the hypothesis that the A^+ cells contribute to the formation of embryonic CG neurons.

352.6

ONSET AND PATTERNING OF THYROTROPIN RELEASING HORMONE mRNA EXPRESSION IN THE DEVELOPING FROG BRAIN AND RETINA. Y.P. Loh and W.P. Hayes (SPON: E.B. Van Deusen). Laboratory of Developmental Neurobiology, NICHD, Bethesda, MD 20892.

To explore how the initiation of the neuropeptide phenotype is regulated in the vertebrate CNS, the development of thyrotropin releasing hormone (TRH) gene expression was examined in developing Xenopus laevis using *in situ* hybridization. Coronal sections of embryos, as well as of brains and retinae from pre- and post-metamorphic frogs were hybridized with sense and antisense radiolabeled 48-mer oligonucleotides encoding regions of Xenopus TRH mRNA (Richter, K.,et al., EMBO J. 3:617, 1984).

TRH mRNA expressing cells were first detected in discrete regions of the forebrain and hindbrain of the embryonic CNS on developmental day 1.5 (Stage 29/30). This basic pattern of TRH expression was maintained through day 2 (Stage 33/34). Anteriorly, labeling was restricted to a bilaterally paired group of cells in the lateral wall of the diencephalon. Posteriorly, labeling was organized in repeated pairs of laterally positioned clusters in the rhombencephalon. By day 2.5 (Stage 39), the diencephalic labeling was more complex. Each of these bilateral groups, still seen just anterior to the level of the optic stalk, was now extended and bifurcated caudally forming two distinct groups of cells in the dorsal and ventral wall of the infundibulum. The more widely distributed pattern of TRH expressing cells seen in adult frog was found in tadpoles by day 4.5 (Stage 46). This included discrete labeling of retinal cells in the innermost lamina of the inner nuclear layer, indicating they may be amacrine cells.

We are currently investigating whether the adult-like distribution seen by day 4.5 can be accounted for by these two initial groups of TRH expressing cells, or whether the new cells seen in the telencephalon and mesencephalon arise from independent TRH cell groups. (W.P.H. is supported by an Associateship from the National Research Council.)

352.7

CLONAL ANALYSIS OF GABAERGIC NEURONS IN FROG EMBRYO SPINAL CORD. S.A. Moody and K.S. Kersey. Department of Anatomy and Cell Biology, University of Virginia, Charlottesville, Va 22908

In the embryonic spinal cord of Xenopus laevis, there are 7 classes of interneurons that express GABA-like immunoreactivity: ascending (**as**), midhindbrain reticulospinal (**mhr**), vestibular commissural (**vc**), rostral hindbrain commissural (**rh**), rostral midbrain (**rm**), optic tract (**ot**) and rostral forebrain (**rf**) (Roberts et al. '87 JCN 261:435). Because Xenopus CNS descends from several embryonic blastomeres(Moody '87 DB 119:560) we tested whether clonal membership is correlated with neurotransmitter phenotype. Single blastomeres of the 16-cell embryo were injected with 10% Texas red-dextran-amine. At late tailbud stages they were fixed, and the entire CNS was processed for indirect immunofluorescence of GABA.

Blastomere V1.1 (see Moody '87 for nomenclature) gives rise to cells in the dorsal spinal cord. None of these were GABAergic. Two specimens had V1.1 progeny in the dorsal part of the rostral forebrain, a few of which were GABAergic. Blastomere V1.2 gives rise to cells in dorsal brain and dorsolateral spinal cord. GABAergic cells in the clone were found sparsely in **rf**, **ot** and **rm**; they were found in great numbers in **mhr** and among the **as** neurons of the spinal cord. Blastomere D1.2 populates dorsolateral brain and ventrolateral spinal cord. Most of the GABAergic neurons of the brain were progeny of this blastomere, but very few GABAergic neurons of the spinal cord descended from it. Blastomere D1.1 gives rise to large regions of the brain and ventral spinal cord. The only GABAergic cells in its clone were occasional cells in the **ot** and ventral forebrain. Thus, GABA expression is not associated with specific blastomeres at this stage. Those that populate lateral regions of the brain and spinal cord produce most of the GABAergic neurons.

352.8

A METHOD FOR DETERMINING EMBRYONIC ORIGINS OF XENOPUS NEUROSECRETORY CELLS. K.M. Conway, NINDS, Bethesda, MD 20892.

The fluorescent dye quinacrine indicates host versus donor origin in chimerae made by grafts between Xenopus laevis and Xenopus borealis frog embryos. Fixatives for use with quinacrine staining (e.g. Carnoy's) impair neuropeptide immunocytochemistry (ICC) used to identify neurosecretory cells. A simple detergent treatment allowed the two protocols to be combined. Brain tissue was immersed in either Carnoy's fix or a paraformaldehyde-picric acid fix used in ICC. A monoclonal antibody (PS-45) which heavily stains mesotocinergic neurons in Xenopus laevis was used in rhodamine labelled ICC. Some slides were then detergent treated (1% Triton-X-100 in phosphate buffered saline (PBS), pH 7.4, overnight). After quinacrine staining, cover slips were mounted with 1:1 glycerol:PBS, pH 4.0. Carnoy's fixed tissue gave strong quinacrine staining, but weak ICC. Tissue fixed for ICC gave strong ICC, but weak quinacrine staining. Detergent treated tissue fixed for ICC gave strong ICC and quinacrine staining with moderate backround. To test this protocol in context, grafts comprising large regions of left anterior neural plate were exchanged between laevis and borealis embryos; these animals were then raised for 4 weeks to swimming tadpole stages (45-47) and processed as described. Individual immunoreactive cells of donor and host origin were identified. This demonstrates the feasibility of future studies using smaller grafts to accurately locate neural plate precursors of neurons with specific peptidergic phenotypes.

352.9

EVIDENCE THAT CELLS EXPRESSING LHRH mRNA IN THE MOUSE ARE DERIVED FROM PROGENITOR CELLS IN THE OLFACTORY PLACODE. S. Wray, P. Grant and H. Gainer, LNC, NINDS, Bethesda, MD 20892

In situ hybridization histochemistry and immunocytochemistry (ICC) were used to study prenatal expression of LHRH cells in the mouse. Cells expressing LHRH mRNA and peptide product were first detected on E11.5 in the olfactory pit (OP). On E12.5, there was a dramatic increase in the number of LHRH cells, with more than 90% located in nasal regions, on 'tracks' extending from the OP to the base of the telencephalon. From E12.5 to E15.5, LHRH cells were detected in a rostral to caudal gradient in forebrain areas. Prior to E12.5, cells expressing LHRH mRNA were not detected in forebrain areas known to contain LHRH cells postnatally. Quantitation of cells expressing LHRH mRNA showed that the number of cells on E12.5 equalled the total number of LHRH cells in postnatal animals. Between E12.5-E15.5, the location of LHRH cells shifted; the number of LHRH cells in the forebrain increased, while the number of LHRH cells in nasal regions decreased. These findings establish that cells first found in the OP and thereafter in forebrain areas produce LHRH mRNA, and confirm the identity of cells detected by ICC. To further examine the ontogeny of the LHRH system, ICC was combined with ^{3}H-thymidine autoradiography to determine when LHRH cells left the mitotic cycle. We show that LHRH neurons exhibit a discrete time of birth, suggesting that they arise as a single neuronal population between E10.0-E11.0. Postnatal LHRH neurons were 'birth-dated' shortly after differentiation of the olfactory placode and before LHRH mRNA was expressed in the OP. These studies support the hypothesis that both nasal and forebrain LHRH cells arise from a discrete group of progenitor cells in the olfactory placode.

352.10

FLUORESCENTLY LABELED TISSUES INCLUDING ENDOTHELIUM IN XENOPUS TADPOLES. C.M. Rovainen Dept. Cell Biol. and Physiology, Washington Univ., St. Louis, MO 63110

The goal of this work has been to mark endothelial cells in vivo and to follow their migration, cell divisions, and possible degeneration during angiogenesis in the optic tectum of transparent tadpoles of albino Xenopus laevis. Rhodamine-dextran was injected into single blastomeres, and cell progeny were observed sequentially with epifluorescence and a SIT camera. A variety of cell types and mixtures were labeled, including brain, eye, olfactory sac, cranial ganglia, epidermis, gut, myotomal muscle fibers, extraocular muscles, notochord, and heart. Some clones included circulating fluorescent blood cells and walls of blood vessels. The fluorescent label initially was distributed through the cytoplasm of cells in each clone. Later it was packaged in vesicles. Both forms of label can be followed in case histories of development. These preliminary results indicate that intracellular fluorescent labeling may be a feasible approach for following the behavior of individual endothelial cells during angiogenesis. Supported by USPHS Grant HL41075.

352.11

COATED PITS AS A CELLULAR MARKER FOR MUSCLE IN DROSOPHILA WITH THE TEMPERATURE-SENSITIVE MUTATION SHIBIRE. M.R. Hummon and W.J. Costello. Dept. Zool. Biomed. Sci./Col. Osteopathic Med., Ohio University, Athens, OH 45701.

Pupal heat pulse (HP: 30C, 6h) induces abnormal muscle (fused or deleted fibers) in Drosophila bearing the ts mutation shi. In bilateral mosaic flies (+/shi) exposed to pupal HP, each indirect flight muscle (DLM; DVM I; DVM II and III) is normal (+) or exhibits the induced shi phenotype. These mosaic flies can exhibit different phenotypes on a single side, suggesting more than one cell lineage for flight muscle. We tested a phenotype of shi membranes, heat-induced coated pits (CP), for use as a possible cellular marker in the mosaic flies. $In(1)w^{vc}$ (Ring X) females and y w sn^3 shi males were used, to represent genotypes present in mosaic flies. Adults 1-2 days old were exposed to non-permissive temperature (30C) for 20 min., dissected in fix at 30C, and processed for TEM. Identified DLM fibers were scanned (7,000-12,000X) for CP. The perimeter of each fiber is about 500 um and was measured to obtain the number of CP per membrane unit. CP were numerous in shi DLM (12-25/500 um, mean=18.9; 7 fibers from 2 flies); in Ring X DLM, CP were rare (0-2/500 um, mean=0.9; 7 fibers from 2 flies). Thus, the heat-induced occurrence of CP in shi tissue can be an effective marker at the cellular level. Incidence of CP may indicate the genotype of the muscle in mosaics and permit further assessment of cell lineage of DLM and DVM. NIH-NRSA (MRH)

352.12

EXPRESSION OF NEURONAL PHENOTYPE BY THE TE671 CLONAL CELL LINE. H. Anne Ahmad[1,2,*] and Ronald J. Lukas[2] (SPON. D.L. Glanzman). Department of Psychology, Arizona State University, Tempe AZ 85287[1] and Division of Neurobiology, Barrow Neurological Institute, Phoenix AZ 85013[2].

Controversy has evolved regarding the relationship between the TE671 cerebellar medulloblastoma and the RD embryonal rhabdomyosarcoma human clonal cell lines and the expression by the TE671/RD line of glial vs. neuronal character. We have utilized immunocytochemical (IC) and enzyme-linked immunosorbent assay (ELISA) approaches with a variety of monoclonal or monospecific antibodies raised against neuronal or glial proteins to address these issues. By both IC and ELISA criteria, levels of TE671 cellular expression of neuron-specific enolase, neurofilament proteins, and choline acetyltransferase are higher than those for glial fibrillary acid protein and the S-100 antigen, which are labeled at levels marginally above background. Positive IC staining and ELISA results are also observed for TE671 cells labeled with antibodies against vimentin and alpha- and beta-tubulin. Taken together with our other results indicating that TE671 cells adopt a neuronal morphology on treatment with specific drugs, express functional muscarinic and nicotinic receptors, and exhibit specific GABA and choline uptake, the present IC and ELISA results demonstrate that the TE671/RD line expresses at least some characteristics of cells of neuronal lineage.

352.13

EXPRESSION OF GLIAL FIBRILLARY ACIDIC PROTEIN GENE IN IMMATURE OLIGODENDROGLIA IN-VITRO: A CORRELATIVE IMMUNOCYTOCHEMICAL AND IN-SITU HYBRIDIZATION STUDY. B.H. Choi, P. Amodei*, J. Geddes*, M. Suzuki* and R.C. Kim*. University of California at Irvine, Irvine, CA 92717.

In previous studies, we presented evidence to suggest, in the developing human and rodent CNS, that the earliest glial forms within the developing CNS are radially organized; that these cells contain glial fibrillary acidic protein (GFAP); that "transitional" cells possessing the features of both oligodendrocyte (OC) and astrocyte (AS) appear just prior to the onset of myelination; and that these early radially organized glial cells may be the ultimate source of all macroglial cell types including AS, OC and ependymal cells. We have also shown the presence of immature OC that co-express myelin basic protein (MBP) and GFAP in culture. In order to further define this phenomenon, a correlative study was carried out with in-situ hybridization using biotinylated cDNA probe for GFAP (provided by Dr. L. Eng) and single and double immunofluorescent labeling for GFAP, MBP and galactocerebroside (GC) in immature OC (obtained by McCarthy and de Vellis method). The cells that express both AS and OC markers in these cultures with or without addition of serum are identified as early as 5 hours following the isolation of immature OC. Such cells are also found in long term cultures of astrocytes derived from the original mixed glial cell culture maintained in serum containing medium. In-situ hybridization using biotinylated cDNA probe for GFAP demonstrates localization of GFAP gene within the processes and cytoplasms of cells with OC morphology. These data suggest not only a close and dynamic ontogenetic relationship between, but also a common cell lineage for AS and OC.

(Supported in part by NIH grant ES 02928)

NUTRITION AND PRENATAL FACTORS

353.1

PRENATAL DIETARY SUPPLEMENTATION WITH N-3 FATTY ACIDS ACCELERATES EYE-OPENING IN MICE. B.Bulman-Fleming and P.Wainwright. Dept. of Health Studies, Univ. of Waterloo, Waterloo, ON. N2L 3G1.

Evidence from our laboratory suggests that prenatal dietary supplementation with n-3 fatty acids (FA) accelerates sensory development in mice, particularly eye-opening. These results occurred in the context of prenatal ethanol effects, and were therefore accompanied by slight undernutrition. Thus the present study sought to replicate this in a well-fed population and to compare pre- with postnatal supplementation. Pregnant mice were fed either of two liquid diets, each providing 20% of the calories from oil and of these 3% were in the form of n-6 FA. The supplemented group also received 1.8% from n-3 FA. The study consisted of four dietary groups, with all pups fostered at birth. These groups were: (PRE/POST) 1.N3/N3, 2.N3/N6, 3.N6/N6, 4.N6/N3. A fifth group (unfostered) was fed lab chow throughout the study. At day 32 post conception pups were assessed using a standardized battery of tests measuring neurobehavioral development. Prenatal n-3 supplementation resulted in a significant but small acceleration of behavioral development overall; individual tests showed a similar effect on eye-opening. There was no difference in body weight between the n-3 and n-6 groups, but they were heavier than the lab chow group. Despite this, their behavioral development was retarded relative to the lab chow control, with this being less severe in the n-3 group.

353.2

PRENATAL TRYPTOPHAN DIET EFFECT ON RAT DEVELOPMENT FROM WEANING TO ADULT. M.Sakuma, L.M. Hryhorczuk,* Dept. of Biol. Sci., Wayne State Univ., Detroit, MI 48201: Lafayette Clinic, Detroit, MI 48207

The long-lasting effect of tryptophan diet fed during the latter half of pregnancy was investigated by open field behavior of offspring. Pregnant females were placed on a diet containing either 0.03% (F), 0.30% (M) or 3.0% (H) of tryptophan during the second half of pregnancy. Offspring were tested at 3, 6, 10, and 15 weeks for seven items in the open field test. The items included rearing count, rearing latency, corner crouch duration, corner crouch latency, face washing count, face washing latency and defecation. Body weights were also measured. Two-way analysis of variance with repeated measures for diet (F, M, H) and age (3, 6, 10, 15 weeks) revealed that all variables showed an age dependency at $p<0.01$ and only defecation differed with respect to diet ($F=6.515$, $p<0.01$). Defecation has been used as an index of emotionality. Significant diet dependent body weight differences were obtained at 3 weeks ($F=4.256$. $p<0.05$) and 6 weeks ($F=6.231$, $p<0.01$). Although the F diet group recorded the lowest weights and the H diet group the highest weights at 10 and 15 weeks, the differences were not statistically significant. This warrants further investigation with a larger number of animals.

353.3

INOSITOL TRANSPORT IN PC12 CELLS: EFFECTS OF INOSITOL DEPRIVATION. Z.W. Yang, G.A. Paleos, and J.C. Byrd. Developmental Neurobiology Program, University of Pittsburgh School of Medicine, W.P.I.C., Pittsburgh, PA 15213.

Inositol triphosphate (IP_3) is an important second messenger in the mediation of the effects of many growth factors. We have studied the transport of myo-inositol, a precursor of IP_3, in the NGF-responsive cell line PC12. We have found that the PC12 inositol transporter closely resembles the comparable carrier found in non-transformed cells. Specifically, this transporter is saturable; sodium dependent; inhibited by cytochalasin B, phloridzin, and PCMB; and shows a low affinity for D-glucose. PC12 cells survive deprivation of inositol as long as 4 days without showing signs of overt toxicity. However, following this period, there is a marked increase compared to controls in the labeling of cellular membranes following brief exposure to [^{3}H]myo-inositol, indicating a state of intracellular phosphatidylinositol deficiency. Furthermore, the Km of the inositol transporter is decreased from 76 μM to 54 μM, suggesting an ability of these cells to modify the affinity of the inositol transporter during periods of inositol deprivation.

353.4

CHANGES IN CORTICAL EVOKED POTENTIALS FOLLOWING INFANT MALNUTRITION. I.Weiss, A. Barnet*, CHNI, Washington, D.C. J.M.Flinn, R.Holt*, S.Lydick*, George Mason, Fairfax, VA.

Cortical evoked potentials (EPs) were recorded from severely malnourished infants at admission to hospital (group MA), at discharge (group MD), and from age-matched controls (group CA and CD). We report the results of a principal component analysis of the EPs recorded from C_3 and C_4 to patterned flash. Eight factors were identified with eigenvalues > 1. Four rotated factors (1,3,5 and 8) showed significant differences between groups. For factors 1 and 3, the scores of the malnourished infants changed in the same direction as those of the age-matched controls between admission and discharge. E.g., for factor 1 ($F(3,108)=6.93$, $p<.001$) the factor scores significantly increased between admission and discharge: -.477 for MA & +.476 for MD, -.115 for CA and +.348 for CD. In contrast, for factors 5 and 8 the changes in the malnourished group were in the opposite direction from the controls. E.g., for factor 8 ($F(3,108)=4.47$ $p<.05$), the scores were -.072 for MA, -.434 for MD, +.157 for CA and +.544 for CD. MA differed significantly both from MD and CD, which differed significantly from each other. These results show that infant malnutrition appears to impair some aspects of brain development and functioning but not others.

353.5

PRENATAL PROTEIN MALNUTRITION CAUSES INCREASED RESISTANCE TO EXTINCTION OF LEARNED SPATIAL ALTERNATION IN RATS. *J. Tonkiss Ph.D, *J.R. Galler M.D., *R.R. Timm and *R.N. Formica (SPON: O. Resnick, Ph.D). Cntr. Behav. Dev., M921, Boston Univ. School of Med., Boston, MA 02118.

This study formed part of an ongoing project to assess the effects of prenatal protein malnutrition on hippocampal function. Sprague-Dawley rats whose mothers were provided with a low (6%) or adequate (25%) protein diet during pregnancy were fostered at birth to adequately nourished dams, and continued to receive the 25% protein diet after weaning. At 90 days of age, 10 previously undernourished and 12 well-nourished males (each with different biological and foster mothers) were selected for testing of spatial working memory on an elevated T-maze, susceptibility to interference and resistance to extinction. There were no differences between nutritional groups in spatial learning or working memory, and both groups were equally susceptible to the effects of a source of proactive interference. However, rats with histories of prenatal malnutrition required a significantly greater number of days (mean ± SEM:12.2 ± 1.0 versus 9.5 ± 0.8) to eliminate the previously learned response. Although it is clear that complete hippocampal dysfunction did not result from the prenatal treatment, the increased resistance to extinction (often noted in hippocampectomized animals) could indicate one specific dimension of hippocampal dysfunction worthy of further investigation.

353.6

LOCOMOTOR ACTIVITY IN SPONTANEOUSLY HYPERTENSIVE RATS (SHR) AND WISTAR KYOTO RATS (WKY) FED LOW PROTEIN DIETS J.T. Earnhardt* and D.J. Mokler (SPON: N. Cross). Dept. Pharmacol., Univ. New England, Biddeford ME 04005.

Protein malnutrition during development alters motor activity and levels of neurotransmitters in the central nervous system. SHR display enhanced motor activity although the effects of changes in dietary protein on this behavior have not been studied. The dams of SHR and WKY were fed a diet of either 8% or 25% casein beginning 7 days postpartum. The 8% casein diet was methionine supplemented and made isocaloric with the 25% casein diet by adjusting carbohydrate. After weaning, animals were fed the diet previously given the respective dams. After six months on the diet, individual animals were placed in plexiglass cages and motor activity was monitored for 30 min. Motor activity in SHR fed 8% casein and housed individually from weaning was higher than 25% SHR, 8% WKY or 25% WKY. Motor activity in SHR and WKY fed 25% casein did not differ. In contrast, 8% casein reduced motor activity in WKY. Clonidine (.03 - 1.0 mg/kg ip) suppressed accommodated motor activity in all groups, although a dose response relationship was not apparent. SHR were more sensitive to the effects of clonidine which were not altered by changes in dietary casein. Thus, motor activity is differentially affected in mature SHR and WKY by reduction of dietary casein. (Supported by NHLB 37738)

353.7

PRENATAL PROTEIN MALNUTRITION AFFECTS VIGILANCE STATE MODULATION OF DENTATE RESPONSIVENESS. C.M. Beiswanger*, K. Austin*, J. Bronzino and P.J. Morgane. Worcester Foundation for Experimental Biology, Shrewsbury, Mass. 01545.

The paired-pulse technique was used to assess the effect of prenatal protein malnutrition on vigilance state modulation of responsiveness in the dentate gyrus of adult rats. Pups born to dams on either 25% or 6% casein diet were fostered at birth to females on 25% diet, providing control (25/25) and prenatally malnourished (6/25) groups. Evoked potentials were recorded in the dentate in response to paired stimulus pulses to the perforant path across 4 vigilance states (AW,IW,SWS,REM). The first response (p1) of each pair was used to generate a stimulus-response (S-R) curve. The ratio of the two responses (p2/p1: paired-pulse index or PPI) was used as an indicator of the relative level of inhibitory or facilitatory activity in the dentate. Both 6/25 and 25/25 rats exhibit PPI responses dependent on IPI: early inhibition (20-40 ms), facilitation (40-100 ms) and late inhibition (100-1000 ms). Vigilance state modulation of the PPI is seen in early inhibition in both groups. However, malnutrition abolishes the increase in p1 amplitude in SWS/REM relative to IW/AW seen in 25/25 S-R curves. These results suggest that two systems modulate dentate responsiveness across vigilance states. One affects transmission of signals (S-R curves) and is sensitive to prenatal protein malnutrition. The other affects inhibition/facilitation (PPI), but is not sensitive to prenatal dietary insult. (Support: NIH grants HD-23338 and HD-22539).

353.8

EFFECTS OF PRENATAL PROTEIN MALNUTRITION ON KINDLING-INDUCED ENHANCEMENT OF DENTATE GRANULE CELL ACTIVITY. J.D. Bronzino, R.J. Austin-LaFrance, R.J. Franceschini* and P.J. Morgane. Trinity College, Hartford, CT 06106.

Several studies indicate that hippocampal kindling enhances transmission at the level of the perforant path/dentate synapse. Single and paired-pulse stimulations were used to assess the effects of prenatal protein malnutrition on dentate granule cell activity before and after kindling. Animals born to dams fed either a low (6%) or normal (25%) casein diet were fostered at birth to lactating dams of the 25% casein diet. At 90-120 days of age, field potentials evoked by single and paired-pulse stimulation of the perforant path were recorded from the granule cell layer of the dentate gyrus before, during and after kindling. Input/output (I/O) and paired-pulse response measures of EPSP slope, population spike amplitude (PSA) and PSA/slope ratio were analyzed to evaluate the effect of kindling on granule cell response. The prenatal nutritional insult results in a significantly higher degree of synaptic enhancement, as measured by I/O curves, and a significantly higher degree of enhanced granule cell inhibition, as measured by paired-pulse index curves. (Supported by NIH Grant #HD-22539)

353.9

EFFECTS OF PROTEIN MALNUTRITION ON THE DENTATE GYRUS IN RATS OF THREE AGE GROUPS. L. Cintra, S. Diaz-Cintra, A. Galvan*, T. Kemper* and P. J. Morgane. Dept. Physiol., Inst. Invest. Biomedicas. UNAM. Mexico D.F. 04510, and Worcester Foundation for Expt. Biology. Shrewsbury, MA 01545.

The hippocampal formation is especially vulnerable to many forms of neuronal insults such as alcohol, heavy metals and various forms of malnutrition. We have studied morphometrically the effects of protein malnutrition (8% casein diet), instituted before mating and continued during gestation and into postnatal life, on the dentate gyrus. Our physiological studies have shown alterations in hippocampal long-term potentiation (Austin et al., 1986) and kindling (Bronzino et al., 1986) produced by protein malnutrition. Using the rapid Golgi method we studied the dentate gyrus in malnourished rats at 30, 90 and 220 days of age. We found a significant reduction in apical dendritic branching in the molecular layer of the dentate gyrus at 90 and 220 days of age as well as a significant reduction in dendritic spines on the outer 2/3 of the apical dendritic tree of dentate granule cells at 30, 90 and 220 days of age. These findings point to a marked effect of protein malnutrition on the molecular layer of the dentate gyrus which may account, in part, for some of the physiological changes we have previously reported following protein malnutrition. (Supported by CONACyT Fellowships Nos. 20518 and 27234, Mexico D.F. and NIH Grants HD-23338 and HD-22539).

353.10

QUANTITATIVE STUDY OF DENDRITIC SPINES FROM THE LARGE PYRAMIDAL CORTICAL NEURONS IN SEVERE INFANTILE MALNUTRITION. L. Benitez-Bribiesca*; I. De La Rosa*; R. Freyre* and A. Feria-Velasco (SPON: G. Tapia-Arizmendi). Unidad Inv. Clinica en Enfermedades Oncologicas, I.M.S.S., Mexico, D.F.; Univ. Autonoma de Guadalajara; and Unidad Inv. Biomed. Occte., I.M.S.S., Guadalajara, Jal. MEXICO.

It is known that protein-calorie malnutrition can alter the development of the central nervous system, both in experimental animals and men, provided that it occurs before the brain has completed its full growth. Dendritic spines, the main site of cortical synapsis, are altered in various pathologic conditions, such as Down syndrome and other chromosomal anomalies; likewise those structures are abnormal in senecence and chronic vascular disease. These abnormalities have been linked to the mental deficiency occurring in those conditions. Our objectives were to study the morphology and numerical distribution of spines in the apical dendrite of the V layer-pyramidal cells of the cortex from infants dying of severe malnutrition (SM), 2 to 24 months old and to compare them with a control group of eutrophic infants of similar ages dying of other causes. Material was obtained from fresh autopsies from the pre and retrorolandic areas (PR and RR), as well as occipital cortex (OC); cortical blocks were processed by the rapid impregnation Golgi method. Dendritic spines were counted in 150 µm-thick sections under a light microscope. Apical dendrites were significantly shorter ($p<0.01$) in the SM group. The total number of spines was diminished in the SM group: 33.3% in the PR, 21.7% in the RR and 16% in OC areas in comparison to the control group. Striking anatomical malformations and fusion of dendritic spines were observed. Our results suggest that severe neurological and physiological damage in malnourished infants can be related in part to the deficient synaptic cortical networks and impared brain plasticity that results from abnormal dendritic spine development.

LEARNING AND MEMORY: ANATOMY V

354.1

SPINAL TRIGEMINAL PROJECTIONS TO THE MAGNOCELLULAR REGION OF THE MEDIAL GENICULATE NUCLEUS IN RABBITS. P.M. McCabe, J.M. Hitchcock, C.G. Markgraf, M.A. Tucker*, R.W. Winters*, and N. Schneiderman. Dept. of Psychology, University of Miami, Coral Gables, FL 33134.

The magnocellular region of the medial geniculate nucleus (mMGN) has been demonstrated to be important in classically conditioned cardiovascular responses (CRs) to acoustic conditioned stimuli (CS) and shock unconditioned stimuli (US). Lesions of mMGN prevent acquisition and abolish the retention of heart rate CRs. It has been demonstrated that cells in mMGN receive somatosensory as well as auditory input, and therefore may be a site of convergence of CS and US information. In the current paradigm the US consists of periorbital shock, and therefore the US pathway involves trigeminal input. The present study examined the connections from the trigeminal nuclei to mMGN in an attempt to identify the potential US inputs in this paradigm.

New Zealand albino rabbits were injected with either the retrograde fluorescent tracer Fluorogold, or the retrograde/anterograde tracer horseradish peroxidase conjugated with wheat germ agglutinin (HRP-WGA). Injections were given using glass micropipettes (tip o.d. 15-25u) connected to a micro-pressure injection system. Initially, injections (10-30 nl) were placed in mMGN or in nearby control sites. After retrograde labeling was identified in a trigeminal region, HRP-WGA was injected into the labeled region and the anterograde labeling was observed.

Injections were centered in mMGN, but due to spread often included portions of the suprageniculate region, ventral subnucleus of MGN, posterior intralaminar nucleus, and posterior thalamus. Cell body labeling was observed in the contralateral caudal subnucleus of the spinal trigeminal nucleus (ST) about 1-2 mm caudal to obex. These cells were located both in the marginal layer (subnucleus Z) of the caudal subnucleus, and in deeper regions of the same subnucleus. More sparce labeling was also seen in subnuclei interpolaris and oralis of ST. Injections of HRP-WGA into the caudal subnucleus of ST led to anterograde labeling in mMGN, as well as in the ventrobasal thalamus. Currently, studies are being conducted to assess whether this pathway is involved in the relay of US information to mMGN in the classically conditioned heart rate response paradigm. Supported by NS 24874, HL 07426, HL 36588.

354.2

EFFECT OF LESIONS OF THE SPINAL TRIGEMINAL TRACT ON ACQUISITION OF CLASSICALLY CONDITIONED BRADYCARDIA IN THE RABBIT. J.M. Hitchcock, T.L. Reed*, P.M. McCabe, and N. Schneiderman. Dept. of Psychology, University of Miami, Coral Gables, FL, 33124.

The purpose of the present study was to investigate the neural pathway that carries unconditioned stimulus (US) information in the classically conditioned bradycardia response paradigm in the rabbit. Because periorbital shock is used as the US, it was hypothesized that spinal trigeminal (ST) input would be involved.

New Zealand albino rabbits received bilateral electrolytic lesions of the ST tract at the level of the facial nerve or control lesions in surrounding areas. Following recovery from surgery, animals received 30 trials of a tone conditioned stimulus (CS) (560 Hz, 90 dB) paired with the periorbital shock US (0.5 sec, 3 mA). A group of unoperated animals served as pseudoconditioned controls. Comparisons with the pseudoconditioned group demonstrated that the control lesion group exhibited conditioned bradycardia responses (CRs), but the ST tract lesion group did not exhibit CRs. In addition, the ST tract lesion decreased the orienting response (OR) to the first presentation of the tone, and attenuated the initial bradycardiac component of the biphasic unconditioned response (UR).

The present results suggest that the ST tract may be part of the US pathway in this paradigm. Concurrent neuroanatomical studies (McCabe et al., this volume) indicate that the ST has direct projections to the magnocellular region of the medial geniculate nucleus (mMGN), which is part of the CS pathway. Thus, the mMGN may be a site of convergence of CS and US inputs in this paradigm.

Some of the lesion effects in this study may have been due to damage to nearby auditory fibers, so future studies will address this question. Future studies will also investigate the effect of lesions of the caudal subnucleus of the ST, which contains the cells of origin of the projection to the mMGN. These studies may delineate the part of the ST nucleus that carries US information in this paradigm. Supported by NIH grants NS 24874, HL 07426, and HL 36588.

354.3

DISRUPTED CLASSICAL EYEBLINK CONDITIONING IN PATIENTS WITH ALZHEIMER'S DISEASE. P.R. Solomon, E. Levine*, T. Bein* and W. W. Pendlebury. Dept. of Psychology, Williams College, Williamstown, MA 01267 and Dept. of Pathology, Univ. of Vermont College of Medicine 05405.

Healthy human subjects ranging in age from 18-85 and patients with Alzheimer's Disease (AD) underwent delay classical conditioning of the eyeblink (EB) response to a tone conditioned stimulus (CS) and a corneal air puff unconditioned stimulus (UCS). In healthy subjects, there was a decline in the percentage of conditioned responses with age that was most pronounced in subjects over age 50. Compared to aged matched controls, AD patients showed a statistically significant further decline. These conditioning deficits could not be attributed to nonassociative factors such as changes in sensitivity to the tone CS or the air puff UCS or to changes in spontaneous blink rate. The results are discussed in terms of using the classically conditioned EB response in humans as a model system for studying age-related deficits in learning and memory.

This work was supported by NSF grants BNS 8616814 and CSI 8650488 and NIA grant AG 00258-03.

354.4

HIPPOCAMPECTOMY PREVENTS TRACE EYE-BLINK CONDITIONING IN RABBITS. J. R. Moyer, Jr., R. A. Deyo* and J. F. Disterhoft. Dept. of Cell Biology and Anatomy, Northwestern Univ. Med. Sch., Chicago, IL 60611.

Hippocampectomy retards extinction but not acquisition of eye-blink conditioning with a short (300ms) trace interval (Moyer et al., 1988). In the present study, a longer (500ms) trace interval was used to evaluate the effects of hippocampectomy on acquisition of trace eye-blink conditioning.

Young adult male albino rabbits received aspiration lesions of either the hippocampus plus neocortex (n=6), the overlying neocortex only (n=6), or a sham control lesion (n=6). Rabbits were trained (using a trace paradigm employing: a 100ms tone CS, a 500ms trace interval and a 150ms corneal air puff UCS) to a behavioral criterion of 80% CRs or for a maximum of 25 days.

Surgical Condition	Trials to Criterion	Percent CRs 1st day	Percent CRs Last day	Percent SLCRs 1st day	Percent SLCRs Last day
Hippocampals	2000 ± 0	11.9 ± 4.6	22.3 ± 5.27	55.8 ± 13.99	43.3 ± 2.06
Neocorticals	760 ± 140	8.8 ± 2.9	82.7 ± 1.04	22.2 ± 9.4	3.5 ± 1.31
Sham controls	733 ± 112	16.7 ± 5.4	82.7 ± 0.99	27.0 ± 6.78	3.5 ± 1.32

Hippocampectomy resulted in: 1) an inability to acquire the task within the allotted 25 sessions ($F(2,15)=48.997$, $p < .0001$), and 2) a sustained increase in the percentage of "non-adaptive" short-latency CRs (SLCRs) on those trials during which a CR was given. These associative learning deficits occurred in the absence of any significant differences in UCR amplitudes ($F(2,15)=3.395$, n.s.). That is, these were learning rather than non-specific performance deficits. These data suggest that the hippocampus is necessary for establishing the appropriate temporal relationship between the CS and UCS during associative learning.

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354.5

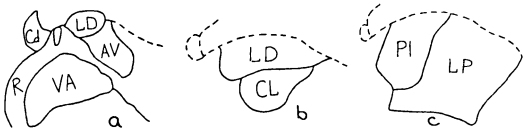
RESPONSES OF CELLS OF LATERODORSAL AND LATERAL POSTERIOR THALAMUS TO AUDITORY STIMULI BEFORE AND AFTER CONDITIONING OF A PAVLOVIAN EYEBLINK REFLEX. O. Melamed* and C.D. Woody (SPON:M.Jarvik). UCLA Med. Ctr., Los Angeles, CA 90024.

Recordings were made from 586 units of the lateral thalamic nuclei and adjacent regions of cats to determine the response to click CS after conditioning relative to levels in the naive state. The pattern recorded from the laterodorsal and central lateral (intralaminar) nuclei was characterized by increased activity in the period 54-480 ms after the CS with repetitive increases in later periods. The pattern recorded from the lateral posterior and anterior pulvinar nuclei was characterized by increased activity in the period 12-320 ms after the CS. Components of unit activity preceding some components of the CR were found at each area. The blink CRs were produced rapidly by pairing click CS with glabella tap and hypothalamic electrical stimulation (Hirano et al Br Res 1987; 570-10ms ISI). The CR depended on the order and interval of stimulus pairing. We have not yet determined if the changes in unit activity in the above regions are associatively induced, but they appear to support discrimination of a forward paired CS from a backward paired DS of comparable intensity. The magnitude of unit response to click was greater than that to hiss after conditioning. Intracellular recordings from 142 units measured excitability by depolarizing current required to produce spiking: naive 0.84±.44 nA SD, cond. 0.82±.40 nA and resting potentials: naive 55.1±10.5 mV, cond. 55.3±8.8 mV. (Support: NS25510.)

354.6

ANATOMICAL STUDIES OF ANTERIOR AND POSTEROLATERAL THALAMIC REGIONS SUPPORTING CONDITIONING OF A PAVLOVIAN EYEBLINK REFLEX IN CATS. V. Chizhevsky* and C.D. Woody (SPON:W.Wyrwicka). UCLA Med. Ctr., Los Angeles, CA 90024.

Intracellular and extracellular applications of PHA-L (with added current passage) were used to define, anatomically, regions of the thalamus from which unit responses to a click CS were increased after eyeblink conditioning. One area (a) was located anteriorly at the borders of the reticular (R), lateral dorsal (LD), ventroanterior (VA), anteroventral (AV), caudate (Cd) and anterior intralaminar nuclei. Another area (b) included the LD and central lateral (CL) nuclei. A third area (c) was located in the lateral posterior (LP) - anterior pulvinar (Pl) complex.



Within these regions three major types of neurons were identified: 1) medium to large (300-500 um^2) size cells with radially arranged, bushy dendrites; 2) small (150-200 um^2) cells with locally ramifying axons; and 3) various (150-500 um^2) size cells with thick, primary dendrites that gave rise to smaller secondary branches. (Supported by NS25510.)

354.7

EXPRESSION OF C-FOS AND PKC IN RAT BRAIN AREAS FOLLOWING SINGLE TRIAL LEARNING. F. Milan*, M.G. Nunzi, A. Negro*, I. Martini*, A. Zanotti* and G. Toffano. Fidia Research Laboratories, 35031 Abano Terme (PD), ITALY

Expression of c-fos proto-oncogene seems to participate in the control of genetic events coupling neuronal activation to information storage. In this study, we investigated c-fos expression in rats after behavioural training in a memory task. Young rats were submitted to the passive avoidance test (PA) and sacrified 1hr after the single learning trial. Sagittal brain sections were processed for in situ hybridization autoradiography of c-fos messenger RNA (mRNA) using a 32P-cDNA probe. In PA animals the hybridization signal was detected in the cerebellum and cortex (frontal and fronto-parietal). C-fos mRNA was not detected in the hippocampus, thus indicating that c-fos expression might be differentially regulated in different areas in relation to behavioural training. In view of the role of protein kinase C (PKC) in mechanisms involved in information transfer and c-fos expression, we further evaluated on the same experimental paradigm the expression of α and β PKC subspecies. While there is no detectable PKCs expression in control rats, both α and β subspecies are expressed in cerebellum and cortex of PA trained animals, their pattern thus overlapping the c-fos expression. Further work is requested to elucidate the biological significance of c-fos expression in memory storage and its relationship with PKCs.

354.8

RED NUCLEUS UNIT ACTIVITY DURING TEMPORARY COOLING OF THE CEREBELLAR INTERPOSITUS NUCLEUS IN CLASSICAL CONDITIONING, D.G. Lavond, S.A. Kanzawa*, R.M. Adams* & A.A. Zhang, Department of Psychology, University of Southern California, Los Angeles, CA 90089-1061.

Unilateral lesion of the cerebellar interpositus nucleus (IP) abolishes ipsilateral classical conditioning. The plasticity associated with learning may be localized in the IP or in its afferents. Neural unit recordings of cerebellar afferents may be used to detect surviving activity associated with learning.

In the present study, the IP is temporarily inactivated by cooling using fuel-injected Freon to a probe with a heated shaft and a thermocouple at the tip. This system allows for precise temperature regulation and for long durations of inactivation.

Rabbits were well trained on classical conditioning (108 paired trials per day; the CS is a 1KHz, 85 dB SPL, 350 msec tone coterminating with 2.1 N/cm^2, 100 msec airpuff UCS, and measuring nictitating membrane extension as the CR/UCR). The IP was alternately cooled or allowed to warm to normal temperature while recording from the red nucleus. Results indicate that unit activity and behavior are abolished for the duration of cooling. These results support the observations of Chapman (1988) using local infusion of lidocaine into IP.

Supported by USC Faculty Research & Innovation Fund.

354.9

SOME PROPERTIES OF TRANSFER OF TRAINING AFTER UNILATERAL CEREBELLAR LESION, S.A. Kanzawa* & D.G. Lavond [SPON: W. McClure], Department of Psychology, University of Southern California, Los Angeles, CA 90089-1061.

Rabbits first trained for classical conditioning on one side then lesioned in the ipsilateral interpositus nucleus show no retention/relearning but quickly learn when switched to contralateral training. Possible explanations are that the memory survives the lesion, that nonspecific memory hastens learning, or that initial learning occurs on both sides.

One group of rabbits was first given unpaired training with CS alone (1 KHz, 85 dB SPL, 348 msec) and UCS alone (2.1 N/cm^2, 98 msec) trials (108/day) for 5 days, lesioned ipsilaterally, then trained with paired trials on the contralateral side. The rabbits took as long or longer to learn than normally, suggesting that nonspecific factors do not account for the savings.

A second group was first given paired conditioning and learned normally, lesioned ipsilaterally, and not retrained ipsilaterally but trained contralaterally instead. There was no evidence of savings.

Savings appears to occur only after specific training after the lesion and does not represent access to a memory that survives the lesion.

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354.10

ROLE OF THE DORSAL AND VENTRAL COCHLEAR NUCLEI IN THE ACQUISITON AND RETENTION OF CLASSICAL CONDITIONING, A.A. Zhang & D.G. Lavond, Departments of Neurobiology & of Psychology, University of Southern California, Los Angeles, CA 90089-1061.

The purpose of this study was to detect whether the dorsal (DCN) or ventral cochlear nucleus (VCN) differentially conveys the auditory conditioned stimulus (CS) to the cerebellar circuitry known to be essential for classical conditioning of the rabbit nictitating membrane response by making lesions and by monitoring auditory evoked potentials.

In acquisiton groups, we first lesioned either DCN or VCN, and later trained the animals by pairing an auditory CS (352 msec, 85 dB SPL, 40 cycle/sec train of clicks) with an airpuff UCS (100 msec, 2.1 N/cm^2). Lesion of either nucleus alone significantly delays learning.

In retention groups, we first trained the rabbits. Early in training the evoked potentials in both nuclei habituated within each trial; after learning there was less intratrial habituation. We hypothesize this might involve the efferent cochlear bundle. After learning, DCN or VCN were lesioned (with NMDA or electrolytically). Lesions of either nucleus alone does not retard subsequent retraining/relearning.

Support: NINCDS NS21853-03 to DGL & NSF BNS8106648, ONR N0001483K0238 & McKnight to R.F. Thompson.

354.11

NEURAL UNIT ACTIVITY IN CEREBELLAR INTERPOSITUS NUCLEUS MODELS CLASSICALLY CONDITIONED EYELID RESPONSES IN THE RAT, R.M. Adams*, A.A. Zhang, & D.G. Lavond (SPON: D. Mitchell). Department of Psychology, University of Southern California, Los Angeles, CA 90089-1061.

In the rabbit, neural unit activity in the cerebellar interpositus nucleus (IP) has been shown to "model" the conditioned nictating membrane response. The present study found that similar activity occurs in the IP of rats during classical eyelid conditioning.

Male hooded rats were prepared with subcutaneous EMG electrodes for recording left eyelid responses and bipolar recording electrodes implanted into the left IP. To help insure proper electrode placement, stimulation current was applied until discrete left eyelid movements were evoked. After one week of recovery, each animal was adapted to the restraining device, then trained to criterion by pairing a white noise CS (350 msec, 85 dB SPL) with a left corneal airpuff UCS (100 msec, 2.1 N/cm^2).

Multiple unit recordings from the left IP revealed the development of activity which paralleled and preceeded the conditioned EMG response. These results suggest that findings regarding the role of the IP in the classical conditioning of rabbits may be generalized to other species.

Supported by USC Biomedical Research Support Grant.

354.12

BILATERAL TRANSFER OF THE RABBIT NICTITATING MEMBRANE RESPONSE UNDER NONASYMPTOTIC UCS SHIFTS. J.C. Greiner*, L.A. Wilson*, and M.M. Patterson. Dept. of Psychology and Coll. of Osteopathic Medicine, Ohio Univ., Athens, OH 45701.

We have examined bilateral conditioned responding (CRs) of the rabbit nictitating membrane reflex to a tone conditioned stimulus (CS) and airpuff unconditioned stimulus (UCS) in a classical conditioning delay procedure. We have found that CRs occur bilaterally, with the level of responding significantly lower in the untrained eye. Also, after conditioning one side to asymptote then moving the UCS to the other eye, the pattern of responding gradually reversed with the now trained eye reaching a high level of CRs and CR frequency in the previously trained eye decreasing (Patterson, Greiner, Hutchins, & Wilson, Neuro Abst., 14:395, 1988; Polenchar, Aluko, Donahue, & Patterson, Neuro Abst., 12:181, 1986). This study determined the pattern of bilateral behavior when the USC was switched prior to asymptotic conditioning. Subjects were given 5 conditioning sessions with the UCS applied to the left eye, 10 sessions with UCS on the right, then 10 sessions with the UCS again on the left. Over the initial 5 sessions the level of CRs for the untrained eye were significantly lower than for the trained eye. After switching the UCS (sessions 6-15), the now trained right eye quickly reached a CR level comparable to initial left eye responding and proceeded to asymptote, while CR percentage dropped dramatically in the left eye. The UCS was then switched to its initial position (sessions 16-25). The CRs for the now trained left eye quickly rose to asymptote while CR level gradually decreased in the untrained eye.

R.F. Thompson and associates have determined the cerebellar dentate/interpositus nuclei to be critical for the formation of the CR (McCormick & Thompson, J. Neurosci., 4:2811, 1984). The present results suggest that learning does not occur independently in both eyes, but that a high degree of communication exists between the dentate/interpositus nuclei of the cerebellar hemispheres.

354.13

RECIPROCAL CONNECTIONS BETWEEN RED NUCLEUS AND INTERPOSED CEREBELLAR NUCLEI IN RABBIT REEXAMINED. M.E. Rosenfield* and J.W. Moore. Dept of Psychol, Univ of Mass, Amherst, MA 01003.

Dry WGA-HRP (Sigma L3892) was implanted by pipette into the right cerebellar anterior interpositus nucleus (NIA) of albino rabbits (method of Mori, J., et al, *Brain Res Bull*, 6:19, 1981) and left *in situ* for 43-52 hours before sacrifice with T61. Animals were perfused transcardially (descending aorta clamped) with approximately 2 L of .9% saline followed by 1 L of 10% formalin and then 3 L of 12% sucrose solution at 4 degrees C. Brains were blocked immediately on extraction (saving only the brain stem and cerebellum), placed in 30% sucrose in .1 M phosphate buffer (pH = 7.2), and stored for 24 hours at 4 degrees C for 24 h. Brain stem and cerebellum were embedded in gelatin; frozen sections were cut transversely at 60 μ, mounted on subbed slides, and reacted with tetramethylbenzidine (TMB). In contrast to our previous report (*Soc Neurosci Abstr*, 14:493, 1988), we made large implants of the label into NIA avoiding cerebellar cortex. We observed fiber and terminal labeling of contralateral red nucleus (RN) but virtually no retrograde cell labeling. Retrograde cell labeling was observed in the contralateral dorsal and medial accessory olivary nuclei (cf. Weiss, C., et al, *Soc Neurosci Abstr*, 14:493, 1988). Implantation of the label in cerebellar cortex (e.g., HVI) resulted in retrograde labeling in contralateral RN and inferior olive. These findings extend those of our previous report and also agree with a study of RN-NIA connections in cat by Walberg and Dietrichs (*Brain Res*, 397:73, 1986) which also used the Mori et al procedure of implanting WGA-HRP. They disagree with studies that do not use this technique (e.g., Conde, F., *J Neurosci Meth*, 21:31-43, 1987). The evident absence of a RN-IP projection is relevant for studies of neural circuits underlying classical conditioning of the rabbit nictitating membrane.

This work was supported by grant AFOSR 86-0182.

354.14

PREFERENTIAL INVOLVEMENT OF THE AMYGDALOID COMPLEX IN ONE-TRIAL AVERSIVE COMPARED TO ONE-TRIAL APPETITIVE LEARNING. Larry Cahill and James L. McGaugh, Center for the Neurobiology of Learning and Memory and Dept. Psychobiology, University of California, Irvine.

This study examined the relative involvement of the amygdaloid complex (AC) in aversive and appetitive learning tasks. We attempted to equate the relative difficulty of the two situations by designing tasks for both that could be learned in only one trial. Male Sprague-Dawley rats received either NMDA-induced lesions of the AC or control surgery one week prior to placement on a water deprivation schedule. This schedule reduced and maintained their weight at approximately 85% of normal. The training apparatus was a Y-maze consisting of a darkened start arm, plus one darkened and one illuminated choice arm, with a water spout at the end of the illuminated arm. On each of four successive days, the thirsty rats were placed in the start arm and allowed to search until they found the water spout. The decrease in latency to find the water between the first and second days served as the one-trial appetitive measure. On the third day, footshock was given when the rat licked the drinking spout, and the rat was immediately returned to its home cage. The subsequent increase in drinking latency between days 3 and 4 was used as the one-trial aversive measure. Results from two separate experiments showed that while the AC lesioned rats showed no impairment in the appetitive portion, they were greatly impaired in the aversively motivated task. This direct comparison of performance by the same subjects in both learning situations suggests a preferential involvement of the AC in aversive over appetitive learning.

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354.15

BILATERAL LESIONS OF THE CENTRAL NUCLEUS OF THE AMYGDALA BLOCK LOUD BACKGROUND NOISE INDUCED STARTLE ENHANCEMENT. S. Campeau* and M. Davis. (SPON: C.A. Sorenson). Dept. of Psychiatry, Yale Univ. Sch. of Med., 34 Park St., New Haven, Ct 06508.

High levels of noise elicit stress-like responses in a variety of species. Lesions of the central nucleus of the amygdala block the ability of various stressors (footshocks, immobilization, loud noise) to alter autonomic, hormonal or behavioral measures of stress. High levels of background noise markedly elevate the amplitude of the startle reflex. It was therefore of interest to examine whether amygdala lesions would block startle facilitation by high levels of background noise. Twenty rats (10 with bilateral lesions of the central nucleus of the amygdala, 5 sham-operated controls, and 5 unoperated controls) were presented with seventy 50-msec startle eliciting air puff stimuli (ISI = 30 sec). After the first 30 stimuli (baseline), an 80-db low frequency background noise was turned on for the rest of the test session. Air puffs rather than noise bursts were used to elicit startle to minimize possible auditory-auditory interactions between background noise and an acoustic startle-eliciting stimulus. Baseline startle response amplitude was comparable across groups, whereas noise enhancement of startle observed in the controls was significantly reduced in amygdala- lesioned animals. These results are consistent with other literature showing that high levels of noise elicit stress-like effects which are dependent upon the central nucleus of the amygdala.

354.16

GLUTAMATE IS PRESENT IN MEDIAL GENICULATE BODY NEURONS THAT PROJECT TO LATERAL AMYGDALA AND IN LATERAL AMYGDALA PRESYNAPTIC TERMINALS. C. Farb, J. LeDoux, and T.A. Milner. Div. Neurobiology, Cornell Univ. Med. Coll., NY, NY 10021.

Projections from medial geniculate body (MGB) to lateral amygdala (AL) have been implicated in the classical conditioning of emotional responses to acoustic stimuli. Recent physiological evidence implicates glutamate (Glu) as a transmitter in this pathway. To identify this anatomic substrate, an antibody to hemocyanin-conjugated Glu was localized in the AL using the peroxidase-antiperoxidase method. By light microscopy, Glu-like immunoreactivity (Glu-LI) was in perikarya and varicose processes throughout the AL. By electron microscopy, Glu-LI was in perikarya, dendrites, axons and axon terminals. Terminals with Glu-LI (0.2-1.5 μm) contained numerous small, clear and 1-3 large dense-core vesicles and formed primarily asymmetric junctions on distal (small) dendrites and dendritic spines. To determine the sources of Glu terminals, retrograde transport of WGA-HRP following an injection into the AL was combined with Glu immunocytochemistry. Within the MGB, many cells containing both Glu-LI and retrogradely transported WGA-HRP were present in the medial MGB, posterior intralaminar nucleus, and suprageniculate nucleus. Electron microscopic analysis of the AL following injections of WGA-HRP into the MGB demonstrated that anterogradely labeled terminals were similar in morphology and types of synaptic associations to terminals with Glu-LI. These data suggest that Glu may be the transmitter in the geniculo-amygdala emotional conditioning circuit. (Supported by NIH 18974 and NIMH 38774).

354.17

LONG-TERM POTENTIATION (LTP) IN THE LATERAL AMYGDALA (AL) IN RESPONSE TO STIMULATION OF THE MEDIAL GENICULATE BODY (MGB). *C. Clugnet, J.E. LeDoux*, Div. of Neurobiology, Cornell Univ. Med. Coll., NY, NY 10021

Projections from the MGB to AL have been implicated in emotional learning. Recent *in vitro* studies have produced LTP in AL by stimulation of the external capsule. In the present study we examined whether LTP could be produced in AL *in vivo* by stimulation of the MGB. Rats (N=12) were anaesthetized with chloral hydrate (7%, ip) and immobilized with d-Tubocurarine chloride (0.12 mg/kg, iv). Extracellular unit responses were recorded in AL during MGB stimulation. Test pulses were single shocks (500 μsec., 300 to 500 μA, 0.3 Hz). Ten train-stimuli consisting of 30 pulses (bipolar, 2 ms apart, 250 μsec half-width, twice the intensity of the test stimulus) at 400 Hz were delivered at 1 Hz. This was repeated five times, once every 5 min. LTP, measured by extracellular recordings of a population spike, was induced in 6 out of 12 rats. The effect lasted from 20 min. to more than 1 hour. Measures of the maximal amplitude of the evoked population spike (average latency 6 ms) showed increases of 100% to 300% over baseline. In two additional rats, kynurenic acid (0.5 μM, icv), a broad spectrum excitatory amino acid antagonist, suppressed responses to test stimuli for 45-60 min. These studies show that LTP can be induced *in vivo* in lateral amygdala by stimulation of the MGB and that normal transmission in this pathway is mediated by excitatory amino acid receptors. Further studies will examine effects of excitatory amino acid receptor blockade (NMDA and non-NMDA) on LTP. Supported by MH38774 and NYHA.

354.18

THE AMYGDALA CENTRAL NUCLEUS AND APPETITIVE PAVLOVIAN CONDITIONING: LESIONS IMPAIR ONE CLASS OF CONDITIONED BEHAVIOR. M. Gallagher, P.W. Graham, and P.C. Holland. Department of Psychology, University of North Carolina, Chapel Hill, NC 27599 and Duke University, Durham, NC 27706.

Rats with bilateral lesions of the amygdala central nucleus (CN) were trained in an appetitive Pavlovian conditioning task. conditioned responses (CRs) that are representative of two classes of behavior were monitored. One type of CR resembled the orienting responses that were elicited by the conditioned stimuli (CSs) prior to pairing with food reinforcement; the other type of CR resembled the behavior elicited by food reinforcement itself. These are referred to as CS-generated and US-generated CRs, respectively. Relative to the control group, the group with CN lesions was impaired in acquisition of CS-generated CRs to both auditory and visual CSs. Orienting responses and habituation to the CSs were, however, comparable for the lesion and control groups. Moreover, the group with CN lesions readily acquired the US-generated CRs. Thus, a specific class of conditioned behavior was impaired by CN damage. Although the effect of CN damage is often characterized as an impairment in fear conditioning, this interpretation cannot account for the present findings. These results, in combination with those of prior investigations, suggest that the CN is part of a neural system which underlies conditioning-dependent potentiation of orienting/alerting behavior.
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MEMBRANE COMPOSITION AND CELL SURFACE MACROMOLECULES II

355.1

LOCALIZATION OF THY-1-LIKE IMMUNOREACTIVITY IN THE RAT AND MOUSE TASTE BUD. R. S. Lasher and P. F. Erickson. Depts. of C and S Biology and Medicine, Univ. Colorado Med. Sch., Denver, CO 80262.

Thy-1 is a major cell surface-associated glycoprotein in neurons and some non-neuronal cell types in vertebrates. The objective of this study was to determine whether or not Thy-1 was associated with the sensory cells in mammalian taste buds. Adult rats and Balb/c mice were perfused with 4% paraformaldehyde, and the tongues were sectioned either on a cryotome or vibratome. Sections of rat tongue were incubated with either OX-7, a monoclonal antibody specific for Thy-1.1, or 1,9C, a monoclonal antibody specific for rat Thy-1. Sections of mouse tongue were incubated with a monoclonal anti-Thy-1.2. Binding of primary antibody was visualized using an indirect immunoperoxidase procedure. Controls included omission of the primary antibodies and use of other monoclonals not specific for Thy-1.

In both rats and mice Thy-1-like immunoreactivity was found to be associated with the plasma membranes of cells in taste buds but not of other epithelial cells. It was also associated with nerve fibers within the epithelium and basal region of the taste buds. In addition, intense immunoreactivity was associated with the lamina propria within valate and fungiform papillae and underlying adjacent epithelium which contained taste buds, but it diminished markedly in more lateral regions of lamina propria. Mast cells also were intensely immunoreactive, and often were found in the lamina propria in the region of the taste buds.

As both 1,9C and OX-7 produced the same pattern of immunoreactivity in rats, it is very likely that the results truly reflect the presence of Thy-1 and are not a result of cross-reactivity with the core protein of rat heparan sulfate proteoglycan which shares a single determinant with Thy-1.1 (Greenspan and O'Brien, 1989, *J. Neurogenetics* 5:25). Thus, Thy-1 is associated with cells in both the adult rat and mouse taste bud. This has not been observed for other types of adult mammalian sensory cells, including olfactory epithelium, hair cells, and rods and cones (eg., Terkelsen et al., 1989, *Anat. Embryol.* 179:311). However, it remains to be determined whether (a) Thy-1 is associated with all the cells in a taste bud, (b) it is developmentally regulated, and (c) the Thy-1 associated with the lamina propria underlying the taste buds plays any role either in the development of the papilla or in directing nerve fibers to the site of the papilla during development and regeneration.

355.2

IMMUNOCYTOCHEMICAL LOCALIZATION OF NEURITIC MONOCLONAL ANTIBODY ON THE CELL SURFACE OF CULTURED RAT DORSAL ROOT GANGLIA. G. E. Goode[1], T. Russell[2], and G. H. DeVries[2], Department of Anatomy and Cell Biology, Eastern Virginia Medical School[1], Norfolk, VA 23501, and Department of Biochemistry and Molecular Biophysics, Medical College of Virginia[2], Richmond, Virginia 23298.

The objective of this study is to test several monoclonal antibodies raised against cultured rat dorsal root ganglion cells for their specificity to surface antigens of cultured neurons, cultured Schwann cells and fibroblasts. Two-week cultures of rat dorsal root ganglion cells, one week cultures of Schwann cells, fibroblasts, and mixed cultures of all three were grown, fixed and immunoreacted in twenty-four well plastic dishes and examined at the light and ultrastructural levels. Four of nine neuritic monoclonal antisera were specific for surface antigens on dorsal root ganglia cell bodies, their neurites and appendages. Electron dense reaction produce (DAB) was most concentrated in associated with neurites and their multivesiculated beaded enlargements. Two of the nine antisera reacted with surface antigen on Schwann cells, but not fibroblasts. A neuron specific antibody will be used to quantify the membrane of neuronal origin derived from axolemma membrane fractionations. Supported by NIH grant NS10821 and National Multiple Sclerosis Society grant RG1818.

355.3

ULTRASTRUCTURAL EXAMINATION OF TOR 23 BINDING IN THE RAT CORTEX AND ANTERIOR PITUITARY E.N. Garrett*, J.M. Kuby*, and P.D. Kushner, ALS Res Center, Pacific Presbyterian Medical Center, San Francisco, CA 94115

The monoclonal antibody *Tor* 23 binds the apparent surface of a subset of neurons in the rat CNS (J. Neurosci. 8: 3035, 1988). We have used pre- and post-embedding techniques for TEM examination. Pre-embedding immunoperoxidase localization confirmed that *Tor* 23 binds the external membranous surface of rare somata in the cortex. At the soma surface *Tor* 23 staining is associated with many but not all neighboring structures, including synapses. Other stained structures in the gray matter are synaptic profiles and fine, unmyelinated processes. An interpretation of the pattern of stain around the soma is that *Tor* 23 stains a soma's environment. Interestingly, *Tor* 23 also binds the cytoplasm of a subset of gonadotrophs (Neurosci. Abs. XIV: 442. 1988). Post-embedding immunogold colocalization of *Tor* 23 and luteinizing hormone (LH) revealed that *Tor* 23 localizes to secretory granules that contain LH. This result suggests that like LH, *Tor* 23 antigen of the pituitary may be secreted. One interpretation of the selective binding specificity of *Tor* 23 in the cortex on rare neurons and in the pituitary within secretory granules is that *Tor* 23 antigen is a secreted molecule with a neurocommunicative function. Funded by the ALS and Neuromuscular Research Foundation and the Department of Health Services, State of California.

355.4

TOR ANTIBODIES BIND SK-N-SH CELLS P.D. Kushner, D. T. Stephenson, E. N. Garrett*, S. Wright* and K. Eagle*. ALS Res. Center, Pacific Presbyt. Med. Center, San Francisco, CA 94115

An important complement to the investigation of novel molecules is a cell line that expresses these molecules. We are using a collection of monoclonal antibodies (MAbs) made to nerve terminals of the electromotor organ of the Torpedo ray (designated *Tor*) to investigate potentially novel neuronal surface molecules. In the present study we report that the human neuroblastoma cell line, SK-N-SH, binds many *Tor* MAbs. SK-N-SH cultures express two cell phenotypes: a small spiny (neuronal) cell and a larger epitheloid-like (flat) cell. Both cells have some biochemical properties of neurons in the undifferentiated state and can interconvert spontaneously. Conditions of differentiation reduce cell division and induce the neuronal cells to have elaborate processes. Differentiated with DMSO, cultures have no flat cells. Differentiated with retinoic acid (RA), cultures are a mixture of flat cells below and neuronal cells on top. Viewed with scanning electron microscopy, neuronal cells have smooth limiting membranes and long, extended processes that branch at defined points and frequently end in growth cones. In RA treated cultures, the neuronal cells express membrane associated acetylcholin-esterase and bind *Tor* 23, a *Tor* MAb of special interest (J. Neurosci. 8:3035, 1988). In conclusion, differentiated SK-N-SH cultures are a system for in vitro expression of *Tor* antigens. Supported by the ALS Association and the ALS and Neuromuscular Research Foundation (San Francisco).

355.5

GLIAL HYALURONATE-BINDING PROTEIN (GHAP) IS BOUND TO HYALURONATE IN VIVO. R. Asher* and A. Bignami. Harvard Medical School and VA Medical Center, Boston, MA 02132.

Glial hyaluronate-binding protein (GHAP) is a CNS-specific glycoprotein of 60 Kd known to bind hyaluronate (HA) in vitro. This latter property formed the basis of its isolation from human white matter (Perides et al., J. Biol. Chem. 264:5981, 1989). Frozen sections of human and dog spinal cord were digested with Streptomyces hyaluronidase in order to determine whether or not GHAP is bound to HA in vivo. Digestion with Streptomyces hyaluronidase prior to conventional staining by indirect immunofluorescence led to a pronounced reduction in the intensity of the staining reaction. Similarly, the protein could be released into the soluble fraction of brain homogenates by hyaluronidase. In these extracts, however, the immunoreactive band was 70 kd rather than 60 kd as purified human GHAP. This band was not detected in identical brain homogenates lacking the enzyme. Also, hyaluronidase digestion had no effect on purified GHAP. In conjunction with immunoelectronmicroscopy showing an extracellular localization of GHAP (Vanderhaeghen et al., in preparation), the finding suggests that a GHAP-HA complex forms the matrix of brain white matter. Supported by NIH grant NS 13034 and the Veterans Administration.

355.6

ABSENCE OF BRAIN SPECTRIN(240/235) IN DENDRITES OF MAMMALIAN BRAIN. S.R. Goodman and I.S. Zagon. Dept. Structural and Cellular Biology, Univ. South Alabama, Mobile, AL 36688 and Dept. Anatomy, Penn. State Univ. Coll. Med., Hershey, PA 17033.

The discovery of non-erythroid spectrin by our laboratory has led to the identification of at least 2 isoforms: brain spectrin(240/235) located in neuronal cell bodies and axons, and brain spectrin(240/235E) located in neural cell bodies and dendrites. Recently Ivy et al. (*Synapse* 2:329, 1988) has reported the presence of brain spectrin(240/235) in Purkinje cell dendrites if tissues were fixed in 4% paraformaldehyde. In experiments examining Ivy et al.'s report, we have utilized rat brain perfused with and without 4% paraformaldehyde, and sections stained with brain spectrin(240/235); both peroxidase and rhodamine served to visualize the primary antibody. Our results reveal *no staining of dendrites* in tissues perfused with and without 4% paraformaldehyde, but neuronal cell bodies and axons were immunoreactive in both preparations. These results confirm earlier light (Riederer et al., *J. Cell Biol.* 102:2088, 1986) and electron (Zagon et al., *J. Neurosci.* 6:2977, 1986) microscopic studies demonstrating that brain spectrin(240/235) is a compartment-specific isoform of spectrin which is enriched in axons and cell bodies but is not present in dendrites.

Supported by NIH Grant NS-21246.

355.7

BIOCHEMICAL MICROHETEROGENEITY OF Cat-301 AND VC1.1 HIGH MOLECULAR WEIGHT ANTIGENS Sam Zaremba[1] Janice Naegele,[2] Dan Geschwind,[1] Colin Barnstable,[2] and Susan Hockfield[1]. [1]Section of Neuroanatomy and [2]Department of Ophthalmology, Yale University School of Medicine.

Monoclonal antibodies Cat-301 and VC1.1 have previously been shown to recognize surface antigens on subsets of mammalian CNS neurons. A 680 kd chondroitin sulfate proteoglycan is responsible for the surface staining by Cat-301 (Zaremba et al., Neuron 2:1207). Three polypeptide bands, of MW 95-105, 140 and 170 kd, were reported to react with VC1.1 (Arimatsu, et al.,J Neurosc 7:1250). We now report that VC1.1 also recognizes a 700 kd antigen. Experiments indicate that Cat-301 and VC1.1 recognize distinct epitopes on partially overlapping sets of high molecular weight (HMW) molecules: (i) Cat-301 immunoprecipitates contain VC1.1 immunoreactivity (and vice versa); (ii) following precipitation to apparent depletion with Cat-301, VC1.1 immunoreactivity remains (and vice versa). The biochemical diversity in these HMW antigens is consistent with immunocytochemical results. Double label immunocytochemistry demonstrates the presence of three different populations of antibody-positive neurons in various CNS regions: Cat-301$^+$/VC1.1$^+$, Cat-301$^+$/VC1.1$^-$, and Cat-301$^-$/VC1.1$^+$. The Cat-301$^+$/VC1.1$^+$ neurons may carry either a single proteoglycan with both epitopes or independent molecules, each with one or the other epitope. These results raise the possibility that these HMW antigens are members of a family of surface molecules unique to particular neuronal subsets.

Supported by grants EY-06511 (SH) and EY-07119 (CB)

355.8

BIOCHEMICAL ANALYSIS OF CORTICAL CELL SURFACE MOLECULES RECOGNIZED BY ANTIBODIES VC1.1 and VC5.1
C. J. Barnstable and J. R. Naegele (SPON. W.H. Miller) Department of Ophthalmology, Yale University School of Medicine, PO Box 3333, New Haven, CT 06510.

The surfaces of subpopulations of CNS neurons are stained by monoclonal antibodies VC1.1 and VC5.1. In cerebral cortex, these correspond to identified subpopulations of GABAergic interneurons. Each antibody recognizes a limited number of different polypeptides. We have now investigated the biochemical nature of these polypeptides.

Deglycosylation of cat cortex membranes with N-glycanase abolished VC1.1 immunoreactivity on Western blots indicating that this epitope is part of an N-linked carbohydrate. Neither the intensity nor the mobility of VC5.1 polypeptides was altered by this treatment.

Species and developmental comparisons of VC1.1 staining on tissue sections and Western blots suggested that the 95-105 kD band recognized by this antibody probably corresponds to a myelin associated molecule such as MAG.

Two other VC1.1 immunoreactive bands with M_r 130-140 kD and 160-170 kD also reacted with a monoclonal antibody against N-CAM polypeptides (5D12). Higher molecular weight VC1.1 immunoreactive bands may include some of the 680 kD chondroitin sulphate proteoglycan also recognized by antibody Cat-301 (see Zaremba et al., this meeting).

Our results demonstrate that specific CNS cell types can create unique cell surface molecular arrays by controlling the carbohydrates expressed on common polypeptide backbones.

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355.9

DISTRIBUTION OF PEANUT AGGLUTININ (PNA) BINDING MOLECULES AT DENERVATED NEUROMUSCULAR JUNCTIONS IN FROGS. T.Somasekhar* C.-P.Ko Dept. Biol. Sci., Univ. So. Calif., Los Angeles CA 90089.

PNA selectively binds to glycoconjugate(s) in the extracellular matrix at neuromuscular junctions (NMJs) and myotendinous junctions (MTJs) in the frog. To examine whether innervation plays any role in the distribution of PNA binding molecules, denervated skeletal muscles of the frog (R.pipiens) were double-stained with rhodamine-PNA and FITC-α-Bungarotoxin. Short-term denervation (1-4 wks) does not affect PNA binding at either NMJs or at MTJs. There was no increase in PNA staining at extrajunctional sites of the muscle, even after 5 months of chronic denervation. However, long-term denervation (3-5 months) results in significant loss of PNA staining at NMJs but not at MTJs. Electron microscopy of long-term denervated muscles shows the absence or depletion of HRP-PNA reaction products at NMJs. HRP reaction products are completely absent at NMJs lacking both nerve terminal and Schwann cell but are present at denervated junctions with persisting Schwann cells. Thus, while denervation does not have an immediate effect, Schwann cells may have a role in the long-term maintenance of PNA-binding molecules at neuromuscular junctions.

355.10

A COMPARISON OF TENASCIN AND PEANUT AGGLUTIN- BINDING MOLECULE (PNA-BM) DISTRIBUTIONS IN FROG SKELETAL MUSCLES. C.-P. Ko, T. Somasekhar*, D.B. Folsom*, & C.-M. Chuong[1]. Dept. Biol. Sci., [1] Dept. Pathol., Univ. So. Calif., Los Angeles CA 90089

Tenascin (J1; cytotactin) is an extracellular matrix molecule found at myotendinous junctions (MTJs) and some neuromuscular junctions (NMJs) (see Sanes, 1989, Ann. Rev. Neurosci. 12:491). We have shown that PNA selectively stains NMJs and MTJs in frogs (Ko, 1987, J. Neurocytol. 16:567). To study if PNA-BMs are related to tenascin and to examine the role of tenascin at NMJs, normal and denervated (1-2 mos.) muscles of frog and developing tadpole muscles were double-stained with fluorescently tagged anti-tenascin antibodies and PNA. In normal frog muscles, tenascin stains MTJs well but only faintly stains some NMJs, as compared with intense PNA staining of all MTJs and NMJs. In denervated muscles, more than 50% of NMJs were brightly stained with anti-tenascin; PNA staining was unchanged. The interstitial areas near denervated junctions were also labelled with anti-tenascin, but no PNA staining was seen. In tadpole muscles, both NMJs and MTJs were stained clearly with both anti-tenascin and PNA. Results suggest that tenascin is not identical to PNA-binding molecules in frog muscles. Furthermore, tenascin distribution at NMJs is related to the state of innervation and development.

355.11

THE DEGRADATION RATES OF TWO CLASSES OF ACETYLCHOLINE RECEPTORS DIFFER IN RESPONSE TO REINNERVATION. S.-L. Shyng* and M.M. Salpeter, Neurobiology & Behavior, Cornell University, Ithaca, NY 14853.

This study compares the responses of two classes of junctional acetylcholine receptors (AChRs) to reinnervation. The two classes are: AChRs which are predominantly synthesized before muscle denervation (referred to as original or slow AChRs), and AChRs which are predominantly synthesized after denervation (referred to as new or fast AChRs). It has been shown in the mouse sternomastoid muscle that the degradation half life (t1/2) of the original AChR decreases after denervation from ~8d to ~3d (Levitt & Salpeter, Nature, 291:239, 1981) and reverts back to ~8d after reinnervation (Salpeter et al., J. Cell Biol., 103:1399, 1986). The new AChRs, on the other hand, have a t1/2 of ~1d (Shyng & Salpeter, J. Cell Biol.,108:647, 1989).

The present study investigates the response of the new (or fast) AChRs to reinnervation. Mouse sternomastoid muscles were denervated by nerve crush and the denervation was maintained by recrushing the nerve every second day for 14 days. All junctional AChRs were inactivated with non-radioactive α-bungarotoxin (BGT) 4 days after the first crush. Newly inserted AChRs were labeled with ^{125}I-α-BGT two days after the last crush. Experimental nerves regenerated 3 to 6 days after the last crush while the control nerves were cut and ligated to prevent regeneration. We found that unlike the original AChRs, once the new AChRs have been inserted into the postsynaptic membrane, their degradation rate (t1/2~1d) does not change in response to reinnervation. The control degradation curve also indicates that although the vast majority (>90%) of AChRs synthesized in denervated muscle are fast AChRs having a t1/2 of ~1d, there may be a small component (<10%) that has a t1/2 of ~3-4d and thus may be slow AChRs. We suggest that innervation and denervation may regulate the ratio of original (or slow) and new (or fast) AChRs that are synthesized, and that the structure of these two classes of AChRs determine whether, once in the postsynaptic membrane, they can further respond to the nerve by modifying their degradation rates.

355.12

DIFFERENTIAL EXTRACTION OF AXONALLY TRANSPORTED PROTEOGLYCANS, J.S. Elam, Florida State University, Tallahassee, FL 32306

This study was designed to differentially solubilize axonally transported proteoglycans by a sequence of extractions that would infer their relationship to nerve terminal membranes. Groups of 15-20 goldfish were injected in one eye with 100 μCi of $^{35}SO_4$ and contralateral optic tecta removed and pooled 16 h later. Tecta were homogenized in 0.25 M sucrose containing protease inhibitors and 1 mM sodium phosphate pH 7.4. Following centrifugation at 100,000 xg for 1 h, the particulate fraction was sequentially suspended and re-centrifuged in (i) homogenization buffer lacking sucrose (lysis wash), (ii) homogenization buffer containing 1 M NaCl, and (iii) homogenization buffer containing 1% Triton-X100 or homogenization buffer containing 1% Triton X100 - 1 M NaCl. Populations of proteoglycans in each fraction were isolated on DEAE ion exchange columns. Results showed that the distribution of transported proteoglycan $^{35}SO_4$ was: original supernatant 24%, lysis wash 15%, 1 M NaCl wash 12%, Triton wash 7% or Triton-NaCl wash 46%, unsolubilized after triton-NaCl 3%. The solubilization pattern obtained suggests that axonally transported proteoglycans are approximately 39% soluble or loosely attached to membrane (original sup+lysis wash), 12% extrinsic membrane proteins (NaCl wash) 7% integral membrane (Triton wash) and 39% integral membrane with salt perturbable attachments [e.g., to cytoskeleton] (Triton-NaCl minus Triton).

355.13

REGULATION OF GLYCOLIPID SYNTHETIC ENZYMES DURING MYOGENESIS IN VITRO. L.D. Cambron* and K.C. Leskawa* (SPON: J. Porter). Dept. of Anatomical Sciences and Neurobiology, University of Louisville, Louisville, KY 40292

During myoblast differentiation in vitro there is a transient increased synthesis of total neutral glycolipids and/or gangliosides during contact and membrane fusion (Leskawa and Hogan, J. Neurochem. 49S:59B, 1987). Using [^{3}H]-Ser as a precursor, clonal murine myoblasts (E63) showed similar increased synthesis during fusion. To better understand regulatory mechanisms involved, glycosyltransferases were assayed during myogenesis. Certain enzyme activities were maximal during myoblast membrane fusion, including GlcCer synthase (GlcT) and GM3 synthase (SAT-1), which then decreased in myotubes. Others sharply increased at cell contact and then decreased during fusion (LacCer synthase (GalT-2) and $LcOse_3Cer$ synthase (GlcNAcT-1)). In contrast, $GbOse_3Cer$ synthase (GalT-6) gradually increased as myotubes formed. Fusion-defective varients (fu-1), cloned from the same parental line (L8), showed similar changes with two exceptions: the contact-related increase in LacCer synthase was absent and GM3 synthase increased 2 days earlier than that seen with E63 cells. The results support suggestions that LacCer is involved in cell contact and that ganglio series structures are minor components in muscle, and further demonstrate that glycolipid synthesis is under complex regulation during differentiation. Supported by NIH grant NS 21057.

355.14

ANTIBODY AND COMPLEMENT MEDIATED DEMYELINATION OF DORSAL ROOT GANGLION CULTURES REQUIRES TERMINAL COMPLEMENT COMPLEXES. S.MANE* and C.KOSKI* (SPON:G.BERGEY.)UNIVERSITY ofMARYLAND SCHOOL of MEDICINE,Baltimore,MD,21201.

In the present work we examined whether terminal complexes of the complement cascade(TCC) were required for in vitro demyelination of dissociated rodent dorsal root ganglion(DRG)myelinated by rodent Schwann cells,by anti-peripheral myelin antibody(anti PNM Ab) from patients with Guillain Barre' Syndrome(GBS). IgM from an acute phase GBS plasma(anti PNM Ab titre,84U/ml),diluted 10-fold in media containing 50μg/ml ascorbic acid was incubated with sets of comparable myelinated DRG cultures in the presence of 20% normal human serum depleted of complement component C7 (C7D-NHS), C7D-NHS+C7, heat-inactivated C7D-NHS+C7 or C7D-NHS in the absence of IgM anti PNM Ab. Demyelination was quantitated by counting damaged and intact myelin segments in 20 high power fields of gluteraldehyde-fixed,Sudan Black stained cultures. Only treatment of cultures with IgM+C7D-NHS+C7 resulted in maximum damage,52.7%,of myelin segments which was significantly greater than the 12-18%seen with C7D-NHS without C7(to inhibit TCC formation),in the absence of Ab, when heat-inactivated C7D-NHS was used to prevent activation of the early complement cascade, or with the media alone.

In summary, our work suggests that the formation of TCC is required for Ab and complement mediated demyelination of this in vitro system.This may be an important mechanism involved in demyelination seen in GBS.

355.15

MONOCLONAL ANTIBODIES DEVELOPED AGAINST MEMBRANE PROTEINS ALTER THE GROWTH OF CULTURED ASTROCYTES. E.E. Geisert, Jr. Department of Cell Biology and Anatomy, Neurobiology Research Center, University of Alabama at Birmingham Medical Center, Birmingham, AL 35294.

In previous studies, a rabbit polyclonal antiserum developed against dissected rat brain white matter was found to alter the growth of cultured neonatal rat astrocytes. There was a dramatic decrease in the mitotic activity of the cells and type 2 astrocytes developed long highly branched processes. To begin defining the antigens responsible for these effects, antibodies were affinity purified from western blots of white matter membrane proteins. Antibodies pulled from proteins ranging in molecular weight from approximately 100 kDa to 160 kDa had similar effects as the original antiserum. These affinity isolated antibodies cross reacted with proteins outside the molecular weight range from which they were isolated. Thus, it was not possible to define a specific antigen responsible for the altered growth of cultured astrocytes using this method. Once the molecular weight range of the antigens was defined, monoclonal antibodies were developed against astrocytic membrane proteins within this molecular weight range. The cell lines resulting from seven fusions were screened for their abilities to bind to living astrocytes and then for their abilities to alter astrocytic growth. This report defines the effects of two of the monoclonal antibodies produced (AMP1 and AMP2). When cultured astrocytes were treated with both of these monoclonal antibodies, the effect was similar to that observed with the original anti-white matter antiserum. On western blots AMP1 stains a band at 120 kDa and AMP2 stains a protein at 190 kDa. Both of these antibodies stain the external surface of cultured astrocytes. Supported by PHS grant NS23613 and the Whitehall Foundation.

355.16

EFFECT OF EXOGENOUS FATTY ACIDS ON MUSCARINIC ACETYLCHOLINE RECEPTORS IN THE NEUROBLASTOMA-GLIOMA HYBRID NG108-15 CELL CULTURE. P. RAY* and W. MIDDLETON* (SPON: J.V. WADE). Dept. of Biol., Div. of Exptl. Theraps., Walter Reed Army Institute of Research, Wash. DC 20307-5100.

The clonal neuroblastoma-glioma hybrid NG108-15 cell line was used to investigate changes in ligand binding of muscarinic acetylcholine receptors (mAChRs) due to addition of fatty acids into the growth medium. Levels of mAChRs were monitored by binding of [^{3}H]-quinuclidinyl benzilate ([^{3}H]QNB) in cell homogenate. Culturing NG108-15 cells for 4-5 days in medium containing unusual proportions (40-80 uM) of polyunsaturated fatty acids (arachidonic, linolenic, linoleic) caused a concentration-dependent decrease (40-70%) in total cellular mAChRs. Upon withdrawal of fatty acids from the medium, the concentration of receptors returned to control levels. Treatment of cells with the antilysosomal agent NH_4Cl (5 mM) along with the fatty acids blocked the decrease of mAChRs. We hypothesize that under the experimental conditions the polyunsaturated fatty acids are incorporated into the cell membrane increasing its fluidity and thereby facilitating the transport of mAChRs to their degradation sites in the lysosomes.

356.1

THERMOGENIC RESPONSES FOLLOWING ADRENALECTOMY IN GENETICALLY LEAN AND OBESE MICE. Cheryl Chancellor-Freeland*, P. Dubuc* and H. Carlisle. (Spon. H. Carlisle). Dept. of Psychology, University of California, Santa Barbara, CA 93106.

Adrenalectomy reverses obesity in genetically obese mice (ob/ob) presumably by correcting deficient energy expenditure. Recent work, however, suggests that adx alone is not sufficient to restore normal energy expenditure, but that dietary restriction must accompany adx. In the present study, obese-adx, obese-intact and lean-intact mice were maintained on a 2.6g/day diet and were then tested in a gradient layer calorimeter for 2 hrs at 5 C to determine whether heat production (O_2 consumption) as well as heat loss were normal.

Contrary to predictions, results did not indicate improved thermogenesis following adx in obese mice. Moreover, there were no significant differences between groups. A follow-up test, however, indicates that both obese-intact and obese-adx groups show higher levels of heat production in the afternoon (12 noon-4 pm) relative to the morning (7-11:30am). Lean mice, on the other hand, seem to generate heat at a constant level independent of a circadian influence.

356.2

RESPONSE OF THE NEURONS OF THE GLOBUS PALLIDUM TO GASTRIC DISTENTION AND EMPTYING. F. Vázquez-Pereyra* and E. Tirado Casique*. (SPON: J.A. Roig). Dept. of Physiology, School of Medicine, U.N.A.M., Apdo. Postal 70-250, 04510 México.

In a previous work, aphagia and adipsia were observed in cats after surgical implantation of chronic electrodes in the globus pallidum (GP). Other authors have reported deaths caused by aphagia and adipsia secondary to bilateral lesions of the GP in rats. These data suggest that the GP might be involved in the behavior oriented towards food ingestion. To dilucidate if the GP receives information about the degree of gastric distention we performed a series of experiments in 9 cats anesthetized with urethane. The unitary activity of the GP was recorded for control and during distention and emptying of the stomach. The recorded activity was plotted as frequency histograms. Of the 39 recorded units, 23 responded to either or both maneuvers; 74% responded to both, distention and emptying of the stomach, and the other 24% responded only to either one. These results support the suggestion that the GP is involved in the processing of the information generated by the degree of gastric distention.

356.3

EFFECT OF NEUROPEPTIDE Y (NPY) ON FOOD INTAKE IN FASTED RATS AND SITE OF ACTION. R.R. Schick, V. Schusdziarra*, B. Mertens*, Ch. Nussbaumer*, M. Classen*, Dept. of Internal Medicine II, Technical Univ. of Munich, D-8000, F.R.G.

Central administration of NPY induces food intake in satiated animals and this effect is mediated by hypothalamic sites. Little is known, however, about the effect and site of action in fasted animals. To examine this further, rats were fitted with brain cannulae aimed at the lateral ventricle (ICV; N=8), the ventromedial (VMH) and lateral hypothalamus (LH) (N=44). After recovery, 24-hr fasted rats were injected with either NPY (10µg/10µl ICV; 1µg/0.5µl tissue) or saline and food intake (F) was recorded. Results: During ICV saline F was 5.9 ± 0.4 g (0-60 min) and 8.3 ± 0.6 g (0-120 min), which was significantly augmented by ICV NPY to 8.7 ± 0.9 g and 14.4 ± 1.5 g ($p<0.05$). This increase in F was due to a 2-fold increase in the time period spent with feeding. Following intracerebral injection of NPY, in 50 % of the histologically identified LH and VMH sites an increase in F (≥20%) was observed (10/20 LH; 5/11 VMH sites). This was statistically not significant, however. Mean F 0-60 min was 4.6 g vs. 3.9 g (LH) and 6.4 g vs. 5.5 g (VMH). Outside the LH or VMH regions, NPY-induced increase in F was observed only in 2/13 sites. Conclusion: NPY effectively stimulates food intake not only in satiated, but also in fasted rats. While this effect may in part be mediated by VMH and LH sites, brain areas other than these have to be examined in view of the highly potent action of ICV NPY.

356.4

THE EFFECT OF NEUROPEPTIDE Y (NPY) ON INTAKE OF FLAVORED SOLUTIONS. M. Grace, W. Welch, C.J. Billington and A.S. Levine VA Medical Center and the University of Minnesota Minneapolis, MN 55417 (SPON: M. Dysken).

Central administration of NPY induces voracious eating, but only limited drinking. We evaluated the effect of intraventricular (icv) NPY injection (5µg) on intake of sucrose (2%), saccharin (0.2%), saline (0.8%) and HCl (0.1M) solutions compared to intake of tap water. There were significant main effects of flavor [$F_{(4,43)}=3.77$, $p=.01$] and drug [$F_{(1,43)}=8.239$, $p=.006$] and a significant interaction [$F_{(4,43)}=2.76$, $p=.04$] over a 4 hour period.

Solution	*Vehicle (ml)*	*NPY (ml)*
Tap Water	1.5 ± 0.2	2.4 ± 0.6
Sucrose	2.3 ± 1.2	10.9 ± 4.2* $p < 0.05$
Saccharin	3.3 ± 1.5	8.8 ± 3.7
Saline	2.5 ± 0.8	4.6 ± 1.5
HCl	0.6 ± 0.1	0.8 ± 0.1

When food was returned to the cage 4 hours after icv NPY injection there was a main effect of drug [$F_{(1,43)}=17.8$, $p=.0001$], but not of flavor on food intake, although the latter approached significance. Food intake (4-6 hr) was not significantly increased after rats ingested tap water, saccharin or saline; but was increased after sucrose and HCl intake. These data confirm the finding that NPY does not have a major effect on water intake in the absence of food; however, NPY does seem to elevate the imbibition of a 2% sucrose solution in the absence of food. (Supported by VA Medical Research and NIDA 1RO1-DA03999)

356.5

THE EFFECT OF INTRACEREBROVENTRICULAR (ICV) NPY ON FOOD REINFORCED BEHAVIOR. D.C. Jewett*, A.S. Levine, J. Cleary*, D.W. Schall* and T. Thompson*. (SPON: G. Maletta). University of Minnesota and VA Medical Center, Minneapolis, MN 55417.

Central administration of NPY results in voracious eating in satiated rats. In the present study we evaluated the ability of NPY to maintain a high level of responding for food. Six rats were trained to press a lever for 45 mg food pellets. The number of responses required for each food pellet was slowly increased to 40 responses (FR 40). A stable low level of responding was maintained in satiated rats. One administration of vehicle (icv) was followed by five successive days of NPY injections in rats trained under the above schedule. There was a main effect of NPY on responding. ($F=4.1$, $p<.01$, $d.f.=5,25$) across days. NPY significantly increased responding, compared to vehicle (10.00 ± 7.4 responses/4 hr), on all but the first day ($p< .05$). Responding peaked on day three of NPY injection at a mean of 5095 (±1159) responses. We then compared these data to those obtained from rats food deprived to 80% of their free feeding body weight. This level of food deprivation resulted in a pattern of responding over the four hour session that was similar to that observed when NPY was given to satiated rats. However, mean total responses were greater under food deprivation (10,194. ± 1040) than under NPY. These data suggest that NPY is a potent stimulator of food intake under conditions requiring a high response cost for each 45 mg food pellet. Patterns of NPY induced responding for food are similar to those produced under conditions of substantial food deprivation.

356.6

A CRITICAL SITE FOR NEUROPEPTIDE Y-INDUCED EATING LIES IN THE CAUDOLATERAL PARAVENTRICULAR/ PERIFORNICAL REGION OF THE HYPOTHALAMUS. B.G. Stanley, W. Magdalin*, and S.F. Leibowitz. The Rockefeller Univ., New York, NY.

Previous work has shown that neuropeptide Y (NPY) stimulates a marked eating response when injected directly into various hypothalamic areas. However, the most effective site has not yet been ascertained. To clarify this, we tested NPY in a wide range of hypothalamic regions using an extremely small injection volume. In satiated adult male rats with implanted guide cannulas, NPY (78 pmol/10 nl) or artificial CSF vehicle (10 nl) was injected through a 33-gauge needle directly into one of 45 evenly-spaced hypothalamic sites (n=3 to 11 rats/site). The results demonstrate that NPY's effect on eating behavior is site specific, with by far the largest responses obtained along the caudolateral edge of the paraventricular nucleus and medial perifornical region. The average food intake obtained from this region was 12.5±1.9 g in 1 hr and 23.0±3.7 g in 4 hrs. NPY injection into sites bracketing this region, less than 1 mm from the critical area, yielded reduced responsiveness, 30% to 67% lower in magnitude, with even smaller responses at greater distances. This suggests that the receptors activated by NPY to induce eating are centered in the lateral paraventricular/perifornical region of the hypothalamus.

356.7

A STRUCTURE-ACTIVITY ANALYSIS OF NEUROPEPTIDE Y-INDUCED EATING BEHAVIOR. W. Magdalin*, B.G. Stanley, A. Fournier[1] and S.F. Leibowitz. (SPON: N.E. Miller). The Rockefeller Univ., New York, NY and [1]INRS, Blvd. Hymus, Point Claire, Quebec.

Porcine neuropeptide Y (pNPY), a 36 amino acid peptide, stimulates a marked eating response when injected into the paraventricular hypothalamus (PVN). To clarify the amino acid sequence required to obtain this response, we compared the effectiveness of various doses of pNPY, human NPY, hNPY free acid, and several C-terminal pNPY fragments (2-36, 5-36, 16-36 & 25-36). Groups of satiated adult male rats, with indwelling guide cannulas in the PVN, were injected with peptide, in 0.3 μl of artificial CSF, and food intake was measured 1 and 4 hrs later. Consistent with our previous results, pNPY (24-235 pmol) elicited a dose-related eating response. Surprisingly, eliminating the N-terminal tyrosine (fragment 2-36) actually increased the potency 2- to 4-fold, while the 5-36 fragment had only a small effect, and the other fragments were ineffective. Human NPY was about 3-fold less potent than pNPY, and its free acid form was ineffective. These findings suggest that the hypothalamic receptors activated by NPY to induce eating may not be identical to those underlying some of NPY's other effects and that these receptors are sensitive to structural changes in both the C- and N-terminal portions of this peptide.

356.8

CONTINUOUS INSULIN INFUSION INTO LATERAL VENTRICLES OF SHEEP FOR THREE DAYS DID NOT EFFECT FOOD INTAKE. L.A. Foster*, K.N. Ames*, R.S. Emery*(SPON. C. Sisk).Dept. of Anim. Sci. Michigan State Univ. E. Lansing MI 48824

In monogastrics insulin infusion into CSF decreases intake. Our objective was to determine if ruminants respond similarly.

Lateral ventricles were cannulated in 14 sheep. Surgeries (Sx) were done on two sheep at a time. Following Sx both sheep were infused with artificial CSF (ACSF) at the same rate for a minimum of one week via either osmotic mini-pumps (134 ul/day) or autosyringes (.7 -1.7ml/day). At the end of the baseline period, porcine insulin (3-5mU/kg/day) was added to the ACSF of one sheep for three days and the other sheep remained on ACSF. There were no differences for intake means between treated and controls (paired T test; $p<.7$; SED = .3kg/day). Concentrations of insulin in a CSF sample from the cistern magna from: control sheep was .0013mU/ml (sensitivity of assay);a treated sheep was 1.2mU/ml.

356.9

INSULIN AND 2-DEOXYGLUCOSE HAVE DIFFERENTIAL EFFECTS ON GASTRIC MOTILITY IN RATS. Robert K. Cato*, Loretta M. Flanagan, Joseph G. Verbalis, Edward M. Stricker. Departments of Behavioral Neuroscience and Medicine, University of Pittsburgh, Pittsburgh, PA 15260.

Pharmacological doses of insulin (3-25 U/kg, sc) elicited feeding and increased gastric motility in rats. In contrast, 2-deoxy-D-glucose (2-DG) had dose-dependent effects on gastric motility; doses of 100 and 200 mg/kg ip, which do not affect food intake, caused pronounced increases in gastric motility, whereas 500 mg/kg 2-DG, which reliably elicits food intake, caused a virtual elimination of gastric motility. Thus, unlike insulin, 2-DG does not produce parallel effects on food intake and gastric motility in rats. Like cholecystokinin (CCK) and LiCl, which also abolish gastric motility in rats, 500 mg/kg 2-DG increased pituitary oxytocin (OT) secretion whereas neither the smaller doses of 2-DG nor insulin-induced hypoglycemia had this effect. Also, pretreatment with 5 mg/kg naloxone, which potentiates the inhibition of gastric motility and the stimulation of OT secretion induced by CCK and LiCl, similarly enhanced both of these effects of 2-DG but had no effects in insulin-treated rats. These results suggest that 2-DG has a dual effect on gastric motility, consisting of an insulin-like excitatory component and a CCK/LiCl-like inhibitory component that appears only at the higher doses and is mediated by the paraventricular nucleus of the hypothalamus.

356.10

CIRCADIAN RHYTHMS OF INSULIN SECRETION RATES AND GROWTH HORMONE IN EATING DISORDERS. J. Licinio, K.A. Halmi, P.E. Stokes. Department of Psychiatry, Cornell University Medical Center, White Plains, NY 10605.

Insulin is known to participate in the regulation of eating behavior and body weight in animals. We will present data from 4 eating disorder patients and 2 normal controls studied under the following four protocols: a) analysis of C-peptide and insulin decay curves following bolus injection of those peptides to assess individual distribution parameters of each peptide; b) using C-peptide as a marker of insulin secretion, the parameters obtained in protocol "a" are used in an open two-compartmental mathematical model to estimate insulin kinetics in response to mixed meals. The 24-hour circadian rhythms of pancreatic insulin secretion and insulin systemic distribution are then compared to the simultaneously measured circadian rhythms of growth hormone and cortisol sampled every 15 minutes; c) measurement of insulin kinetics in response to a standard oral glucose load; d) intravenous glucose tolerance test, which will be used to assess insulin sensitivity with the use of Richard Bergman's minimal model. Our acutely-ill normal-weight bulimic patients were more glucose intolerant and had higher insulin secretion rates and higher systemic distribution rates as well as higher number of peaks of growth hormone than recovered bulimics or normal controls.

356.11

HIGH LEVELS OF DIETARY FAT REDUCE FEEDING RESPONSES TO BOTH 2-DEOXY-D-GLUCOSE (2DG) AND MERCAPTOACETATE (MA). J. Taylor* and S. Ritter (SPON: F.Young). Dept. of VCAPP, Washington State University, Pullman, WA 99164-6520.

The level of fat in a rat's maintenance diet appears to alter feeding responses evoked by blockade of either glucose utilization ("glucoprivic feeding") or fatty acid oxidation ("lipoprivic feeding"). It has been suggested that elevation of dietary fat enhances lipoprivic feeding, but attenuates glucoprivic feeding. To understand more fully the relationship of dietary fat to the expression of these metabolic controls of food intake, we systematically examined the effect of dietary fat on feeding responses to 2DG-induced glucoprivation and MA-induced lipoprivation. Adult male rats adapted to one of 3 levels of dietary fat (10%, 40% or 60% of caloric content) for at least 2 wks were tested with 2DG (0, 50, 100, 200 and 400 mg/kg, s.c.) and MA (0, 400, 600 and 800 umol/kg, i.p.). Intake of the maintenance diet was measured hourly for 6 hrs during the light portion of the photoperiod. We found that total calories ingested in response to 2DG was inversely related to dietary fat content. In rats ingesting 60% fat, 2DG did not stimulate feeding significantly at any dose. MA stimulated food intake only in rats ingesting 40% fat but did not stimulate feeding in rats maintained on either the 10% or the 60% fat diets. Thus, high levels of dietary fat appear to suppress both glucoprivic and lipoprivic feeding, but the mechanisms responsible for these effects are not yet understood.

356.12

GLYCEMIC, INSULIN AND FEEDING RESPONSES TO DUODENAL GLUCOSE INFUSION IN THE RABBIT. P.J. Geiselman, L. O'Farrell, D.S. Gray*, A. Acevedo-Cruz*, T. Teruya* and Y. Teruya*. Dept. Psychol and Neurosci. Prgm., UCLA; Div. Diab., USC Sch. Med., Los Angeles, CA 90024.

Rabbits were given duodenal infusions of 0.3M glucose or 0.15M NaCl at a fast (3ml/min) or a slow (1ml/min) rate, and serial samples of blood were collected from jugular cannulae.

Fast duodenal glucose infusion produced a sharp increase in insulin levels followed by a precipitous decline in glycemic levels when food was not presented. When food was present, rabbits increased their food intake and, thus, did not show a decrease in glycemic levels. After slow duodenal glucose infusion, insulin levels showed only a moderate increase, which failed to decrease glycemic levels. When food was present in this condition, rabbits suppressed feeding.

These data are consistent with the hypothesis that hunger can be mediated by an insulin-induced decrease in blood glucose. Whereas, insulin levels that are insufficient to produce a significant reduction in glycemic levels appear to mediate satiety.

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356.13

OPIOID RECEPTOR SUBTYPE MEDIATION OF HYPERPHAGIA IN RATS FOLLOWING EXPOSURE TO A HIGH FAT DIET . A. K. Islam and R. J. Bodnar, Dept. of Psychology, Queens College, CUNY, Flushing, NY 11367.

Selective antagonists can specify the roles of different opioid receptor subtypes in various feeding models. By comparing the relative efficacies of naloxone (NAL), a short-acting, nonspecific opioid receptor antagonist, and naloxonazine (NAZ), an irreversible μ-1 opioid receptor antagonist, our laboratory has implicated the μ-1 receptor subtype in the opioid modulation of free feeding, deprivation-induced feeding and morphine hyperphagia, but not opioid hyperphagia induced by glucoprivation or agonists of kappa and delta receptor subtypes. The present study evaluated the opioid receptor subtype mediating short-term (2 h) hyperphagia following exposure to a high fat diet (67% Purina mash, 33% Crisco) by comparing the inhibitory effects of NAL (0.1-10mg/kg, IV; 1-5 μg, ICV, 20 min and 24 h pre), NAZ (10 mg/kg, IV, 24 h pre), and ß-funaltrexamine (ß-FNA: 10 μg, ICV, 18 h pre), an irreversible μ receptor antagonist, relative to vehicle injections. Significant dose-dependent inhibitions of high fat diet intake occurred following systemic (68% decrease) and central (51% decrease) NAL injections. While ß-FNA significantly reduced ingestion of the palatable diet by 37%, NAZ (8% increase) failed to alter intake. These data indicate that central μ-2 and non-μ opioid receptor subtypes are involved in the opioid mediation of hyperphagia following exposure to a high fat diet.

356.14

CENTRAL MEDIATION OF TASTE AVERSIONS PRODUCED BY MORPHINE, NALOXONE, LITHIUM, METHSCOPOLAMINE, AND SCOPOLAMINE IN RATS. G. Carre, R.E. Filyer*, V.L. Grant*, G.M. Martin*. Dept. of Psychology, Memorial Univ. of Newfoundland, St. John's, NF, A1B 3X9

Eight experiments studied conditioned taste aversions (CTA's) produced by drugs (apomorphine, lithium chloride, methscopolamine, morphine, naloxone, and scopolamine) administered to the ventricles. Control injections were delivered intraperitoneally (IP) and infused in the cerebellum, with the exception of apomorphine which was only given IP. When infused in the cerebellum, only a high dose of methscopolamine produced a reliable CTA. When administered IP, all drugs induced CTA relative to saline controls. When infused in the fourth ventricle all drugs with the exception of apomorphine produced CTA relative to saline controls. These findings, combined with those from area postrema ablation studies, suggest that the area postrema is one chemosensory site at which lithium chloride and methscopolamine induce CTA. Morphine, naloxone, and scopolamine may also induce CTA at sites neighboring the fourth ventricle. Apomorphine appears to induce CTA at a site distant from the fourth ventricle.

356.15

INJECTION OF OPIOID ANTAGONISTS INTO THE PARAVENTRICULAR NUCLEUS : EFFECTS ON DEPRIVATION-INDUCED FEEDING. A.S. Levine, M. Grace*, W. Welch*, C.J. Billington* and P.S. Portoghese. VA Medical Center and University of Minnesota, Minneapolis, MN 55417.

Previously, we and others have demonstrated that PVN injection of opioid agonists increases feeding and PVN injection of naloxone decreases feeding. In the present study we administered opioid antagonists of the kappa, delta and mu receptors into the PVN of 24 hour food deprived male rats. Nor-binaltorphimine (nor-BNI) had a main effect on food deprivation-induced feeding [$F_{(3,67)} = 6.93$, $p = .004$]. Doses of 15 (68.4 ± 6.5%), 10 (76.4 ± 9.2%) and 1 nmol (81 ± 9.9%) nor-BNI significantly decreased food intake compared with vehicle injected controls during the first hour of the study ($100 \pm 2.9\%$, $p < 0.05$). There was no significant main effect of the delta antagonist naltrindole (1 nmol: 108 ± 10.7%) on deprivation-induced feeding (100 ± 10.9%). There was a tendency for the antagonist ß-funaltrexamine (ß-FNA) (10 nmol: 76.8 ± 4.9%, $p = 0.057$) to decrease feeding when injected 24 hours prior to presentation of food. The non-specific receptor antagonist ß-chlornaltrexamine dihydrochloride (ß-CNA) (10 nmol: 48 ± 9.7) potently decreased deprivation-induced feeding (100 ± 14.9, $p = .012$) when injected into the PVN 24 hours prior to administration of food. These data support the concept that the PVN and the kappa opioid receptor play important roles in opioid-induced feeding. (Supported by VA Medical Research and NIDA 1RO1-DA03999)

356.16

CENTRAL INJECTIONS OF MU AND DELTA OPIOID AGONISTS STIMULATE SALINE INTAKE IN NON-DEPRIVED RATS. B.A. Gosnell, D.D. Krahn and M.J. Majchrzak. Dept. of Psychiatry, Univ. of Michigan, Ann Arbor, MI 48109-0116.

Endogenous opioid peptides are thought to play a role in mediating the palatability or rewarding aspects of sweet tastes. There is some evidence, however, which suggests that opioids may also influence the preference for the taste of salt (Bertino et al., Pharmacol. Biochem. Behav. 29:617, 1988; Cooper and Gilbert, Psychopharmacol. 84:362,1984). In the present studies, we measured the effects of central administration of the mu agonist [D-Ala^2,$MePhe^4$, Gly-ol^5]enkephalin (DAGO) and the delta agonist [D-Thr^2]Leucine enkephalin-Thr^6 (DTLET) on the ingestion of salt solutions. In non-deprived rats given a choice of water and 0.6% saline, icv injections of DAGO (1 and 3 nmols) significantly increased the intake of 0.6% saline; mean 2 hr saline intakes were 1.6, 6.6 and 8.5 mls for the 0, 1 and 3 nmol doses, respectively. Baseline water intake was minimal and was unaffected by DAGO. DTLET (1 and 3 nmols) also significantly increased 0.6% saline intake; mean 2 hr intakes were 1.1, 6.2 and 8.3 mls, for the 0, 1 and 3 nmol doses, respectively. In rats given a choice between water and 1.7% saline, DAGO and DTLET stimulated both water and saline intake; total volume ingested (water + 1.7% saline) was equal to or less than the amount ingested when 0.6% saline was available. The effect of naloxone (icv) was measured in rats on a deprivation schedule in which water and 0.6% saline were available for only 2-3 hrs/day. Naloxone (20 and 50 ug) significantly decreased 0.6% saline intake (1 hr intakes were 27.5, 19.5 and 14.8 mls, respectively, for the 0, 20 and 50 ug doses); baseline water intake was low (3-5 mls) and was unaffected by naloxone. These results suggest a role for central mu and delta opioid receptors in mediating the preference for salt solutions.

Supported by NIDA Grant DA05471.

356.17

LATERAL HYPOTHALAMIC STIMULATION-INDUCED FEEDING THRESHOLDS ARE INCREASED FOLLOWING CHRONIC EXPOSURE TO NALTREXONE. *S. Uysal* and E. E. Coons.* Department of Psychology, New York University, New York, NY 10003.

Several laboratories have demonstrated that chronic opioid receptor antagonism results in increased numbers of brain opioid receptors and supersensitivity to exogenous opiates and endogenous opioids. We expected that subsequent to chronic naltrexone treatment (two 30 mg pellets implanted s.c. for nine days), thresholds for inducing feeding by electrical stimulation of the lateral hypothalamus (LH) would be reduced since a) LH stimulation-induced feeding (SIF) is opioid mediated (Carr & Simon, 1983), b) opiates injected into the ventral tegmental area (VTA) potentiate LH SIF (Jenck et al., 1986), and c) VTA opioid receptors upregulate greatly in response to chronic naltrexone treatment (Tempel et al., 1984).

SIF thresholds were assessed for each animal under the following conditions: 1) baseline, 2) acute naloxone, 3) acute naltrexone (NTX), 4) during chronic NTX treatment, 5) after chronic NTX treatment, and 6) during acute NTX challenge on the first day after NTX pellet removal. Thresholds were determined four times during each feeding test.

Acutely, naloxone and NTX both increased mean SIF thresholds, naloxone being more potent. Within a test session, thresholds progressively increased, as previously demonstrated (Carr et al., 1987). Mean SIF thresholds also were elevated during chronic NTX treatment. However, unlike acute NTX, chronic NTX uniformly elevated thresholds within a test. After removal of NTX pellets, SIF thresholds were elevated uniformly within a test; during acute NTX challenge thresholds progressively increased. Furthermore, the rate at which acute NTX elevated SIF thresholds was increased following chronic NTX treatment. Within 3-4 days of NTX pellet removal mean SIF thresholds returned to baseline levels.

356.18

CNS REGIONAL CHANGES IN ^{3}H-DIPRENORPHINE BINDING FOLLOWING ELECTRICALLY ELICITED FEEDING IN THE RAT. E.A. Stein[1], K.D. Carr[2] and E.J. Simon[2]. Depts. of Psychiatry, Medical College of Wisconsin, Milwaukee, WI 53226[1] and NYU Medical Center, New York, NY 10016[2].

Feeding induced by both natural cues, as well as lateral hypothalamic electrical stimulation (ESLH) can be inhibited by opiate antagonists and antibodies to opioid peptides. In contrast, opiate agonists have been reported to increase food intake. The central sites at which opioid peptides modulate feeding and how they play a role in its maintenance is not well understood. To address these issues, we compared regional ^{3}H-Diprenorphine (^{3}H-Dpr) binding in rats a) getting ESLH and allowed to eat, b) ESLH in the absence of food, and c) implanted rats receiving no ESLH. On the final day of testing, ^{3}H-Dpr (0.002 mg/kg) was injected IV following trial 5 of a 10-trial session. Rats were subsequently sacrificed and brains prepared for autoradiography. Since we utilized an *in vivo* procedure, peptide released as a consequence of the behavior will occupy receptors and lead to a decrease in ^{3}H-Dpr binding. Independent of food intake, ESLH caused increases in binding in the frontal cortex, cingulate, bed nucleus of the stria terminalis, medial habenula and zona incerta, and decreases in the claustrum, nucleus accumbens, lateral hypothalamus, ventral tegmentum and caudate. In addition, eating decreased binding in the medial prefrontal cortex and basolateral amygdala and increased in gustatory cortex. Results are discussed in view of the role of opioids in consummatory behavior as well as structures known to be involved in motivated behaviors. Supported by grants NS08265 (EAS), DA03956 (KDC) and DA0016 (EJS).

356.19

ROLE OF PARAVENTRICULAR NUCLEUS MEDIATING OPIATES' ACTION ON POLYDIPSIA: STUDY OF INBRED MICE. I. Nagatomo*, T. Katafuchi* and K. Koizumi. Dept. of Physiology, SUNY Health Science Center at Brooklyn, NY 11203.

We have previously reported that excessive drinking (5-8 times of normal mice) of inbred mice, STR/N, which is not caused by a lack of vasopressin or kidney dysfunction, is related to abnormality in the brain opiate system (1). In the present study effects of direct injections of an opiate antagonist, naltrexone methobromide, to the PVN were found to be more specific than that of the drug given to the lateral ventricle; a much smaller dose was effective in attenuating spontaneous drinking only in STR/N, and even at a higher dose the drug did not affect food and water intake of control mice. In brain slice preparations effects of opiates on activity of PVN neurons were examined. Morphine added to the bathing medium (10^{-6}M) supressed neural activity of 44% of PVN cells tested in STR/N, while in control mice 62% were inhibited. Threshold for the inhibitory action was 10^{-7} to $^{-8}$M for STR/N compared to 10^{-8} to $^{-9}$M in controls. No neuron was excited by the opiate in both strains. Naloxone blocked morphine's action on PVN neurons, but had no direct effect. Dynorphin added to the bathing medium at 10^{-6}M inhibited only ¼ of PVN neurons in polydipsic strain, while ½ of cells were inhibited in controls. Our results indicate that the PVN, rather than the AV3V (1), may be more involved in the primary polydipsia. (Supported by a grant from USPHS NS- 00847). (1) Abst. Soc. Neurosci. 1988 14: 1106.

REGULATION OF AUTONOMIC FUNCTIONS IV

357.1

NEURAL PATHWAYS INVOLVED IN THE CONTROL OF PERIPHERAL ENERGY HOMEOSTASIS. AB Steffens*, B Balkan*, PGM Luiten, and AJW Scheurink*. (SPON: AJ Sipols). Dept of Animal Physiology, University of Groningen, 9750 AA Haren, The Netherlands. Dept of Psychology, Univ of Washington, Seattle WA, 98195 USA.

Areas within the CNS that contribute to the regulation of food intake are also involved in the regulation of blood glucose and plasma FFA levels in resting and exercising rats. Particularly hypothalamic areas such as the ventromedial (VMH), the lateral (LHA) and the paraventricular (PVN) hypothalamus play an important role. The regulation of peripheral glucose and FFA levels is mediated both directly via neural pathways and indirectly via hormones; e.g., glucagon, insulin, epinephrine, norepinephrine, corticosterone, and CCK.

Neuroanatomical data reveal the intrahypothalamic connections and pathways between the hypothalamus and the motor areas of both the sympathetic system (the intermediolateral column of the spinal cord) and the parasympathetic system (the dorsal motor nucleus of the vagus and the nucleus ambiguus in the brain stem).

Physiological data suggest that the hypothalamus can regulate blood glucose, plasma FFA, and insulin independently of each other. For example noradrenergic stimulation of the VMH leads to a concomitant increase of glucose, FFA, and insulin levels into the blood whereas noradrenergic stimulation of the LHA causes an increase in glucose and insulin and a decrease in plasma FFA. Experiments dealing with selective adrenoceptor blockade in distinctive hypothalamic areas of exercising rats, and glucose tolerance tests in pair-fed VMH-lesioned rats, provide additional evidence for the important role of the hypothalamus in the control of peripheral energy homeostasis. (Supported by grants from the Netherlands Organization of Scientific Research).

357.2

MELATONIN PROTECTS HAMSTERS AGAINST THE HYPOCLYCEMIC EFFECT OF D-AMPHETAMINE. B.G. Ortega - Corona, N. Esparza -Avalos* and F. Antón-Tay. Unidad de Investigación Biomédica, IMSS, Instituto de Biología, UNAM and Biología de la Reproducción, UNAM, Iztapalapa, MEXICO 06760.

Amphetamine (AMPH) LD_{50} was determined in golden hamster, Cricetus auratus, kept for two weeks previously under standard control conditions. The effect of either a single melatonin dose (1 or 10 mg/Kg 2 ½ hs before experiments or a similar dose given for 3 days previous to the experiment was studied. Glucose plasma levels were enzymatically determined and animal survival rate was assesed 3 hs after AMPH administration. AMPH treated animals showed low levels of plasma glucose (12 $\pm$ 1.2 mg/dl) and a lower survival rate than the melatonin treated ones. Glucose levels in the melatonin treated animals at either 1 or 10 mg/Kg dose and under experimental conditions were 73 $\pm$ 6.6 mg/dl, $p < 0.001$. Our results suggest that dead after a LD_{50} dose of AMPH is at least partially due to hypoglycemia and that melatonin could exert its protective effect by counteracting this lowering of the plasma glucose.

357.3

ONLY THE μ OPIATE RECEPTOR MEDIATES HYPERGLYCEMIA AFTER 3rd VENTRICULAR OPIOID PEPTIDE INFUSIONS IN RATS. M.W. Gunion, M.J. Rosenthal, J.E. Morley, S. Miller, and B. Zib. GRECC, Sepulveda VAMC, Sepulveda, CA 91343.

Three experiments were performed. In all experiments, hypothalamic 3rd ventricle infusions were given through chronic cannulae implanted at least one week prior to testing; blood samples (120 ul) were taken from the tail tip 0, 15, 30, 60, 90, and 120 min postinfusion. In Expt. 1, the agonists DAGO (μ), β-endorphin ($\mu+\epsilon$), DSLET (δ), and dynorphin (k) (0,.3,1,3,10 nmol/rat in 0.9% NaCl+0.1% BSA) were tested for effects on glucose, free fatty acids, and corticosterone. Both DAGO and β-endorphin increased glucose and corticosterone; neither affected free fatty acids. Dynorphin transiently elevated glucose, and caused prolonged increases in free fatty acids and corticosterone. DSLET had absolutely no effect on any measure. In Expt. 2, naloxone (1 mg/kg, sc, 20 min prior to 3rd ventricle infusion) suppression of the effectiveness of DAGO (3 nmol), β-endorphin (10 nmol), and dynorphin (10 nmol) was tested. While the elevations of glucose and corticosterone caused by DAGO and β-endorphin were blocked, there was no alteration of the effects of dynorphin; that is, the dynorphin effects were not mediated by opiate receptors. Expt. 3 tested whether the ϵ receptor mediated the β-endorphin effects. The μ-specific antagonist β-funaltrexamine (20 μg, 3V) was given 24 hr prior to testing with DAGO (3 nmol) or β-endorphin (10 nmol). Both peptides produced significant hyperglycemia in vehicle-pretreated rats, but neither had any discernible effect in rats pretreated with the μ-receptor blocker. In sum, although there may be at least four opiate receptor types in rat brain (μ, ϵ, δ, k), only the μ-receptor mediates hyperglycemia after infusions of opioid peptides into the 3rd ventricle. [Supported by Veterans Administration funds (MWG, MJR), NS20660 (MWG), and AG04793 (MJR).]

357.4

ELECTROPHYSIOLOGICAL RECORDING FROM NTS NEURONS WHICH RECEIVE AFFERENT INPUT FROM ABDOMINAL CHEMO- AND MECHANORECEPTORS W.B. Laughton and L.A. Campfield Department of Neurobiology and Obesity Research, Hoffmann-La Roche Inc., Nutley, NJ 07110

Extracellular single-unit recordings have been obtained from afferent neurons in the nucleus of the solitary tract (NTS) of chloralose/urethane anesthetized male, Sprague-Dawley rats (350g-450g). Recordings were made through single and multi-barreled glass micropipettes (tip diameter 1-2u). Specific, transient alterations in hepatic-portal glycemia, systemic glucose levels, and intragastric pressure were experimentally induced. NTS neurons were identified which responded either to one of these experimental manipulations, or in some cases, to more than one manipulation. Both increases and decreases in neuronal firing rate were seen (in different neurons) following alterations in these parameters; the two types of responses were seen with approximately equal frequency. These changes in patterns of firing in afferent pathways provide the substrates for central nervous system monitoring of peripheral metabolic state. The integration of these signals by the central nervous system plays an important role in the overall regulation of energy balance.

357.5

AREA POSTREMA LESIONS ALTER GLUCAGON RESPONSES TO GLUCOSE LOADS. B.J. Davis, W.Williams and M.L. Mangiapane, Departments of Neurology, Neurobiology and Anatomy, and Pharmacology, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642.

The area postrema (AP) is thought to be part of the autonomic circuitry of caloric homeostasis. Lesions of the AP alter body weight maintenance levels and glucoprivic feeding responses (Contreras, et al, Fed. Proc. 43, 1984). In the present study we assessed the effects of bilateral AP lesions on plasma glucose, insulin and glucagon responses to an oral glucose load (OGL). Young adult male Sprague-Dawley rats were given electrolytic lesions of the AP. Sham lesioned rats served as controls. All rats were tested on day 7 and again on day 25 after surgery. After an overnight fast, rats were given150 mg glucose/100 gm body weight. Blood was withdrawn at 0 minutes (fasting level), and at several time points after OGL. Plasma glucose, insulin and glucagon levels were determined for each time point.

At 7 days following lesions, sham and AP rats showed comparable glucose levels at 0,2, 60 and 120 minutes following OGL. However, at 30 minutes, glucose levels of AP rats were significantly higher than in shams. Likewise, insulin levels were comparable in AP rats and shams at 0,2, 60 and 120 minutes with a higher insulin level at 30 minutes in AP rats. At 7 days, glucagon responses of the two groups differed only at 2 minutes following OGL with AP rats showing significantly lower levels.

At 25 days, AP rats showed fasting hypoglycemia, but glucose and insulin responses were comparable between shams and AP rats at all other time points. In contrast, AP rats showed significantly elevated glucagon levels with respect to shams at all time points following OGL except at 2 minutes. We conclude that removal of the AP alters the endocrinology of glucose homeostasis and that metabolic alterations following AP lesions may be related to altered glucagon responses. Supported by AG05937 and HL32981.

357.6

BROWN ADIPOSE TISSUE (BAT) ACTIVATION OF RATS FOLLOWING VENTROMEDIAL HYPOTHALAMIC (VMH) STIMULATION. J.A. Thornhill, I. Halvorson, L. Gregor and M. Desautels. Dept. of Physiology, Univ. of Saskatchewan, Saskatoon, Sask., Canada S7N 0W0.

Interscapular brown adipose tissue (T_{IBAT}), colonic (T_C), surface tail temperatures (T_t), and blood pressure (BP) were monitored in urethane-anesthetized male Sprague-Dawley rats before and after repeated (3) electrical stimulation of the VMH. The VMH of various age-matched, cold-acclimated (C.A., kept at 4°C 3 weeks prior to testing) or warm-acclimated (W.A., kept at 22±1°C) was stimulated (120 μA, 50 Hz, 0.5 msec duration pulses) for 30 sec. Intravenous (iv) propranolol HCl (2.5 mg/kg) was given 10 min prior to the last VMH stimulation. VMH stimulation to the W.A. groups did not significantly increase T_{IBAT}s or T_Cs, compared to pre-injection control values, though T_{IBAT}s were significantly raised in other W.A. rats following iv infusion of norepinephrine HCl (40 μg/ml). C.A. rats showed a significant rise in T_{IBAT}s, above those of T_Cs, following VMH stimulation, effects abolished upon the third VMH stimulation following propranolol administration. GDP binding of isolated BAT mitochondria of C.A. groups was significantly increased from age-matched W.A. animals. Results indicate that C.A. increases the thermogenic capacity of BAT such that VMH stimulation can evoke a significant rise in T_{IBAT} above that of T_C, effects not detected in W.A. rats. Supported by MRC of Canada.

357.7

THERMOGENESIS IN BROWN ADIPOSE TISSUE IS MODULATED BY THE RETINOHYPOTHALAMIC SUPRACHIASMATIC COMPLEX. S. Amir, CSBN, Concordia University, Montreal, Canada, H3G 1M8.

The hypothalamic suprachiasmatic nuclei (SCN) regulate several functions concerned with the maintenance of energy balance, including feeding, corticosterone secretion and glucose metabolism. Another mechanism of energy balance regulation that might be under SCN control is heat generation in brown adipose tissue (BAT). The present study shows that stimulation of SCN neurons by microinjection of the excitatory amino acid glutamate (100 mM-1 M, in 0.25 ul), activates interscapular BAT (IBAT) thermogenesis in rats. Similarly, electrical stimulation of the retinohypothalamic projection (RHP) (a 30 sec train of square wave pulses, 50 pulses per sec, pulse duration 0.5 msec, intensity 100-400 uA), which leads to release of glutamate in the SCN, activates IBAT thermogenesis. The effect of RHP stimulation on IBAT activity can be blocked by injecting the excitatory amino acid blocker, kynurenic acid (0.5-2 ug in 0.25 ul) or the NMDA receptor antagonists, gamma-D-glutamylaminomethyl phosphonic acid (0.5-2ug), into the contralateral SCN; injecting the preferential kainate/kuisqualate receptor blocker, gamma-D-glutamylaminomethyl sulphonic acid (0.5-5ug) has no effect. Together, the results show that stimulation of the RHP can activate thermogenesis in BAT and that the signal for such activation involves action of glutamate at NMDA receptors in the SCN.

357.8

THERMAL STIMULATION, THERMAL SENSATIONS AND CHANGES IN DIGITAL PULSE VOLUME: A METHOD FOR STUDYING INTEGRATIVE FUNCTIONS OF CNS: I. V. Kojo (SPON: European Neuroscience Association), Dept. of Physiology, Univ. of Helsinki, Helsinki, Finland.

Changes in digital pulse volume (DPV) were measured during thermal stimulation of the glabrous skin of human hand. The aim of the study was to search if those changes are associated with changes in physical stimulus parameters or thermal sensation.

The skin was stimulated with a contact stimulator which allows the subjects to control the temperature during experiments. The subjects were told to increase or decrease the temperature of the stimulator until they feel a thermal sensation and to keep the sensation constant for two minutes by adjusting the stimulus as required. DPV was measured with a plethysmography.

Innoxious warming increased and cooling decreased DPV. However, changes in DPV began before the stimulus temperature began to change. Heat pain caused a decrease in DPV.

With stimuli producing innoxious thermal sensation the DPV changes seem to be dependent on physical temperature parameters. The changes are also associated with the psychological factors (e.g. expectancy) of the subjects. This method allows the study of CNS receptive, integrative and afferent functions by a simple but effective tool.

357.9

THE HYPOTHERMIZING EFFECT OF NOREPINEPHRINE (NE) MICRODIALYZED INTO THE PREOPTIC AREA (iPO) OF GUINEA PIGS IS INDEPENDENT OF ENVIRONMENTAL CONDITIONS. N. Quan, C.M. Blatteis, and R.D. Howell.* Dept. of Physiol. Biophys., Univ. of Tennessee, Memphis, TN 38163

NE microinjected iPO produces different thermal effects, depending on the ambient conditions. However, microinjection per se introduces artifacts which may confound the results. As microdialysis obviates these problems, we examined whether the thermal response to NE microdialyzed iPO is also affected by the environment. Previously prepared, conscious guinea pigs were exposed to 24±1, 33±1 or 15±2°C at 8:30 a.m.; other animals were exposed to 24±1°C at 8:30 p.m. in the dark. After their T_{CO} had stabilized, NE was administered (10 ug/ul at 2 ul/min for 3 h). NE induced a fall in T_{CO} under all conditions. It was, however, significantly smaller in the animals in 33 and 15° than in those in 24°C. The latency of the T_{CO} fall and its recovery after perfusion were longer at night than during the day. Thus, the ambient conditions did not affect the direction of the T_{CO} response to NE but modified its magnitude and course. (Supported by NS 22716.)

357.10

VOLATILE ANESTHETIC ACTION ON THERMOREGULATORY RESPONSES IN THE NEURAXIS OF CATS. W.T. Schmeling, J.P. Kampine and D.C. Warltier*. Depts of Anesthesiology & Pharmacology, Medical College of Wisconsin, Milwaukee, Wisconsin 53226

Normal thermoregulatory (TR) function is modulated by the preoptic region (POR) of the anterior hypothalamus and at other sites including the spinal cord (SC). Volatile anesthetics disrupt normal TR function and produce post anesthetic "shivering" (SH). The present investigation examined the action of halothane (H) on TR responses produced at the POR and SC of cats. Cats were chronically instrumented with bilateral cannulae in the POR. Double lumen thermodes allowed selective heating (HT) and cooling (CO) of the POR. Electrodes were implanted in hindlimb and forelimb muscles. Animals underwent selective HT and CO thermal challenge of the POR in the awake state, during H and during emergence. Pontine transected cats underwent alternate HT and CO cycles of the spinal cord. HT and CO was performed in the unanesthetized state, at 0.25-2.0% H and during emergence. EMG responses were quantitated and analyzed for power spectral density. HT and CO of the POR in the conscious state resulted in appropriate TR responses, including increased EMG SH activity to CO. H abolished these SH responses. During emergence from H, typical spontaneous SH responses were significantly attenuated by HT of the POR and augmented by CO. CO of the SC produced graded SH like responses. H dose dependently diminished this response. During emergence, CO of the SC induced a SH response similar to controls. The ability of the POR to modulate post anesthetic SH implies that there may be a resetting of the normal gain and an imprecision in the TR responses of the rostral CNS. The attenuation of SH responses at the SC implies a significant blunting action of H on SC TR mechanisms. Spectral analysis of EMG responses during emergence, were similar to the true SH responses to the TR challenge observed in the unanesthetized state, without.evidence of tonic--clonic type activity.

357.11

ELECTRICAL PROPERTIES OF NEURONS IN THE GUINEA PIG GALLBLADDER. G M. Mawe. Department of Anatomy and Neurobiology, The University of Vermont, Burlington, VT 05405

Recent studies indicate that the ganglionated plexus of the gallbladder is similar in structure, connections, and transmitter content to the ganglionated plexuses of the small intestine. Given these similarities, and the fact that the gallbladder originates as an evagination of the duodenum, intracellular recording techniques were used to determine the basic membrane properties and action potential characteristics of gallbladder neurons, for comparison with those of neurons in the small intestine.

The mean resting membrane potential of gallbladder neurons, with overshooting spikes, was -50.0 ± 1.0 mV, with values ranging from -40 to -58 mV and the mean input resistance was 75.6 ± 5.4 MΩ (n = 41). These neurons responded with single or multiple fast EPSPs following fiber tract stimulation. No rundown of the fast EPSPs was observed during stimulation at frequencies up to 10 Hz. Depolarizing current pulses (≥ 200 ms) elicited only single or double spikes regardless of the amplitude of the pulse beyond the spike threshold. The stimulus evoked spikes were abolished by TTX (0.5 μM). Spikes reappeared when TEA (20 mM) was added to the TTX-containing solution. The mean duration of afterspike hyperpolarizations (AHP) was 179.9 ± 11.9 ms. The amplitude of the AHP was decreased by 20 mM TEA or 100 μM curare. Anodal break action potentials could be elicited in some of the gallbladder neurons, but only when rather large hyperpolarizing currents pulses (producing 20-30 mV hyperpolarization) were injected into the cells.

Results from this study indicate that the neurons located in the gallbladder represent a class of neurons with properties that differ from those of neurons studied in the small intestine. Supported by NS26995.

357.12

NEURAL CONTROL OF MOTILITY IN SUBSEGMENTS OF THE DOG COLON WALL. K.D. Keef*, R.J. Stevens* and S.M. Bowen*. (SPON: J. Peacock) Dept. of Physiol., Univ. of Nevada, Reno, 89557.

The contractile response to enteric nerve stimulation was investigated in subsegments of the colon wall including the outer half of the circular muscle (OHCM) and myenteric plexus; the inner half of the circular muscle (IHCM) and submucosal plexus; the longitudinal muscle (LM) and myenteric plexus; and "bulk" circular muscle (BCM) with both plexus regions removed. All subsegments of the colon with the exception of the BCM exhibited ongoing rhythmic activity although the frequency and pattern of activity varied markedly from layer to layer. Rhythmicity could be induced in the BCM with acetylcholine (10^{-7}M). Tetrodotoxin (5×10^{-7}M) did not abolish rhythmic activity in any subsegment and in the OHCM and IHCM led to enhancement of rhythmic activity suggesting a tonic release of inhibitory transmitter substance. Transmural stimulation (20 Hz, 10 s) of the OHCM or IHCM gave a large initial contraction which was largely eliminated with atropine (5×10^{-7}M) and in the case of the OHCM was followed by an inhibitory phase. Neurally induced inhibition of contraction in the IHCM was revealed in the presence of atropine. Only 1 of 8 BCM preparations contracted with transmural nerve stimulation suggesting that neural innervation is concentrated in those regions near the myenteric and submucosal border. The results indicate that both the basal contractile activity of colonic subsegments and their neural control differs throughout the colon wall.

357.13

HISTOCHEMICAL IDENTIFICATION OF NEURONS INNERVATING BOTH THE JEJUNUM AND ILEUM OF THE RAT. X. Zhang*, R. Fogel* and W. Renehan. (SPON: J. Elkes) Depts. of Medicine and Anatomical Sciences and Neurobiology, University of Louisville School of Medicine, Louisville, KY 40292.

Most mammalian sensory and somatic motor systems are topographically organized. Furthermore, a viscerotopic organization of vagal cells innervating the rat stomach has been suggested. We explored the organization of the innervation of the rat intestine by determining whether individual afferent and efferent neurons innervate both the jejunum and the ileum. Five Sprague Dawley rats were injected with Fast Blue and WGA-HRP. One tracer was injected into the jejunum and the other into the ileum. Five days later the brainstem, prevertebral ganglia (PG), nodose ganglia (NG), selected dorsal root ganglia (DG) and the injection sites were removed. In the brainstem, labeled cells were seen only in the dorsal motor nucleus of the vagus (DMNV). Of these cells, 19.3 ± 0.4 % contained both labels. In contrast, only 2.9 ± 0.5 % of nodose cells were double labeled. In the PG and DG, 8.9 ± 0.4 % and 19.7 ± 0.6 % of the cells were double labeled, respectively. Our results suggest that a substantial percentage of DMNV and DG neurons innervate a large portion of the intestine. In contrast, the afferent information reaching the DMNV via the NG, as well as the sympathetic innervation, is very specific. The significance of this pattern of innervation in the coordination of intestinal function requires further study. VA Merit.

357.14

INNERVATION OF THE PANCREAS BY NEURONS IN THE GUT. A. L. Kirchgessner, and M. D. Gershon, Department of Anatomy and Cell Biology, Columbia Univ. P & S, New York, NY 10032.

Enteric neurons project out of the gut to innervate neurons in prevertebral ganglia and the gallbladder. We therefore investigated whether neurons in the bowel send functional axonal projections to the pancreas. The retrograde tracer, FluoroGold (FG) was injected into the pancreas. In addition, the intercalating fluorochrome DiI, was microinjected into myenteric ganglia. FG labeled neurons in the myenteric, but not the submucosal plexus, of the duodenum (the first 6 cm) and the antrum of the stomach. The density of FG-labeled neurons was higher in the stomach (9.2 ± 0.9/ganglion) than in the duodenum (3.8 ± 0.3/ganglion; $p < 0.001$). No labeling was observed in controls in which inadequate time was allotted for retrograde transport. Myenteric neurons were found in both duodenum and antrum that were doubly labeled by FG and anti-5-HT sera. In addition, thick bundles of 5-HT-immunoreactive nerve trunks ran between the duodenum and the pancreas. Most 5-HT-immunoreactive axons in the pancreas terminated in ganglia, although some fibers were also observed near acini, ducts, vessels, and islet cells. Following injection of DiI into myenteric ganglia, fluorescent fibers were seen to collect into bundles that entered the pancreas at many locations through the intervening connective tissue. Veratridine (10 μM) was perfused through the lumen of duodenal segments to which pancreas was left attached. The cytochrome oxidase (CO) activity of pancreatic ganglion cells was demonstrated histochemically and measured by computer-assisted video microdensitometry. Duodenal veratridine increased pancreatic neuronal CO activity; this effect was antagonized by tetrodotoxin (1.0 μM), hexamethonium (10 μM), and mechanical ablation of the myenteric plexus of the gut. It is concluded that there is a functional entero-pancreatic innervation, that pancreatic ganglia are targets of this innervation (*via* a nicotinic synapse), and that some of the entero-pancreatic fibers are serotonergic. Supported by NIH grants NS 12969 and NS 07062.

357.15

BOMBESIN (BB) MICROINJECTED INTO THE PARAVENTRICULAR NUCLEUS (PVN) INHIBITS GASTRIC ACID SECRETION (GAS) STIMULATED BY TRH MICROINJECTED INTO MEDULLARY NUCLEI. H. Yang* and Y. Taché. CURE, West VA Medical Ctr., Dept. of Med. and Brain Res. Inst., UCLA, Los Angeles, CA 90073.

Microinjection of BB into the PVN inhibits GAS stimulated by pylorus ligation or electrical vagal stimulation in the rat (Brain Res. 422:118, 1987;Jpn. J. Pharmacol. 45:129, 1987). In the present study we investigated medullary nuclei and autonomic pathways involved in mediating the inhibitory effect of BB injected into the PVN. Male rats (SD, 220-350 g) fasted for 24h were anesthetized with urethane and implanted with gastric fistula. GAS was measured by flushing the stomach and titrating the flushed perfusate. Microinjections were performed by pressure ejection over 2 min of 50 nl of peptides or vehicle. After 30 min of basal GAS, BB or vehicle was microinjected bilaterally into the PVN (30 pmol/site) and immediatly after the stable TRH analog, RX 77368, was microinjected unilaterally either into the dorsal vagal complex (DVC, right side) or nucleus ambiguus (NA, left side). Basal GAS expressed per 2 h was 21 ± 2 μmol (n=70). Microinjection of BB into the PVN, unlike into the lateral hypothalamus inhibited by 69% the acid response (129 ± 12 μmol/2h) to RX77368 (8 pmol) microinjected into the DVC. Also, BB injected into the PVN, inhibited by 53% the GAS response (120 ± 12 μmol/2h) to intracisternal injection of TRH (550 pmol) and by 100% gastric acid response (73 ± 10 μmol/2h) to RX77368 (8 pmol) microinjected into the NA. The inhibitory effect of bombesin against RX 77368 microinjected into the NA was completely abolished by cervical cord transection. BB in PVN inhibited GAS induced by medullary vagal stimulation through spinal pathways. (supported by NIH DDK-30110).

357.16

ROLE OF GABA IN CONTROL OF GASTRIC MOTILITY IN THE CAT DORSAL VAGAL COMPLEX. C.D. Rossiter*, P.J. Hornby, R.L. White*, J.W. Harmon*, R.A. Gillis* (SPON: L.M. Petrucelli). Depts. of Pharmacology and Surgery, Georgetown University, Washington, D.C. 20007.

Immunocytochemical techniques have demonstrated a remarkable density of GAD immunoreactivity in the dorsal vagal complex, specifically the dorsal motor nucleus of the vagus (DMV) and the medial subnucleus of the tractus solitarius (mNTS). These nuclei are known to influence gastric function since excitation of the DMV and mNTS results in increases and decreases in gastric motility, respectively. Therefore, to evaluate the role of GABA in control gastric motility we microinjected 20-100ng of bicuculline ($GABA_A$ antagonist) into the DMV and mNTS in alpha-chloralose anesthetised cats while monitoring pyloric motility and blood pressure. Microinjection of bicuculline into the DMV (N=7) resulted in statistically non-significant increases in pyloric minute motility index (MMI) of 4.5±1.9. However, microinjection of bicuculline into the mNTS (N=5) evoked a marked increase in pyloric MMI of 11.7±3.7 ($P<0.05$). This occurred with no significant increase in mean blood pressure or heart rate. The increase in pyloric motility was reversed by vagotomy or by microinjection of 50-100ng of muscimol ($GABA_A$ agonist) into the mNTS during the peak response. These data indicate that GABA present in the DMV may have, at most, a minor role in control of gastric motility; however, GABA present in the mNTS exerts a tonic inhibitory effect on neurons controlling excitatory vagal outflow to gastric smooth muscle. Supported by NIH grant AM29975

357.17

TRH INHIBITS THE ACTIVITY OF GASTRIC INFLATION-RELATED NEURONS IN THE SOLITARY NUCLEUS.
R.C. Rogers and M.J. McCann. Dept. Physiology, Ohio State University College of Medicine, Columbus, OH 43210.

Thyrotropin releasing hormone (TRH) has been identified as a potent central regulator of gastrointestinal function. It has been hypothesized that TRH-containing neurons in the central nervous system influence gastric function by modulating the effectiveness of gastric vago-vagal reflexes. To test this hypothesis, we studied the effects of small amounts of TRH (10-100 femtomole in 10 to 100 picoliters) on the firing rate of single neurons in the nucleus of the solitary tract (NST). Of 23 NST neurons responsive to gastric distension (2 ml) 11 or 48% were inhibited by micropressure applications of TRH; none were excited. These results provide evidence that a part of the TRH effect on the regulation of gastric function involves the suppression of NST responses to gastric vagal afferent input from the vagus nerve.

[Supported by NINCDS Grant 24530 to RCR]

357.18

OXYTOCIN (OT) EXCITES GASTRIC DISTENTION-RELATED NEURONS IN THE NUCLEUS TRACTUS SOLITARIUS (NTS). M.J. McCann and R.C. Rogers. Dept. of Physiology, Ohio State University, Columbus, OH 43201.

Previous reports indicate that central OT neurons can influence the vagal control of gastric function. OT neurons from the paraventricular nucleus project to vagal sensory (NTS) and motor nuclei in the brainstem. We determined whether OT could modulate the activity of NTS neurons that were specifically related to gastric function. A triple-barreled glass recording electrode/injection pipette array was used to record single extracellular potentials and eject OT or vehicle solutions. The array was positioned in the medial NTS and gastric-related neurons were identified by inflating the stomach with a balloon. Responsive cells were either excited (ON), or inhibited (OFF) by gastric inflation. When OT (100-300 fm in 100-300 pl) was pressure ejected onto these cells, the following effect on spontaneous activity was found:

	ON	OFF
excite	60%	36%
inhibit	0%	21%
no effect	40%	43%

The predominant effect of OT was excitatory. 82% of the cells responding to OT were excited; ON cells were preferentially stimulated by OT. Thus, OT may influence gastric function by modulating gastric-related sensory neurons in the NTS, which then affect the vagal output to the stomach. (Supported by NS 24530).

357.19

TYROSINE MODULATES STRESS GASTRIC ULCERATION IN RATS:
R.Murison*, D.Hellhammer, H.Lehnert. Depts. Physiological Psychology,Univ.Bergen/Norway, University Trier/FRG and Internal Medicine, University Mainz/FRG.
The aim of the present study was to explore whether increasing available tyrosine under a stress exposure would modulate the extent of gastric ulceration developing in response to that stress. Earlier studies have demonstrated that tyrosine supplementation is able to modulate endocrine and neurochemical responses to other stressors.

Food-deprived male rats were subjected to a stress of restraint in room temperature (17.5°C) water for 75 minutes. Experimental animals were injected i. p. with 200 mg/kg tyrosine 30 minutes before the initiation of stress procedures. After removal from the restraint, animals were returned to their home cages for 15 minutes prior to sacrifice. Prior injection with tyrosine at this dose level clearly led to a reduction in the amount of bleeding gastric ulceration when compared to saline-injected rats subjected to the same stressor.

These data provide further evidence for the potential of tyrosine to modulate stress responses and suggest a role for this amino acid in protection against stress pathology, although the mechanism remains undetermined.

357.20

THE GASTROINTESTINAL CORRELATES OF MOTION SICKNESS IN THE CAT. I.M. Lang, M. Steensrud*, and S.K. Sarna* Dept. of Surg. & Physiol., Med. Coll. Wisc., Zablocki VAMC, Milw., WI 53295.

The aim of this study was to determine the motor and myoelectric activity of the GI tract associated with motion sickness in the cat. Four cats were preselected for their susceptibility (1 vomit in < 10 min) to motion sickness induced by vertical oscillation (VO) at 0.5 Hz. These cats were chronically instrumented with strain gauge force transducers on the fundus, antrum duodenum, and ileum; bipolar electrodes on the antrum and small intestine; and catheters in the jugular vein and gastric corpus. The effect of VO on GI activity was determined and compared to that activated by $CuSO_4$ (5-10 mg, ig) or UK 14304(1-5 µg/kg, i.v.). In all experiments VO inhibited motor activity of the stomach and upper small intestine within 5 min of onset which lasted until approximately 5 min after VO ended. During some experiments the cats exhibited a short lasting antral dysrhythmia which was accompanied by profuse salivation but no change in GI motor activity. At other times the cats vomited and every vomiting episode was accompanied by a characteristic set of motor and myoelectric events (e.g., the retrograde giant contraction (RGC)) similar to that observed previously in the dog. The RGC activated by VO was not significantly different from that activated by $CuSO_4$ or UK14304. Tachygastria was not observed during VO. Conclusions: 1) Motion-induced vomiting, like other forms of vomiting, is accompanied by a stereotypic set of GI motor and myoelectric events including the RGC. 2) The prodromal signs of vomiting (e.g. salivation), which have been equated with nausea in man, are correlated with gastric dysrhythmias other than tachygastria. Therefore, nausea may not be associated with either tachygastria or the gastrointestinal motor correlates of vomiting.

BASAL GANGLIA AND THALAMUS III

358.1

SYNAPTIC ACTION ON SUBSTANTIA NIGRA COMPACTA NEURONS BY SUBTHALAMIC INPUTS STUDIED BY A SPIKE TRIGGER AVERAGING METHOD. Y. Kang*, Y. Kubota, and S. T. Kitai. (SPON: L.D. Partridge) Dept. Anatomy & Neurobiology, College of Medicine, The University of Tennessee, Memphis, Memphis, TN 38163.

The nature of synaptic inputs from the subthalamic nucleus (STH) to the substantia nigra compacta (SNc) were studied by a spike trigger averaging method in an *in vitro* slice preparation in the rat. In a saggital slice containing both SNc and STH, EPSPs were produced in SNc neurons by activation of STH neurons and by microstimulation. First, extracellular antidromic unitary spikes were recorded from STH neurons following microstimulation (< 5 uA) of a discreet site of SNc to identify the area of STH projection. Subsequently, intracellular potentials were recorded from SNc neurons located in the stimulated site. Glutamate induced or spontaneously firing extracellular spikes recorded from STH neurons were used to trigger a computer which averaged synaptic potentials recorded from SNc neurons. Microstimulation was applied through tungsten microelectrodes (DC resistance of 1-3 MΩ). To exclude the possibility of stimulating passing fibers, microstimulation was also applied to multiple sites in STH and its surrounding areas. Biocytin was injected to identify the morphology and location of recorded SNc neurons, and their transmitter phenotype was examined by TH immunoreactivities. STH spike triggered averaging of SNc intracellular responses clearly indicated that STH inputs exert excitatory synaptic action on SNc neurons. Based on the rise time of STH induced EPSPs, the synaptic site was considered to be located on the proxymal dendrites of SNc neurons. The amplitude and falling phase of both unitary and composite EPSPs were markedly attenuated depending on the holding potential at which A-current was active. These data indicate that STH neurons exert excitatory action on SNc neurons and the theshold for spike generation of SNc neurons by this input can be greatly modified by the state of membrane potential. [Supported by NIH NS 20702 and NS 23886 to STK].

358.2

TWO TYPES OF SUBSTANTIA NIGRA COMPACTA NEURONS BASED ON ELECTROPHYSIOLOGICAL MEMBRANE CHARACTERISTICS.
Y. Kubota, Y. Kang*, and S. T. Kitai. Dept. Anatomy & Neurobiology, College of Medicine, The University of Tennessee, Memphis, Memphis, TN 38163.

Electrophysiological membrane properties of dopaminergic (DA) neurons in the substantia nigra compact (SNc) were studied in an *in vitro* slice preparation in the rat. Intracellular potentials were recorded from SNC neurons using conventional techniques. Biocytin was intracellularly injected to study the morphology of the recorded SNc neurons and their TH immunoreactivity for identification of DA neurons. SNc TH positive neurons were classified into two types based on their electrophysiological membrane properties (i.e., Type I and II). Type I neurons were characterized by (1) having a low threshold calcium spike, (2) moderate slow return to base line after the offset of intracellularly applied hyperpolarizing current, and (3) no pronounced IS-SD inflection on the rising phase of fast action potential. Type II neurons, on the other hand, were characterized by (1) having no low threshold calcium spike, (2) a prominent and prolonged slow return to base line after the offset of hyperpolarizing current, and (3) a prominent IS-SD inflection. Ramp-like slow depolarizing potential followed by rapid repolarization can be produced in both Type I and Type II neurons. IS-SD spikes can be triggered in both Type I and II neurons by depolarizing current injection. The amplitude and threshold of the spikes linearly decreased with a hyperpolarization of the membrane potential. However, the range of threshold changes was much greater in Type I neurons. These findings indicate that Type I neurons can change their timing of firing (e.g., from rhythmic firing to bursting mode) much easier than Type II neurons. [Supported by NIH NS 20702 and NS 23886 to STK].

358.3

THE REGULATION OF RHYTHMIC FIRING OF SUBSTANTIA NIGRA COMPACTA (SNc) DOPAMINERGIC NEURONS. S. T. Kitai, Y. Kang*, and Y. Kubota. Dept. Anatomy & Neurobiology, College of Medicine, The University of Tennessee, Memphis, Memphis, TN 38163.

It has been reported that SNc DA neurons can fire in a regular pacemaker like pattern. We have attempted to investigate the underlying electrical membrane factors responsible for regular rhythmic firing of SNc neurons in an *in vitro* slice preparation. Intracellular potentials were recorded from SNc DA neurons using a conventional technique. To identify the morphological and transmitter phenotype of the recorded neurons, biocytin was intracellularly injected and tyrosine hydroxylase immunocytochemistry was performed. All of the recorded DA neurons displayed rhythmic firing and the firing frequency was dependent on the holding potential. A bath application of 4-AP increased its frequency. An addition of TTX in the bath abolished Na spike and revealed the underline pacemaker- like potential (PLP) which was characterized by a slow ramp-like depolarization followed by a rapid repolarization. PLP frequency was also voltage dependent and was activated at holding potentials between -55 to -45 mV. PLP could also be activated following the offset of intracellularly injected hyperpolarizing current pulses. The rate of rise of PLP was related to the intensity of the injected current. Intracellular injection of EGTA blocked the repolarizing phase of PLP, prolonged its time course (< 1 sec), and markedly reduced its frequency. These data suggest that calcium current may be involved in the depolarizing phase of PLP and that PLP is responsible for rhythmic firing of SNc neurons. The threshold of PLP and its rate rise are dependent on the spike after-hyperpolarization, which are influenced by calcium dependent potassium current and A-current. [Supported by NIH NS 20702 and NS 23886 to STK].

358.4

ELECTROPHYSIOLOGICAL COMPARISON BETWEEN SNC CELLS OF YOUNG AND AGED WISTAR RATS. Lavín, M. A. and R. Drucker Colín. Instituto de Fisiología Celular, UNAM, Apdo.Postal 70-600, 04510 Mexico, D.F.

There is evidence indicating that some characteristics of substantia nigra compacta (SNC) are altered in aged animals (McGeer et al, Arch. Neurol. 34: 33, 1977).

Young male Wistar rats (3 months old) and aged (18 and 20 months old) were anesthetized with halothane, tracheostomized and fixed to a steretotaxic apparatus. Extracellular activity was recorded using microelectrodes filled with 2% pontamine blue in 0.5 M sodium acetate (12-20 Mohms). 102 SNC cells were recorded in young rats. These cells had a frequency rate of 3.38±2.14 spikes/seg (X± SD) a spike interval of 0.97±0.24 seg and a mean duration of 3.59±1.71 mseg. In 18 months old rats 16 cells were recorded, the frequency rate was 3.47±0.99 spikes/seg, the spike interval of 0.75±0.55 and the mean duration of 2.5±1.02 mseg. In 20 months old rats, 25 cells were recorded and their frequency rate was 1.75±1.31 spikes/seg their spike interval was 1.66±2.06 seg and the mean duration of 3.27±1.28 mseg. The difference in the frequency rate was significant. There was also a significant difference in the frequency distribution pattern between young and old rats since the latter showed more activity than the former. In conclusion there are electrophysiological changes in the aged rats, which may reflect either altered membrane properties or changes in dopaminergic activity.

358.5

CORRELATION OF DOPAMINE AGONIST INDUCED GLUCOSE UTILIZATION INCREASES AND INHIBITION OF SUBSTANTIA NIGRA PARS RETICULATA NEURONAL ACTIVITY. J.M. Trugman[1], B.G. Weick[2], J.R. Walters[2], and G.F. Wooten[1].
[1]University of Virginia, Charlottesville, VA. 22908 and [2]NINDS, Bethesda, MD. 20892.

[^{14}C]-2-Deoxyglucose (2-DG) autoradiographic studies of dopamine agonist-induced turning in rats with unilateral 6-hydroxydopamine lesions of the nigrostriatal pathway have shown that D1 agonists (SKF 38393 5.0-25.0 mg/kg) markedly increase glucose utilization in the substantia nigra pars reticulata (SNr) ipsilateral to the lesion whereas D2 agonists (quinpirole 0.1-5.0 mg/kg) do not (J. Neurosci. 7:2927, 1987). In studies using extracellular single unit recording in paralyzed ventilated lesioned rats, SKF 38393 (10 mg/kg) was more effective than quinpirole (1.0 mg/kg) in inhibiting the activity of SNr neurons (Brain Res. 405:234, 1987). We have now studied the effect of SKF 38393 (10 mg/kg) and quinpirole (1.0 mg/kg) on glucose utilization in paralyzed ventilated rats. SKF 38393, but not quinpirole, increased 2-DG uptake in the SNr ipsilateral to the lesion resulting in marked left/right asymmetry (55% increase compared to the contralateral side, $p<0.01$). The results support a positive correlation between increased glucose utilization and inhibition of SNr neuronal activity in this animal model. Since both D1 and D2 selective agonists induce turning, the results do not support a correlation between increased glucose utilization or inhibition of SNr activity and turning behavior. Glucose utilization increases observed in awake behaving rats following D1 stimulation likely represent a primary drug effect and not a consequence of movement or sensory feedback.

358.6

NOVEL BRANCHING OF SINGLE DOPAMINERGIC NIGROTECTAL CELLS IN THE RAT. K.J. Campbell, M. Takada and T. Hattori. Dept. of Anatomy, Univ. of Toronto, Toronto, Ontario M5S 1A8, Canada.

Our previous work has reported that single cells in the substantia nigra pars reticulata (SNr) send divergent axon collaterals ipsilaterally to both the striatum and superior colliculus (SC) in the rat. These bifurcating SNr cells, many of which express dopaminergic traits, are located predominantly in the ventrolateral portion of the nucleus at its rostal level. Employing a combination of fluorescent retrograde tracing and immunofluorescence histochemistry for tyrosine hydroxylase, we examined further characteristics of this specific SNr cell population. More than 50% of cells projecting ipsilaterally to both the striatum and SC also issued third collateral branches to the contralateral SC. The vast majority (greater than 85%) of these multi-collateralized cells were dopaminergic. Selective chemical lesions of the medial forebrain bundle with MPTP or 6-hydroxydopamine, did eliminate multi-branched SNr cells under study, whereas similar lesions of the SC failed to induce noticeable degeneration of the cells. This suggests that dopamine may preferentially be transported only towards the striatal terminals of parent SNr cells, while another substance (possibly GABA) only towards their SC counterparts. Supported by the MRC of Canada.

358.7

PROJECTIONS OF THE NUCLEUS TEGMENTI PEDUNCULOPONTINUS TO CRANIAL NERVE NUCLEI IN THE RAT. S. Keane and I. Grofova, Dept. of Anat., Mich. State Univ., E. Lansing, MI 48824.

Several lines of evidence have suggested that the basal ganglia (BG) may influence movement through projections to the nucleus tegmenti pedunculopontinus (PPN). Previous studies from our laboratory have demonstrated prominent descending PPN projections to the pontine and medullary reticular nuclei as well as sparse direct projections to the spinal cord. The present study utilizes anterograde transport of the lectin Phaseolus vulgaris (PHA-L) to determine whether the PPN also projects to the motor nuclei of the cranial nerves.

Following large PHA-L injections centered in the PPN, labeled fibers exhibiting preterminal and terminal varicosities were observed bilaterally in the 7, 12, Amb, and occasionally in the Mo5 nuclei. The dorsal nucleus of 10 and the solitary nucleus also contained a moderate number of labeled fibers. The densest terminal plexus, presenting two different distribution patterns, was observed in the 7 nucleus. The first pattern was characterized by prominent concentration of labeled fibers in the contralateral rostromedial 2/3 of the nucleus, and the second by diffuse bilateral distribution with greater ipsilateral density.

These observations support a concept that the PPN is an anatomically and functionally diversified nucleus which may mediate BG influences on motor, autonomic, and sensory functions. (Supported by N.I.H. Grant NS25744).

358.8

PUTATIVE KAPPA OPIOID-SENSITIVE NEURONS INCLUDE NIGROTECTAL AND NIGROTHALAMIC CELLS. L.A. Thompson and J.M. Walker. Dept. of Psychology, Brown University, Providence, RI 02912.

Since prodynorphin peptides are virtually isolated from other opioid peptide families in the substantia nigra pars reticulata (SNR), the striatonigral prodynorphinergic projection is a model system for the investigation of opioid peptide function. A group of SNR output neurons that increase their spontaneous firing rate in response to a mechanical pressure stimulus applied to the hindpaw are particularly sensitive to the kappa opiate U50,488. While 67% of these pressure-sensitive cells were inhibited by the iontophoretic application of U50,488, less than 21% of other SNR neurons were affected by this compound. In order to better characterize these neurons, extracellular single unit recordings were carried out in anesthetized rats and the targets of these neurons were determined by antidromic activation. These pressure/kappa-sensitive neurons project to the tectum and to the ventromedial thalamus; some are branched neurons projecting to both structures. These cells comprise approximately 30% of spontaneously active SNR neurons and appear predominantly in the lateral aspect of the SNR. These results suggest that prodynorphin peptides may influence motor responses to sensory inputs through actions on these nigrotectal and nigrothalamic neurons.

358.9

DIFFERENTIAL EXPRESSION OF CCK AND NEUROTENSIN (NT) mRNAS IN THE SUBNUCLEI OF RAT VTA. A. Jayaraman, T. Nishimori, P. Dobnar*, and G.R. Uhl. Dept of Neurol, LSU Sch of Med, New Orleans, LA 70112, ARC/NIDA, Depts of Neurol & Neurosci, Johns Hopkins Sch of Med, Baltimore, MD 21224; and U of Mass Med Sch, Worcester, MA 01605.

Cytoarchitecture and neural connections suggest a structural basis for functional heterogeneity among the subnuclei of VTA. Many of the dopaminergic neurons of VTA also coexpress CCK and NT. To further characterize the neurochemical organization of VTA, we studied the pattern of expression of mRNAs encoding CCK & NT in each of the subnuclei of VTA using quantitative in situ hybridization techniques. Studies using 35-S labeled oligonucleotides with specificity for CCK & NT mRNAs show that they are distinctly distributed in the different nuclei of VTA. CCK mRNA is expressed maximally in parabrachialis pigmentosus (14.2% of neurons), interfascicular (12.9), & rostral linear nuclei (12.6%), and just above background levels in caudal linear (4.1) and paranigral (3.2) nuclei. In contrast, NT mRNA is expressed maximally in caudal linear nucleus (15.6%) and nucleus parabrachialis pigmentosus (12.4%), moderately in rostral linear nucleus, minimally in nucleus paranigralis, and only weakly in interfasicular nucleus.

Our results indicate that the different subnuclei of VTA display surprising differences in their neuropeptide gene expression. These differences could have importance considering the key roles played by these neurons in several functions of the brain, including locomotion and central mechanisms of behavioral reinforcement.

358.10

THE NIGROSTRIATAL PROJECTION SYSTEM IN PIGEONS: AN LM AND EM IMMUNOHISTOCHEMICAL STUDY USING ANTISERA AGAINST TYROSINE HYDROXYLASE. E. Karle, K.D. Anderson, and A. Reiner (SPON: M. Shibata). Dept. of Anatomy and Neurobiology, Univ. of Tennessee, Memphis, TN, 38163

In all amniotic vertebrates studied, the striatum receives a massive dopaminergic input from the substantia nigra. A number of studies have used immunocytochemical techniques with antisera against tyrosine hydroxylase (TH) to characterize the LM and EM features of this input in a variety of mammalian species. Since very little ultrastructural data are available on the nigrostriatal system in nonmammals, we investigated this system in pigeons using anti-TH.

At the LM level, numerous TH+ terminals were observed throughout the striatum. LM comparisons to sections labeled for dopamine beta hydroxylase (which labels noradrenergic terminals) revealed that the vast majority of TH+ terminals in the pigeon striatum were dopaminergic. EM examination revealed that a significant percentage of the synaptic terminals in the striatum were labeled for TH. The TH+ terminals contained numerous densely-packed, round and pleomorphic, medium-sized vesicles, some of which were labeled. TH+ terminals typically made symmetric synapses, primarily with the dendritic shafts of striatal neurons, but also on the dendritic spines of striatal neurons.

These results suggest that there are major similarities in the organization of the nigrostriatal projection system in birds and mammals. Dopaminergic terminals in the striatum in pigeons appear similar in terms of their abundance, morphology, and postsynaptic targets to that previously reported in rats. Supported by NS19620 (A.R.) and Huntington's Disease Society of America (K.D.A.).

358.11

LM AND EM STUDIES OF SUBSTANCE P-CONTAINING TERMINALS ENDING ON NIGRAL NEURONS IN PIGEONS. K.D. Anderson, E. Karle and A. Reiner. Dept. Anatomy and Neurobiology, Univ. of Tennessee, Memphis, TN

In all amniotes studied, a large population of striatonigral projection neurons contains substance P (SP) and is by far the major source of SP+ terminals in the nigra. The ultrastructural features of SP+ terminals in the nigra have been studied in rats and monkeys, but whether features observed in these two mammalian species are typical of those present in other amniotes is unknown. Using immunohistochemical techniques for electron microscopy, we examined SP+ terminals in the substantia nigra in pigeons. SP+ terminals contained densely-packed unlabeled small vesicles, and often also contained several labeled large dense-core vesicles. Reaction product was also present in the cytoplasm of the terminals and surrounding the vesicles. Observed synaptic membrane specializations were of the symmetric type. Dendrites contacted by SP+ terminals were densely lined with terminals, about half of which were SP+. To determine whether SP+ terminals contact the subpopulation of nigral neurons that contain dopamine, we used LM double-label immunohistochemical techniques to simultaneously label dopaminergic nigral neurons (using an antiserum against tyrosine hydroxylase, TH) in combination with labeling of SP+ terminals. SP+ varicosities in close association with large and small TH+ dendrites were abundant. SP+ varicosities in contact with TH+ perikarya were also common but less numerous. These results indicate that in pigeons SP+ striatonigral projection neurons form axodendritic synapses having features similar to those in mammals and suggest that the targets of many of these synapses are dopaminergic nigral neurons. Supported by NS19620 (A.R.) and Huntington's Disease Society of America (K.D.A.)

358.12

TOPOGRAPHY OF STRIONIGRAL PROJECTIONS IN THE NORTH AMERICAN OPOSSUM. O.B. Gbonegun and J.C. Hazlett. Department of Anatomy and Cell Biology, Wayne State University, Detroit, MI 48201.

In a recent retrograde tracing study (Gbonegun and Hazlett, '89), WGA-HRP was used to determine the origin of projections to the opossum substantia nigra. Our findings suggested that the strionigral connections might be topographically organized in the medial-lateral dimension. In order to clarify these data, we injected WGA-HRP in portions of the caudate nucleus or putamen and examined the distribution of strionigral fibers. Labeled fibers resulting from caudate injections ramified in the medial two thirds of the pars reticulata throughout its length, while those observed after putamen injections occupied the lateral half of this subdivision throughout much of its length. Reacted fibers within the pars compacta were sparse following injections in the head and body of the caudate nucleus and absent after placements in the putamen. Little if any orthograde transport was seen among the pars lateralis neurons after any striatal injections. Therefore, as in the rat, the opossum pars reticulata is the primary terminus for striatal projections. These projections exhibit a rudimentary topography in which the fibers from the caudate and putamen overlap in the middle of the pars reticulata but otherwise distribute to medial (from caudate) or lateral (from putamen) aspects of this subdivision.

358.13

SOMATOSENSORY MAPPING AND MICROSTIMULATION OF PRIMATE SUBTHALAMIC NUCLEUS (STN). T. Wichmann*, H. Bergman*, and M.R. DeLong (SPON: L. Fechter). Dept. of Neurology, The Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

The STN likely has a role in motor control since it receives topographic input from the pre-central motor fields and projects to the globus pallidus. While lesions of STN result in hemiballismus, the effect of microstimulation on movement is uncertain. In a green monkey, we examined the effects of STN microstimulation, and also mapped neuronal responses to somatosensory examination to characterize the stimulation sites and extend previous studies which suggested a somatotopic organization of STN.

Microelectrode penetrations were made from an oblique anterior approach in the parasagittal plane in a 500 um-grid. Cells responding to deep palpation and movement of individual joints of the contralateral arm were found mainly in anterior dorsal STN, whereas cells responding to similar manipulations of the contralateral leg were found mainly in the central and posterior STN. Cells responsive to orofacial movements were located more laterally. Cells with similar responses to passive stimulation were often clustered along individual penetrations. Most ventral STN cells did not respond to somatosensory examination. These findings provide new evidence for a somatotopic organization of STN.

STN microstimulation (trains of 400 ms; biphasic pulses; 40 uA; 300 us/phase; 400 pulses/s) was carried out during the same penetrations at 200 um intervals. Stimulation within STN did not evoke movements. However, stimulation in and near the lateral internal capsule induced contralateral limb movements. Stimulation of areas above, below and rostral to the STN produced contralateral saccades. The lack of microexcitability sets STN apart from motor cortex and some of its efferent targets such as putamen.

358.14

ELECTRO-PHYSIOLOGICAL STUDIES OF THE CONNECTIONS BETWEEN THE SUBTHALAMIC NUCLEUS (STN) AND GLOBUS PALLIDUS (GP) IN BEHAVING MONKEYS. H. Bergman* and M.R. DeLong. Dept. of Neurology, Johns Hopkins Univ. Sch of Med., Baltimore, MD 21205

Earlier lesion and electrical stimulation studies suggested that the STN projections to GP are inhibitory, while more recent anatomical, physiological and pharmacological studies suggest their excitatory (glutamatergic) nature.

Microelectrode recordings were carried out simultaneously in the STN and both segments of GP in two awake behaving Rhesus monkeys. The STN electrode was used interchangeably for microstimulation and single unit recording. STN-GP connections were studied by observing the responses in GP neurons to microstimulation of the STN, as well as by crosscorrelating the spontaneous activity of GP and STN neurons (usually recorded in the same place where the stimulation was delivered). The internal connectivity of GP was studied by correlating the activity of distant (recorded by two microelectrodes) and neighboring (recorded by one electrode) GP neurons.

Microstimulation (bi-polar single pulse, max amplitude 40 uA) in the STN resulted in short latency (<10 ms) complete inhibition (mean duration 40 ms) of spontaneous activity in 40% of the recorded GP neurons (n= 180). However, none of the neuron-pairs studied showed correlated activity. Neighboring GP neurons yielded a flat correlogram in 80% of cases (n=74), and a short latency inhibition in 20%. No one of the distant GP neuron-pairs (estimated distance between 0.5 to 4 mm) showed correlated activity (n=49).

In conclusion, the inhibitory responses of GP to STN stimulation are more likely not due to activation of the STN projection neurons. Furthermore, the lack of crosscorrelation between STN and GP and the lack of common input to simultaneously recorded GP neurons indicate that GP afferents are highly topographic and specific.

358.15

ORGANIZATION OF VENTRAL STRIATAL EFFERENT PATHWAYS WITH THE DOPAMINERGIC NEURONS OF THE PRIMATE SUBSTANTIA NIGRA. E.L. Lynd*, S.N. Haber (SPON: T. Begenisich). Department of Neurobiology and Anatomy, University of Rochester Medical Center, Rochester, New York, 14642.

We undertook a study to examine the pattern of efferent projections from the ventral striatum to the substantia nigra in relation to the dopamine containing neurons. We also examined the relationship of these striatal terminals with specific nigro-striatal projections.

Injections of tritiated amino acids , PHA-L, and WGA-HRP were placed in various striatal regions including the nucleus accumbens, ventromedial caudate, rostral medial caudate, and ventral putamen. Sections were double stained using an antibody to tyrosine hydroxylase. Results demonstrate that the entire rostral-caudal extent of the substantia nigra receives inputs from these striatal regions. Rostrally the terminal labeling for all ventral striatal injections converges in the medial aspect of the substantia nigra. More caudally labeling extends out laterally. Terminal labeling overlapped with the cell bodies of the dopamine neurons as well as their dendrites. Results indicate that nigral neurons projecting to the striatum receive inputs from a different region of the striatum. For example some dopamine neurons in the medial portion of the substantia nigra project in part to the dorsal caudate and receive some inputs from the ventral striatum. These studies suggest that the connections of the striatum and the substantia nigra are complex with both reciprocal and non- reciprocal pathways.

358.16

VASOACTIVE INTESTINAL PEPTIDE (VIP) INNERVATION OF THE EXTENDED AMYGDALA IN THE RAT: A LIGHT AND ELECTRON MICROSCOPIC IMMUNOCYTOCHEMICAL STUDY. H.T. Chang and Q. Tian*. Department of Anatomy and Neurobiology, The University of Tennessee - Memphis, College of Medicine, 875 Monroe Ave., Memphis, TN 38163.

The concept of the "**Extended Amygdala**" was proposed recently by Alheid and Heimer (Neurosci. 27:1,'88) as an area which includes the centromedial amygdala, the sublenticular part of the substantia innominata, and the bed nucleus of stria terminalis (BNST). A region along the posterior limb of the anterior commissure is also included in the extended amygdala based on its apparent intimate relationship with BNST and the central amygdaloid nucleus, and was earlier identified as the interstitial nucleus of the posterior limb of the anterior commissure by de Olmos ('72). This region, however, is not easily distinguished from the ventral striatum in Nissl preparations, and is referred here as the caudal ventral striatum (VSc). In support of VSc as a part of the extended amygdala, we report here that in contrast to the rest of the ventral striatum, only VSc is densely innervated by vasoactive intestinal peptide immunoreactive (VIP+) axons. The dense plexuses of VIP+ axons in VSc appeared to be continuous with those in BNST. A few VIP+ neurons were also found within these regions. Preliminary electron microscopic analysis revealed that VIP+ boutons formed asymmetrical synapses with mainly dendrites and spines of VSc neurons.

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358.17

ADRENERGIC INNERVATION OF THE RAT SUBSTANTIA INNOMINATA: A LIGHT AND ELECTRON MICROSCOPIC IMMUNOCYTOCHEMICAL STUDY. H. Kuo and H.T. Chang, Department of Anatomy and Neurobiology, The University of Tennessee - Memphis, College of Medicine, 875 Monroe Ave., Memphis, TN 38163.

Adrenergic and noradrenergic inputs to the rat substantia innominata (SI) were studied by immunocytochemical localization of phenylethanolamine N-methyltransferase (PNMT) and dopamine ß-hydroxylase (DBH), the synthetic enzymes for adrenaline and noradrenaline, respectively, using rabbit antibodies to PNMT and DBH (Eugene Tech International). While many PNMT-like immunoreactive (PNMT+) axons were found in SI, sequential double-labeling experiments indicated that PNMT+ axons were much less numerous than DBH-like immunoreactive (DBH+) axons in SI. In order to ascertain that the PNMT+ axons were adrenergic fibers, double-immunofluorescence labeling experiments were performed using a mouse monoclonal antibody to tyrosine hydroxylase (TH) (Boehringer Mannheim), the synthetic enzyme for dopamine. The result indicated that all PNMT+ axons in SI were also immunoreactive for TH. Preliminary electron microscopic analysis revealed that PNMT+ boutons in SI formed asymmetrical synapses with dendrites of SI neurons.

(This study was supported by USPHS Grants NS21003, AG05944, and a grant from the Alzheimer's Disease and Related Disorders Association.)

358.18

NON-CHOLINERGIC CORTEX-PROJECTING NEURONS IN THE RAT BASAL FOREBRAIN: A LIGHT AND ELECTRON MICROSCOPIC STUDY. J.A. Whittaker*, K.A. Williams*, H. Kuo, N.G.F. Cooper, S.T. Kitai, and H.T. Chang, (SPON: S.K. Bhattacharya) Department of Anatomy and Neurobiology, The University of Tennessee - Memphis, College of Medicine, 875 Monroe Ave., Memphis, TN 38163.

Previous studies have shown that some of the cortex-projecting neurons in the rat basal forebrain were non-cholinergic. In this study we sought to further characterize their morphology and synaptic relationships. The retrograde tracer Fluoro-Gold was injected into the frontal cortex. Sections containing retrogradely labeled neurons in the substantia innominata (SI), the globus pallidus (GP) and the nucleus basalis (NB) were processed for immunofluorescence reactions using antibodies raised against choline acetyltransferase (ChAT). The results indicated that most of the retrogradely labeled neurons were immunoreactive for ChAT. The few non-cholinergic cortex-projecting neurons were found in GP and SI. Some of these cells appeared smaller, while others were comparable in sizes as the cholinergic cortex-projecting neurons. Work is in progress to examine the ultrastructural morphology and the synaptic relationships of these non-cholinergic retrogradely labeled neurons using an immunoperoxidase reaction with a mouse antibody raised against Fluoro-Gold.

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358.19

IDENTIFICATION OF ZINC-CONTAINING EFFERENTS TO THE NEOSTRIATUM BY RETROGRADE TRANSPORT OF ZINC SELENIDE G.A. Howell, P. Mandava*, M.K. Christensen*, and C.J. Frederickson, Lab for Neurobiology, Univ. Texas/Dallas, Richardson, Texas 75083

Prior denervation studies have indicated that the zinc-containing efferents to the neostriatum originate within the cerebral cortex. In the Present work, Se^{-2} was iontophoresed into the striatum precipitating ZnSe in zinc-containing axonal boutons. The resulting retrograde transport of the reaction product from the labeled boutons was studied to determine the specific cells of origin.

The Se^{-2} was stereotaxically delivered from glass micropipettes (1-2 uA; 100-300 sec; 10-20 Mohm tips) and the rats were sacrificed 24 h later. Cryostat sections (20 um) were developed in Timm silver solution to render the ZnSe visible.

Silver grains were found in the zinc-containing neuropil at the injection sites and in the perikarya and proximal dendrites of cortical neurons located primarily in superficial layers and distributed topographically according to the rostro-caudal placement of the injection. Labeled perikarya were not found in the substantia nigra or medial thalamus, indicating that those efferents to the striatum are not zinc-containing.

358.20

TARGETS OF HIPPOCAMPAL AXONS IN NUCLEUS ACCUMBENS (VENTRAL STRIATUM). G. E. Meredith, F. G. Wouterlood, and A. Pattiselanno* Dept. Anatomy and Embryology. Free University Medical School, Amsterdam, THE NETHERLANDS.

Nucleus accumbens (acc) receives an extensive projection from the hippocampus. The neuronal targets of these axons are not known but may include GABAergic or cholinergic neurons. We examined the synaptic relationship between hippocampal terminals and their targets in Acc by combining fornix lesions or iontophoretic injections of *Phaseolus vulgaris*-leucoagglutinin (PHA-L) in the subiculum with immunocytochemistry for glutamate decarboxylase (GAD) or choline acetyltransferase (ChAT).

In dual-labelled light microscopic sections we found GAD- or ChAT-immunoreactive perikarya and dendrites embedded in a web of PHA-L-labelled hippocampal fibers. An electron microscopic examination of over 200 PHA-L-labelled or degenerated terminal boutons revealed that 100% of them form asymmetric synaptic specializations; 96% make contact with spines; and 4% contact dendritic shafts. We never found a labelled or degenerated bouton in contact with a ChAT-immunoreactive element even though they were sometimes juxtaposed. Degenerated boutons did form synapses with GAD-labelled elements, particularly labelled spines. These findings suggest that most hippocampal inputs to nucleus accumbens monosynaptically activate GABAergic neurons but probably have little relationship with cholinergic cells.

358.21

THE ORGANIZATION OF THE EFFERENT PROJECTIONS FROM THE PRIMATE VENTRAL PALLIDUM. S.N. Haber*, E. Lynd*, D. Wolfer*, and S. Mitchell+, Department of Neurobiology and Anatomy, University of Rochester School of Medicine, Rochester, NY 14642, and +VA Medical Center, Syracuse, NY.

The ventral pallidum is considered to be the limbic-related portion of the globus pallidus by virtue of its afferent connections with the nucleus accumbens, and its efferent projection to the dorsomedial nucleus of the thalamus. In the rat, the ventral pallidum projects to the subthalamic nucleus, the lateral habenular nucleus and the substantia nigra, the ventral tegmental area, as well as to the dorsomedial nucleus of the thalamus (Haber et al., J.C.N., 235:322). Little is known of the ventral pallidal efferent connections in the monkey. We report here a study of the organization of ventral pallidal efferent connections in the primate to determine whether efferent pathways differ from those observed in the rat.

Injections of 3H amino acids, WGA-HRP, or PHA-L were placed centrally into the ventral pallidum, in the region directly below the anterior commissure. As expected, a dense terminal field is observed in the subthalamic nucleus and the substantia nigra. However only the most medial portion of the subthalamic nucleus receives this innervation, while terminals are not limited to the medial nigra. A densely labeled fiber bundle travels in the stria medularis to terminate in the lateral habenular nucleus. Fibers from these injections also reach the dorsomedial nucleus and the ventral anterior nucleus of the thalamus, the lateral and medial parts of the hypothalamus, and the striatum. Although the regions to which the projections from the primate ventral pallidum do not appear to differ substantially from those of the rat, the density and organization of these projections are significantly different in a number of regions.

358.22

ACCUMBENS PROJECTIONS TO DORSAL AND VENTRAL PALLIDUM AND TO THE EXTENDED AMYGDALA IN THE MONKEY USING PHA-L. G.F. Alheid[1], C. Haselton[2], L. Heimer[2,3] 1) Depts. of Behav. Med. and Psychiat., 2) Otolaryn., & 3) Neurosurg., Univ. Virginia, Health Sci. Ctr., Charlottesville, Va. 22908.

We have recently advocated that the nucleus accumbens could be considered a mixed structure with similarities to both the striatum and the extended amygdala. The latter structure includes the centromedial amygdala, the bed nucleus of the stria terminalis (BST) as well as interconnecting cell columns beneath the globus pallidus. To further evaluate this view we have re-examined accumbens projections with the anterograde tracer phaseolus vulgaris leucoagglutinin (PHA-L) in marmoset monkeys.

As previously observed, the accumbens projects to the ventral pallidum but also to the medial tip of the internal pallidal segment. Moreover, terminals appear to form a double layer in the ventral pallidum as is the case for striatal projections to dorsal pallidum. Injections medial, central and caudal in the accumbens resulted in terminal labeling in the extended amygdala with the most extensive terminals from the central or caudal injections in nucleus accumbens. Terminals were observed particularly in subpallidal areas but also in the BST. Additional axons continued caudally along the medial surface of the peduncle entering the substantia nigra at its medial edge. Scattered axons were also observed in the paraventricular nucleus of the thalamus. Supported by USPHS #NS 17743.

BASAL GANGLIA AND THALAMUS IV

359.1

DISTRIBUTION AND REGULATION OF TYROSINE HYDROXYLASE (TH) mRNA IN MESENCEPHALIC DOPAMINE (DA) NEURONS, Linda T. Weiss-Wunder and M.-F. Chesselet, Dept. of Pharmacology, Med. Coll. Pennsylvania, Philadelphia, PA 19129.

Subpopulations of mesencephalic DA neurons have different electrophysiological, pharmacological and anatomical characteristics. We have measured the level of mRNA for TH, the rate-limiting enzyme in the synthesis of DA, in subgroups of DA neurons in rats and mice using in situ hybridization histochemistry with an ^{35}S-labelled cRNA probe (D. Chikaraishi). In addition, we have analyzed the changes in levels of of TH mRNA induced by alterations in the activity of DA neurons in the mouse. Levels of mRNA were quantified in emulsion-coated slides using computer-assisted grain analysis. In controls, the mean cellular labelling for TH mRNA was 30% lower in lateral and mid portions of the A9 DA cell group than in medial A9 and A10. In mice treated with fluphenazine-N-mustard (FNM; 4 µmol/kg), an irreversible antagonist of DA D2 receptors, DA turnover was increased in the striatum, but TH mRNA was unchanged in A9 and A10 DA neurons 3, 5, and 8 hours after a single injection of FNM. Striatal DA turnover was still increased after 1 and 2 days, but not 4 and 6 days of FNM (2 injections/day). TH mRNA was decreased in A9 DA neurons after 2 and 4 days of treatment, but unchanged at 4 days in neurons of the A10 group. The results suggest that subpopulations of mesencephalic DA neurons are differentially regulated at the level of TH gene expression and that activation of A9 DA neurons by sustained blockade of DA D2 receptors results in a compensatory decrease in TH mRNA which may participate in the molecular adaptation of DA neurons to chronic neuroleptic treatment. (Supported by BNS 86-07645, MH 44894 and the Dystonia Med. Res. Found.)

359.2

6-HYDROXYDOPAMINE (6OHDA)-INDUCED CHANGES IN LEVELS OF STRIATAL mRNAs ENCODING SOMATOSTATIN (SOM) AND GLUTAMIC ACID DECARBOXYLASE (GAD) AS DETECTED BY IN SITU HYBRIDIZATION HISTOCHEMISTRY (ISHH). J.J. Soghomonian and M.F. Chesselet. Dept. of Pharmacology, Medical College of Pennsylvania, Philadelphia, PA 19129.

The mRNA for GAD and SOM were visualized on coronal sections of rat neostriatum using ISHH with ^{35}S-labeled cRNA probes (A.J. Tobin and R. Goodman) and radioautography. The nigrostriatal dopamine pathway was lesioned by one injection of 6OHDA (6 µg in 2 µl) in the substantia nigra after desipramine pretreatment. A higher intensity of GAD labeling was observed in striatal neurons on the lesioned as compared to the contralateral side. This marked asymmetry was observed in a large proportion of labeled neurons at three coronal levels of the striatum corresponding to plane A10, A9.5 and A9[1]. Few intensely GAD-positive cells could however be observed in both ipsi- and contralateral striata. ISHH for SOM mRNA revealed densely labeled cells scattered throughout the striatum. Using computerized grain analysis, the mean labeling associated with individual striatal cells was significantly lower on the ipsilateral side of the lesion than the contralateral side, with a maximum difference of 35%. This effect in mRNA content was observed at coronal level A9.5 but not A9[1]. Comparison with sham operated animals, showed that the SOM labeling asymmetry in 6OHDA treated rats was solely related to an increase of mRNA levels in the striatum on the contralateral side of the lesion. These results substantiate a different role of dopamine in the regulation of GAD and SOM mRNA in rat striatum. (Supported by BNS 16841, MH 44894-01 and PMAF fellowship to J.J.S.). [1]Paxinos, G. and Watson, C., Acad., Sidney, '86.

359.3

NMDA GLUTAMATE RECEPTORS STIMULATE TYROSINE HYDROXYLASE ACTIVITY IN NIGROSTRIATAL DOPAMINE TERMINALS. J.A. Arias-Montaño*, D. Martínez-Fong* and J. Aceves*. (SPON: J. Villarreal). Dept. of Physiology, Biophysics and Neurosciences, CINVESTAV-IPN. Ap. Postal 14-740, 07000 México, D.F.

Glutamate receptors have been demostrated on nigrostriatal dopamine terminals (Roberts, P.F. et al. Brain Res. 235: 83, 1982). Here we have studied the role of glutamate receptors modulating tyrosine hydroxylase (TH) activity in these terminals.

Experiments were performed in striatal slices of the rat brain; TH activity was evaluated determining by HPLC l-Dopa accumulation following Dopa decarboxylase inhibition with NSD-1015 (0.5 mM).

l-Glutamate increased TH activity in a dose-dependent manner (EC_{50} = 3 µM); this effect was blocked by 10 µM 2-amino-5-phosphonovaleric acid, a selective NMDA receptor antagonist. The stimulatory effect was calcium-dependent and tetrodotoxine insensitive.

These results suggest that glutamate stimulates striatal dopamine biosynthesis by activation of NMDA receptors coupled to calcium channels presumably localizated on nigrostriatal dopamine terminals.

359.4

EFFECTS OF DOPAMINE RECEPTOR STIMULATION ON SUBSTANCE P RELEASE IN SUBSTANTIA NIGRA OF RATS. **D. Orosz* and J.P. Bennett, Jr. Departments of Neurology, Psychiatry and Pharmacology, University of Virginia Medical School, Charlottesville, VA. 22908.**

Basal ganglia outflow is mediated by both Substance P- and GABA-utilizing pathways in the striatonigral system. We have monitored release of Sub P into the extracellular fluid (ecf) of rat substantia nigra, utilizing brain microdialysis in rats with unilateral lesions of the ascending nigrostriatal pathway (nsp). Nsp lesions were made with 8 µg of 6-hydroxydopamine injected perinigrally, followed 2 weeks later by implanataion of Carnegie-Medicin dialysis probe holders into the substantia nigra reticulata (snr). Dialysis was performed in awake animals before and after systemic treatment with apomorphine, 1 mg/kg.

Substance P was assayed by a modified RIA with sensitivity of detection of 0.2 fmol. Using ^{3}H-Sub P, we found that the 2 mm Carnegie-Medicin probes had an efficiency of 5-6% transfer of Sub P at 1 µL/min perfusion rate. Basal Sub P ecf levels averaged 0.2 nM in lesioned snr and rose 2-3 fold after apomorphine treatment. No rise in Sub P ecf levels was observed after vehicle injection.

We conclude that the Sub P-utilizing component of the striatonigral pathway is activated by stimulation of supersensitive DA receptors.

359.5

DOPAMINE D1 HETERORECEPTORS ON STRIATONIGRAL AXON TERMINALS ARE NOT STIMULATED BY ENDOGENOUS DOPAMINE EITHER TONICALLY OR AFTER AMPHETAMINE: EVIDENCE FROM TERMINAL EXCITABILITY

L.J. Ryan, M. Diana*, S.J. Young* and P.M. Groves, Dept. Psych., Oregon State Univ., Corvallis, OR 97330 and Dept. Psychiat., Univ. California San Diego, La Jolla, CA 92093.

The role of dopamine D1 heteroreceptors on striatonigral axon terminals was investigated in rats. Direct infusion (1 and 10 uM; 300 nL in 5 min) of the direct acting, specific dopamine D1 agonist, R-SKF 38393, into the substantia nigra terminal field of antidromically identified neostriatal projection neurons decreased the electrical excitability of these terminals whereas infusion into nonterminal regions did not. The effect could be at least partially reversed by subsequent infusions of the specific D1 antagonist, R-SCH 23390 (10 uM). In contrast, systemic administration of SCH-23390 (0.3 and 0.6 mg/kg, iv), of the non-specific antagonist, haloperidol (0.2 mg/kg, iv) and of the indirect acting dopamine agonist, amphetamine (1.0 and 5.0 mg/kg, iv) were without effect. Since activation of D1 receptors caused a decrease in excitability, the lack of effect of antagonists indicates that endogenous dopmaine is not tonically activating these receptors. Furthermore, the lack of effect of amphetamine indicates that even high levels of released dopamine in substantia nigra do not affect neostriatal D1 heteroreceptors. Thus it is unlikely that endogenous dopamine modulates neostriatal control of the substantia nigra through these presynaptic terminal D1 heteroreceptors.

359.6

NEURONAL LOCALIZATION OF CANNABINOID RECEPTORS IN THE BASAL GANGLIA.

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Cannabinoid receptors have recently been characterized and localized using a potent analog, [^{3}H]CP55,940, in section binding assays. In rat brain the highest densities are in the globus pallidus (GP) and substantia nigra pars reticulata (SNr). Selective lesions of either the striatonigral or nigrostriatal pathway were respectively made by unilateral infusion of ibotinic acid into the striatum or 6-OHDA into the medial forebrain bundle (mfb). After 2 or 4 week survivals, slide-mounted brain sections were incubated with ligands selective for cannabinoid, dopamine D-1 ([^{3}H]SCH-23390) and D-2 ([^{3}H]raclopride) receptors, and the dopamine uptake site ([^{3}H]GBR-12935), and exposed to ^{3}H-sensitive film. Resulting autoradiography showed ibotinate-induced losses of cannabinoid, D-1, and D-2 receptors in the striatum and topographic losses of cannabinoid and D-1 receptors in GP and SNr at both survivals. Four weeks after mfb lesions (which resulted in amphetamine-induced rotations), there was loss of GBR binding in the striatum and substantia nigra pars compacta but no change in cannabinoid receptor binding. The data show that cannabinoid receptors in the basal ganglia are neuronally located and are co-localized with D-1 receptors on striatal projection neurons, including their axons and terminals. Cannabinoid receptors are not localized on dopaminergic nigrostriatal cell bodies or terminals.

359.7

DOPAMINERGIC FEEDBACK CONTRIBUTES TO FUNCTIONAL DIFFERENCES BETWEEN TYPE I AND TYPE II STRIATAL NEURONS.

E.S. Nisenbaum, A.A. Grace, and T.W. Berger. *Departments of Behavioral Neuroscience and Psychiatry, University of Pittsburgh, Pittsburgh, PA 15260*

Type I and Type II striatal neurons can be identified based on their paired impulse responses to stimulation of the corticostriatal pathway. We have examined the extent to which dopaminergic (DA) feedback contributes to functional differences between these two striatal cell classes.

Destruction of DA afferents increased the spontaneous activity of both Type I and Type II neurons *in vivo*. Such denervation also increased the range of facilitation displayed by Type I neurons to interstimulus intervals (ISIs) of <50 ms, and decreased the magnitude of inhibition to ISIs of 50-250 ms. In contrast, responses of Type II cells were unaffected.

Using a corticostriatal slice preparation, paired impulse stimulation of cortical afferents produced a facilitation of striatal cell discharge to ISIs of 10-400 ms. DA (10 µM) did not affect the facilitatory responses of *in vitro* neurons to short ISIs. However, in a small percentage of these cells, DA produced a pronounced inhibition to ISIs of 30-480 ms which was similar to that found for Type I neurons *in vivo*. This effect was mediated by activation of D_1 but not D_2 receptors.

Potentiation of GABAergic activity using pregnanolone (5 µM) selectively enhanced short-interval (10-20 ms) inhibition of approximately 50% of the *in vitro* neurons sampled; the paired impulse profiles of these *in vitro* cells parallelled those displayed by Type II neurons *in vivo*. When low doses of DA (5 µM) and pregnanolone (5 µM) were combined, separate populations of neurons displayed either short- or long-interval inhibitory responses. These results define conditions under which Type I- and Type II-like neurons can be identified *in vitro*, and suggest that long ISI inhibition observed *in vivo* is mediated by activation of D_1 receptors. Supported by NS19608, MH00343, MH09717.

359.8

CHARACTERIZATION OF LUCIFER YELLOW-LABELED RAT STRIATAL NEURONS IDENTIFIED BY THEIR RESPONSE TO PAIRED PULSE CORTICAL STIMULATION.

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In vivo intracellular recording and Lucifer yellow staining were used to differentiate the properties of Type I and Type II striatal neurons categorized by their inverse responses to paired pulse stimulation (Nisenbaum et al, J. Neurosci. 8: 4138, 1989). Type I cells exhibited a more variable morphology. The multipolar to fusiform soma (22-35 µm long) gave rise to 2-3 comparatively long and thick primary dendrites, which divided into sparsely spined or heavily spined processes extending distally. The axons arose from the soma and ramified locally, but only axons of densely spinous neurons could be followed for long distances. These neurons had higher rates of spontaneous activity (2.6-17.7 Hz) and fired predominantly in a bursting pattern. In the majority of Type I cells examined, the spikes did not exhibit prominent IS-SD breaks.

Type II neurons were similar in morphology to the medium spiny neurons, with the 14-22 µm long soma giving rise to 3-6 primary dendrites with densely spinous, angular or recursive secondary and tertiary processes. The axon arose from the soma and arborized extensively within the dendritic field, with the main axonal branch extending for long distances. These neurons were silent or slow firing (0-7.4 Hz), with spikes exhibiting prominent IS-SD delays. Most neurons fired individual spikes, but faster firing cells (>3-4 Hz) often fired in bursts. Systemic administration of apomorphine (0.1 mg/Kg i.v.) was observed to facilitate spike firing in Type II neurons in response to frontal cortical stimulation. This could be reversed by the administration of haloperidol (0.1 mg/Kg i.v.). In contrast, apomorphine had no observed effect on the physiology of two Type II neurons that were not firing spikes spontaneously. Supported by USPHS NS19608, NS08288, & MH00343.

359.9

DOPAMINERGIC MODULATION OF STRIATAL SENSORY PROCESSING

T.I.Lidsky, A.M.Cosentino* and S.Banerjee CUNY Med

Sensory processing in the striatum changes during changes in behavior (1). The present study assessed the possible role of dopamine in striatal sensory plasticity. Striatal field potentials were evoked by facial stimulation in urethane anesthetized rats. Dopamine release due to stimulation of the substantia nigra (SN) attenuated striatal sensory responses. Surprisingly, the dopamine receptor blocker Haldol (H) also attenuated sensory responses. Further work suggested that the attenuation is due to increased dopamine release caused by blocking presynaptic receptors. Paradoxically, H reversed the inhibitory effect of the SN: after H, SN stimulation enhanced striatal sensory responses. The latter effect, since it was blocked by picrotoxin, may be due to inhibitory striatonigral feedback triggered by the enhanced dopamine release brought about by SN stimulation in combination with presynaptic receptor blockade.

The present findings indicate that the synaptic effects of dopamine may differ from the functional effects. While dopamine inhibits striatal firing, its effects on striatal processing can be either suppression or facilitation as a function of dopaminergic tone.

1. Manetto, C. & Lidsky,T.I. Neuroscience Letters 1989, 96:295-299 (NS21418)

359.10

ON-LINE MORPHOMETRY OF TYROSINE HYDROXYLASE-IMMUNOREACTIVE AXON TERMINALS IN CAUDATE PUTAMEN AND NUCLEUS ACCUMBENS IN RAT WITH A FOCUS ON SYNAPSES.

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On-line analysis of tyrosine hydroxylase immunoreactive striatal boutons was executed with the EM analogue of a **camera lucida** (Carl Zeiss) supported by morphometric functions of hardware and software dedicated to 3-D reconstruction (Eutectic). We report on a preliminary sample of every fifth of 1,864 intact boutons generated by a "gating" procedure utilizing the x-y traverse controls of the microscope. Boutons were located in dorsolateral (dl, 777) or dorsomedial (dm, 150) caudate-putamen (Cpu) and in rostral (r, 254) or medial (m, 559) accumbens (Acb) or the accumbal "core" (124). Patch and matrix compartments were not distinguished. Skewed-right, monomodal frequency distributions of profile area in CPu and Acb with medians of 2.6 and 2.3 µm^2, respectively, reflected the sums of bimodal plots that contrasted dm vs. dl CPu and m vs. r and core accumbens. Some profiles in CPu (7.3% of 927) and Acb (8.9% of 937) exhibited synaptic appositions which, excepting less than 0.5%, were symmetric and ranged from 0.05 to 3.5 µm in length. Postsynaptic targets of synapses in mAcb were skewed toward dendrite shafts (72%) as opposed to spines (11%). Those in CPu were distributed equally among shafts (43%) and spines (46%) as were those in rAcb and core Acb (41% shafts, 41% spines). Suppported by USPH NIH NS-23805.

359.11

BREAKDOWN OF DOPAMINE D1/D2 RECEPTOR SYNERGISM IS INDEPENDENT OF RECEPTOR DENSITY. G.J. LaHoste, M. Andreini*, L.C. Brunner* and J.F. Marshall. *Department of Psychobiology, University of California, Irvine 92717.*

Recent evidence indicates that in neurologically intact animals dopamine (DA) receptor subtypes act synergistically; i.e., DA receptor-mediated events (either behavioral or electrophysiological) require concomitant stimulation of D1 and D2 receptors to be manifest. Following 6-hydroxydopamine-induced DA denervation or subchronic reserpine treatment, however, there is a breakdown in synergism; D1 and D2 receptors act independently and with greatly increased sensitivity to agonists. We tested the hypothesis that treatments which alter receptor density would modify D1/D2 synergism. Four groups (n = 8) of adult male rats were given daily injections of saline (1.0 ml/kg), SCH 23390 (SCH; 0.5 mg/kg), haloperidol (HAL; 0.5 mg/kg), or both SCH and HAL (0.5 mg/kg each) for 21 days. Seventy-two h after the last drug injection, animals were observed for motor stereotypies in response to stimulation of D1 receptors alone (SKF 38393, 20 mg/kg, 30 min after the D2 antagonist eticlopride, 0.1 mg/kg), D2 receptors alone (quinpirole, 3.0 mg/kg, 30 min after the D1 antagonist SCH 23390, 0.1 mg/kg) or both D1 and D2 receptors (SKF 38393, 20 mg/kg + quinpirole, 3.0 mg/kg). This procedure was repeated at 48 h intervals until all subjects had received each of the test conditions in counterbalanced order. The data from all four groups were virtually identical; subjects displayed a pattern of complete D1/D2 synergism (i.e., stereotypies were observed only after concomitant stimulation of D1 and D2 receptors). By contrast, when independent groups of rats were treated for 5 d with reserpine (1.0 mg/kg/day), stereotypies were observed following stimulation of either D1 or D2 receptors alone, indicating a breakdown in D1/D2 synergism. Since previous receptor binding studies have shown that chronic treatment with SCH or HAL increases D1 or D2 receptor densities, respectively, whereas five days of reserpiine treatment does not alter D1 or D2 receptor binding, the present data indicate that D1/D2 synergism is unrelated to D1 or D2 receptor density.

359.12

STRIOSOMAL AND GRADIENT PATTERN OF DOPAMINE HIGH-AFFINITY AND MONOAMINE SYNAPTIC VESICLE SITES IN THE RABBIT CAUDATE. J.F. Marshall, R. Navarrete* and S.J. O'Dell, Dept. of Psychobiology, Univ. of Calif., Irvine, CA 92717.

The dopaminergic afferents to the neostriatum appear distributed heterogeneously, reflecting the patch-matrix organization of this structure as well as topographic gradients. For example, the tyrosine hydroxylase (TH) immunoreactivity is lower in striosomes of the caudate nucleus (CN) than in surrounding matrix (Graybiel et al, *PNAS 84*:303-7). Yet, it is unclear whether such heterogeneities mark differing densities of dopamine (DA) innervation or populations of DA terminals differing in their characteristics. In rabbit neostriatum we have used film autoradiography to quantify the binding of [^{3}H]mazindol (MAZ), which recognizes the high-affinity catecholamine transporter of DA neurons, and [^{3}H]ketanserin (KET), which labels the monoaminergic synaptic vesicles of this structure, in relation to acetylcholinesterase (AChE) histochemistry and [^{3}H]hemicholinium-3 (HC-3) labeling on adjacent sections. The AChE-poor striosomes of the rabbit CN correspond to [^{3}H]HC-3-poor regions (Rhodes et al, *Br. Res. 412*:400-4). A patchy distribution of both [^{3}H]MAZ and [^{3}H]KET binding was observed in the anterior CN, with circumscribed zones of 20-40% lighter binding of these ligands corresponding with regions sparse in AChE and [^{3}H]HC-3 binding. At the level of the body of the CN, the patchy distribution of [^{3}H]MAZ and [^{3}H]KET binding is less evident, but there is a striking three-fold dorso-ventral decreasing gradient of [^{3}H]MAZ binding, with lowest levels found in nucleus accumbens (NAc). However, [^{3}H]KET binding is almost (75-80%) as high in NAc as in dorsal CN. The lighter binding of both [^{3}H]MAZ and [^{3}H]KET in striosomes, together with the lower TH immunoreactivity of this compartment, suggests a less dense innervation by DA fibers of striosomes than matrix. But we hypothesize that the dorsoventral gradient of [^{3}H]MAZ binding sites may reflect a NAc population of DAergic terminals having a paucity of high-affinity DA transport sites.

359.13

ULTRASTRUCTURAL CHANGES IN RAT BRAIN ASSOCIATED WITH NEUROLEPTIC DRUG TREATMENT. C.K. MESHUL, A. JANOWSKY*, D.E. CASEY*, AND R.K. STALLBAUMER*. V.A. MEDICAL CENTER AND DEPTS. OF PATHOLOGY, PSYCHIATRY, AND NEUROLOGY, OREGON HEALTH SCIENCES UNIVERSITY, PORTLAND, OR. 97201.

We have previously shown that treatment with haloperidol (0.5 mg/kg/day, 14 d) causes an increase in the number of synapses containing perforated postsynaptic densities (PSDs) in the caudate but not the nucleus accumbens (Meshul and Casey, 1989). This effect was reversed after discontinuation of the drug. There was no effect on total synapse density. We have now examined the effects of haloperidol (0.5 mg/kg/day, 14 d and 30 d), a dopamine D-2 receptor blocker, SCH 23390 (1 mg/kg/day, 14 d), a D-1 receptor blocker, or clozapine (30 mg/kg/day, 14 d), a weak D-1/D-2 receptor blocker, on the number of perforated PSDs and the total number of synapses in the caudate. One day after treatment, the rats were perfused with fixative and the caudate prepared for electron microscopic analysis. Both haloperidol (14 d) and SCH 23390 (14 d) caused a 40% increase in the number of synapses with perforated PSDs as compared to the controls, while clozapine had no effect. In addition, there was a 30% increase in the number of perforated PSDs following haloperidol treatment for 30 days There was no change in total synapse density following haloperidol or SCH 23390 treatment for 14 days, but clozapine (14 d) and haloperidol (30 d) caused a decrease in the total number of synapses. The lack of perforated PSD change with clozapine may correlate with its low incidence of extrapyramidal side effects. Supported by the V.A.

359.14

DEVELOPMENT OF THE STRIATAL PATCH-MATRIX ORGANIZATION FOLLOWING PERINATAL DOPAMINE DEPLETION.

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Nigrostriatal dopamine (DA) projections are early markers for the patch/matrix organization of the striatum. During the first postnatal week in the rat, patches of DA fibers overlap with clusters of striatal neurons (Snyder-Keller, Neurosci. Lett. 91: 136 (1988)), projecting back to the substantia nigra (Fishell & van der Kooy, J. Neurosci. 7: 1969 (1987)). In order to determine the influence of DA on the formation of striatal patches, the organization of immunocytochemically-identifiable cell types was examined during development, in normal rats and in rats DA-depleted as infants (0 or 3 days) or in utero (E17). During the first week of life, corresponding patches of DA afferents and substance P (SP)-immunoreactive neurons exist in the dorsal striatum of normal animals. AChE-positive zones found primarily in the lateral striatum also show partial overlap with both SP and DA patches. Injection of 6-HDA into the lateral ventricles of fetal (E17) or infant rats produced a dramatic loss of striatal DA terminals, but did not disrupt the patchy distribution of SP-immunoreactive neurons nor the pattern of AChE staining. The distribution of cells immunoreactive for enkephalin or calcium-binding protein was also not noticeably changed by pre- or postnatal DA depletion. These experiments suggest that the patchy distribution of DA afferents is secondary to the early clustering of striatal neurons forming the patch compartment, and is not necessary for the maintenance of striatal patch/matrix organization. [Support MH-45342]

359.15

MULTIPLE CHALLENGES WITH APOMORPHINE INCREASE ROTATIONAL BEHAVIOR IN 6-OHDA OR KAINIC ACID LESIONED RATS L.M. Wyatt, A.B. Norman, M. Kulmonpunporn, C.A. Moody*, P.R. Sanberg, and M.N. Lehman. Departments of Anatomy & Cell Biology, Neurology, and Psychiatry, Univ. Cincinnati Coll. Med., Cincinnati, OH 45267

Rotational behavior following injection of the dopamine agonist apomorphine is a commonly used test of nigro-striatal dopamine function in rats. The present study assessed the stability of this measure over time. Male Sprague-Dawley rats either received a unilateral injection of 6-OHDA to the medial forebrain bundle or substantia nigra or kainic acid (KA) lesions to the striatum. After a minimum of 10 days, rats were placed in an open-field arena, allowed to habituate, and then injected with apomorphine (0.1 or 0.25 mg/kg s.c.). The number of complete, tight rotations in either direction was counted visually and expressed as the difference between the two directions. Data were grouped by 5 minute periods. Identical tests were then repeated at intervals of 3-5 days. Preliminary results indicate a significant increase in the total number of rotations between the first and subsequent trials for 6-OHDA rats ($p<.05$) and in both total rotations and peak number of rotations in a 5-minute period for KA lesioned rats ($p<.05$). Latency to the peak number of rotations did not change between trials. Duration of rotational behavior was not significantly increased for either 6-OHDA or KA lesioned rats. This phenomenon indicates a sensitization to the effects of repeated administration of apomorphine and has implications for the use of rotational behavior to quantify drug interactions following surgical interventions with dopaminergic systems. [supported by NIH NS25647 (PRS) and NIH NS24292 (MNL)]

359.16

NEURAL MECHANISMS MEDIATING DOPAMINE AGONIST-INDUCED DYSTONIA IN THE PARKINSONIAN MONKEY A.R. Crossman*, I.J. Mitchell* and M.A. Sambrook*. (SPON: Brain Research Association)
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Dystonia refers to a class of movement disorders which is characterised by a syndrome of abnormal sustained postures. Relatively little is known about the pathophysiology of dystonia though the basal ganglia have long been implicated in the underlying pathology. This report describes an experiment which investigates the neural mechanisms which mediate experimental dystonia in a novel model of dopamine agonist-induced dystonia in the macaque monkey. This condition was produced by chronic dopamine agonist treatment of an animal with a unilateral parkinsonian syndrome induced by infusion of MPTP into the right carotid artery. The treatment resulted in the appearance of axial and contralateral limb dystonia at peak dose. The 2-deoxyglucose (2-DG) metabolic mapping technique was applied to the animal during the expression of active dystonia. Densitometric analysis of the resultant autoradiographs revealed large asymmetries in 2-DG uptake between the lesioned side of the brain (which gave rise to the dystonic symptoms) and the control side. Increases in optical density on the lesioned side relative to the unlesioned side were seen in the subthalamic nucleus (36.1%), lateral pallidal segment (12.4%), medial pallidal segment (16.7%) and substantia nigra (18.4%) In contrast, large decreases in optical density were seen in the ventral anterior/ventral lateral nuclei of the thalamus (10.6%) and lateral habenula (14.9%) ipsilateral to the lesion. These findings are interpreted as suggesting that dystonia results from over-activity of the projections from the putamen to the medial pallidal segment and from the lateral pallidal segment to the subthalamic nucleus but decreased activity in the subthalamopallidal and pallidothalamic pathways.

359.17

NEONATAL DEVELOPMENT OF NIGRAL DOPAMINERGIC NEURON ELECTROPHYSIOLOGY *IN VIVO* J.M. Tepper, F. Trent*, and S. Nakamura, Center for Molecular and Behavioral Neuroscience, Rutgers University, Newark, NJ 07102

Neuroanatomical data indicate that dopaminergic (DA) neurons are among the earliest in the brain to differentiate, migrate, and send axons to neostriatal terminal fields, but data concerning the development of their physiological properties are lacking. Extracellular single unit recordings were obtained from antidromically identified nigrostriatal (NS) DA neurons in urethane-anesthetized rat pups ranging in age from postnatal day (PD) 1 to PD28. NSDA neurons from PD1-3 rats fired spontaneously at low rates (0.96±.1 spikes/s), in an irregular pattern lacking bursts, with long periods of inactivity. Mean firing rates increased over the next week (PD4-6, 1.3±.2; PD7-9, 1.5±.2), and bursts typically consisting of 2 spikes with interspike intervals less than 100 ms were observed, giving rise to bimodal interspike interval histograms. Spontaneous firing rates and patterns reached adult values between the 3rd and 4th postnatal weeks. Spike waveforms were wider in PD1-3 rats (4.95±.7 ms) than in adults (3.36±.1), and often exhibited a delay between the initial segment (IS) and somadendritic (SD) components > 1 ms. In PD1-7 neonates, the IS spike amplitude was often ≥ that of the SD spike.

The mean latency of striatal-evoked antidromic (AD) responses did not change significantly from PD1-3 (15.6±.9 ms) through adulthood (14.6±.6), although the proportion of AD responses that consisted of the full IS-SD spike decreased from PD1-3 (50.3±8%) through maturity (25.3±3%). IS-SD AD responses in neonates showed long delays between the 2 spike components, often > 2 ms. Equivalent proportions of NSDA neurons in neonates and adults displayed multiple, discrete AD latencies, and AD threshold currents did not differ significantly with age, suggesting that NSDA axons can function at least as early as PD1. Intracellular recordings did not reveal striatal-evoked synaptic responses in NSDA neurons although all non-DA nigral neurons responded with IPSPs whose mean onset latency (9.8±.4 ms) did not differ from adults.

Thus, rat NSDA neurons are physiologically competent at least from PD1, although the rate and pattern of spontaneous activity do not mature to adult values before PD21-28. Several physiological indicators suggest that neonatal NSDA neurons exist in a more depolarized state than in the adult rat. This may be due to a combination of immature membrane properties and a relative lack of physiologically functional neostriatal and/or pallidal inhibitory afferents.

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359.18

3-ACETYLPYRIDINE RESULTS IN DEGENERATION OF BOTH EXTRAPYRAMIDAL AND CEREBELLAR MOTOR SYSTEMS: LOSS OF THE DORSOLATERAL STRIATAL DOPAMINE INNERVATION. A. Y. Deutch, J. Elsworth, D. Rosin, S. Bruce*, M. Goldstein[1], and R. H. Roth. Depts. of Psychiatry and Pharmacology, Yale Univ. School of Medecine, New Haven, CT 06508 and [1]Dept. of Psychiatry, NYU School of Med, NY, NY 10016.

Although 3-acetylpyridine (3-AP) is well known to effect degeneration of the olivocerebellar climbing fiber system, another pyridine, MPTP, effects degeneration of the nigrostriatal dopamine (DA) system; it has only recently been shown that MPTP results in some cerebellar toxicity. We have examined the effects of 3-AP treatment of rats on the nigrostriatal DA system. 3-AP resulted in degeneration of the DA innervation of the dorsolateral striatum (CP), and the emergence of a distinct patchy distribution of surviving tyrosine hydroxylase (TH)-immunoreactive fibers. 3-AP treatment decreased DA content in the dorsolateral but not central CP, and did not alter DA levels in the NAS or PFC. 3-AP treatment also decreased striatal TH activity. *In vitro* studies showed that 3-AP inhibited MAO_B but not MAO_A activity; however, deprenyl pretreatment did not prevent the 3-AP-induced decrease in striatal DA. These data indicate that 3-AP results in degeneration of both extrapyramidal and cerebellar motor systems, and that 3-AP-induced sequelae may serve as a useful animal model of olivopontocerebellar atrophy-associated parkinsonism.

BASAL GANGLIA AND THALAMUS V

360.1

GABAERGIC INTERNEURONS, RATHER THAN SPINY CELL AXON COLLATERALS, ARE RESPONSIBLE FOR THE IPSP RESPONSES TO AFFERENT STIMULATION IN NEOSTRIATAL SPINY NEURONS. C.J. Wilson, H. Kita and Y. Kawaguchi, Dept. of Anat. and Neurobiol., Univ. of Tenn. Sch. Med., Memphis, TN 38163.

Cortical or local stimulation evokes a powerful EPSP followed by less prominent GABAergic IPSP in neostriatal slices, and corresponding response components occur *in vivo*. Because many spiny projection neurons contain GABA, it is usually assumed that the IPSPs arise from recurrent axon collaterals of the spiny neurons. Recently, GABAergic interneurons have been demonstrated in the neostriatum. These offer an alternative explanation for the IPSPs.

We examined the responses of identified spiny neurons to antidromic stimulation in slices and intact rats. Antidromic spike afterpotentials were distinguished from synaptic potentials by preventing the antidromic spike in the recorded cell by collision with a previous spike or application of strong hyperpolarizing current. No IPSPs comparable to those associated with excitatory orthodromic stimulation could be observed under these conditions.

To determine whether orthodromic activation could occur in the absence of action potentials in the spiny neurons, cortical stimulation was applied in a slice with intact connections from a portion of the cerebral cortex. This orthodromic stimulus does not directly activate any neostriatal neurons or their axons. It was adjusted so that it evoked small subthreshold synaptic potentials in spiny neurons, but did not cause any spiny neurons to fire action potentials. Under these conditions we were still able to detect the IPSP.

Thus, excitation of spiny projection neurons is neither necessary nor sufficient for the observation of the GABAergic IPSPs observed in spiny cells. The GABAergic IPSPs must therefore arise from the action of another GABAergic cell type in the neostriatum.

360.2

THE MORPHOLOGY OF PARVALBUMIN IMMUNOREACTIVE NEURONS IN THE RAT NEOSTRIATUM. H. Kita, T. Kosaka, and C. W. Heizmann, Dept. of Anat. & Neurobiol., Univ. of Tenn. Sch. Med., Memphis, TN 38163 USA, Natl. Inst. for Physiol. Sci., Okazaki, 444 Japan, and Inst. for Pharmacol. and Biochem., Univ. of Zurich-Irchel, Zurich, Switzerland.

Studies have indicated that a small population of neurons in the neostriatum contain a Ca-binding protein parvalbumin (PV) and that these neurons are strongly immunoreactive for GABA and glutamate decarboxylase antibodies. We have conducted more detailed light and electron microscopy on the PV-immunoreactive neurons.

Rats were prefused with 4% paraformaldehyde, 0.1% glutaraldehyde, and 0.2% picric acid in 0.12M phosphate buffer. The brains were removed and sectioned on a Vibratome. The brain sections were processed for immunocytochemistry for PV using avidin-biotin-peroxdase method.

Light microscopy revealed that PV-neurons correspond with medium size aspiny neurons described in previous Golgi studies. The medium sized somata were fusiform or polygonal in shape and aspiny dendrites were smooth and cylindrical at the proximal portion but were varicosed at the distal portion. Electron microscopy revealed that the somata of PV-neurons contain a deeply intended nucleus and an intranuclear rod. Many synaptic boutons were found on the proximal and distal dendrites and only a few were on the somata. While a small number of the boutons on the somata and proximal dendrites formed symmetrical synapses, the majority formed asymmetrical synapses. Many PV stained boutons were found on the somata and proximal dendrites of medium spiny neurons which were identified by their morphological features.

The data indicate that immunohistochemistry for parvalbumin selectively stains medium, aspiny, GABAergic interneurons in the neostriatum. The electron microscopy suggested that the neurons are incorporated in a feed-forward inhibitory circuit that they receive excitatory afferent inputs and their outputs are to inhibit neostriatal projection neurons. (Supported by NIH grants NS 25783 and NS 26473.)

360.3

EVIDENCE THAT THE EXTRASTRIOSOMAL MATRIX OF THE PRIMATE STRIATUM CONTAINS A MOSAIC OF NEURONAL CELL-CLUSTERS PREFERENTIALLY PROJECTING TO THE EXTERNAL AND TO THE INTERNAL PALLIDUM. J.-M. Gimenez-Amaya* and A.M. Graybiel.

Dept. of Brain and Cognitive Sciences, MIT, Cambridge, MA 02139, USA.

In a preceding report (Gimenez-Amaya and Graybiel, Neurosci. Abstr. 14:156) we presented retrograde tracer evidence that striatal neurons projecting to the pallidum in the primate are concentrated in the extrastriosomal matrix of the putamen (P) and caudate nucleus (CN), and that different populations of neurons project to the two pallidal segments (GPe, GPi). We also suggested that neurons projecting to each pallidal segment may aggregate in clusters, some at a distance from the main fields of striatal labeling. In this study we have analyzed this clustering phenomenon, and have investigated the topography of the striatopallidal projection in 41 hemispheres of 24 adult squirrel monkeys in which we placed HRP-WGA as a retrograde tracer in GPe, GPi or both, or HRP-WGA and [35S]methionine as anterograde tracers in CN, P or both. The findings suggest: (1) there is a global, three-dimensional, largely radial topography of the striatopallidal projection, in which the CN as well as the P participates (e.g. anterograde and retrograde evidence suggests a sizable projection from the rostral CN to the rostral GP, especially GPe); (2) there is a highly ordered mosaic of projection-neuron clusters in the extrastriosomal matrix. In fact, no injection in the pallidum, whether in GPe or in GPi, failed to evoke labeling of some cell clusters. For GPi injections (with a lesser fiber-of-passage problem), clustering was a dominant pattern. The relative weakness of labeling of striosomes accounted for some but by no means all of the patchiness. We conclude that clustering of striatopallidal projection neurons may help to account for functional heterogeneity of striatal units studied physiologically and for functional specificity of different striatal output paths. Supported by Javits Award 2 RO1 2552901A1, The Seaver Institute, and FISSS 87/1890.

360.4

SENSORY AND OCULOMOTOR PROPERTIES OF SINGLE NEURONS IN THE CAUDATE NUCLEUS OF THE ALERT CAT : EVIDENCE FOR REGIONAL ORGANIZATION W.A.Thomas*, C.R. Olson, and E.M. Bowman. Department of Psychology, Princeton University, Princeton, N.J. 08544.

The caudate nucleus of the cat is anatomically heterogeneous. At a gross level, it consists of connectionally distinct sectors: a lateral (L) zone (with input from somesthetic and skeletomotor cortical areas), a dorsomedial (DM) zone, (with input from visual association and oculomotor cortical areas) and a ventromedial (VM) zone (with input from prefrontal and other complex areas). At a fine level, it consists of chemoarchitecturally and connectionally distinct compartments: the striosomes and matrix.

We have searched for signs of functional heterogeneity in the caudate nucleus by recording from 135 neurons in 3 cats trained to execute saccadic eye movements and maintain fixation on visual targets. We have found: (a) that caudate neurons exhibit robust task-related activity in relation to the appearance of the target (32%), the execution of the saccade (10%) and the maintenance of fixation (57%); (b) that neuronal properties vary by subregion, with phasic sensory and saccadic signals most common in the DM zone, tonic fixation-related signals most common in the VM zone, and task-related firing rare in the L zone. We are currently investigating the relation between neuronal physiology and striosomal-matrix organization.

360.5

MORPHOLOGY OF NEURONS IN CELL CLUSTERS IN THE CAUDATO-PUTAMEN AND NUCLEUS ACCUMBENS OF THE RAT. V.B. Domesick, P.A. Paskevich* and H.K. Evans*. Mailman Research Center, Mclean Hospital, Belmont, MA 02178 and Harvard Medical School, Boston, MA.

In the present study, several recently observed differences between cell clusters composed of serially contiguous cells in the caudato-putamen (C-P) and nucleus accumbens (NA) are reported. The arrangement of individual cells in the clusters were visualized by three-dimensional reconstructions (software by John Kinnamon, 1987) from serial one micron sections. Clusters in the C-P frequently include up to 25 neurons, while the number in the nucleus accumbens was often higher, up to 50. The morphology of the neurons was examined in serial semi-thin sections and in ultrathin sections. In the C-P, most cells in a single cluster appear to be of the small-to-medium type with smooth unindented nuclei corresponding to the spiny projection neurons of Graveland and Difiglia (1985). In the NA, small cells with one or two processes form clusters; these clusters are solely composed of neurons with either smooth or indented nuclei. At the ultrastructural level, the profiles of cytoplasmic membranes are typically separated by a 6nm space with intercalated zonulae occludens and wider spaces. In the C-P, these appositions are usually. soma-somatic, while in the NA they also are dendro-somatic. These cell clusters are included within larger neuronal groups which resemble the striatal cellular islands described in the primate by Goldman-Rakic, 1982. (This work is supported by 1R01 MH43018.)

360.6

EXPRESSION AND SECOND MESSENGER RESPONSES OF MUSCARINIC RECEPTORS IN PRIMARY CULTURES OF RAT NEOSTRIATUM. K. Nishi*, P.T. Akins, D.J. Surmeier, S.T. Kitai (SPONSOR:William L. Byrne), Dept of Anatomy and Neurobiology, College of Medicine, The University of Tennessee, Memphis, Memphis, TN 38163

One action of acetylcholine in the neostriatum is the inhibition of dopamine-stimulated increases in adenylate cyclase activity. We employed the primary culture model of rat neostriatum to investigate the role of post-synaptic receptors in this action.

Primary neostriatal cultures were established and maintained from embryonic (E17) rat neostriata as previously described (Surmeier et al. Dev. Brain Res., 42:265-282,1988). Binding studies were carried out with 3H-N-methyl-scopolamine (NMS) on intact cultures maintained 3, 7, and 14 days in vitro. The neuronal population was estimated from cell counts and protein was measured for each experiment. The Bmax at 3, 7, and, 14 days was 138±17, 212±68 and 187±43 fmol/mg protein or 1060±55, 2061±420 and 32000±3000 sites/neuron. These results indicate muscarinic receptors rapidly increase in cultured neostriatum.

Previous studies determined that receptors were predominantly of the M1 subtype and linked to phosphoinositide turnover. We tested their linkage to adenylate cyclase. Cultures (12 DIV) were labeled with [3H]adenine and cAMP levels were stimulated with 10 uM forskolin (76%) or 10 uM dopamine (93%) in the presence of 1 mM isobutylmethylxanthine. Carbachol (50 μM) had no effect on these stimulated levels. The results suggest that the M1 receptor expressed by neostriatal neurons does not directly inhibit adenylate cyclase. This work was supported by P.H.S. grants NS 20702 to S.T.K., NS 26473 to S.T.K. and D.J.S.

360.7

MUSCARINIC MODULATION OF A TRANSIENT OUTWARD CURRENT IN RAT NEOSTRIATAL NEURONS. P. Akins, D. J. Surmeier and S.T. Kitai, Dept. of Anatomy and Neurobiology, College of Medicine, The University of Tennessee, Memphis, Memphis,TN 38163.

Cholinergic interneurons are important elements in the circuitry of the neostriatum. However, little is known about the modulatory actions of acetylcholine on voltage-dependent conductances in neostriatal neurons. Current-clamp studies (Misgeld et al., Pflugers Arch., 407:482-487, 1986) of striatal neurons have suggested that muscarinic agonists modulate the conductances controlling the duration of the calcium-dependent plateau potential. To quantitatively characterize the specific conductances mediating this effect, we have investigated the actions of muscarinic agonists on ionic currents in rat neostriatal neurons using the whole cell voltage clamp technique.

Neurons were cultured from embryonic rat neostriatum and recorded from as previously described (Surmeier et al., Brain Res., 473: 187-192, 1988). Preliminary experiments have shown that in roughly half of the neurons, bath application of carbachol in the presence of a nicotinic receptor antagonist (mecamylamine) increases the transient outward current evoked by depolarizing test pulses. This transient current exhibited activation and inactivation characteristics similar to the A-current previously described in these neurons. In a smaller percentage of neurons, carbachol application increased the sustained outward currents evoked by depolarization. No consistent effects were observed on holding current at -70 mV or on the outward currents evoked by holding the cell at -40 mV. These results suggest that acetylcholine may depress excitability in neostriatal neurons through enhancement of the A-current. This work was supported by P.H.S. grants NS 20702 to S.T.K., NS 26473 to S.T.K. and D.J.S.

360.8

MULTI-CHANNEL RECORDING OF NEURONAL ACTIVITY IN HISTOLOGICALLY DEFINED COMPARTMENTS OF NEOSTRIATUM IN FREELY MOVING RATS. S.F. Sawyer, J.Y. Chang, C.D. Myre and D.J. Woodward, *Dept. of Cell Biology & Anatomy, Univ. Texas Southwestern Medical Center at Dallas, Dallas, TX 75235.*

Anatomical studies have revealed a heterogenous organization in the mammalian neostriatum consisting of a patchwork of spatially congruent neurochemical and hodological compartments ("patches") that are embedded within a more voluminous "matrix". To date, electrophysiological evidence concerning the functional aspects of patch and matrix in awake behaving animals is lacking. We report here our initial electrophysiological findings concerning neurons located in the matrix. Sixteen microwires were implanted in the dorsolateral neostriatum of adult male Long-Evans rats. Subsequently, multi-channel extracellular single unit recordings were obtained from the freely moving animals, including during an auditory-cued treadmill paradigm. Localization of recording sites to patch or matrix was inferred on-line by positive responses (i.e., 5-10 ms latency orthodromic excitation and/or 100-200 ms duration post-stimulus inhibition) to electrical stimulation of prelimbic or motor cortex, respectively. Microlesions of selected recording sites, in conjunction with radiolabeling of histological sections with ^{3}H-Naloxone to label patches, permitted verification of the location of recording sites to either patch or matrix. Neostriatal neurons typically exhibited enhanced spike activity during treadmill-induced locomotion, as previously described (West et al., Soc. Neurosci. Abstr. *13*:979, 1987). These neurons were found to be located in the matrix. Furthermore, many of these units were identified as "Type II" neurons (based on responses to twin pulse cortical stimulation). In contrast, a smaller sample of "Type I" neurons in the matrix did not exhibit pronounced treadmill-related changes in firing rate. The activity of neurons in the patch compartment are currently under investigation to examine the general issue of functional heterogeneity within the neostriatum. *Supported by NIDA grant DA-05352.*

360.9

EFFECTS OF PILOCARPINE ON THE DORSAL IMMOBILITY RESPONSE IN THE RAT. G.A. Cottrell, T. Potter*, M.E. Meyer* and C. Van Hartesveldt. Dept. of Psychology, Univ. of Florida, Gainesville, Fl, 32611 U.S.A.

Cholinergic agonists, such as pilocarpine, are known to increase immobility latencies on a number of catalepsy tests in rats. These agents elicit catalepsy when injected into the mesencephalic reticular formation but have no effect when administered alone to the striatum. The effect of pilocarpine on the Dorsal Immobility Response (DIR), a response elicited by grasping a rat by the dorsal skin at the nape of the neck and lifting it off its feet, was tested on male and female Long-Evans rats. All surgery was done a minimum of 2 weeks prior to testing. Guide cannulae were implanted above the anterior dorsal striatum. In Exp. 1, pilocarpine (90 mg/kg, sc) increased bar catalepsy latencies but decreased DIR latencies. Exp. 2 found that pilocarpine, in doses as low as 1.25 mg/kg sc, was effective. This effect appears to be cholinergic since scopolamine (1 mg/kg, sc) pretreatment blocks it. In Exp. 3, the DIR was decreased following bilateral pilocarpine injection (5 ug/0.2 ul/side) into the striatum. Striatal scopolamine (10 ug/0.4 ul/side) pretreatment blocked the effect of pilocarpine on the DIR. Thus DIR is exquisitely sensitive to cholinergic manipulation but the cholinergic control and neural site of action are different for catalepsy and DIR.

360.10

DOPAMINE AND CALCIUM BINDING PROTEIN-CONTAINING NEURONS AND FIBERS OF THE NIGROSTRIATAL SYSTEM FOLLOWING ADULT AND NEONATAL 6-OHDA LESIONS. L. M. Grimes, H. Criswell, M. Sar*, W.E. Stumpf, R.A. Mueller, G.R. Breese. Bio. Sci. Res. Center, UNC-Chapel Hill, NC 27599.

Rats treated ICV with 6-OHDA as neonates,but not as adults, exhibit self-injurious behavior (SIB) when given L-DOPA. We compared the destruction of the nigrostriatal dopamine system in adult- and neonatal-lesioned rats. Rats were perfused with 4% paraformaldehyde and 0.2% glutaraldehyde. Serial Vibratome sections (30 μm) were immunostained for tyrosine hydroxylase (TH), 28kD calcium binding protein (CaBP), and for colocalization of both antigens. Radioimmunocytochemistry for TH was performed on 4μm frozen sections in striatum. 30% of TH-containing neurons in ventral tegmental area (VTA) and 10% in substantia nigra pars compacta (SNPC) were preserved in adult-lesioned rats, 10% of VTA cells and none in SNPC were preserved in neonatal-lesioned rats; cells preserved in adult but not neonatal-lesioned rats were in the lateral VTA and dorsal SNPC. In VTA and dorsal SNPC, neurons containing CaBP and TH were destroyed in the same proportions as neurons containing TH alone. Some fibers were preserved in the nucleus accumbens (NA) and olfactory tubercle (OT) of neonatal and adult-lesioned rats; in adult-lesioned rats, some fibers were also preserved in ventral caudate-putamen (CP). These results suggest that neurons in the lateral VTA and medio-dorsal SNPC are the source of fibers which project to the ventral CP and protect against SIB. (Supported by NS-21345 and HD-23042.)

360.11

DISTRIBUTION OF THE LIMBIC SYSTEM ASSOCIATED MEMBRANE PROTEIN (LAMP) IN THE CAUDATE NUCLEUS (CN) AND SUBSTANTIA NIGRA (SN) OF THE CAT M.-F. Chesselet, C.Gonzales and P. Levitt. Med. Coll. Pennsylvania, Philadelphia, PA 19129.

LAMP is a cell surface 64-68 kD glycoprotein associated, in the adult, with brain areas innervated by the limbic system. We have analyzed the distribution of LAMP in the basal ganglia of the adult cat using immunoperoxidase staining and a monoclonal antibody. In CN, areas of intense LAMP staining were in register with areas of weak acetylcholinesterase activity (striosomes), which receive inputs from the amygdala and prefrontal cortex. In contrast, the extrastriosomal matrix, which receives afferents from the cingular cortex, A10, A8, and the densocellular zone of A9, was devoid of LAMP labelling. In the SN, an intense labelling for LAMP in the cell sparse zone of the pars compacta contrasted with the absence of immunostaining in areas containing dense clusters of dopaminergic neurons (densocellular zone). The results show that subsets of neurons of the CN and SN share a common antigenic determinant with the limbic system, further revealing a segregation of limbic inputs in the basal ganglia.

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360.12

ORIGIN OF THE AMYGDALOID PROJECTION TO THE LATERAL SUBSTANTIA NIGRA AND RETRORUBRAL FIELD. C. Gonzales and M.-F. Chesselet. Dept. of Pharmacology, Medical College of PA, Phila. PA 19129

In an attempt to identify the site of origin of the projection of the central nucleus of the amygdala (CeN) to the lateral substantia nigra (SN), small iontophoretic injections of the anterograde tracer PHA-L were made in discrete areas of the CeN. PHA-L was detected immunohistochemically in 30 um thick sections using an antibody to PHA-L (Vector Laboratories) and the avidin-biotin system with diaminobenzidine as the chromogen. In addition, adjacent sections were processed for somatostatin (SOM) immunoreactivity using an antibody (R. Benoit) against SOM 1-12. Only injections of PHA-L into the dorsal and rostral CeN resulted in heavily labeled bouton-like swellings in the lateral SN pars compacta and SN pars lateralis. These injections also gave rise to a dense labeling in an area of the retrorubral field which partially overlaps with the A8 dopaminergic cell group and contains a dense plexus of SOM-positive nerve terminals. Similar labeling was observed after injections rostral, or dorsal and medial to the CeN. These results suggest that the cells of origin of the amygdaloid projection to the lateral SN are located in a restricted area of the CeN but are also present in a region dorsal and rostral to the CeN within the sublenticular substantia innominata (extended amygdala).

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360.13

MORPHOLOGICAL CHARACTERS OF INTRACELLULAR LABELED SPINY NEURONS IN RAT NEOSTRIATAL GRAFTS. Z.C.Xu, C.J.Wilson and P.C. Emson Department of Anatomy and Neurobiology, School of Medicine, U. T. Memphis. Memphis, TN. and AFRC Institute of Animal Physiology and Genetics Research, Cambridge, U.K.

A cell suspension prepared from 15-18 day embryonic striata was implanted into neostriata of adult rats two days after kainic acid lesion. Intracellular recording *in vivo* was performed 2-6 months after implantation. After recording, neurons were stained by intracellular injection of biocytin.

The somatic sizes of the labeled spiny neurons in the grafts were larger than in normal neostriatum, with cross sectional areas ranging from 112.6-375.6 μm^2 (as opposed to 112-197 in the host neostriatum). The dendritic fields were similar in size and complexity to those of normal neostriatum. Dendrites of graft spiny neurons near the edges of the grafts crossed the boundary , with some dendrites remaining in the graft and some entering the host. The pattern of spines along the dendrites was similar to normal cells (i.e. the first 20 µm from somata had few spines, the peak spine density was between 40-100 µm), but the overall density of spines on graft spiny neurons was lower than the host cells , especially in the center of the grafts.

Axons of the graft spiny neurons branched repeatedly to form a dense network throughout the grafts, and mostly confined to the grafts. A few axon collaterals could be traced a short distance outside the grafts. Electron microscopic analysis revealed synaptic contacts both in the grafts and in the host neostriatum. These synapses in the grafts were similar in structure to those in the hosts. About 40% of the labeled terminals contacted dendrites, with the remainder contacting dendritic spines or spine stalks.

360.14

AXONAL PROJECTION PATTERNS OF IENTIFIED NEOSTRIATAL MATRIX SPINY CELLS REVEALED BY INTRACELLULAR INJECTION OF BIOCYTIN IN RATS. Y. Kawaguchi, C.J. Wilson and P.C. Emson. Frontier Research Program, RIKEN, Wako, Japan 351-01, Dept. Anatomy and Neurobiology, Univ. of Tennessee, Memphis, Memphis, TN, and AFRC Institute of Animal Physiology and Genetics Research, Babraham, Cambridge, U.K.

Cytochemical studies have shown that substance-P containing neostriatal spiny neurons project primarily to the substantia nigra (SN) and entopeduncular nucleus (EP), while enkephalin-containing cells project primarily to the globus pallidus (GP). A thorough classification of cells based on their axonal projections is not possible using population labelling techniques, but requires examination of the complete axonal projections of single neurons.

We have found that complete visualization of the axonal projections of single neostriatal neurons can be achieved using intracellular injection of biocytin. Labeled cells were visualized by avidin-biotin-HRP complex with DAB reaction. Neurons residing in the matrix compartments were identified by counterstaining sections containing the injected cells using immuno-histochemistry for calbindin. In the matrix, we have found at least three subtypes of spiny neurons: (1) cells that project only to (GP), (2) cells projecting to GP and SN, (3) to GP, EP and SN. All three kinds of neurons have axonal branches in GP. The shapes and densities of the GP arborizations varied in the three cell types, with the cell projecting only to GP projecting more heavily there than the other two. These results indicate that spiny neurons are more heterogeneous in their efferent projection patterns than previously suspected, and that population studies of efferent projections and peptide content may not reveal the the full range of efferent cell types.

360.15

DEVELOPMENTAL EXPRESSION OF CALBINDIN-28KD IN STRIATUM OF POSTNATAL RATS. F.-C. Liu, A.M. Graybiel, P.C. Emson and C. Gerfen. Dept. Brain & Cognitive Sci., MIT, Cambridge, MA 02139, USA; A.F.R.C., Inst. Animal Physiology, Cambridge, UK; NIMH, Bethesda, MD, USA.

Calbindin-28KD-like immunoreactivity (CB-LI) is known to be a marker for medium-sized neurons in the extrastriosomal matrix in mature striatum of rat and other species. Here we sought to learn whether CB is expressed selectively by matrix neurons during development, and whether during development its expression is restricted to medium-sized striatal neurons. CB-LI was studied in sections through the caudoputamen (CP) of P0, P3, P7 and P15 rats. For all but P0 tissue, serial-section comparisons with tyrosine hydroxylase-like immunoreactivity (TH-LI) were made. CB-LI was expressed by two neuronal types in the P0 CP: small-to-medium cells and medium-to-large cells with well-stained dendrites. The smaller cells appeared in patches and lattice-works in a ventral arc stretching from mediocaudal to laterorostral CP, mostly ventral to regions with the darkest TH-positive dopamine (DA) islands. With increasing age, CB-LI invaded a progressively larger ventral zone that by P7 included about half, and by P15 nearly three-quarters, of the CP. The early lattice-pattern filled in so that at P7 and P15 there were only relatively circumscript CB-poor zones in otherwise CB-rich fields. Nearly all DA islands detected ventrally occurred in CB-poor patches. Often these islands were smaller than the CB-poor patches. There were many CB-poor zones for which DA islands could not be detected. The larger CB-positive neurons were mostly in the dorsal CP, where fields of small-to-medium CB-positive neurons were lacking. These larger CB-positive neurons were prominent at P0 but were rare at P7 and P15. We conclude that CB-LI is a marker of medium-sized matrix neurons during postnatal development; that its compartmental expression may occur independently of, and along different gradients from, the DA island system; and that different matrix neurons may express CB-LI at different times. The transient population of larger CB-positive neurons may either migrate out of the striatum, die, or cease expressing the CB antigen. Supported by NSF BNS 8720475.

360.16

CYTOCHROME c OXIDASE: A STRIATAL MATRIX MARKER S.J. Augood*, D.E. Lawson* and P.C.Emson MRC Group, AFRC, Institute of Animal Physiology & Genetics Research, Babraham, Cambridge, CB2 4AT.

Cytochrome c oxidase (ferrocytochrome c, oxygen oxidoreductase, E.C 1.9.3.1) is present as a trans-membrane protein of the inner mitochondrial membrane in all eukaryotes and forms part of the cell membrane in some prokaryotes. In this study we have compared the distribution of cytochrome oxidase (CO) activity with the pattern of immunocytochemical staining for the enzyme tyrosine hydroxylase (TH) and the calcium binding protein, calbindin 28K (CaBP), in the adult rat striatum at the light microscopic level.

The heterogeneous distribution of CO activity observed in the rat striatum using immunoperoxidase histochemistry was found to be analogous to the immunocytochemical staining pattern seen for TH and CaBP. Within the rat striatum areas of high TH and CaBP- like immunoreactivity (termed the matrix) corresponded to areas of high CO reaction product. Conversely, areas of low TH and CaBP-like immunoreactivity (termed striosomes or patches) corresponded to areas of low CO reaction product. We therefore propose that in addition to the established markers acetylcholine esterase, TH and CaBP, the enzyme CO may be a useful striatal matrix marker.

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360.17

LONG TERM SURVIVAL OF FETAL STRIATAL GRAFTS: LOCALIZATION OF CALBINDIN, ENKEPHALIN, NADPH-DIAPHORASE AND GABA. R.C. Roberts and M. DiFiglia. Lab. of Cell. Neurobiology, Mass. Gen. Hosp. East, Charlestown MA 02129.

We examined whether neurons in 16 month fetal striatal grafts retained similar morphological characteristics to those seen in 2 month old grafts. We also compared the morphology and frequency of grafted spiny neurons to adult neostriatal spiny neurons, by localizing immunoreacive (i) calbindin-D28k (CAL), a selective marker of spiny neurons. Grafts from 17 day fetal striata were injected into lesioned caudate in 5 adult rats. After 16 months, the neostriatum was processed for the localization of GABA, CAL, enkephalin (ENK) and NADPH-diaphorase (-d) activity. The frequency of iGABA, iENK and NADPH-d neurons in the 16 month grafts (25±6, 13±4, and 3±3, % of total neurons±SD, respectively) was similar to findings in 2 mo. grafts (Roberts & DiFiglia, 1988), indicating long-term survival of these cell types in the grafts. The density of iCAL neurons in the graft vs the host was 58±8%. The nuclear morphology of grafted iCAL cells was much more varied than in spiny neurons from normal caudate. Although approximately 1/3 of grafted iCAL cells had unindented nuclei, and thus resembled neostriatal spiny neurons, the remaining nuclei were markedly indented, notched or ruffled. This indicates a change in phenotype of many grafted spiny neurons. Supported by NS-08308-02 to RCR and NIH NS-16367 to MD.

360.18

ULTRASTRUCTURAL LOCALIZATION OF IMMUNOREACTIVE GAP-43 IN THE ADULT RAT CAUDATE NUCLEUS. M. DiFiglia[1], R.C. Roberts[1] and L.I. Benowitz[2] Lab. of Cell. Neurobiology[1], Mass. General Hosp. East, Charlestown MA 02129 and Mailman Research Ctr[2], McLean Hosp., Belmont MA 02178.

Growth-associated protein (GAP)-43 is a neuron-specific phosphoprotein implicated in neuronal development, regeneration and plasticity. In the adult, the neostriatum is among those brain areas with a high content of GAP-43. GAP-43 immunoreactivity was examined in the adult rat caudate (n=5) using a sheep polyclonal antibody. At the light microscopic level immunoreactive (i) GAP-43 was heavily localized throughout the caudate neuropil, but was absent from neuronal somata. At the ultrastructural level, labeling was most prevalent in unmyelinated axons of small diameter (0.12-0.15um). Reaction product was distributed along fibers in discrete patches about 1 um apart and in preterminal sites from which vesicle-filled boutons arose. Staining was also present in small (0.35um) axon terminals which contained round vesicles and formed asymmetric synapses mostly with thin spines. Labeled terminals resembled cortical afferents. Unexpectedly, iGAP-43 was also occasionally found in the heads of thin dendritic spines that received asymmetric contacts. We speculate that GAP-43 may be important in the remodeling of synapses onto medium spiny neurons in the adult caudate nucleus. Supported by NIH NS-16367 to MD, NS-08308-02 to RCR.

360.19

THE MORPHOLOGIC BASIS OF A MEDIAL TO LATERAL GRADIENT IN RAT STRIATAL CHOLINERGIC MARKERS. RE Burke, A Karanas,* N Kenyon*. Dept. Neurology, Columbia Univ., NYC, NY, 10032.
The striatum is organized into both distinctly bounded striosomal compartments and gradients of neuronal markers (Graybiel and Ragsdale, 1983). Several studies have shown that cholinergic (CAT) biochemical markers show an increasing medial to lateral gradient in the rat. The morphologic basis of this gradient is unknown. We therefore studied whether CAT perikarya or fibers, or both, are distributed in such a gradient. CAT perikarya were stained by immunohistochemistry using a monoclonal antibody (B-M) and PAP. The striatum was divided into medial and lateral compartments by a line along the greatest dorso-ventral axis. Stained neurons were counted at 400x in the two compartments in 2 representative sections from each rat. The area of each compartment was determined with an image analyzer and the density (neurons/mm^2) was determined. There was no significant difference between lateral and medial density of neurons either at 3 weeks (Lat x=22.2±2.2; Med x=18.6±1.7, p=0.2, NS; N=8 planes in 4 rats) or at 9 weeks (Lat x=17.5±0.7; Med x=18.6±1.0, p=.5,NS; N=8 planes in 4 rats). However, fiber staining in frozen sections in both 3 week and adult rats, showed an increased density in the lateral compartment, especially dorsally. The increased density was apparent both as number of fibers per field at 1000x, and, at the population level, as increased density of staining. NINDS #1K07NS00746, DMRF, PDF.

BASAL GANGLIA AND THALAMUS VI

361.1

EXPRESSION OF STRIATAL RECEPTORS AND CHANNELS IN RNA-INJECTED XENOPUS OOCYTES. S. Mundamattom*, S. Sealfon* and B. Gillo* (SPON: N. Levin). Dept. of Neurology and Neurobiology. Mt. Sinai Medical School. New York, N.Y. 10029.
The GABAergic neurons of the striatum (STR) play an important role in voluntary movement. We studied the expression of receptors and channels in Xenopus oocytes injected with RNA extracted from rat STR microdissections. Cells were voltage clamped 3-4 days after RNA injection. In STR RNA injected oocytes, responses to ACH (1257±167 nA, n=13), GABA (111±40 nA, n=4), NMDA (24±3 nA, n=20), quisqualate (34±12 nA, n=2), kainate (8±2 nA, n=14), CCK (103±10 nA, n=3), Substance P (233±180 nA, n=3) and neurotensin (95±41 nA, n=3) were recorded. Expression of the voltage-activated Ca current (recorded via Ca-sensitive Cl current, Tout) was increased in STR RNA injected cells. By measuring the high threshold Tout, a cAMP sensitive current, we found augmentation of the response by forskolin (25±3%, n=3) and by SKF38393 (53±29%, n=5) in STR RNA injected cells, indicating expression of STR D1 receptors. This system suggests a way to study the interaction of receptors and channels subserving the striatal contribution to motor control.

361.2

PHOSPHOLIPASE-C AND ENKEPHALIN IN THE NUCLEUS ACCUMBENS. P.Voorn* and C.R.Gerfen. Lab of Cell Biology, NIMH, Bethesda, MD 20892.

Enkephalin (ENK)-immunoreactivity (IR) in the nucleus accumbens (NA) is distributed in a heterogeneous pattern, consisting of areas with heavy, moderate or light immunostaining. This pattern is closely related to the distribution of other markers and thalamic afferents. The question is how the mosaic structure of the NA is related to that of the caudate-putamen (CP). In the present study the distribution of Phospholipase-C isozymes (PLC-II and -III, Rhee, Science, i.p.) was examined in the striatum of young (P5) and adult rats, in relation to that of ENK. A comparison was made between the occurrence of ENK and ENK mRNA in the different areas observed in the IR-pattern of ENK in the NA. The results show that in young animals PLC-II is preferentially localized in the patch compartment of the CP, whereas in adults the distribution of the enzyme is rather homogeneous, although heavy staining in the patches can still be recognized. The distribution of PLC-III in the adult is homogeneous. Medially in the NA of adults, areas heavily immunostained for PLC-II are present, which coincide with the lightly immunostained ENK areas. In turn these areas correspond to the so-called cell clusters. This feature makes PLC-II, together with μ-opiate receptors, a specific marker of the cell cluster "compartment". No correspondence was observed between the previously described heavily stained ENK areas laterally in the NA and the distribution of PLC-II. Combined immunocytochemistry and "in situ" for ENK showed that the lightly immunostained areas medially in the NA are occupied by cells lightly labeled for ENK mRNA, whereas the surrounding, moderately immunostained areas consist of cells expressing higher amounts of mRNA. In conclusion,heavy immunostaining for PLC-II is a shared feature of the patch compartment in the CP (best appreciated during development) and the cell cluster compartment in the NA. Furthermore, in contrast to the CP, where no difference is observed in the distribution of cells expressing ENK mRNA in patch or matrix compartment, the heterogeneous pattern of ENK-IR in the NA is reflected in the distribution of ENK mRNA. On the other hand, in the CP patches are characterized by heavy ENK-immunostaining, whereas the cell clusters in the NA are lightly stained and, in addition, express little ENK mRNA.

361.3

DIFFERENTIAL EFFECTS OF CHRONIC HALOPERIDOL AND CLOZAPINE TREATMENT ON EXPRESSION OF SOMATOSTATIN (SOM) mRNA IN THE STRIATUM, NUCLEUS ACCUMBENS AND FRONTAL CORTEX OF THE RAT. P. SALIN, M. MERCUGLIANO and M.F. CHESSELET, Dept. of Pharmacology, The Medical College of Pennsylvania, Philadelphia PA 19129.

Quantitative in situ hybridization with an ^{35}S-labeled cRNA probe (R. Goodman) was used to measure SOM mRNA levels in adult rat brain. In normal animals, the number of neurons containing SOM mRNA was higher in the nucleus accumbens (NAc) (11.7 ± 0.5 cells/mm^2) than in the striatum (St) (4.5 ± 0.2 cells/mm^2) or the Frontal cortex (FCx) (6.7 ± 0.7 cells/mm^2). In contrast, SOM mRNA per individual neuron was more abundant in the St than in the Nac or the FCx suggesting that SOM mRNA expression in these brain structures is differentially regulated. Chronic administration of haloperidol (1mg/kg for 28 days) induced a marked decreased of SOM mRNA contents in the NAc, FCx and in the medial but not the lateral St. Clozapine treatment (20mg/kg for 28 days) increased SOM mRNA in the NAc, but not in the St and the FCx when compared to normal animals. This difference between the effects of haloperidol and clozapine (an atypical neuroleptic) on SOM mRNA expression suggests that alterations in the activity of striatal somatostatinergic neurons may be critical for the induction of extrapyramidal side effects. (Supported by grants BNS 16841, MH 448934-01 and training grant MH 14654 to M.M.)

361.4

EFFECTS OF CHRONIC NEUROLEPTIC TREATMENT ON THE LEVEL OF GLUTAMIC ACID DECARBOXYLASE (GAD) mRNA IN THE GLOBUS PALLIDUS (GP) OF THE RAT AS REVEALED BY IN SITU HYBRIDIZATION HISTOCHEMISTRY (ISHH). M. Mercugliano,[1] M.F. Chesselet,[1] C. Saller,[2] A. Salama [2], and D. U'Pritchard [2]; Med. Coll. Penn., Philadelphia, PA.[1] and ICI America, Inc., Wilmington, DE.[2]

GABAergic neurons constitute the major output pathways of the basal ganglia and are likely to participate in the overall effects of agents which modify striatal neurotransmission. We have compared the effects of two antipsychotic drugs with different pharmacologic profiles and side effects on the level of GAD mRNA in the efferent neurons of the GP. Rats were treated with either 1 mg/kg/day of haloperidol, 20 mg/kg/day of clozapine, or vehicle for 28 days. After sacrifice by decapitation, brains were frozen, cryostat-cut into 10 µm sections, and stored at -70° C. ISHH was carried out using an ^{35}S cRNA probe (A.J. Tobin) on paraformaldehyde post-fixed sections for 3 1/2 hours at 50° C. The intensity of labeling on emulsion-coated slides was measured using computer-assisted grain analysis (Morphon). In control rats, GAD mRNA levels were similar in the dorsal, ventral, medial, and lateral GP. Sections of the GP in clozapine-treated rats showed an increase in labelling for GAD mRNA (average 42%) in all GP regions when compared to corresponding levels in control rats. No change, or a slight decrease, was seen in GP sections of haloperidol-treated rats. The results show that the level of GAD gene expression in the basal ganglia can be altered by pharmacological treatment and suggest that the atypical neuroleptic clozapine, but not haloperidol, may increase GABAergic output from the GP.

Supported in part by BNS16841, MH44894-01, Tourette Syndrome Association, ICI America, Inc. and training grant MH14654 (MM).

361.5

ULTRASTRUCTURAL EXAMINATION OF ENKEPHALIN AND SUBSTANCE P INPUT TO CHOLINERGIC NEURONS IN THE RAT NEOSTRIATUM. M. Martone*, S. J. Young*, D. M. Armstrong and P.M. Groves, (SPON: J. C. Hansen) University of California, San Diego.

In the present study, we use ultrastructural double-labeling immunocytochemical techniques to examine the interaction of enkephalin and substance P containing terminals with cholinergic perikarya in the rat neostriatum. Previous work from this laboratory has shown that in the cat, cholinergic neurons appear to concentrate within substance P rich- and enkephalin-rich patches in the dorsal-lateral caudate nucleus. While it has been demonstrated previously that cholinergic neurons receive input from substance P containing terminals (Bolam et al, Brain Res. 397, 1986), it is not known whether enkephalin interacts in a similar fashion. Both enkephalin and substance P are found in subpopulations of common spiny striatal neurons.

Adult male Sprague-Dawley rats were perfused transcardially using acrolein as the primary fixative. Vibratome sections were processed immunocytochemically for choline acetyl transferase (ChAT) (antibody provided by L. Hersh) using a silver intensified colloidal gold procedure. Sections were then processed for either enkephalin (mouse monoclonal antibody-Sera Labs) or substance P (rat monoclonal antibody-Sera Labs) using peroxidase as the second label. Following the double-labeling procedure, sections were osmicated and embedded for sequential light and electron microscopy.

Preliminary results suggest that cholinergic neurons receive a sparse enkephalinergic input onto their cell bodies and proximal dendrites. Enkephalin-positive profiles, some containing vesicles, were found apposed to 3 out of the 5 cholinergic neurons examined thus far. On two of these neurons, only one such profile was observed. No synaptic specializations were seen although serial section analysis has not yet been attempted. Enkephalin synapses were seen onto unlabeled perikarya and dendrites which formed symmetrical synaptic specializations. Three cholinergic neurons were examined in sections double-labeled for substance P and ChAT. Two of these neurons received input from substance P terminals: as many as five substance P synapses were observed onto a single longitudinally sectioned dendrite. Substance P staining terminals contained large round or pleomorphic vesicles and formed symmetrical synapses.

This work was supported in part by the Office of Naval Research.

361.6

SPECIFIC BILATERAL ALTERATIONS IN LOCAL CEREBRAL GLUCOSE UTILIZATION FOLLOWING INTRANIGRAL INJECTION OF MUSCIMOL AND SUBSTANCE P. C. Dermon*, M. Tzagrounissakis*, P. Pizarro* and H.E. Savaki Lab. of Physiology, Div.Medicine, Univ. of Crete, 71409 Iraklion, Greece.

The local cerebral glucose utilization (LCGU) of 70 bilateral anatomically discrete brain structures was investigated by means of the autoradiographic ^{14}C-deoxyglucose method, in conscious awake rats, following unilateral intranigral injection of either muscimol or substance P. Intranigral injection of the GABAergic agonist muscimol induced: (1) increased glucose consumption within the substantia nigra with the exception of the intermediate zone in the SN reticulata which remained unaffected, and (2) bilateral effects in basal ganglia and associated thalamo-cortical components with the contralateral structures more active. Intranigral injection of substance P induced: (1) enhanced glucose consumption locally in the SN, and (2) bilateral increases in LCGU within the basal ganglia and associated thalamocortical components. It is suggested that stimulation of the intermediate SN-reticulata zone is required for the activation of the ipsilateral reticularis, motor and intralaminar thalamus.

361.7

DISTRIBUTION OF SOMATOSTATIN IN THE BASAL FOREBRAIN OF THE SQUIRREL MONKEY. C. Desjardins*, A. Sadikot and A. Parent, Lab. of Neurobiol., Fac. of Med., Laval Univ., Québec, Canada.

The distribution of somatostatin (SS)-like immunoreactive fibers was studied in three squirrel monkeys (*Saimiri sciureus*) with polyclonal antibodies (SS 309 and SS 320; R. Benoit). A moderately dense network of SS-positive fibers was observed throughout the striatum in a patch-like organization. The putamen showed a greater density than the caudate. Its fibers were larger and its staining stronger peripherally than centrally. At its medial margin, a well defined limit with the pallidum was observed, the later being almost devoid of SS. The ventral striatum stood out by its very strong reactivity, coupled with a marked heterogeneity conferred by non reactive diagonal band of Broca and islands of Calleja. The accumbens nucleus was moderately stained. The amygdalo-striatal transitional zone surrounding the anterior commissure was strongly reactive. Substantia innominata was weakly stained except for some dense plexuses that appeared in register with the cell clusters of the basal nucleus of Meynert. The nuclei of the amygdala showed a wide range of staining. The central nucleus was lightly reactive while the intercalated and medial nuclei were moderately stained. A dorso-ventral gradient of staining was seen in the ventral lateral portion; the staining in the lateral nucleus was moderate to dense whereas it was very light to light in the basal nucleus. The most intensely stained area of the basal forebrain was the ventromedial portion of the amygdala especially the periamygdaloid cortex and the accessory basal nucleus. These findings will serve as a framework for studies of SS modulation in animal models of neurodegenerative diseases. (Supported by MRC, FRSQ and FCAR)

361.8

IMMUNOHISTOCHEMICAL STUDY OF BASAL GANGLIA IN PARKINSONIAN MONKEYS. B. Lavoie, P.J. Bédard and A. Parent. Lab. of Neurobiol., Fac. of Med., Laval Univ., Québec, Canada.

Antibodies against tyrosine hydroxylase (TH), substance P (SP) and enkephalins (ENK) were used to study basal ganglia of cynomolgus monkeys (*Macaca fascicularis*) in normal and parkinsonian states. Five monkeys were treated with MPTP and kept for periods ranging from 1 to 21 months after the onset of bradykinesia. One untreated animal served as a control. The TH denervation of the striatum followed a pattern strikingly similar to that in parkinsonian patients. Caudal putamen and dorsolateral regions of rostral striatum were most severely affected. Depletions in ventromedial striatum were noted only when TH cell loss in midbrain involved ventral tegmental area. A few long and linear TH-positive fibers were still present in severely depleted striatal areas, whereas the patch/matrix compartmentalization remained intact in less affected striatal areas. In contrast, the pattern of distribution of TH-positive fibers at pallidal level was not significantly altered in parkinsonian monkeys. A remarkably dense plexus a TH fibers occurred in the internal pallidum (GPi) and TH neurons remaining in substantia nigra/ventral tegmental area were distributed according to a pattern similar to that found after retrograde tracer injection in GPi in a previous study. These findings indicate that the nigropallidal dopaminergic projection is selectively spared in parkinsonian monkeys. There was also a significant increase in ENK- and a decrease in SP-immunostaining in striatopallidal neurons in MPTP-treated monkeys. These changes were particularly obvious in animals with relatively short (1 to 3 months) survival periods. (Supported by grants from the MRC, FRSQ and FCAR).

361.9

LOSS OF STRIATAL SOMATOSTATIN NEURONS FOLLOWING PRENATAL METHYLAZOXYMETHANOL. J.M. Radke, K. Semba, A. Jakubovic, H.C. Fibiger and S.R. Vincent. Kinsmen Laboratory of Neurological Research, Dept. of Psychiatry, The Univ. of British Columbia, Vancouver, V6T 1W5, Canada.

In the rat striatum, somatostatin is contained in a population of aspiny interneurons, together with neuropeptide Y, and the enzyme NADPH-diaphorase. During development, these neurons undergo their final mitotic division on gestational days G15-16 (Semba et al., *J. Neurosci.* 8:3937, 1988). Methylazoxymethanol (MAM) kills dividing cells by alkylating nucleic acids, and i.p. injections of MAM into rat dams on G15 produces a marked reduction in telencephalic size of the offspring. In the present study we examined the effect of such treatment on the somatostatin system in the rat striatum. Injection of 25 mg/kg MAM into pregnant rats on G15 had no effect on body weight, but resulted in microencephaly of the offspring, when examined at 6 months of age. This was reflected in reductions in the wet weight and protein concentration of the striatum. The concentrations of dopamine and serotonin were increased in proportion to the reduction in striatal tissue, however, the total amounts of these amines per striatum did not differ from control. In contrast, the striatal concentration of somatostatin was reduced by half, and the total amount of somatostatin in the striatum was reduced by over 60%. This reduction was paralleled by a loss of half the somatostatin neurons in the striatum. Although this MAM treatment had a significant effect on the neurochemical organization of the striatum, opiate receptor autoradiography indicated that there was no effect on the patch-matrix organization of the striatum.

361.10

SYNAPTIC CONNECTIONS OF SUBSTANCE P IMMUNOREACTIVE TERMINALS IN THE MARGINAL DIVISION OF THE STRIATUM IN THE RAT. S.Y. Shu, X. Zhang, X. Bao J.F. McGinty*and G.M. Peterson‡ The Fourth Medical College, Xian, China. *East Carolina University,Greenville,NC 27858.U.S.A.

Based on the cellular morphology, immunohistochemistry and projection pattern, a new subdivision, the marginal division has been found in the striatum of rats(S.Y. Shu et al. 1988). The marginal division is more densely filled with Met-enkephalin-,substance P-(SP), dynorphin B-, neurotensin- and cholestokinin-immunoreactive terminals. Electron microscopic study has shown several types of synapses of SP positive terminals onto neurons in the marginal division. One SP containing terminal was often seen to make synaptic contacts with two dendrites or two spines. Two SP-immunoreactive terminals,or one SP positive terminal and one or more SP negative terminals are sometimes observed to make synaptic contacts with one dendrite of the neuron in the marginal division. It is suggested the possibility that imputs from SP-immunoreactive fibers converge or diverge onto neurons in the marginal division.

BASAL GANGLIA AND THALAMUS VII

362.1

PECKING AND ASSOCIATED STEREOTYPED OROFACIAL MOVEMENTS EVOKED BY ELECTRICAL STIMULATION OF THE PIGEON STRIATUM. I.J. Goodman. Departments of Psychology, and Behavioral Medicine & Psychiatry, West Virginia University, Morgantown WV 26506-6040.

Striatal mechanisms of orofacial motor activity in birds were studied, using electrical brain stimulation (ESB) in unanesthetized, awake pigeons with stereotaxically-implanted electrodes. ESB (30 Hz, biphasic waves, 0.1 ms, 0-0.6 mA) evoked repetitive pecking (not feeding) and/or stereotyped non-pecking orofacial movements from histologically confirmed dorsolateral (paleostriatum augmentatum, PA) and rostromedial (parolfactory lobe, LPO and nuc. accumbens, Ac) striatal electrode placements. Birds at 80% of normal body wt. displayed lower peck-evoking thresholds than when at normal wt. ESB-evoked pecking rates tended to be positively related to intensity (current), except when interfering movements were simultaneously evoked. Patterns of stereotyped head movements evoked with ESB were similar to those previously reported in pigeons with systemic or direct substantia nigra injections of a dopamine agonist (apomorphine). Similarities in ESB-evoked stereotyped head movements observed from PA, LPO and Ac sites alone do not rule out a functional differentiation among these avian striatal structures, particularly since other studies have provided some anatomical and physiological findings that suggest differentiation. Further striatal studies are required.

362.2

MOTOR IMPAIRMENTS PRODUCED BY MODULATION OF GABAERGIC AFFERENCES OF THE PALLIDUM IN THE CAT. Amalric M., Schmied A.*, Farin D.*,Dormont J.F.* Lab.de Neurobiol.et Neuroph. du Développement, CNRS UA 1121, Bât.440,UPS 91405, Orsay France.

Striatal outputs towards the pallidum (Globus Pallidus, GP, and Entopedoncular nucleus, EP) are mostly GABAergic. To evaluate their role in motor control, we examined the effects of unilateral local microinjections of muscimol or bicuculline into the GP or the EP on the performances of cats trained to release a lever in a simple Reaction Time (RT) task. Opposite effects of muscimol were observed in the 2 structures. In the GP, muscimol at low doses (5-10 ng) induced an increase in RT, followed by contralateral rotations at higher doses. Conversely, in the EP, 10 ng of muscimol induced a decrease in RT and increased the number of anticipatory trials. Higher doses were followed by an arrest of the performance. After muscimol injection, the force exerted on the lever was lowered in the two structures. Bicuculline (20 to 50 ng) slightly decreased RT's, when injected in the GP but had no effect, when injected in the EP. Impairment of performances following GABA receptor activation in the GP was similar to the blockade of dopamine function in the striatum, suggesting a possible modulation of the dopamine on GABAergic Pallidal output. The decrease of RT observed after the activation of EP GABA receptor could result in the blockade of inhibitory outputs of the EP towards the thalamus and/or the midbrain.

362.3

EFFECTS OF NON-STRIATAL GRAFTS AND POST-LESION DELAY ON REVERSAL OF LOCOMOTOR ABNORMALITIES AFTER INTRASTRIATAL KAINIC ACID LESIONS. M. Giordano, E.M. Zubrycki, A.B.Norman, and P.R. Sanberg Div. of Neuroscience, Dept. Psychiatry, University of Cincinnati Medical Center, Cincinnati, OH 45267-0559.

After bilateral intrastriatal kainic acid lesions (KA) Sprague-Dawley male rats received intrastriatal grafts of striatal ridge, dorsal mesencephalon (tectum), and frontoparietal cortex (E 15-16) seven or thirty days after lesion. Subjects were then evaluated in a variety of motor tasks every 4-6 weeks over a period of six months. A differential trend in results was found for haloperidol-induced catalepsy and amphetamine-induced activity tests for different tissue grafts. With regards to catalepsy both striatal groups (early and late post-lesion) had consistently higher scores than both cortical groups. The lesion-only group showed the highest increase in rearings over sixty minutes after amphetamine (1 mg/kg i.p.). The early tectal and cortical groups showed a qualitatively similar pattern, whereas the striatal groups paralleled the response of the sham group. Gross behavioral abnormalities such as convulsions and hyperreactivity were observed in a variety of animals regardless of graft type. These responses maybe the result of further degeneration induced by the lesion and by possible negative effects of the grafts.
These results support the notion that tissue specificity is important for behavioral recovery.

362.4

KYNURENIC ACID-INDUCED BLOCKADE OF EXCITATORY AMINOACID TRANSMISSION IN THE STRIATUM OF RAT PRODUCES A REGIONALLY SELECTIVE IMPAIRMENT OF FORELIMB REACHING. M. Pisa, S. Anwar* and C.A. Booth*. Dept. of Biomed. Sci., McMaster University, Hamilton, Ont., L8N 3Z5.

This research was part of a project aimed at elucidating the regional behavioral roles of striatal excitatory-aminoacid (EAA) activity. Rats (N=10) with chronic intracerebral implants of guide cannulae were trained in a forelimb reaching task (M. Pisa, Neurosci., 24:453,1988) and tested 30 min after bilateral injections of kynurenic acid, an antagonist of EAA transmission (either 0, 24, 48 or 72 nmoles in .4 ul PBS, pH 7.4), into either the dorsolateral striatum or the ventromedial striatum (nucleus accumbens). Striatal region and dose were between- and within- factors, respectively. Only injections of kynurenic acid in the dorsolateral striatum produced a statistically significant, dose-dependent impairment of reaching performance, a regionally selective effect consistent with the findings of previous lesion studies (Pisa and Schranz, Behav. Neurosci. 102:429,1988). Since the excitatory aminoacid glutamate is a major transmitter at corticostriatal synapses, the present results suggests a critical role of corticostriatal glutamatergic transmission in reaching efficiency. (Supported by the MRC of Canada. M. Pisa is a Research Associate of the Ontario Mental Health Foundation).

362.5

ELECTROPHYSIOLOGICAL ACTIONS OF NICOTINE ON ANTERIOR THALAMIC AND NEOSTRIATAL NEURONS IN FREELY MOVING AND TREADMILL LOCOMOTING RATS. D.J.Woodward, J.Y.Chang, S.F.Sawyer and R.-S.Lee, *Dept. of Cell Biol., UT Southwestern Med. Ctr., Dallas,TX 75235.*

The anterior thalamic nuclei (ATN) have the highest density of nicotinic receptors in the brain, and nicotine elicits large changes in metabolic activity in these nuclei. The aim of the present study was to investigate the electrophysiological responses to nicotine of neurons in the ATN. Teflon insulated stainless steel microwires (5-16 of 25-65μm diameter) were implanted in ANT or neostriatum of adult male Long-Evans rats. One week later, of the units that were histologically confirmed to be in the ATN, 69% (22/32) exhibited an increase in firing rate following nicotine with a mean±SEM increase of 139.6±14.7% and 148.4±16.7% following the 20μg/kg and 40μg/kg i.v. doses, respectively. The responses started a few seconds after injection and typically returned to pre-drug levels in 1-2 min. Mecamylamine (1.0mg/kg, i.v.), effectively blocked the response to nicotine. In some experiments, gross behavior was entrained by placing the animals on a treadmill (TM) that cycled off and on. 20μg/kg nicotine (i.v.) given during the TM-induced locomotion resulted in a 60.2±10.5% increase in unit activity. In contrast, 40μg/kg nicotine (i.v.) was infused during the TM-off period, causing a 112.3±21.8% increase in unit activity (similar to freely moving). On the fully TM trained rats, 0.1-0.3mg/kg nicotine (i.p.) caused a prolonged suppression of firing rate during TM on phase. Neurons in neostriatum exhibited altered firing patterns for up to 60 min after 0.3mg/kg (i.p.). Subcutaneous nicotine (0.4mg/kg) elicited equivalent increases in firing rate (123±17.1%) and lasted for 5-8 min. Consistent with receptor binding and metabolic studies, these experiments provide evidence that low doses of nicotine activates ATN neurons and that long term changes in receptor mediated events in ATN and neostriatum may induce the increase of glucose uptake. (*Supported by DA2338, NIAAA3901, R.J.R. Tobacco Co. and Biological Humanics Foundation*)

362.6

PHYSIOLOGY OF HUMAN CAUDATE NEURONS: I. BASIC MEMBRANE PROPERTIES AND INTRACELLULAR STAINING. C. Cepeda*, J.P. Walsh, W. Peacock* and N. Buchwald. MRRC, UCLA School of Medicine, Los Angeles, CA 90024.

Our laboratory has studied the physiological properties of neocortical neurons in specimens taken from 16 children suffering from intractable epilepsy in which partial or complete hemispherectomies were made. In 5 of these (ages 8 mo. to 8 yr.) caudate nucleus tissue was also taken for pathological study. A small piece of this tissue was made available to us for in vitro physiological and morphological examinations. In brain slices of this tissue intracellular recordings were made with K^+ acetate electrodes (50-100MΩ). Basic membrane properties were determined in 20 neurons. Resting membrane potentials ranged from -65 to -80mV. Action potentials evoked by depolarizing current pulses usually ranged from 70 to 80mV. Most cells showed rectification when hyperpolarizing current pulses were applied. Spike adaptation to the depolarizing current pulses was not observed. The input resistance of these neurons varied between 13 and 26 MΩ. Excitatory postsynaptic potentials were evoked in all cells by intracaudate stimulation. In addition, 19 cells were injected with Lucifer Yellow. All but one of the cells proved to be medium-sized spiny neurons. Dye-coupling was observed in 2 cases. The action potentials amplitudes and input resistances of these human neurons were similar to those obtained in our laboratory from cat and rat brain slices. These results indicate the viability of caudate brain slices obtained from pathological human specimens and establish the feasibility of performing electrophysiological and morphological examinations of this tissue.

362.7

ELECTROPHYSIOLOGICAL EFFECTS OF NMDA RECEPTOR ACTIVATION IN THE DEVELOPING NEOSTRIATUM OF THE CAT. M. Bertolucci-D'Angio*, S.M. Siviy, M.S. Levine C.D.Hull and N.A. Buchwald. MRRC, UCLA, Los Angeles, CA 90024.

It is believed that excitatory corticostriatal inputs are glutamatergic. There is increasing evidence that these postsynaptic receptors are of the kainate/quisqualate variety. Although n-methyl-d-aspartate (NMDA) receptors do not appear to mediate corticostriatal transmission in adult cats and rats, they may be more important during early stages of development. In this study, an in vitro slice preparation was used to assess the effects of NMDA receptor activation in the developing cat neostriatum. Neostriatal slices (400 μm) were obtained from kittens 16-54 days of age. To demonstrate NMDA contributions to evoked excitatory postsynaptic potentials (EPSPs), Mg^{2+} free Ringers was used as the bathing medium. Bicuculline (10 μM) was added in order to block inhibition mediated by $GABA_A$ receptors. Under these conditions, the amplitude and duration of EPSPs evoked by local stimulation was increased. This enhancement was maximal in kittens under 32 days of age, with local stimulation producing long duration EPSPs with bursts of action potentials. This enhancement was reversibly blocked by AP-5 (10-50 μM), a selective NMDA antagonist. To date we have been unable to evoke this bursting response in older cats. These findings demonstrate that NMDA receptors play a prominent role in early development of the cat neostriatum. Supported by USPHS Grant HD 5958.

362.8

PHYSIOLOGY OF HUMAN CAUDATE NEURONS: 2. EXCITATORY AMINO ACIDS AND DOPAMINE. J.P. Walsh, C. Cepeda*, W. Peacock* and N.A. Buchwald. MRRC, UCLA, Los Angeles, CA 90024

In the course of hemispherectomies designed to alleviate intractable epilepsy in children (ages 8 mo. to 8 years), specimens of caudate nucleus were obtained. Using in vitro slice procedures, intracellular recordings revealed that local extracellular stimulation elicited short duration excitatory post-synaptic potentials (EPSPs) in caudate neurons. The general excitatory amino acid antagonist, kynurenic acid, blocked these EPSPs without effecting cell excitability or input resistance. To investigate the possible activation of post-synaptic NMDA receptors during synaptic excitation, slices were bathed in a solution of Mg^{2+} free Ringers. This solution served to eliminate the Mg^{2+} block of NMDA receptors which occurs in normal medium. Synaptic excitation was examined in isolation by adding bicuculline (10 μM) which blocked GABA mediated inhibition. Bicuculline, by itself, had little effect on EPSPs. Bicuculline in Mg^{2+} free Ringers solution increased the amplitude and duration of EPSPs. Supra-threshold extracellular stimulation of slices bathed in Mg^{2+} free saline elicited long duration depolarizations and bursts of action potentials. This enhancement of synaptic input and bursting was blocked reversibly by the NMDA antagonist AP-5 (1-50 μM). In a separate set of experiments, dopamine (DA) was tested for its ability to modulate synaptic input and cell firing in caudate neurons. Two effects were seen: 1) DA (10-100 μM) decreased the number of action potentials elicited by depolarizing current injection and 2) DA decreased EPSP amplitude in a dose dependent manner. These data demonstrate that, in agreement with results from animal models, human caudate neurons receive synaptic input mediated by excitatory amino acids and that DA decreases the efficacy of this input.

362.9

VOLTAGE-CLAMP ANALYSIS OF CALCIUM CURRENTS IN RAT NEOSTRIATAL NEURONS. D. J. Surmeier, J. Bargas and S. T. Kitai, Dept. of Anatomy and Neurobiology, College of Medicine, The University of Tennessee, Memphis, Memphis, TN 38163.

Calcium currents play an important role in regulation of neuronal excitability and integration. Previous current-clamp studies have suggested that only high-voltage activated calcium currents are expressed by rat neostriatal neurons. Using the the whole-cell variant of the patch clamp technique, we have found that these neurons express both low-voltage activated (LVA) and high-voltage activated (HVA) calcium currents.

Neurons from embryonic rat neostriatum were dissected and maintained in vitro as previously described (Surmeier et al., Brain Res. 473: 187-192, 1988). Neurons were maintained in culture for from 7-21 days prior to study. Whole cell currents were recorded using conventional techniques. Calcium currents were recorded in media containing (in mM): 120 TEA-Cl, 10 CaCl2, 6 4-AP, 10 Cs-HEPES, 3 CsCl, 11 glucose, 0.01 TTX (pH=7.3). Patch pipettes contained (in mM): 60 CsF, 60 CsCl, 20 TEA-Cl (or F), 10 EGTA, 10 HEPES, 2 Mg-ATP, 0.2 GTP (pH=7.3).

An LVA transient component of the calcium current was evoked by step depolarizations above -40 mV from negative holding potentials (-100 to -70 mV). The LVA current was blocked by Ni (100 μM) but only slightly affected by Cd (50-100 μM). Step depolarizations above -20 mV evoked a more long-lasting, slowly inactivating inward current. This component of the calcium current was preferentially blocked by Cd (100 μM) and was enhanced by replacing Ca with Ba as the charge carrier.
This work was supported by P.H.S. grants NS 20702 to S.T.K., NS 26473 to S.T.K. and D.J.S.

362.10

SEROTONIN EFFECTS ON RAT STRIATAL NEURONS IN *IN VITRO* SLICE PREPARATION. A. Stefani and S.T. Kitai, (Spon: A. Del Bo) Dept. of Anat. and Neurobiol., College of Medicine, The Univ. of Tenn. Memphis, TN 38163.

Anatomical studies indicated that 5-HT axons originating from the dorsal raphe nucleus terminate on the dendrites of striatal neurons in the rat. Although the 5-HT innervation in the striatum is largely "non-junctional" (Soghomonian et al., Brain Res. 481:67, 1989), previous electrophysiological studies indicated that electrical stimulation of the dorsal raphe nucleus induces excitatory postsynaptic potentials. We have examined the effect of bath application of 5-HT in a striatal slice preparation in the rat. Neostriatal slices (300-350 uM thick) were preincubated for 1-2 hours in oxygenated (95% O_2 -5% CO_2) Krebs Ringer buffer (NACl 124, KCl 5, Mg SO_4 1.3 KH_2 PO_4 1.22, $NAHCO_3$ 25.5, $CaCl_2$ 2.3, glucose 10) then transferred to submerged recording chamber at 35° C. Intracellular recordings were obtained with electrodes (50-70 Mega ohm) filled by 3M Methylsulfate. Serotonin was bath applied by adding freshly prepared stocks to the saline and by switching the perfusion from control saline to drug-containing solution. Complete exchange of the chamber volume took 90-150 seconds. Intracellular recordings were fed into a Neurodata (1R-183) preamplifier, displayed on digital oscilloscope and stored on magnetic tape. Records were obtained from 16 neurons with the RMP ranging from -65 to -80. Intracellularly applied depolarizing pulses evoked repetitive tonic firing with little adaptation. The action potential was triggered from a slow ramp-like depolarizing potential. Fast action potential was followed by after-hyperpolarization. Bath application of 5-HT (20-40 um) produced in 85% of recorded neurons a clear increase in firing rate with an increase in membrane resistance during depolarization current injections. An initial transient small hyperpolarization was produced in 6 out of 16 neurons immediately following 5-HT applications, subsequently these neurons also responded with excitations. (Supported by NIH NS 20702 and NS 23886 to STK).

362.11

LONG-TERM, MULTI-CHANNEL RECORDING OF SINGLE UNITS IN RAT NEOSTRIATUM DURING AN ON-OFF TREADMILL PARADIGM

D.Y. Sanchez, R.-S. Lee, S.F. Sawyer, and D.J. Woodward. *Cell Biology and Anatomy, Univ. Texas Southwestern Medical Center, Dallas, TX 75235.*

Previous studies in this laboratory (West et al., 1987) have demonstrated altered modes of activity in rat neostriatal neurons during a fixed interval, on-off treadmill (TM) exercise paradigm. The response of these neurons changed markedly as the rat learned to anticipate the regular cycle of the TM. The objective of the present study was to quantitatively characterize the response of neurons in the neostriatum to TM exercise, over a period of 2 - 7 weeks. Twenty microwires (25-45 μm diameter) were surgically implanted in the neostriatum of adult, male, Long-Evans rats. Days later, TM-naive rats were induced to walk on a treadmill (30 sec on/ 30 sec off). A tone served as a cue for the onset and offset of TM locomotion. Single unit, multi-channel activity of 2 - 6 neostriatal neurons was recorded extracellularly in each animal. We have found that many neurons increased their firing rate during the TM-on phase of each cycle (TM-on response) while a small percentage of neurons showed increased activity during the rest phase (TM-off response). Additionally, some neurons decreased the "on" or "off" response to the TM cycle within the initial 30 min of the rat's first TM session. In contrast, other units did not change their TM response even after several weeks of daily, single-hour trials. Among the neurons that exhibited decreased response to TM over time, some resumed their original TM response after a 10 to 20 min rest period. These results demonstrate that the time course of changes in TM response is highly variable in rat neostriatal neurons. Clearly, the population of neuronal responses even within a small area of neostriatum is highly heterogeneous. We postulate that the pattern of activity of neostriatal neurons corresponds to attentional, anticipational, or context states which change during long term exposure to a sensory motor task.

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362.12

LOCALIZATION OF THE CORPUS STRIATUM SUBREGION RESPONSIBLE FOR A PAW TREADING CHOREA IN THE RAT.

H.C.Cromwell* and K.C.Berridge. The University of Michigan, Department of Psychology, Ann Arbor, MI. 48109.

Human striatal lesions are often accompanied by choreic movement patterns. Previous studies have shown that large striatal lesions in the rat result in a paw treading chorea to oral taste infusions (Berridge et al,1988). This exaggerated paw treading was elicited by a wide range of oral stimuli after such lesions. The response was a large amplitude, long duration forepaw movement that is not produced by intact rats.

In the previous large lesion study, the treading was most pronounced following posterior striatal lesions. To determine whether a crucial subregion exists within the posterior striatum responsible for this pathological behavior, small excitotoxic lesions were made. Microinjections of kainic acid (KA), quisqualic acid (QA), quinolinic acid (QUIN), or ibotenic acid (IBO) were made bilaterally into either the posterior ventromedial striatum (including globus pallidus) or the posterior dorsolateral striatum, or the posterior dorsomedial striatum. Potential treading was elicited by oral infusions of either palatable (eg., sucrose) or unpalatable (eg., quinine and HCL) solutions. Paw treading and other elicited behaviors were scored in a slow motion taste reactivity analysis.

The choreic response was most pronounced after lesions in the ventromedial sector. The dorsomedial group also showed infrequent paw treading. The choreic pattern was not seen following dorsolateral damage. The aversive evaluation of the taste seemed to be an important aspect of the triggering mechanism. The choreic treading was only seen to infusions of unpalatable solutions (e.g., quinine). Thus medial posterior damage to the striatum in the rat appears to specifically potentiate treading as a motor pattern elicited by unpalatable oral stimuli.

MOTOR SYSTEMS: REFLEX FUNCTION I

363.1

PHASE-DEPENDENT HUMAN FLEXOR REFLEX CHANGES DURING CYCLING.

D. A. Brown,* C. G. Kukulka. Graduate Program in Physical Therapy, University of Iowa, Iowa City, IA 52242.

This study examined phase-dependent changes in the Human Flexor Reflex (HFR) during cycling. Human studies have not addressed the problem of demonstrating reflex changes independent of the motoneuron pool excitability level. This study investigated the HFR during cycling while controlling ankle muscle activation. Ten healthy, male subjects were each involved in a set of three experiments. All experiments involved cycling on an ergometer at a set rate and workload. A 333 Hz, 15 ms pulse train of electrical stimulation was randomly delivered to the tibial nerve. First, subjects were stimulated at six hip positions while cycling with normal, phasic ankle muscle activity. Next, under static conditions, the HFR was elicited while controlling either the soleus or tibialis anterior (TA) background EMG at a target level. While cycling, stimulation was applied as the subjects maintained similar levels of background contraction. Responses were analyzed by averaging 5-18 stimulation trials triggered at similar hip angles. Trials with distinctively different M-wave amplitudes and baseline EMG activity were eliminated. Results demonstrated changes in response patterns, latency, and mean response peak areas, during cycling, that persist under conditions of steady background muscle activity. It is concluded that the HFR may be modulated by mechanisms independent of motoneuron pool excitability levels.

363.2

DIFFERENTIAL MODULATION OF CUTANEOUS REFLEXES DURING SLOW RHYTHMIC FINGER MOVEMENTS.

A.M.S. Poon*, I.C. Bruce, P.W.F. Poon* and J.C.C. Hwang. Department of Physiology, University of Hong Kong, Hong Kong.

Cutaneous reflexes were elicited in the first dorsal interosseous muscle by electrical stimulation of the digital nerves at specific points in each cycle of 0.5Hz adduction-abductions of the index finger. Reflex EMG components were compared with control values obtained during tonic contractions. After correcting for the voluntary activity and individual variations in timing, EMG activity relative to background was computed (mV/ms) for each component.

Two separate excitatory-inhibitory sequences, one early and one late (onsets 26 & 58 ms), were recorded. During adduction the amplitudes of all components decreased in parallel with the voluntary EMG. In abduction, while the amplitude of the late excitatory component continued to drop below background as voluntary EMG increased, the values for the early components rose. Around the abduction-adduction transition, the late excitatory component switched to maximum amplitude above background, then once again matched the voluntary EMG during the next adduction.

This "switched" modulation of the late component may reflect a change in motor set during slow rhythmic finger movements.

363.3

THE EFFECT OF NON-PAINFUL CUTANEOUS STIMULI ON HUMAN MOTOR PERFORMANCE.

E. Logigian* and D. Cros* (Spon: J. Donoghue) New England Medical Center and Massachusetts General Hospital, Boston, MA.

We investigated modulation of first dorsal interosseous (FDI) motor performance by cutaneous stimuli adjacent to and remote from this muscle. During tonic, and prior to "ballistic", isometric contraction of FDI, trains of non-painful cutaneous stimuli (stim) were applied over the palmar surface of the second digit (PD) or the lateral aspect of the foot (LF) in 4 normal subjects. Rectified surface electromyographic activity (EMG) and force of FDI were recorded. During tonic contraction, both PD and LF stim evoked a consistent late excitatory EMG response (E2) and a less consistent early response (E1). PD, but not LF stim, evoked a long silent period prior to E2. The respective tonic force profiles were therefore different. PD stim produced a relatively large lapse in force followed by a rebound to baseline, whereas LF stim produced a slight increment in FDI tonic force output. In contrast, the amplitude of "ballistic" force pulses, voluntarily produced after an auditory tone, was enhanced when the tone signal was combined with either PD or LF stim. In summary, there are remote effects of LF stim which are excitatory for both tonic and "ballistic" FDI motor tasks. PD stim is inhibitory for tonic motor tasks, but may be excitatory for "ballistic" tasks.

363.4

REFLEX ACTIVATION OF HUMAN WRIST EXTENSOR MUSCLE MOTOR UNITS BY TENDON VIBRATION.

P. Romaiguère*, J.P. Vedel, and S. Pagni* LNF-U3, CNRS,Marseille cedex 9, France.

As required by the Helsinki declaration all subjects gave their informed consent to the experimental procedure.

Recruitment thresholds of motor units (MU) of the extensor carpi radialis muscles were compared during volontary and reflex isometric contractions characterized by similar force increase velocity. MU recruitment thresholds were strongly decreased during reflex contractions. This effect was explained by the almost selective activation of the extensor carpi radialis muscles by their tendon vibration while during volontary contractions the radialis muscles and their synergist, the extensor carpi ulnaris muscle, contracted with the same intensity. This observation suggests a weak diffusion of the Ia motoneuronal activation from the extensor radialis muscles to the extensor ulnaris muscle.

Analysis of MU reflex activity using "post stimulus time histograms" showed that slow MU were mainly monosynaptically activated by tendon vibration while fast MU were activated both monosynaptically and polysynaptically. Slight superficial cutaneous stimulations induced total inhibition of all MU monosynaptic activation without modifying fast MU polysynaptic activation. This differential effect suggests a presynaptic inhibition of the Ia monosynaptic connection with motoneurons by low threshold cutaneous afferents.

(work supported by a DRET grant).

363.5

SUCCESSIVE MUSCLE CONTRACTIONS DECREASE RECRUITMENT FORCE THRESHOLDS IN SINGLE MOTOR UNITS. S Suzuki*, A Hayami*, M Suzuki*, S Watanabe*, and R S Hutton Sch of Human Sci., Waseda University, Japan Women's College of PE, Kinjo Gakuin University, Kyorin University, and Dept. of Psych., University of Washington.

Recruitment force thresholds (FTs) in single motor units (SMUs) are known to decrease as speed of contraction increases (Desmedt, TINS, 3:265, 1980). Preliminary evidence suggests that successive muscle contractions also lower the FT in SMUs (Suzuki, et al., Med. Sci. Sports Exerc., 20:S4, 1988). The purpose of this study was to examine further the latter observation. FTs of 30 biceps brachii SMUs were recorded from 6 male subjects (Ss) using fine wire coiled copper electrodes. Ss rested their upper arm horizontally on a table with their elbow angle at 90 degrees in flexion. With the forearm in supination, Ss gripped a handle attached to a force transducer to generate isometric flexion forces. Visual force feedback was provided by an oscilloscope. The initial mean FT for 30 SMUs was 20.8 N (range 1.8 - 54.5 N). FTs were found to decline from < 10% to 100% over the course of 5 additional successive contractions. The initial mean FT was significantly higher ($p < 0.05$) than the mean FT values observed during the final 3 contractions. SMUs exhibiting lower initial FTs generally showed larger percent decreases in FT over successive contractions. Intermittent muscle stretch (90 degrees of extension) tended to reset the SMU FT toward the initial value. This suggests that the source of greater excitatory current in lowering SMU FTs during successive contractions may reside in contraction-induced potentiation of stretch reflex pathways (Hutton et al., J Physiol. (Lon.), 393:247, 1987).

363.6

MECHANICAL AND REFLEX CONTRIBUTIONS TO JOINT STIFFNESS DURING COCONTRACTION. R.R. Carter, P.E. Crago and M.W. Keith. Depts. of Biomedical Engineering and Orthopaedics, Case Western Reserve Univ., Cleveland, OH 44106.

Cocontraction of antagonists can increase joint stiffness independently of joint torque. We are estimating the magnitudes of the passive, intrinsic active, and reflex contributions to the total response during single muscle contraction and during cocontraction of the flexor and extensor pollices longus in human subjects. The total response at the thumb interphalangeal joint is measured while the subjects exert both a net torque and a level of cocontraction. Subjects are instructed to 'not react' to a sudden joint extension. The passive response is measured with the muscles relaxed completely. Each muscle's intrinsic response is estimated separately during electrical stimulation. The total intrinsic component is calculated as the sum of the separately scaled intrinsic responses of the two antagonists. Scaling parameters are estimated by an ordinary least squares fit of the two normalized intrinsic components to the total active response prior to reflex onset, and are unique since torques subtract but stiffnesses add. The passive and total intrinsic components are then subtracted from the total response, leaving only the net reflex component. Reflex components are then compared during single muscle activation and during cocontraction.

(Supported by NIDRR Grant H133E80020)

363.7

LONG LATENCY MUSCLE RESPONSES FOLLOWING RECURRENT LARYNGEAL NERVE STIMULATION IN HUMANS. C. L. Ludlow, S. G. Yin* and M. Hallett. Speech and Voice Unit, NIDCD and Human Motor Control Section, NINDS, Bldg.10/5N226, Bethesda, MD 20892.

Adductor laryngeal muscle responses occur between 22 and 24 ms following stimulation of the superior laryngeal nerve sensory branch. In this study, long latency muscle responses were found in normal volunteers following recurrent laryngeal nerve (RLN) stimulation. Hooked wire recordings from the right and left thyroarytenoid (TA) and cricothyroid (CT) muscles and surface recordings from the posterior cricoarytenoid were made during quiet respiration, effort closure and sustained vowel. Stimulation needle electrodes were placed in the region of the right RLN at the third tracheal ring. Ten single or triple pulse stimulations were administered at different current levels between threshold and supramaximal direct responses. Triggered averages as well as individual muscle responses were measured from each set of ten stimulations. A late response starting between 50 and 60 ms at an amplitude of between 30 and 50% of maximal activity was consistent in the right TA in 80% of subjects tested. This polyphasic response peaked between 60 and 70 ms and lasted 20 to 30 ms. The amplitude increased with higher levels of muscle activation such as during effort closure and with triple pulse stimulation. In some subjects a simultaneous response was seen in either the contralateral TA or the ipsilateral or contralateral CT. An earlier shorter single low amplitude response between 20 and 30 ms occurred inconsistently in 50% of subjects. This only occurred in the right TA, had a duration of less than 10 ms, was enhanced at lower stimulation levels and during respiratory activation. These characteristics are suggestive of a mechanism analogous to a flexor reflex.

363.8

MODULATION OF THE UNLOADING REFLEX DURING VOLUNTARY MOVEMENT: R.R.Young, A.W.Wiegner and L.Davies*. Spinal Cord Injury Unit. Veterans Administration Medical Center. Boston. MA 02132.

During tonic contraction, muscle spindles provide excitatory input to α-motoneurones via Ia afferent fibres. An abrupt decrease in activity in these fibres produces an unloading reflex. This reflex may be useful in maintaining a desired muscle output against small force perturbations but might be counter-productive during very fast movements. We studied the unloading reflex in the biceps of human subjects during voluntary elbow flexion at various speeds.

Three variants of elbow flexion were examined: slow ramp movement under fine control (peak velocity 29±8 deg/s Mean±SD), intermediate speed movements of the type used for everyday limb placement activities (56±18 deg/s) and rapid ballistic movements (356±28 deg/s). While unloading reflexes were observed throughout slow and intermediate speed movements, the reflex could not be demonstrated during ballistic movements until the target had been attained. This suppression of a monosynaptic reflex during ballistic movements may serve to prevent confounding afferent input from reaching the spinal motoneurone pool.

363.9

METHODOLOGIES FOR DIFFERENTIATING BETWEEN "RII" AND "RIII" REFLEX EMG RESPONSES IN HUMANS. R.G. Durkovic and S.G. Nord. Depts. of Physiol. & Anat., SUNY Health Sci. Ctr. and Neurology Service, VAMC, Syracuse, NY 13210.

The "RII" and "RIII" components of sural nerve/biceps reflex EMG responses are customarily defined in terms of latencies from stimulus onset ("RII" 40-80 ms; "RIII" 85-150 ms). However, within/between subject variability can sometimes obscure this distinction. As a consequence, we sought additional criteria to help differentiate these two components.

Because of its effectiveness in enhancing lower-limb reflexes, we tested the effect of the Jendrassik maneuver on the reflex components. Innocuous percutaneous electrical stimulation (200 Hz, 4 pulses) of the sural nerve was presented 1/min while recording EMG activity from the ipsilateral biceps femoris muscle. For most subjects "RII" was enhanced and "RIII" was depressed during the maneuver. Recordings from additional muscles also proved useful in that "RIIs" tended to be restricted to muscles near the site of stimulation, while "RIIIs" were widespread throughout the body, like startle responses. Notably, "RIII" onset latencies exhibited a cephalo-caudal sequence, like startle responses. In contrast to reports of some investigators, painful stimuli were not required for eliciting "RIIIs" in most subjects. Supported by NSF Grant BNS 8808495.

363.10

DOES ASYMMETRICAL MODULATION OF SEGMENTAL INPUT ALTER ABDOMINAL EXPIRATORY ACTIVITY (AEA) IN HUMANS? B. Bishop, E. Aurnou*, F. Cerny* and J. Hirsch. SUNY/B. Buffalo, NY 14214.

Feedback from proprioceptors in lungs and abdominal wall is essential for AEA and tilting from supine to headup augments AEA. Does changing from supine to the left lateral decubitus position cause a bilateral modification in AEA? To answer this question, we had subjects (Ss) turn from supine to side-lying, as a way of shifting the visceral stretch on the abdominal wall. We expected such a shift to cause regional changes in AEA. EMGs were recorded bilaterally from the internal and external (EO) oblique abdominal muscles while S, wearing a nose clip and breathing through a mouthpiece, expired against an ETL (i.e. a water column of preset heights), a reliable stimulus for AEA. Thresholds for AEA in the four muscles were lower when left-lying than when supine. Regardless of body position, normalized EMG amplitudes of each muscle increased in concert with each increment in ETL. Only in left EO did a change in AEA correlate with the change in body position: its increase in AEA with each increment in ETL was less in the left-lying than in the supine position. These results suggest that an asymmetrical input from abdominal receptors has but minor effects on AEA.

363.11

MYOTATIC REFLEXES IN HEALTHY AGING HUMAN SUBJECTS. BM Myklebust, JB Myklebust. Laboratory of Sensory-Motor Performance, Zablocki VA Medical Center & Medical College of Wisconsin, Milwaukee, WI 53295.

In studies of "healthy" elderly subjects (ages 65 years and older) Albert[1] reports that the patellar tendon jerk reflex time is increased, and the Achilles tendon jerk reflex is diminished or absent in approximately 10% of the population. Whether these changes are a concomitant of "normal aging" is uncertain.

We tested myotatic reflexes of the lower extremities in 30 healthy active subjects, ages 65-75 years, and identified the following: 1] Differences in myotatic reflexes, when compared with normal young adult subjects, were recorded in every subject. 2] Achilles and/or patellar tendon jerk reflexes were absent in 3% and delayed in 55% of the subjects. 3] Responses not previously identified in normal healthy subjects were evoked by tendon tap, including responses of the soleus muscle to tibialis anterior tendon taps (*hyperreflexia*), responses of quadriceps and/or hamstrings muscles to Achilles and/or tibialis anterior tendon taps (*reflex irradiation*) , and *reciprocal excitation* of agonist and antagonist muscles following patellar and Achilles tendon taps. 4] Tap-to-tap variability was identified in onset and amplitude of reflex responses of the agonist and antagonist muscles, as well as in responses from limb muscles at a distance from the tapsite.

Previous work suggests that there is plasticity in the CNS of aging humans.[2] Structural changes have been identified in the motoneuron pool which may affect the quality and precession of performance.[3] Our current studies demonstrate a greater variability in myotatic reflex patterns in healthy aging subjects than has been identified previously. Caution must be exercised when using myotatic reflex changes as diagnostic criteria in aging patients with neurologic disease.

1. Albert ML (Ed): Clinical Neurology of Aging. New York, Oxford, 1984.
2. Myklebust *et al.*: Soc Neurosci Abstr #186.66, 1986.
3. Schiebel AB: Clin Geriatr Med 1:671, 1985.

This work has been supported by research funds from VA Rehabilitation R&D.

363.12

EVIDENCE FOR PROPRIOSPINAL ACTIVITY IN PATIENTS WITH SPINAL CORD INJURY. B. Calancie, J. Difini* M. Traad* and D.R. Ayyar* The Miami Project & Dept. of Neurol. Univ. of Miami, Miami FL 33136

Severe spinal cord injury (SCI) results in a complete motor deficit below the lesion, due to the loss of excitatory input onto spinal motoneurons. Descending inhibitory input is also lost, however. We have observed examples of short latency interconnections between upper and lower extremity muscle groups following peripheral nerve stimulation in quadriplegic patients.

EMG activity was recorded from up to 12 muscles simultaneously. Constant-current electrical stimulation of nerves was applied through hand-held surface probes. Responses were amplified and recorded on digital tape for analysis.

Approximately 30% of the patients examined demonstrated responses in upper extremity muscles to stimulation of both contra- and ipsilateral lower extremity mixed nerves. Response latencies were inversely proportional to current strength. The minimum latency in thenar muscles to tibial nerve stimulation at the knee was typically less than 50 ms, consistent with these responses being mediated by rapidly conducting propriospinal pathways.

363.13

THE TONIC STRETCH REFLEX IN SPASTICITY. A.M. Sherwood, T. Bacia*[1], M.R. Dimitrijevic and W.B. McKay*, Baylor Coll. Med., Houston, TX 77030, and [1]Medical Acad. Warsaw.

While both phasic and tonic stretch reflexes are exaggerated in spasticity, the tonic reflex is of greater clinical significance. The tonic reflex can be elicited by passive movement or by muscle spindle vibration. We compared these two methods in 50 (unselected) spinal cord injury subjects, in whom we applied vibration to the achilles and the patellar tendons, and passive movement of the ankle or the hip and knee joints. To elicit the vibration reflex (VR), we used an air-driven motor with an eccentric weight, which provided 100 Hz vibration of 2-3 mm displacement. Surface EMG responses were recorded over quadriceps, hamstrings, tibialis anterior and triceps surae muscles. From 13% of the sites, stimulation elicited a spasm. The tonic response to vibration spread to non-vibrated muscles in 32% of the sites. A much more evident form of irradiation is in the vibration-induced spasm (VIS) response, which occurred 39% of the time. These were typically much larger responses in amplitude, in duration and in number of responding muscles (mean of 5), with 41% of the VIS responses appearing bilaterally. In comparisons with passive movement, vibration elicited a response in fewer muscles, although the number of muscles responding to passive movement and to vibration was positively correlated. We conclude that the VR can be used to assess the tonic stretch reflex in spastic patients.

363.14

STRETCHED AND ABERRANT OPPOSING MUSCLE TENDON TAP REFLEX RESPONSES AS INFLUENCED BY MOVEMENT IMPAIRMENT AND TENDON IMPACT FORCE. N.J.LAMBERT and W.P. KROLL* Motor Control Lab, Univ. of Denver, Denver, CO 80208

Patellar tendon tap reflex EMG and peak FORCE response to a minimum (MIN), mid-range (MID) and maximum (MAX) impact force were compared in an able-bodied group (AB,n=12) and a disabled group (DA,n=12) for a stretched (STR) rectus femoris muscle and (NSTR) opposing biceps femoris muscle. DA subjects were spinal cord injured with no voluntary movement (n=3) and impaired movement (n=3) or with cerebral palsy and impaired movement (n=6). STR and NSTR peak to peak EMG, and FORCE responses increased with the 3 conditions of impact force (STR: MIN 43uv,MID 249uv,MAX 506uv, $p<.01$; NSTR: MIN 8uv,MID 58uv,MAX 97uv,$p<.01$; FORCE:MIN 3N,MID 16N,MAX 26N). A greater gain of response to increased impact force is observed for STR than NSTR muscle (difference: STR 463mv, NSTR 89uv,$p<.01$); the EMG signals therefore represent responses of different muscles. STR and NSTR, but not FORCE, responses were greater for DA than AB (difference:STR 275uv,$p<.01$;NSTR 89mv, $p<.01$). STR, NSTR and FORCE responses were greatest for subjects with impaired movement. NSTR response is contrary to reciprocal inhibition.

363.15

THE BILATERAL AMPLITUDE IMBALANCE IN THE JAW-JERK REFLEX. H.W. van der Glas, R. Buchner*, F. Lobbezoo* and F. Bosman. Lab. of Oral Pathophysiology, Univ. of Utrecht, Padualaan 14, 3584 CH Utrecht, The Netherlands.

The bilateral amplitude asymmetry of the jaw-jerk reflex has been studied in the surface EMG of the masseter and the anterior temporal muscles of 20 patients with myogeneous craniomandibular disorders and 20 symptom-free subjects.

Constant transient vertical loads given to a central mandibular point while the subjects maintained 10% MVC of a masseter muscle, caused a displacement up to 0.2 mm with a rise time of 3.5 ms. The resulting reflex amplitude was corrected for the effect of the excitability level of the motor neurons by taking into account the background muscle activity. Furthermore, in 3 subjects, the mandibular displacement was successively controlled for each side at 0.05 mm while the reflex was studied for a range of activity levels (4-20% MVC) of the muscles on the displacement side.

With a constant load, significant bilateral reflex asymmetries occurred in 53-75% of the subjects in both samples. The reflex asymmetry was, in general, not correlated with an asymmetry in the background muscle activity, which might cause an asymmetric muscle stiffness. Hence, the reflex asymmetry is predominantly related to an imbalance in reflex gain. The experiments with a constant displacement, where the effect of muscle stiffness is compensated, also indicate the involvement of a different reflex gain.

363.16

Stretch reflex latency changes following repetitive reciprocal and hetero-segmental electrical stimulation in spastic hemiplegic subjects. M.F.Levin and C.W.Y. Chan. School of Physical and Occupational Therapy, McGill University, Montreal, Canada H3G 1Y5.

Repetitive electrical stimulation of the spinal cord reportedly decreases spasticity. In a previous study (Hale & Chan 1986), 30 min of transcutaneous electrical nerve stimulation (TENS) applied segmentally to the low back had no immediate effects on lower limb reflexes in spastic hemiplegics. In the present investigation, we extended the study to include changes in stretch reflex (SR) latencies following longer duration (45 min) of reciprocally and hetero-segmentally applied TENS.

Ten spastic hemiplegics and 7 age-matched normals participated in the study. Spasticity was evaluated by clinical scales and electromyographic measurements consisting of: 1) ratio of maximum H-reflex to M response, 2) inhibition of H-reflex during vibration, and 3) soleus SR at rest. Comparison was made of data obtained before and at 3x20 min intervals after 1) placebo stimulation, and TENS applied: 2) reciprocally to the common peroneal nerve, or 3) hetero-segmentally to the contralateral wrist.

Our findings showed that stretch reflex onset latencies were shorter in hemiplegics (45.4±SD 11.8 msec) than normals (54.6±17.3 msec). Both TENS treatments significantly increased SR latencies when compared to placebo stimulation. The mean percentage change in SR latencies was 90.8±7.7% for placebo compared with 109.8±9.2% for TENS to the leg, and 110.5±4.5% for TENS to the wrist ($p<.005$). This increase in latency was evident for up to 60 min post-TENS. No consistent changes were found in other measures.

These results indicated that TENS may reduce spasticity as shown by increased SR latencies via reciprocal and/or non-segmental mechanism(s).

363.17

PRELIMINARY STUDIES ON MODIFICATION OF HYPERACTIVE SPINAL STRETCH REFLEXES IN STROKE PATIENTS. R.L. Segal, P.A. Catlin*, R.Cooke*, L.Johnson*, A.McCallum*, D.Murzin*, K.Rubel* and S.L.Wolf. Human Movement Lab. and Div. Phys. Ther., Dept. Rehab. Med., Emory Univ. Sch. Med., Atlanta, GA 30322.

The biceps brachii spinal stretch reflex(SSR) was downtrained in 6 stroke patients(mean age, 58yrs.; mean time postlesion, 5.3 yrs.). Each subject received two baseline sessions without feedback of SSR amplitude. Subjects were then randomly assigned to one of two sequences: 1)Training (9 sessions with feedback & operant conditioning)-control (9 sessions without feedback & operant conditioning)(TC); 2) Control-training (CT). Baseline, control and training sessions consisted of 250 random stretches which occurred 1-5 secs. after maintaining the elbow joint at 90°±5° and biceps EMG at a level to resist a small extension torque. No sequence effect was found between TC and CT. CT median SSR's drifted below baseline in the control phase, but training had a greater effect. TC median SSR amplitudes reduced from baseline during the training phase more than CT amplitudes dropped during CT control phase, suggesting that downtraining occurred. However, an initial smaller decrease in SSR amplitude independent of training may also occur. Percent changes from baseline were greater during the training phases for both sequences ($p<.007$). The average reduction in amplitude was 40.3%. However, 1 subject was unable to downtrain. These preliminary results support the notion that a hyperactive SSR can be downtrained.

363.18

PRELIMINARY STUDIES ON MODIFICATION OF HUMAN SPINAL STRETCH REFLEXES. S.L. Wolf, M.L. Evatt* and R.L. Segal, Human Movement Lab. Div. of Phys. Ther., Dept. Rehab. Med, Emory Univ. Sch. of Med., Atlanta, GA 30322,

The biceps brachii spinal stretch reflex (SSR) was uptrained (N=4) or downtrained (N=5) in normal human subjects (age range: 25-70 years; median age: 31.5 years). Each subject received two baseline sessions without feedback of SSR amplitude. The median values of these pre-training sessions was used to determine the starting point for 8-9 training sessions when subjects' responses were "shaped" through visual cues that showed each response relative to a criterion level.

Each baseline, training session and a single follow-up evaluation (19.4 days post-training) consisted of 400 random stretches which occurred at 1-5 seconds after maintaining the elbow joint at 90°± 5° and biceps EMG within a "window" approximating 15% of M.V.C. Uptrainers were able to increase SSR amplitudes by 63.7% and downtrainers could reduce SSR amplitude by 21.5% from the median baseline values. Differences between the group's averages of median percent changes across sessions were significant ($p<.002$). In 8 of 9 subjects the follow-up SSR changes remained in the appropriate direction without any visual feedback. These findings compliment data on SSR mutability in monkeys as demonstrated by Wolpaw and support the notion of applying this approach to determine if SSR's can be downtrained in humans with hyper-reflexia.

MOTOR SYSTEMS: REFLEX FUNCTION II

364.1

DO NOCICEPTIVE AFFERENTS BELONG TO THE GROUP OF "FLEXOR REFLEX AFFERENTS" ? E.D. Schomburg, H. Steffens and P.F. Schmidt (SPON: J. Noth). Univ. Inst. of Physiology, D-3400 Göttingen, F.R.Germany

In order to analyze a possible specificity of nociceptive cutaneous afferents in spinal motor control the reflex effects of these afferents (activated by radiant heat) were investigated in high spinal cats in comparison and in combination with the effects from low threshold cutaneous, joint and group II muscle afferents (non-nocicepotive "flexor reflex afferents", FRA).

In many respects the results revealed a wide congruence between the motor effects of nociceptive afferents and non-nociceptive FRA: the synaptic effects in lumbar α-motoneurones were similar; there was a wide convergence onto common segmental interneurones; the sensitivity to depressive actions of enkephalins (δ- or μ-opioid receptor agonists) onto the transmission in the reflex pathways was not fundamentally different for both types of afferents. However, beside the reflex pathways presumably used in common a special excitatory nociceptive pathway from the central foot pad to plantaris and foot extensors was observed. This pathway did not follow the flexor reflex pattern, did not show a convergent action with non-nociceptive FRA and was less effectively depressed by enkephalins than an excitatory nociceptive FRA pathway.

Thus nociceptive cutaneous afferents seem to contribute to spinal motor control generally in a similar way as non-nociceptive FRA, which also may evoke reflex effects via private non-FRA pathways.

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364.2

THE ELECTRICALLY-STIMULATED FLEXOR REFLEX IN PITHED RATS IS A TEST MODEL FOR DIFFERENT CENTRAL RECEPTOR TYPES. T. Skarsfeldt*. (Spon: I. Divac). H. Lundbeck A/S, Ottiliavej 7-9, DK-2500 Copenhagen-Valby, Denmark.

Andén et al. found that DOPA increased the muscle tension (flexor reflex) in the hind limb of pithed rats (Nature 202, 1344, 1964). Recently, I described that the dopaminergic (DA) D-1 agonist SK&F 38393 and the DA D-2 agonist pergolide incerased the flexor reflex elicited by electrical stimulation. This effect could be selectively blocked by DA D-1 and DA D-2 antagonists, respectively (Skarsfeldt, Pharmacology & Toxicology 64, 298, 1989). In a recent report we found that not only DA compounds but also the α_1-adrenoceptor agonist St 587 facilitated the flexor reflex (Skarsfeldt and Hyttel, Eur. J. Pharmacol. 125, 333, 1986). The activity of St 587 was selectively blocked by α_1-adrenoceptor antagonists. Additionally, we have observed that clonidine (an α_2-adrenoceptor antagonist) increased the flexor reflex. We have also reported that the 5-HT_{1A} agonist 8-OHDPAT and the 5-HT_1 agonist TFMPP induced a dose dependent increase of the reflex (Skarsfeldt and Larsen, Eur. J. Pharmacol. 148, 389, 1988). The serotonin precursor l-5-HTP had a similar effect which was attenuated by 5-HT_2 antagonists (manuscript submitted). In conclusion the described flexor reflex model can be used as a test for multiple receptors in the spinal cord of pithed rats.

364.3

EFFECTS OF A NORADRENERGIC BLOCKER ON POLYSYNAPTIC SPINAL REFLEXES IN THE CAT. J.F. Miller, C.J. Heckman, M. Munson* and W.Z Rymer. Dept. of Physiol., Northwestern University, Chicago, IL, 60611.

Dorsal hemisection of the spinal cord in the decerebrate cat alters the recruitment and frequency modulation of motoneurons in a way that is similar to some changes seen in spastic human patients. The monoaminergic fibers in the dorsal lateral funiculus may play a role in these changes. Our preliminary results on the effects of the noradrenergic alpha-2 blocker, yohimbine, on 2 polysynaptic spinal reflexes (sural and crossed extension) are consistent with this possibility.

Results: 1) No changes were observed in tendon vibration or stretch reflexes, which are largely dependent upon the monosynaptic Ia pathway. However, single shocks to the ipsilateral sural nerve produced a small excitatory reflex in medial gastrocnemius (MG), as measured by force and EMG. Yohimbine potentiated this presumably polysynaptic response (120-200% of control). This is contrary to the previous work of Engberg et al. (Acta physiol. scand. 72: 142, 1968), but we used a slightly different decerebrate preparation (pre- instead of mid-collicular). 2) In contrast to the single shock response, sustained stimulation of the sural nerve (100 Hz for 1-2 s) produced a powerful activation of MG that tended to be unaltered or decreased (70-80% of control) by yohimbine. 3) Similar sustained activation of the contralateral tibial nerve generated a powerful crossed extension reflex in MG that was clearly decreased by yohimbine (40-50% of control). These alterations in sustained reflexes are consistent with the potentiating effect of noradrenaline upon long latency flexion and crossed extension reflexes seen in spinalized preparations.

Discussion: The reductions in the sustained reflexes were similar to, but not as large as, those produced by dorsal hemisection (sural: 10-30% of control, contralateral: 5-30%). Thus, loss of noradrenergic input may be responsible for some of the effects of dorsal hemisection. We are presently investigating further monoaminergic agents. Future studies will include direct measurements of the effects of these agents on the recruitment and frequency of single motor units.

364.4

THE RELATIVE ROLES OF MUSCLE SPINDLE AFFERENTS AND MOTONEURON POOL ORGANIZATION IN FORCE DEVELOPMENT DURING RAMP STRETCH. Dj. Boškov* and W. Z. Rymer (Spon. C. J. Heckman). Dept. of Physiol., Northwestern Univ., Chicago, IL 60611.

Sudden loss in stiffness (yield) that occurs when active, areflexive muscle is stretched can be compensated by recruitment of new motor units or, to a lesser extent, by increasing the firing rate of already active units. To explore the behavior of various components of stretch reflex, we measured spindle afferent activity, EMG, reflex and mechanical forces in the soleus muscle of the decerebrate cat, starting at different initial forces and applying different stretch velocities.

The time of yielding (ranging from 40 to 80 msec) increased with the velocity of stretch and with initial force. If the muscle was activated after the onset of stretch, the force developed during stretch was significantly higher than that of the muscle tonically active before the stretch. Reflex force contribution during and after stretch and responses of spindle afferents were virtually independent of initial forces, for the same stretch. However, EMGs had a very pronounced dynamic responses at low initial forces, which became very small even at moderate forces. The initial burst of EMG, which always occurred well in advance of yield (30-70 msec), increased with velocity and initial force.

In order to study the behavior of EMG, a computer model of motoneuron pool was produced, based on the model described by MacGregor and Oliver (Kybernetik 16:53, 1974), with increasing firing thresholds and inputs corresponding to central and muscle spindle afferents. For a particular velocity, spindle afferent input was not changed, and the central input was varied to simulate different initial forces. The simulated EMG (a weighted sum of bipolar waveforms), was quite realistic and included features described above for the real EMG. The initial burst was roughly proportional to a sum of synchronized already recruited and newly recruited units. Little new recruitment occurred during stretch, except at very low central drive.

These results suggest that the spindle afferents serve only as simple transducers signalling the disturbance to the motoneurones which are, therefore, responsible for computing a corrective signal, depending on the combination of central and spindle afferent inputs. Although this computation appears to be very crude, it seems suitable to mechanical properties of muscle units and apparently follows the size principle. Predictive reflex action, based on computation of corrective signal from disturbance signal is consistent with the principle of feedforward control.

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364.5

DIFFERENCES IN FLEXION REFLEX IN DECEREBRATE VS. SPINAL CATS. J.A. McMillan, W.A. Yuhas* and L.L. Morgan.*. Dept. of Biol. and WAMI Program, State University, Bozeman, MT 59717

Excitability of the flexion reflex (FR) is known to be greater in spinal than in decerebrate (DCER) cats. We report here that such differences are more complex than has been previously considered.

Excitability of the FR, evoked by stimulating the left tibial nerve at the ankle and monitored by recording isometric tension from the left semitendinosus, was tested in DCER cats. The thoracic spinal cord was then cut and the same tests were repeated.

In the DCER preparation, the FR was evoked by A-delta fibers at 10 Hz but required input over C fibers at 1 Hz. In the spinal preparation a brisk FR was evoked by A-delta's at 1 Hz; no increase in the reflex was seen after adding C's to the volley. Furthermore, there was no long-latency (>350 msec) response and no prolonged central summation when stimulating C's at 0.5 Hz; both are commonly seen in the DCER preparation.

These results suggest that descending influences which suppress the FR in the DCER preparation affect inputs over A-delta and C fibers differentially. This has implications for our understanding of motor organization and for using the FR as a model of pain perception. (Supported by NSF BNS 86-19148 and NIH RR08218-06)

364.6

ALTERED REFLEX ORGANIZATION UNDERLYING CLASP-KNIFE RESPONSES IN CHRONIC SPINAL CATS. T.R. Nichols and T.C. Cope. Dept. of Physiology, Emory Univ., Atlanta, GA 30322, and Dept. of Physiology and Biophysics, Hahnemann Univ., Philadelphia, PA 19102-1192 (TCC).

A striking symptom which follows spinal transection is the clasp-knife response, a flexion-like reflex evoked by muscle stretch (Rymer,W.Z. & Cleland,C.L., Soc. Neurosci. Abstr., 9:529, 1983). We studied clasp-knife responses in chronic spinal cats (T13X, 1-4 weeks) using stretch-evoked reflexes among soleus (S), gastrocnemius (G) and tibialis anterior (TA) muscles in decerebrated preparations. Stretch of G, S or TA produced powerful inhibition in S, prolonged excitation of TA and modest inhibition of G. The inhibition of S and G due to stretch of S was superseded by the tonic stretch reflex and synergistic excitation, respectively, at higher forces in S. The strength of the inhibition increased with length of S. These properties indicate that clasp-knife is due to a mechanism different from the inhibition from G to S regularly observed in control decerebrates (Nichols, T.R., J. Physiol., 410:463, 1989), and that soleus, a pure extensor, is affected preferentially. The clasp-knife pattern contrasts sharply with the absence of autogenic inhibition and poverty of heterogenic reflexes in long-term (11 mo.) lesioned animals (Cope,T.C.,& Nichols,T.R., Soc. Neurosci. Abstr., 14:795, 1988).

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364.7

RECOVERY OF SPINAL CORD EXCITABILITY FOLLOWING EXPOSURE TO A HYPEROSMOTIC SOLUTION. D.A. Lake. Dept Physical Therapy, Northeastern University, Boston, MA 02115.

Spinal cords of Rana Pipens were isolated and superfused with a modified glucose-Ringer's solution (NaCl, 100 mM; KCl, 2.5 mM; Na2HPO4, 2.5 mM; NaH2PO4, 0.45 mM; CaCl2, 1.9 mM; NaHCO3, 12 mM; and glucose, 2.8 mM) aerated with a 95% O2 and CO2 gas mixture (pH 7.4). The tenth dorsal root (DR 10) was stimulated supramaximally (1-5 mA) using 0.1 ms duration pulses delivered at 0.2 Hz with a bipolar hook electrode. The reflex responses were recorded using bipolar electrodes from the tenth ventral root (VR 10) and passsed through a preamplifier with the low frequency filter set at 10 Hz and the high frequency filter at 30 Hz. Measurements were taken from averages of 16 recorded potentials. The control medium was replaced by the control medium with varying concentrations of the non-electrolyte mannitol (25, 50, 75 and 100 mM). After 30 minutes of exposure to the hyperosmotic medium, the control medium was reintroduced for 60 minutes to measure recovery. All of the hyperosmotic solutions decreased both the amplitude and area of the evoked potentials. Progressive increases in osmolarity progressively decreased the evoked potentials. The decrease in the evoked potential occurred within the first 5 minutes of exposure to the hyperosmotic medium. There was no further significant decrease in the evoked potential over the next 25 minutes. There was a recovery of the evoked potentials after the reintroduction of the control medium. However, the evoked potentials did not return to control levels even after 60 minutes of exposure to the control medium.

364.8

THE EFFECT OF RECURRENT INHIBITION ON SHORT-TERM SYNCHRONIZATION OF MOTONEURONS DURING FICTIVE LOCOMOTION. M.L. McCurdy and T.M. Hamm Div. of Neurobiology, Barrow Neurological Insitute, Phoenix, AZ 85013.

The effect of recurrent inhibition on synchronization of motoneuron discharge was examined during fictive locomotion produced in paralyzed, decerebrate cats by stimulation of the mesencephalic locomotor region. Spike trains of single motor axons recorded with glass microelectrodes were used to collect spike-triggered averages of rectified neurograms, providing a quantifiable estimate of synchronization between the single motor axons and the motor axon populations in several hindlimb muscle nerves. Preliminary results showed short-term synchronization between motoneurons supplying anterior biceps femoris, medial gastrocnemius and lateral gastrocnemius-soleus. Synchronization was decreased (5 of 6 cases), or unchanged, by intravenous administration of mecamylamine given to reduce recurrent inhibition, indicating that recurrent inhibition has a synchronizing effect on motoneuron discharge during fictive locomotion. This finding contrasts with the effects of recurrent inhibition during reflex activity (Adam et al., Biol. Cybern. 29: 229, 1978; Buahin and Rymer, Soc. Neurosci. Abstr. 10: 329, 1984), suggesting that the action of recurrent inhibition on motoneuron synchronization may differ in reflex and centrally generated motor activities.

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364.9

POST-TETANIC EFFECTS ON MONOSYNAPTIC REFLEXES, DORSAL ROOT REFLEXES AND DORSAL ROOT POTENTIALS IN KITTENS. P. Bawa Kinesiology, Simon Fraser University, Burnaby, B.C., Canada, V5A 1S6.

Tetanic stimulation of muscle afferents results in post-tetanic potentiation (PTP) of monosynaptic reflexes (MSRs) in adult cats. Similar stimulation in new born kittens results in post-tetanic depression (PTD). Percent PTP increases and PTD decreases gradually with age of the animal (Bawa, Canad. J. Physiol., Pharm., 65:328-336, 1986).

Concomitantly recorded dorsal root reflexes (DRRs) and dorsal root potentials (DRPs) exhibited similar pattern of PTP and PTD when muscle nerves were stimulated to elicit these responses i.e. predominantly PTD of DRRs and DRPs in kittens and PTP in adult cats. However, when cutaneous nerves were stimulated to elicit DRRs and DRPs, PTP was rarely observed at any age. Both in kittens and adults PTD of DRRs and DRPs was observed.

The mechanisms underlying these differential post-tetanic effects of muscle and cutaneous nerves could be due to differences in 1) depletion of transmitter, 2) extracellular K^+ accumulation and 3) transmission failure at cutaneous and muscle afferent terminals.

Supported by NSERC of Canada.

364.10

COMPARISON OF RECRUITMENT ORDER BETWEEN PAIRS OF MOTOR UNITS IN THE CAT MEDIAL GASTROCNEMIUS MUSCLE. B.R. Botterman and K.E. Tansey. Dept. of Cell Biol., UT Southwestern Med. Ctr., Dallas, TX 75235.

The order in which motor units are recruited is found to be highly correlated with motoneuron size, as judged by the conduction velocity (CV) of its axon, and with several properties of its muscle unit, such as its contractile strength and fatigability. Among fast twitch (type F) units, it has been shown by others that in the cat plantaris muscle tetanic tension is a better predictor of recruitment order than CV. Therefore, we wished to confirm this finding in another large, hindlimb muscle containing a high proportion of type F units.

Direct comparisons of recruitment order were made in pairs of type-identified medial gastrocnemius (MG) motor units by recording from and stimulating their axons in the MG nerve. Motor units were recruited by monosynaptic reflexes evoked by stimulation of the L_7+S_1 dorsal roots in decerebrate, unanesthetized animals spinalized at C_1. Of the 74 units studied, 77% were recruited by either a single stimulus (n=37) or by a single stimulus following facilitation of the reflex by a 500-pps conditioning train. Of the 17 units not recruited, all but two were type FF units. In 41 of 44 pairs studied, the least forceful unit had the lower threshold for recruitment (1/2 for S-S pairs, 18/18 for S-F pairs, 22/24 for F-F pairs). For the two exceptions in the F-F group, the lower threshold unit was the more fatigue resistant in both pairs and had a slower CV in one pair. In the one S-S pair in which the more forceful slow twitch unit (type S) was recruited first, unit tensions differed by 1 g. Among S-F and F-F pairs, in only 1 pair did the unit with the greater resistance to fatigue have the higher threshold for recruitment. By contrast, the lowest threshold unit had the slowest conducting axon in only 8/15 cases for F-F pairs and 0/2 cases for S-S pairs. However, the correlation between functional threshold and CV was much better for S-F pairs (16/17). In 8 pairs, the CVs were indistinguishable between units.

The present work confirms the findings of others that the relative tension and fatigability of a unit is a better predictor of a unit's threshold for recruitment than its conduction velocity, especially among type F units. NIH grant NS17863.

365.1

A SLICE PREPARATION OF THE VERTEBRATE SPINAL CORD FOR NETWORK AND SINGLE CELL STUDIES. Evelyne Sernagor* and Michael O'Donovan (Spon: J.McIntosh) Lab. of Neural Control, NINDS, NIH, Bethesda, MD, 20892.

We have developed a slice preparation of the embryonic 12-14 day old chick spinal cord for studies of network function and single cell membrane properties. Slices (200-750 μM thick) were cut from the spinal cord using scissors with fine (50 μM) blades. Slices could be made in which roots and individual muscle nerves were preserved intact. Individual motoneurons could be easily visualized in these slices using a microscope equipped with Hoffman optics.

The slices contain a substantial amount of functional synaptic circuitry. Dorsal root stimulation produces synaptic potentials in motoneurons closely resembling those recorded in the hemisected spinal cord. In addition, some components of the circuitry underlying motor activity persist in thicker slices because bath application of the excitatory amino acid NMDA produces rhythmic motor activity similar to that recorded in the intact cord. Further studies are in progress to establish whether or not the phasing of flexor and extensor motor activity is normal in the slices.

On some occasions it has been possible to obtain whole-cell patch clamp recordings from motoneurons in slices which had been subjected to a mild enzymatic treatment. Preliminary results indicate that motoneuron input impedances are in the range of 0.3 to 0.6 Gohm and action potential amplitudes > 80 mV. A prominent feature of the motoneurons' electrical behavior is a potent anodal break response appearing at -40 pAmp and which can produce repetitive firing with larger currents. This preparation should be useful for studying the development of intrinsic neuronal properties and the formation of synaptic connections in the spinal cord.

365.2

THE DEVELOPMENT OF RECURRENT MOTONEURONAL CONNECTIONS IN THE SPINAL CORD OF THE CHICK EMBRYO. Michael O'Donovan. Lab. of Neural Control, NINDS, NIH, Bethesda, MD, 20892.

The development of recurrent motoneuronal connections was studied in the *in-vitro* lumbosacral cord of stage 30-38 chick embryos. Motoneuron axons were activated by ventral root (VR) stimulation and recurrent responses recorded either from the adjacent ventral root (all ages) or intracellularly from motoneurons (stage 36-38 embryos) using K citrate or KCL electrodes. VR stimulation produced depolarizing synaptic potentials in motoneurons, which were largest using KCL electrodes, often had multiple components and fatigued rapidly during repetitive stimulation. Following antidromic stimulation of motoneurons the latency of the intracellular potential averaged 10 ms (minimum 5.5 ms). This latency is compatible with a disynaptic pathway because the preparation is at room temperature and afferent evoked monosynaptic potentials have a latency of 5 ms. In the majority of experiments the recurrent VR potentials were reversibly abolished by bath application of curare (10 μM) or strychnine (100 μM). Surprisingly, the potentials were also blocked by the GABAergic antagonists, picrotoxin and bicuculline (100μM).

These results suggest that the recurrent potentials may be mediated by inhibitory interneurons similar to mammalian Renshaw cells. The early appearance of this pathway during development (stage 30-32) reveals that motoneurons have neuronal *and* peripheral synaptic targets during the cell death period, raising the possibility that both targets may influence motoneuron survival.

365.3

AN INTERSEGMENTAL SPINAL CORD REFLEX IN GUINEA PIG. A. R. Blight. Center for Paralysis Reseach, Purdue University, West Lafayette, IN 47907.

The cutaneous trunci muscle reflex was studied in guinea pigs, using electromyography, motion analysis, selective lesions, retrograde HRP and microscopy. As in the rat (Theriault, A., and Diamond, J., *J. Neurophysiol.* 60: 46-462, 1988) the muscle covers much of the trunk as a subdermal sheet. The dorsal half of the muscle contracts locally in response to tactile or electrical stimulation of the skin. The reflex consists of a brief twitch centered 1-2 cm rostral of the stimulus, and the receptive field includes much of the thoracic and lumbar dorsal surface. Sensory input is carried by segmental cutaneous nerves, the receptive fields of which form overlapping strips on either side of, and perpendicular to the midline. The lateral thoracic nerves from the brachial plexus innervate the muscle on each side. Motoneurons are in the lower cervical ventral horn (C7-T1). The ascending limb of the reflex involves projections in the ventral half of the thoracic lateral funiculus. These axons convey information primarily from ipsilateral skin, together with a limited input from contralateral skin, which crosses at segmental levels. Electromyographic responses to skin stimulation are 15-30 msec bursts at latencies of 15 - 20 msec. The reflex does not readily habituate or fatigue at stimulus frequencies below 10 Hz, and it persists under light pentobarbital anesthesia. This combination of characteristics makes the reflex useful for a variety of physiological and pathophysiological studies of spinal cord function. Currently, it is being used to monitor conduction in traumatically injured spinal cord, allowing repeated measurements in unanesthetized or lightly sedated animals.

365.4

EFFECT OF QUANTITY OF ACTION POTENTIALS ON MOTONEURON OXIDATIVE CAPACITY G. R. Chalmers, R. R. Roy, and V. R. Edgerton. Dept. of Kinesiology and Brain Research Institute. UCLA, Los Angeles, CA 90024.

Two models were used to examine the effects of 6 months of reduced action potential (AP) activity on motoneuron (MN) oxidative capacity. Model 1: 9 female cats were spinalized (SP, T12-T13), to reduce electrical activity in the MNs of the lumbar cord. Based on EMG activity of selected hindlimb muscles, MN AP activity was reduced by approximately 30% (Alaimo et al., *J. Appl. Physiol.* 56:1608, 1984). The hindlimb muscles of 5 of the 9 SP cats were trained (weight support) 30 min/day, 5 days/week. Model 2: 9 female cats were spinal isolated (SI) i.e., complete spinal cord transection at T12-T13 and at L7-S1 and all dorsal roots between transection sites severed. EMG indicated that the generation of APs in MNs in the lumbar cord was virtually eliminated (Roy et al., *Soc. Neurosci. Abstr.* 14:948,1988). The right leg in each cat received cyclic passive stretching for 30 min/day, 5 days/week. Nine normal cats served as control (C). Succinate dehydrogenase (SDH) activity, an aerobic marker enzyme, of MNs in the lumbosacral enlargement was measured using quantitative histochemistry (Chalmers and Edgerton *J. Histochem.* Cytochem, In Press). The results were similar in the exercised and non-exercised limbs, thus these data were pooled. There were no significant differences in MN SDH activity (mean ±SD, n) among the C (0.0148 ±0.0035 OD/min, 1057), SP (0.0135 ±0.0025 OD/min, 1144), and SI (0.0137 ±0.0027 OD/min, 1107) groups. Soma size was determined for MNs in which there was a visible nucleus. There were no significant differences in soma area among the C (4035 ±535 μm^2, 437), SP (4279 ±520 μm^2, 447) and SI (3985 ±516 μm^2, 356) groups. These data indicate that the soma size and SDH activity of lumbar MNs are unaffected by a marked reduction in the number of generated APs for up to 6 months, suggesting that AP activity may not be a major determinant of MN oxidative capacity. *Supported in part by NIH Grant NS16333.*

365.5

LOCOMOTION INDUCED BY ELECTRICAL STIMULATION OF THE MAMMALIAN SPINAL CORD. E. Garcia-Rill, Y. Atsuta* and R.D. Skinner, Department of Anatomy, University of Arkansas for Medical Sciences, Little Rock, AR 72205

Electrical and chemical activation of the medioventral medulla (MED), the main descending projection target of the mesencephalic locomotor region (MLR), induces locomotion in decerebrate cats and rats. In the present study, an attempt was made to induce locomotion by activating the descending projection sites of the MED, the cervical and lumbar spinal cord, using electrical stimulation. Adult cats (n=23) and rats (n=8) were anesthetized with halothane and decerebrated at a precollicular level. Stimulation was applied epidurally and subdurally. In 6 cats, a transection was made at the T6 level of the spinal cord and the lumbar enlargement stimulated. Repetitive stimulation of the cervical (C4-T1) as well as lumbar (L3-S1) cord epidurally or subdurally induced low threshold, actual and fictive locomotion as long as long duration, low frequency stimuli were used. Stimulation of the surface of the cord was found to be effective when applied to the dorsal columns or to the dorsal root entry zone. Stimulation of the lumbar enlargement induced locomotion in spinalized animals beginning about 4 hrs after transection. EMGs and neurograms during induced actual and fictive locomotion showed a normal-type locomotor pattern and was changed to a trot and gallop by increasing stimulus intensity. These results suggest that spinal stepping generators can be activated in a controlled manner by local electrical stimulation of the spinal cord in the decerebrate and spinalized mammal. Supported by USPHS grant NS20246 (Patent Pending).

365.6

RECRUITMENT ORDER AND DISCHARGE PATTERNS AMONG PAIRS OF MOTOR UNITS EVOKED BY BRAINSTEM STIMULATION. K.E. Tansey and B.R. Botterman. Dept. of Cell Biol., UT Southwestern Med. Ctr., Dallas, TX 75235.

The progressive recruitment of motor units and the subsequent modulation of their discharge rates are the two primary processes involved in the gradation of muscle force. To begin to assess the significance of rate modulation in the control of muscle force, pairs (n=24) of medial gastrocnemius (MG) motor units (n=46) were recorded from the MG nerve during their activation by stimulation in and below the mesencephalic locomotor region in high decerebrate cats. Graded contractions of up to 2 kg, lasting for up to 30 s, were evoked in MG. Motor-unit type (S,FR,FF), axonal conduction velocity (CV) and maximum tetanic tension (P_o) were determined for pairs of units and correlated with their recruitment order and discharge properties.

In 1 S-S, 10 S-F and 13 F-F pairs, recruitment proceeded by motor-unit type (S>FR>FF: 24/24 pairs), and by increasing P_o (21/23 pairs) and CV (7/10 pairs) when differences within pairs could be reliably measured. In 8 pairs, both units were recruited and their discharge patterns were analyzed. In most pairs (7/8), the motor unit recruited first discharged at a lower mean rate. The range of mean firing rates for evoked activity lasting 0.5-10 s was 11-24 pps for 5 S units, 32-51 pps for 3 FR units, and 36-58 pps for 8 FF units. These 16 units discharged with brief intervals (2.3-15.9 ms), either at the beginning of (initial doublet, n=4), or elsewhere in their discharge. Brainstem stimulation evoked a widely varying response in these units. In some cases, changes in unit discharge rate closely followed changes in muscle force, while at other times rate appeared to plateau as muscle force continued to rise. Pairs containing units of similar type and recruitment threshold often discharged with similar patterns.

This data confirms other work showing that motor-unit recruitment proceeds by increasing P_o and CV. In addition, the discharge rates of recruited units display varying degrees of modulation and generally fall on the steep portion of frequency-tension curves for similar type units. (NIH grant NS1783)

365.7

STUDY OF MOTONEURONAL ADAPTATION AND MOTOR-UNIT FATIGUE BY EXTRACELLULAR ACTIVATION OF SINGLE MOTONEURONS. J.M. Spielmann, G.A. Robinson, Y. Laouris, R.M. Reinking and D.G. Stuart. Dept. of Physiology, Univ. of Arizona, Tucson, AZ 85724.

Motoneuronal adaptation refers to the slowing of discharge rate during sustained or repetitive activation at a constant stimulus strength. Beyond the work of Kernell and Monster (Exp. Brain Res., 46:191 and 197, 1982) little is known about the nature of such adaptation and its relation to functional muscle unit properties and Henneman's size principle. While a few relationships were reported by these workers, many expected correlations were not. Technical difficulties inherent in intracellular work may have confounded their results. We have begun experiments to test for associations between motoneuron adaptation and muscle unit mechanical properties, including fatigability. We expect to resolve some of the previous problems by stimulating single cat motoneurons with extracellularly applied current (Gustafsson and Jankowska, J. Physiol. (Lond.), 258:33, 1976) rather than using the traditional intracellular approach. Individual medial gastrocnemius motoneurons were stimulated with a microelectrode positioned near the motoneuron to ensure a low threshold of activation. Muscle units were characterized by twitch contraction time, peak force, and sag. Each motoneuron was stimulated with sustained current for up to 4 min. After a short rest, the same cell was stimulated with repetitively applied current (600 ms @ 1/s), again for up to 4 min. Motoneuronal responses to sustained stimulation showed a rapid early adaptation followed by a more slowly developing late adaptation (cf. Kernell and Monster, 1982). Repetitive stimulation revealed intra-train adaptations in the majority of cases. In addition, inter-train adaptations were marked by a progressive decline in both firing rate and the number of discharges per train. These results support our "extracellular" approach in the study of motoneuronal adaptation. Supported by USPHS grants NS 07309, HL 07249, NS 25077, NS 08363, RR 05675.

365.8

SINGLE-UNIT RECORDING OF LUMBAR LAMINA IX NEURONS IN SPINAL CORD SLICES FROM ADULT RATS. L.-M. Kow and D.W. Pfaff. The Rockefeller University, New York, NY 10021.

Slices (400-500u) of lumbar cord were prepared from rats (b.wt. 120 to 414g), with a Vibratome and with artificial cerebrospinal fluid that contained sucrose in place of NaCl (Aghajanian and Rasmusson, 1989). Single-unit activity was recorded from medial and lateral lamina IX under visual guidance. Bipolar stimulating electrodes were placed at ventral rootlet exit in attempts to activate motoneurons antidromically.

A total of 108 units were recorded in 21 experiments. When applied between repeated serotonin infusions, TRH could modulate the excitatory action of serotonin (15/24 units), even when TRH had no effect by itself. At rest, the proportion of active neurons was higher ($p<.01$) among medial (40/49) than lateral neurons (16/35). More medial than lateral cells fired with regular interspike intervals. More lateral than medial neurons responded to electrical stimulation or transmitters. Thus, medial neurons (where motoneurons innervating lordosis-relevant epaxial muscles are located) and the lateral cell group (which contains limb motoneurons) appeared to behave differently both at rest and upon stimulation.

365.9

RHYTHMICAL MOTOR ACTIVITY IN NEO-NATAL RAT; COMPARISON BETWEEN INTACT ANIMAL AND IN VITRO PREPARATION. J.R. Cazalets*, P. Grillner*, I. Menard*, J. Crémieux*, F. Clarac. Lab. Neurosciences Fonctionnelles (LNF2) 31 chemin J. Aiguier, BP71 13402 Marseille CEDEX 9, FRANCE.

Swimming behavior in the neo-natal rat was compared with locomotor-like activity induced in vitro. The swimming behavior was studied by electromyographic recordings of the four limbs. From day 1 to day 20 the swimming frequency increased from 1 to 5 Hz. The isolated preparation was a brain stem-spinal cord preparation from new born rats (0-6 days). The activity was recorded on ventral roots and hind limb muscles. Bath application of 5-HT (10^{-5}M) and norepinephrine (NE, 10^{-5}M) elicited rhythmical bursting activity (5 to 8 Hz). The temporal characteristics (phase relation and burst duration of either antagonistic or agonistic muscles) recorded in vitro, were directly comparable to those recorded in vivo. Occasionally 5-HT also elicited a pattern consisting of a slow modulation (between .5 and .2 Hz and alternating on right and left lumbar segments) of the high frequency rhythm. Rhythmic patterns were also obtained by bath application of 5-HT and NE when lumbar segments were isolated from the rest of the spinal cord.

365.10

ELECTROPHYSIOLOGICAL ASSESSMENT OF DESCENDING MOTOR PATHWAYS FOLLOWING SPINAL CORD INJURY IN RAT. J.H.Kim, J.S Cheon, Y.G. Park*, R. Prado, and W.J.Levy. Dept. of Neurological Surgery, Univ. of Miami, Sch. of Med., Miami,FL 33136.

Following partial spinal cord injury in the adult cat, the resultant motor deficit can be predicted by monitoring the motor evoked potential(MEP). However, the adult rat with complete transection of the corticospinal tract, the major component conducting the MEP, still recovers hindlimb locomotion within 2-3 weeks. The objective of this study is to electrophysiologically assess the substrate of the recovery of locomotor function after partial spinal cord injury in rat. The evoked potentials were monitored epidurally at T6 and L1 during electrical stimulation of red nuclei(RN; magnocellular division), lateral vestibular nuclei(LVN) and gigantocellular reticular nuclei (RetN). Varying degree of spinal cord injuries were photochemically induced at T8 level of cord in rat(Prado et al., J. Neurosurg.,67:745). The evoked potentials were monitored 1-4 weeks postlesion. The evoked potentials were analyzed as the amplitude ratio between the first peak recorded on the lumbar and thoracic cord (L/T ratio). Upon increasing stimulus intensity, the L/T ratio of LVN evoked potential(LVEP) and RNEP increased to 3 times the value obtained at threshold, but L/T ratio of the RetN evoked potential (RetEP) declined gradually with increased stimulus intensity. Unlike LVEP and RNEP, the L/T ratio of RetEP increased after partial section of dorsal cord. The L/T ratio of the RetEP returned to normal 3 weeks after partial spinal cord injury which was coincide with the recovery of motor function of the hindlimbs.

365.11

NON-DEPENDENCE OF PSP'S FROM SURAL NERVE ON ITS PERIPHERAL INNERVATION. H Nishimura & JB Munson. Depts of Neurosurg & Neurosci, Univ of FL Coll of Med, Gainesville, FL 32610.

Stimulation of caudal cutaneous sural (CCS: commonly "sural") or of lateral cutaneous sural (LCS) nerve in pentobarbital-anesthetized cats each elicits PSP's of characteristic EPSP/IPSP configuration in triceps surae motoneurons (TS MN's). We showed that cross-connection (x-conn) of CCS to medial gastrocnemius (MG) nerve did not alter these PSP's (Soc Neurosci Abstr 14: 696, 1988). To extend this result we investigated the ability of similar targets (cutaneous-cutaneous) to sustain or alter input organization in the spinal cord. CCS and LCS nerves were cut and x-conned distally. 1-2 years later, exchange of innervation territory was confirmed by recording each nerve's activity in response to skin stimulation. Intracellular recording from TS MN's showed that stimulation of the distal portion of the CCS nerve (x-conned proximally with LCS nerve) gave PSP complexes appropriate for normal LCS nerve. Stimulation of distal LCS gave the analogous result. Thus CCS-LCS x-conn, like CCS-MG x-conn, did not respecify the input organization of the LCS or CCS nerve in the spinal cord. We further tested target-dependence of CCS synaptic function by axotomizing and capping CCS. After 1-2 years, electrical stimulation of CCS (lacking evident peripheral innervation) elicited apparently normal PSP's in TS MN's. Thus CCS afferents may differ from large muscle afferents in not requiring peripheral innervation to sustain synaptic efficacy in TS MN's. Support: NS15913.

365.12

Concordant depolarizing responses produced by locus coeruleus stimulation and norepinephrine in individual spinal motoneurons. S.J. Fung, D. Manzoni*, J.Y.H. Chan, and C.D. Barnes. Dept. of VCAPP, Washington State Univ., Pullman, WA 99164, USA.

Locus coeruleus (LC) stimulation (Fung and Barnes, Brain Res., 402:230, 1987) and norepinephrine (NE) (Fung, White and Barnes, Soc. Neurosci. Abstr., 14:214, 1988) have been shown independently to cause depolarization in spinal motoneurons of cats. The present study was undertaken to compare the membrane response, recordable from the same cell, due to these two causes when applied individually and in combination. In decerebrate cats, stable recordings from motoneurons with antidromic spikes above 60 mV were used in this analysis. Parallel depolarizing changes were evident, within the same cell, following tetanic LC stimulation (4-54 pulses at 500 Hz, 15-50 uA) and iontophoretically applied NE (50-80 nA, 60-90 sec). The NE-induced depolarization was typified by a slow time course, whereas the LC-induced EPSP was briefer in duration, with weak ensuing IPSP in some cells. Both LC stimulation and NE relieved the partial block of the antidromic activation. Upon interaction the LC-induced EPSP summated with the NE-induced depolarization, resulting in motoneuron discharge. H^+ application was without effect on the motoneuron excitability. These data reveal that LC stimulation produced EPSP is mimicked in part at least by the NE application in lumbar motoneurons of cats. Supported by NIH grant NS 24388.

365.13

MULTISITE OPTICAL RECORDING OF NEURON ACTIVITY IN ORGANOTYPIC SPINAL CORD SLICE CULTURES OF THE RAT. M.G. Rioult* and H.-R. Lüscher (SPON. M. Wiesendanger). Dept. of Physiology, University of Bern, CH-3012 Bern, Switzerland.

Optical recordings were used to study the electrical activity of different populations of neurons in an organotypic coculture of embryonic rat spinal cord (SC), dorsal root ganglia (DRG) and skeletal muscle. In this culture system functioning synaptic contacts involved in the spinal reflex arc have been shown to exist by conventional electrophysiological technique (Spenger et al. Neurosci. Abstr. 1989).

The cultures, grown for 14 days in vitro, were stained with the fluorescent voltage-sensitive styryl dye RH-237 at concentrations of 5 - 15 µM for 30 min. Changes in fluorescence intensity were recorded by a 12 x 12 element photodiode (PD) array mounted in the image plane of an inverted microscope in epifluorescent configuration. 20x, 0.8 n.a. and 40x, 1.3 n.a. glycerin immersion objectives were used giving a spatial resolution of 28 x 28 µm and 14 x 14 µm respectively. A 100-W mercury lamp was used as a light source. The signal from each PD could be sampled at a maximum rate of 3.3 kHz for 300 msec.

Large DRG cells (diameter approx. 40 µm) were impaled by microelectrodes and stimulated with depolarizing current pulses. The intracellular electrical signal and the fluorescence signals were recorded simultaneously. Action potentials as well as subthreshold depolarizations could be resolved in the optical recordings from the PDs covering the cell body of the stimulated neuron without signal averaging. Extracellular focal stimulation of "dorsal roots" evoked fast as well as slow optical signals recorded from the ventral part of the SC. These signals may arise from synaptic excitation of the motoneurons.

Optical recording in combination with organotypic slice cultures of SC may provide a promising tool for studying local neuronal circuits in the spinal cord. (Supported by SNF No 3.265-0.85)

365.14

MORPHOLOGICAL AND PHYSIOLOGICAL EVIDENCE FOR A FUNCTIONAL SPINAL REFLEX ARC IN AN ORGANOTYPIC COCULTURE SYSTEM. C. Spenger*, J. Streit*, U.F. Braschler* and H.-R. Lüscher (SPON: J. Allum), Department of Physiology, University of Bern, CH-3012 Bern, Switzerland

To investigate the spinal reflex arc in vitro, an organotypic coculture of embryonic dorsal root ganglion (DRG), spinal cord (SC) and skeletal muscle (SM) was developed using a modified roller tube technique. Evidence for the differentiation of functioning synaptic contacts involved in the spinal reflex arc in this culture system is presented. HRP filling of DRG cells usually revealed 3 stem dendrites (1-7), one of which was T shaped in about half of the cases. Its peripheral branch extended towards the skeletal muscle explants, and its central branch extended into the ventral region of the SC. Synaptic boutons of HRP filled DRG cells on AChE counterstained motoneurons were seen. Motoneurons were identified by their shape (5.8 ± 1.0 stem dendrites, minor diameter = 22.6 ± 4.9 µm, major diameter = 37.3 ± 10.1 µm, ± S.D. n = 72), location, by AChE staining and by retrograde tracing with HRP or rhodamine beads applied onto the coexplanted skeletal muscle. Skeletal muscle regenerated and formed regularly striated fibres. Neuromuscular contacts were revealed as AChE positive motor endplates, singly or in clusters. Extracellular focal stimulation of DRG cells evoked EPSPs in the motoneurons and action potentials followed by contractions in the muscle fibres, with latencies of 23 and 35 ms respectively (22 °C). The EPSP's had a mean amplitude of 15 ± 5mV (S.D. n=20) and evoked action potentials in 75% of the cases. The EPSP's were blocked by Cd (0.5mM) and kynurenic acid (1mM), showing that they were mediated by glutamate. Pancuronium bromide blocked muscle contractions evoked by stimulation of DRG cells or motoneurons, and in higher doses, spontaneously occurring contractions. This suggests that innervated muscle fibres with differentiated endplates coexisted with undifferentiated hypersensitive fibres. Supported by S.N.F. (Grant No 3.265-0.85)

365.15

DIFFERENTIAL INHIBITION OF BACLOFEN ON MONOSYNAPTIC RESPONSES OF MOTONEURONS PRODUCED BY Ia AND DESCENDING FIBERS IN THE CAT SPINAL CORD. P. Rudomín, I. Jiménez and M. Enríquez*. Dept. Physiol. CINVESTAV, México, D.F.

Recent studies in humans with spinal cord trauma (Exper. Neurol. 103: 165, 1989) show that intrathecal baclofen reduces mono- and polysynaptic reflexes and spasticity without greatly affecting voluntary movements. This could be due to a low density, or absence, of GABA-B receptors in the intraspinal terminals of descending fibers, in contrast with Ia fibers. We used cats anesthetized with chloralose (70 mg/kg), paralyzed and artificially respired. The right spinal cord was hemisected at low thoracic level and the left lumbosacral spinal roots sectioned. Intravenous injection of 1.5-2 mg/kg of baclofen markedly depressed (4.7-14% of control), and in some cases abolished, the monosynaptic responses of Ia fibers intracellularly recorded from motoneurons or electrotonically recorded from ventral roots with the sucrose gap technique, as well as the monosynaptic extracellular field potentials recorded in the motor nucleus. The monosynaptic responses produced by stimulation of descending fibers in the ipsilateral ventromedial fasciculus were on the other hand more resistant to baclofen than Ia monosynaptic responses and were depressed to 36-64% of control. They persisted after additional injections of baclofen (up to 3.5 mg/kg), which completely supressed Ia monosynaptic responses. Experiments in progress are aimed to disclose the origin of the descending fibers producing baclofen-sensitive and baclofen-resistant monosynaptic responses in motoneurons. Partly supported by grants NIH NS 09196 and CONACyT PCEXCNA-041739. Baclofen was a gift of CIBA-GEIGY CO.

365.16

EFFECTS OF SELECTIVE STIMULATION OF NUCLEUS RAPHE MAGNUS AND ADJACENT RETICULAR FORMATION ON THE INTRA-SPINAL EXCITABILITY OF Ia AND Ib MUSCLE AFFERENTS. I. Jiménez, J. Quevedo, J.R. Eguibar & P. Rudomín CINVESTAV and ICUAP, México, 07000.

Studies of the effects of raphe-spinal fibers on the effectiveness of afferent fibers are limited to cutaneous afferents. We now report the effects of selective stimulation of raphe- and reticulo-spinal pathways on group I muscle fibers. In cats with right hemisection at T13, single pulses (20-100 uA) applied to the nucleus raphe magnus (NRM; 4 mm rostral to obex, midline, 5-6 mm depth) produced negative cord dorsum potentials (CDP) with latency of 5.4±0.5 ms, lasting 45.4±10 ms (n=14). Stimulation of the adjacent reticular formation (RF; 4 mm rostral to obex, 2 mm lat, at 4 mm depth) produced instead a positive CDP with latency of 2.4±0.2 ms, lasting 7.4±4 ms (n=14). Section of the ipsi- dorso-lateral fasciculus abolished the NRM but not the RF responses. Analysis of field potentials showed that NRM fibers end dorsally (0.6-1.8 mm depth) whereas RF fibers synapse with ventrally located neurons (2.8-3.8 mm). Trains (16-18 pulses, 300 Hz, 20-50 uA) applied to NRM and RF reduced the intraspinal threshold of single PBSt Ib afferent fibers to 82.1±15.4% and 79.8±13.6% of control (n=7). The resting threshold of single Ia fibers (n=15) was unchanged by NRM and RF stimulation, which however inhibited the PAD produced by group I PBSt volleys. In two cats the monosynaptic PAD of Ia fibers produced by intraspinal microstimulation was inhibited by RF but not by NRM stimulation. It is suggested that, although raphe- and reticulo-spinal fibers have similar spinal actions, they act on different interneurons along the pathways mediating PAD of Ia and Ib fibers. Grants NIH NS 09196 and CONACyT PCEXCNA-041739.

365.17

PGO-RELATED HYPERPOLARIZING POTENTIALS IN LUMBAR MOTONEURONS ARE MEDIATED BY AN INHIBITORY AMINO ACID. F. López*, F.R. Morales, P.J. Soja and M.H. Chase. *Depts. of Physiology and Anatomy, and the Brain Research Institute, UCLA School of Medicine. Los Angeles, CA 90024.*

Inhibitory postsynaptic potentials occur in lumbar motoneurons immediately following the occurrence of pontogeniculooccipital (PGO) waves during active sleep (AS) (*Sleep Res., 18:* 20, 1989). During AS motoneurons also receive a sustained barrage of inhibitory postsynaptic potentials (AS-IPSPs) (*Exp. Neurol., 98:* 418-435, 1987) that are blocked by strychnine (*J. Neurosci., 9:* 743-751, 1989). The present study was undertaken to examine the neurotransmitter control of the PGO-related potentials and to determine whether they are mediated by the neurotransmitter that is responsible for the active sleep-specific IPSPs. In three chronic cats that were implanted for intracellular recording of lumbar motoneurons during sleep and wakefulness, strychnine (15 mM, pH 6.5) was applied juxtacellularly by microiontophoresis using currents of 100 to 200 nA. The intracellular recording electrodes were filled with K-citrate and had resistances of 5 to 15MΩ. PGO waves were recorded from the contralateral lateral geniculate nucleus (LGN) with bipolar electrodes. The membrane potential of lumbar motoneurons was averaged preceding and following PGO activity using specially designed computer software. Prior to the administration of strychnine, the PGO-related IPSP exhibited the following characteristics (which are expressed in terms of their means): latency (measured from the foot of the PGO wave to the initiation of the averaged IPSP), 26 ± 3.7 ms; latency-to-peak, 47.8 ± 3.1 ms; half-width, 25.03 ± 2.9 ms; amplitude, 1.3 ± 0.2 mV; duration, 48.9 ± 11.5 ms. These values are similar to those previously reported for IPSPs following ipsilateral primary PGO waves (*Sleep Res. op cit.*). After the microiontophoretic juxtacellular application of strychnine, the IPSPs were either no longer present in the averaged motoneuron membrane potential, or there were very small hyperpolarizing shifts that were difficult to distinguish from the background noise. These results indicate that the previously described PGO-related IPSPs are mediated by an inhibitory amino acid, presumably glycine, as are the AS-IPSPs. Supported by grant NS 23426.

365.18

LUMBAR MOTONEURON BASIC PROPERTIES DURING THE ATONIA OF ACTIVE SLEEP ARE STRYCHNINE-SENSITIVE.

P.J. Soja, F. López*, F.R. Morales and M.H. Chase. *Departments of Physiology and Anatomy and the Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.*

Recently, we reported that the spontaneously occurring IPSPs which inhibit lumbar motoneurons during the atonia of active sleep (AS) are mediated principally by glycine because they are abolished by strychnine, but not by picrotoxin or bicuculline (*J. Neurosci.* 9: 743-751, 1989). In the present study, we further explored the role of glycine in mediating the inhibitory shunt that occurs in these cells during AS. Accordingly, certain basic electrical properties of lumbar motoneurons were determined during quiet sleep (QS) and AS prior to and in conjunction with the microiontophoretic administration of strychnine. Under control drug-free conditions, lumbar motoneuron excitability decreased during AS when compared to preceding episodes of QS, as indicated by a 91.7% mean increase in rheobase (R_h), a 40.9% decrease in input resistance (R_{in}) and a 52.8% decrease in membrane time constant (τ_m) (all $P<0.001$, paired Student's t-test). Following sustained juxtacellular microiontophoretic applications of strychnine, the dramatic changes in these basic properties that occur during AS were suppressed. Motoneuron R_h increased by only 14.3% from QS to AS ($P>0.05$); R_{in} and τ_m were decreased by 11.4% ($P>0.05$) and 20.8% ($P<0.05$), respectively. In addition, in control paired experiments performed during QS, strychnine did not exert any changes in R_h, R_{in} or τ_m ($P>0.05$). Spontaneous IPSP activity normally present during AS (*ibid.*), and IPSPs evoked during QS by low-threshold peripheral nerve stimulation, were either abolished or markedly reduced following juxtacellular strychnine. These results suggest that the decreased motoneuron excitability which occurs during the atonia of AS is due to an enhanced inhibitory synaptic drive that is generated at motoneuronal somata and proximal dendrites. This synaptic drive is mediated primarily by a strychnine-sensitive inhibitory amino acid, such as glycine. Supported by grants NS23426, MH43362 and MH43862 (PJS).

365.19

THE ROLE OF THE NUCLEUS RETICULARIS GIGANTOCELLULARIS (NRGC) IN THE CONTROL OF MOTONEURONS AS REVEALED BY POPULATION POTENTIAL ANALYSIS WITH THE SUCROSE GAP TECHNIQUE. J. Yamuy*, I. Jiménez*, F.R. Morales, P. Rudomín and M.H. Chase. *Departments of Physiology and Anatomy, and the Brain Research Institute, University of California, Los Angeles and Centro de Investigación y de Estudios Avanzados del IPN, Mexico, D.F. and D. de Fisiología, Facultad de Medicina, Montevideo, Uruguay.*

The sucrose gap technique was utilized to record the postsynaptic population potentials of spinal cord motoneurons in five cats following stimulation of the medullary NRGc. The preparation was the precollicular decerebrate cat in which NRGc stimulation was performed prior to and after the induction of atonia by the intrapontine administration of carbachol (0.25–1.0 µl, 16 mg/ml). Following stimulation of either the ipsi- or contralateral NRGc (1–4 pulses, 400 Hz, 0.8 ms pulse duration, ≤10 µA), an early depolarizing (i.e., positive) potential was observed whose amplitude varied according to the intensity of stimulation and number of pulses applied. After the intrapontine microinjection of carbachol (L1.3, P2, H-5.5), the depolarizing potential decreased in amplitude and, in addition, NRGc stimulation evoked a longer latency hyperpolarizing (i.e., negative) potential at a time-to-peak of 40 to 54 ms. At the higher levels of current and 4 pulses, hyperpolarizing potentials of 152 to 229 µV were observed. Their duration, measured at their half-width, was between 16 and 25 ms. Because the hyperpolarizing potential was induced specifically during the atonia elicited by carbachol injection, and because it exhibited a latency following NRGc stimulation similar to the IPSP evoked in the chronic preparation during AS (*Br. Res., 386:* 237-244, 1986), we suggest that both potentials are produced by the activation of the same inhibitory pathway. In addition to the data presented in this report, the present experiment demonstrates the usefulness of the sucrose gap technique in studying state-dependent patterns of motor control that are exerted by supraspinal systems. Supported by grants MH43362, NS23426 and by NS09196.

365.20

SOMATIC SHUNTS IN NECK MOTONEURONS OF THE CAT. P.K. Rose and P. Brennan*, Department of Physiology, Queen's University, Canada, K7L 3N6.

Recent studies of the electrotonic structure of spinal motoneurons have concluded that specific membrane resistivity (R_m) is not uniform. Instead, R_m of the somatic region (R_{ms}) is lower than R_m of the dendritic tree (R_{md}). A low R_{ms} may be a consequence of damage caused by electrode penetration. If so, the observation that these neurons have "healthy" membrane potentials is surprising since damage caused by electrode penetration should lead to a sharp depolarization. In the present experiments, we have examined the possibility that electrode damage may provide a route for Ca to enter motoneurons, activate calcium dependent K (K(Ca)) channels and cause a hyperpolarization that masks the depolarization due to damage. Two populations of neck motoneurons were studied. In one group, K (Ca) channels were inactivated by intracellular injection of EGTA. Each group of motoneurons had similar electrotonic characteristics. Furthermore, the relationship between the ratio R_{md}/R_{ms} and the resting membrane potential was the same for both populations of motoneurons. These results indicate that activation of K (Ca) channels do not mask the effects of damage caused by electrode penetration. Thus, regional differences in R_m must be attributed to factors other than damage, for example, local tonic synaptic activity. (Supported by MRC)

RETINA III

366.1

ELECTRORETINOGRAM B-WAVE AND SLOW PIII COMPONENTS ARE PRODUCED BY MÜLLER CELL RESPONSES TO RETINAL POTASSIUM. R. Wen and B. Oakley II, University of Illinois, Urbana, IL 61801

We tested the hypotheses that light-evoked changes in K^+_o lead to changes in Müller cell membrane voltage, which in turn generate the ERG b-wave and slow PIII currents. Using *B. marinus* isolated retinas and K^+-selective microelectrodes with fine tips, we recorded a light-evoked decrease in K^+_o in the receptor layer and a distinct, light-evoked increase in K^+_o near the OPL; this "distal" K^+ increase was found over <20 µm depth, had a max. amplitude of >0.2 mM, and could not have been an electrical artifact, due to the small field potential at this depth. We also recorded the ERG and the light-evoked responses of rods and Müller cells. Superfusion with 200 µM Ba^{2+} increased the amplitude of rod photoresponses and the light-evoked decrease in K^+_o, but nearly abolished the Müller cell hyperpolarization and slow PIII. 200 µM Ba^{2+} attenuated both the Müller cell depolarization and the b-wave by ~50%, *but this treatment had no effect upon the distal K^+ increase*; under other conditions (*eg* low pH_o), all 3 of these responses varied in unison. Since Ba^{2+} reduces K^+ conductance of Müller cells *in situ* (Newman, 1985), these results are very strong support of the K^+/Müller cell hypotheses of the origin of the ERG b-wave and slow PIII components; the light-evoked changes in K^+_o still are present during superfusion with Ba^{2+}, but the K^+_o-evoked Müller cell responses (and thus the ERG components) are decreased in amplitude, since their K^+ conductance is reduced. Our experiments have demonstrated that K^+-mediated changes in Müller cell membrane voltage are a direct step in the sequence of events leading to the generation of the ERG b-wave and slow PIII components. Supported by EY04364.

366.2

WASH-OUT OF GLUTAMATE RESPONSES IN RETINAL DEPOLARIZING BIPOLAR CELLS. S. Nawy and C.E. Jahr. Vollum Institute, Oregon Health Sciences University, Portland OR 97201.

We have investigated the actions of glutamate and several of its analogs on depolarizing bipolar cells (DBCs) under whole-cell voltage clamp using the tiger salamander retinal slice preparation. Drugs were applied with a multi-barreled perfusion system which rapidly exchanged solutions around the slice. Synaptic transmission was blocked by adding 2 µM strychine, 100 µM picrotoxin and 1 mM cobalt to all of the barrels. Glutamate (1 mM) decreased the membrane conductance and evoked outward currents of 10-60 pA in DBCs that were held at -50 mV. Although L-2-amino-4-phosphonobutyrate (APB) (50 µM) mimicked the action of glutamate, neither quisqualate (5 µM) nor kainate (50 µM) elicited any outward current at concentrations that produced large responses in hyperpolarizing bipolar cells. Both glutamate and APB responses washed-out. The rate of wash-out following break-in varied from cell to cell. The time to half-decay ranged from 3 to 24 minutes. Loss of the glutamate response was associated with a decrease in membrane conductance in 10 of 13 cells. The magnitude of the decrease was equal to or greater than that produced by glutamate. We propose that wash-out of the glutamate response is due to the loss of a second messenger(s) which is required to maintain glutamate channels in an open state. As this substance is dialyzed out of the cell, the number of open channels that are available to be closed by glutamate is reduced, and the glutamate current decreases.

366.3

TRANSMITTER SENSITIVITIES, RECEPTOR DISTRIBUTION AND CONDUCTANCE MECHANISMS OF MAMALIAN RETINAL NEURONS. J.E.G. Downing*, M. Kaneda*, A. Kaneko and S. Suzuki* National Institute for Physiological Sciences, Okazaki 444, Japan.

To discover the molecular bases for the receptive field properties of retinal output, we characterized the ionic conductances of cells contained within the inner retina. Using enzyme digestion (papain) and mechanical dissociation we separated the complete range of cell types from cat, rabbit and mouse. Cells retaining high degree of morphological differentiation were consistently prepared. Morphologies of bipolar cells can easily be discriminated. Ganglion cells (GC) were identified by retrograde labelling with DiI injected into the superior colliculus, and/or FITC-dextran into the lateral geniculate nucleus (LGN), 3 days before enucleation. Soma size and projection pattern were used to further categorize GC subclasses. Amacrine cells (AC) were identified by their multipolar form, lack of retrograde label and the use of AC-specific fluorescent markers (*eg.* DAPI).

Bipolar cells obtained from the mouse responded to both glycine and GABA. Both glycine-induced currents and GABA-induced currents were almost entirely carried by Cl ions. Since the chloride equilibrium potential in intact cells is assumed to be -55 mV, these transmitter substances are thought to generate hyperpolarizing responses in cells maintained at their resting potential (-45mV). The sensitivity to both glycine and GABA was the highest at the axon terminal bulb, the structure on which amacrine cells make feedback synapses. Pharmacological study revealed that glycine and GABA worked on separate receptor molecules.

366.4

POTASSIUM (K) AND CALCIUM (Ca^{++}) CURRENTS (I) IN RETINAL HORIZONTAL CELLS (HC) OF WHITE BASS (Roccus chrysops) J.M. Sullivan* and E.M. Lasater (SPON: J.W. WOODBURY). Depts of Physiol. and Ophthalmol., Univ. of Utah, Salt Lake City, Utah, 84108

I_K's and I_{Ca}'s were isolated, using TTX and either cobalt (I_K's) or 22mM Ca^{++}/4AP (I_{Ca}'s) and studied using whole-cell patch-clamp in enzymatically and mechanically dissociated HCs maintained for 1 to 7 days in culture. I_K's were studied primarily in HC subtypes H1, H2 and H4. They have an inwardly rectifying I_K which activated at membrane potentials below -70mV, and a delayed rectifier which activated above -30mV and was unaffected by up to 50mM TEA. H2's and H4's possess a transient A-type I_K which activated above -40mV in H2's (809±371 pA max at around +70mV), and above -30mV in H4's (380±271 pA max at around +70mV). I_A was fully inactivated at -10mV holding potential and reversibly blocked by 2.5mM 4AP. I_A was small in H1's. I_{Ca}'s were investigated in H2's and H4's which exhibited transient (TR) and sustained (S) currents. I_{Ca}TR was activated above -50mV, while I_{Ca}S was activated above -10mV. Both I_{Ca}'s are fully and reversibly blocked by 10mM cobalt. Supported by EY 05972.

366.5

IONIC CURRENTS OF SOLITARY HORIZONTAL CELLS ISOLATED FROM TURTLE RETINA. A. G. Golard* and P. Witkovsky (SPON: S. Stone). Dept. of Ophthalmology, NYU Medical Center, New York, NY 10016.

Horizontal cell bodies (HCB) and axon terminals (HCAT) were isolated from retinas of the turtle Pseudemys scripta elegans. The cells were maintained in Leibovitz (L-15) medium for 2-4 days. Whole cell patch recordings were obtained in a standard manner.

An anomalous rectifier was present in the HCB and the HCAT. It activates below E_K, its magnitude depends on the K gradient, and its activation kinetics depend on voltage in a complex way.

A transient inward current was also present in both the HCB and the HCAT. This current was blocked by 5uM TTX and is thus presumably carried by sodium.

In addition we found an A current in the HCAT, but not in the HCB. This current shows typical voltage dependent activation and inactivation. However its temporal characteristics are unusual: after being expressed, a long (typically 2 min) pulse interval is needed for full recovery. This current is blocked by 5mM 4-AP and or 20uM Capsaicin.

A small sustained inward current was observed in the HCAT when [Ca]out=20mM. This current was blocked by Co and enhanced by Bay-K 8644. It activated above -30mV and reached a peak near 0mV. These characteristics suggest it is an L-type calcium current.

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366.6

UNITARY CONDUCTANCE AND MODULATION OF GAP JUNCTION CHANNELS IN WHITE PERCH HORIZONTAL CELLS. D.G. McMahon, A.G. Knapp, and J.E. Dowling, The Biological Laboratories, Harvard University, Cambridge, MA. 02138.

Dopamine modulates electrical synaptic transmission between teleost retinal horizontal cells. Based on noise analysis we have previously reported that dopamine alters the open probability of junctional channels (SNS Abstr., 14, 161). We have now examined the effects of dopamine on single channel gating events in pairs of voltage-clamped white perch (Roccus americana) horizontal cells.

Dissociation, culture, and recording methods were as previously described. In poorly coupled cells, unitary junctional events were evident as equal and opposite changes in the two clamp circuits. Events were recorded from 18 pairs, 11 with standard solutions and 7 with Cs^+-substituted pipette solutions. The standard solution group; 4 uncoupled with dopamine, 4 with octanol, and 3 untreated pairs, all had unitary conductances of 50-60 pS (mean=54.4 pS ±9.6 SD), while the Cs^+ group (4 dopamine-treated, 3 untreated) had events of 30 pS (mean=30.9 pS ±5.7). Comparison of the duration of junctional channel openings at maximum uncoupling to open durations during onset or recovery from dopamine revealed that dopamine acts by reducing the duration of junctional events (mean at max uncoupling= 7.5 ms vs 15.2 ms for partial uncoupling, 7 pairs). Thus dopamine apparently alters the open time of cell-to-cell channels.

366.7

ON Ca AND Na REGULATION IN RETINAL HORIZONTAL CELLS: Na-Ca EXCHANGE, Na-K ATPase, AND INTERNAL Ca STORES. S.Yasui*. (SPON: M. Tachibana). Kyushu Inst. of Tech., Iizuka, 820 Japan.

This study is concerned with Ca^{2+} and Na^+ homeostasis in horizontal cells (HCs) which are second-order retinal neurons. Whole-cell voltage-clamp experiments were made using patch pipettes on solitary HCs isolated from the goldfish eye. HCs were pre-treated with L-glutamate for > 1 hr. Right after the GΩ-seal was ruptured under current-clamp mode, V_m was ≃ 0 mV; the HCs had been kept depolarized about that much. The unit was then immediately put under whole-cell voltage-clamp at the preceding V_m value. The cobalt test revealed the voltage-gated Ca^{2+} influx (I_{Ca}) whose rate exceeded as much as 1 μM/sec: g_{Ca} of HCs does not completely inactivate with time. But, I_{Ca} was absent when Na^+ was replaced by Li^+ in the external medium. I_{Ca} was also abolished when the depolarization-inducing pretreatment was done with a high-K and low-Na Ringer. Furthermore, the switch from normal to Na-free caused the current to shift slowly outward before arriving at the same plateau as attained by cobalt application. These results suggest that Na-Ca exchanger operates to compensate for I_{Ca}, and that blocking it ultimately eliminates I_{Ca} due to Ca-dependent Ca-channel innactivation. Caffeine application induced an outward-going phasic change in current, indicating the presence of Ca stores. Their storage capacity is probably quite high; Ca^{2+} of sevaral mM (cytosolic equivalent) was found to flow into single units before Ca-channel inactivation was completed during block of the Na-Ca exchange. An ouabain experiment showed that Na-K ATPase is present, presumably to balance synaptic and non-synaptic Na-influx including the exchange component.

366.8

NETWORK MODELS OF THE HORIZONTAL CELL LAYER IN FISH RETINA. R. L. Winslow*, S. Ma*. (SPON: R. Purple). Dept. of Physiol., University of Minnesota, Minneapolis, MN 55455.

Experimental and modeling studies of horizontal cell response properties in intact retina provide evidence that these cells respond in an approximately linear fashion to a variety of stimuli. In contrast, studies of horizontal cells isolated from fish retina demonstrate the existence of five different voltage-dependent macroscopic membrane currents, including a spike generating Ca^{++} current. We are attempting to understand the physiological basis of differences in isolated cell versus network properties through the use of computer models. We have previously shown that two factors tend to linearize responses of horizontal cells in the intact retina: a) the inward Ca^{++} must be partially inactivated at rest; b) tonic synaptic input from photoreceptors in backgrounds of dark prevents bistable, or spiking behavior (Winslow, J. Neurophysiol., in press). We extend these analyses to address the role played by gap junctional coupling between neighboring horizontal cells in determining network properties. Newtons iteration coupled with conjugate-gradient descent methods are used to compute steady-state responses of large scale networks of horizontal cells, each with a full complement of voltage dependent channels, to arbitrary spatial patterns of synaptic input. Coupling conductances (G_s) two orders of magnitude smaller than that known to exist between pairs of cultured horizontal cells (Lasater and Dowling, PNAS, 82: 3025, 1985) provide a current shunt sufficiently large to block bistable behavior in the network. G_s values in the physiologically observed range yield a network input resistance of 15-30$M\Omega$, and highly linear network I-V curves. The predicted network space constant and dc transfer function are in close agreement with physiological data. Gap junctional coupling between neighboring horizontal cells is therefore a powerful factor acting to linearize properties of the horizontal cell network (supported by The Whitaker Foundation, and NIH #PO1NS17763-07).

366.9

MODELLING OF ELECTRICAL PROPERTIES OF VARICOSE DENDRITES IN RETINAL RADIATE AMACRINE CELLS. G.M.Ratto* and C.Usai* (SPON: G.Maguire) Ist. di Neurofisiologia, Pisa and I.C.B., Genova, Italy.

Radiate amacrine cells display a star-like morphology with long, unbranched dendrites. These processes are very thin (0.1 - 0.3 um) and are interlaced with many large varicosities (about 2 um in diameter).

We simulated the effects of the presence of varicosities on the passive spread and on the size of the postsynaptic potential (PSP) generated by a stationary change of conductance at the synaptic site. Input resistance (R_i) and electrotonic attenuation (A_V) have been computed for a thin cylindrical dendrite, on which a variable number of varicosities has been positionated randomly.

Varicosities reduced R_i for an injected current and increased A_V. The smaller R_i widens the linear range of the PSP-conductance relationship, helping impedance matching with other elements of the network and reducing the effect of synaptic noise. Due to the higher attenuation, a localized conductance change affects a shorter portion of the dendrite, thus increasing the number of local micro-circuits present on a single cell.

366.10

NMA: AN INTERACTIVE GRAPHICS APPLICATION FOR GENERATING AND MANIPULATING COMPARTMENTAL MODELS.
M. Tiller*, R. L. Winslow*, R. F. Miller. (SPON: C. Terzuolo). Dept. of Physiol., University of Minnesota, Minneapolis, MN 55455.

NMA, for Neural Modeling Application, is an application which overcomes bottlenecks inherent in the development and execution of large-scale compartmental models of single neurons with voltage-dependent membrane. NMA reads data files generated by a Eutectics Neuronal Reconstruction System (Capowski, J. Neurosci. Meth., 8: 353, 1983) containing information on the length, diameter, and branching pattern of neuronal dendritic trees. Given passive membrane parameters, NMA uses the morphological data to automatically partition the dendritic tree into a number of cylindrical segments of arbitrary electrotonic length. A Schol plot of the dendritic tree is then displayed on a SUN-4 workstation. Users may alter membrane parameters at either the single compartment, branch, primary dendrite, or whole neuron level by selecting a neuron structure using the graphics terminal mouse, and then modifying the structure's parameter list in a pop-up menu. Compartmentalization of the dendritic tree and the displayed Schol plot are immediately updated to reflect parameter changes. Voltage-dependent channels may be inserted at any structural level of the model by clicking on icons representing various channel templates. These presently include templates for synaptic conductances, a delayed rectifier K^+ current, a Na^+ current, a Ca^{++} modulated K^+ current, and a Ca^{++} current. Once a model is specified, NMA automatically generates a simulation program which can be executed by SABER, a general purpose electrical circuit simulation package developed by Analogy Inc. SABER has proven to be a powerful tool for executing compartmental models with non-linear membrane (Flach et al., Soc. Neurosci. Abstr., 13(1): 159, 1987). SABER output can be displayed and manipulated using PITool (Analogy, Inc.) or by passing SABER data structures to S. (NIH Grant PO1NS17763-07).

366.11

GABA-ACTIVATED CURRENTS IN RAT RETINAL GANGLION CELLS. M.L. Veruki* and H.H. Yeh, Program in Neuroscience and Dept. Neurobiology and Anatomy, Univ. Rochester School of Medicine, Rochester, NY 14642.

Gamma-aminobutyric acid (GABA) is a major inhibitory transmitter in the vertebrate retina. In the inner retina, GABA has been reported to affect third order neurons by both $GABA_A$ and $GABA_B$ receptor-mediated mechanisms. Here, we examined GABA-activated currents (I_{GABA}) in freshly dissociated rat retinal ganglion cells. A major goal was to determine whether GABA activated in these cells a conductance which involved one or more ionic species and which could be attributed to the activation of $GABA_A$ or $GABA_B$ receptors, or both.

Freshly dissociated ganglion cells were identified by fluorescent retrograde tracers (rhodamine-labeled microbeads or fast blue). GABA agonists and antagonists were applied by pressure and the resulting current was recorded under whole-cell voltage clamp. All ganglion cells recorded thus far (n=58) responded to GABA (0.1-10 uM). In a few cases, differential sensitivity to GABA between the soma and processes could be demonstrated. The response was typically monophasic, hyperpolarizing, and associated with a conductance increase. Prolonged or successive brief applications caused a rapid partial fading of I_{GABA}. The reversal potential for I_{GABA} shifted with varying pipet $[Cl^-]$ in a manner which approximated that predicted from the Nernst equation for Cl^-. Muscimol (5 uM) induced a current which mimicked I_{GABA}. Picrotoxin and bicuculline (200 uM) blocked I_{GABA}. Baclofen (up to 200 uM), in the presence or absence of bicuculline, produced no apparent conductance change (15 cells).

Thus, under our recording conditions, our data to date suggest that the I_{GABA} in rat retinal ganglion cells appears to be predominantly, if not exclusively, a Cl^- current due to $GABA_A$ receptor activation. However, the possibility cannot be excluded that a bicuculline-insensitive, baclofen-activated current exists in the extreme distal dendrites which may have been lost in the dissociation procedure.

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366.12

A SLOWLY INACTIVATING K^+ CURRENT IN POSTNATAL RAT RETINAL GANGLION CELLS. Nikolaus J. Sucher and Stuart A. Lipton, Dept. of Neurology, Children's Hospital & Harvard Medical Schl, Boston, MA.

K currents in rat retinal ganglion cells have been separated into components resembling I_A, I_K, and $I_{K(Ca)}$ (Lipton & Tauck, *J. Physiol.* 385:361, 1987). Recently, slowly inactivating K currents with distinct biophysical and pharmacologic properties have been described in rat hippocampal cells (I_D; Storm, *Nature* 336:379, 1988) and ganglion cells from tiger salamander retina (I_B; Lukasiewicz & Werblin, *J. Neurosci.* 8:4471, 1988). Here we characterize a slowly inactivating K current not previously described in mammalian retinal ganglion cells. Postnatal ganglion cells (P4-9) were fluorescently labeled *in situ*, dissociated from the retina, and maintained in culture. K currents were recorded using whole-cell recording with patch electrodes containing a KCl-saline. The slowly decaying K current was pharmacologically isolated by suppressing I_{Na} with 1μM TTX, I_A with 5 mM 4-AP, I_K with 20 mM TEA and I_{Ca} and $I_{K(Ca)}$ with 3 mM Co, all added to the bath. The slowly decaying K current was activated from -80 mV by steps positive to –40 mV and was present in 90% of all ganglion cells tested (n = 39). The mean amplitude at 10 mV was 327 ± 222 pA (mean ± S.D.). The current activated in less than10 ms and inactivated with a time constant of about 130 ms at 23°C. Inactivation was voltage dependent, almost complete at 0 mV, and half-maximal at -55 mV. The current was blocked by internal Cs and TEA or by external 1 mM Ba, but not by 250 μM Cd, 3 mM Co, 5 mM 4-AP, or 20 mM TEA. The tail currents reversed at –84.6 ± 2.6 mV (n = 5; r = 0.98; 140 mM K inside, 5.8 mM K outside, equimolar Cl, 35°C). Comparison of this current with I_D and I_B, both of which have been implicated in regulating firing patterns, show that its properties resemble more closely those of I_B in amphibian retina than those of I_D in rat hippocampus. It may thus be important for specific aspects of information processing in the retina.

366.13

CRYOPRESERVATION OF POSTNATAL GANGLION CELLS FROM RAT RETINA: PERSISTENCE OF VOLTAGE- AND LIGAND-GATED IONIC CURRENTS. Paul Sforza*, Nikolaus J. Sucher, Toni P.O. Cheng*, and Stuart A. Lipton (SPON: H. Blume). Dept. of Neurology, Children's Hospital & Harvard Med. School, Boston, MA. 02115.

Despite heightened interest in cryopreservation of central nervous system tissue, little is known about its effects on the electrophysiology of neurons. Therefore, we used the patch-clamp technique to study retinal ganglion cells cryopreserved from 1 week-old rats. Following dissociation, retinal cell suspensions containing fluorescently labeled ganglion cells were frozen in 8% DMSO and stored at –80°C for up to 45 days prior to culture. The cryopreserved cells appeared morphologically normal, grew processes, and cleaved fluorescein from fluorescein diacetate, indicating their viability. The cells maintained resting potentials of –60 mV and fired action potentials upon depolarization. Under voltage-clamp, depolarizing pulses activated Na+-, K+- and Ca++-currents. K+-currents were separated into I_A, I_K, $I_{K(Ca)}$, and a slowly decaying I_B (Sucher & Lipton, these abstracts). GABA (20 μM) and glycine (100 μM) activated chloride selective currents that were blocked by bicuculline (10 μM) and strychnine (5 μM), respectively. Glutamate (100 μM), NMDA (200 μM), kainate (125 μM), and ACh (20 μM) activated cation selective currents. The NMDA response was blocked by APV (100 μM), the kainate induced current by CNQX (10 μM), and the ACh evoked current by hexamethonium (20 μM). Comparison of fresh and cryopreserved cells showed that the pharmacological and biophysical properties of these voltage- and ligand-activated ionic currents were largely unaffected by cryopreservation. Hence, this method may help reduce the number of research animals used in a series of experiments by allowing collection of a large number of neurons from a single animal to be used at later times.

366.14

EFFECTS OF NOREPINEPHRINE ON GANGLION CELLS AND THE ERG IN THE RABBIT RETINA. Ethan D. Cohen and Robert F. Miller, Dept. Physiol. Univ. of Minn., Minneapolis, MN 55455

We examined the effects of norepinephrine (NE) and other adrenergic agonists and antagonists on ganglion cells in the rabbit retina using extracellular recording in a superfused eyecup preparation. Bath application of NE at low concentrations (1-10μM) produced an increase in the spontaneous activity of all types of ganglion cells studied. Sustained cells were more sensitive to low doses of NE than transient cells. At concentrations above 15 μM, NE was inhibitory to on-sustained cells. We were unable to block the excitatory actions of NE with the β receptor antagonists timolol, propranolol, or the α antagonist phentolamine. The adrenergic agonists phenylephrine and UK-14304-18 had little effect on rabbit ganglion cells. Isoproterenol produced only a weak excitation while only epinephrine duplicated the excitatory effects of NE. We examined the possibility NE was acting through a dopamine receptor. Neither D-1 nor D-2 dopamine agonists SKF38393 or LY171555 were able to completely mimic the effects of NE. However, we were able to completely block the excitation of NE on off-center ganglion cells with the D1 antagonist SCH23390. We conclude that NE appears to be acting as a mixed agonist at dopamine receptors. NE also induced changes in the standing potential and suppressed the C wave of the ERG. These latter actions were blocked by prazosin, suggesting these effects are mediated by α_1 adrenergic receptors. (Supported by EY03014)

366.15

REPETITIVE SPIKING IN GANGLION CELLS OF THE TIGER SALAMANDER RETINA. P.A. Coleman, J.F. Fohlmeister and R.F. Miller. Univ. of Minnesota, MN 55455.

The individual contributions of the intrinsic, membrane currents to ganglion cell spike entrainment was assessed with a computer model based on the descriptions of Lukasiewicz & Werblin (J. Neurosci., 1988) and Lipton & Tauck (J. Physiol., 1987). The five ionic currents considered were: 1) Na^+, 2) Ca^{2+}, 3) non-inactivating K^+ [delayed rectifier], 4) fast-inactivating K^+ [A type], and 5) Ca^{2+} mediated K^+.

The model was compared with physiological data obtained from whole cell recordings of ganglion cells in the intact tiger salamander retina-eyecup preparation. A close correspondence between the model and physiological data was observed for both the waveform of individual impulses and the relationship between spike frequency and intracellular polarization. In addition, pharmacological manipulations of ionic currents were compared with the simulated absence of one or more ionic channels. To date, the close correspondence between model and experimentally obtained data suggests that the functional roles for each of the ionic currents can be understood under a wide variety of conditions. This research was supported by EY03014 and EY07376 to RFM.

366.16

IMPULSE INITIATION IN RETINAL GANGLION CELLS. P.N. Steinmetz*, J.F. Fohlmeister and R.F. Miller Dept. of Physiology, Univ. of Minn. Mpls. MN 55455

The axons of mudpuppy retinal ganglion cells narrow to a diameter approaching 0.1 μm for a length of 50 to 110 μm, beginning some 10-15 μm from the soma. In contrast, the initial segment and more distal axon are about 1 μm in diameter. In a companion abstract (Coleman, Fohlmeister and Miller,1989), we have analyzed the contributions of 5 different ionic channels to the control of impulse initiation and frequency, by comparing simulated and physiological data. In this study we have used compartmental models to evaluate the function of the thin axonal segments in regulating impulse firing behavior. The neuron included some portion of dendrite, the soma and axon which was compartmentalized into 5 μm segments for numerical integration of the kinetic equations. The density of each channel subtype was altered from compartment to compartment to explore the interaction of asymmetric geometry and non-uniform ion channel distribution. The results of these simulations were constrained by physiological observations which suggest that impulse initiation probably occurs at the initial segment and subsequently activates somatic and axonal spikes within the thin segment. The functional advantages of this arrangement are evaluated in terms of impulse synchronization and reduced shunting by the axon provided by the thin segment. (Supported by NEI grant R01EY07376).

366.17

SYNAPTIC INPUT TO A PHYSIOLOGICALLY IDENTIFIED X-CELL IN THE CAT RETINA. A.J. Weber, M.A. McCall, and L.R. Stanford. Department of Comparative Biosciences and the Waisman Center, University of Wisconsin, Madison, WI 53706.

Intracellular injection of horseradish peroxidase (HRP) was used to label a retinal X-cell recorded in an in vivo preparation. This cell, located 4.5° from the area centralis, showed linear spatial summation, and responded tonically to a visual stimulus of standing contrast. After histochemical processing, the cell was embedded in resin, drawn at 1000X, and sectioned tangential to the retinal surface. Approximately 85% of the dendritic arbor of this cell was then reconstructed from photographic montages (23,000x) of alternate serial thin sections. Amacrine and bipolar cell inputs were identified according to the criteria established by Kolb ('79; J. Neurocytol. 8:295). The dendritic field of this "ON-center" X-cell (soma= 360um^2) was approximately 52um in diameter and was confined to sublamina b of the inner plexiform layer (IPL). This cell was found to receive approximately the same number of synaptic contacts from bipolar (60) and amacrine cells (82). Few contacts (5 from bipolar cells) were found on the primary dendrite. Most of the bipolar cell input was found on 2° and 3° dendrites; the majority of the amacrine cell input was onto 2°, 3°, and also 4° dendrites. Both types of input also could be found on more distal dendrites. Although amacrine and bipolar cell inputs were distributed evenly over much of the dendritic field, we did find that the proportion of amacrine cell input increased on dendritic processes that approached the border between sublaminae a and b of the IPL.

The data from this X-cell will be compared with data from Y- and W-retinal ganglion cells in order to determine whether the relative contributions of amacrine and bipolar cell inputs contribute to the physiological differences seen among different classes of retinal ganglion cells. Equally important is the possibility that retinal ganglion cells with very different morphological features, but common physiological characteristics, share similar patterns of synaptic input. Supported by NIH grants EY 04977 and EY 05869.

366.18

COMPARISON OF CAT RETINAL GANGLION CELL RESPONSES TO LIGHT STIMULI AND EYEBALL DEFORMATION AFTER INJECTION OF APB AND MUSCIMOL. A.W. Przybyszewski, H.T. Chung*, O.-J. Grüsser and M. Hagner*. Dept. of Physiol., Freie Universität Berlin, Arnimallee 22, 1000 Berlin 33, (West) Germany.

We compared the effects of APB (2-amino-4-phosphonobutyric acid) and GABA-agonists: MU (muscimol) and IBO (ibotenic acid - metabolized into MU) injected (0.5-3 mg pro eye) into the vitreous body on ganglion cell activity evoked by diffuse short (10 ms) or longer (1 s) light stimuli or eyeball deformation. The experiments were performed in pentobarbital anaesthetized cats.

After APB, IBO and MU injection, light-induced responses in on-ganglion cells were strongly reduced in a very similar manner. In contrast, off-ganglion cell light-evoked activity changed only slightly after APB and completly disappeared after GABA-agonists. With all substances, an excitation phase was observed in most ganglion cells. During the excitation phase induced by APB, ganglion cell responses to eyeball deformation were at first reduced, later disappeared completely and significantly faster than the responses to light. In contrast, after MU and IBO injection, responses to eyeball deformation were abolished simultaneously with the reduction in light responses.

We assume that eyeball deformation affects primarily horizontal cell activity and it could be a useful method to determine in which part of the retina APB, IBO or MU interact with synaptic transmission.

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366.19

TEMPORAL RESPONSE CHARACTERISTICS OF CAT RETINAL GANGLION CELLS. M.H. Rowe. Department of Zoology, College of Arts and Sciences & Basic Science Unit, College of Osteopathic Medicine, Ohio University, Athens, OH 45701.

A method is described which allows a relatively quick and accurate assessment of the temporal frequency characteristics of retinal neurons. The method is similar to one widely used in the analysis of electronic systems, and is based on the response of a linear, time invariant system to a temporal impulse containing a wide range of frequencies at equal amplitudes. However, instead of an impulse, the stimulus is presented in the form of a temporal 'step', by flashing a grating on for a fixed duration. The response to this step is then differentiated and subjected to Fourier analysis which yields information about the spectrum of temporal frequencies contained in the response. When compared to the actual responses of X- and Y-cells to sinusoidal gratings which either drift or alternate over a range of temporal frequencies (see also Frishman, et al., J. Gen. Physiol., 89: 599-628, 1986), the method yields a function which fits these data points very well. Thus, it can be applied confidently in situations where data collection time is at a premium, such as recording from W-cells or intracellular recording from non-spiking retinal interneurons. Supported by grants EY06013 and EY08038 from the National Eye Institute.

366.20

TEMPORAL ALIASING IN OPTIC NERVE FIBERS

Michael A. Crognale* and Gerald H. Jacobs (SPON: Loy Lytle), University of California at Santa Barbara, Santa Barbara, CA 93106

Investigations of neuronal temporal response properties have often utilized trains of regularly repeating stimuli. Attempts to relate the neuronal activity to behavioral observations have typically focused upon the response of the cell at the frequency of stimulation. The response of single optic nerve fibers to temporally modulated stimuli (square wave: 5,10,20,30,40 and 50 Hz) were recorded from the ground squirrel (Spermophilus beecheyi). The stimuli were positioned so as to completely cover the receptive field of the cell. Under these conditions, most ganglion cells produced a phase-locked response to modulation frequencies as high as 50 Hz. This was true for both spectrally opponent cells and spectrally non-opponent cells. At these high frequencies, many cells also produced large modulated responses at half of the stimulus frequency, in addition to the response at the stimulus frequency. The relative amplitudes of these components were dependent on stimulus frequency. As stimulus intensity was increased, the response of the cell at the stimulus frequency increased relative to the response at half of the stimulus frequency. For most cells, there was a range of intensities for which the response at half the stimulus frequency was large while the response at the stimulus frequency could not be detected. These results suggest that behavioral threshold detection and supra-threshold resolution of rapidly modulated stimuli may be mediated by responses from neurons that individually fail to produce a response at the stimulus frequency.

366.21

WHITE NOISE ANALYSIS OF RABBIT GANGLION CELL LIGHT RESPONSES. S.C. Mangel, H.M. Sakai* and K-I. Naka. Department of Ophthalmology, University of Alabama School of Medicine, Birmingham, AL 35294.

To characterize the light responses of ganglion cells in the rabbit retina using white noise analysis, we cross-correlated the ganglion cell spike discharges (transformed into unitary pulses) against the analog light input white noise. Sakuranaga et al.(1987) have shown that first and second order kernels from ganglion cell spike trains are proportional to those from intracellular ganglion cell recordings. Because amacrine amd bipolar cells produce kernels of characteristic signature, presynaptic inputs to a given ganglion cell can be inferred by observing the kernel waveforms or signatures.

Ganglion cell light responses evoked by white noise-modulating a full field LED stimulus around a mean luminance were more bandpassed than those evoked by flashing stimuli. At the light levels used, brisk sustained and transient ganglion cells produced well-defined first order but not second order kernels. Large field unit ganglion cells, however, displayed a prominent second order nonlinearity, suggestive of an input from transient on-off amacrine cells. Direction-sensitive cells were specifically tuned to a full field stimulus modulation rate of 20 Hz.

366.22

SYNAPTIC INTERACTIONS OF RETINAL DIRECTIONAL GANGLION CELLS. C. Brandon, Department of Cell Biology and Anatomy, Chicago Medical School, North Chicago, IL

In the rabbit retina, ON-center directionally selective ganglion cells (ON-DS GC's) project to nuclei of the accessory optic system; their directional selectivity appears to be generated by GABAergic neurons and modified by cholinergic input.

To study these synaptic interactions, Nuclear Yellow (NY) was injected into the rabbit medial terminal nucleus. Retrogradely labeled GC's were impaled and filled with Lucifer, then studied by immunocytochemical localization of choline acetyltransferase or glutamate decarboxylase, or serial-section EM.

Dendrites of presumed ON-DS GC's branched at an inner plexiform depth of 70%, precisely overlapping cholinergic IPL sublamina b, and occupying the zone between the two most proximal GABAergic strata. In general, there was not more than one or two microns separating these three strata, an arrangement that would permit extensive synaptic interaction among them. ON-DS GC dendrites received synaptic input 1) from many small, clustered amacrine processes that were probably cholinergic; 2) from bipolar cell axon terminals; and 3) from larger amacrine processes with a very low electron density; this last type may be the source of GABAergic input onto the ON-DS GC. It appeared that there was a segregation of synaptic input onto a given ON-DS dendrite, such that clusters of presumed cholinergic terminals, and the larger, pale amacrine processes, impinged on different regions of that dendrite. Such spatial segregation may underlie synaptic mechanisms of directionality.

366.23

COMPUTER SIMULATION OF RETINAL MOTION DETECTION. F.H. Eeckman, M.E. Colvin* and T.S. Axelrod*. Lawrence Livermore Natl. Laboratory, Livermore, CA 94550.

We present a three-dimensional simulation of the motion detection circuitry in the vertebrate retina. The model consists of five layers of cells in a closely packed hexagonal array. All cells are modeled as single compartment, leaky-integrator neurons. The following features of retinal organization are present in the model:

1) Coupling and overlap between the receptor-elements. The range of coupling can be changed interactively.
2) Nonlinear gain and thresholding is present in all layers.
3) Difference-of-gaussians type receptive fields.
4) Transient calculation is independent from directional selectivity.
5) Directionally selective inhibition using shunting interactions.
6) Convergence. The ratio of convergence can be changed interactively.
7) Feedback between the inner and the outer retina. The feedback controls the gain in the outer retina and is driven by the amount of inhibition present at the ganglion cell level.

The network presented here is capable of detecting small moving targets against a noisy background. The model performs well down to a corrected signal-to-noise ratio of 2.6.

CHEMICAL SENSES: OLFACTORY PATHWAYS AND PROCESSING

367.1

RAT OLFACTORY BULB GLOMERULI STAIN NON-UNIFORMLY WITH CYTOCHROME OXIDASE AND ACID PHOSPHATASE: POSSIBLE CAUSES AND IMPLICATIONS. C. Weinberg and E. Meisami. Physiology Dept., Univ. of Illinois, Urbana, IL, 61801.

The glomeruli (GL) of the rat olfactory bulb show a marked staining for cytochrome oxidase and acid phosphatase, marker enzymes for mitochondria and lysosomes respectively. Careful comparison of the staining patterns for these two enzymes in serially sectioned bulbs reveals that across the GL layer, the various GL do not stain uniformly, some staining more intensely than others. This non-uniformity is evident even within a particular GL. Based on the assumption that cytochrome oxidase staining indicates neural activity whereas acid phosphatase indicates neural degeneration/turn-over, we have analyzed the patterns of staining for these two enzymes, in 40 um adjacent frontal sections, which are then compared quantitatively. The results indicate that the GL staining darkly for cytochrome oxidase tend to stain lightly for acid phosphatase and vice versa. Also, even within a GL, cytochrome oxidase positive areas tend to be acid phosphatase negative and vice versa. The results imply that in the adult rat, various olfactory GL show differential patterns of activity and degeneration and that this spatial variation in activity may be present even within the same GL. We will also report the effects of olfactory nerve transection and chemical deafferentation on these staining patterns.

367.2

LECTIN LABELING OF RAT OLFACTORY BULB GLOMERULI M.J. RIGGOTT and J.W. SCOTT (Department of Anatomy and Cell Biology, Emory University School of Medicine, Atlanta, GA 30322)

Functional subclasses of sensory neurons can be identified by the carbohydrates they express (Dodd et al, 1984, Nature 311:469). We examined the carbohydrate composition of olfactory bulb glomeruli. The lectins Ulex europaeus 1 (UEA1), and Soybean agglutinin (SBA) bind specifically to subsets of olfactory bulb glomeruli in the adult rat. Individual glomeruli are uniformly labeled, and lightly and darkly labeled glomeruli can be found next to each other. The most densely labeled glomeruli are found in the ventrolateral regions of the main olfactory bulb. Lectin binding was blocked with competing sugars and indicates that UEA1 recognizes fucose and SBA binds to galactose and N-acetylgalactosamine in main bulb glomeruli. It appears that the lectin binds to the afferent components of the glomeruli because denervation severely reduces UEA1 binding and, in partially denervated glomeruli, the remaining afferent fibers continue to bind UEA1. The heterogeneous binding of the lectins UEA1 and SBA indicates that subclasses of receptor cell afferent terminals can be identified by the carbohydrates they express.

This research is supported by NIH grant NS-12400

367.3

CAUDAL-ROSTRAL GRADIENTS OF SYNAPTOGENESIS IN THE NEONATAL RAT OLFACTORY BULB. C.A. Greer, C.K. Kaliszewski* and H.A. Cameron*. Sections of Neurosurgery & Neuroanatomy, Yale University School of Medicine, New Haven, CT 06510.

Local circuits in the olfactory bulb (OB) external plexiform layer (EPL) consist almost exculsively of reciprocal dendrodendritic synapses between interneurons, granule cells, and the output neurons, mitral and tufted (M/T) cells. Prior evidence suggests that M/T cell systems may be parallel circuits that are segregated within sublamina of the EPL. Light microscopy of afferent and efferent projections as well as 2DG studies have suggested that caudal portions of the OB mature first, perhaps in response to early functional requirements. To investigate these issues further we studied gradients of local circuit synaptogenesis in rostral and caudal divisions of the EPL. Rats at 0, 4, 8, 16 and 32 days postnatal were processed for 100 kV transmission electron microscopy. Montages of micrographs, passing radially through the EPL sublaminae, were used to classify dendrodendritic synapses as either Gray type I or type II. The total number of synapses increased by a factor of 4 between 0 and 32 days postnatal. Gray type I synapses led the Gray type II synapses by a ratio of approximately 2:1 throughout development, although this may reflect the difficulty in identifying Gray type II's in single sections. The results showed clear topographic gradients in the appearance of synapses. The caudal OB was most precocious, leading the rostral OB in density of synapses/um^2 by approximately 4 days. Similarly, the superficial EPL led the deep EPL by approximately 2 days. The data, in showing that local circuits in the caudal region are more precocious in their development, support the hypothesis that the caudal regions of the OB mature on an earlier timecourse than the remainder of the OB.

367.4

THE GEOMETRY AND CONNECTIVITY OF DENDRODENDRITIC MICROCIRCUITS IN THE OLFACTORY BULB. T.B. Woolf[1], G.M. Shepherd[1] and C.A. Greer[1,2]. Sections of Neuroanatomy[1] and Neurosurgery[2], Yale Univ. Sch. Med., New Haven, CT 06510.

The external plexiform layer (EPL) of the olfactory bulb (OB) contains reciprocal dendrodendritic synapses between granule cell spines (G_{sp}) and mitral or tufted (M/T) dendrites. The extent to which these circuits may contribute to a divergence or convergence of information within the EPL is not well understood. Moreover, computational modeling has been limited due to the lack of morphometric data on the geometry of these circuits. To address these issues we initiated ultrastructural studies of the EPL in which serial sections were used to reconstruct the 3-dimensional geometry of dendritic processes and their connections. Sixty-six G_{sp}'s have been reconstructed in full and 14 M/T dendritic processes reconstructed to depths of 2 um. The volume of G_{sp} heads was highly variable ranging from 0.03 - 1.6 um^3. Similarly, the G_{sp} head surface area varied from 0.32 - 8.3 um^2. G_{sp} necks were less variable with volume ranging from 0.03 - 0.34 um^3 and surface area from 0.31 - 2.0 um^2. Most of the M/T profiles reconstructed established reciprocal synaptic connections with G_{sp}'s; the density of synaptic connections ranged between 0.5 - 6 per linear um of M/T dendrite. Moreover, in a volume of 530 um^3 the great majority of G_{sp}'s connected to individual M/T dendrites were from different granule cell dendrites, supporting the concept of divergence within EPL microcircuits. In addition, the morphometric results are providing a basis for biophysical computational models in which the functional implications of divergence in local circuit connectivity can be examined.

367.5

EVIDENCE FOR CA++ INFLUX INVOLVEMENT IN THE LONG-LASTING DEPOLARIZATION OF MITRAL/TUFTED CELL DENDRITES IN THE SALAMANDER OLFACTORY BULB. A.R. CINELLI* K. A. HAMILTON and J.S. KAUER. Neuroscience Program and Dept. of Neurosurgery. Tufts Medical School-New England Medical Center, Boston, MA. 02111.

Optical recording methods using several different voltage-sensitive dyes have revealed relatively long-lasting depolarizations in the external plexiform layer of the olfactory bulb after both electrical and odor stimulation. In the present study, real-time video imaging at 30 frames/sec, of calcium-sensitive dyes injected into single mitral/tufted cells has permitted observation of changes in dendritic and somatic Ca++ levels after electrical stimulation of the input and output pathways of the bulb. Orthodromic stimulation elicits long-lasting signals with complex, non-uniform spatial and temporal distributions. Antidromic stimulation showed similar patterns of activity, although generally with faster time courses. These observations are correlated with intracellular electrode recordings and voltage-sensitive dye images from the same cells and agree with the hypothesis that the late depolarization in mitral/tufted dendrites seen with voltage-sensitive dyes are Ca++ related.

Supported by PHS Grants NS-20003, NS-22035 and the Department of Neurosurgery, Tufts-New England Medical Center.

367.6

MEMBRANE CONDUCTANCE CHANGES DURING ODOR-INDUCED POTENTIALS IN RAT OLFACTORY BULB NEURONS. D.P. Wellis and J.W. Scott. Dept. Anatomy and Cell Biol., Emory Univ. Sch. of Med., Atlanta, GA 30322.

Intracellular recordings show that the generation of odor responses in olfactory bulb (OB) neurons involves the differential activation of both excitatory and inhibitory synaptic inputs (Wellis et al., in press; Hamilton and Kauer, in press). As a step toward understanding the biophysical processes underlying odor-induced membrane potential changes, we measured membrane conductance during odor stimulation in rat OB mitral/tufted cells. Odor-induced hyperpolarizations were associated with increases in membrane conductance relative to average resting conductance during spontaneous activity. For example, long-duration, odor-induced IPSPs correlated with a 20 to 65% increase in somal membrane conductance in 5 cells. Granule cells, GABAergic interneurons of the OB, mediate similar long-duration hyperpolarizations and increases in Cl conductance in mitral cells following electrical stimulation of the olfactory tract or nerve. Odor-induced depolarizations, on the other hand, increased (50% and 55% in 2 cells) and decreased (20%, 35%, and 80% in 3 cells) membrane conductance, suggesting that different synaptic mechanisms can depolarize an output neuron. Removal of granule cell-mediated tonic inhibition, for example, could result in a decrease of membrane Cl permeability and depolarization. In addition to the changes measured within a single sniff period, slow decreases in conductance occur across a series of sniffs, suggesting that activation of longer paths of circuitry within the bulb also determine mitral/tufted cell responses to odor.

Supported by NIH NS-12400.

367.7

ODOR RECOGNITION AFTER REMOVAL OF A BULBAR AREA IDENTIFIED WITH 2-DG. X. M. Lu and B. M. Slotnick. The American University, Washington, D.C. 20016.

A reliable focus of 2-DG activity occurs in the dorsomedial olfactory bulb of rats exposed to propionic acid (PA) vapor but removal of the area has no effect on detection of PA (Slotnick et al., Brain Res, 417:343, 1986). To test for odor recognition, rats were trained using operant conditioning for the concept 'respond if the odor is not propionic acid' (e.g. responses to any odor except PA are reinforced). Surgery was performed when rats reliably responded to the initial presentation of novel odors (60 odors tested). In postoperative tests conducted under extinction controls, rats with lesions of the lateral bulb or of the PA focal area had perfect or near perfect performance on this task. Further, there were no differences among groups in discrimination between PA and acetic acid or between mixtures of these odors. Thus, the 2-DG identified bulbar area is not essential for detection, recognition, or discrimination of propionic acid. Supported by NSF grant BNS-8319872 to B.M.S.

367.8

PHASE GRADIENTS AND SPATIAL AMPLITUDES OF OLFACTORY EEG SEGMENTS ARE FROZEN AT STATE TRANSITIONS DRIVEN BY RECEPTOR INPUT TO BULB. Walter J Freeman, Univ. California, Berkeley

Measurement of multiple EEG traces recorded simultaneously from 64 sites on the olfactory bulb, nucleus or cortex shows that neurons in each structure generate essentially the same time series in all parts over its surface, though differing between the three parts. With each inhalation there recurs a state transition between the low-level interburst chaotic state and a high-amplitude oscillatory state or burst. The spatial patterns of phase and amplitude modulation reflect a learned attractor, which is accessed through a basin that is defined by an odor CS. The correlation dimension of the burst state appears to be lower than that of the basal interburst state. Both appear to be chaotic, but the burst state has one or more sharp peaks in its spectra, and it may conform reproducibly to learned spatial patterns, whereas EEGs between bursts display 1/f spectra and no reproducible patterns. One interpretation is that a bifurcation occurs with each inhalation from chaos to a learned limit cycle state. A more plausible interpretation is that a stable chaotic attractor characterizes the waking dynamics in both the interburst and burst states, that learning modifies the shape of this attractor, and that odorant recognition is based on the forced relocation to one part of this attractor under impress of an odorant, which places the system in the basin of the appropriate subsection of the attractor. If so, odor perception is not due to bifurcation but to stimuli constraining the system into one wing of a global attractor.

367.9

TOPOGRAPHICAL PROJECTION FROM VOMERONASAL ORGAN TO ACCESSORY OLFACTORY BULB. MARY WISGIRDA* & MICHAEL MEREDITH (SPON: P. BORRONI). FLORIDA STATE UNIVERSITY, TALLAHASSEE, FL.

The vomeronasal organ (VNO) is an accessory chemosensory structure found in most terrestrial vertebrates which is involved in reproductive behaviors in many species, including the male hamster. It is an elongated tubelike structure which lies long the base of the nasal septum. Receptor cell axons form 3 nerve bundles which terminate in the accessory olfactory bulb (AOB). Horseradish peroxidase (HRP) was injected into one of these nerve bundles in separate series of hamsters to trace the connections from anterior, central and posterior sets of receptors into the AOB. The HRP was transported retrogradely to the receptor cell bodies and anterogradely to their terminals in the AOB. Frozen sections of the AOB and VNO were processed using TMB histochemistry and examined for HRP reaction product (RP). Anterior nerve injections labeled cells only in the anterior portion of the VNO. Posterior nerve injections labeled cells only in the posterior end of the VNO. Density of RP in 3 areas of each AOB section was determined with computer assisted image analysis. Each nerve had labeled terminals throughout the glomerular layer of the AOB, but their distribution varied significantly. Injection of the most posterior (ventral) nerve produced a much greater density of RP in the ventral than in the dorsal half of the AOB with little variation medio-laterally. In contrast, anterior nerve injections had significantly more RP laterally than medially in the AOB in addition to a ventro-dorsal gradient. These results suggest a degree of topographic organization to this projection which is more complex than a simple point to point connection.

Supported by NSF grants 8412141 and 8615159.

367.10

OLFACTORY BULB CONNECTIVITY IN A GYMNOTOID FISH, WITH SPECIAL REFERENCE TO TELENCEPHALIC, LOCUS COERULEUS AND RAPHE INPUTS. E. Sas and L. Maler. Dept. of Anatomy, University of Ottawa, Ottawa, Ontario, Canada K1H 8M5.

The connectivity of the olfactory bulb (OB) of Apteronotus leptorhynchus, was investigated following wheat germ agglutinin-conjugated horseradish peroxidase injections within the OB. Most bulbopetal cells receive reciprocal connections from the OB, with the exceptions of: 1) telencephalic cells at the border of area dorsalis medialis pars dorsalis/area dorsalis centralis, area dorsalis lateralis pars posterior and caudal area dorsalis centralis, 2) nucleus raphe centralis, 3) locus coeruleus. The OB projects bilaterally, with a major ipsilateral component via a medial and lateral olfactory striae. The olfactory target areas are: 1) Telencephalic areas: ventralis pars ventralis, medial olfactory terminal field (between areas ventralis partes ventralis and dorsalis) areas ventralis centralis, ventralis supracommissuralis, ventralis intermedia, dorsalis lateralis pars ventralis, dorsalis posterior and nucleus taenia. 2) Diencephalon: habenula, thalamus, N. preopticus periventricularis posterior and N. tuberis posterior. Distribution of NAergic, serotonergic, substance P and somatostatin fibers in the OB will be discussed as well as the topography of area dorsalis centralis cells projecting to the OB, optic tectum and torus semicircularis.

367.11

HYPOTHALAMIC EFFERENTS TO THE RAT OLFACTORY BULB REVEALED BY AN IMPROVED CHOLERA TOXIN B-HORSERADISH PEROXIDASE (CTB-HRP) CONJUGATE. P.M. Young* & F. Mullin*, School of Psychology, Lancashire Polytechnic, Preston, PR1 2TQ. U.K.

The use of periodate to oxidize the carbohydrate shell of HRP produces a high level of coupling leaving little unreacted CTB after conjugation, and no need for further purification. CTB-HRP produced by this technique is an exceptionally sensitive anterograde and retrograde neuronal tracer and it was used to investigate the bi-directional communications of the main and accessory olfactory bulbs. 100 nl injections of CTB-HRP were stereotaxically placed into the required component of the olfactory bulb, 24-72 hours later the animals were sacrificed and 80μm frozen sections were processed using tetramethyl benzidene as the chromogen. Forty two definable brain nuclei contained retrogradely labelled neurones, and twelve of these were in the hypothalamus. These mostly unique findings, demonstrate the sensitivity of CTB-HRP conjugate as a neuronal tracer. The labelled cells in so many parts of the hypothalamus provide a morphological basis for the direct centrifugal pathways required to explain a body of electrophysiological data. Given the significance of the olfactory sense in the rat, it is not too surprising to find direct reciprocal connections with those parts of the brain functioning in the maintenance of internal homeostasis, reproduction and reinforcement.(SPON: BRA)

367.12

DiI LABELED, NERVUS TERMINALIS AFFERENTS DISTRIBUTE TO MANY BRAIN REGIONS IN *XENOPUS LAEVIS*. E.F. Perkins* and G.D. Burd. Depts. of Molecular and Cellular Biology and Anatomy, University of Arizona, Tucson, AZ 85721.

In many vertebrates, three types of afferent fibers project from the nasal mucosa to the CNS via the olfactory nerve--axons of the olfactory receptor cells, the vomeronasal receptor cells, and the terminal nerve (nervus terminalis, NT). Using dil (0.1% dil, 90% EtOH, 10% DMSO), we labeled all three fiber systems in *Xenopus laevis* tadpoles and young frogs (stages 43-66) by placing the dye solution into the nasal capsules of paraformaldehyde-fixed animals. After 14 days, bright staining was observed in the olfactory nerve, vomeronasal nerve, and the nerve and glomerular layers of the main and accessory olfactory bulbs (MOB,AOB). We also observed a loose bundle of fibers that projected beyond the glomerular layer. These fibers, which are presumed to be part of the NT, projected caudally through the medial MOB, medial pallium, and septum. At this point in the pathway, individual axons projected into piriform cortex. The fiber bundle then divided with many axons crossing to the contralateral side through the anterior commissure while the majority continued to project caudally on ipsilateral side of the brain. A small group of the contralateral fibers coursed rostrally into the OB and terminated at the edge of the glomerular layer. The other group of the contralateral fibers projected caudally and traveled either along the lateral surface of the diencephalon or along the midline through the preoptic area. The termination of both the ipsilateral and contralateral fibers that project caudally beyond the preoptic area could not be determined. Others have shown that NT fibers are immunoreactive for luteinizing hormone releasing hormone (LHRH). In our study, adult frogs contained LHRH-like immunoreactivity in many of the same regions that were labeled with the dil. Support: NIH-NINCDS #NS25596.

367.13

THE AFFERENT CONNECTIONS OF THE MAIN OLFACTORY BULB (MOB) IN THE ARMADILLO (CHAETOPHRACTUS VELLEROSUS). A RETROGRADE TRACING STUDY WITH HRP, FLUORESCENT TRACERS AND SODIUM SELENITE. J.S. de Olmos*, H. Ferreyra-Moyano, C. Beltramino*, E. Barragan* and D. Scapellato*. Inst. Inv. Med., M. y M. Ferreyra, C.C. 389, 5000 Cordoba, Argentina.

The afferent connections of the MOB of the armadillo were examined by injecting horseradish peroxidase (HRP), fluorescent tracers (FT) and sodium selenite (NAS) into this structure, either by microelectrophoresis, hydraulic pressure or implantation of solid pellets. Twenty four to forty eight hours after unilateral injections of HRP or FT to the MOB, tracer-labeled perikarya appeared in the following telencephalic structures on the ipsilateral side: all portions of the anterior olfactory nucleus (AON), except its external part (AONe) the lateral transition field (LT), the piriform cortex (PIR), the nucleus of the horizontal (HDB) and vertical (VDB) limbs of DBB and the nucleus of the lateral olfactory tract (LOT).Labeled cells were also seen in the dorsal and medial transition fields, the ventral and intermediate precommissural hippocampus,ventral preorbital cortex (PRO), the sublenticular substantia innominata, the anterior amygdaloid area, the posterolateral cortical amygdaloid nucleus (PLCO) and the amygdalo-piriform transition area and in the lateral entorhinal cortex. In the contralateral side, labeled neurons were found in all parts of AON with heavy labeling of AONe. Labeled cells were also found in the ventral PRO, LT, and in LOT whereas fewer neurons were also found in the frontal PIR. In the diencephalon and brain stem tracer labeled neurons were observed in the dorsal, perifornical, mamillo-infundibular and lateral hypothalamic areas as well as in locus coeruleus and the dorsal and medial rapne nuclei. When NAS was used as a retrograde tracer for showing zinc-rich bulbopetal projections, this marker was detected only in the paleo and periallocortical formations listed above and in PLCO and LOT. No selenite-labeled neurons could be identified in HDB, VDB or any of the diencephalic and tegmental cell groups labeled with HRP or FT

367.14

ANATOMY OF THE MITRAL/TUFTED CELLS OF THE ACCESSORY OLFACTORY BULB IN THE ADULT RAT. S. Takami* and P.P.C. Graziadei. Dept. Biol. Sci., Florida State Univ., Tallahassee, FL 32306-3050.

The output neurons of the accessory olfactory bulb (AOB) have been recognized by HRP retrograde labeling injected into the medial amygdaloid nucleus. Their soma is located in two layers, the glomerular and the external plexiform layers (GL) and (EPL). Here we report on the morphology of the output neurons whose soma is located in the EPL. A rapid Golgi method was used and the neurons studied in serial sections of the AOB. These neurons have soma of 10-25 microns in diameter and apical long dendrites branching in the GL. The neurons can be classified according to the number of their apical dendrites; the larger have as many as 4 dendrites and the smaller only 1. The dendrites expand into the glomerular domain either by tufts or by loop-like, previously unreported, baskets. Not only each dendritic arbolization of the same neuron terminates in different glomeruli, but also each tuft or basket occupies either a relatively small volume of each glomerulus or most of the glomerular volume. Thus each glomerulus may be composed of many compartments provided by different dendrites from different neurons. This arrangement is likely to have some significance in the coding mechanism of the vomeronasal system. Supported by grant NIH NS 20699 to PPCG.

367.15

TUFTED CELLS OF THE INTRA- AND INTERBULBAR ASSOCIATIONAL PATHWAYS ARE IMMUNOPOSITIVE FOR CHOLECYSTOKININ OCTAPEPTIDE. T. Jang, T.A. Schoenfeld, R.M. Kream*, and F. Macrides. Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545, Tufts University Schools of Medicine, Boston, MA 02111.

Previous studies have shown that two distinct populations of tufted cells in the main olfactory bulb (MOB) of hamsters participate in topographically organized associational pathways. One population mediates point-to-point interconnections between the left and right sides of each MOB. The axons of tufted cells in this intrabulbar pathway travel in the internal plexiform layer (IPL) and terminate in this layer. The other population of tufted cells projects to the superficial plexiform layer of pars externa (pE) of the anterior olfactory nucleus. The neurons of pE in turn project topographically via the anterior commissure to the contralateral MOB. This interbulbar pathway interconnects homotopic longitudinal strips in the left and right MOBs. The somata of both populations are distributed in regions of the external plexiform layer (EPL) that contain substance P (SP), tyrosine hydroxylase (TH) or cholecystokinin octapeptide (CCK) immunopositive tufted cells. Combined retrograde and immunohistochemical analyses, using punctate injections of rhodamine impregnated latex microspheres and the avidin-biotin complex (ABC) or indirect (fluorescein) immunofluorescence procedure, were conducted to assess possible correspondences between these connectionally versus immunohistochemically defined populations of tufted cells. Both populations of associational tufted cells were found to contain CCK-like immunoreactivity. The projections of the SP and TH positive tufted cells appear to be predominantly intrabulbar and restricted to the same side of the MOB as the parent somata. The ultrastructural features of the CCK positive somata and dendrites in the superficial layers and processes in the IPL were examined with a modification of the ABC method followed by conventional electron microscopic procedures. The CCK positive tufted cells receive axo-dendritic peripheral input in the glomeruli and make both pre- and postsynaptic dendro-dendritic contacts in the EPL and interglomerular regions. Their apparent axon terminals in the IPL synapse on immunonegative dendrites. The CCK-like immunoreactivity in terminals is associated predominantly with highly granulated or dense core vesicles.

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367.16

EFFECTS OF NMDA AND NON-NMDA RECEPTOR ANTAGONISTS ON SYNAPTIC TRANSMISSION IN PIRIFORM CORTEX. J. Larson*, M.W. Jung, and G. Lynch. CNLM, Univ. of Calif., Irvine, CA 92717

Study of the pharmacology of synaptic transmission in piriform cortex has been hampered by a lack of potent and selective antagonists for excitatory amino acid receptors. Here we report effects of the non-NMDA antagonist, DNQX, and the NMDA antagonist, D-AP5, on excitatory transmission in this region.

Slices of rat piriform cortex cut perpendicular to the cortical surface were maintained in vitro. Population EPSPs were recorded in response to lateral olfactory tract (LOT) stimulation or activation of associational (ASSN) fibers.

DNQX (20 uM) completely abolished EPSPs generated by LOT or ASSN inputs. At less than 0.1 uM, DNQX was ineffective and 20 uM was sufficient to completely block either response. The dose-response curves for antagonism of both LOT and ASSN responses were similar with a half-maximal effect at about 2-5 uM. D-AP5 (50 uM) had very little effect on LOT or ASSN responses in medium containing 2.5 mM Mg. Perfusion of Mg-free medium caused a small increase in the peak amplitude of both responses as well as a large prolongation of the decay of EPSPs. D-AP5 reduced the late component of the response with little effect on the increase in EPSP amplitude. In Mg-free medium, application of 20 uM DNQX did not abolish either synaptic response; however, the time course of the remaining EPSP was much slower than the typical response. These responses most likely represent EPSPs mediated solely by NMDA receptors.

These results indicate that low frequency transmission at LOT and ASSN synapses are mediated by non-NMDA (quisqualate/kainate) receptors. However, NMDA receptors in both systems are activated by transmitter release in the absence of extracellular Mg. Recent data (Jung, et al., this meeting) also indicate that NMDA receptors participate in induction of LTP at these synapses. (Supported by ONR #N00014-89-J-1255.)

367.17

DO NMDA RECEPTORS PARTICIPATE IN NORMAL SYNAPTIC TRANSMISSION IN THE PIRIFORM CORTEX? D. K. Patneau and J. S. Stripling. Department of Psychology, University of Arkansas, Fayetteville, AR 72701.

Although the role of excitatory amino acids (EAA) in synaptic transmission in the piriform cortex (PC) slice has been extensively investigated, the effects of EAA antagonists on PC function in vivo have not previously been explored. This study examined the role of NMDA receptors in PC synaptic transmission utilizing the non-competitive NMDA antagonist ketamine and evoked potential techniques in male Long-Evans rats with chronically implanted electrodes.

The effects of a sub-anesthetic dose of ketamine administered by slow i.v. infusion on the PC potential evoked by olfactory bulb stimulation was monitored continuously during and at 5 and 15 min following ketamine administration. Analysis primarily focused on distinguishing between ketamine's effects on the monosynaptic component of the population EPSP, reflecting activation of PC pyramidal cells via the lateral olfactory tract (LOT) and the disynaptic component, reflecting activation via the PC association fiber system.

Ketamine had relatively specific effects on the disynaptic component of the population EPSP, reducing its amplitude by over 60%. Other effects included a small, but significant, decrease in the monosynaptic component of the population EPSP. These findings are consistent with the interpretation that NMDA receptors in the PC are primarily found at association fiber synapses, but the possibility that ketamine's effects on the disynaptic EPSP were due to decreased cell excitability could not be excluded.

367.18

AFFERENT AND ASSOCIATION FIBER DIFFERENCES IN SHORT-TERM POTENTIATION IN RAT PIRIFORM CORTEX. M.E. Hasselmo and J.M. Bower, Div. of Biology 216-76, Caltech, Pasadena, CA, 91125.

For several years, we have been developing a structurally based model of the piriform cortex as a self-organizing associative network for the classification of olfactory stimuli (Wilson and Bower, 1988, Neural information processing systems, D. Anderson, ed. AIP Press, N.Y., pp. 114-126). We have used this model to explore the possible functional relationships between the afferent input to the cortex and the extensive system of intrinsic association fiber connections. The computer modeling suggests interesting associative properties result if the excitatory synapses associated with these two fiber systems have different forms of modification in response to persistent activity .

To test this possibility, intracellular recordings of 46 pyramidal cells in 25 rat brain slices were made using standard procedures. Afferent and association fiber systems were differentially stimulated with electrodes placed in layer 1a or layer 1b respectively. To quantify synapse modifiability, the height of PSPs to paired pulse stimulation (100ms interval) was averaged over a 50 second period before and after a set of 10 stimulus trains (10 pulses each, 20 Hz).

The results indicate that synapses associated with these two fiber systems are influenced differently by trains of stimuli. Specifically, while long-term changes in synaptic strength appeared in very few cells, short-term potentiation characteristics differed significantly on a paired t-test ($T=5.5$, $df=23$, $p<.00001$). Over the 50 second post-train period, Layer 1b showed an average potentiation of 23.2% in the first pulse of the stimulus pairs, while layer 1a showed an average response depression of 10.9%. Layer 1b potentiation decayed with an average time constant of 72 msecs and was decreased by the NMDA receptor antagonist 2-amino-5-phosphonovaleric acid.

Supported by ONR Contract N00014-88-K-0513 and NIH postdoctoral training grant NS07251.

367.19

APV DEPENDENT INDUCTION OF LONG TERM POTENTIATION IN PIRIFORM (OLFACTORY) CORTEX SLICES. E.D.Kanter and L.B.Haberly. Neurosci. Training Prog. and Dept. of Anatomy, Univ. of Wisc., Madison, WI 53706.

Several groups have reported that 1sec duration, 100Hz shock trains that induce a robust long-term potentiation (LTP) in hippocampus, induce only a brief post-tetanic potentiation of monosynaptic EPSPs in piriform (olfactory) cortex in vivo and in vitro. However, Roman et al (Br. Res. 418:221) recently reported that LTP of afferent fiber responses in piriform cortex can be induced by brief (4 shock) 100 Hz bursts repeated at the theta frequency when animals are trained to respond to this stimulation. We report here that this theta-pattern stimulation paradigm induces LTP in both afferent and association fiber systems in slices of piriform cortex maintained in vitro.

Extracellular field potentials were recorded in layer Ia (afferent fiber layer) and layer Ib (association fiber layer) of slices of rat piriform cortex cut perpendicular to the surface. Stimulus trains and test pulses were delivered through tungsten microelectrodes placed under direct vision in either layer Ia or layer Ib. Trains for induction of LTP consisted of 10 bursts of 4 pulses at 100Hz delivered at 160ms intervals.

Theta-pattern stimulation induced increases in strength of EPSPs evoked by afferent fiber stimulation by up to 30% (typically 10-20%) and EPSPs evoked by association fiber stimulation by up to 35% (typically 20-30%). The 2 pathways could be independently potentiated in the same slice. Potentiation of the afferent fiber response typically decayed at a slow rate (time constant much greater than 1h); potentiation of the association fiber response decayed at a faster rate (typical time constant of 30m to 1h). Addition of 30μm DL-2-amino-5-phosphonovalerate (APV) to the bath blocked induction of LTP in both pathways. LTP could be induced after washout of the APV. Supported by grant NS 19865 to LBH.

367.20

INDUCTION OF SELECTIVE LONG-TERM POTENTIATION IN THE PIRIFORM CORTEX REQUIRES AN INCUBATION PERIOD. J. S. Stripling and D. K. Patneau. Dept. of Psychology, University of Arkansas, Fayetteville, AR 72701.

Repeated high-frequency stimulation that directly activates association fibers in the piriform cortex (PC) selectively potentiates late components of potentials evoked in the PC (Soc. Neurosci. Abstr. 14: 1189, 1988). In previous research we have seen potentiation appear in the PC only after two or more sessions of high-frequency stimulation spaced 24 hr apart. This could be due either to an incubation period necessary for the establishment of potentiation, or to a requirement for more stimulation than is provided by 1 stimulation treatment.

To address this issue, male Long-Evans rats received repeated high-frequency stimulation of PC association fibers in 4 sessions separated by intervals of 1, 4, or 24 hr. Animals receiving stimulation at 24-hr intervals exhibited significant potentiation 5 min and 24 hr after the last stimulation. Animals receiving stimulation at 4-hr intervals showed potentiation only 5 min after the last stimulation, and animals in the 1-hr group showed no significant potentiation at either time. These results suggest that the mechanisms underlying the induction of potentiation in the PC require several hours to be established, and that additional stimulation during this time does not substantially accelerate the process. (Supported by NSF Grant BNS 85-19700.)

CHEMICAL SENSES: GUSTATORY PATHWAYS

368.1

IN VITRO RECORDING FROM SLICES OF THE VAGAL GUSTATORY LOBE OF GOLDFISH. T.E. Finger & T.V. Dunwiddie. Dept. Cell. & Struct. Biology and Dept. Pharmacology, U. Colo. Med. Sch., Denver, CO 80262.

The vagal lobe of goldfish is a laminated evagination of the midmedulla which contains the reflex circuitry required to carry out intraoral food sorting (Finger; *Brain, Behav. Evol. 1988*). Primary gustatory afferents course through the deep fiber layer to terminate in superficial layers while motoneurons innervating the orobranchial apparatus lie in the deep layers. The vagal lobe was removed from cold-anaesthetized goldfish and sliced on a tissue-chopper at 500 μm and placed in artificial CSF. The tissue slices then were placed in a slice recording chamber and permitted to recover for at least 30 minutes prior to recording. A bipolar stimulating electrode was used to stimulate small fascicles in the primary afferent fiber layer; a glass pipette recording electrode was positioned under visual control at different loci in the vagal lobe. Clear evoked population responses were observed at restricted locations in the vagal lobe at 4 msec following stimulation. This negative-going response was recorded in layers 6-8 -- the principal layers of termination of the primary sensory fibers. The maximal response was recordable only for a distance of 0.5 - 0.7 mm across the surface of the lobe confirming anatomical results showing that each fascicle of primary afferent fibers ramifies only within a limited expanse of the lobe. Further, the waveform of the response was labile being sensitive to both the interstimulus interval and voltage applied to the stimulating electrodes. In addition, this response disappeared upon removal of calcium from the bathing solution and reappeared following the reintroduction of calcium. Thus this negative wave appears to reflect synaptic currents and not simply the incoming volley in the primary afferent fibers. Future studies will explore the neurotransmitters involved in the transmission of the primary gustatory inputs.

368.2

CONVERGENCE OF GASTRIC AND GUSTATORY INPUTS IN THE MEDULLA OF THE CHANNEL CATFISH, *ICTALURUS PUNCTATUS*. J.S. Kanwal and T.E. Finger, Dept. of Cellular & Structural Biology, Univ. of Colorado Sch. Med., Denver, CO 80262.

Coelomic (general) visceral inputs ascend through the CNS in parallel to those of the gustatory system. Behavioral and physiological data suggest that gustatory and gastrointestinal inputs may, however, interact at one or more sites within the brain. While "downstream" reflex-type pathways exist for the gustatory modulation of vagal motor output, a locus in catfish where gastric and gustatory inputs converge is unknown.

Electrophysiological recordings were obtained from ventral medullary regions in the vicinity of the facial motor nucleus. HRP-filled, glass microelectrodes (impedance < 1 megohm) were utilized to record in catfish in which stimulating electrodes had been implanted in the stomach. Once the recording electrode encountered neurons responsive to taste and/or tactile stimulation, the stomach was stimulated electrically. If the neurons responded both to gustatory and to electrical stimulation, the recording site was marked with an iontophoretic injection of HRP. Single unit analysis indicated the presence of at least two types of neurons which responded both to chemical stimulation of the oral cavity and to electrical stimulation of the stomach. One neuron type exhibited a spontaneously rhythmic pattern of activity (average firing rate of approx. 10 spikes/s), while the other had a relatively low rate of spontaneous activity (approx. 1 spike/s). Subsequent anatomical analysis confirmed that the gastrogustatory convergence site is situated in the dorsolateral reticular formation at the level of the facial motor nucleus. Several small (≈30 μm) and a few large (≈70 μm) neurons were labeled at the recording site.

368.3

ELECTRON MICROSCOPIC OBSERVATIONS OF GLOSSOPHARYNGEAL AFFERENT TERMINALS IN THE HAMSTER SOLITARY NUCLEUS. S. K. Brining, M. N. Lehman, and D. V. Smith. Depts. Anat. & Cell Biol., Otolaryngol. & Maxillofac. Surg., Univ. Cincinnati Coll. Med., Cincinnati, OH 45267.

Light microscopic investigations in several species show rostrocaudally overlapping terminal fields within the nucleus of the solitary tract (NTS) from gustatory fibers in cranial nerves VII, IX and X. In the present study, afferent terminals of the IXth nerve within the NTS were examined with electron microscopy (EM). Crystalline HRP (Sigma Type VI) was placed in the region of the petrosal ganglion of the IXth nerve. After a 2-day survival, hamsters were perfused with 1% paraformaldehyde, 1.25% glutaraldehyde and the brains removed and processed for HRP histochemistry using DAB as the chromagen. Periganglionic placement of HRP resulted in dense labeling of afferent fibers and terminal boutons within the NTS, clearly observable at the light microscopic level. At the EM level, HRP-labeled myelinated and unmyelinated axonal processes were observed in the vicinity of the NTS. Labeled *en passant* and terminal boutons contained small, round, clear vesicles (20 - 40 nm diam) and formed asymmetrical synapses onto small, unlabeled dendritic processes within the NTS. Labelled terminal boutons often had a scalloped appearance due to indentation by surrounding, unlabeled processes. Occasionally, both labeled and unlabeled terminals were found synapsing upon the same dendrite. We are currently in the process of quantifying these observations so that comparisons can be made with the morphology of VIIth nerve afferent terminals (Whitehead, M. C., *J. Comp. Neurol. 244*: 72, 1986). Supported by NS23524 to D.V.S.

368.4

INTRINSIC CHARACTERISTICS OF GUSTATORY NEURONS IN RAT SOLITARY NUCLEUS. R.M. Bradley and R.D. Sweazey. Sch. Dent., Univ. Michigan, Ann Arbor, MI 48109.

To investigate the intrinsic membrane properties of second order gustatory neurons in the nucleus of the solitary tract (NTS), we are using an in vitro slice preparation of the rat brainstem.

Intracellular recordings have been made from 25 NTS neurons. These neurons had stable resting potentials, from -37 to -70 mV ($\bar{x}$ = 52.5 ± 8.7 SD), membrane resistances of at least 50 megohms, and little or no spontaneous activity. Mean membrane resistance was 81.6 ± 28.5 megohms and membrane time constants were 9 msec or less. Action potential amplitudes ranged from 38 to 80 mV ($\bar{x}$ = 60.6 ± 12.9) and spike duration was usually 2 msec or less. Most neurons also exhibited a hyperpolarization after each action potential. These hyperpolarizations ranged from 1 to 16 mV ($\bar{x}$ = 5.5 ± 4.7). Based on responses to an intracellular injection of 0.5 nA, 100 msec depolarizing current pulse, the neurons could be separated into two groups. The first group, which contained most of the neurons, responded with a burst of 4-5 action potentials, whereas the second group exhibited a burst of 12-14 action potentials. These results represent the first intracellular recordings from rostral NTS. Compared to non-gustatory neurons located in caudal NTS, neurons in gustatory NTS are less spontaneously active, are not intrinsically rhythmic and exhibit fewer action potentials to a depolarizing current pulse.

Supported by N.I.H. Grant NS21764.

368.5

Morphology of NTS Cells in Sodium Deprived and 'Recovered' Rats. C. Tessitore King* and David L. Hill. Dept. Psychology, Univ.Virginia, Charlottesville, VA

Sodium deprivation instituted early in prenatal development produces suppressed responses of the chorda tympani nerve (CT) to lingual applications of NaCl. Taste responses of rats fed a sodium replete diet for at least 15 days after deprivation 'recover' to control levels. HRP experiments have revealed that CT terminal fields in deprived rats are the same size as that of controls; however, a rearrangement of the field within the NTS occurs. In 'recovered' rats, the terminal field area is 3x that of controls and the deprivation-induced pattern of innervation persists. Golgi-Cox filled cells from control, deprived and 'recovered' rats were examined. Preliminary data suggest that no differences in soma area or in the number of 1^o dendrites are apparent in any cell type across experimental groups. Fusiform cells of deprived rats show an increase in length of 1^o dendrites, number and length of 2^o dendrites and an increase in the percentage of cells possessing 3^o dendrites. Similar changes were found in multipolar cells of deprived rats except no changes were seen in 1^o dendrites. Ovoid cells were similar in deprived and control rats. In most cases, cell morphology in 'recovered' rats is intermediate to that of control and deprived rats. These results indicate that sodium deprivation and 'recovery' differentially affect the morphology of taste responsive cells in the NTS.

Supported by NIH grants NS24741 and NS01215.

368.6

ORGANIZATION OF TASTE-RESPONSIVE ACTIVITY IN THE PARABRACHIAL NUCLEUS IN THE GOLDEN HAMSTER. C.B. HALSELL and M.E. FRANK (SPON: M. McPheeters) Center for Neurological Sciences/Dept. of BioStructure & Function, UCONN Health Center, Farmington CT. 06032.

The parabrachial nucleus (PBN) is the obligatory third-order relay for the ascending gustatory system in rodents. Only part of the PBN, the pontine taste area (PTA), is responsive to taste stimuli. Although PTA physiology has been examined in hamsters, its location has not been correlated with PBN anatomy. Multi-unit taste-responsive activity was mapped in the PBN and recording sites marked by iontophoretically deposited horseradish peroxidase (HRP). Taste stimuli (0.03 M NaCl, 0.1 M KCl, 0.1 M sucrose and a mixture) were applied to the anterior tongue. The PBN is composed of six subdivisions based on cytoarchitectural criteria and cytochrome oxidase activity. The PTA for the anterior tongue is located within only two of these subdivisions, the medial and ventral-lateral. HRP injections into the rostral pole of the nucleus of the solitary tract preferentially labels the PTA. Connections between the PTA and a physiologically defined gustatory cortical area are being examined. These results suggest that there is a well-defined area of gustatory function in the PBN and provide information required for tract-tracing studies aimed at establishing gustatory, as distinct from visceral, pathways. NIH Grant #NS16993

368.7

PARABRACHIAL NEURAL ACTIVITY DURING LICKING OF SAPID STIMULI BY RATS. H. Nishijo* and R. Norgren Dept. of Behavioral Science, College of Medicine, The Pennsylvania State University, Hershey, PA 17033.

Previously we reported pontine taste responses in behaving rats (Neurosci. Abst.14: 473.4, 1988). In the present study pontine gustatory neural responses were compared when rats ingested fluids by licking or by infusion via an intraoral cannula. Tongue movements were monitored from genioglossus EMG electrodes and tongue contact by a lickometer. A total of 51 single neurons was recorded from the parabrachial nuclei of 3 rats. Of these, 39 neurons were tested during both licking and intraoral infusion of sapid stimuli. When compared with distilled water, 35 responded differentially to one or more of the 4 sapid chemicals. The remainder responded equivalently to both water and taste stimuli. Of the taste neurons, 10 responded best to sucrose (0.3M), 24 to NaCl (0.1M), and 1 to citric acid (0.01M). The best-stimulus categories remained the same regardless of the route of fluid delivery. The side-band responsiveness of some neurons, however, did vary as a function of the method of ingestion. When the rats were licking stimuli, 9 taste neurons responded significantly to only one sapid chemical, but were more broadly tuned during intraoral infusions. Conversely, 3 taste neurons that responded specifically during intraoral infusions were not specific when the animal licked the same stimuli. These results suggest that a taste neuron's best-stimulus category is characteristic, but that second order responses are more variable. Supported by PHS grants NS 20397 and MH 00653.

368.8

CODING OF AMINO ACIDS IN THE GUSTATORY CORTEX OF THE ALERT CYNOMOLGUS MONKEY. T.R. Scott, C.R. Plata-Salaman and V.L. Smith*. Dept. Psychol., Univ. Delaware, Newark DE 19716.

We have completed analyses of gustatory quality and intensity coding in the cortex of the alert monkey based on responses to an array of basic taste stimuli (Plata-Salaman, Scott and Smith, SNA, 1989; Scott and Smith, SNA, 1988). The present study extends this line of research to include stimuli of greater molecular complexity and biological relevance: the amino acids. Chemicals were deionized water, fruit juice, the four prototypical taste stimuli (glucose, NaCl, HCl, quinine) and 16 amino acids selected for their nutritional significance and for the availability of published psychophysical data on their perceived qualities.

Response profiles to the stimulus array were determined for 54 gustatory neurons. The mean spontaneous rate of these cells was 2.4 spikes/s; the mean breadth-of-tuning coefficient was 0.71. Amino acids most effective in activating cortical taste neurons were MSG, PRO and GLY. The least effective stimuli were TYR, THR, PHE and TRP. An analysis of taste quality indicated that the clearest distinction was between sweet and non-sweet stimuli. Among non-sweet chemicals, amino acids were either insipid (allied with water) or sour-bitter (related to HCl and quinine). These findings conform well with electrophysiological results from the peripheral taste nerves of rats and with human psychophysical data.

Supported by NSF grant BNS 8514202.

368.9

INTENSITY CODING IN THE GUSTATORY CORTEX OF THE ALERT CYNOMOLGUS MONKEY. C.R. Plata-Salaman, T.R. Scott and V.L. Smith*. Dept. Psychol., Univ. Delaware, Newark, DE 19716.

Single neuron responses to taste stimuli in the primary gustatory cortex of two alert cynomolgus monkeys were analyzed. Chemicals were deionized water, fruit juice and several concentrations of the four prototypical taste stimuli: 10^{-3} to 1.0 M glucose, 10^{-3} to 1.0 M NaCl, 10^{-4} to $3X10^{-2}$ M HCl and 10^{-5} to $3X10^{-3}$ M quinine HCl. Responses could be recorded from a cortical area that extended 4.5 mm A-P, 3.0 mm M-L and 6.0 mm D-V. Taste-responsive neurons constituted 3.7% of the cells tested. Intensity-response functions were determined for 62 gustatory neurons. Thresholds were, glucose: 10^{-1} M, NaCl: 10^{-2} M, HCl: 10^{-3} and quinine: 10^{-4} M. These are similar to psychophysical thresholds determined in humans. Mean discharge rate was a direct function of stimulus concentration for glucose, NaCl and quinine. Other characteristics of cortical taste neurons were: 1) breadth of responsiveness: this was moderate compared to taste cells at other relays. The mean breadth-of-tuning coefficient was 0.65. 2) Topographic organization: there was no clear evidence for a topographic distribution of response either by stimulus quality or intensity. 3) Neuron types: an analysis of response profiles permitted most taste cells to be assigned to a small number of groups, each statistically independent but within which the constituent neurons were not identical. The monkey appears to provide an appropriate neural model for human intensity perception.

Supported by NSF grant BNS 8514202.

DEGENERATIVE DISEASE: OTHER I

369.1

CLINICAL AND PATHOLOGICAL CORRELATES OF LOCUS COERULEUS PATHOLOGY IN PARKINSON'S DISEASE AND HUNTINGTON'S DISEASE. R.M. Zweig*, C.A. Ross, J.C. Hedreen, C. Peyser*, J.E. Cardillo*, M. Cohen*, S.E. Folstein*, and D.L. Price (SPON: P.J. Whitehouse). University of Nevada, Johns Hopkins, and Case Western Reserve Schools of Medicine.

Numbers of neurons at selected anatomical levels of the locus coeruleus (LC) were counted in 11 patients with idiopathic Parkinson's disease (PD) without concurrent Alzheimer's disease (AD), 16 patients with Huntington's disease (HD), and age-matched controls. In PD, counts at rostral, middle, and caudal levels were 47%, 37%, and 32% of control values, respectively. Unlike results from AD patients, level-to-level differences were not significant. Neuronal counts in PD correlated negatively with age ($p<.05$ rostrally). Individuals with dementia (determined from blinded chart reviews) had significantly fewer neurons at all levels than those without ($p<.02$). In HD, neuronal counts were similar to controls; however, patients with severe dementia (prospective mini mental state scores <3) had significantly fewer neurons than patients with mild or no dementia (scores >15) at rostral and middle levels ($p<.02$). Rostral LC counts also correlated negatively with impairment of voluntary motor function (scored prospectively) and neostriatal atrophy (macroscopic scoring) in HD patients ($p<.05$). Our findings may reflect relationships between dementia and involvement of the LC or of other structures in which severity of pathology correlates with LC pathology.

369.2

MEMORY DYSFUNCTION AS A CONSEQUENCE OF HYPERTENSION AND HIGH CHOLESTEROL DIET IN THE MONKEY M.B. Moss, T. Kemper*, D.L. Rosene, and W. Hollander*. Boston University School of Medicine, Boston, MA 02118.

As part of the development of a primate model of cerebrovascular disease, the behavioral and neuropathological effects of hypertension, alone or in combination with a hypercholesterolemic diet, were studied in the cynomolgous monkey. An assessment of visual recognition memory using the delayed non-matching to sample task (DNMS) was performed in monkeys that received either coarctation of the thoracic aorta alone (HYP), or in combination with maintenance on an atherogenic diet (HYP + ATH) for 12 months prior to testing. Post-treatment performance on the DNMS task was compared to that of operated contol animals maintained on an atherogenic diet (ATH) and operated control animals maintained on a normal diet (OC) for the same pretesting period. Monkeys with HYP + ATH were significantly impaired as a group in the acquisition of the DNMS task. In contrast, monkeys in the ATH group were unimpaired on the task. Though not impaired as a group, individual monkeys with HYP alone obtained elevated scores relative to monkeys in either the OC or ATH groups. Initial histological assessment revealed multifocal neuropathologies in monkeys in the HYP+ATH and HYP groups, including perivascular hemorrhage, mineral deposits, demyelination and ischemic infarction. The data suggest that hypertension and a high cholesterol diet can act synergistically to produce both CNS damage and impaired higher cortical function. (Supported by Grant HL13262)

369.3

DEFICITS IN PROCEDURAL LEARNING IN AN ANIMAL MODEL OF HUNTINGTON'S DISEASE. J. E. Kelsey and M. M. Sault*. Dept. Psychology, Bates College, Lewiston, ME 04240.

Several reports suggest that patients with Huntington's Disease (HD) are deficient in acquiring skill tasks that require procedural learning. It has also been argued that injection of the neurotoxin, quinolinic acid (QA), into the striatum of rats can reproduce the major histological and neurochemical changes observed in HD. The intent of this study was to determine if QA injections produce deficits in procedural learning. In Experiment 1, ten rats injected with 150 nm QA on each side of the striatum were initially deficient in finding a visible platform in a Morris water tank from a single starting position. Indicating that this deficit was not simply the reflection of a motor deficit that caused them to swim more slowly, Experiment 2 demonstrated that these rats were also deficient in acquiring a position habit in a Y maze. Three rats injected bilaterally with 75 nm were not deficient in either task, indicating that the effects were dose dependent. Thus, this study indicates that a neurotoxin that produces the major histological and neurochemical features of HD also produces one of the major behavioral symptoms, a deficit in procedural learning.

369.4

COGNITIVE DEFICITS IN MOTOR NEURON DISEASE. O.L. Lopez, F.J. Huff and A.J. Martinez. Univ. of Pittsburgh School of Medicin, Pittsburgh, PA 15213

We reviewed the clinical and neuropathological features of a 63 year old man with Motor Neuron Disease (MND) with progressive dementia and without parkinsonism. A battery of neuropsychological tests revealed that language, attention, orientation, visual scanning and problem solving were the cognitive areas affected. Neuropathological examination showed moderate astrocytosis and neuronal loss in the frontotemporal cortex, chiefly in the frontal lobes. Spongiform changes were seen in the upper layers of the temporal cortex. Minimal neuronal loss and astrocytosis were observed in the parietal and occipital lobes, caudate nuclei, putamen, thalamus and substantia nigra. Degenerative changes were seen in the corticospinal tract, spinal anterior horns and hypoglossal and vagus nuclei. This case offers evidence that cortical lesions associated with MND may produce dementia, not always clinically distinguishable from other cortical dementias, and with neuropsychological deficits maily in functions related to the frontal lobe.

369.5

NEUROMUSCULAR FATIGUE IN MULTIPLE SCLEROSIS PATIENTS. C. L. Rice*, F. Furbush*, T. Vollmer*, C. K. Thomas, and B. Bigland-Ritchie. John B. Pierce Foundation & Neurology Dept., Yale University, New Haven, CT 06519.

For MS patients, fatigue is the major symptom limiting physical performance. Contributing factors are being studied by methods used in normal subjects (Bigland-Ritchie et al, J. Appl. Physiol. 61:421, 1986). Preliminary results from one patient showed near normal strength in one leg for maximum voluntary quadriceps contractions (MVC), with complete twitch occlusion. For the other leg, the MVC force was reduced by about 50%, always with large superimposed twitches; but MVC force, calculated by extrapolation, was nearly normal. For both muscles, twitch contraction times and MVC motor unit (MU) firing rates were slow (83 ± 5ms & 14 ± 2Hz) compared to controls (71 ± 7ms & 24 ± 6Hz). Endurance times for repeated 50% MVCs were longer (10-12 min.) than normal (4-5 min.) and twitch occlusion, during brief test MVCs, showed little further decline. Similar results were seen in another less severely affected patient. Since objective signs of abnormal neuromuscular fatigue were absent, and the normal pain and stiffness after exhaustive exercise were not felt, these patients' symptoms may be mainly due to impaired sensory function. Slow muscle properties may result from chronic low frequency MU firing rates due to the limited capacity of demyelinated fibers to carry high rates (Bostock & Grafe, J. Physiol. 365:239, 1985).

Supported by the MS Society and USPHS grant HL 30062.

369.6

KINEMATIC ANALYSIS OF STEREOTYPIC FACIAL MOVEMENTS IN TARDIVE DYSKINESIA. R. E. A. van Emmerik*, R. L. Sprague* and K. M. Newell. Department of Kinesiology, University of Illinois at Urbana-Champaign, Urbana, IL 61801.

A method has been developed to digitize the space-time properties of abnormal stereotypic movements from videotape so that one may analyze the movement dynamics of stereotypic behaviors, such as abnormal lip and jaw movements. These data are presented graphically in representations of different dynamical control spaces to help understand the dynamical properties of stereotypic movements. This orientation provides a more sensitive measurement for the assessment of movement disorders and the basis for a physical account of the dynamics of tardive dyskinesia. Our preliminary data on lip, tongue and jaw motions of patients with tardive dyskinesia suggest: (1) that on drug withdrawal, the space-time characteristics of lip, tongue and jaw movements are highly coupled; and (2) that on re-instatement of psychotropic medication, this highly constrained behavior is not present, although it can be induced in the presence of a secondary task. It appears that tardive dyskinetic movements are consequences of a high degree of constraint imposed on the highly flexible motor apparatus limiting it to a few degrees of freedom that oscillate in a stable mode. Reinstatement of a psychotropic medication releases the ban on the motor apparatus and generates more independently controlled and adaptive behavior.

369.7

COGNITIVE DEFICITS IN NONINSULIN DEPENDENT DIABETICS. W. Lichty, Psychology Dept., Washington Univ., St. Louis, MO 63130

Cognitive performance was studied in 178 individuals ages 40-69y: 60 diabetics with NIDDM (noninsulin dependent diabetes) and 118 nondiabetics matched for age ($\underline{M}$=55y) and education ($\underline{M}$=13y). The cognitive tasks evaluated immediate memory (verbal span), long-term memory (paired-associates learning), reasoning (analogies), speed of processing (digit symbol substitution), perceptual identification (number comparison), and frequency coding. Diabetics were significantly impaired on measures of long-term memory, speed of processing, and perceptual identification, with the most severe deficit being on the memory task (M_D=19%, M_{ND}=32%). Performance of diabetics and nondiabetics was equivalent on measures of frequency coding, immediate memory, and reasoning. When the influence of cardiovascular problems was considered in additional analyses, none of the initial findings changed, thus cardiovascular problems do not account for the cognitive decline. Performance scores did not correlate with any diabetic variables such as glycemia, glycemic control, and disease duration. Although diabetics with NIDDM show task-specific, nonglobal cognitive alterations, the cause of the deficits remains to be elucidated. The possibility that CNS deterioration is involved needs to be investigated.

369.8

DIABETIC NEURONAL DISTROPHY OF THE GUT. A.M.Di Giulio, B.Tenconi*, R.La Croix*, P.Mantegazza*, F.Cattabeni& and A.Gorio&. Dept. of Medical Pharmacology, School of Medicine, Via Vanvitelli 32, & Inst. of Pharmacol. Sciences, School of Pharmacy, Via Balzaretti 9, Milano.

Experimental and clinical reports show that diabetes mellitus is associated to functional impairments of the nervous system affecting electrical conduction parameters as well as synaptic transduction mechanisms (see Abbracchio et al., same abstract volume). In the gut of diabetic animals the changes are gradual and develop much more slowly than in the motor and sensory peripheral nerves. 14 weeks following the induction of experimental diabetes noradrenaline hyperinnervates the duodenum, while in the jejunum and large intestine the amine levels appear significantly decreased. Also vasoactive intestinal polypeptide hyperinnervates the small intestine, while the levels of two intrinsic peptides, met-enkephalin and substance P, are markedly reduced throughout the small and large intestine. Serotonin does not appear to be affected in the gut of diabetic animals. The complex alterations involving the neuronal network innervating the gut might be responsible of the gastrointestinal dysfunctions present in the vast majority of diabetic patients.

369.9

RELATIONSHIP OF INSULIN-LIKE GROWTH FACTOR I GENE EXPRESSION TO DIABETIC NEUROPATHY. D.N. Ishii, D.M. Guertin*, G. Huitt* and L.R. Whalen* Physiology Dept., and Anatomy and Neurobiology Dept., Colorado State University, Ft. Collins, CO 80523.

Our previous studies indicate that insulin and insulin-like growth factors (IGFs) may act in concert to support neurite growth and survival in sensory and sympathetic neurons, which are cell populations afflicted in diabetic neuropathy. A decline in insulin activity alone may be insufficient to precipitate neuropathy. The hypothesis that IGF-I gene expression is reduced was tested in streptozotocin diabetic rats. IGF-I mRNA content was sharply decreased relative to total and poly(A)$^+$ RNA in diabetic liver and adrenal glands, whereas histone and tubulin mRNA content were not. The loss in IGF-I mRNAs in spinal cord after 1 and 2 weeks correlated with the early onset of conduction velocity deficits seen in spinal cord and peripheral nerves. Streptozotocin toxicity was not involved because insulin infusion opposed the decline in IGF-I mRNAs. These results support the interpretation that abnormal IGF-I gene expression may contribute to the syndrome of diabetic neuropathy. (Supported by NIH grant RO1 NS24327).

369.10

ALTERATIONS OF CENTRAL NERVOUS SYSTEM G-PROTEINS IN EXPERIMENTAL DIABETES. M.P. Abbracchio*, F. Cattabeni, M. Di Luca*, A.M. Di Giulio^, B. Tenconi*^ and A. Gorio (SPON.: M. Motta) Inst. Pharmacol. Sci., Sch. of Pharm., and ^Dept. Med. Pharmacol., Sch. of Med., Univ. of Milano, 20133 Italy.

A single alloxan injection to rats causes the development of experimental diabetes within a week. Besides the relatively well characterized peripheral neuropathy, both experimental and clinical data suggest that signal transduction mechanisms might also be altered in the CNS of diabetics. We have previously shown that in the c. striatum of alloxan-treated rats Gs-mediated processes (dopamine stimulation of membrane adenylate cyclase) are increased, whereas Gi/Go activity (measured as bromocriptine inhibition of adenylate cyclase) is significantly reduced.

In this study, the Gi/Go subpopulation of G-proteins was directly evaluated in several brain areas, including the retina, of diabetic rats by means of pertussis toxin induced ADP-ribosylation. ^{32}P-ADP ribose incorporation into retinal Gi/Go proteins was dramatically reduced; less marked decreases were also observed in the cortex, hippocampus and hypothalamus of diabetic rats. All together, these results suggest either a reduced expression or post-translational structural modifications of Gi/Go proteins in the CNS of diabetics; moreover retinal changes likely preceed similar brain alterations and might represent the earliest sign of diabetic encephalopathy.

369.11

AN ELECTRON MICROSCOPIC STUDY OF THE GRACILE NUCLEUS IN ALLOXAN-INDUCED DIABETIC RATS. S.S.W. Tay, & W.C. Wong, Department of Anatomy, National University of Singapore, Kent Ridge, Singapore 0511.

Alloxan-induced diabetic rats showed drastic changes in the gracile nucleus. At 3-7 days post-diabetes, both electron dense and electron lucent types of degenerating axons, axon terminals and dendrites were present. Degenerating axon terminals contained swollen mitochondria and clustered small spherical agranular vesicles. Degenerating dendrites contained swollen mitochondria, dilated rER, small vacuoles and randomized ribosomes. Some synaptic glomeruli formed by axon terminals and dendrites were also affected. Neuronal cell bodies appeared normal. At 3-6 months, degenerating neuronal cell bodies contained multigranular bodies, neurofilaments, tubulovesicular elements and swollen mitochondria. Macrophages and glial cells were actively removing degenerating neural elements. At 9-12 months, numerous freshly degenerating axons, axon terminals and dendrites were found. Several degenerating neuronal cell bodies were highly vacuolated and filled with amorphous materials. There appeared to be some neuronal cell death at later time intervals. It is concluded that alloxan-induced diabetes has a drastic and prolonged effect on the gracile nucleus.

369.12

BULBOSPINAL AXONAL DAMAGE PRECEDES HINDLIMB PARALYSIS IN RATS WITH EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS (EAE). G. Samathanam*, M. Wessendorf, R.M. Bowker and S.R. White. Dept. of VCAPP, Washington State University, Pullman, WA 99164-6520 and Dept. of Cell Biol. and Neuroanat., University of Minnesota, Minneapolis, MN 55455.

The autoimmune disease, EAE, is generally characterized as an inflammatory and demyelinating disease with relative sparing of axons. However, recent studies have noted that extensive damage to bulbospinal monoamine- and peptide-containing axons accompanies the hindlimb paralysis stage of EAE in Lewis rats. The present study investigated the time course of development of this axonal damage.

Male Lewis rats, inoculated for EAE, were selected for analysis at each of four stages in the development of clinical signs of disease: weight loss, flaccid tail, partial paralysis and complete hindlimb paralysis. Longitudinal spinal cord sections from rats in each group were stained with serotonin, tyrosine hydroxylase or pre-pro-$TRH_{160-169}$ antisera. By the flaccid tail stage of disease, multiple foci of markedly distorted, swollen axons were found that were positive for serotonin-, catecholamine- or pre-pro-$TRH_{160-169}$ like immunoreactivity. A few distorted axons were also found in sections from rats in the weight loss stage of EAE. We conclude that bulbospinal axonal damage precedes the most severe clinical signs of EAE and may contribute to the severity of clinical signs. (Supported by NS 24388).

369.13

PRESERVATION OF MOTOR NERVE FUNCTION DURING EARLY DEGENERATION BY PRETREATMENT WITH THE 21-AMINOSTEROID U74006F. P.A. Yonkers* and E.D. Hall (SPON: P. Ho). CNS Diseases Research, The Upjohn Co., Kalamazoo, MI 49001.

The effects of 5 days of pretreatment with the lipid peroxidation inhibitor U74006F have been examined on the rate of functional degeneration of cat soleus motor nerve terminals after axon section. Female cats were dosed for 5 days with either 6.0, 10.0 or 35.0 mg/kg of U74006F p.o. twice daily followed by unilateral sciatic nerve section at the hip level on day 5. On day 7, the bilateral in vivo soleus nerve muscle prep. was set up to assess the neuromuscular functional status of the 48 hr. degenerating soleus nerve terminals in comparison to the contralateral non-sectioned preparation. In untreated cats, the ratio of the nerve-evoked (0.4 Hz) contractile tension of the 48 hr. nerve-sectioned to that of the contralateral non-sectioned was only 52±8%. U74006F pretreatment produced a dose-related improvement with the 10.0 dose having the best effect; the ratio was 86±5% ($p<0.01$ vs. untreated). The maintenance of tetanic tension during a 10 sec period of 100 Hz nerve stimulation was also improved by the 10.0 dose from only 54.0±5.2% in untreated animals to 72.2±5.7 ($p<0.02$). These results show a preservation of motor nerve function during early degeneration by the anti-oxidant U74006F which suggests a role for lipid peroxidation in the anterograde degeneration process.

369.14

SPINAL CORD PROTEIN IN BOVINE CEREBROSPINAL FLUID RISES IN EXPERIMENTAL ALLERGIC ENCEPHALITIS. C.F. MacPherson and L. Maxie*. Dept. of Psychiatry, Univ. of Western Ontario, London, Ont. N6G 2K3, and Univ. of Guelph, Guelph, Ont.

The anti-encephalitogenic spinal cord protein (SCP) is a normal component of bovine and human cerebrospinal fluids (CSF) and sera. (Soc. Neurosci. Abstr. 1987 13, 1686). The CSF-SCP rose in some animals that developed experimental allergic encephalitis (EAE). To ascertain 1) if the elevated SCP levels in EAE could be confirmed, and 2) if the CSF-SCP/serum-SCP ratio might be useful to monitor human demyelinating diseases, EAE was induced in three Holstein heifers with two i.c. injections of 250 mg of bovine spinal cord in complete Freund's adjuvant. Cisternal CSF was obtained every 4th day and serum every 2nd day. SCP was measured by a competitive inhibition enzyme immunoassay (EIA) using a rabbit anti-BSCP antiserum and purified BSCP. The three heifers developed severe EAE from 7 to 11 days after the 2nd injection of BSC. The average CSF total protein (TP) was elevated twofold after signs of EAE appeared. The average CSF-SCP level before EAE developed was 47 ng/mL. After EAE developed, CSP rose to 108 ng/mL in one animal. The average serum-SCP was unchanged at 645 ng/mL.

369.15

EXTENSIVE ECTOPIC DENDRITE GROWTH IS FOUND ON CORTICAL PYRAMIDAL NEURONS IN A RECENTLY DISCOVERED PUTATIVE MODEL OF TYPE C NIEMANN-PICK DISEASE IN THE CAT. **A.C. Lowenthal* and S.U. Walkley (SPON: P. Model). Dept. Neuroscience, Albert Einstein College of Medicine, Bronx, NY**

A variety of neuronal storage diseases in man and animals have been found to be characterized by ectopic dendritic sprouting. Systematic studies evaluating newly discovered models of these diseases are being pursued as a means to determine if a common metabolic defect underlies this phenomenon. Golgi staining was performed on cerebral cortex of a 10-week old kitten with a partial sphingomyelinase deficiency, and elevated levels of cholesterol, glucosyl- and lactosylceramide, sphingomyelin, and GM2 and GM3 gangliosides identical to that observed in human Niemann-Pick disease type C (D.A. Wenger, et al., unpubl.). Golgi-impregnated pyramidal cells displayed widespread and prolific growth of axon hillock neurites (dendrites), ectopic somatic spine-like processes, and rarely, spiny meganeurites. Nonpyramidal cells were normal-appearing or possessed aspiny meganeurites. The degree of ectopic neurite growth on pyramidal neurons appeared to exceed that seen in all other storage diseases in cats at this age. (NS18804)

369.16

CEREBRAL CORTEX CULTURES FROM POSTNATAL GM2 GANGLIOSIDOSIS CATS. K. Dobrenis*, J. Becker* and M.C. Rattazzi* (SPON: J. Arezzo). Dept. of Pediatrics, North Shore Univ. Hosp. - Cornell Univ. Med. Coll., Manhasset,NY 11030

In order to test enzyme and gene replacement strategies for neurodegenerative lysosomal storage disorders and investigate pathogenetic mechanisms in Tay-Sachs and Sandhoff disease, we have developed methods for culturing cerebral cortex from 2-3 day cats with genetic GM2 gangliosidosis.

Tissue is digested in hyperosmotic solution with trypsin, hyaluronidase, chondroitinase ABC and DNase I, mechanically dissociated, and centrifuged through 4% BSA. Cells are plated on polylysine + collagen in a defined medium (neurons), plus low serum (mixed cell types), or on non-coated vessels in basal medium with high serum (astrocytes). GM2 ganglioside (GM2) storage was detectable by immunofluorescence by 4 days in vitro (DIV) in marker-identified neurons and astrocytes, as well as other cell types. At 120 DIV, GM2 was present in neuronal perikarya and processes, and in meganeurite-like structures, as seen in vivo. Initial observations on astrocytes suggest alterations in growth patterns and neuritogenic properties.

This is the first time GM2 gangliosidosis neurons have been maintained in long-term culture. Our findings indicate these cultures will be useful in our studies and suggest that astroglial abnormalities may be present in GM2 gangliosidosis. Supported by NS21404-04.

369.17

PHORBOL ESTER RECEPTORS IN CEREBRAL CORTEX OF CATS WITH GM1 GANGLIOSIDOSIS. G. Shanker and H. Baker*. Dept. of Comparative Medicine, Bowman Gray School of Medicine, Winston-Salem, NC 27103.

GM1 Gangliosidosis is an inherited disease of lysosomal metabolism which is characterized clinically by severe, progressive neurological dysfunction. Lysosphingolipids inhibit protein kinase C suggesting a possible pathogenetic role in these neurological diseases. We measured Phorbol ester binding in cerebral cortex of cats with GM1 gangliosidosis (mutant) compared with age and sex matched normal siblings. Cerebral cortex homogenates were prepared and the binding of [^{3}H] phorbol-12,13-dibutyrate was assayed by the method of Raizada, et al (Neurochemical Res, 13, 51, 1988.) Binding was found to be highly specific as well as time and concentration dependent in both normal and mutant brain, but binding was greater in mutants. Sphingosine inhibited binding to approximately the same extent in normal and mutant brain. Quantitation of binding sites and affinity are in progress. Supported by NIH grant NS 10967.

369.18

NEW EXPERIMENTAL THERAPEUTIC APPROACHES IN HEPATIC ENCEPHALOPATHY INDUCED IN RATS BY GALACTOSAMINE. M.Baraldi, P.Zanoli*,^M.L.Zeneroli*,"R.A.Fano*,"R.Fante*,"G.P.Trenti ni*.Chair of Pharmacology and Pharmacognosy, Sch. of Pharm., ^III Dep. of Medicine and "Morbid Anatomy, Sch. of Med., Modena University, Italy.

Hepatic encephalopathy (HE) seems to be the result of an imbalance between an increased inhibitory and a decreased excitatory neurotransmission since GABA-a receptor complex undergoes to a denervation supersensitivity phenomenon. This finding together with the described supersensitivity to sedatives administration of patients with HE prompted us to use benzodiazepine antagonists which counteract temporarelly HE both in animals and men. Degenerative processes have been described in glia and neurons of animals and man with HE. Peripheral toxins such as ammonia could interfere in synthesis and release of metabolic and neuronal pool of glutamate and GABA. Herein we report that there is an increase of glutamate binding sites in brain areas of rats in mild stage of HE galactosamine-induced while in the severe stage there is a decrease. Thought that excitatory aminoacids might play a functional role in the hyperexcitability present in the early stage of HE and a neurotoxic effect we infused intracerebrally the isosteric antagonist of glutamate receptors APH to rats with HE. This infusion however precipitated the rats in severe stage of coma. By the contrary the use of allosteric modulator GM1 associated with NGF seems to counteract the symptoms of HE.

DEGENERATIVE DISEASE: OTHER II

370.1

NIGRAL DOPAMINE TYPE 1 RECEPTORS ARE REDUCED IN HUNTINGTON'S DISEASE. F. Filloux, M.V. Wagster, S. Folstein, J. Hedreen, T. Dawson, J. Wamsley (SPON: T. Cook) Dept. Psychiat., Univ. Utah Sch. Med., SLC, UT 84132 and Neuropath. Lab and Dept. Neurol., Johns Hopkins Univ. Sch. Med., Baltimore, MD 21205.

Intrastriatal injection of quinolinic acid has been proposed as an animal model of Huntington's disease (HD). Such neurotoxic lesions of the caudate-putamen result in marked dopamine type 1 (D1) receptor losses in the injected nucleus as well as in the ipsilateral substantia nigra reticulata. In vitro receptor autoradiography was employed with post-mortem human brain to determine whether HD results in a similar loss of nigral D1 receptors. 14 micron, slide-mounted tissue sections of SNR from 7 patients dying of HD and from 6 non-neurological-diseased controls were incubated with [^{3}H]SCH 23390 in the presence of 5 μM serotonin to label D1 dopamine receptors and with [^{3}H]forskolin to label adenylate cyclase. Autoradiograms were quantitated by computerized microdensitometry. Nigral [^{3}H]SCH 23390 binding in HD brains was reduced to 29% of control values (3.79 fmoles/mg tissue in HD brains compared with 12.93 fmoles/mg tissue in controls, $p<0.001$). There was also a moderate correlation ($r=0.78$, $p<0.001$) between [^{3}H]SCH 23390 and [^{3}H]forskolin binding. The results support the interpretation that, in the human as in the rat, neuronal degeneration in caudate and putamen is accompanied by loss of presynaptic nigral D1 receptors, and at these D1 receptors are linked to adenylate cyclase.

370.2

ALTERNATION OR CHANGE IN SOMATOSTATIN AND CHOLINE ACETYL-TRANSFERASE IN RAT BRAIN FOLLOWING QUINOLINIC ACID ADMINISTRATION INTO EACH LATERAL VENTRICLE. H. Kaneda*, Z. Susel*, O. Shirakawa*, T.N. Chase, C.A. Tamminga, (SPON. M.D. Johnson). Maryland Psychiatric Research Center, University of Maryland, Baltimore, MD 21228; NINDS, NIH, Bethesda, MD.

Central neurodegeneration, with a pattern characteristic of Huntington's chorea or Alzheimer's disease, may result from heightened levels of endogenous quinolinic acid (QA). In attempt to link elevated CNS QA with one of the neurodegenerative diseases, we have analyzed SRIF content and CAT activity after QA administration to rats in vivo using a subchronic intraventricular infusion. Eighty ug QA was administered into each lateral ventricle for 14 days. The QA infusion resulted in a significant reduction in CAT activity in the striatum (4.2 $\pm$ 0.27 moles/min/ugprot (QA) VS 5.2 $\pm$ 0.16, $p<0.05$). Subchronic QA administration followed by 2 or 4 weeks of withdrawal did not produce any significant CAT reduction. A reduction in SRIF content was not present in any of the regions. We also studied 2-deoxy-glucose utilization using the same QA administration technique. Alternations in glucose utilization will be reported. These results provide little evidence that low dose infusions of QA produces prolonged neurotoxicity. Moreover, they suggest intraventricular QA produces CAT lesions and SRIF sparing, as has been previously found with intratissue injections, reminiscent of postmortem pathology in Huntington's, not Alzheimer's disease.

370.3

THE EFFECTS OF STRIATAL QUINOLINIC ACID LESIONS AND FETAL STRIATAL TRANSPLANTS ON NADPH-DIAPHORASE AND METABOLIC ACTIVITY. M.E. Ragozzino, E.M. Zubrycki, S.Y. Lu, A.B. Norman, M.T. Shipley and P.R. Sanberg, Div. Neurosci., Depts. Psychiat. and Anat., Univ. of Cinti. Col. Med.,Cinti.,OH 45267.

It was reported that striatal quinolinic acid (QA) lesions produced similar neurochemical changes seen in Huntington's disease, with sparing of somatostatin, neuropeptide Y and NADPH-diaphorase (NADPH-d) neurons (Beal et al., Nature 321:168-171, 1986). Other investigators were unable to demonstrate selective sparing of these neurons (Davies and Roberts., Nature 327:326-328, 1987). The present study investigated NADPH-d sparing and metabolic activity in long-term striatal QA lesions and fetal striatal transplants.

Rats received bilateral injections of 150 nmol of QA into each striata. One month following lesion, transplant animals were injected with 4 ul of Day 17 fetal striatal tissue. Eight months later, alternate striatal sections were examined by Nissl staining, cytochrome oxidase (CO) for determining metabolic activity, and NADPH-d histochemistry.

CO staining was absent in the striatum of animals receiving QA lesions. These areas of diminished CO activity corresponded to areas of neuronal loss. NADPH-d neurons were seen only in areas where metabolic activity resembled normal striatum. The transplants exhibited normal CO staining even when surrounded by areas lacking CO activity. NADPH-d neurons were present in the transplant.

These results do not support a long-term sparing of NADPH-d neurons ater QA lesions as suggested by other investigators. However, they do demonstrate that striatal transplants do contain NADPH-d neurons and are metabolically active over the long-term.

370.4

CHRONIC INJECTION OF L-PYROGLUTAMATE INTO RAT STRIATUM SELECTIVELY SPARES ASPINY II NEURONS: NEUROPATHOLOGY SIMILAR TO HUNTINGTON'S DISEASE. G.K. Rieke and J. Semenya*. Dept. of Anatomical Sciences, Meharry Med.College, Nashville, TN 37208.

The neurotoxic hypothesis for Huntington's disease (HD) suggests that endogenous compounds may produce the neuropathology in HD. Recent studies of postmortem HD brains revealed a selective sparing of NADPH diaphorase positive, somatostatin-like immunoreactive aspiny neurons in the striatum. Individual toxins with possible etiological roles in HD would be expected to produce similar lesions in the striatum of rats receiving intracerebral injections of the toxin. Since HD is a long onset progressive disease and neurons are thought to be exposed over time to elevated tissue levels of the toxin(s), the neurotoxic amino acid L-pyroglutamate (L-PGA) was injected into the striatum through an implanted cannula coupled to an Alzet mini-osmotic pump. The pumps were loaded with one of three concentrations of L-PGA: 10.5, 17.6 or 45.8 mg/ml, buffered to pH=7.1 (3-13x amount of L-PGA/gr wet weight rat forebrain). Histological examination of the striatum revealed that the peripheral spongiose region of the lesion contained dis-persed NADPH diaphorase positive, somatostatin-like immunoreactive neurons. Morphologically these cells are the aspiny II neurons with a large soma, several dendrites and an asymmetrically positioned nucleus. L-pyroglutamate, like quinolinic acid, is another endogenous neurotoxin with an etiological potential in HD. The potential role of L-PGA in HD in specific families is strengthened by occurrence of increased amounts of L-PGA in the plasma of family members with HD and some of their offspring "at-risk". The potential role of L-PGA in HD needs to be more thoroughly examined. Supported by Grant No. NSF BNS-8710184 and NIH MBRS 2S06RR08037.

370.5

QUINOLINIC ACID INCREASES SOMATOSTATIN mRNA AND PEPTIDE LEVELS IN CULTURED RAT CORTICAL NEURONS. S.C. Patel*, D.N. Papachristou* and Y.C. Patel* (SPON: H.H. Zingg). Fraser Labs, McGill University, Montreal, Quebec and Newington VAMC, CT.

Striatal atrophy in Huntington's disease (HD) is characterized by selective preservation of a subclass of neurons colocalizing NADPH-diaphorase, somatostatin (SS) and neuropeptide Y (NPY) which show 3-5 fold increases in immunoreactive SS (SS LI) and NPY content. Since quinolinic acid (QUIN) is produced in excess in HD striatum, and since experimental QUIN lesions show neuronal loss with sparing of NADPH-d/SS/NPY neurons, the excitotoxin has been implicated in the pathogenesis of HD. In the present study we determined whether QUIN (a NMDA receptor agonist) stimulates SS gene transcription in cultured cortical cells (which are rich in NADPH-d/SS/NPY neurons). Cultures of dispersed fetal rat cortical cells were exposed to QUIN (1 mM) (with or without the NMDA antagonist 2-APV, 0.5 mM) and glutamate (GLU, 0.5 mM). Cells were extracted for determination of SS LI (by RIA) and SS mRNA (by Northern analysis with a cRNA probe).

	SS-LI (% of Control)	SS mRNA (% of Control)
QUIN	165 ± 8*	254 ± 12*
2-APV	89 ± 6	105 ± 11
QUIN + 2-APV	81 ± 5	122 ± 18
GLU	113 ± 6	117 ± 15

Compared to control, QUIN induced significant (*$p < 0.01$) 1.6 and 2.5 fold increases in SS LI and SS mRNA accumulation respectively which were abolished by 2-APV. By contrast, GLU was without effect on SS LI and SS mRNA. These results demonstrate a QUIN-induced selective NMDA receptor-mediated stimulation of SS gene transcription and SS biosynthesis in vitro, and suggest a similar mechanism for the augmented striatal SS LI in HD.

370.6

CELLULAR COMPOSITION OF STRIATAL PATCH AND MATRIX COMPARTMENTS IN HUNTINGTON'S DISEASE. R.J. Ferrante, N.W. Kowall, E.P. Richardson, Jr.* Mass. General Hospital, Boston, MA

We have previously demonstrated that the patch-matrix pattern, a normal feature of striatal architecture, persists in Huntington's disease (HD) striatum. The pattern is altered, however, in that the matrix zone is significantly reduced in area, while the total area of the patches is unchanged as compared to contols. In addition we have found that there is a relative preservation of immunoreactive CCK terminal fibers in patches and a reduction in matrix cytochrome oxidase activity in HD. Using combined enzyme (NADPH-d and acetylcholinesterase) and routine (cresyl violet and hematoxylin) staining to identify patch and matrix and cell types, we examined the total cell density and numbers of neurons (N), astrocytes (A), and oligodendrocytes (O) within a 0.25 mm2 area in striatal patch and matrix compartments of 6 HD brains (grade 3) and 6 age-matched controls. The dorso-ventral extent of the caudate nucleus (CN) of stained sections was divided into 4 equal areas. Patch and matrix cell counts were made in each area. In the control striatum, the total number of patch and matrix cells (patch=133±12; matrix= 140±9) and their component percentages (patch N=64%, A=15%, O=21%; matrix N=69%, A= 13%, O=18%) were not significantly different. There were no dorso-ventral differences. In HD there were no significant differences between the total cell numbers and individual cell percentages within each of the four areas defined. There was, however, a marked ventro-dorsal gradient of neuronal loss (67%) and astrocyte (280%) and oligodendrocyte (200%) increase. The vulnerability of striatal neurons in HD is not dependent upon their placement within or out of patch and matrix compartments.

370.7

TRANSFORMING GROWTH FACTOR (TGF) I IMMUNOCYTOCHEMISTRY OF HUNTINGTON'S DISEASE STRIATUM. K. Harrington*, A.C.McKee and N.W.Kowall (SPON: B. A. Eckstein). Neurology Service, Mass. General Hospital, Boston MA 02114.

Transforming growth factors are polypeptides that induce the phenotypic transformation of normal cell lines. TGF I (or alpha) shares structural features with epidermal growth factor and is expressed during early fetal development. Recently, cells synthesizing TGF I mRNA have been localized in adult mouse striatum and immunoreactive fibers have been found in rodent globus pallidus. We examined the distribution of TGF I in human striatum using a commercial antibody against TGF I (Peninsula) and standard immunoperoxidase procedures. In normal striatum TGF I is distributed in a patchy pattern surrounded by a matrix of lower intensity staining which is more prominent dorsally. Individual immunoreactive neurons are not resolved. A dense plexus of terminal staining is seen in the external segment of the globus pallidus. In Huntington's disease striatum the patch-matrix pattern is lost and dystrophic subependymal fibers are seen. Rarely, single medium sized neurons are visualized. The terminal staining pattern persists in the globus pallidus. The pattern of TGF I immunostaining strongly resembles that of enkephalin. The functional significance of TGF I in the striatopallidal enkephalinergic projection system is unknown but our findings confirm that this system degenerates in Huntington's disease.

370.8

SELECTIVE SPARING OF NEUROPEPTIDE-Y NEURONS IN HUNTINGTON'S DISEASE CEREBRAL CORTEX. M. Cudkowicz* and N.W. Kowall (SPON: C.E. Poletti). Neurology Service, Mass. General Hosp., Boston MA 02114.

It is well established that neuropeptide Y (NPY) concentrations are elevated in the basal ganglia and cerebral cortex of Huntington's disease (HD) patients. We recently showed that NPY local circuit neurons are preserved while non-phosphorylated neurofilament (SMI 32) pyramidal projection neurons are depleted in the cingulate gyrus and superior frontal cortex.To determine whether similar histological changes occur in other regions of the cerebral cortex, we examined the distribution of NPY neurons and SMI 32 neurons in the occipital cortex of 13 HD and 6 age matched control brains. Adjacent cortical sections stained with cresyl violet were normal. We quantitated the number of NPY and SMI 32 reactive neurons per cortical transverse (neurons/mm) and found no significant difference between the number of NPY neurons in the HD and control brains (unpaired t-test). The mean number of NPY neurons in the occipital cortex of control brains was 35.95 ± 1.81/mm compared to 38.03 ± 2.43/mm in HD. In contrast, the number of SMI 32 immunoreactive neurons was significantly reduced in the occipital cortex in HD. The mean number of SMI 32 positive neurons fell from 27.33 ± 1.93/mm to 19.50 ± 2.20/mm (p=.04). Our findings show that NPY immunoreactive local circuit neurons are spared in HD occipital cortex while SMI 32 pyramidal projection neurons are depleted. The resistance of NPY neurons to degeneration in HD cortex supports the possibility that an glutamate-like excitotoxin is responsible for the pathogenesis of neuronal degeneration in HD.

370.9

DEGENERATIVE AND REGENERATIVE CHANGES ARE FOUND IN HUNTINGTON'S DISEASE STRIATUM WITH NEUROFILAMENT AND NEURAL CELL ADHESION MOLECULE (NCAM) HISTOCHEMISTRY. N.W.Kowall and A.C. McKee, Neurology Service and Dept. Neuropathology, Mass. General Hospital, Boston MA 02114.

Golgi studies have shown dendritic degeneration and regeneration in Huntington's disease (HD) striatum. We used histochemical markers of somatodendritic (non-phosphorylated neurofilament antibody, SMI 32) and axonal (phosphorylated antibody, SMI 31) compartments and axonal growth cones (NCAM, courtesy of Dr. K.S. Kosik) to further investigate this phenomena. SMI 32 labelled many medium sized neurons scattered throughout the normal striatum. In HD the density of SMI 32 positive neurons decreased dramatically and remaining neurons showed dendritic proliferation, tortuosity and fragmentation most pronounced in the subependymal zone. Dendritic spines and growth cone-like filopodia, not apparent normally, were evident. SMI 31 labelled thin, uniform fibers in normal striatum which increased in density in HD and became more coarse, shorter and fragmented. NCAM immunoreactivity in normal striatum was more dense in the striatal matrix compartment. In HD striatum, many short thread-like processes were seen most prominently in the subependymal zone. Our findings show that neurons and axonal fibers degenerate in HD in conjunction with aberrant proliferation of both dendrites and axons.

370.10

CYTOPATHOLOGICAL FEATURES,UBIQUITIN,AND HEAT-SHOCK PROTEINS IN AMYOTROPHIC LATERAL SLEROSIS P.N.Leigh,O.Garofalo,P.G.E.Kennedy*,G.B.Clements*, J.Martin,M.Swash,and B.H.Anderton.Departments of Neurology and Neuroscience,Institute of Psychiatry, and Department of Neurology,The London Hospital,London;and*Department of Neurology,Institute of Neurological Sciences,Glasgow,U.K.(SPON: BRA)

Antibodies to ubiquitin detect characteristic neuronal inclusions in vulnerable neurons in ALS. The aims of this study were(1)to clarify the relationship between ubiquitin-immunoreactive(Ub-IR) inclusions and inclusions identified by conventional stains,and (2) to investigate the localisation of other heat shock proteins (HSPs) in damaged anterior horn cells in ALS.Serial sections from ALS and control cases were labelled with affinity-purified ubiquitin antibody,monoclonal antibodies to HSPs, or stained by haematoxylin and eosin.Ub-IR inclusions were associated with a wide range of cytological abnormalities,such as loss of Nissl substance(but not true chromatolysis),small 1-4μm eosinophilic inclusions(Bunina bodies),and large eosinophilic inclusions(Lewy-like bodies)as well as ill-defined vacuolar areas. HSP antibodies did not label neuronal inclusions in ALS.We conclude that Ub-IR inclusions reflect abnormal protein breakdown in damaged neurons.

370.11

INCREASED NUMBERS OF ALZ-50 REACTIVE NEURONS MAY PRECEDE SENILE PLAQUE (SP) FORMATION IN DOWN'S SYNDROME (DS).
D. Larry Sparks and John C. Hunsaker, III* Sanders-Brown Center on Aging, Depts. of Pathology and Neurology, Univ. KY Med. Center; and Kentucky Medical Examiner's Program, Lexington KY 40536.

In contrast to SIDS infants (Sparks et al., A.A. Forensic Sci., 1989), no more ALZ-50 reactive neurons are found in Down's infants than control infants (Wolozin et al. PNAS, 1988). We have studied 10 controls: 3 between 10 and 15 mos. of age; 4 between 5 and 27 yrs; and 3 subjects between 5 and 27 yrs dying after being in coma. 3 DS patients between 1 and 25 yrs of age were investigated. Sections of parahippocampal gyrus were processed with ALZ-50 antibody; adjacent tissue was stained by the Bielschowsky method.

ALZ-50 reactive neuron density was similar in control and Down's infants, each absent Bielschowsky positive pathology. No ALZ-50 or Bielschowsky positive features were observed in either the control group or in the coma cases. ALZ-50 reactive neurons were found without SP in a 19 yr old DS patient. Abundant ALZ-50 positive neurons were found with diffuse form SP in a 25 yr old DS patient. The data indicate the presence of ALZ-50 reactive neurons may antedate SP formation in Down's syndrome.
(Supported by ADRDA (II-078-87) and NIH Grants 1-P01-AG05119 and 1-P50-AG05144)

370.12

ABSENCE OF AMYLOID IN NEURITIC PLAQUES IN YOUNG CASES OF DOWN'S SYNDROME. R.C. Switzer III, S.K. Campbell* and A.P. Osmand*+. R.H. Cole Neuroscience Lab., Depts. of Pathology and Medicine+, Univ. of Tennessee Med. Ctr., Knoxville, TN 37920.

The cortical neuritic plaques that characterize and are diagnostic of Alzheimer's Disease (AD) are also found in virtually all cases of Down's Syndrome (DS) that are 35 years of age or older. Inexplicably, few cases of DS in their 20s have been examined neuropathologically. It is in this age range of DS that we find evidence that contradicts the commonly held conception that the pathological changes of the neurites in neuritic plaques are accompanied by the presence of amyloid. Using a highly sensitive silver stain (Soc. Neurosci. Abstr., 13(1): 678), as well as standard Thioflavin-S (TS) and Congo red (CR) staining techniques on freeze-cut sections, we examined two DS cases ages 21 and 24 yrs. and compared them with 17 and 59 yr old DS cases. No plaques were found in the 17 yr old with any method. In the 21 and 24 yr old cases, argyrophilic plaques were abundant, but no plaques were revealed by the TS or CR methods. In the 59 yr old case, plaques were readily demonstrated by each method, and appeared indistinguishable from those seen in AD. In AD cases, careful comparison of adjacent sections revealed that fewer plaques stained with TS than with silver. These results indicate that advanced neuritic plaque formation can occur in the absence of detectable amyloid. If a plaque is less of a neurophysiologic perturbation without amyloid, then strategies to prevent amyloid deposition could be of value in the treatment of both AD and DS.

Supported by the Robert H. and Monica Cole Neuroscience Foundation, the NIH (NINCDS NS23634) and Alzheimer's Disease Research, a program of the American Health Assistance Foundation.

370.13

TREATMENT OF BRAIN TUMORS BY CHLORAMBUCIL-TERTIARY BUTYL ESTER (TBC). N.H. Greig*, S. Genka*, H.U. Shetty*, T.T. Soncrant, V. John*, I. Lieberburg*, and S.I. Rapoport. (SPON: C.R. Lake) Laboratory of Neurosciences, NIA, NIH, Bethesda, MD 20892, and Athena Neurosciences, Inc., S. San Francisco, CA 94080

We have developed a lipophilic derivative, TBC, of the widely used anticancer alkylating drug chlorambucil for the treatment of brain tumors. TBC reaches and maintains high concentrations in brain and possesses intrinsic alkylating activity. Following its i.v. administration to rats, TBC (13 mg/kg) achieves a brain/plasma concentration versus time ratio of approximately 30. This compares favorably with a ratio of 0.02 for chlorambucil. Pharmacokinetic studies have demonstrated that TBC remains primarily unaltered in brain, and is metabolized to chlorambucil and phenylacetic mustard systemically. In vitro studies indicate that the rate of metabolism is species-dependent. Additionally, TBC has demonstrated significant anticancer activity against intracerebral implants of Walker 256 carcinosarcoma tumor in rats. Median survival time was increased to 175% of controls, which is significantly greater than that achieved after chlorambucil. Further, quantitation of brain tumor size has demonstrated that TBC causes in excess of 90% inhibition of intracerebral tumor growth. Preclinical pharmacology is presently being undertaken to develop the agent for clinical testing.

370.14

HYDROXYL RADICAL ADDUCTS OF DOPAMINE ARE POTENT INHIBITORS OF ARYLSULFATASE-C. T.A. CAWLEY, JR and T.J. SHICKLEY, Dept. of Pharmacology and Toxicology, Philadelphia College of Pharmacy and Science, Philadelphia, PA. 19104

It has been previously shown that dopamine (DA) is subject to attack by hydroxyl radicals to form a mixture of three possible ring-monohydroxylated products. The products: 2-hydroxydopamine (2OHDA), 5-hydroxydopamine (5OHDA) and 6-hydroxydopamine (6OHDA) are formed in a ratio of 3:2:1.

Arylsulfatases (EC 3.1.6.1) occur in nature in three distinct forms: A/B and C (ARS-C). The deficiency or absence of one or more of the arylsulfatases results in metachromatic leukodystrophy (MLD), a progressive demyelinating disease. In one form of MLD a schizophreniform behavior has been observed.

This laboratory has reported that DA as well as the DA agonist, apomorphine inhibit ARS-C (Soc. Neurosci. Abstr. (14)2 p.1075, 1988). In the present study we examine the effects of three ring-monohydroxylated adducts of DA on ARS-C activity to determine the potential role of free-radicals on inhibition of arylsulfatases.

Partially purified ARS-C (Sigma, S-1629) activity was assayed spectrophotometrically using p-nitrophenyl sulfate (p-NPS) as substrate by measuring enzymatically liberated p-nitrophenol. Inhibition was analyzed by varying DA-adduct concentration in the presence of fixed concentrations of p-NPS.

Analysis of inhibition revealed the following K_i(app): 6OHDA=10uM, 2OHDA=25uM, 5OHDA=55uM and DA=350uM. Kinetic studies disclosed the inhibition for all compounds to fit a model for simple competitive inhibition.

These results suggest a potential role of free-radicals in the generation of inhibitors of arylsulfatases which may have relevance in the heterogeneity of schizophrenia and other neuro-degenerative disorders.

(Supported by USPHS Grant NS-26040 to TJS)

370.15

HUMAN AND EXPERIMENTAL NEUROAXONAL DYSTROPHY: PRESENCE OF UBIQUITIN IN DYSTROPHIC AXONS. E. Cochran*, A. Patton*, B. Bacci*, T. Mizutani*, L. Autilio-Gambetti, and P. Gambetti. Division of Neuropathology, Case Western Reserve University, Cleveland, OH 44106.

Ubiquitin, a protein involved in intracellular proteolysis, has recently been demonstrated in a variety of non-membrane bound filamentous cytoplasmic inclusions such as neurofibrillary tangles, Pick bodies, and Lewy bodies. We now report the presence of ubiquitin in dystrophic axons of a case of infantile neuroaxonal dystrophy (IND) as well as of rats intoxicated with p-bromophenylacetylurea (BPAU), which induces the formation of dystrophic axons in the central nervous system following chronic intraperitoneal injection. Immunohistochemistry of the case of IND with antiserum to ubiquitin showed specific and intense staining of numerous dystrophic axons in the nucleus cuneatus, nucleus of the spinal trigeminal nerve, tractus solitarius and dorsal motor nucleus of the vagus nerve. In the BPAU-intoxicated rats, the dystrophic axons present in the nuclei and fasciculi cuneatus and gracilis, nucleus and tract of spinal trigeminal nerve and nucleus dorsalis of Clarke were also immunostained with antiserum to ubiquitin. Immunoelectron microscopy performed in both conditions confirmed that ubiquitin is present in various structures contained within the dystrophic axons as well as in axons and dendrites that appear normal. The presence of ubiquitin in axons and dendrites suggests that the ubiquitin system is active not only in the neuronal cell body but also in its processes.
Supported by NIH grants AG 00795 and NS 14509.

370.16

IMMUNOCYTOCHEMICAL DEMONSTRATION OF ANTIOXIDANT ENZYMES IN THE NERVOUS SYSTEM
F. J. Denaro, UCSD Med Ct, San Diego Ca. and J. S. Schneider, Hahnemann Univ. Phil., Pa

The superoxide anion and other reactive intermediaries, pose a constant threat to biological systems. Extensive biochemical evidence demonstrates that all aerobic organisms contain enzymes which protect the cells against such destructive agents. Crucial to such a protection system are the enzymes Superoxide Dismutase (SOD), Catalase (Cat), and Glutathione Peroxidase (GPX). However, biochemistry can only give a partial picture of the variation in cellular enzyme concentration. Anatomical relationships cannot be revealed by that approach. Yet, such information is key to our understanding of oxidative stress in the CNS. Immunocytochemical evidence is now accumulating on the cellular distribution of these enzymes. Immunocytochemistry has shown a considerable variation in the distribution and concentration of these enzymes among cell types. This fact, together with the other biological importance of these enzymes suggests a possible new cell classification.

370.17

THE RELATIVE INCIDENCE OF ECTOPIC DENDRITE GROWTH ON CORTICAL PYRAMIDAL CELLS VARIES WIDELY IN DIFFERENT NEURONAL STORAGE DISEASES AND MAY BE CORRELATED WITH THE PRESENCE OF GM2 AND/OR GM3 GANGLIOSIDES. S.U. Walkley. Dept. Neuroscience, Rose F. Kennedy Center, Albert Einstein College of Medicine, Bronx, NY 10461

An explanation for the occurrence of the ectopic dendritogenesis which characterizes certain neurons in neuronal storage diseases remains unknown. The availability of a large variety of these diseases in a single species (cat) has allowed for comparative studies on the incidence of neurite growth relative to known biochemical defects. Golgi studies of cortical pyramidal neurons in 7 types of storage disease (GM1 and GM2 gangliosidosis, Niemann-Pick disease types A and C, mucopolysaccharidosis type I, and inherited and swainsonine-induced α-mannosidosis) were evaluated for evidence of ectopic dendritogenesis. At end-stage disease, GM2 gangliosidosis cats revealed the greatest incidence of neurite growth and NPD type A the least. The single case of NPD type C available for study demonstrated the most significant early growth of neurites. A metabolic defect common to all of these diseases appears to be the accumulation of GM2 and GM3 gangliosides. (Supported by NS18804)

370.18

SEPARATION, CHARACTERIZATION & QUANTITATION OF CHOLINESTERASE ISOZYMES IN RAT PLASMA. A. Korenovsky*, H. Laev and S. Mahadik (SPON: M. Stanley). Div. Neurosci. NYS Psychiat. Inst., Depts Psychiat, and Biochem & Mol Biophys, P & S, Columbia U., NY, NY 10032.

Plasma cholinesterase (ChE) consists of over 15 isozymes. Reported studies have determined only 2 types of ChE activities (pseudo- and true-) by conventional assay. Levels of individual ChE isozymes can be more useful as markers for cholinergic dysfunction.

Eleven ChE isozymes were detected on slab gel by their activity with either acetylthiocholine (AcTCh) or naphthyl acetate (NA) as substrates. Enzymatic product formed by each isozyme was quantitated by densitometric scanning. Isozymes 3 & 11 (1 being the slowest to migrate) hydrolyzed AcTCh more than NA and were inhibited by BW284C51. Isozymes 1, 2 & 9 hydrolyzed NA more than AcTCh and were inhibited by IsoOMPA. Other minor isozymes showed similar activities with both substrates and were inhibited by IsoOMPA. Isozymes 3 & 11 hydrolyzed NA more (62 & 38% respectively) in presence than in absence of IsoOMPA. The densitometric area of enzymatic product formed by each isozyme was linear with increasing levels of plasma (10-60ul). Using known amounts of standard ChE (horse ChE, Sigma) each isozyme activity was determined. Levels of isozymes remained stable with repeated bleeding but varied (up to 100%) among animals. Quantitative slab gel electrophoresis procedures are applicable for determining changes in individual ChE isozymes.

370.19

HUMAN BRAIN TUMORS HAVE ALTERED PHORBOL ESTER BINDING CAPACITY. F. Battaini, A. Leggio*, S. Govoni*~, L. Frattola*~, I. Apollonio*~, C. Ferrarese~, R. Piolti*~ and M. Trabucchi*. II Univ. of Rome, Dept. Exp. Med. and Bioch. Sci., Chair Toxicol; ~ Univ. of Bari, Dept. of Pharmacobiology; ~ Dept. of Neurology, S. Gerardo Hospital, Monza, Italy.

Increasing evidence points to the involvement of protein Kinase C (PKC) in tumor cell growth; cells with an umbalanced expression of PKC show in vitro altered growth and in vivo tumorigenicity. We have studied PKC expression by means of the binding of 3H-phorbol 12,13 dibutyrate (3H-PdBu) in human brain normal tissue taken from patients operated on for aneurysma and in tumors removed during surgery for cerebral neoplasia. Normal white matter has less than 50% 3H-PBu binding capacity in comparison to gray matter (59.4 versus 136.4 pmoles/mg prot). Glioblastoma and astrocytoma have a lower 3H-PdBu binding capacity (27.9 and 21.1 pmoles/mg protein respectively) in comparison to normal white matter. Tumors of non glial origin, such as meningioma and neurilemmoma, have even less availability of binding sites (12.8 and 11.4 pmol/mg prot). Metastatic specimens have the lowest binding capacity (8.7 pmol/mg prot). No significant modification was observed in all specimens in 3H-PdBu affinity parameters being all in the 10-20 nM range. The data indicate the possibility of an unbalance of the regulatory domain of PKC in brain tumors with possible influence on the catalytic enzymatic function. Moreover considering that PKC exert a negative control on Epidermal Growth Factor receptors (EGFR), our observation may be related to previously reported data on EGFR overexpression in brain tumors.
The results support the importance and the involvement of PKC in the process of malignant brain cell transformation.

DEGENERATIVE DISEASE: PARKINSON'S III

371.1

TIME-SERIES ANALYSES OF PARKINSONIAN RESTING TREMOR. C.J. Hunker*, G.E. MacLeod*, and J.H. Abbs (SPON: R. Sufit) Speech and Motor Control Labs, Waisman Center, Univ. Wisconsin, Madison, WI 53705

Hunker & Abbs (1983, 1988) observed uniform average resting tremor frequencies in the lip, jaw, tongue, and finger. However, cross-correlations yielded insignificant coefficients. It was proposed that the CNS may have a somatotopically organized pacemaker commonly activated but independently effecting parkinsonian tremor. From this prospective, the stochastic properties of resting tremor recorded simultaneously from the index fingers and the lower lip of seven subjects were examined. Frequency histograms showed identical, dominant frequencies at each recording site in the same subject, with remaining values densely distributed around this central frequency. The distribution pattern was different for each body part denoting the subtle independent fluctuations in tremor period; the auto- and cross-correlations documented the overall constancy of the tremor frequency as well as the independent shifts in period, respectively. The peak-to-peak amplitudes varied considerably across structures. For some sites, the amplitudes were concentrated around a structure-specific central value, while for others the amplitude distribution decreased monotonically. A tremor amplitude trend analysis at each site indicated that adjacent observations were not independent; rather, there were structure-specific amplitude sequences of variable duration.

Parkinsonian resting tremor is a narrow band, randomly ordered phenomena. While the dominant frequency is invariant across structures, the tremor period independently fluctuates at each site. The peak-to-peak amplitude of the tremor waveform is unrelated across structures as are the modulated amplitude sequences. Resting tremor appears to be a continuous process variably adjusted by an underlying second process. These results support a hypothesized somatotopically organized tremor pacemaker, a related but independently functioning source for each tremorous structure. (NIH Grants NS-13274, HD-03352)

371.2

QUANTIFICATION OF POSTURAL STABILITY IN PARKINSON'S DISEASE. S. Glatt*, J. Wash*, S. Cho*, M. Melnick, R. Dubinsky*, J. Redford* and W. Koller. Center on Aging, University of Kansas Med Ctr., Kansas City, KS 66103

Assessment of efficacy of dopaminergic agonists in Parkinson's disease (PD) are dependent on subjective evaluation. While therapy results in decreased bradykinesia and improved gait, the effect of dopaminergics on postural stability is unclear. We have developed a quantitative method to measure postural stability using a force platform (AMTI, Newton, MA) which can measure forces in three orthogonal planes and movements about these axes to calculate position of the center of pressure (CP). The area traversed by CP during quiet standing was measured in 11 young control subjects (YNS) (age 26.1 ± 3.4 yrs), 7 elderly controls (ENS) (age 62.0 ± 6.3) and 35 patients with PD for 15 seconds. Patients were stratified according to Hoehn and Yahr stage. There were 14 patients in stage 1 (PT 1) (age 68.3 ± 10.2), 12 in stage 2 (PT 2) (age G5.2 ± 7.1) and 9 in stage 3 (PT 3) (age 68.0 ± 11.4). Mean area measurements in cm^2 were YNS 1.280 ± .46, ENS 1.62 ± .394, PTI 1.603 ± .307 PT2 2.356 ± .931, and PT3 4.239 ± .967. Significant differences were seen between PT3 and YNS, ENS and PT1. The data from this pilot study suggest that this method can be used to quantify postural stability in PD and that instability increases with disease severity.

371.3

PRIMING METHOD AND REACTION TIME IN PARKINSON'S DISEASE. F. Viallet, M. Borg, E. Legallet and E. Trouche. LNF 3, CNRS, 13402 Marseille Cedex 9, France.

The priming method consists of introducing a probabilistic bias between two possible stimulus-triggered motor responses : one (frequent) stimulus (75 % of the trials) induced the so-called "primed response", whereas the other (rare) stimulus (25 % of the trials) triggered a non primed response. This procedure was adapted here to a pointing task. A luminous stimulus constituting the target could be placed in either the "primed" (75 %) or the "non-primed" position (25 %), the choice being between two possible trajectories with different amplitudes (large vs small) which were successively and alternately primed. Eleven parkinsonians undergoing therapy were compared with ten age-matched controls. In general, the RTs were significantly longer in the patients. In normal subjects, the RTs of non-primed responses were significantly and constantly longer than those of the primed ones. In parkinsonians, the same pattern was observed with some differences depending on the series : the priming effect increased in the RTs preceding the large trajectory, whereas it decreased in the RTs preceding the small one. These data suggest that the cognitive processes detecting a probabilistic bias in a choice RT task seem to be partly preserved, depending on the amplitude of the forthcoming movement.

371.4

DIFFERING TIME COURSES OF DOPAMINERGIC STIMULATION AND CLINICAL EFFECTS IN PARKINSONISM. S.T. Gancher*, W.R. Woodward, J.G. Nutt, B. Boucher* Dept. Neurol. & Biochem., Oregon Health Sciences University, Portland, OR 97201

The pharmacological basis of the acute response to dopaminergic stimulation in parkinsonism is not completely understood. While it is generally assumed that the clinical response reflects concurrent dopaminergic stimulation, it is also possible that receptor activation initiates events that outlast receptor occupancy. To distinguish between these two possibilities, we administered ineffective and effective doses of the dopamine agonist, apomorphine, to parkinsonian patients and monitored clinical responses and plasma drug levels. The response to an effective dose was divided into a suprathreshold phase, the period while plasma drug levels exceeded peak ineffective levels, and a persistence phase, the period after plasma drug levels had fallen below peak ineffective levels. Greater than 75% of the response occurred during the persistence phase. Since apomorphine rapidly diffuses into brain and is not stored, we conclude that the clinical effects are triggered by but do not require continued receptor stimulation. (Supported by Oregon Medical Research Foundation and NIH Grants NS21062 and R00334.)

371.5

MODULATION OF THE STRETCH REFLEX DURING SINUSOIDAL TRACKING IN PARKINSONISM. M. Johnson*, A. Kipnis*, M. Lee*, T.J. Ebner (SPON: D. Knopman). Depts. Neurosurgery and Neurology, Univ. of MN, EMPI, Mpls., MN 55455.

Disordered sequencing of reflex activity during reciprocating volitional wrist movement was hypothesized to underlie bradykinesia. For 14 Parkinson's and 10 age matched normal subjects, a visually tracked sinusoidal wrist movement was perturbed with a torque transient at 0,90,180,270°. Reflex EMG, volitional EMG, and deflections were recorded. Indices reflecting the ratio of EMG magnitude during phases of tracking produced by homonymous muscle shortening (assisting movement) and lengthening (braking movement) were derived for the volitional and reflex EMG. Indices of reflex and volitional EMG coactivation were also computed. A decrease in extensor reflex assistive/braking (A/B) ratio was found in the Parkinson group (T-test, $p < 0.015$). The parkinsonian patients demonstrate primary defects in reflex modulation in extensors (n=8), flexors (n=3), or both (n=3). The assumption that flexor or extensor reflex defects contribute to bradykinesia is supported by an A/B index comparison ($p < 0.001$). Volitional EMG modulation and coactivation indices show no significant differences between groups. Shift of reflex activity into braking phases without a corresponding shift in volitional EMG occurs in parkinsonism, contributing to symptomatic bradykinesia.

371.6

MOTOR LEARNING IN PARKINSONIAN PRIMATES. C.L. Ojakangas, T.J. Ebner. Depts. Neurosurgery and Physiology, Neuroscience Grad. Prog., Univ. of MN, Mpls., MN 55455.

We have studied the motor learning strategies in primates performing visually guided two-dimensional arm movements. The animals placed a cursor using a manipulandum in target boxes under control conditions and when the relationship between hand and cursor (gain) was varied. As we showed previously, when adapting to a novel gain normal primates gradually scale velocity to the new distance the hand must travel to place the cursor in the target and the time to peak velocity remains constant. In primates receiving a unilateral internal carotid injection of MPTP, velocity profiles were frequently not bell-shaped, even after adaptation. Maximum velocities decreased in relation to normal for all gains ($p < .025$), but did not vary proportionately with distance. Total movement duration increased for all conditions ($p < .001$) as did reaction times for most conditions. Time until peak velocity was not held constant when the animal was subjected to a new gain. Results indicate that the parkinsonian primates utilize a different motor learning strategy than normal primates. A fundamental deficit is seen in scaling velocity to movement distance during the initial phase of movement. Instead, the strategy involves slower velocities and a prolonged deceleration phase during which visual feedback control is available. Supported by NSF/BNS-8707572 and NIH/NS-18338.

371.7

KAINIC ACID LESIONS IN THE PONTINE TEGMENTUM CAUSE DISTANT LESIONS OF THE SUBSTANIA NIGRA THAT ARE ATTENUATED BY MK-801. M. Mintz, B.J. Knowlton and A.J. Annala. Dept. of Psychology, Univ. of Southern California, Los Angeles, CA 90089.

In 8 rats lesions in the left pontine reticular formation were made by injection of 1.5 nmol/ul kainic acid. Three of these rats were treated with 5mg/kg MK-801 (MK), an NMDA receptor antagonist, 1/2 hr before injection. All rats that did not receive MK showed twisted posture to the left noted from the time of recovery from anesthetic after injection. After 3 weeks, the rats were sacrificed, their brains fixed, sectioned through the pons and midbrain, and stained with cresyl violet. Areas of sections of left and right substania nigra (SN) were computed for each rat by 2 observers, one unbiased, with good correlation between them. The region of cell loss and gliosis at the injection site was confined to the pontine reticular formation, 1-2 mm caudal to SN. Among rats not receiving MK, the left SN was significantly smaller than the right (correlated t-test, $t=4.742$, $df=4$, $p<.01$). The rats receiving MK did not show a significant difference between left and right($t=.063$, $df=2$). The left-right differences were significantly lower for the MK group ($t=3.396$, $df=6$, $p<.02$). These results suggest that SN cell death in Parkinsonism could be related to the NMDA receptor.
Supported by NSF grant (BNS-8718300) to R.F. Thompson.

371.8

RELATIONSHIP BETWEEN SEVERITY OF SYMPTOMS OF PARKINSON PATIENTS AND CONCENTRATIONS OF *pros*-METHYLIMIDAZOLEACETIC ACID IN LUMBAR CSF. J. P. Green, G. D. Prell, J. K. Khandelwal*, and R. S. Burns* (SPON: J. Thornborough) Dept. Pharmacology, Mount Sinai School of Medicine, CUNY, New York, NY 10029 and Section on Experimental Therapeutics, NIMH, Bethesda, MD 20205

Patients who met clinical criteria for Parkinson's disease (57.9±9.3 SD years old; n=13) and healthy controls (63.3±4.0 SD years old; n=9) gave informed consent for participation in the study. Severity of Parkinson symptoms of all patients was rated by a single observer using the Columbia University Rating Scale (CURS) and the Hoehn-Yahr Rating Scale (HYRS). Many patients had not received L-DOPA in the past; all but one had been drug-free for at least six months. All subjects were on a diet low in monoamines; they were fasted and recumbent from midnight until 9 am when lumbar punctures were performed. Aliquots (14-17th ml) were collected and immediately frozen. Concentrations of *pros*-methylimidazoleacetic acid (p-MIAA) were measured by gas chromatography-mass spectrometry. There was a strong positive correlation between scores on the CURS and levels of p-MIAA in CSF from all patients together (Spearman's $\rho = 0.749$, $p < 0.005$) and males and females analyzed separately. There was a significant correlation ($\rho = 0.67$, $p < 0.02$) between scores rated on CURS and HYRS. However, mean (±SEM) levels of p-MIAA (pmol/ml) from controls (61.1±10.9) and patients with Parkinson's disease (50.6±7.2) were not significantly different ($t=0.8$, $p > 0.1$). The data suggest that levels of p-MIAA, while not pathognomonic of Parkinson's disease, may be associated with the severity of its symptoms. Since levels of p-MIAA in brain are raised by administration of *pros*-methylhistidine (p-MeHis) (J. Neurochem. 52: 561, 1989), a normal component of diet, it is possible that increased p-MIAA in CSF is reflective of p-MeHis or another substance derived from diet. Alternatively, it may reflect aberrant metabolism of an endogenous substance such as free p-MeHis or its conjugate, anserine. [Supported by NIMH grant 31805.]

371.9

LEWY BODIES AND EXTRAPERIKARYAL OVOID BODIES IN STRIATONIGRAL DEGENERATION AND PARKINSON'S DISEASE.
T. Yamada*, H. Akiyama*, S. Itagaki* and P.L. McGeer.
(SPON: J.G. Sinclair). Kinsmen Lab. of Neurol. Res., Univ. British Columbia, 2255 Wesbrook Mall, Vancouver, B.C., V6T 1W5, Canada.

Striatonigral degeneration (SND) often produces symptoms indistinguishable from parkinsonism (PD) although the pathology is much more widespread, especially in the striatum. We describe similar pathological features in the substantia nigra (SN) in a case of SND and several cases of PD. In common with PD cases, the SND case showed loss of melanin containing cells. HLA-DR positive reactive microglia were plentiful in the SN in both conditions. Lewy bodies in melanin-containing neurons were also a common finding. These stained positively for tyrosine hydroxylase (TH) and microtubule associated proteins (MAPs). In addition, a previously undescribed pathological entity was found in both conditions. Ovoid structures, up to 7.5 µm in diameter and up to 15 µm in length, were scattered in the neuropil. These bodies are difficult to see by conventional histological methods, lack nuclei but also stained positively for TH and MAPs. They would therefore appear to be swollen neurites, at least some of which are associated with catecholamine neurons. Both types of abnormal bodies stained positively with antibodies to several components of the complement system, indicating they were undergoing degeneration.

371.10

THE ROLE OF S-ADENOSYL-METHIONINE IN CHRONIC L-DOPA ADMINISTRATION. B. Crowell,Jr.; R. Benson* and C.G. Charlton. Dept. of Physiology, Meharry Medical College, Nashville, TN 37208.

When injected in the brain S-adenosyl-methionine (SAM) caused Parkinson's Disease (PD)-like symptoms. This suggests that excesses of SAM-dependent methylation may be involved in PD and that the success of l-dopa therapy in PD may be due to the ability of l-dopa to deplete SAM. This will concomitantly increase the synthesis of SAM. It follows that the lack of efficacy from prolonged l-dopa therapy may be due to an increase in the activity of methionine-adenosyl transferase (MAT), the enzyme that synthesizes SAM from methionine and ATP. We tested this hypothesis by administering l-dopa and studying its effects on MAT in the liver and the brain of rats and on the SAM-induced motor abnormalities. Rats were chronically treated with 100mg/kg or 500mg/kg of l-dopa or with saline before sacrificed for enzymatic assay or challenged with SAM.

The results showed that about 73% and 53% of the activity of MAT was in the insoluble fraction of the brain and liver, respectively. L-dopa increased the activity of MAT in the brain by 32% in the 100mg/kg and 37% in the 500mg/kg as compared to the saline treated rats. No significant changes occurred in the liver. As contrast to acute l-dopa (Charlton and Way, 1978) the chronic l-dopa did not significantly alter the SAM-induced motor abnormalities. The decreased efficacy of chronic l-dopa in PD may be explained by an increased MAT activity, which will increase SAM. SAM will react with l-dopa to produce 3-O-methyldopa. This may further explain the high plasma levels of 3-O-methyldopa observed in l-dopa treated PD patients, especially those exhibiting the on-off phenomenon. If the effects of l-dopa in PD is due to its ability to reduce SAM then chronic l-dopa will offset its own effects and this explains why chronic l-dopa did not antagonize the effects of SAM.
Supported by: NIH RR03032 and NSF RII-H704121.

371.11

EXCESSIVE BIOLOGICAL METHYLATION IN THE BRAIN: A POSSIBLE CAUSE OF PARKINSON'S DISEASE. C.G.Charlton, Dept. of Physiology, Meharry Medical College, Nashville, TN 37208.

Nigrostriatal degeneration, depletion of tyrosine hydroxylase (TH), dopamine, other biogenic amines and melanin are related to the symptoms of tremors, hypokinesia, rigidity and motor freezing in Parkinson's disease (PD). An increase in methylation can cause parallel changes in those biochemicals and can produce cell-damaging metabolites. Therefore, the methyl donor S-adenosyl-methionine (SAM), the limiting factor in methylation, may be involved in the genesis of PD. To test this hypothesis the effects of SAM on motor functions, neuronal degeneration and on TH were studied in the rat. The rats were cannulated for lateral ventricular injection.

SAM caused tremors, hypokinesia, rigidity, motor freezing and abnormal posture and reflexes. Tremors occurred at rest and mainly in the snout and extremities. The rats made attempts before movements were achieved and when propped remained motionless for an extended period. SAM caused damage to the caudate nucleus, septum and hippocampus and a reduction in size of and degeneration in the substantia nigra (SN). These aberrations were accompanied by a reduction in the immunoreactivity and disruption in the cellular compartments for TH in the SN.

The motor abnormalities caused by SAM are physiologically identical to those occurring in PD. The specific degeneration in the SN and the depletion of TH, the synthesis enzyme for dopamine, also parallel the neurological findings in PD. This study and other evidence showed that excesses of SAM-dependent biological methylation in the brain can cause the cascade of reactions that may result in PD and probably other degenerative neurological disorders, depending on the sensitivity of the cellular and molecular substrates for methylation.

Supported by: NIH RR03032 and NSF RII-H704121.

371.12

ENKEPHALIN AND NEUROPEPTIDE Y ARE DECREASED IN ADRENAL MEDULLA OF PARKINSONIAN PATIENTS. S.L. Stoddard, J.E. Ahlskog*, D. Lucas*, D. Roddy* and S.W. Carmichael. Dept. of Anatomy, Indiana U. Sch. of Medicine, Fort Wayne, IN 46805 and Depts. of Neurology and Anatomy and GI Hormone Research Lab, Mayo Clinic, Rochester, MN 55905.

Adrenal medullary catecholamines have previously been shown to be significantly decreased both in autopsy specimens from parkinsonian individuals and in tissue collected from Parkinson's disease patients who have undergone autologous transplantation of the adrenal medulla to the caudate nucleus (Stoddard et al. *Exp. Neurol. 104*:22-27 and *104*:in press, 1989). To determine the extent of this diminished secretory capability, we measured levels of several neuropeptides in adrenal medullary tissue collected at autopsy from control (N=38) and parkinsonian (N=8) populations. [Met]enkephalin (ENK) and neuropeptide Y (NPY) were measured by radioimmunoassay. Levels of ENK were significantly less ($p=0.016$) in the parkinsonian group ($x \pm SEM = 3597 \pm 690$ ng/gm tissue) than in the control population (9754 ± 1547 ng/gm). Levels of NPY were also notably lower in the parkinsonian group, although the difference did not reach significance (2540 ± 1015 vs. $11,359 \pm 5231$ ng/gm, respectively; $p=0.068$). These results suggest that the adrenal medullary dysfunction associated with Parkinson's disease is not limited to catecholamine content alone, but also includes decreased content, and perhaps secretion, of neuropeptides.

371.13

HUMAN NEUROMELANIN: FINE CHEMICAL STRUCTURE AND CHARACTERIZATION. A Vescovi*, L Zecca*, P Traldi**, G Malanca, C Goi**, E A Parati. Neurol Inst C Besta, Milan *CNR, Milan *CNR, Padova. "Forensic pathology , Milan, Italy.

A crucial role of neuromelanin (NM) in Parkinson's disease (PD) ethiopatogenesis has been proposed In fact previous papers clearly report non covalent binding between neuromelanin and several pharmacological active molecules including both the parkinson-mimetic toxins 1-methyl-4-phenylpyridinium ion and Manganese

However, little is known about biosinthesis and chemical structure of neuromelanin and about its putative phisiological functions We report here a study on neuromelanin, performed by electron impact-mass spectrometry studies. By pyrolising NM in the ion source, the main pyrolisis products were identified by their exact masses as pyrrole, alkylcatechols, alkylphenols, 5 OH-indole, 1,4-benzothiazine; the same compounds are observed with natural and sinthetic 5-S-cysteynildopa derived pheomelanins

This comparative analisis is the first step in understanding the chemical composition of neuromelanin and in turn to clarify its biosinthetic pathway and its role in PD ethiopatogenesis

371.14

CSF FROM PARKINSON'S DISEASE PATIENTS IS CYTOTOXIC TO VENTRAL MESENCEPHALIC CULTURES AND THE SUBSTANTIA NIGRA OF THE RAT. P.M. Carvey, L.C. Kao, E.S. Lo*, H.L. Klawans*, A. Dahlstrom (1), and A. McRae (1).
Rush University, Chicago, IL, 60612; (1) U. Goteborg, Goteborg, Sweden.

The CSF of Parkinson's disease (PD) patients contains an antibody (Ab) which immunocytochemically reacts with the substantia nigra (SN) of the rat. We have previously reported that the Ab disappears in 8/9 patients exhibiting clinical improvement following adrenal medulla-to-brain transplantation. This might suggest that the Ab participates in the PD process. We examined this hypothesis in ventral mesencephalic cultures as well as in vivo.

The CSF from 3 transplant patients was incubated for 7 days with rat ventral mesencephalic cultures. Immunopositive CSF, taken at the time of transplantation, was overtly cytotoxic to the cultures resulting in a significant diminution of anti-DA immunoreactivity. In contrast, immunonegative CSF taken from the same patients 6-12 months following surgery was without effect. In a second experiment, immunopositive CSF taken from a patient with idiopathic PD or immunonegative CSF taken from a non-parkinsonian patient, was loaded into Alzet minipumps and infused for 14 days (5 ul/hr) into the rat SN (n=4 in each group). Both samples were immunoabsorbed in whole rat blood prior to infusion. Animals infused with immunopositive CSF exhibited cotraversive posturing after 5 days and ipsilateral amphetamine-induced rotations on both the 7th and 14th day of infusion. Immunonegative CSF was without effect. Although the data must be considered preliminary, it may suggest that the immunologic response to nigral degeneration in PD patients may contribute to progression of the disease and further, that the moderate improvement observed in transplant patients may result from loss of this secondary autoimmune component as a result of adrenal medulla-to-brain transplantation.

371.15

THE CSF OF PARKINSON'S DISEASE PATIENTS CONTAINS AN ANTIBODY WHICH IMMUNOCYTOCHEMICALLY REACTS WITH THE SUBSTANTIA NIGRA OF THE RAT. L.C. Kao, A. McRae (1), R.D. Penn, A. Dahlstrom (1), H.L. Klawans*, and P.M. Carvey.
Rush U.,Chicago, IL, 60612; (1) U. Goteborg, Sweden.

Although the CNS is historically considered an immunologically privileged region, many investigators have reported that neurodegenerative disorders are associated with the production of antibodies directed against the CNS. We therefore examined the CSF of Parkinson's disease (PD) patients for antibodies reacting with the substantia nigra (SN).

CSF (diluted 1:1 with tris buffer) was incubated overnight with 10 micron sections of rat SN, as well as other CNS regions, which had been perfusion fixed with allyl alcohol in a cocydylate buffer. An avidin-biotin-peroxidase reaction was employed to visualize immunocytochemical reactivity of the CSF against the CNS. Thus far, 131 patient samples with a variety of neurologic and non-neurologic diagnoses have been examined. 42 of 53 PD patients (79%) have been blindly identified from within the 131 patient series using this technique. Cell bodies are predominantly marked although processes are occasionally observed to react. Postive immunocytochemical reactivity was observed in the SN and VTA, and to a lesser extent, in the retrorubral area and striatum. 3 of 4 (75%) symptomatic MPTP patients were also immunopositive. In addition, 6 non-parkinsonian patients with movement disorders potentially involving the SN, were also immunopositive. In contrast, the CSF 68 patients with a variety of other neurologic disorders, as well as patients undergoing routine myelography were immunonegative. Taken together, this data suggests the degeneration of the SN, whatever its cause, is secondarily associated with the production of an antibody directed against DA rich regions of the brain, and further, that evaluation of this antibody may be useful as a marker for nigral degeneration.

371.16

COGNITIVE IMPAIRMENT IN PARKINSON'S DISEASE: WIDESPREAD AMYLOID PLAQUES IN THE CEREBRAL CORTEX AND CIRCUMSCRIBED NEUROFIBRILLARY CHANGES IN THE ENTORHINAL REGION. H. Braak and E.Braak. Dept. Anatomy, J.W.Goethe University, Frankfurt, Fed. Rep. Germany.

Sensitive silver methods for amyloid and neurofibrillary changes were employed to examine the cortical pathology in Parkinson's disease. Amyloid plaques were found in varying densities in most areas of the cerebral cortex while neuritic plaques were rare or absent. Neither the Ammon's horn nor the neocortex revealed a sufficiently large number of tangles to permit diagnosis of Alzheimer's disease. An abundance of tangles was found only in layer II of the entorhinal cortex. This layer gives rise to the perforant path and via this bundle information from isocortical association areas is transmitted to the hippocampal formation. The affection of layer II consequently isolates the hippocampal formation. In conclusion the impairment of cognitive functions frequently seen in patients afflicted with Parkinson's disease might possibly be associated with the wide spread deposition of amyloid plaques and the severe pathological alteration of layer II of the entorhinal cortex. (Supported by the DFG).

371.17

TYROSINE HYDROXYLASE mRNA EXPRESSION IN THE SURVIVING NIGRAL DOPAMINE NEURONS IN PARKINSON'S DISEASE. F. Javoy-Agid*, E.C. Hirsch, S. Dumas*(1), J. Mallet*(1), and Y. Agid* (SPON: A. Prochiantz). U289 INSERM - Hôpital Salpêtrière - 75013 PARIS - (1) CNRS UPR11 - 91190 GIF S/YVETTE - France

Parkinson's disease (PD) is associated with a massive dopamine (DA) cell death in the substantia nigra resulting in a severe striatal DA denervation. Though an increased striatal DA turnover is observed in the surviving neurons, continuous progression of DA nerve cell death occurs in the course of the disease.

This study was undertaken to see whether the remaining nigral cells were still functionnal. Tyrosine hydroxylase (TH) messenger RNA (mRNA) was detected by in situ hybridization histochemistry using a 35-S-labelled cDNA probe complementary to TH mRNA. The level of expression of TH mRNA in TH-immunostained neurons was determined by estimating the density of grains (detected by nuclear emulsion autoradiography) over a neuron, using the computer assisted system from BIOCOM (France).

In PD, not only did the number of cells expressing TH mRNA decrease dramatically, but the cellular level of TH mRNA was markedly reduced when compared to controls. The data are compatible with immunocytochemical observations and suggest a reduced TH content in surviving cells.

EXCITATORY AMINO ACIDS: ANATOMY AND PHYSIOLOGY II

372.1

DEMONSTRATION OF PUTATIVE GLUTAMATERGIC/ASPARTATERGIC ASSOCIATIONAL CONNECTIONS IN THE OLFACTORY CORTEX OF THE RAT. L.E. White*, K.M. Carnes, & J.L. Price. Department of Anatomy & Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

Studies of excitatory phenomena (e.g. long term potentiation, seizure discharges) in the olfactory cortex suggest that excitatory amino acids play a central role in these processes. In the present study, small injections of a combined solution of ^{3}H-D-aspartate (^{3}H-D-Asp) and wheat germ agglutinin-horseradish peroxidase (WGA-HRP) were placed in the anterior piriform cortex in order to identify presumptive glutamatergic and/or aspartatergic (Glu/Asp) inputs to this region.

The findings suggest that associational projections in the olfactory cortex are Glu/Asp, with the exception of the fibers from the tenia tecta. Neurons retrogradely labeled by ^{3}H-D-Asp were primarily found in the anterior olfactory nucleus, the surrounding anterior piriform cortex, the posterior pirform cortex, the endopiriform nucleus, the anterior cortical amygdaloid nucleus, the periamygdaloid cortex, and the entorhinal cortex. In addition, small numbers of labeled cells were seen in the olfactory bulb, the agranular insular cortex, the claustrum, the magnocellular basal forebrain, the nucleus of the lateral olfactory tract, and the midline nuclear complex of the thalamus.

Fewer neurons were retrogradely labeled by ^{3}H-D-Asp than by WGA-HRP in all of the regions except the posterior piriform cortex, the endopiriform nucleus, and the midline nuclear complex of the thalamus. Except for an occasional cell, neurons in the tenia tecta, the raphe nuclei, and the locus coeruleus were labeled by WGA-HRP but not by ^{3}H-D-Asp. (Supported by NIH NS09518 and ADRC AG05681.)

372.2

ASPARTATE-LIKE IMMUNOREACTIVITY IN CELL BODIES AND TERMINALS OF PRIMARY AFFERENTS. S. De Biasi, D.J. Tracey and A. Rustioni, Cell Biol. & Anat., Univ. North Carolina, Chapel Hill NC 27599.

Glutamate (glu) and aspartate (asp) may act as excitatory transmitters within the central nervous system. We have previously demonstrated glu-like immunoreactivity in central terminals and cell bodies of primary afferents; we present evidence here that aspartate may also be a transmitter in primary afferents, using an antibody raised against asp.

For pre-embedding EM-ICC, rats were perfused with 4% carbodiimide, and 50 µm sections taken from the superficial laminae of the dorsal horn were treated with primary antibody (1:10K) which was visualised using the ABC technique. For post-embedding EM-ICC, rats were perfused with 2.5% glutaraldehyde and 0.5% paraformaldehyde, and thin sections treated with primary antibody (1:5K) which was visualised using colloidal immuno-gold particles. Electron microscopy revealed labelled terminals, including some with scalloped borders characteristic of primary afferents, indicating the presence of aspartate-like immunoreactivity.

In further experiments, rats were perfused with 4% carbodiimide followed by 4% paraformaldehyde. Cryostat sections of dorsal root ganglia were treated with primary antibody against asp (1:5K), visualised using the ABC technique. Light microscopy showed that many of the small cell bodies (20-30 µm diameter) were intensely stained. Comparison of these sections with adjacent sections treated with anti-glu showed that in most small cells, asp and glu were co-localised. Pre-absorption of the aspartate antibody with free asp blocked staining in a dose-dependent manner, while pre-absorption with glu, NAAG, and NMDA did not affect staining of primary afferent cell bodies. These data support the hypothesis that some primary afferents release aspartate as a neurotransmitter.

372.3

SPINOCERVICAL TRACT TERMINALS ARE ENRICHED IN GLUTAMATE-LIKE IMMUNOREACTIVITY: AN ANTEROGRADE TRANSPORT - QUANTITATIVE IMMUNOGOLD STUDY IN THE CAT. J. Broman*, J. Westman* and O.P. Ottersen. Department of Human Anatomy, Uppsala University, S-751 23 Uppsala, Sweden (J.B., J.W.), and Anatomical Institute, Karl Johans Gate 47, N-0162 Oslo 1, Norway (O.P.O).

The present study was undertaken to investigate the possibility that glutamate may be a neurotransmitter in the lateral cervical nucleus (LCN), and special interest was focused on spinocervical tract (SCT) terminals. To identify such terminals, WGA-HRP was injected into the spinal cord of cats. After 3-5 days survival the animals were perfused and the upper part of the spinal cord was removed. Vibratome section containing the LCN were processed with tetramethylbenzidine and H_2O_2 and embedded in Durcupan. Ultrathin sections were then cut, collected on formvar coated nickel grids and processed for immunogold labeling with an antiserum produced against glutamate-glutaraldehyde-BSA conjugate.

The density of gold particles was assessed over 104 SCT terminals and compared with the gold particle density over terminals containing flattened or pleomorphic vesicles. The gold particle density over the SCT terminals was found to be significantly higher (by a factor of 2.44) than that over the terminals containing flattened or pleomorphic vesicles. Neuronal cell bodies in the LCN were also labeled by the glutamate antiserum.

The above findings suggest that glutamate may be a transmitter in the synapses between SCT terminals and LCN neurons. The labeling of neuronal cell bodies in the LCN is more difficult to interpret because of possible interference of metabolic pools of glutamate.

372.4

TWO CLASSES OF GLUTAMATE-IMMUNOREACTIVE SYNAPTIC TERMINALS IN THE GUINEA PIG SUPERIOR OLIVARY COMPLEX. R.H. Helfert, J.M. Juiz, S.C. Bledsoe, Jr., J.M. Bonneau*, R.J. Wenthold+, and R.A. Altschuler. Kresge Hearing Research Institute, Univ. of Michigan, Ann Arbor, MI, 48109; +Lab. of Neuro-Otolaryngology, NIDCD, NIH, Bethesda, MD, 20892.

The excitatory amino acid neurotransmitter glutamate is thought to play an important role in processing auditory information in the brain stem. To provide evidence for its presence and determine its localization, colloidal gold immuno-electron microscopy was used to identify glutamate-like immunoreactivity (GLU-LI) in specific classes of synaptic terminals in two nuclei of the superior olivary complex, the lateral superior olive (LSO) and the medial nucleus of the trapezoid body (MNTB).

The synaptic terminals exhibiting the most intense GLU-LI were those that contained large, electron-lucent vesicles. A second type of terminal, which contained flattened pleomorphic vesicles, exhibited a moderate level of GLU-LI. Only background labeling was found in terminals with oval pleomorphic vesicles and those with small, round vesicles.

In previous studies we have demonstrated that, in the LSO, terminals containing flattened pleomorphic vesicles exhibit intense glycine (GLY)-LI, and that they appose postsynaptic areas immunopositive for the glycine receptor. Thus, the possibility now exists that flattened pleomorphic vesicle terminals may actually co-contain GLU and GLY as neurotransmitters and/or neuromodulators. Studies are currently in progress to determine if co-containment of GLU and GLY in these terminals does indeed occur.

Supported by NS 24369 and NS 07106.

372.5

COMBINATION OF D-[3H]ASP RETROGRADE LABELLING AND IMMUNOCYTOCHEMICAL DETECTION OF L-GLU AND L-ASP IN THE PAG PROJECTION TO RAPHE MAGNUS. M. MULLETT*, S. ARANEDA*, G. GHILINI*, L. WIKLUND*, AND A. BEITZ. Dept. Vet. Biol., Univ. of Minn., St. Paul, MN 55108 and Lab. Physiol. Nerv., CNRS, Gif-sur-Yvette, France.

Neuronal connections using excitatory amino acids (EAA) can be identified with selective D-[^{3}H]ASP retrograde labelling. EAAs have been proposed as putative transmitters in the periaqueductal gray (PAG)-raphe magnus (RM) projection. The present study combines D-[^{3}H]ASP retrograde labelling with immunocytochemistry (ICC) to define which EAAs are involved in this pathway. In rats, D-[^{3}H]ASP (0.01M, 50 nl) was injected into the nucleus RM. After 17 hr. survival, the perfused brains were cut on a freezing microtome. Alternate floating sections were treated for ICC with mouse anti-L-Glutamate or rabbit anti-L-Aspartate serum. The tissue was subsequently processed for autoradiography. L-GLU immunoreactivity was found in 97% of the D-[^{3}H]ASP PAG cells in GLU reacted sections, while 94% of the D-[^{3}H]ASP cells contained L-ASP immunoreactivity in alternate sections. This study shows that autoradiographic D-[^{3}H]ASP retrograde labelling can be combined with ICC detection of EAA on the same sections. EAA transmission in the PAG projection to RM has been suggested, and the present results indicate that these neurons contain L-GLU and/or L-ASP. Supported by NSF grant BNS8607520 and NIH grants DE06682 and NS19208.

372.6

RELEASE OF EXCITATORY AMINO ACIDS (EAA) IN HIPPOCAMPUS: FROM K^+- INDUCED STIMULATION OF SLICES TO ELECTRICAL STIMULATION OF PATHWAY IN ORGANOTYPIC CULTURES. K.Q.Do, F.X.Vollenweider*, Z.P.Jian-Zhang*, B.Gähwiler and M.Cuénod. Brain Research Institute, University of Zürich, Switzerland.

Release of endogenous EAA induced either by 50 mM K^+ - depolarisation in rat hippocampal slices (A) or by electrical stimulation in organotypic cultures (B) was investigated. In (B), Schaffer's collateral (SC) pathway was stimulated by monopolar field stimulation (4 Hz, 1msec/pulse, 20-40 μA, 4 min) and the population response recorded in area CA1. The perfusate was collected with a cannula, the tip of which was centered above the stratum radiatum or the pyramidal cell layer of area CA1. The released materials from (A) or (B) was analyzed by precolumn derivatization HPLC to detect amino acids at the femtomole level. In (A), K^+ increased the efflux of Glu from 5.9 ±1.7 to 536.1 ±132.0 pmol/mg /min and of Asp from 2.6 ±0.5 to 66.0 ±11.7 pmol/mg /min. In (B), the resting efflux of Glu and Asp in area CA1 ranged from 0.3 to 7 pmol/min and 0.3 to 2 pmol/min respectively. Following stimulation of SC, Glu and Asp efflux was increased by 60-600 %. These results suggest that both Glu and Asp or a precursor of these amino acids are released from synaptic terminals of the SC. Moreover, in (A) the efflux of homocysteic acid which is a NMDA receptor preferring agonist in cat striatum was also increased from 0.08 ±0.01 to 0.5 ±0.2 pmol/mg /min. The combined analysis with sensitive methods of the released material induced by K^+ in slices and by electrical stimulation of pathways in cultures has proved to be an useful approach for the search of transmitters in identified neuronal pathways.

372.7

DISTRIBUTION OF GLUTAMINASE AND GLUTAMIC ACID DECARBOXYLASE IN THE HUMAN CEREBRAL CORTEX. H. Akiyama*, T. Kaneko*#, H. Tago*, P.L. McGeer, E.G. McGeer, H. Kimura*, N. Mizuno#. (SPON: E. Manuelidis). Kinsmen Lab., Univ. British Columbia, 2255 Wesbrook Mall, Vanc., B.C. V6T 1W5, Canada, #Dept of Anatomy, Kyoto Univ. Japan.

The glutamate-glutamine cycle has been considered to play a major role in generation of transmitter glutamate as well as of γ-aminobutyric acid (GABA) precursor glutamate. Phosphate activated glutaminase (PAG) catalyses conversion of glutamine to glutamate. In GABAergic structures, the glutamate is further synthesized into GABA by glutamic acid decarboxylase (GAD). The distribution of PAG and GAD were studied immunohistochemically in postmortem human cerebral cortex. Large to medium-sized pyramidal neurons in layer III and V stained intensely for PAG, while the majority of smaller pyramidal neurons were labeled either lightly or moderately. Modified pyramids, such as Betz cells, pyramidal cells of Meynert and solitary cells of Ramón y Cajal also stained intensely for PAG. Fusiform cells in layer VI showed moderate to intense labeling. In addition, a number of non-pyramidal neurons stained moderately to intensely. Observations of GAD stained nearby sections indicated that many and perhaps PAG(+) non-pyramidal neurons were GABAergic. Together with other evidence, PAG positive projecting neurons are considered to be glutamatergic, while PAG positive interneurons are GABAergic.

372.8

HOMOCYSTEATE-LIKE IMMUNOREACTIVITY IS LOCALIZED IN CEREBELLAR CLIMBING FIBERS. P. Grandes*, M. Cuénod and P. Streit. Brain Research Institute, University of Zürich, CH-8029 Zürich, Switzerland

Homocysteate has recently been proposed as transmitter candidate in the olivocerebellar climbing fiber pathway (Cuénod et al., 1988, Exp. Brain Res. Series 17:161, also for review). To investigate this issue further, we attempted to localize this sulphur containing, excitatory amino acid by means of immunohistochemistry.

Mice were immunized with glutaraldehyde-linked homocysteate-albumin conjugates, and polyclonal antibodies were obtained in ascitic fluids. These antibodies exhibited a high specificity for homocysteate in experiments involving various aminoacid-albumin conjugates and absorption (ELISA and immunohistochemistry) as well as staining tests (conjugates embedded; sections processed together with tissue). Immunohistochemistry at the level of light microscopy was performed on semithin sections of perfusion-fixed rat cerebellar tissue by means of a silver-intensified peroxidase-antiperoxidase method (Liu et al., 1989, Histochem. 90:427).

The most conspicuous elements containing homocysteate-like immunoreactivity (HCA-LI) were terminal-like dots and beaded fibrous profiles mostly in close association with Purkinje cell perikarya and dendrites. Occasionally, small labeled elements were noticed in the granule cell layer and in the white matter. HCA-LI was totally abolished in climbing fiber deprived cerebellar cortex 10 days following treatment with 3-acetyl-pyridine. This observation in cases with a lesioned inferior olive and the immunohistochemical findings in normal tissue strongly indicate that HCA-LI is contained in a considerable population of climbing fibers projecting to hemispheres and vermis of the rat cerebellum. Additional evidence, thus, has been obtained for the role of homocysteate as a climbing fiber transmitter.

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372.9

TRANSECTION OF THE OPTIC NERVE CAUSES LARGE DECREASES IN N-ACETYLASPARTYLGLUTAMATE, BUT NOT GLUTAMATE OR ASPARTATE IN SELECTED AREAS OF THE VISUAL SYSTEM IN THE RAT. L.Williamson, J.R.Moffett, D.Garrison, #A.C.Murphy, +M.Palkovits, J.H.Neale and M.A.A.Namboodiri. Department of Biology, Georgetown University, Washington, DC 20057, +Lab. of Cell Biology, NIMH and #Lab. of Cell Biology, NHLBI, NIH, Bethesda, MD 20892.

Several lines of evidence have been presented in recent years that support the hypothesis that N-acetylaspartylglutamate (NAAG) may be a transmitter of the optic tract in mammals. Glutamate has also been considered for a long time to be a transmitter of the optic system in many species. In the present study, we have determined the relative distribution of these two potential transmitters in the optic projections of the rat using the disappearance of their levels on transection as a reliable index.

The levels of NAAG, aspartate and glutamate were measured in ten selected areas of the rat visual system ten days following bilateral optic nerve transection and were compared with sham operated controls. Brain regions were microdissected by the punch technique and NAAG levels were measured by RIA (IC50: NAAG = 2.5 nM, NAA = 100 uM; smallest detectable amount = 1-2 pg/assay), while aspartate and glutamate levels were determined using an amino acid analyzer.

Large decreases (50-60%) in NAAG levels were detected in the suprachiasmatic nucleus, lateral geniculate and superior colliculus, while moderate losses (25-50%) were noted in the pretectal nucleus, nucleus of the optic tract, parietal cortex and occipital cortex 18. Little or no changes were detected in the paraventricular nucleus, pretectal area and occipital cortex 17. Also, no significant changes in glutamate or aspartate levels were detected in any of the above areas. These results support a transmitter role for NAAG but not for glutamate or aspartate in the visual system of the rat. (Supported by NIH Grant DK 37024 to MAAN and NIDA grant DA 02297 to JHN).

372.10

A POSSIBLE EXPLANATION FOR THE PURPORTED EXCITATION OF PIRIFORM CORTICAL NEURONS BY N-ACETYL-L-ASPARTYL-L-GLUTAMIC ACID (NAAG). Edward R. Whittemore and James F. Koerner. Department of Biochemistry and Neuroscience Grad. Program, Univ. of Minn., Minneapolis, MN 55455.

The excitation of piriform cortical neurons by iontophoresis of NAAG isolated from rat brain is frequently cited as major support for the possible neurotransmitter role of NAAG in the CNS (ffrench-Mullen, et al., PNAS 82 (1985) 3897). However, using synthetic NAAG, we are unable to reproduce this observation, and we offer an alternative explanation. Specifically, 23 neurons from 16 slices of rat piriform cortex which responded vigorously to iontophoresis of L-glutamate did not respond to iontophoresis of synthetic NAAG. Responses to NAAG were never observed despite extensive probing for NAAG-sensitive sites and ejection of NAAG using negative currents (10 mM NAAG in H_2O, pH=8) and positive currents (10 mM NAAG in 100 mM NaCl, pH=6.2). In contrast, iontophoresis of the potassium salt of synthetic NAAG (10 mM NAAG/20 mM KCl/100 mM NaCl), or of potassium ions (20 mM KCl/100 mM NaCl) using positive currents evoked single unit spiking. Iontophoretic responses to NAAG/K^+ and K^+ were inhibited by 200 μM L-2-amino-4-phosphonobutanoic acid (L-AP4) and desensitized rapidly, as reported previously. This suggests that residual potassium ions, remaining after the original purification, were responsible for the excitation attributed to NAAG. Supported by NIH NS 23935.

372.11

N-Acetylaspartylglutamate (NAAG): A Possible Modulator of [^{3}H]MK801 Binding in Rat Hippocampal Membranes. P.S. Puttfarcken and J.T. Coyle. Depts. of Neuroscience and Psychiatry. The Johns Hopkins School of Med., Balto., MD 21205.

Although NAAG is localized to putative glutamatergic pathways, its role as an excitatory neurotransmitter remains controversial because of variable neurophysiologic effects. The effect of NAAG on the specific binding of [^{3}H]MK801, the noncompetitive NMDA receptor antagonist, was examined in rat hippocampal membranes. To inhibit NAALADase activity, the assay was performed at 25°C in 20 mM Tris/Acetate buffer/1mM EGTA. The homogenates were incubated for 90 min with 5 nM [^{3}H]MK801; and the reaction was terminated by rapid filtration. Glutamate (GLU) has previously been reported to stimulate [^{3}H]MK801 binding by 100-200% with an IC_{50} of 250 nM. Preliminary data suggest that NAAG may be more potent, yet not as efficacious as GLU. NAAG stimulates [^{3}H]MK801 binding by approximately 40% above control levels with an IC_{50} of 50 nM. However the addition of NAAG to maximally stimulatory concentrations of GLU inhibited the augmentation of [^{3}H]MK801 binding by 55%. While N-acetyl aspartate exhibited activity in these two conditions, it was 20-fold less potent than NAAG. These results suggest that NAAG acts as a mixed agonist/antagonist at NMDA receptors.

372.12

HPLC ANALYSIS OF RADIOLABEL FOLLOWING RECEPTOR BINDING ASSAY WITH N-ACETYLASPARTYL-[3H]-GLUTAMATE REVEALS [3H]-GLUTAMATE TO BE THE BOUND ENTITY. H.M. Valivullah. Dept. of Biology, Georgetown University, Washington, D.C. 20057.

Studies of peptidase sensitivity of NAAG have called into question equilibrium binding data, which provided evidence of receptor-ligand-like interaction of radiolabeled N-acetylaspartyl-[3H]-glutamate with brain cell membranes.

Incubation (45 min, 37°C) of brain cell membranes and [^{3}H]-NAAG was followed by HPLC analysis of membrane bound radioligand. About 95% of the bound radiolabel comigrated with glutamate while only 5% comigrated with NAAG on HPLC. Assay of radioligand binding, in the presence of peptidase inhibitors, resulted in 85-95% reduction of radiolabel binding, relative to binding in the absence of peptidase inhibitor. These data suggest that speculation on the existance of high affinity binding sites for NAAG are not warranted in the absence of more rigorous analyses than previously reported. (Supported by NIDA grant DA 02297)

372.13

SPATIALLY RESOLVED TRANSIENT AND SUSTAINED [Ca^{2+}]i SIGNALS EVOKED BY EXCITATORY AMINO ACIDS IN CULTURED RAT HIPPOCAMPAL PYRAMIDAL NEURONS. Steven R. Glaum, Wendy K. Scholz and Richard J. Miller. Dept. Pharmacol. and Physiol. Sci., Univ. of Chicago, 947 E. 58th St., Chicago IL, 60615.

We examined $[Ca^{2+}]_i$ signals evoked by excitatory amino acids in cultures of hippocampal pyramidal neurons (see Abele et al., this volume) using a fura-2 based digital imaging technique. In HEPES/Hank's balanced salt solution, depolarization with 50mM K^+ or addition of NMDA (500μM in Mg^{2+}-free, 1μM glycine supplemented solution) increased $[Ca^{2+}]_i$ throughout the cell soma as well as in proximal and distal dendrites. Addition of glutamate (GLU, 500μM, normal medium) increased $[Ca^{2+}]_i$ primarily in the cell soma and in proximal dendrites. In Ca^{2+}-free medium, 50mM K^+ was ineffective, but GLU and caffeine (10mM) still produced a single, transient increase in $[Ca^{2+}]_i$. When challenged with GLU or NMDA more than once, cells exibited long lasting increases in $[Ca^{2+}]_i$ that persisted >20 min after drug washout. These sustained increases in $[Ca^{2+}]_i$ were due to maintained Ca^{2+} influx, as they were rapidly abolished in Ca^{2+}-free medium or reduced (25-50%) by the addition of nitrendipine (1μM) or Ni^{2+} (500μM). Tetrodotoxin (1μM), however, produced no inhibition. Replacement of extracellular Cl^- with SO_4^{2-} during and after addition of agonist prevented sustained Ca^{2+} influx, indicating that it may involve GLU-induced cell swelling. Thus GLU can differentially increase $[Ca^{2+}]_i$ in different parts of the neuron by activating both Ca^{2+} influx and mobilization from intracellular stores. Furthermore, GLU can produce long term changes in $[Ca^{2+}]_i$ that may be important for synaptic plasticity and/or excitotoxicity.

372.14

VARIATION IN GLUTAMATE RECEPTOR DENSITY IN CENTRAL NEURONS. L.O. Trussell & G.D. Fischbach. Dept. of Anatomy & Neurobiology, Washington U. Sch. of Med. 660 S. Euclid, St. Louis, MO.

Responses of NMDA (G1) and non-NMDA (G2) receptors to glutamate were examined in outside-out patches isolated from chick spinal neurons. Mg^{++}-insensitive channels of 18 pS maximal conductance were activated by 1 mM glutamate with an ensemble rise time of 1.2 msec and a rapid desensitization (τ = 2.2 msec). G1 receptors exhibited a much slower desensitization (τ > 100 ms) and a much higher agonist affinity than G2 receptors (K_d < 3 μM versus 500 μM).

G2 receptor response amplitudes varied considerably among patches, from 2 to 95 pA at -60 mV, corresponding to 2 to 110 channels/μm^2 (patch area, 0.8 μm^2). The variation in receptor density over the neuronal surface was examined in patches as well as in whole-cell ionophoretic studies. Some membrane regions exhibited G2 responses that were 5-13 fold greater than background (modal) values. These areas may contain subsynaptic clusters.

Removal of Mg^{++} block of G1 receptors in patches increased the steady-state glutamate current 6-fold. Taking this added current as a measure of G1 activity, we found a direct relation between G1 and G2 currents. However, the estimated number of active G2 receptors varied from 2 to 88, while G1 receptors in the patches varied only from 0 to 5. Thus, either G1 receptors have a lower opening probabililty than G2 receptors or are present at much lower density. Furthermore, the distribution of G1 receptors is apparently more uniform than that of G2 receptors.

372.15

GLUTAMATE AND GABA RECEPTORS ON BASAL FOREBRAIN NEURONS IN TISSUE CULTURE. A.A. Khan and R.W. Baughman. Department of Neurobiology, Harvard Medical School, Boston MA 02115.

The basal forebrain receives descending glutaminergic input from neocortex and gabaergic input from local circuit neurons. Cholinergic basal forebrain neurons are selectively damaged in some neurodegenerative diseases, and they are very sensitive to excitotoxins. We investigated the excitatory and inhibitory receptor profile of large (20-40 um) basal forebrain neurons in tissue cultures prepared from 8-10 day-old Long-Evans rat pups. Neurons were enzymatically dissociated (Baughman and Huettner '86) and plated on glial feeder layers. Whole-cell-patch voltage clamp recordings were made from cells in cultures 20-40 days old. The medium contained 0.5 μM TTX, 1 μM Gly and no added Mg^{2+}. Pipettes 1-1.5 μm ID were used for pressure application of glutamate, kainate, quisqualate, NMDA and Gaba. Kainate (20 μM-1.0 mM), quisqualate (1-50 μM) and NMDA (50 μM-1.0 mM) produced inward currents; the kainate and quisqualate responses were blocked by CNQX (20 μM), and the NMDA response was blocked by APV (50 μM). With focal application of glutamate, at a distance of 2-5 μm from the cell, a rapidly adapting response was observed (Trussell and Fischbach '88). Gaba application (100 μM) produced an outward current that was blocked by bicuculline (20 μM). These results indicate that magnocellular basal forebrain neurons have NMDA, non-NMDA and $Gaba_A$ receptors. (Supported by NIH NS26422 and ADRDA)

372.16

HETEROGENEOUS DISTRIBUTION OF EXCITATORY AMINO ACID RECEPTORS ON POSTNATAL NEURONS ACUTELY DISSOCIATED FROM RAT DORSAL HORN. O. Arancio, K. Murase, M. Yoshimura and A.B. MacDermott Dpt. Physiology and Center for Neurobiology and Behavior, Columbia Univ., NYC, NY 10032

Mapping of glutamate receptors on the soma and neurites of rat spinal cord neurons in culture has revealed heterogeneity of receptor distribution for each receptor subtype activated by its selective agonist, kainate, quisqualate or NMDA. Furthermore, areas of high sensitivity to one agonist do not always correspond to high sensitivity to the other two agonists. We have tested whether such differential patterns of receptor distribution occur on neurons allowed to develop *in vivo* rather than in culture. Neurons with long dendrites were obtained from 6–11 day old rats by mechanical dissociation following enzyme treatment and placed in a rapidly flowing bath. Recordings were made in voltage clamp using whole cell recording. Drugs were applied by pressure through thin–tipped (<1um id) pipettes. Responses to the three agonists were always present on both the soma and the neurites in all cells tested. In some cells, agonist sensitivity fell off with distance from the soma while in others, response to agonists occurred in an irregular pattern along the neurite with hot spots (areas of membrane with higher amplitude responses than adjacent areas on the same neurite). The percentage of cells with hot spots was 70%, 72% and 80% respectively for kainate, quisqualate and NMDA. In addition, comparison of the responses for two agonists at the same site on the neurite showed that hot spots for different agonists did not necessarily correspond. Thus, data from acutely isolated neurons support the results previously obtained with cells in culture.

372.17

SOMADENDRITIC BUT NOT AXONAL PRESYNAPTIC GLUTAMATE RECEPTORS MEDIATE GLUTAMATE-INDUCED STIMULATION OF GABA RELEASE IN DISSOCIATED CELL CULTURES FROM THE RAT SUPERIOR COLLICULUS. M. Perouansky* and R. Grantyn* (SPON: Y. Yaari). Max Planck Inst. Psychiatry, Martinsried, F.R.G. After 1 week in vitro cultured neurons from the superficial gray layer of the neonatal rat superior colliculus form synaptic networks where about half of the terminals display GAD-immunoreactivity (Warton, Perouansky and Grantyn, Dev.Brain Res.1989). Application of glutamate (Glu) to these cultures increased the frequency of spontaneous bicuculline-sensitive synaptic Cl currents, even if a TTX- and Cd-induced block of voltage-activated Na and Ca channels prevented generation and conduction of action potentials along the axons. To answer the question whether Glu receptors on GABAergic axon terminals would account for this effect we used a push-pull type microsuperfusion system for selective chemical stimulation of voltage clamped somata and attached terminals. Under these conditions stimulation of GABA release was still observed with 30 mM KCL, while Glu and its agonists failed to increase the frequency of bicuculline-sensitive synaptic Cl currents. A role of axonal Glu receptors in the regulation of GABA release is therefore unlikely.

EXCITATORY AMINO ACIDS: ANATOMY AND PHYSIOLOGY III

373.1

MK-801: EFFECTS ON PIRIFORM EEG AND EVOKED RESPONSES. Tim Otto, John Larson, Michel Baudry, and Gary Lynch. Center for the Neurobiology of Learning and Memory, Univ. Cal., Irvine, CA 92717.

MK-801, a non-competitive NMDA receptor antagonist, was administered (ip, 1 mg/kg) to rats implanted with a bipolar stimulating electrode in the LOT and a monopolar recording electrode in the ipsilateral superficial plexiform layer (1a) of piriform cortex. Piriform EEG was recorded continuously for several hours after administration of MK-801. EPSPs evoked by single pulse activation of the stimulating (LOT) electrode every 15 sec were recorded also, and were subsequently analyzed for slope and amplitude.

The effects of MK-801 on piriform physiology can be best described as a repeating cycle of three distinct stages. During Stage 1, EEG consisted of smooth, rhythmic, high frequency (200 Hz) activity. EPSPs during Stage 1 remained unchanged relative to baseline. Stage 2 EEG was composed of dramatically depressed low voltage activity and was accompanied by severely attenuated EPSPs. Stage 3 was characterized by high voltage seizure-like discharges and EPSPs approximately 200% of baseline. The cycle period remained consistent within subjects, but varied across subjects (10 -24 min). Within a cycle, Stages 2 and 3 lasted not longer than 1 min each, with Stage 1 comprising the remaining time. Eight to ten cycles, each composed of these three stages, were typically observed. An extended period (approximately 1 hr.) of highly attenuated EEG and EPSPs followed, succeeded by baseline EEG activity and EPSPs.

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373.2

THE EFFECTS OF THE NON-COMPETITIVE NMDA ANTAGONIST, MK-801 ON MONO AND POLYSYNAPTIC REFLEXES IN THE SPINALIZED CAT. M.E. Mann and P.A. Boxer. Parke-Davis Pharm. Res. Div., Warner-Lambert Co., Ann Arbor, MI 48105.

The dissociative anesthetic, ketamine, has been shown to selectively decrease spinal cord polysynaptic reflexes in cats at doses from 1 to 10 mg/kg, IV (Campbell et al., 1987). Ketamine has a variety of pharmacological actions, including non-competitive antagonism of the NMDA receptor through an interaction at the PCP site in the channel. The present experiments were designed to determine if the more potent and specific non-competitive NMDA antagonist, MK-801, had similar effects on reflex activity. Experiments were performed in C1-sectioned mongrel cats (2-4 kg) maintained under artificial respiration. Following isolation and cutting, a dorsal root (L6-S1) was stimulated (10X threshold) and reflex responses were recorded from a ventral root. The area under the curve of the monosynaptic reflex was unaffected by MK-801 at cumulative doses from 0.2 to 2.0 mg/kg IV, while the polysynaptic reflex showed a significant dose and time dependent decrease. The effects of 2.0 mg/kg MK-801 were similar to those seen with 10 mg/kg ketamine, indicating that in this system the potency ratio between these 2 drugs is very different from that predicted from binding at the PCP receptor. These data support the hypothesis that NMDA receptors are not involved in spinal cord monosynaptic reflexes, but do partially mediate polysynaptic reflexes.

373.3

SUSTAINED DEPOLARIZATIONS DURING SEIZURE-LIKE ACTIVITY IN CULTURED RAT HIPPOCAMPAL NEURONS. W.J. Koroshetz and E. J. Furshpan. Department of Neurobiology, Harvard Medical School, Boston, MA 02115.

Hippocampal neurons cultured for weeks or months in the presence of 1mM kynurenate and 11.3 mM Mg^{2+} showed, after withdrawal of these blocking agents, intense spontaneous activity including runs of paroxysmal depolarization shifts and, often, large (25-60 mV) sustained depolarizations (SDs); when prolonged, this seizure-like activity killed many of the neurons. SDs were often reversed by kynurenate or by APV (Furshpan, E. J. & Potter, D. D., 1989, Neuron, in press). SDs may be an indicator or precursor of impending cell death; we have studied the role of glutamate receptors and of action potentials in their generation and maintenance. We now report that, unlike APV, the non-NMDA antagonist, CNQX (10-20 μM; plus glycine, 500μM) was usually ineffective in reversing SD's although it often enhanced repolarization when added to APV; large SDs sometimes arose in the presence of CNQX, but not in the presence of APV. These observations emphasize a special role for NMDA receptors in the generation and maintenance of SDs. Tetrodotoxin (TTX; 1 μM) sometimes caused full reversal of SDs, especially when applied soon after SD onset; in other cases it failed to cause repolarization despite the continued effectiveness of APV or of kynurenate plus elevated Mg^{2+}. In addition, some neurons generated large APV-reversible SD's in the continuous presence of TTX. These observations suggest the presence, under some circumstances, of substantial glutamate release in the absence of Na-dependent action potentials. Cultures chronically grown in the presence of APV ($[Mg^{2+}]$ = 1.3 mM) also displayed SDs after washout of APV; however, paroxysmal depolarization shifts were less prominent in these cultures.

373.4

IN-VIVO INTERACTIONS OF PCP-RECEPTOR AGONISTS WITH MOUSE CEREBELLAR PURKINJE AND DOPAMINERGIC PROJECTIONS. J. CLER*, T.S. RAO, S. MICK*, S. IYENGAR and P.L. WOOD. (SPON: J. MICHEL). CNS Diseases Research, G.D. Searle & Co., St. Louis, MO 63198.

A number of pharmacological studies indicate a tight functional coupling between N-methyl-D-aspartate [NMDA] and phencyclidine [PCP] receptors. However, recent studies indicate the presence of a sub-population of PCP receptors which modulate dopamine [DA] metabolism in rat pyriform cortex to be uncoupled from NMDA receptor. The present study examined the effects of PCP agonists on mesolimbic, nigrostriatal and mesocortical DA metabolism, as well as cerebellar cyclic GMP in mouse. PCP agonists, (+)-MK-801 [5 mg/kg], ketamine [50 mg/kg], PCP, TCP and dexoxadrol [5 mg/kg each] potently decreased cGMP while (-)-MK-801 [0.3 mg/kg] and levoxadrol [5 mg/kg] were less effective than their active isomers. (+)-MK-801, ketamine and dexoxadrol increased striatal DA metabolism without affecting its release, while TCP, PCP, levoxadrol and (-)-MK-801 were ineffective. In olfactory tubercle, (+)-MK-801 and ketamine increased DA metabolism, while TCP, PCP, dexoxadrol, levoxadrol and (-)-MK-801 were ineffective. In pyriform cortex none of the PCP agonists were effective at increasing DA metabolism. These studies indicate differential interactions of PCP agonists with dopaminergic pathways and also suggest that mouse pyriform cortex is less responsive to PCP receptor agonists than rat pyriform cortex.

373.5

SYSTEMICALLY ADMINISTERED NMDA PRODUCES TOLERANCE IN RAT EEG. GEORGE P. ALBERICI, JACQUI-LINN GASKILL AND GEORGE F. STEINFELS. E. I. DuPont de Nemours & Co., Med. Prod. Dept., Wilmington, DE 19880-0400 USA.

NMDA has been implicated in a variety of physiological processes including learning and memory and neuronal cell death. Behavioral effects following systemic administration have been observed in drug discrimination and learning/memory paradigms. This study examined the effects of NMDA, administered subcutaneously (sc) on rat EEG. Two major EEG changes were noted: first, there was a large decrease (over 50%) in EEG amplitude approx. 6 minutes after dosing that returned to control levels within 6 minutes: second, there was a dose dependant (6.8,15.0 and 48.0 mg/kg) increase in relative spectral power of EEG delta band frequencies with the peak increase occurring approx. 15 to 30 minutes after dosing. When NMDA was re-administered 2 days later to previously dosed rats (15.0 mg/kg) , no EEG changes were seen, which suggested tolerance. In a second experiment 15.0 mg/kg NMDA was administered to 2 groups of rats. Two days later rats received either 15.0 or 48.0 mg/kg NMDA. Again no EEG changes occurred in the 15.0 group however EEG changes were seen in the 48.0 mg/kg group which suggests that the tolerance could be overcome by a higher dose. Pre-treatment with the NMDA antagonist CPP, prevented both the immediate EEG effects and tolerance development. The time course of tolerance was also evaluated. The onset of tolerance began 4-24 hours and was maximal at 48 hours. Quite surprisingly tolerance persisted up to 8-12 weeks.

373.6

NMDA-STIMULATED RELEASE OF TAURINE FROM ELECTRO-PHYSIOLOGICALLY-MONITORED RAT HIPPOCAMPAL SLICES. K.R. Magnusson, J.F. Koerner, and A.J. Beitz. Dept. of Veterinary Biology, Univ. of Minnesota, St. Paul, MN 55108.

The amino acid taurine has been shown to protect against neurotoxicity caused by certain excitatory amino acid analogs and has been shown to be released *in vivo* in response to high doses of N-methyl-D-aspartate (NMDA). The present study was undertaken to determine whether taurine release could also be stimulated by low (μM) concentrations of NMDA. Rat hippocampal slices were bathed with an artificial CSF media in an open flow-through chamber. The electrical field potential of the CA1 region was monitored throughout the experiment. The slices were exposed to varying concentrations of NMDA for a 4-6 minute period. With the use of 15μM NMDA, taurine release, measured by HPLC, peaked at 127% of prestimulation baseline levels between 2-6 min. following the initial exposure to NMDA and returned to baseline release levels by 8 minutes. The CA1 field potential recovered fully following exposure to 15 μM NMDA. With the use of 30, 60 and 120μM NMDA, taurine release peaked at 500%, 1000%, and 1300%, respectively, during the 4-6 min. sample and returned to baseline only after 30 minutes, 24 minutes after NMDA was totally cleared from the chamber. The CA1 field potential did not recover fully at these higher NMDA concentrations. The pattern of recovery resembled the events which occur during spreading depression. Taurine release may in part be due to direct stimulation by NMDA, however, taurine also appears to be released as a consequence of recovery events which follow NMDA exposure. Supported by NIH grant AG00329.

373.7

INTERACTIONS BETWEEN GLYCINE (GLY) AND N-METHYL-D-ASPARTATE (NMDA) ON RAT RED NUCLEUS CELLS RECORDED IN VIVO. J.M. Goldstein, L.M. Koch*, L.C. Litwin* and A.I. Salama. Dept. of Pharmacology, ICI Pharmaceuticals Group, ICI Americas Inc., Wilmington, DE 19897.

Recent in vitro electrophysiological studies have shown that GLY markedly potentiates NMDA responses in mammalian neurons and this effect is blocked selectively by the putative GLY antagonists 1-hydroxy-3-aminopyrrolidone-2 (HA-966) and 7-chlorokynurenic acid (7-CL KYN). We now report that this GLY modulatory site is functional in the intact adult rat brain. Extracellular single unit recording techniques and iontophoresis were employed in chloral hydrate anesthetized rats to measure the response of red nucleus cells to NMDA, GLY, d-serine, HA-966 and 7-CL KYN. Iontophoretic application of NMDA (50 mM) caused a marked excitatory effect which could be greatly enhanced by coiontophoresis of either GLY (100 mM) or d-serine (200 mM). Both HA-966 and 7-CL KYN antagonized the excitatory effects of NMDA, but there were differences in potency and efficacy. Whereas 7-CL KYN produced near maximal inhibition of NMDA at 25 mM, the inhibitory effects of HA-966 plateaued at less than maximal at concentrations up to 64 mM. The inhibitory effects of HA-966 and 7-CL KYN were reversed by subsequent iontophoretic application of GLY or d-serine. These results show that agonists and antagonists acting at the GLY modulatory site of the NMDA receptor complex interact similarly under in vivo or in vitro conditions.

373.8

WITHDRAWN

373.9

VASOPRESSIN INDUCED EXCITATIONS IN THE RAT LATERAL SEPTUM ARE DISPLAYED VIA ACTIVATION OF THE NMDA RECEPTOR. P. van den Hooff, M. Peppelenbosch and I.J.A. Urban. (SPON: D. Bradford). Rudolf Magnus Institute for Pharmacology, University of Utrecht, Vondellaan 6, 3521 GD Utrecht, The Netherlands.

The excitatory effect of vasopressin (VP) on lateral septum neurons was studied with intracellular micro-electrodes in brain slices. Recordings were made different membrane potentials using current injection through the electrode. At membrane potentials at least 10 mV below the firing level (which is around -55 mV), bath application of $10^{-6}/10^{-8}$ M VP in only 10% of the cells resulted in a reversible membrane depolarization accompanied by the generation of action potentials. At membrane potentials around the firing level 90% of the cells responded to VP by increased firing rates. These effects could be prevented by the V1 receptor-antagonist and be reversed with $5*10^{-5}$ M 2APV, a specific antagonist for the NMDA receptor. In 5 slices superfused with with a "zero" magnesium medium 1uM VP caused in 3 neurons a membrane depolarization of 7 ± 0.7 mV. We conclude that magnesium ions interfere with the excitatory action of VP, presumably in a voltage-dependent way that may involves NMDA receptors.

373.10

EFFECTS OF POLYAMINE SPIDER TOXINS ON NMDA RECEPTOR-MEDIATED TRANSMISSION IN RAT HIPPOCAMPUS *IN VITRO*. A.L. Mueller[1], A.H. Ganong[2], B.C. Albensi[1]* and H. Jackson[1]. [1]Natural Product Sciences, Inc., Salt Lake City, UT 84108 & [2]Pfizer Central Research, Groton, CT 06340.

Previous reports have demonstrated that polyamine-containing toxins from spider venoms block glutamate-mediated synaptic transmission in the mammalian CNS. Further, such toxins have more recently been reported to selectively suppress transmission mediated by non-NMDA (quisqualate and kainate) type glutamate receptors (Ashe et al., Brain Res. 480:234, 1989; Saito et al., Brain Res. 481:16, 1989). Our results, however, suggest that at least some of the polyamine-type spider toxins are selective NMDA receptor antagonists. Standard techniques were used for preparing and recording from submerged hippocampal slices. Stratum radiatum was stimulated every 60 sec and the effects of bath applied toxins on population spike (PS) and field EPSP responses in CA1 were monitored. Responses mediated primarily via non-NMDA receptors were largely insensitive to the spider toxins. Argiotoxin636 (ARG636), from *Argiope aurantia*, NSTX3, from *Nephila maculata*, and three polyamine toxins from the funnel-web spider *Agelenopsis aperta* had no significant effect on the PS at concentrations up to 100 µM. However, the induction of long-term potentiation, an NMDA receptor-mediated phenomenon, could be blocked by application of the *Agelenopsis* toxins (100 µM) or ARG636 (10-50 µM). This effect was variable and is under further investigation. Similarly, the normal field EPSP was unaffected by 10 µM ARG636 or ARG659. However, the NMDA receptor-mediated EPSP, recorded in the presence of DNQX and lowered Mg^{2+}, is suppressed about 70% by 10 µM ARG636 or ARG659. These results suggest that at least some of the polyamine-type spider toxins selectively suppress NMDA receptor-mediated synaptic transmission in the mammalian CNS.

373.11

EFFECTS OF CALCIUM ANTAGONIST PEPTIDE SPIDER TOXINS ON HIPPOCAMPAL SYNAPTIC TRANSMISSION STUDIED *IN VITRO*. B. C. Albensi*, A. L. Mueller and H. Jackson. Natural Product Sciences, Inc., Salt Lake City, UT 84108.

A peptide fraction termed AG1 from funnel web spider (*Agelenopsis aperta*) venom suppresses synaptic transmission in the CNS by blocking voltage-sensitive calcium channels (VSCC) (Jackson and Parks, Ann. Rev. Neurosci. 12:405, 1989). We have examined the ability of purified peptides from *A. aperta* to suppress synaptic transmission in rat hippocampal slices maintained *in vitro*.

Rat hippocampal slices were submerged and superfused with oxgenated aCSF (recirculating system, volume 2 ml). Stratum radiatum was stimulated every 60 sec, and the effects of bath applied toxins on population spike (PS), field EPSP, and afferent volley (AV) responses in CA1 were monitored.

Two peptides, tentatively designated K and 19, totally suppressed the PS at concentrations of 2 µM or less. This effect is quite similar to that seen with 1-5 µM omega-conotoxin. The effects of peptide K were largely reversible over a 30-60 min wash whereas no significant recovery from peptide 19 was seen. These peptides also blocked the field EPSP with no significant effect on the AV. Results of changing extracellular $[Ca^{2+}]$ as well as results from other experimental approaches [e.g. Artman et al., this meeting] indicate that these toxins are novel VSCC antagonists in the mammalian CNS.

373.12

EVIDENCE FOR A ROLE OF GLYCINE IN AREA TEMPESTAS FOR TRIGGERING CONVULSIVE SEIZURES J. Wardas*, J. Graham* and K. Gale Dept. Pharmacology, Georgetown University Medical Center, Washington, D. C. 20007

Previous studies have demonstrated that convulsions induced by the unilateral microinjection of bicuculline in area tempestas (AT; an epileptogenic site in the deep prepiriform cortex) are prevented by the focal application of competitive NMDA receptor antagonists in the same site. If glycine is involved in the activation of NMDA receptors in AT, then blockade of the glycine site should also prove anticonvulsant in this region. Therefore, we evaluated the effect of 7-chloro-kynurenic acid (7-CLKYN) in AT on bicuculline-evoked convulsions. Bicuculline methiodide (118 pmol in 120 nl) was unilaterally microinjected into AT 5 min after the application of 7-CLKYN into the same locus. 7-CLKYN (2.5 nmol in 250 nl) protected 100% of the animals against convulsions; a lower dose (1.25 nmol) protected 33% of the animals. Kynurenic acid (KYN) was also effective, but 6.25 nmol was required in order to protect the majority of animals against convulsions. Thus, 7-CLKYN was at least four-fold more potent than KYN for preventing convulsions triggered from AT. As 7-CLKYN is known to be significantly more potent than KYN as an antagonist at the glycine regulatory site, but is of comparable potency to KYN as a direct antagonist at NMDA receptors, our results implicate glycine antagonism as a mechanism for preventing convulsions triggered via NMDA-receptor activation. This would suggest that endogenous glycine participates in the process of seizure induction from AT.

373.13

SEX DIFFERENCES AND INVOLVEMENT OF THE DOPAMINE SYSTEM IN BEHAVIORAL RESPONSIVENESS TO MK-801. H.E. Criswell, R.A. Mueller and G.R. Breese. Univ. of North Carolina School of Medicine Chapel Hill, NC 27514.

Dose response curves for the effect of the NMDA antagonist MK-801 on activity were shifted to the left when female rats were compared to males indicating that females were more sensitive. Furthermore, repeated administration of doses as low as 0.03 mg/kg to females resulted in behavioral sensitization to MK-801, while a dose of 0.3 mg/kg was required for a similar effect in males. Several lines of evidence suggest that the dopamine system was only minimally involved in these actions of MK-801. Neither neonatal- nor adult- 6-OHDA lesions blocked the effects of MK-801. In fact, neonatal-6-OHDA lesions augmented its activating effect. When individual behaviors were examined in neonatal-6-OHDA lesioned rats, MK-801 did not produce the same behaviors as a dopamine agonist. There was, however, some interaction between the NMDA and dopamine systems as MK-801 blocked some of the behaviors including self-biting induced by L-DOPA in these rats. The increased activity level following MK-801 administration was only partially blocked by pretreatment with α-methyltyrosine or the combination of a D_1- and D_2-dopamine antagonist. neither dopamine antagonist was effective alone. These data suggest that there is a functional link between dopamine and NMDA receptor mediated systems but that other neural systems contribute to behavioral changes accompanying NMDA receptor blockade. Supported by USPHS grants NS21345, HD23042 and HD03110.

373.14

EVIDENCE FOR IN VIVO POTENTIATION BY GLYCINE OF THE ACTIVITY OF A GLUTAMATE-SENSITIVE SYMPATHOINHIBITORY AREA IN CAT MEDULLA. C.W. Dempesy, D.E. Richardson, and C.J. Fontana*. Lab. of Neurosurgery, Tulane Univ. School of Medicine, New Orleans, LA 70112.

We have previously detected in cat anesthetized with pentobarbital sodium a sympathoinhibitory area located adjacent to the rostral end of the compact division of the ambiguus nucleus (Neurosci. Abst. 14:192, 1988). Microinjections of glutamic acid (Glu, 5 nmol) into this area induce hypotension and bradycardia, and bilateral lesions of this area eliminate the sympathetic component of baroreflex induced by phenylephrine (PeSBR). We now report that, although serial microinjections of glycine (Gly, in increments of 50 ng) in this area will finally inhibit its activity, in approximately half the cases tested the initial doses of Gly potentiated its activity. Within the range of 50-200 ng of Gly: 10 of 23 sites yielded enhanced hypotension (in mmHg, 30 ± 5 before to 45 ± 6 after, from baseline 125 ± 8) and bradycardia (in bpm, 13 ± 3 before to 24 ± 4 after, from baseline 197 ± 8) in response to Glu; 13 of 20 sites (treated bilaterally) led to enhanced PeSBR (as a ratio of heart rate fall to blood pressure rise, .30 ± .08 before to .54 ± .07 after). Cats were vagotomized or prepared with atropine (1 mg/kg) to ensure sympathetic activity only. Further work is needed to establish that this Gly-potentiation is similar to that seen in cultured cell preparations (J.W. Johnson and P. Ascher, Nature 325:529, 1987).

373.15

PHENCYCLIDINE AND ITS ANALOGUES INTERACT SELECTIVELY WITH NMDA RECEPTORS TO DEPRESS THE POLYSYNAPTIC REFLEX IN NEONATAL RATS, *IN VITRO*. Y. Ohno and J.E. Warnick. Dept. of Pharmacol. & Exp. Ther., Univ. of MD Sch. of Medicine, Baltimore, MD 21201.

The differential sensitivity of mono- and polysynaptic reflexes to phencyclidine (PCP) and its analogues was examined in a Mg^{2+}-free physiological solution using an *in vitro* spinal cord preparation. The cords were removed from male Wistar rats (6-9 days old), hemisected and superfused with oxygenated physiological solution. Stimulation of an L_{3-5} dorsal root evoked a response in the corresponding ventral root which was recorded with a suction electrode. The monosynaptic reflex was relatively resistant to NMDA antagonists [Mg^{2+}, 2-amino-5-phosphonovalerate (APV) and 2-amino-7-phosphonoheptanoate (AP7)] while the polysynaptic reflex was markedly reduced in a concentration-dependent manner. The magnitude of the monosynaptic reflex only decreased 20-30% at concentrations of Mg^{2+} (1.3 mM), APV (10 μM) and AP7 (10 μM) which completely depressed the polysynaptic reflex. PCP and its analogues also selectively depressed the polysynaptic reflex in a concentration-dependent manner and had relative potencies consistent with those for the PCP receptor [*i.e.*, 1-(1-*m*-aminophenylcyclohexyl)piperidine = MK-801 [(+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a,d*]-cyclohepten-5,10-imine maleate] > 1-[1-(2-thienyl)cyclohexyl]piperidine $\geq$ PCP > > (+)-*N*-allylnormetazocine > > 1-(1-*m*-nitrophenylcyclohexyl)piperidine. The latter compounds depressed the monosynaptic reflex to the same extent as Mg^{2+}, APV and AP7 at concentrations which completely depressed the polysynaptic reflex. Furthermore, the depression of the reflexes by PCP was unaffected by haloperidol and methiothepin precluding the involvement of sigma and serotonin receptors in PCP-induced depression of the polysynaptic reflex. Our results suggest that PCP and its analogues selectively depressed the polysynaptic reflex through PCP receptors associated with the NMDA-aspartate receptor complex. (Supported in part by USPHS grant DA-02804.)

EXCITATORY AMINO ACIDS: ANATOMY AND PHYSIOLOGY IV

374.1

KAINIC ACID ACTIVATES FUNCTIONAL PATHWAYS FROM CORTEX AS SHOWN BY ACUTE 2-DG AUTORADIOGRAPHY IN THE GERBIL. H. Scheich, H. Steffen*. Inst. Zool., Univ. Darmstadt, FRG. Local injections of Kainic acid (KA) have been widely used to produce selective brain lesions. We have mapped the primary KA induced neuronal activation in 2-deoxyglucose (2DG) sessions shortly after unilateral small injection of KA into auditory, visual or somatosensory cortex (2 nMol KA in 1 µL saline injected under halothane, injection i.p. of 20µCi 14-C-fluoro-2DG in awake animals 15 min after KA, 45 min 2 DG exposure in lighted sound proof box). Beside local increase of metabolic activity around injection sites through the depth of cortex specific patterns of activation and suppression occurred in distant ipsilateral brain areas. Largely independent of modality of injected cortex the limbic structures med. prefrontal, entorhinal and olfactory cortex, basolat. n. of amygdala, n. accumbens, tuberculum olfactorium and parts of the striatum showed massive increase. Specific thalamic nuclei, some neocortical areas, and hippocampus showed regional activation depending on modality of injected cortex while other thalamic structures were suppressed. The lack of mirror foci of activation contralateral to injected cortices was remarkable. It is assumed that neuron classes in sensory cortices have different responses to global saturation of KA type glutamate receptors. This could lead to preferential activation of limbic output from cortex over several synapses, to differential activation of cortico-thalamic and cortico-striatal feedback and to sparing of callosal and other connections.

374.2

DIFFERENTIAL EFFECTS OF NMDA, QUISQUALATE AND KAINATE ON [^{3}H]DOPAMINE RELEASE FROM MESENCEPHALIC CELL CULTURES P. Boksa, H. Mount, I. Chaudieu*, J. Kohn* & R. Quirion. Douglas Hospital Res. Ctr., Depts. Psychiat. & Pharmacol. McGill Univ., Verdun, PQ, Canada H4H 1R3.

NMDA, quisqualate (QA) and kainate (KA), respective agonists for 3 excitatory amino acid (EAA) receptor subtypes, stimulated [^{3}H]dopamine ([^{3}H]DA) release from rat dissociated mesencephalic cells in culture (Mount et al. Soc. Neurosci. 13:937, 14:481). Release evoked by all 3 agonists was Ca^{2+}-dependent and inhibited by broad-spectrum EAA antagonists (cis-2,3-PDA, & kynurenate). However, kynurenate and cis-2,3-PDA were more potent against KA than QA and only KA-evoked release was blocked by GAMS (IC_{50} 700µM). NMDA-stimulated [^{3}H]DA release was selectively inhibited by competitive (CPP & APV) and non-competitive (PCP & MK-801) NMDA-receptor antagonists. In 1.2mM Mg^{2+}, NMDA-stimulated [^{3}H]DA release was Na^+-dependent and inhibited by tetrodotoxin (TTX). However, in 0 Mg^{2+}, NMDA-evoked release was neither TTX-sensitive, nor Na^+-dependent. Thus, TTX-sensitivity of the NMDA response in 1.2mM Mg^{2+} apparently occurs because Na^+-action potentials are required to alleviate a Mg^{2+} blockade. Neither QA-, nor KA-evoked release was affected by Mg^{2+} or TTX. In low Na^+ buffer, the QA response was increased more than 4X, while KA-evoked release was unaltered.

It is concluded that NMDA, QA and KA may release [^{3}H]DA via separate receptor subtypes. TTX-insensitivity of EAA-stimulated release suggests that interneurons do not mediate EAA-stimulated DA release. Supported by FRSQ.

374.3

PHENCYCLIDINE EVOKES RELEASE OF [^{3}H]DOPAMINE FROM MESENCEPHALIC CELL CULTURES H. Mount, P. Boksa, I. Chaudieu* & R. Quirion. Douglas Hospital Research Centre, Depts. Pharmacol. & Psychiat., McGill Univ., Verdun, PQ, Canada, H4H 1R3.

Phencyclidine (PCP) inhibits dopamine (DA) release stimulated by excitatory amino acids at the NMDA-receptor. Conversely, PCP may also enhance DA release, by inhibiting DA reuptake or directly stimulating release. We examined the effect of PCP and related compounds on [^{3}H]-DA release from rat dissociated mesencephalic cells in culture. PCP and MK-801 inhibited NMDA-evoked [^{3}H]-DA release (IC_{50}s of 2.3µM & .25µM, respectively). At concentrations $\geq$ 100µM, PCP, MK-801 and TCP stimulated Ca^{2+}-independent [^{3}H]-DA release. A competitive NMDA-receptor antagonist, dl-APV, had no such effect on basal DA release. The effect of PCP was not mediated by inhibition of DA uptake, since MK-801, which has low potency in blocking DA uptake (Snell et al. '88), evoked release while the DA uptake inhibitor, GBR 12783 (.01µM-1mM), did not. Also, TCP-evoked release was not inhibited by the σ-opiate antagonist, haloperidol (100µM). It is unlikely that [^{3}H]-DA release was mediated by an anaesthetic effect of PCP since naloxone (0.1µM-1mM), which has been reported to release DA via an anaesthetic action, failed to evoke release from the cells. These data concur with an earlier suggestion (Rao et al. Soc. Neurosci. 14:1049) that PCP-receptor agonists may evoke DA release by a mechanism independent of effects on DA uptake, or on NMDA- or σ-opiate-receptors. Supported by FRSQ.

374.4

INHIBITION OF EXCITATORY AMINO ACID STIMULATED [^{3}H]DOPAMINE RELEASE BY CYCLOHEXYLADENOSINE IN CELL CULTURES OF RAT MESENCEPHALON. I. Chaudieu*, H. Mount, J. Diorio*, R. Quirion & P. Boksa. (SPON: A. Fournier) Douglas Hosp. Res. Ctr., Depts. Psychiat. & Pharmacol., McGill Univ., Verdun, PQ., Canada.

Numerous reports suggest a close relationship between A1 adenosine receptors and excitatory amino acid (EAA) neurotransmission. For e.g., adenosine A1 and NMDA receptors are similarly distributed in rat brain, and both adenosine agonists and NMDA antagonists protect against neuronal death following seizures or ischaemia. To test for a functional interaction between adenosine and EAA receptors at the cellular level, the present study examined the effects of cyclohexyladenosine (CHA), an A1 receptor agonist, on [^{3}H]dopamine ([^{3}H]DA) release stimulated by EAAs from cell cultures of fetal rat mesencephalon. CHA (100µM) inhibited release evoked by NMDA, quisqualate and kainate (preferred agonists at 3 EAA receptor subtypes) and glutamate by 90%, 39%, 37% and 50%, respectively. Neither K^+-evoked, nor basal [^{3}H]DA release were affected by CHA, indicating that the effect of CHA is somewhat specific for the EAA-receptor mediated response. [^{3}H]DA release evoked by the Na^+ channel activator, veratridine, was also inhibited by CHA. These results indicate that A1 adenosine receptors and EAA receptors (mainly but not exclusively of the NMDA subtype) may interact to modulate DA release. Supported by FRSQ.

374.5

EFFECTS OF THE EXCITATORY AMINO ACIDS ON NEURONS ISOLATED FROM SPINAL TRIGEMINAL NUCLEI. Y.-P. Gu* and L.-Y.M. Huang (SPON: F.A. Kutyna). Marine Biomedical Institute, University of Texas Medical Branch, Galveston, TX 77550.

L-glutamate is a major excitatory transmitter involved in synaptic transmission between primary sensory neurons and projection neurons in trigeminal nuclei. In order to identify various glutamate receptors mediating synaptic transmission in this system, we have studied the actions of quisqualate (QA), kainate (KA) and N-methyl-D-aspartate (NMDA) in acutely dissociated neurons isolated from spinal trigeminal nuclei using whole cell patch recordings. Under voltage clamp conditions, QA, KA and NMDA elicited currents in all the cells tested. The current voltage relations of QA and KA were relatively linear. In the presence of Mg^{++}, the NMDA receptor mediated current voltage curve had a negative slope conductance between -90 and -50 mV. The NMDA responses were abolished 2mM D(-)-2aminophosphovaloric acid (APV). The dose-response experiments were also performed for the three agonists. The apparent dissociation constants were 9 μM for QA, 48 μM for NMDA and 260 μM for KA. Among the three agonists, QA had the highest affinity and KA had the lowest affinity for their respective receptors. The maximum response produced by KA was 3-4 times that produced by QA. Glycine greatly potentiated the magnitude of NMDA responses but only produced a small reduction of the apparent dissociation constant for NMDA. Supported by NS23061 and NS01050.

374.6

THYROTROPIN RELEASING HORMONE INDUCED GLUTAMATE RELEASE FROM THE LATERAL HYPOTHALAMUS IN VIVO. A.-L. Sirén, K. Xu* and G. Feuerstein. Dept. of Neurol., USUHS, Bethesda, MD 20814.

We recently reported that the glutamate (GLU) n-methyl-d-aspartate receptor blocker MK-801 attenuated the pressor and vasoconstrictor responses produced by hypothalamic microinjections of thyrotropin releasing hormone (TRH). To prove that GLU mediates the hemodynamic responses to TRH we studied the effect of TRH on GLU release from LH in conscious male Sprague-Dawley rats (325-350 g, n=5-7). Twenty-four to 48 hrs after unilateral implantation of a microdialysis probe into the LH, the rats were perfused with artificial CSF for 3 hrs; TRH (20 mg/kg i.p.) was administered and perfusion fluid collected and analyzed by HPLC for several amino acids. The baseline levels of GLU, aspartate (ASP) and glutamine (GLN) before TRH were 1.9±0.4 pmol/μl, 1.2±0.2 pmol/μl and 23.4±2.9 pmol/μl, respectively. TRH increased ASP by more than 4-fold and GLU by 1.5 fold with no significant effect on GLN output. Perfusion of LH with K^+ (20 mM, 80 mM) increased ASP and GLU levels in the perfusate in a dose-related manner but had no effect of GLN, alanine or histidine release. Together with the previous findings the present results imply a role for GLU and ASP in the hypothalamus in mediation of the cardiovascular effects of TRH.

374.7

ROLES OF NMDA AND NON-NMDA EXCITATORY AMINO ACID RECEPTORS IN THE COMPUTATION OF MOTION BY NEURAL CIRCUITS. P.S. Ulinski, L.J. Larson-Prior, and N.T. Slater. Dept. of Physiology, Northwestern University Medical School and Dept. of Organismal Biology and Anatomy, Univ. of Chicago, Chicago, IL U.S.A.

While excitatory amino acids (EAAs) are known to play central roles in synaptic transmission in a variety of neural systems, there are few examples where their functional role in a *defined* behavior has been demonstrated. We have used data on the anatomy and synaptic physiology of turtle visual cortex to develop a model that links the functional properties of EAA receptors in cortical circuitry to the turtle's ability to follow moving objects in visual space. Single unit studies indicate that cells in turtle visual cortex respond well to small moving stimuli anywhere in binocular visual space and likely participate in visually guided tracking behaviors. Anatomical studies explain how a convergence of points in visual space onto such cortical cells generate these receptive field properties. They show that the azimuth lines of visual space are represented in isoazimuth lamellae that extend from lateral to medial in visual cortex. Cells within a lamella receive inputs from geniculate neurons responsive to stimuli anywhere on a given vertical meridian. A stimulus on the horizontal meridian at time t=0 excites neurons throughout the appropriate isoazimuth lamella in visual cortex via fast geniculocortical responses mediated by non-NMDA receptors. Intracortical systems then excite neurons in adjacent isoazimuth lamellae through both non-NMDA and NMDA receptors with delays of several msec. As the stimulus moves past the turtle, these same neurons are activated continuously for several hundred msec, resulting in a short-term potentiation of synaptic transmission that lasts up to 1.8 sec and is NMDA receptor-mediated. Consequently, cortical neurons respond robustly to moving stimuli, but only weakly to stationary stimuli. An ensemble of such neurons can code for objects moving continuously past the animal for distances much larger than the receptive fields of individual retinal ganglion or geniculate cells. Supported by NS17489 & NS25682.

374.8

EXCITATORY AMINO ACID RECEPTOR-MEDIATED TRANSMISSION IN GENICULOCORTICAL AND INTRACORTICAL PATHWAYS IN VISUAL CORTEX. L.J. Larson-Prior, P.S. Ulinski, and N.T. Slater. Dept. of Physiology, Northwestern University Medical School and Dept. of Organismal Biology and Anatomy, Univ. of Chicago, Chicago, IL. U.S.A.

Excitatory amino acid (EAA) receptors have been shown to play important roles in both synaptic transmission and plasticity in visual cortex. However, the EAA receptor subtypes that mediate transmission in the geniculocortical and intracortical pathways have not been clearly differentiated. Such a differentiation is of considerable importance in understanding how cortical circuitry mediates visual function. We have used a preparation of turtle brain in which the integrity of the visual cortex and geniculocortical inputs are maintained *in vitro* to compare the EAA receptor subtypes involved at geniculocortical and intracortical synapses. Stimulation of the geniculocortical fibers at subcortical loci produces monosynaptic EPSPs in visual cortical neurons which are blocked by the broad spectrum EAA receptor antagonist kynurenate (1-2 mM). These fast responses are also blocked by the non-NMDA antagonist DNQX (10 μM), but not by the NMDA antagonist DL-AP-5 (100 μM). Stimulation of intracortical fibers evokes multicomponent EPSPs. An early component is reduced by kynurenate and DNQX, but not DL-AP-5. An EPSP of intermediate latency and time course remains in the presence of any of the EAA receptor antagonists tested. The late component is blocked by DL-AP5, but not by DNQX. These results provide evidence that the fast, monosynaptic EPSP arising from activation of geniculocortical fibers is mediated via non-NMDA receptors. The multicomponent intracortical EPSP is more complex, and is mediated by non-NMDA, non-EAA, and NMDA receptors. These components of the intracortical EPSP are likely to arise from both mono- and polysynaptic elements which play a role in motion analysis in turtle visual cortex. Supported by NS17489 & NS25682.

347.9

AN *IN VIVO* EVALUATION OF NMDA AND QUISQUALATE RECEPTOR-MEDIATED TRANSMISSION AT THE COMMISSURAL SYNAPSE ON CA1 PYRAMIDAL CELLS. Edda Thiels and Donald J. Weisz, Department of Behavioral Neuroscience and Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260.

The electrophysiological responses of hippocampal CA1 pyramidal cells following application of L-glutamate are mediated by various receptor subtypes, most notably the quisqualate (QUIS) and *N*-methyl-D-aspartate (NMDA) receptors. As part of the development of a method for the identification of compounds that selectively modulate NMDA receptor-mediated transmission at the commissural-CA1 synapse in the intact hippocampus, we determined the effects of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), a selective QUIS antagonist, and D-2-amino-5-phosphonovalerate (APV), a selective NMDA antagonist, on CA1 dendritic field excitatory postsynaptic potentials (EPSP) elicited by either low or high frequency stimulation in vivo. Pressure injections (0.4 μl at a distance of 300 μm from the recording tip) of CNQX reduced EPSP amplitude, measured at 2 ms after EPSP onset, in a dose-dependent fashion, whereas APV had no effect on EPSP topography elicited by low frequency stimulation. Both CNQX, at doses that completely suppressed the EPSP, and APV blocked the induction of long-term potentiation (LTP) following high frequency stimulation that reliably produced LTP in control rats. Using the same test arrangement, we delineated the novel compound azetidine-2,4-dicarboxylic acid (AZT) to function as a selective NMDA agonist: low doses (≤ 100 μM) produced spontaneous bursting, whereas high doses caused local seizures; EPSP amplitudes subsequently were suppressed in a dose-dependent fashion. These effects of AZT were blocked by prior administration of either APV or MK-801, a noncompetitive NMDA receptor antagonist. (Supported by a gift from the Johnson and Johnson Focused Giving Program.)

347.10

EVIDENCE FOR AN EXCITATORY AMINO ACID PROJECTION FROM THE VENTROMEDIAL HYPOTHALAMUS TO THE MEDIAL ZONA INCERTA. M.J. Eaton and R.L. Moss Dept. of Physiol., Univ. Texas Southwestern Med. Ctr., Dallas, TX 75235

Anatomical studies have shown afferent input to the medial portion of the zona incerta (ZI) from the ventromedial hypothalamic nucleus (VMH). In previous *in vitro* electrophysiological studies, we have demonstrated that VMH neurons project to and terminate in the medial ZI. The predominant orthodromic response is excitation. The present study was designed to elucidate the identity of the transmitter mediating this excitatory response, by attempting to block the orthodromic excitation of medial ZI neurons with kynurenic acid (KYN), an excitatory amino acid antagonist. Transverse vibratome sections (425 microns) through the ZI and VMH were obtained from Sprague-Dawley female rats. Extracellular single cell recordings were made from 17 orthodromically excited neurons located in the medial ZI. Using peristimulus time histograms, a neuron was considered to be orthodromically excited, if it showed a reproducible and a variable, short latency increase in firing rate following ipsilateral VMH stimulation. If three successive histograms showed good consistency, the normal artificial cerebrospinal fluid (ACSF) superfusing the slice was switched to ACSF containing 100 or 500 uM KYN. Peristimulus time histograms were used to determine if KYN blocked the orthodromic response and to check for recovery after replacement with normal ACSF. In 12 of the 17 cells tested, the orthodromic response was either totally abolished or partially blocked by application of KYN. The time course for this response was 4 to 10 minutes and varied according to the position of the tissue in the in vitro slice chamber and to the depth in the brain slice of the neuron being recorded. In all cases, recovery occurred within minutes. These data suggest that an excitatory amino acid, such as, glutamate or aspartate, may be the transmitter mediating the orthodromic response. Supported by HD09988-V and HD07062.

374.11

ELECTROPHYSIOLOGY AND PHARMACOLOGY OF THE CORTICOTHALAMIC RESPONSE. M. Deschênes and B. Hu*, Lab. of Neurophysiology, Laval Univ. Sch. of Med., Québec, Canada.

Though most electrophysiological results suggest that the corticothalamic (CT) pathway is excitatory, inhibitory effects have also been reported. A methodological problem in these studies, however, was that corticofugal effects could not be separated from those mediated by collateral activation of reticular thalamic neurons. In the present study, the reticular complex was lesionned by kainic acid and the CT response of thalamic (T) cells was recorded intracellularly in cats under pentobarbital or urethane anesthesia. Results show that (1) CT pathway activation depolarized T cells and that this response potentiated when stimulation rate exceeded 2 Hz. (2) Potentiation was completely abolished by ketamine injection (5 mg/kg i.c.) or by local pressure application of APV. (3) Though CT pathway stimulation could facilitate subthreshold EPSPs of cerebellar origin, this facilitation resulted from simple summation rather than from an heterosynaptic potentiating mechanism. It is then concluded that CT neurons depolarizes T neurons, partly through the activation of NMDA receptors. The numerical importance of the CT neuronal population and the autopriming mechanisms operating at CT synapses indicate that the role of the thalamus is not only to transfer peripheral information toward the cortex, but also and mainly to relay to the cortex a fac-simile of its own neuronal constructs.Supported by the Canadian MRC.

374.12

EFFECTS OF KYNURENIC ACID ON VISUALLY EVOKED POTENTIALS IN THE ALBINO RAT. D.F. Sisson, J. Siegel and S.J. Grant. School of Life and Health Sciences and Department of Psychology, University of Delaware, Newark, DE 19716.

The putative transmitter of neurons that project from LGN to visual cortex (OC1) is thought to be an excitatory amino acid (EAA). Since early components of the VEP are indicative of geniculo-cortical activity, we investigated the effects of kynurenic acid (KYN), a non-selective EAA antagonist, directly applied to OC1 on flash VEPs of chloral hydrate anesthetized albino rats.

A cortical cup was used for superfusion of CSF over left OC1. VEPs were recorded from this cortical surface and simultaneously from the intact right cortex.

Within one minute of the addition of 0.1 mM KYN to the superfusate, VEP components N_1 and those after P_2 were abolished. P_1 was lost by 5 min, and an anomalous P_2 remained after 10 min. After 1 hr or more of CSF wash, all VEPs returned and were again comparable to VEPs recorded from right OC1.

The elimination of all components of the VEP by KYN except P_2 indicates that an EAA is the transmitter in the geniculate afferents and that P_2 is generated either by commissural afferents from contralateral OC1 or by non-geniculate afferents. The lack of a P_2 effect in right OC1 argues against the commissural explanation.

This work was supported in part by ARO Contract DAAL 0388K0043.

374.13

CYSTEINE REDUCES SPIKE AFTERHYPERPOLARIZATION OF CA3 PYRAMIDAL CELLS IN HIPPOCAMPAL SLICE CULTURES S. Charpak*, T. Knöpfel, K.Q. Do* and B.H. Gähwiler (Spon:G.Kato). Brain Research Institute, University of Zürich, CH-8029 Zürich (Switzerland)

Recently, it has been described that endogenous cysteine (Cys) could be released from various rat brain regions upon potassium stimulation in a calcium-dependent manner (Keller et al. J. Neurochem. 1989, in press). In the present work, we investigated whether Cys affects membrane properties of hippocampal CA3 pyramidal cells.

Organotypic cultures of rat hippocampus were prepared as described previously. After 2-5 weeks in vitro, the cultures were superfused with a balanced salt solution containing 1 µM TTX and single-electrode voltage-clamp techniques were used to record currents from CA3 pyramidal cells.

Cys (500 µM to 1 mM) induced an inward current and reversibly reduced slowly decaying outward tail currents induced by short lasting depolarizing voltage jumps. These tail currents reversed at the potassium equilibrium potential and were blocked by the Ca^{++} antagonist Cd^{++} suggesting that they are produced by the Ca^{++}- activated potassium conductance underlying spike afterhyperpolarization and accommodation.

These observations support the hypothesis that Cys is involved in chemical neurotransmission.

374.14

c-fos AND HEAT SHOCK (hsp72) GENE EXPRESSION FOLLOWING INTRAVENTRICULAR ADMINISTRATION OF QUISQUALATE AND QUINOLINIC ACIDS. J.W. Sharp, S.M. Sagar, P. Jasper*, T. Marsh*, and F.R. Sharp, Depts. Neurology & Physiology, UCSF and VA Med Ctr, San Francisco, CA 94121.

Since systemic kainic acid induces HSP72 in brain (Gonzalez et al., Mol. Brain Res., in press), the effects of 5ul of 50mM intraventricular quisqualate (QQ) and quinolinic (QL) acid, which acts at the NMDA receptor, were studied. Expression of the c-fos proto-oncogene and the inducible heat shock gene, hsp72, were examined 24 hours later. QQ induced Fos bilaterally in limbic structures, including hippocampal pyramidal neurons, amygdala, piriform cortex, basal forebrain, and septal neurons, but not in neocortex or hippocampal granule cells. QQ markedly induced HSP72 in Golgi like patterns bilaterally in hippocampal pyramidal neurons, dentate hilus, amygdala, and parvocellular PVN. QL induced Fos in ipsilateral neocortex, septum, basal forebrain, amygdala, and piriform cortex, and bilaterally in hippocampal pyramidal neurons and dentate granule cells. QL minimally induced HSP72 in a few CA4 pyramidal neurons and in scattered patches of cortical neurons. The data show that excitatory amino acids induce c-fos and hsp72 genes in brain, that different receptors may mediate induction in different populations of neurons, and that a subset of neurons in which Fos is induced express the stress protein, HSP72.

374.15

EXTRACELLULAR pH and Ca SHIFTS EVOKED BY GLUTAMATE AND ASPARTATE IONTOPHORESIS IN TURTLE CEREBELLUM. **M. Chesler[1,2] & M.E. Rice[2].** Depts. of Neurosurgery[1] and Physiology & Biophysics[2], NYU Med. Ctr, 550 1st Ave, NY, NY 10016.

Parallel fiber (PF) stimulation causes an extracellular alkaline shift (AS) that is blocked by Mn (Kraig et al. J. Neurophys. 49:831) and kynurenate (Chesler & Chan, Neuroscience 27(3):941). We studied the pharmacologic sensitivity of the AS and its relationship to Ca_o shifts in the isolated turtle cerebellum. pH_o and Ca_o shifts, recorded with ion-selective microelectrodes, were evoked by afferent stimulation, or iontophoresis of aspartate (asp) or glutamate (glu).

Stimulation of PF's or peduncular afferents evoked AS's in only the molecular layer (ML). Kynurenate (0.5-4 mM) and Cd (0.1 mM) reversibly blocked the PF-evoked AS. APV (.02-.10 mM) had no effect. GABA (1 mM) blocked the PF-evoked AS, with no effect on pH_o. The AS is therefore not generated by NMDA or GABA-gated channels. With asp or glu iontophoresis, AS's occured in all layers (up to 0.4 pH units). Ca_o transients and AS's corresponded in time course and spatial profile, however, in the GL, application of 5 mM Mn only partially (50%) blocked the AS. In the ML, 100 mM Cd blocked synaptic transmission with no effect on the iontophoretic-evoked AS.

The correspondence between Ca_o and pH_o shifts suggests a relationship between Ca currents and the AS. However, the small effect of Mn and Cd suggests that voltage-dependent calcium channels are not necessary. The block of the afferent response by GABA indicates that the AS is not generated by acid flux through excitatory post-synaptic channels. Assuming GABA "clamps" dendritic membrane potential, its block of the AS suggests that the response is triggered by dendritic depolarization. Whether the AS arises due to acid-base flux across a channel is still undetermined. Supported by USPHS grant NS-13742.

374.16

COMPUTER SIMULATION OF EXCITATORY POSTSYNAPTIC CURRENTS DUE TO VARYING RATIOS OF NMDA TO NON-NMDA RECEPTORS. W.R. Shankle*, Matthew Wilson, D.R. Stevens, Don Perkel, James Bower, Carl W. Cotman(SPON:C.W. Cotman). Cotman Lab., Dept. of Psychobiology, UC Irvine, Irvine, CA 92717. Bower Lab,Division of Biology, California Instit.itute of Technology, Pasadena, CA 91125

We have simulated currents resulting from synaptic release of glutamate onto a mixture of NMDA and non-NMDA receptors at the postsynaptic membrane. NMDA activated currents were fit with voltage dependence derived from the voltage clamp results of Mayer and Westbrook(1984) on cultured mouse spinal cord neurons. Depolarizing current injections produced small transient changes in membrane potential(low resistances); in contrast, hyperpolarizing current injections produced much larger transient changes in membrane potential(high input resistances) to voltage clamped membranes over a range of clamped potentials. Synaptic currents were simulated over a range of ratios of NMDA to non-NMDA receptors(1:10 to 3:1). Under voltage clamp conditions, membranes with as low as 10% of their receptors being NMDA showed voltage dependence of the peak current plus a voltage dependent increase in the amplitude and time of the decay phase of the excitatory postsynaptic currents(epsc). In contrast, increasing the NMDA channel conducting time did not affect the peak current but did affect the time course and voltage dependence of the epsc decay phase. NMDA to non-NMDA receptor ratios of 3:1 may be closer to ratios seen in the hippocampus. The epscs changes polynomially with membrane potential at this ratio. The capacity for more temporal summation of epscs and the greater current amplitudes along the decay phase provided by a more heavily weighted NMDA receptor fraction may have fundamental effects on new memory formation.

374.17

AMINO ACID INDUCED MODULATION OF DOPAMINERGIC NEURONS IN THE SUBSTANTIA NIGRA. M.F.Pozza*, S.Margarson*, E. Kueng*, H.-R.Olpe* (SPON: G.E.FAGG). Res. and Dev. Dept. Pharmaceuticals Div., CIBA-GEIGY Ltd., CH-4002 Basel.

The actions of amino acid transmitters were tested on the activity of single dopaminergic neurons in the substantia nigra pars compacta (SNpc). Extracellular recordings were made in 400 um midbrain slices. All drugs were bath-applied. Dopamine (100 μM) reduced the firing rate of all neurons tested by 55 ± 8 % (n = 15).

Effects of GABA-A and -B agonists and antagonists: L-baclofen (1.0 μM) and muscimol (1.0 μM) strongly reduced the spontaneous firing rate of the cells. Bicuculline (1.0 um) potently antagonized the effects of muscimol without affecting the action of L-baclofen. Phaclofen (1 mM) blocked the action of L-baclofen but did not affect the muscimol response. Neither phaclofen nor bicuculline alone had a significant effect on the spontaneous firing rate. These results indicate that GABA-A and GABA-B receptors are present.

Effects of excitatory amino acids: NMDA and kainate increased the firing rate of SNpc cells in a concentration dependent manner (5-20 μM) without causing any burst firing. The NMDA effect was selectively antagonized by the NMDA-receptor antagonist CGP 37 849. Quisqualate had no effect. The results suggest that NMDA and kainate receptors but not quisqualate receptors may be involved in the regulation of nigral dopaminergic cell activity.

374.18

DEVELOPMENTAL EXPRESSION OF A NOVEL EXCITATORY AMINO ACID RESPONSE IN CEREBELLAR PURKINJE NEURONS. C.L. Franklin* and D.L. Gruol (SPON: W. Young). Res. Inst. Scripps Clinic, La Jolla, CA 92037.

Cerebellar Purkinje neurons (PNs) in culture respond to micropressure application of the excitatory transmitter glutamate (Glu) and the selective agonists quisqualate (QA) and kainate (KA). In mature PNs recorded extracellularly, the agonist responses are characterized by an initial excitatory period, a period of burst activity and an inhibitory period. Glu, Quis and KA differ in the effectiveness of evoking the three response components. In intracellular recordings, the excitatory component of the response is associated with a membrane depolarization; the inhibitory phase is associated with a small hyperpolarization or no change in membrane potential; bursting occurs during the repolarizing and inhibitory phases (Yool and Gruol, Soc. Neurosci. Abstr, 1988). In the present study, we have examined the developmental expression of the agonist responses using extracellular recording techniques and micropressure application of agonists. Immature PNs without visible dendritic structure also exhibited the novel response when tested with Glu, QA and KA. However, response components were generally less robust than in mature neurons. Developmental changes in the response properties were agonist dependent, suggesting that multiple excitatory amino acid receptor subtypes were involved The most dramatic developmental changes were observed with QA, which was also the most potent agonist at all culture ages. The novel features of the amino acid responses in the PNs may be due to the involvement of more than one QA receptor subtype. Supported by NIAAA 06665.

374.19

NMDA AND NON-NMDA EXCITATORY AMINO ACID NEUROTRANSMISSION IN CONSTRUCTION OF RECEPTIVE FIELDS OF RAT BARREL-FIELD NEURONS. M. Armstrong-James* (SPON: K. Chapman). Physiology Dept., London Hospital Medical College, London, E1 2AD, England.

Vibrissal excitation of 135 neurons in layers II-V was found to be dominated by NMDA-dependent somato-sensory neurotransmission, as tested against adequate APV iontophoresis, although for all layers, except V, excitatory surround-receptive fields (SRFs) were more depressed by APV than the single - vibrissa centre-receptive field (CRFs). Layer II and upper layer III neurons were almost exclusively NMDA-dependent, both CRFs and SRFs being 80-100% eliminated (20-30 nA APV). CRFs and SRFs of lower layer III and IV neurones were progressively less (60-80%). In contrast to their SRFs however, CRFs of very short latency (6-8 ms) layer IV neurones, were only marginally affected by APV (0-18%). Both SRFs and CRFs of layer V neurones were depressed about 50% by 40-80 nA of APV. Similar results were achieved using kynurenate (blocker of both NMDA and non-NMDA receptors) although (1) greater suppression of RFs was generally achieved and (2) Short latency CRFs of layer IV neurons were additionally suppressed to 50-85%. These results suggest that most intracortical neurotransmission for constructing total RFs of upper layer and lower layer SI cortical neurons, and SRFs of layer IV neurons is dominated by NMDA-receptor activation. CRFs of layer IV/lower layer III neurons, reflecting the thalamo-cortical inputs, are more non-NMDA dependent, (presumably quis, kainate or other unknown receptor types).

NEUROENDOCRINE REGULATION: PHOTOPERIOD/PINEAL

375.1

A SINGLE BLOCK OF PHOTOPERIODIC INFORMATION EACH YEAR CAN SYNCHRONIZE THE ANNUAL REPRODUCTIVE CYCLE OF EWES. C. J. I. Woodfill, B. Malpaux*, J.E. Robinson* and F. J. Karsch*. Reproductive Sciences Program and Department of Physiology, The University of Michigan, Ann Arbor 48109.

Although many species display circannual cycles of biological activity that are synchronized by daylength, the specific photic requirements for synchronizing such cycles are not established for any species. We tested the hypothesis that the circannual reproductive cycle of sheep can be synchronized by just one discrete block of photoperiodic information a year. Ewes were functionally disconnected from their photoperiodic environment by pinealectomy (pnx). Specific photoperiodic signals were restored via replacement of 24-hr patterns of melatonin (mel), the pineal hormone which transmits photic information to the reproductive neuroendocrine axis. Two groups of pnx ewes were kept in a 12-month photocycle which alternated between short (8L:16D) and long (16L:8D) days every 6 months for 5 years. Non-infused controls (pnx) did not exhibit synchronous reproductive cycles. Experimental ewes (pnx+mel) received alternating 70-day infusions of short- and long-day patterns of mel every 6 months each year for the first 2-1/2 years; thereafter, ewes received just one 70-day infusion of long-day mel a year. Infusion onsets coincided with photoperiodic shifts. Pnx+mel ewes exhibited synchronous annual reproductive cycles throughout the study, including the final two years when they received only one block of photoperiodic information (ie., mel infusion). Cycle period was 365 ± 3 days; standard deviation of the date of onset of reproductive induction averaged only 3 days. Our study provides the first direct evidence that a single block of photoperiodic information each year can synchronize a circannual cycle. (NSF-DCB-8316364 & DCB-8710099)

375.2

THE EFFECT OF OVARIECTOMY AND ESTRADIOL TREATMENT ON THE COHERENCE OF CIRCADIAN ACTIVITY RHYTHMS IN CONSTANT LIGHT. E.M.Thomas* and S.M.Armstrong* (SPON: R.F.Mark), Dept. Physiol. Monash Univ. Vic 3168 and Dept. Psych. LaTrobe Univ. Vic 3183, Australia.

Whilst exposure to constant bright light (LL) often leads to the disruption of rat circadian rhythms, in dim LL, rhythms usually persist and free-run. 16 ovariectomized rats (ovx), 8 implanted with estradiol-17β and 8 with empty implants, 8 intact rats and 6 sham operated rats were maintained in dim LL (<20 lux) for 70 days. The circadian activity rhythms of the intact and sham operated animals free-ran as expected but the wheel-running and drinking activity rhythms of the ovx, blank-implanted rats were rapidly disrupted. The ovx, estradiol-implanted rats displayed activity rhythms that were more similar to those of intact rats than to those of ovx rats. Rather than being arrhythmic, the disrupted activity data of the ovx, blank-implanted rats appeared to contain free-running, ultradian components. These data appear consistent with the concept of a multioscillatory basis to the circadian system and suggest a role for ovarian estradiol in the maintenance of synchrony between component oscillators.

375.3

NEURAL PROCESSING OF ENVIRONMENTAL INFORMATION IN THE SIBERIAN HAMSTER (PHODOPUS SUNGORUS). K.S. Matt* and S.R. Morgan*. (SPON: A. Kammer). Dept. of Zool., A.S.U., Tempe, AZ 85287.

Seasonally breeding animals must carefully integrate information from their external environment with information regarding their internal physiological environment. These studies were designed to examine the neuroendocrine mechanisms involved in integrating photoperiodic, behavioral and dietary information. Siberian hamsters are particularly suited to these studies because it seems that reproductive success may be dependent on pair bond formation, and parental care by both the male and female. We examined changes in brain catecholamines in the pre-optic area, medial basal hypothalamus, and cortex in animals housed under various conditions. These studies demonstrate that separation of previously paired animals in a long photoperiod results in a decrease in norepinephrine and dopamine in the medial basal hypothalamus. Similarly, exposure of short photoperiod males to a sexually receptive female mate, or a female mate with young, results in increases in norepinephrine and dopamine in the medial basal hypothalamus. Furthermore, exposure of short photoperiod males to different dietary regimes results in changes in norepinephrine and dopamine concentrations in the pre-optic area. These results strongly suggest that a variety of environmental stimuli can alter and override photoperiod effects on neurotransmitters and that processing of this information may occur in different regions of the brain. Funded in part by United Way and BRSG 2 SO7 RR07112, Division of Research Resources, National Institutes of Health.

375.4

LHRH AND GAP IMMUNOCYTOCHEMISTRY AND LHRH IN-SITU HYBRIDIZATION IN GOLDEN HAMSTERS EXPOSED TO DIFFERENT PHOTOPERIODS. E. Ronchi*, L. Krey*, D.W. Pfaff. (SPON: R. Lustig). The Rockefeller University, New York, NY 10021.

Hypothalamic luteinizing hormone releasing hormone (LHRH) and gonadotropin releasing hormone associated peptide (GAP) biosynthesis and storage were estimated by immunocytochemistry and in-situ hybridization in male golden hamsters maintained in different photoperiods. Intact or castrated male hamsters (with testost. capsules) were exposed to long-day (14:10) or short-day photoperiods (LD 10:14) for four to eight weeks. Brains were rapidly processed using a novel free-floating procedure which allows immunocytochemistry and in-situ hybridization on alternate tissue sections. Exposure to short photoperiod is associated with an increase in the number of LHRH and GAP immunoreactive cells in the diagonal band of Broca/medial septum. This increase is correlated with augmented steady state LHRH mRNA levels, as measured by in-situ hybridization and RNAase protection assay. Furthermore, morphometric analysis revealed that animals exposed to short daylengths display significantly more LHRH but not GAP immunoreactivity in the median eminence as opposed to hamsters exposed to long-day photoperiods. Thus, suppression of release but not synthesis of LHRH may be associated with reproductive quiescence in the golden hamster.

375.5

THYROIDECTOMY BLOCKS TRANSITION TO ANESTROUS SEASON BY SUSTAINING LH-PULSE GENERATING ACTIVITY IN EWES. S.M. Moenter*, C.J.I. Woodfill, F.J. Karsch*(SPON: F.J.P. Ebling) Reprod. Sci. Prog., Dept. Physiol., Univ. Mich., Ann Arbor.

Seasonal reproductive transitions in ewes are endogenously generated and are synchronized by annual changes in photoperiod. Previous evidence implies thyroidectomy prevents the transition to anestrus in ewes maintained in a fixed day length, suggesting the thyroid is needed for endogenously generated reproductive arrest (Reprod Nutr Develop 28: 375, 1988). Here we tested the hypothesis that the thyroid is needed for endogenous seasonal suppression of the neuroendocrine system which generates pulsatile secretion of luteinizing hormone (LH). Ewes were thyroidectomized (THX, n=6) in summer, 6 weeks prior to onset of the breeding season, or left intact (INT, n=6). They were housed in simulated natural photoperiod until the winter solstice; thereafter they remained on that photoperiod (10L:14D). To monitor pulsatile LH secretion, ewes were ovariectomized, implanted with estradiol, and LH was measured in both frequent (6 min) and infrequent (twice/week) blood samples. In this model, high LH indicates reproductive induction, low LH reproductive arrest. Although LH levels (samples twice/week) rose concurrently in both groups in September, levels remained high longer in THX ewes (onset low values INT, 15 Feb ± 14 d; values still high in THX at end of study, mid-April). Frequent sampling in autumn (breeding season) revealed both THX and INT exhibited frequent LH pulses (THX 7.0 ±0.6 vs INT 6.0 ±0.7 pulses/4 hr). In spring (anestrus), THX ewes still had frequent pulses (9.8 ± 1.1 pulses/ 4 hr), whereas INT had no pulses/4 hr. This supports the hypothesis that the thyroid is necessary for endogenous suppression of neuroendocrine systems which generate LH pulses, a suppression crucial for transition to anestrus.(NIH-HD-18337)

375.6

DAILY RHYTHMS OF LUTEINIZING HORMONE IN FEMALE HAMSTERS STERILIZED WITH MONOSODIUM GLUTAMATE. R.S. Donham*, K.M. Ogilvie* and M.H. Stetson*. (SPON:S. Binder-Macleod). Physiol. Sect., Sch. of Life and Health Sci., Univ. Delaware, Newark, DE 19716.

The prepubertal female golden hamster has a daily rhythm of circulating luteinizing hormone (LH) with a maximum at 1700h each afternoon. At puberty, the daily rhythm is replaced by a 4d estrous cycle rhythm. Injection of monosodium glutamate (MSG) into neonatal rodents sterilizes the adult as a consequence of specific degeneration of the retina and arcuate nucleus perikarya. We used MSG to evaluate the hypothalamic components necessary for the expression of the daily rhythms of gonadotropins in female hamsters. We injected MSG (8 mg/g b.w.) into 8d old females. Animals were weaned at 21d of age and blood samples were obtained at 24d of age at 1300 and 1700h. In saline-injected controls, the levels of LH were 0.7 ± 0.46 and 5.5 ± 1.6 ng/ml (1300 and 1700h, respectively, mean ± SEM, n=6, both times; $p<0.05$, Mann-Whitney test) and regular, 4d vaginal estrous cycles were initiated between 29 and 37d of age. MSG-treated animals had levels of LH that were 0.2 ± 0.03 and 3.0 ± 1.1 ng/ml, $p<0.01$); none of these had initiated regular estrous cycles by 50d of age. Thus MSG did not affect daily, circadian-based rhythmicity in LH secretion even though it made the adult-age animals infertile.

375.7

ALTERATIONS IN HYPOTHALAMIC SEROTONIN METABOLISM IN HAMSTERS WITH PHOTOPERIOD-INDUCED TESTICULAR REGRESSION. R.W. Steger*, C. Dennis*, A. VanAbbema*, E. Gay-Primel* (SPON: D. Molfese). Dept. of Physiology, Southern Illinois Univ School of Medicine, Carbondale, IL 62901.

Exposure of male hamsters to a short photoperiod (<12.5h light/day) results in decreased levels of LH, FSH, Prl and atrophy of the reproductive system. These changes are preceded by decreases in hypothalamic norepinephrine and dopamine turnover but the effects of photoperiod on 5-HT metabolism are unknown. In the present study, the rate of 5-HT synthesis in several brain regions of long day (LD, 16:8 light:dark) and short day (SD, 8:16) hamsters was estimated by measuring 5-hydroxytryptophan (5-HTP) accumulation after inhibiting aromatic amino acid decarboxylase with NSD-1015 (150mg/kg, ip). Exposure to SD led to decreases in testicular weight and plasma levels of LH and Prl. Concentrations of 5-HTP in the median eminence (ME), medial basal hypothalamus (MBH), anterior hypothalamus (AH) and olfactory bulbs (OLFB) increased linearly with time after NSD administration in both LD and SD hamsters. The accumulation of 5-HTP in the MBH was significantly greater in the SD than in the LD controls. 5-HTP accumulation did not differ in other brain areas. Similar results were seen in a 2nd experiment where it was also shown that 5-HT synthesis was restored to control levels within 2 days after transferring SD hamsters to LD. ME, MBH, AH and OLFB 5-HT and 5-HIAA levels in uninjected hamsters were not affected by photoperiod but the ratio of 5-HT to 5-HIAA in the MBH was reduced in the SD animals. The significance of the increase in MBH 5-HT synthesis in SD hamsters is not known but may be related to changes in Prl secretion since we have shown that hypophysectomy increases MBH 5-HT synthesis and this effect can be reversed by Prl replacement (Supported by NSF - DCB-8619702).

375.8

EFFECT OF SHORT-DAY INDUCED GONADAL REGRESSION ON THE GnRH NEURON SYSTEM IN THE ADULT MALE DJUNGARIAN HAMSTER. S.M. Yellon, Div. Perinatal Biology, Depts. Physiology, Pediatrics and Anatomy, Loma Linda Univ. Sch. of Med., Loma Linda, CA 92350.

In the Djungarian hamster, exposure to short days inhibits gonadotropin secretion and induces gonadal regression. During sexual maturation, the effect of short days to arrest testes growth is correlated with reduced numbers of immunoreactive-GnRH neurons in the diagonal band of Broca (DBB) and medial preoptic area (MPOA). However, in adult white-footed mice, short-day mediated gonadal atrophy is associated with increased GnRH neuron number. Because similar data are lacking for the adult hamster, the present study determined the effect of short days on the number and morphology of GnRH neurons in the DBB and MPOA, a region that contains the majority of GnRH cell bodies. Males reared in long days (LD, 16L:8D) were either maintained in LD or transferred to short days (SD, 10L:14D) at 30 d of age. Hamsters were killed 10, 30, or 60 d later by intracardiac perfusion (40, 60, or 90 d of age, respectively, n = 4 each). Testes were weighed and brains were sectioned (60 μm) from just rostral to the corpus callosum decussation to the suprachiasmatic nucleus. Every section was processed for GnRH immunocytochemistry (LR1 gift of R. Benoit). Testes weights were significantly reduced by 10 d of SD (398 ± 137 mg) and atrophied by 60 d (44 ± 2 mg) compared to LD controls (about 850 mg). Total number of GnRH immunoreactive perikarya was increased after 10 d of SD (133 ± 8 vs 97 ± 12, $p < 0.05$, SD vs LD). This change was small and restricted to the DBB (both unipolar and bipolar subtypes). At other ages, GnRH neuron number and ratio of unipolar to bipolar subtypes in the DBB and MPOA remained unchanged in SD compared to LD hamsters despite gonadal regression. The findings indicate that a relatively stable population of GnRH immunoreactive neurons are present in adulthood compared to that found in the peripubertal male hamster or white-footed mouse. (Sup. NIH HD22479)

375.9

THE DECREASE OF HYPOTHALAMIC LHRH CONTENT IN CONSTANT LIGHT CANNOT BE REVERSED BY MELATONIN ADMINISTRATION. T.Porkka-Heiskanen, M-L.Laakso*, D.Stenberg*. Inst.Physiology, Univ.Helsinki, 00170 Helsinki, Finland

Adult male Wistar rats were kept in constant (LL) or periodical (LD) light for one week, where they received either saline or melatonin (50 ug/0.1 ml saline) injections s.c. daily at 9 A.M. or 4 P.M. After decapitation the hypothalamus was dissected and the hypothalamic LHRH content was measured with radioimmunoassay. Hypothalamic LHRH content was lower in constant light exposed rats than in periodical light exposed rats, and melatonin given either in the morning or in the evening was unable to inhibit the decrease.

We conclude that the constant light effect on hypothalamic LHRH content is not mediated via melatonin.

375.10

PHOTOPERIODIC HISTORY MECHANISMS IN SYRIAN HAMSTERS. J. D. Karp*, M. E. Dixon*, and J. B. Powers. Dept. Psychology, Vanderbilt Univ., Nashville, TN 37240.

The reproductive response of Syrian hamsters to altered daylengths is affected by their photoperiodic history (PPH). Two features of PPH were evaluated; (a) the melatonin (MEL) signal experienced, and (b) the circadian after-effects on tau. Male hamsters in LD 14:10 were administered single daily injections of MEL (15 μg; n=22) or saline (SAL) (n=10) 1 hr prior to the onset of darkness for 8 weeks. Their subsequent reproductive response to 8 weeks without injections in LD 12:12 or LD 8:16 was evaluated (grps=MEL 12:12; MEL 8:16; SAL 12:12). MEL caused significant testicular regression (TR); SAL did not. Regressed Ss experienced testicular stimulation (TS) in LD 12:12 and this occurred significantly faster than the TS caused by photorefractoriness among the MEL 8:16 males. The SAL 12:12 hamsters exhibited TR, indicating that MEL history and not photoperiod duration per se was important in mediating PPH effects.

In a second study, PPH consisted of daily light cycles (8 hr duration) with periods shorter (T=23.33; n=20) or longer (T=24.67; n=21) than 24 hrs, followed by transfer to LD 12:12. These T-cycles were chosen because both would be stimulatory to the reproductive system, but their after-effects on tau would qualitatively mimic those produced by conventional long or short days. After 5 weeks in the assigned T-cycle, all hamsters maintained large testes. Subsequent exposure to LD 12:12 for 11 weeks caused some TR in both groups, but not nearly as much as was shown by the SAL 12:12 males in the first experiment. Because both groups interpreted LD 12:12 as a short day, the PPH effect of their prior T-cycle exposure was consistent with its being interpreted as a long day by the reproductive system, even though the two T-cycles did generate different circadian after-effects on tau.

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375.11

REGULATION OF GAP JUNCTION EXPRESSION BETWEEN ADULT RAT PINEALOCYTES. V.M. Berthoud* and J.C. Sáez*. (SPON: J.A. Connor). Dept. Neurosci., A. Einstein College of Medicine, Bronx, NY 10461.

Gap junctions mediate electrotonic and metabolic coupling. We previously showed that rat pinealocytes are electrically and dye coupled and that they express at least connexin 26 (Sáez et al., Soc. Neurosci. Abstr. 14: 1175, 1988). We now report the effect of norepinephrine (NE) and second messengers on the incidence of dye coupling between pinealocytes in culture. Pineal glands of Sprague-Dawley rats (ca. 200 g) were used to prepare primary cultures of enzymatically dissociated pinealocytes. After 8 days in culture, the cells were treated with NE (6.6 μM) plus ascorbic acid (66 μg/ml), cAMP (0.1 mM) or DAG (100 nM). Dye coupling was evaluated by injecting Lucifer yellow into one cell of a pair or cluster. NE (plus ascorbic acid) markedly increased the incidence of dye coupling (from 0-20% to 50%-70%), reaching a plateau at 6 h. Four to 6 h after NE was washed out, the incidence of coupling decreased to values near those seen in control cultures. cAMP and DAG treatment also increased the incidence of dye coupling 6 h after its addition, but to a lower and more variable extent than NE. Experiments are underway to determine the extent to which each second messenger contributes to the NE effect on coupling. These data suggest that coupling between pinealocytes is regulated by NE, the neurotransmitter released during darkness by SCG nerve terminals that innervate the epiphysis. Since NE-induced melatonin secretion is maximal at about 6 h of the dark half of a 12:12 h light:dark cycle when NE would increase coupling maximally, we suggest that gap junctions play a role in melatonin secretion induced by NE.

375.12

ULTRASTRUCTURAL AND BIOCHEMICAL COMPARISON OF THE CAT AND RAT PINEAL GLAND. L. Kus*, L.M. Fox*, J.A. McNulty* (SPON: A. LaVelle). Dept. of Anatomy, Stritch School of Medicine, Maywood, IL 60153

The mammalian pineal gland exhibits species variability in morphology and biochemistry. As a potential model for studies on pineal function in a seasonal breeder, this study compared the ultrastructure and biochemistry of the cat pineal with that of the rat. 19 adult cats and 10 Sprague-Dawley rats housed under standard laboratory conditions (L:D 12:12) were sacrificed during the light period and the pineal glands processed for either TEM or HPLC of monoamines and free amino acids. Compared to the rat, the cat pineal was less well vascularized by non-fenestrated capillaries, contained larger bundles of neural elements, and more glia. Important biochemical differences between these species were observed in the ratios (cat/rat per mg protein) of NE (3/1), 5HT (1/20), 5HIAA (1/2), and the neuroactive amino acids glutamate (1/10) and taurine (1/3). A rich sympathetic innervation of the cat pineal (J. Pineal Res.3:15, 1986) is consistent with high levels of NE in this species. Low concentrations of 5HT and 5HIAA in the cat may reflect species differences in indole turnover. Finally, the sparcer vascularity and reduced steady state levels of free amino acids suggests a generally lower metabolic rate in the pineal gland of the cat compared to the rat.

375.13

FREE AMINO ACIDS IN THE PINEAL GLAND: CIRCADIAN CHANGES AND EFFECTS OF SUPERIOR CERVICAL GANGLIONECTOMY. J.A. McNulty*, L.M. Fox*, L. Kus* (SPON: F. LaVelle). Dept. of Anatomy, Stritch School of Medicine, Maywood, IL 60153.

The neuroactive amino acids (aa) glutamate and taurine are present in high concentrations in the pineal gland and may function to regulate melatonin production. To further elucidate possible role(s) of aa in pineal neuroendocrine function, steady state levels of aa were measured over a light: dark cycle in intact and denervated pineals. 108 male Sprague-Dawley rats were equally divided into 3 groups (intact, shams, SCGx), entrained to a 10:14 L:D cycle, and sacrificed at 6 circadian time points. HPLC was used to measure monoamines (HPLC-EC) and aa (OPA Fluor) in individual pineals (6/group/time point). In intact and sham controls, significant circadian differences were found in the monoamines NE, 5HT, 5HIAA, NAS, MEL, and the aa GLU, TAU, ASP, ALA, GLY, ARG, and GLN. SCGx abolished each of the rhythms. SCGx also resulted in a significant (ANOVA) reduction of GLU (52%), ASP (69%), ALA (68%), ARG (67%). Effects of SCGx on TAU and GLY were found only at 1 time point. There was no effect of SCGx on the aa SER and total protein. Our findings that circadian changes in aa depend on an intact innervation are consistent with metabolic demands related to melatonin production. The magnitude of the reduction of GLU after SCGx suggests that this aa is particularly important to these metabolic processes.

Supported by NSF BNS-8801726.

375.14

SUPPRESSION OF PINEAL MELATONIN CONTENT IN LONG-EVANS HOODED RATS: DOSE-RESPONSE CURVE AT 640 NM. Brian J. Broker*, J.P. Hanifin*, M. D.Rollag*, W.A. Thornton*, and G.C. Brainard, Department of Neurology, Jefferson Medical College, Philadelphia, PA, Department of Anatomy, USUHS, Bethesda, MD, and Prime Color Inc., Cranford, NJ.

It has been thought that wavelengths of red monochromatic light above 610 nm would not influence rat pineal physiology due to the belief that rods are relatively insensitive to this part of the spectrum. The purpose of this study was to examine the capacity of red monochromatic light at 640 nm (10 nm half-peak bandwidth) to suppress typically high nocturnal pineal melatonin content in rodents. Groups of adult male Long-Evans Hooded rats were entrained to a 10:14 light:dark (L:D) cycle (lights on 0700-1700 h) and then exposed to different irradiances of 640 nm for five minutes during their dark phase. Matched groups of control animals were handled similarly but were not exposed to 640 nm pulses. Following exposure animals were held in darkness for 15 minutes and then sacrificed. Pineal glands were dissected out, frozen at -20° C, and later assayed for melatonin content by RIA. Irradiances used were; 0.03, 0.3, 3, 6, 18, 30, 136, and 243 uW/cm^2. Control groups all showed typically high nocturnal pineal melatonin contents while experimental groups exposed to 18, 30, 136, and 243 uW/cm^2 showed a significant suppression of pineal melatonin content ($p<0.001$). Groups exposed to lower irradiances showed high melatonin contents which were not significantly different from those of matched control groups. The results demonstrate that wavelengths of monochromatic light at 640 nm are capable of influencing rat pineal physiology and therefore should not be used indiscriminantly for observation of rats during the dark phase of their L:D cycle. NASA grant NAGW 1196 (GCB), UHUHS Protocol C07049 (MDR), and NIH grant #HLO7497-09 (BJB).

375.15

BOVINE PINEAL GLAND SYNTHESIZES TRANSTHYRETIN. R.L.Martone* and J.Herbert*. Dept. of Neurology, Columbia Univ., New York, NY 10032. (SPONSOR: M.L.Shelanski).

Transthyretin (TTR) is a 56 kDa tetrameric protein that plays an important role in the serum transport of thyroxine and retinol. TTR is synthesized by the liver, yolk sac endoderm and choroid plexus (CP) epithelium. Because of the functional homology between the CP epithelium and the retinal pigment epithelium (RPE), we previously investigated and reported specific synthesis of TTR by the RPE. We now report synthesis of TTR by the phylogenetic "vestigial eye" - the pineal gland. Northern blot anlaysis of total RNA isolated from bovine pineal glands revealed the presence of TTR mRNA at levels significantly lower than those present in bovine CP. Western blot analysis of bovine pineal gland confirmed the presence of immunoreactive TTR protein. By immunohistochemical methods, TTR was restricted to the cytoplasm of pinealocytes. While the function of TTR in the CNS is unknown, the expression of TTR by the pineal gland suggests that it may play a role in mediating the effects of thyroxine on pineal neuroendocrine function.
(Supported by the Aaron Diamond Foundation, Florence Irving Assistant Professorship Award, and NINCDS CIDA #NS01155.)

375.16

CIRCANNUAL ANALYSIS OF PINEAL INDOLES IN RATS KEPT ON CONSTANT PHOTOSCHEDULES. M.M. Prechel*, T.A. Dombrowski*, W.H. Simmons, J.A. McNulty*. Depts. of Anatomy and Biochemistry, Loyola Univ., Maywood, IL 60153.

Photoperiod is the primary regulator of circadian and seasonal changes in melatonin synthesis in the pineal gland. The extent to which other inputs are superimposed on this photic control has not been clearly established. This study was undertaken to determine if there are seasonal effects on circadian indole metabolism in rats housed under a constant photoperiod. Male, adult S/D rats were kept in L:D 12:12 with lights on at 0700 h. Pineal glands (n=4-8/time point) were collected over 15 monthly intervals at 4 time points (1200, 1900, 2400, 0500 h) over each L:D cycle. Individual pineal extracts were assayed either for indoles by HPLC-EC or RIA for neurohypophsyeal hormone activity. Results demonstrated circadian cycles in 5HT, 5HIAA, N-acetylsertonin and melatonin (MEL), and the August increase in immunoreactive arginine vasotocin (iAVT). Reduction in the amplitude of the MEL rhythm coincided with elevated iAVT, and 5HT levels tended to be greater during the winter months. Seasonal differences were also observed in correlation coefficients between indole constituents in individual glands. These findings suggest seasonal effects on the pineal melatonin pathway and peptide content even under controlled laboratory photoschedules.

375.17

PROPRANOLOL AND MELATONIN MODULATION OF SHORT PHOTOPERIOD-INDUCED GONADAL REGRESSION. T. H. Champney. Dept. of Med. Anat., Texas A&M Univ., College Station, TX 77843.

Daily propranolol (PROP) injections, given late in the day, block short photoperiod(SP)-induced gonadal regression in male Syrian hamsters, but not gonadal regression produced by daily injections of melatonin (MEL). Since MEL implants block gonadal regression produced by SP or MEL injections, the interaction of PROP, MEL implants and MEL injections was examined. Male hamsters were placed in SP (10L:14D, lights on at 0700 h, n=7 or 8 per treatment) and received daily sc injections (1645 h) of vehicle (ethanolic-saline), PROP (125 µg), MEL (25 µg) or PROP + MEL. Additional hamsters received biweekly implants of MEL (1 mg in 24 mg beeswax), daily PROP + implants, daily MEL + implants or daily PROP + daily MEL + implants. After ten weeks, body weights, testes weights and spleen weights were recorded. Plasma was collected and assayed for testosterone, thyroxine and cortisol levels. PROP injections blocked SP-induced testicular regression ($p<0.001$), but not MEL-induced regression. Hamsters implanted with MEL did not undergo SP- or MEL-induced gonadal regression ($p<0.001$) irrespective of any other treatments. These results indicate that PROP does not alter the ability of MEL implants to block gonadal regression. This adds further support to the hypothesis that PROP acts at the pineal to prevent SP-induced gonadal regression.

375.18

Fluorescent Dye Labeling of Retinal Cells with Projections to the Hypothalamus and Basal Forebrain. T.G. Youngstrom & A.A. Nunez. Dept. Psychology & Neuroscience Prog., Michigan State Univ., E. Lansing, MI 48824-1117.

In the Syrian hamster, several forebrain structures contain stained fibers after eye injections of Cholera toxin conjugated to horseradish peroxidase (Youngstrom, Weiss, & Nunez, *Soc. Neurosci. Abstr.* 14:52, '88). The present experiment sought to confirm some of these retinal ganglion cell projections using pressure injections of rhodamine labeled microspheres (RLM) aimed at several brain targets. Adult male and female Syrian hamsters (*Mesocrecitus auratus*; 130-190 gr) received bilateral pressure injections of 80 nl of RLM while under Equithesin anesthetic. After 7 days, animals were sacrificed with an overdose of the anesthetic. The upper torso was perfused with heparinized saline, the eyes were removed, then the upper torso was perfused with 10% buffered formaline. The retinae were removed from the eyes and fixed overnight in the same fixative. To confirm the location of the injections, the brains were sectioned (80 µm) and alternate sections mounted to subbed slides. Retinae were mounted without attempting to maintain visual field orientation. All tissue was examined with epifluorescence illumination. Because of the nature of the RLM labeling, only the somata and proximal dendrites were consistently labeled. The retinal projection to the paraventricular nucleus previously described (Pickard, *Soc. Neurosci. Abstr.* 14:50, '88) was confirmed in the present study. In addition, retrogradely labeled retinal ganglion cells were seen following injections into the preoptic area/diagonal band of Broca and rostral pole of the thalamus (Anteroventral/Anteromedial thalamic nuclei). Supported in part by NIMH Grant MH 37877 to AAN and by Biomedical Research Funds from Michigan State University.

377

SYMPOSIUM. THE BASAL GANGLIA: STRUCTURE AND FUNCTION. M.R. DeLong, Johns Hopkins Univ. (Chairperson); C. Gerfen, NIMH; S. Kitai, Univ. of Tennessee; O. Hikosaka, National Institute for Physiological Sciences, Okazaki, Japan; G.E. Alexander, Johns Hopkins Univ.

This symposium will be focused on recent developments in our understanding of the structure/function relations of the basal ganglia and the broader role of these structures in cerebral function. DeLong will give a brief overview of the recently proposed scheme of multiple parallel reentrant circuits linking specific regions of the cerebral cortex, basal ganglia and thalamus. Recent data suggest the existence of functionally segregated circuits concerned with skeletomotor, oculomotor, cognitive and limbic functions which selectively influence discrete frontal cortical areas, namely the supplementary motor area, the frontal eye fields, the prefrontal cortex and the anterior cingulate area. Gerfen will discuss recent anatomic findings on the intrinsic patch/matrix organization of the striatum and its relation to striatal afferent and efferent systems. Kitai will discuss recent concepts of the functional organization of the basal ganglia circuits at the cellular level, with emphasis on specific neurotransmitter/neuropeptide actions. Hikosaka will present recent physiologic findings obtained in behaving primates concerning the role of the basal ganglia in oculomotor function. Alexander will discuss recent findings on the physiology of the basal ganglia-thalamocortical 'motor circuit,' focusing on single cell recording studies in behaving primates that indicate this circuit may play a role in some of the highest levels of planning and preparation for movements, as well as in movement execution. DeLong will close the session with a consideration of the role of the 'motor circuit' in the pathophysiology of movement disorders, such as Parkinson's disease and Huntington's disease.

378

SYMPOSIUM. INSTRUCTIVE EFFECTS OF NEURONAL ACTIVITY IN THE DEVELOPING VISUAL PATHWAY. M. Constantine-Paton, Yale Univ. (Chairperson); C. Shatz, Stanford Univ.; M. Stryker, UCSF; M. Sur, MIT; S. Udin, SUNY Buffalo.

A popular explanation for the effects of early activity on the development of vision is that correlations in the patterns of activity carried by young retinal ganglion cells are used to determine the position of labile visual synapses. Only synapses that are simultaneously active remain together in central target zones. Implicit in this hypothesis is the idea that neural activity actually controls the structure of developing neural pathways rather than just permits normal development to occur. This symposium will present recent data relevant to the instructive hypothesis in the retinotectal pathway of developing amphibians and the geniculocortical pathway of developing cats. The presentations will cover experiments in which normal developmental dynamics are well understood and where activity has been manipulated surgically and pharmacologically. M. Constantine-Paton will present data indicating that pharmacologically induced changes in the sensitivity of the NMDA receptor alter the morphology of amphibian retinal ganglion cell terminal arbors and change the degree of segregation between retinal inputs within developing amphibian tecta. S. Udin will demonstrate that correlated patterns of activity working through an NMDA receptor are the critical determinants of registration between the left and right eye maps in *Xenopus laevis* tecta and that registration is achieved by selective afferent terminal arborization in the region of correlated activity. C. Shatz will describe the effects of prenatal activity block on the differentiation of retinal axon terminals and their segregation within the dorsal lateral geniculate nucleus of the fetal kitten. M. Sur will present data illustrating the interactions between X & Y type retinal terminals in the postnatal lateral geniculate and he will outline studies demonstrating activity-dependent aspects of these interactions. M. Stryker will close the symposium with his studies of the effects of activity manipulations and chronic drug infusions in the primary visual cortex of the postnatal cat.

379.1

DOES ENDOGENEOUS NGF PLAY A ROLE AS A NEUROTROPHIC FACTOR DURING REGENERATION? G.Raivich*, R.Hellweg* and G.W.Kreutzberg
Max-Planck Institute for Psychiatry, Munich, FRG

NGF is a well known neurotrophic factor which exherts its effects after retrograde axonal transport together with NGF receptor (NGFR) to the NGF-sensitive neuronal perikarya. Here we have studied changes in this axonal transport of endogeneous NGF, NGFR and NGFR saturation (NGF/NGFR) following axotomy and during regeneration of the rat sciatic nerve using NGF Enzyme-linked Immunoassay (ELISA) and quantitative *in situ* NGFR autoradiography.

The retrograde transport of NGF decreased dramatically to 10% of the normal levels one day after axotomy (1daa) but then returned to a stable plateau of 30-37% 3-13daa. The retrograde transport of NGFR decreased more gradually, reaching a similar plateau of approximately 40% 3-13daa. Starting with 21daa the axonal transport of both endogeneous NGF and its receptor gradually increased, reaching normal levels 45daa. Interestingly, the NGFR saturation dropped precipitously to 15% of the normal values 1daa but then rapidly recovered to normal levels (70-130%) during the whole course of nerve regeneration (6-45daa).

This normal NGFR saturation suggests that the endoneurium of the regenerating sciatic nerve provides the regenerating axons with NGF levels very similar to those in the normal peripheral target tissues. Despite this apparent ability of the axotomised endoneurium to replace the periphery, there is a 3-fold reduction in the axonal transport of NGF due primarily to the strong decrease in axonal NGFR expression and retrograde transport in the regenerating sciatic neurites. These data strongly suggest a similar dramatic decrease in the neuronal sensitivity to the neurotrophic effects of NGF during regeneration.

379.2

β-AMYLOID PROTEIN PRECURSOR GENE EXPRESSION: NERVE GROWTH FACTOR EFFECTS. W.C. Mobley, J. Valletta,* T. Barrall,* J. Long, F.M. Longo, D. Price and R. Neve. Dept. of Neurology, UCSF, San Francisco, CA 94143; Dept. of Pediatrics, Children's Hospital, Boston, MA 02115

The β-amyloid protein (β-AP) is present in the amyloid deposits that serve as a pathological marker of Alzheimer's disease (AD). Interest in discerning a role for β-AP in AD pathogenesis has focused attention on the mechanism of β-AP formation and plaque deposition. β-AP is derived from the β-amyloid precursor protein (APP), a membrane glycoprotein normally expressed in neurons in the central nervous system. APP expression alone is insufficient to explain plaque formation. However, the recent finding of three different APP mRNA species (Nature 331:525, 528 & 530, 1988) has raised the possibility that distinct forms of APP may contribute differentially to β-AP production. If so, controls regulating APP transcripts might influence β-AP and plaque formation.

Previously we examined APP expression in developing hamsters and found regionally specific patterns for APP mRNA (PNAS 85:9811, 1988). To examine further APP regulation we designed APP transcript-specific oligonucleotide probes. APP mRNA levels in the developing rat septum and caudate-putamen paralleled the patterns in hamster; levels were low immediately postnatally and rose to adult values within the first two weeks. APP-695 mRNA predominated throughout development and into adulthood, exceeding the level of APP-751/770 mRNA by at least 10-fold. The developmental increase in APP mRNA was thus due almost exclusively to an increase in APP-695 mRNA. In contrast to the rat brain, the predominant APP mRNA in undifferentiated PC12 cells is APP-751/770. NGF was shown to increase APP mRNA in the developing hamster septum; preliminary data show that NGF also induces an increase in the rat. In addition, PC12 cells treated with NGF showed a 2-fold increase (50 ng/ml for 24 hr in 2% FCS, 1% HS). It will now be possible to determine whether NGF influences APP expression through a differential effect on specific transcripts.

379.3

STUDIES ON THE EXPRESSION OF NGF mRNA AND PROTEIN IN THE DEVELOPING HIPPOCAMPUS IN VITRO. J.D. Roback+, T.H. Large#, U. Otten@, and B.H. Wainer+. +The University of Chicago, Chicago, IL 60637, #UCSF, San Francisco, CA 94143, and @Biocenter of the University, Basel, Switzerland.

Nerve growth factor (NGF) is a putative trophic factor for the basal forebrain cholinergic neurons of the central nervous system. NGF is synthesized at high levels in the target regions of these cholinergic neurons, the hippocampus and neocortex. In order to understand the physiologic signals which regulate NGF expression in the developing hippocampus, we have isolated the hippocampus from E_{15} mouse embryos and grown it in three-dimensional reaggregating culture.

At the developmental stage at which the hippocampus is removed it is "naive" with respect to septal interactions: no synaptic contacts have yet formed with the septal cholinergic neurons. Previously, we reported that NGF protein expression in aggregates from naive hippocampus increases approximately 10-fold between days 7 and 21 in culture. More recently, we have used Northern Blot analysis to quantitate NGF mRNA expression in hippocampal aggregates. Surprisingly, NGF mRNA does not undergo a similar developmental increase over the same culture period. Between days 7 and 21 in culture (a time during which NGF mRNA levels *in situ* increase approximately 12-fold), NGF mRNA levels in hippocampal reaggregates remain approximately constant (ca. 50 fg/25 ug total RNA). The observed increase in NGF protein thus appears to reflect accumulation of the protein within the aggregates rather than a developmental increase in NGF mRNA. Therefore, when the hippocampus is removed from its normal developmental environment, it retains the ability to synthesize NGF mRNA and protein, but has apparently not encountered appropriate signals for NGF mRNA upregulation as observed *in situ*. Supported by NIH grants 5-T32HD070009, NS-25787, and NS-21824 (to LFR).

379.4

Expression of the NGF Gene in Dissociated, Cultured Rat Hippocampal Cells. B. Lu, M. Yokoyama, C. F. Dreyfus and I. B. Black. Div. Dev. Neurol., Cornell Univ. Med. Coll., New York, N.Y., 10021.

We have used the hippocampus (Hi) as a model to study regulation of nerve growth factor (NGF) gene expression in the central nervous system (CNS). Sensitive nuclease protection assays detected Hi gene expression as early as embryonic day 16 (E16) in vivo, well before innervation by afferents. Hi explantation to culture, with consequent isolation, resulted in a time-dependent increase of NGF mRNA, mimicking development in vivo. These results suggest that the Hi has an intrinsic ability to produce NGF, independent of ongoing innervation.

To define the particular cell types that express NGF mRNA, we used dissociated cultures of relatively pure populations. In Hi neuronal cultures, NGF mRNA increased with time, further supporting the contention that the Hi is able to synthesize NGF in the absence of extrinsic innervation. In addition, NGF mRNA was highly expressed in nonneuronal support cell cultures. Levels of message were extremely high in glia-like cells plated at low density, undergoing rapid growth. With confluence, and decreased growth, the levels of NGF mRNA decreased. Transfer to serum-free medium further decreased message expression. These observations suggest that Hi glia-like cells also contribute to the elevation of NGF message in vitro, especially under rapid growth conditions.

To determine whether glial cells normally synthesize NGF in vivo, we measured the message in optic nerve, which presumably consists of astrocytes and oligodendrocytes, but no neuronal cell bodies. No detectable NGF mRNA was observed in adult rat optic nerve. Consequently, glial expression of NGF mRNA in vitro may reflect cell damage, analogous to peripheral Schwann cell expression of the gene after axotomy. We are currently studying glial-neuronal relations in the elaboration of NGF in the brain. (supported by NIH grants HD 22315, NS 10259, and a grant from the Bristol Myers Co. Inc.)

379.5

REGULATION OF NGF EXPRESSION IN RAT HIPPOCAMPAL CULTURES BY INTERLEUKIN-1 AND OTHER INFLAMMATORY MEDIATORS. W.J. Friedman[1,3], L. Larkfors*[3], C. Ayer-LeLievre*[1,2], T. Ebendal*[3], L. Olson*[2], and H. Persson*[1]. [1]Lab. of Molecular Neurobiology, [2]Dept. of Histology and Neurobiology, Karolinska Institute, Stockholm, and [3]Dept. of Developmental Biology, Uppsala University, Uppsala, Sweden

Nerve growth factor (NGF) is known to be expressed in the rat hippocampus, providing trophic support for afferent neurons such as the cholinergic projection from the basal forebrain. Lesions of this input elicit an increase in the level of NGF mRNA in the hippocampus. We have been using a dissociated culture system to examine regulation of NGF mRNA and protein. Rat hippocampi were dissected from embryonic day (E) 19 or 21 and maintained for periods of time up to 1 week in culture.

We have previously demonstrated that treatment of hippocampal dissociates with interleukin-1 (IL-1) elicited significant increases in NGF mRNA and protein. We have now used the culture system to further analyze the influence of IL-1 as well as other factors known to mediate responses to inflammation and injury. We have found that glucocorticoids inhibit expression of NGF, and that prostaglandin E_2 (PGE_2) enhances levels of NGF mRNA. Further studies are in progress to identify which populations of cells in these cultures express NGF and respond to these factors.

379.6

NERVE GROWTH FACTOR (NGF) REVERSES AXOTOMY-INDUCED ATROPHY OF MEDIAL SEPTUM CHOLINERGIC NEURONS. S. Varon, T. Hagg, J.M. Conner*, H.L. Vahlsing*, B. Fass-Holmes, M. Manthorpe. Dept.Biology, M-001, UCSD, La Jolla, CA 92093.

Delayed intraventricular NGF infusion in the adult rat can rapidly reverse the disappearance of most axotomized medial septum cholinergic neurons (MSC) immunostained for choline acetyltransferase (ChAT) or NGF receptor. We have utilized the delayed NGF treatment protocol to evaluate changes in MSC somal size following unilateral aspirative fimbria-fornix transection and subsequent NGF treatment. The cross-sectional size (μm^2) of ChAT-positive MSC neurons was determined in brain tissue sections at a total magnification of 500X using a digitizing computer program. Seven or 14 days post-lesion, the expected reductions in total numbers of ChAT-positive MSC neurons was accompanied by a reduction in average size, and frequency distribution analysis revealed an actual increase in the number of small neurons ($\leq 100\ \mu m^2$). That number increased even further after one day of NGF infusion started on day 7 or 14. The next several days of delayed NGF infusions reversed the average size reduction as well as the apparent reduction in total ChAT-positive neuronal numbers. With 2-week infusions, the average MSC somal size increased beyond normal values reflecting both a numerical increase of large ($>200\ \mu m^2$) and a numerical decrease of small neurons compared to normal.

These results suggest that the loss of endogenous NGF consequent to axotomy leads to a progressive cellular atrophy (rather than to the preferential loss of stainability of a subset of large MSC neurons). Furthermore, NGF treatments can reverse somal atrophy and can even induce hypertrophy of some MSC neurons. The finding that NGF can regulate neuronal size, in addition to the presence of several neuronal proteins, e.g. ChAT and NGF-receptor, confirms the view that NGF has a genuine trophic function for adult basal forebrain cholinergic neurons. Supported by grant NINCDS NS-16349.

379.7

A CELL LINE PRODUCING RECOMBINANT NGF EVOKES GROWTH RESPONSES IN INTRINSIC AND GRAFTED CENTRAL CHOLINERGIC NEURONS. P.Ernfors#[1], T. Ebendal[2], L. Olson[3], P. Mouton[3] J. Strömberg[3], V. Chan-Palay[4], H. Persson[1]. (SPON: O. Johansson) [1]Dept. of Chemistry, Lab. of Molecular Neurobiology and [3]Dept. of Histology and Neurobiology, Karolinska Institute, Stockholm, Sweden, [2]Dept. of Developmental Biology, Uppsala University, Uppsala, Sweden. [4]Dept. of Neurology, University hospital, 8091 Zurich, Switzerland.

The rat β-nerve growth factor gene was inserted into a mammalian expression vector and cotransfected with a plasmid conferring resistance to neomycin into mouse 3T3 fibroblasts. From this transfection a stable cell line was selected that contains several hundred copies of the rat NGF gene and produces excess levels of recombinant NGF. Such genetically modified cells were implanted into the rat brain as a probe for new in vivo effects of NGF on central nervous system neurons. In a model of the cortical cholinergic deficits in Alzheimer's disease (AD), we demonstrate a marked increase in the survival of, and fiber outgrowth from, grafts of fetal basal forebrain cholinergic neurons, as well as a stimulation of fiber formation by intact adult intrinsic cholinergic circuits in the cerebral cortex. Adult cholinergic interneurons in intact striatum also sprout vigorously towards implanted fibroblasts. It is suggested that this model has implications for future treatment of neurodegenerative diseases. To evaluate the possible role of NGF in AD, expression of NGF and NGF receptor were examined in brains from AD patients.

Submitting and presenting author

379.8

NGF REGULATES GABA-ERGIC NEURONS IN THE CULTURED RAT BASAL FOREBRAIN. C. F. Dreyfus and I. B. Black. Div. Devel. Neurol., Cornell Univ. Med. Coll., New York, N.Y. 10021.

Recent studies have indicated that high-affinity NGF receptors are associated with at least two basal forebrain (BF) populations. Radioautographic visualization of high-affinity NGF receptors (NGF-R's) and immunocytochemistry have localized high-affinity NGF-R's to choline acetyltransferase (CAT)+, cholinergic neurons, as well as to gamma-aminobutyric acid-ergic (GABA) cells. However, while biological actions of NGF on the cholinergic population have been extensively described, it has been difficult to define actions of the trophic factor on GABA neurons. The present study was designed to determine whether NGF regulates this BF population.

Dissociated cultures of embryonic day 17 rat BF were grown for up to 3 weeks. GABA neurons were monitored by immunocytochemical visualization of GABA and catalytic assay to detect levels of glutamic acid decarboxylase (GAD), the GABA synthetic enzyme.

Initial studies indicated that GABA neurons underwent dramatic development in control medium. GABA+ cells exhibited extensive neurite elaboration during the first 7 days in culture, accompanied by a significant increase in GAD catalytic activity. To define the actions of NGF, cultures were grown in the presence of growth factor and assayed for GAD activity after 5, 7, 10, 14 or 21 days. After 10 days, NGF elicited a marked 2-fold increase in GAD activity. Our observations suggest that the action of NGF in the BF is not limited to the cholinergic population, but extends to GABA cells as well. We are presently further characterizing the actions of NGF on these different forebrain populations. (Support: NIH grant HD 23315, NS 10259)

379.9

SEXUALLY DIMORPHIC EXPRESSION OF THE NGF RECEPTOR GENE IN RAT BRAIN. D.R. Kornack, B. Lu, and I.B. Black. Div. Devel. Neurol., Cornell Univ. Med. Coll., New York, NY 10021.

While sexually dimorphic traits are well-recognized in brain systems, associated dimorphism in trophic, regulatory molecules has not been defined. We now report evidence for sexually dimorphic gene expression of a trophic receptor molecule in the brain.

Recent work has detected sexually dimorphic development of cholinergic enzyme activity in the rat basal forebrain (BF); females attain adult levels of choline acetyltransferase (CAT) activity earlier than males. Moreover, these neurons are known to express nerve growth factor (NGF) receptors. Further, NGF increases activity of BF CAT. Is the sexually dimorphic development of transmitter phenotype a reflection of dimorphic expression of trophic elements? We have measured levels of NGF receptor (NGF-R) mRNA in rat BF and NGF mRNA in hippocampus, examining sex differences during development.

BF NGF receptor message levels were consistently greater in females than in males during the first two postnatal weeks; by the third week, however, male and female levels did not differ, a finding that parallels sexually dimorphic cholinergic development in the BF. In contrast to the sexually dimorphic NGF-R message development, hippocampal NGF mRNA levels did not differ between sexes, suggesting that regulation of trophic effects in the mammalian brain may occur at the level of trophic receptor gene expression.

Our observations suggest that sex differences in transmitter phenotype in specific brain nuclei are associated with sexually dimorphic gene expression of trophic, regulatory elements. (Supported by NIH grants HD 23315 and NS 10259.)

379.10

CO-LOCALIZATION OF NGF AND ESTROGEN RECEPTORS: IMPLICATIONS FOR THE BASAL FOREBRAIN. C. D. Toran-Allerand[1] and N. J. MacLusky[2]. Dept. Anat. & Cell Biol.[1], Columbia Univ., P&S, New York, NY 10032 and Div. Reprod. Sci.[2], Univ. Toronto, Toronto, Canada M5G IL4.

While research on aging and Alzheimer's disease has focused on the importance of NGF for the basal forebrain, scant attention has been paid to other survival- and growth-promoting substances which influence those neurons such as estrogen. We report for the first time the co-localization of estrogen and NGF receptors to a subset of neurons in the medial septum/nuclei of the diagonal band, using ^{125}I-estrogen autoradiography combined with immunocytochemistry. P12 female rats received a single dose of 2 nmol/kg 16α-^{125}I-iodo-11β-methoxyestradiol (2,200 Ci/mmol), a synthetic estrogen. 4% paraformaldehyde fixed, 10 μm brain sections were processed for steroid autoradiography, followed by NGF receptor immunocytochemistry, using monoclonal IgG 192. Neurons were found interspersed in the medial septum/diagonal band that expressed NGF receptor immunoreactivity with or without discrete localization of ^{125}I-estrogen. Other neurons exhibited estrogen receptors alone or neither marker.

Growth-promoting effects of estrogen have been demonstrated not only during development but in estrogen receptor-containing regions of the traumatized adult CNS as well. Our observations suggest that the potential for interactions between NGF and estrogen exists and that both NGF and estrogen may be trophic for the development and maintenance of basal forebrain circuits, which may have considerable clinical relevance. (Supported by NIH (AG08099) and the American Health Assistance Foundation.)

379.11

PURKINJE CELLS IN THE DEVELOPING RAT CEREBELLUM EXPRESS HIGH- AND LOW-AFFINITY NERVE GROWTH FACTOR RECEPTORS. S. Cohen-Cory, C.F. Dreyfus and I.B. Black. Lab. of Neurobiol., Rockefeller Univ. and Div. of Dev. Neurol., Cornell Univ. Med. Coll., New York, N.Y., 10021.

In the cerebellum, Nerve Growth Factor (NGF) and NGF receptor are highly expressed during late embryonic and early postnatal development, suggesting that NGF may play an important role in cerebellar ontogeny. While receptor has been localized to some cerebellar populations, delineation of receptor subtypes, and potential physiologic function, remain to be defined.

The objective of the present work was to localize NGF binding sites to cell types in the developing cerebellum, and to define receptor subtypes. We employed ^{125}I-NGF binding to delineate low- and high-affinity receptor sites in the postnatal day 10 rat cerebellum. High-affinity receptor sites were detected by displacement of low-affinity binding with a chase of excess unlabeled NGF. We observed both high- and low-affinity receptor sites on developing Purkinje cells immunostained with anti Vitamin D-dependent Calcium Binding Protein antibodies (kindly provided by Dr. S. Christakos). ^{125}I-NGF-generated silver grains were localized to cell bodies, dendrites and axonal processes of all Purkinje cells. On some cells, a higher density of silver grains was localized to the distal dendrites, while in others, the highest density was on the basal pole of the cell body. Grain density per Purkinje cell varied within and among folia, and appeared to be correlated to maturation of Purkinje cells. Our observations indicate that the biologically active, high-affinity sites are localized to cerebellar Purkinje cells. (NIH grant HD23315)

379.12

EVIDENCE FOR A PHYSIOLOGICAL ROLE OF NGF IN THE CNS OF ADULT RATS. M. Fusco*, N. Schiavo*, L. Bigon*, M. Fabris*, A. Leon and G. Vantini. Fidia Research Laboratories, 35031 Abano Terme, Italy.

We have recently shown that intracerebroventricular (i.c.v.) administration of rabbit anti-NGF IgG (6 ug injected daily from postnatal day 2 to 8) significantly reduces choline acetyltransferase (ChAT) activity in the septum and hippocampus of 9-day-old rats. These results provide direct demonstration that endogenous NGF plays a physiological role in the septo-hippocampal cholinergic system of newborn animals. In addition, following the injection protocol described above, a significant reduction in ChAT activity was also observed in the striatum of newborn rats.

To further investigate the extent to which endogenous NGF is involved in maintenance or regulation of function of mature forebrain cholinergic neurons we have examined the effect of continuous infusion of anti-NGF IgG (via miniosmotic pump) in CNS of adult rats. Results indicate that i) i.c.v. (right lateral ventricle) infusion of anti-NGF IgG induces a moderate decrease of ChAT activity in the striatum ipsilateral to the infusion side, ii) intrastriatal continuous infusion of anti-NGF IgG induces a marked decrease of ChAT activity in the area adjacent to the injection side.

These data provide evidence for a physiological role of endogenous NGF in adult mammalian CNS.

380.1

PURIFICATION AND CHARACTERIZATION OF BRAIN L-GLUTAMATE RECEPTOR. J.-Y. Wu,[1,2,3] C.C. Liao,[*2,3] C.J. Lin,[*2,3] Y.H. Lee,[1*] J.Y. Ho,[1*] and W.H. Tsai.[*1] (SPON: B.Dworkin). [1]Inst. Biomed Sci., Academia Sinica, Taipei, Taiwan, [2]Neurosci. Program & Dept. of Anatomy, Penn. St. Univ., Hershey, PA 17033, and [3]Dept. Physiol. & Cell Biol., Univ. Kansas, Lawrence, KS 66045.

L-Glutamate receptor (GluR) was isolated from pig brain by initial homogenization in 0.32 M sucrose, followed by differential centrifugation to obtain the crude mitochondrial fraction (P2 fraction) and finally by hypotonic shock to lyse the P2 fraction resulting in the receptor enriched P2 membrane. The basic binding characteristics of membrane-bound GluR were studied over a wide range of L-Glu from 5.36 nM to 5 μM. The dissociation constant, K_d and the total receptor concentration, B_{max} were obtained from Scatchard plot as 102 nM and 2.23 pmol/mg protein, respectively. A Hill coefficient of 0.99 was obtained from Hill plot suggesting a single site model for the binding of L-Glu to the receptor. GluR could be solubilized by incubating P2 membrane (15 mg/ml) with 10 times of 50 mM Tris-Cl, pH 7.2 containing 0.5% (^{V}/v) of Triton X-100 at 4^{o}C for 30 min. The solubilized GluR has binding characteristics similar to the membrane-bound receptor. Further purification of GluR has been achieved by a combination of ultrafiltration and repetitive column chromatographies, e.g. AcA 34 gel filtration. (Supported in part by grants from National Science Council, Taiwan & grants NS 20978, NS 20922, and EY 05385 from NIH, U.S.A.)

380.2

ANALYSIS OF A KAINIC ACID RECEPTOR IMMUNO-RELATED PROTEIN IN RAT AND FROG BRAIN. D.R. Hampson, C. Banner, C.J. Dechesne, R.J. Wenthold. Faculty of Pharmacy, University of Toronto, Lab of Molecular Biology and Lab of Molecular Otology NIH, Bethesda MD.

Monoclonal antibodies raised against a kainic acid receptor purified from frog brain label a 48 kDa protein on immunoblots of frog brain. One of these antibodies also labels a 99 kDa protein in both frog and rat brain. This protein appears to be brain-specific in both species. On immunoblots of frog brain, reducing agents are capable of modulating the relative intensities of the Mr=48,000 and the Mr=99,000 proteins, suggesting the possibility of a monomer/dimer relationship. However, reducing agents had no effect on the intensity of the 99 kDa protein in rat brain. Immunocytochemical localization demonstrated that the immunoreactivity was widely distributed in frog brain; the pattern of immunoreactivity in frog brain coincides with the pattern of ^{3}H-kainic acid binding sites. In rat brain, the pattern was more restricted. Pyramidal cells in the hippocampus and purkinje cells in the cerebellum stained intensely with this antibody.

Further characterization of the protein in rat brain is being conducted by analysing cDNA clones identified through immunoscreening of rat brain expression libraries. We suggest that the Mr=99,000 protein in rat brain may be part of, or associated with, a mammalian kainic acid receptor or a related receptor subtype.

380.3

INHIBITION OF [^{3}H]MK-801 BINDING BY 7-CHLOROKYNURENIC ACID, A NON-COMPETITIVE INHIBITOR OF THE NMDA RECEPTOR, IS SELECTIVELY REVERSED BY GLYCINE. R. Sircar, M. Frusciante* and S.R. Zukin. Departments of Psychiatry, Neuroscience and Neurology, Albert Einstein College of Medicine, Bronx, NY 10461.

Glycine, D-serine and related amino acids stimulate NMDA receptor mediated neurotransmission and PCP receptor binding via the activation of a non-strychnine-sensitive glycine recognition site situated on the NMDA receptor complex. 7-chlorokynurenic acid (7-CK) has been reported to inhibit [^{3}H]glycine binding and to abolish NMDA-induced currents in rat cortical neurons, effects selectively reversed by glycine but not by NMDA. Here we present evidence that 7-CK inhibits [^{3}H]MK-801 binding to the PCP receptor and that this effect is also selectively reversed by glycine. Aliquots of thoroughly-washed rat forebrain synaptosomal membranes were incubated with 1 nM [^{3}H]MK-801 and graded concentrations of 7-CK in the presence and absence of glutamate and/or glycine. Non-specific binding was determined by addition of 10 μM unlabeled MK-801. Bound radioligand was separated from free by filtration under reduced pressure and radioactivity was measured by liquid scintillation spectrometry. Under all experimental conditions 7-CK inhibited [^{3}H]MK-801 binding. In the absence of any added glutamate and/or glycine 7-CK attenuated the binding with an IC_{50} of 6.8 $\pm$ 1.5 μM. 50 μM glycine, both in the presence and absence of glutamate, significantly shifted the dose response curve of 7-CK to the right. These data support the concept that 7-CK acts as a competitive antagonist at the glycine modulatory site of the NMDA receptor complex. Since [^{3}H]MK-801 binding is an index of NMDA receptor associated ion channel function, reduced [^{3}H]MK-801 binding in the presence of 7-CK is consistent with the concept that glycine is required for channel activity.

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380.4

IN VIVO EVIDENCE THAT D-CYCLOSERINE IS A PARTIAL AGONIST AT THE NMDA-ASSOCIATED, GLYCINE RECEPTOR SITE. M.R. Emmett*, P.L. Wood, T.S. Rao, S. Mick*, J. Cler*, and S. Iyenger. (Spon: D.L. Cheney). CNS Diseases Research, G.D. Searle & Co., St. Louis, MO 63198.

The antibiotic D-cycloserine has been shown to be a partial agonist at the NMDA coupled, strychnine-insensitive glycine receptor by in vitro receptor binding (Hood et al., 1989). This partial agonism was demonstrated in an in vivo system by monitoring NMDA mediated cerebellar cyclic GMP levels in mice.

Both direct intracerebellar and systemic (subcutaneous) injections of D-cycloserine produced a biphasic, partial dose response curve. When intracerebellar co-injections were made with NMDA, D-cycloserine decreased the NMDA elicited increase in cyclic GMP by approximately 50%. D-cycloserine was also able to block D-serine (glycine agonist) induced increases in cyclic GMP. Systemic injections of D-cycloserine elicited a much wider window of agonism than seen with intracerebellar injections. These data are important in the study of NMDA mediated neurotransmission since D-cycloserine is a parenterally bioavailable compound with both agonist and antagonist properties at the NMDA-associated, glycine receptor.

380.5

IN VIVO MODULATION OF CEREBELLAR NMDA RECEPTORS BY THE GLYCINE AGONIST, D-SERINE: SUPPORT FOR COMPARTMENTATION OF GLYCINE IN THE CEREBELLUM. P.L. Wood, M. Emmett*, T.S. Rao, S. Mick*, J. Cler* and S. Iyengar. CNS Diseases Research, G.D. Searle and Co., St. Louis, MO 63198.

Direct intracerebellar injections of NMDA elicited dose-dependent increases in mouse cerebellar cGMP levels. The Hill slope for this logit-log dose relationship suggested that 3 molecules of NMDA are required per NMDA receptor for activation. In contrast, direct intracerebellar injections of D-serine increased cGMP levels with a Hill slope of unity, indicating a unimolecular interaction with its receptor.

The actions of D-serine were antagonized by the competitive NMDA antagonist, CPP and by a partial agonist (D-cycloserine) and competitive antagonist (HA-966) of the NMDA-coupled glycine receptor. These data are all consistent with D-serine activation of the NMDA-coupled glycine receptor resulting in a potentiation of ongoing NMDA-dependent neuronal activity in the cerebellum. These data also argue that the glycine receptor is not saturated at physiological concentrations of glycine.

380.6

DIFFERENT ACTION OF 3-AMINO-1-HYDROXY-2-PYRROLIDONE (HA-966) AND 7-CHLOROKYNURENIC ACID IN THE MODULATION OF N-METHYL-D-ASPARTATE SENSITIVE GLUTAMATE RECEPTORS. W. Danysz*, E. Fadda*, J.T. Wroblewski and E. Costa. (SPON: A.P. Kozikowski). Fidia-Georgetown Institute for the Neurosciences, Georgetown Univ. Sch. of Med., Washington, D.C. 20007.

N-methyl-D-aspartate (NMDA)-sensitive glutamate receptors were reported to be inhibited by HA-966 and 7-chlorokynurenic acid (Cl-KYN) that counteract the positive glycine modulation. In rat brain synaptic plasma membranes Cl-KYN and HA-966 inhibited completely [^{3}H]glycine binding. Moreover, Cl-KYN inhibited completely the binding of [^{3}H]glutamate and of the NMDA antagonist [^{3}H]3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP). In contrast, HA-966 only partially inhibited [^{3}H]glutamate binding, but increased the affiniy of [^{3}H]CPP binding with a potency similar to its inhibition of [^{3}H]glycine binding. This increase was inhibited by glycine and Cl-KYN. The binding of [^{3}H](+)5-methyl-10,11-dihydro-5H-dibenzocyclohepten-5,10-imine maleate (MK-801), used as an index of NMDA receptor activation, was completely inhibited by Cl-KYN, but only partially by HA-966. Moreover, HA-966 antagonized the inhibitory action of Cl-KYN to the level seen with HA-966 alone. In addition, HA-966, but not Cl-KYN, increased the potency of CPP to inhibit [^{3}H]MK-801 binding. One might infer that Cl-KYN is an antagonist of the glycine modulatory site, while HA-966 acts as a negative modulator of NMDA-sensitive glutamate receptors.

380.7

N-METHYL-D-ASPARTATE SENSITIVE GLUTAMATE RECEPTORS IN CEREBELLAR GRANULE CELLS INDUCE A CALCIUM-MEDIATED RELEASE OF ARACHIDONIC ACID. J.W. Lazarewicz*, J.T. Wroblewski and E. Costa. Fidia-Georgetown Institute for the Neurosciences, Georgetown Univ. Sch. of Med., Washington D.C. 20007

In primary cultures of cerebellar granule cells, glutamate, aspartate and N-methyl-D-aspartate (NMDA) induced a dose-dependent release of [^{3}H]arachidonic acid (AA). This release was inhibited by NMDA receptor antagonists and was potentiated by the positive modulator D-serine. The observed effect was selective for NMDA receptor agonists and was not induced by kainate or quisqualate. The agonist-induced [^{3}H]AA release was reduced by quinacrine at concentrations which inhibited phospholipase A_2 but failed to affect the activity of phospholipase C or the hydrolysis of phosphoinositides induced by glutamate or quisqualate. This indicates that the increased formation of AA is due to a receptor-mediated activation of PLA_2 rather than the action of PLC followed by diacylglycerol lipase. The receptor-mediated [^{3}H]AA release was dependent on the presence of extracellular Ca^{2+} and was mimicked by the Ca^{2+} ionophore ionomycin. The pretreatment of granule cells with pertussis toxin failed to inhibit the receptor-mediated [^{3}H]AA release. The results suggest that the stimulation of NMDA receptors in cerebellar granule cells leads to an activation of PLA_2 that is mediated by Ca^{2+} ions entering through the NMDA channel, rather than by a direct coupling involving a pertussis toxin-sensitive GTP-binding protein.

380.8

KAINIC ACID RECEPTORS MEDIATE THE RELEASE OF ALANINE AND GLYCINE THROUGH A MECHANISM THAT DIFFERS FROM THE RELEASE OF GLUTAMATE. M. Ulivi*, E. Costa, and W. J. Wojcik, FGIN, Georgetown University, Washington D.C. 20007

In primary cultures of cerebellar granule cells, kainic acid (KA) induces the release of glutamate (GLU), a putative neurotransmitter for these cells, and two other amino acids: alanine (ALA) and glycine (GLY). These amino acids can be separated by HPLC and measured after derivatization to fluorescent compounds. The KA-receptor mediated release of GLU is Ca^{++} dependent and probably results from kainic acid induced depolarization. In contrast, KA releases ALA and GLY in a Ca^{++} independent, but Na^+ dependent manner with a IC_{50} of approximately 30 uM. The KA receptor antagonists, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), was used and found to inhibit the KA-induced release of ALA and GLY indicating that this effect was KA receptor-mediated. The IC_{50} for CNQX against 100 uM KA was 0.5 uM. Quisqualate was also tested and found to have no action by itself. However, it antagonized KA, 300 uM, with an IC_{50} of 50 uM. The amount of ALA and GLY released into the incubation buffer was greater during the first five minutes after KA exposure than the amount of released GLU. However, the KA induced release of ALA and GLY stops after five minutes due to a depletion of intracellular stores. The KA induced GLU release continues to increase with continuous exposure to KA. An action on ALA and GLY transport may be operative in the KA induced release of these two amino acids.

380.9

A NEW GLUTAMATE RECEPTOR SUBTYPE: THE QUISQUALATE RECEPTOR COUPLED TO THE INOSITOL PHOSPHATE RESPONSE AND C-KINASE. F. A. J. Sladeczek*, O. J. J. Manzoni*, I. Sassetti*, F. Fignels*, C. Le Peuch*, and J. Bockaert* (SPON: B. Rouot). C.C.I.P.E. Rue de la Cardonille, F-34094 Montpellier Cedex 2, France.

In striatal neurons in primary culture we discovered a glutamate receptor of the quisqualate (Q) subtype able to stimulate the formation of inositol phosphates (IPs) (Sladeczek F. et al., *Nature*, 317: 717, 1985). Here we show that quisqualate induced the rapid formation of IP_1, IP_2, IP_3 and IP_4 and potently increased intracellular calcium concentrations ($(Ca^{++})_i$) in the cell bodies of these cells as measured by photometer microscopy with Fura-2. The $(Ca^{++})_i$ increase but not the formation of IPs depended on the presence of extracellular Ca^{++}. AMPA stimulated with higher affinity the $(Ca^{++})_i$ increase (EC_{50} = 2· μM) than the IP-response (EC_{50} = 560 μM). Kainate and Domoate potently stimulated the $(Ca^{++})_i$ increase but had only very small effects on the IP response. CNQX decreased both types of responses, whereas GAMS only affected the $(Ca^{++})_i$ -response. Quisqualate (10 μM) but not AMPA (10 μM) activated C-kinase as assessed by its translocation. 10 nM of the phorbol ester PdBu completely blocked the quisqualate evoked IP-response but had no effect on the $(Ca^{++})_i$ increase. Phorboldidecanoate was without effect and staurosporine (100 nM) completely reversed the PdBu-blockade. These results demonstrate that there exist two different types of quisqualate receptors. A classical ionotropic receptor, increasing $(Ca^{++})_i$ most probably via depolarization and opening of voltage sensitive Ca^{++}-channels and a new metabotropic quisqualate receptor able to stimulate the formation of inositol phosphates, to activate C-kinase and which can be desensitized by protein kinase C activation. In order to distinguish these two receptors we recently proposed the nomenclature Q_i for the ionotropic receptor and Q_P for the metabotropic receptor (Sladeczek et al., *Trends in Neurosc.*, 11: 545, 1988).

380.10

EXCITATORY AMINO ACIDS STIMULATE INOSITOL PHOSPHOLIPID HYDROLYSIS AND CA^{2+} FLUX, AND INDUCE NEURODEGENERATION, IN CULTURED RETINAL CELLS: EFFECT OF GM1 GANGLIOSIDE. S.D. Skaper, D. Milani*, L. Facci and A. Leon. Fidia Research Laboratories, 35031 Abano Terme, Italy.

Excitatory amino acid (EAA) recognition sites located in synaptic membranes have been widely studied. However, little is known concerning the molecular processes operating in the transduction of their chemical signals across the plasma membrane. To investigate whether EAAs activate membrane phosphoinositide breakdown and alter Ca^{2+} homeostasis in neurons of the visual system, cultures of retinal cells were prepared from 8-day chicken embryo, labeled with [^{3}H]inositol at day 5, and at day 7 challenged with various EAA receptor agonists. Kainate stimulated the formation of inositol mono-, bis- and trisphosphates in a concentration and time-dependent manner. The rank order of agonist potency was: glutamate›kainate›NMDA››ibotenate ≈ quisqualate. External Ca^{2+} was required, with Mg^{2+} blocking the action of glutamate and NMDA. EAA agonists induced an increase in $[Ca^{2+}]i$ in selected cells, as measured using Fura-2. Glutamate, NMDA and kainate were also neurotoxic (sparing photoreceptors); ganglioside GM1 pretreatment reduced this neurodegeneration. The role of these biochemical events in the pathophysiological response of retinal neurons to EAA agonists, and the effect of GM1 on their action, is under investigation.

380.11

EXCITATORY AMINO ACID RECEPTORS. NOVEL SELECTIVE AGONISTS AND ANTAGONISTS FOR NMDA AND NON-NMDA RECEPTORS. U. Madsen*, L. Brehm*, J.J. Hansen* P. Krogsgaard-Larsen, Dept. Org. Chem., Royal Danish Sch. Pharmacy, Dk-2100 Copenhagen, Denmark.

The excitatory amino acid (EAA) receptors are at present subdivided into three classes: NMDA, QUIS/AMPA, and KAIN receptors, some of which appear to be heterogeneous.

A number of highly selective NMDA and QUIS/AMPA receptor agonists, derived from ibotenic acid, have been developed. Structure-activity studies on these compounds have disclosed different conformational and stereochemical requirements for activation of NMDA and QUIS/AMPA receptors. The results of electrophysiological studies indicate different agonist characteristics of cortical and spinal NMDA receptors. Further development of these ibotenic acid analogues have led to the two non-NMDA antagonists, AMOA and AMNH. In a rat cortical slice model AMOA was shown to be a selective QUIS/AMPA antagonist (ED_{50}, 200μM), whereas AMNH selectively blocked KAIN receptors (ED_{50}, 100 μM). Using microelectrophoretic techniques the excitatory effects on cat spinal neurones of AMPA and KAIN were equally sensitive to blockade by AMOA or AMNH. Receptor binding and electrophysiological studies have shown that neither AMOA nor AMNH interact with NMDA receptors.

380.12

REGULATION OF GLUTAMATE/ASPARTATE RELEASE BY NMDA RECEPTORS. D. Martin, G.A. Bustos, M.A. Bowe, S.D. Bray* and J.V. Nadler. Depts. Pharmacology and Neurobiology, Duke Univ. Med. Ctr., Durham, NC 27710.

The synaptic release of the excitatory transmitters glutamate and aspartate can be regulated by a variety of neuroactive compounds present in the vicinity of the synapse. We have addressed the issue of regulatory mechanisms by studying the actions of transmitters and modulators upon glutamate/aspartate release from the Schaffer collateral-commissural-ipsilateral associational (SCCIA) pathway in area CA1 of the rat hippocampus.

Transmitter release was evoked from superfused slices of the CA1 area (excluding stratum lacunosum-moleculare) by exposing the slices to two 1-min pulses of 50 mM K^+. Test compounds were present only during the second K^+ exposure, and their effects were determined on the ratio of amino acid released by the second exposure compared to the first. Previous studies showed that under our conditions nearly all the release of glutamate and aspartate is Ca^{2+}-dependent and it originates predominantly from the SCCIA pathway.

TTX (0.1 uM) reduced the K^+-evoked release of glutamate and aspartate by about 40%, suggesting that a portion of the release depended on the generation of action potentials as the K^+ concentration within the tissue was rising. NMDA (100 uM) did not affect glutamate/aspartate release evoked by either 50 or 33 mM K^+. However, the NMDA receptor antagonists CPP (20 uM) and phencyclidine (20 uM) reduced the release of both amino acids by 22-36%. CPP was effective only in the absence of Mg^{2+}, whereas phencyclidine had similar effects in the absence or presence of Mg^{2+}. The action of CPP was entirely confined to the TTX-sensitive component of release. These finding suggest that NMDA receptors regulate glutamate/aspartate release from SCCIA terminals. These receptors appear to be fully occupied by agonist during exposure to elevated K^+ and agonist occupation enhances the further release of transmitter. The mechanism of this enhancement more likely involves a block of K^+ channels than the facilitation of Ca^{2+} influx. (Supported by NIH grant NS 16064.)

381.1

TRANSCRIPTIONAL CONTROL OF NGF GENE EXPRESSION. S.R. D'Mello, J.M. Alexander* and G. Heinrich, Dept of Med, University Hospital and BUSM, Boston, MA 02118.

Transient expression of 5' deletion mutants of the cloned mouse NGF gene promoter in L929 cells indicated that several regions within 250 bps upstream of the transcription initiation site affect basal transcription. To identify transcription factors that bind to the functionally defined regions, DNAse I footprint analysis was undertaken. L929 cell nuclear extracts protected three regions within the 250 bp mouse promoter. A fourth region displayed partial protection. Competition experiments indicated that binding of the factors to these sites is specific. The nucleotide sequence within two of these footprints share a 'GGAGGG' motif, suggesting that these regions are bound by the same factor. An additional footprint was found at the first intron-exon junction. Comparisons of the nucleotide sequences contained within these protected regions with the binding sites of known regulatory factors revealed no significant similarities, suggesting that as yet unknown factors may regulate NGF gene expression. The L929 cell footprint patterns are being compared with those of mouse SMG as a first step towards understanding the mechanisms underlying the high level of NGF gene transcription in that tissue.

381.2

NEURON-SPECIFIC ENOLASE PROMOTER DIRECTS β-GALACTOSIDASE EXPRESSION TO NEURONS IN TRANSGENIC MICE. S.Forss-Petter, P.E.Danielson*, E. Battenberg, J.Price* and J.G.Sutcliffe. Research Institute of Scripps Clinic, La Jolla, CA 92037.

To analyze regulatory elements for neuron-specific gene expression, mouse oocytes were injected with a hybrid gene, composed of 2 kb 5' upstream sequence from the rat neuron-specific enolase (NSE) gene and E.coli β-galactosidase (β-gal) coding sequence. Of seven mice carrying complete trans-gene integrations, four gave rise to offspring with detectable transgene mRNA and β-gal activity (analyzed in situ using the chromogenic substrate X-gal) in the brain. Two of the lines showed a high level of expression in most CNS-neurons and, like endogenous NSE, the amount varies in different neuronal types. Testes were the only other site of expression. A third line showed moderate but widespread neuronal β-gal activity, whereas the fourth line expressed β-gal in a limited set of neurons. Though the integration site affects expression, these results indicate that neuron-specific transcription elements are located within 2 kb upstream from the NSE gene. These mice should be valuable in cell-lineage studies, as neurons of this genotype are easily identifiable.

381.3

GAP-43 GENE STRUCTURE. E. Grabczyk*, M.X. Zuber*, H. Federoff*, S.-C. Ng, A. Pack* and M.C. Fishman. Howard Hughes Medical Institute, Developmental Biology Laboratory of the Massachusetts General Hospital, Boston, MA 02114

GAP-43 is a protein believed to be important to the function of growth cones and to nerve terminal remodelling. Many features of its gene regulation suggest it to be an excellent guide to the molecular biology of the developing neuron. It will be important to determine what causes restriction of its expression to neurons, regional restriction of expression in the adult CNS, and increased expression during periods of axonal elongation and regeneration. In order to begin to pursue the nature of cis- and trans-acting GAP-43 gene control we have isolated genomic GAP-43, and mapped its intron-exon boundaries and transcriptional start sites. The promoter is quite unusual in its structure, containing a repetitive sequence capable of forming unusual conformations and lacking some canonical promoter components. Transcription can initiate from more than one site, and some of the start sites are utilized differently in the central and peripheral nervous systems.

381.4

GENERATION AND REGULATION OF THE DIVERSITY OF MURINE NCAM TRANSCRIPTS DURING BRAIN DEVELOPMENT AND SELF-REPAIR OF THE CNS. W.Wille[1], D.Barthels[1*], A.Jungblut[1*], C.Ruppert[1,*], S.W. Scheff[1,2], M.J.Santoni[3*], and C.Goridis[3*] [1]Inst. f. Genetik, Univ. of Cologne, Köln, **FRG**, [2]Dept. Anat. Univ. of Kentucky, Lexington KY **USA**, and [3]Centre d' Immunol., INSERM-CNRS, Marseille, **France**

The neural cell adhesion molecule (NCAM) of the mouse exists in several isoforms which are selectively expressed by different cell types and at different stages of development. Four isoform-related transcripts are generated from just one primary messenger and code for NCAM-180, -140 & -120. Since the differences between these isoforms are due to alternative splicing in the coding region for the transmembrane and cytoplasmic domains, the extracellular, N-terminal portion of NCAM seemed to be shared by all three protein forms. Extensive studies revealed a number of alternatively spliced-in extra exons generating a complex hinge region in the membrane proximal domain and an altered Ig-like domain IV. The alternative splicing does not only generate a high number of distinctive NCAM transcripts at a given developmental stage, but changes the composition of NCAMs during CNS neurogenesis. This age-dependent process is distinguishable from NCAM gene induction during CNS self-repair.

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381.5

MOLECULAR CLONING AND EXPRESSION OF TYPE II ADENYLATE CYCLASE FROM BOVINE BRAIN. P.G. Feinstein*, H.A.Bakalyar* J.Krupinski*, A.G.Gilman*, and R.R.Reed(SPON:B.Largent). Howard Hughes Med. Inst. and Department of Molecular Biology and Genetics, Johns Hopkins Univ. Sch. of Medicine,Balt., MD 21205. Department of Pharmacology, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd.,Dallas, TX 75235

Adenylate cyclase has been extensively characterized at the biochemical level during the past 15 years. These studies have suggested two basic forms of the enzymes, a calcium/calmodulin sensitive form as well as an insensitive species. Adenylyl cyclase protein has been characterized from several tissue sources by biochemical and immunological techniques. We have undertaken a molecular genetic approach to further our understanding of the basis for the various forms of the adenylate cyclase enzyme. A calcium/calmodulin sensitive bovine brain adenylate cyclase was purified over a forskolin-sepharose column and the sequence of several tryptic peptides determined. An oligonucleotide probe deduced from the sequence of one peptide was used to screen cDNA libraries derived from bovine and rat tissues. Two classes of cDNA clones were isolated. One class of cDNA's encodes a protein predicted to contain all of the tryptic peptides whose sequences were determined. We refer to the protein encoded by this cDNA as adenylate cyclase,Type I. Additional cDNAs were identified and predicted to encode a protein which is similar in size and topological arrangement. The considerable amino acid homology (55% overall) and the extensive similarity in the proposed catalytic domains (80-90%) argues that this cDNA encodes an alternative form of adenylate cyclase (Type II). Northern analysis indicates that Type II adenylate cyclase is expressed in a variety of tissues.

381.6

NEUROEPITHELIAL EXPRESSION OF A THY-1.2/*lacZ* FUSION GENE IN THE DEVELOPING CNS OF TRANSGENIC MICE. K.A. Kelley, G.S. Manjunath* and K. Herrup. The Fishberg Research Center for Neurobiology, The Mount Sinai School of Medicine, New York, NY 10029 and The E.K. Shriver Center, Waltham, MA 02254.

Transgenic mice were prepared from a Thy-1.2/*lacZ* hybrid gene. As previously reported (Kelley and Herrup, Soc. for Neuro. Abst., 14: 622, 1988), neuron-specific expression was observed in adult offspring from one transgenic animal. Embryos from this line were examined to determine the temporal pattern of expression, if any. Intense ß-galactosidase (ß-gal) activity was detected in the neural tube as early as embryonic day 10 (E10). By E13, the neuroepithelial cells lining the ventricular zone are heavily labeled, and this staining persists through later stages. Labeled cells are present that appear to be moving away from the ventricular zone, reminiscent of migrating neurons. In addition to the neuroepithelial expression, ß-gal is also present at very high levels in the dorsal root ganglia (DRG) from E13-E14. The DRG staining is absent at E15, and is never detected in the adult.

Thy-1/*lacZ* transgene activity in cells of the early neuroepithelium, and its persistence in migrating neurons, suggests that the ß-gal staining can be used to trace developing neurons from their genesis in the ventricular zone. At E13, for example, labeled spinal cord motoneurons in the lateral motor column that have migrated out from the ventrolateral region of the ventricular zone are visible; mature adult motoneurons express relatively high levels of transgene activity. The utility of this marker as a tool for the study of cell lineage and cell maturation is thus quite high.

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381.7

FUNCTIONAL ANALYSES OF THE CALMODULIN (CaM)-BINDING DOMAIN OF CAM-KINASE II (CK-II) USING SYNTHETIC PEPTIDES AND SITE-DIRECTED MUTAGENESIS. P. Kelly, T. Honeycutt, R. Weinberger, D. Blumenthal, R. Yip, and N. Waxham, Dept. of Neurobiol. and Anatomy, and Neurology, Univ. of Texas Med. Sch., Houston, TX 77225.

Recombinant DNA and synthetic peptide studies have localized the CaM-binding domain of CK-II to residues 295-309 of the 50 kDa (alpha) subunit (ARRKLKGAILTTMLA). Synthetic peptides encompassing this domain are potent inhibitors (IC_{50}=90 nM) of CaM-dependent activation of CK-II. This regulatory domain has properties similar to many CaM-binding peptides, namely an amphipathic alpha-helix with basic amino acids near one end and hydrophobic residues near the other. A series of deletion peptides with the same C-terminus (Ala-309) were synthesized to more accurately define the minimum CaM-binding domain of CK-II. Removal of the two N-terminal arginines only slightly decreased the CaM antagonist properties (IC_{50}=200 nM), whereas deletion of the N-terminal sequence ARRK produced a peptide (residues 299-309) that displayed very poor CaM antagonism (IC_{50}=75 uM). Similar CaM-antagonistic properties were observed with this series of peptides using myosin light chain kinase, an enzyme activated at much lower [CaM] (K_a=4 nM) then CK-II. Analyses of site-directed mutations of bacterially-expressed CK-II supported results with synthetic peptides and showed the importance of basic amino acids at the N-terminus of its CaM-binding domain with Lys-298 being a critical determinant. Mutational studies further revealed that the CaM-dependent activation of CK-II does not require the hydrophobic sequence ILTTMLA (residues 303-309) near its C-terminus. These results indicate that the important features of CaM-dependent activation of CK-II reside in the basic amino acids KLK (residues 298-300).

381.8

MOLECULAR CHARACTERIZATION OF BRAIN CALMODULIN-DEPENDENT PROTEIN PHOSPHATASE, R. L. Kincaid*, D. Amorese*#, J. Tamura*, S. Higuchi*, S. C. Dixon*, C. A. Marietta*, P. Rathnagiri* and B M. Martin*+ (SPON: M. Billingsley) Immunology Sect., NIAAA, Rockville, MD 20852; #Molecular Genetics Group, DuPont Co., Wilmington, DE 19898; and +Molecular Neurogenetics Sect., NIMH, Bethesda, MD 20892.

Full-length cDNA and partial genomic clones for calcineurin were isolated and characterized from murine and human brain libraries using non-isotopic methods. A biotinylated restriction fragment corresponding to the 5' end of a partial cDNA (Kincaid, et al, PNAS 85: 8983, 1988) was used to identify clones, the gross structures of which were deduced by Southern blot analysis with biotinylated oligonucleotide probes. The DNA sequence for the catalytic subunit, determined by automated sequencing using fluorescent dideoxy nucleotides, showed >40% identity with those of the protein phosphatases 1 and 2A. Analysis of mRNA expression showed a major species of ~4000 bp, present in highest amounts in brain, with minor bands in other tissues such as testis. The 59 kDa subunit was expressed in bacteria and purified in high yield (0.8 mg/liter) by affinity chromatography. Studies using recombinant forms with specific deletions have identified the site of interaction with calmodulin.

381.9

TISSUE SPECIFIC EXPRESSION OF A GENE WHICH IS MUTATED IN A MPP'-SELECTED PC12 MUTANT. S.G. Carlson*, M.J. Kadan, R.C. Douglas*, K.A. Marcus, M.M.S. Lo. NIDA, ARC, Baltimore, MD 21224

Pheochromocytoma (PC12) cells were mutated by infection with a recombinant retrovirus, and selected on 500uM MPP'. The proviral position was examined in 30 drug selected clones. A particular locus was found to contain integrated virus in 3 different mutant clones. The provirus and 12 Kb of flanking genomic DNA downstream to the virus was cloned from one mutant (M39). The flanking genomic DNA contained a specific 0.9 Kb coding region which hybridized to a distinct messages of about 1.5 Kb in normal PC12 cells. This coding sequence was used to isolate cDNA clones from a PC12 cDNA library. A near-full length cDNA of 1.4 Kb was used to screen Northern blots of the MPP' selected mutant lines, and found to be reduced in several lines. The same cDNA was used to screen Northern blots prepared from various rat tissues. Significant levels of this gene were detected in salivary gland, but not in brain, liver or adrenal gland. The DNA sequence of this gene was also determined, and was not found to be related to any other known gene sequences. Elucidating the function of this gene, which is differentially expressed in salivary gland, may provide some clues to its role in MPP' toxicity in PC12 cells.

381.10

MOTOR NEURON-SPECIFIC GENES: A HYBRID CELL cDNA CLONING STRATEGY. S. Pasternak*, N.R. Cashman, K. Hastings*. Dept. of Neurology, McGill University, Montreal, Qc H3A 2B4.

Studies of the cell biology of specific neuronal populations are hampered by the limited purity and yield of primary cultures. In the attempt to provide a potentially unlimited homogenous supply of motor neuron-like cells, we have developed a series of neuroblastoma-spinal cord (NSC) hybrid cell lines (Soc Neurosci Abs, 1987). The subclone NSC-34-6 expresses a variety of differentiated motor neuron characteristics not observed in the neuroblastoma parent N18TG2, including choline acetyltransferase activity and formation of stable neurite-myotube contacts. We employed a cDNA cloning and screening strategy designed to identify cloned copies of mRNAs which are more abundant in NSC-34-6 than in the N18TG2 parent. Differential plaque hybridization yielded 3 such clones; two correspond to a mitochondrial mRNA (cytochrome oxidase subunit 1) and one corresponds to an unknown mRNA containing a copy of the B1 repeat sequence. A separate subtraction hybridization screening yielded one clone whose mRNA was undetectable in the N18TG2 parent. This NSC-34-6 specific mRNA encodes chromogranin B. Because chromogranins are known to be expressed in motor neurons, this result suggests that neural hybrid cell-specific gene expression may reflect a contribution from the non-tumor parent cell. Further subtraction hybridization screening of the library is being undertaken in the hope of identifying additional NSC-34-6 specific and possibly motor neuron-specific mRNA.(Supported by MDA)

381.11

PROTO-ONCOGENE C-FOS IN THE LEECH CNS. Susan A. DeRiemer & Lara Gussman* Columbia University, New York, NY 10027

A majority of proto-oncogenes appear to be elements of signaling pathways and exist in high levels in the brain. The levels of c-fos are modulated in mammalian cells by synaptic activity and calcium influx. In order to study the evolution and function of these genes, we looked for homologues in the simpler nervous system of the leech.

Using monoclonal antibodies raised against peptides from the mouse c-fos protein, we have found a c-fos immunoreactive protein in the leech CNS which shares many properties with that found in mammals. It appears as a doublet on SDS gels with M_rs=52 and 62 kDa. Its levels are increased by high potassium depolarizations and forskolin within 30 min. of stimulation, and there appear to be both nuclear and cytosolic fos-like proteins in intact cells. Comparison of the cross-reactivity with two antibodies recognizing different parts of the protein suggest that the C-terminal region (amino acids 359-378) which is c-fos specific is more highly conserved than a region (amino acids 132-154) found in both c-fos and v-fos.

We are now analyzing the relationship between c-fos levels and the activity of single cells in vivo and in vitro.

This work was begun as a Westinghouse Science Project (L.G.) with support from a Sloan Research Fellowship (SAD).

381.12

WITHDRAWN

382.1

EARLY PROCESS OUTGROWTH OF SPINAL ACCESSORY MOTOR NEURONS STUDIED WITH diI IN FIXED TISSUES. V. Palavali* and W.D. Snider, Dept. of Neurology, Washington Univ. Sch. Med., St. Louis, MO 63110.

Spinal accessory neurons are unique among motor neurons in the spinal cord because their axons do not exit in ventral roots but rather exit dorsally as rootlets of the spinal accessory nerve. A description of axon outgrowth of accessory neurons is thus pertinent to understanding how the axons of spinal motor neurons make correct pathway choices. We have utilized lateral diffusion of diI in fixed tissues (Godement et al.1987) to study early process outgrowth of these neurons in embryonic rats. Growth cones of accessory neurons were labelled on E11 by placing tiny crystals of diI near the ventral commissure of the cervical cord. Accessory growth cones are found in the lateral region of the cord and project dorsally to exit midway betwen the dorsal and ventral poles. Upon exiting the cord, accessory axons immediately turn rostrally to form the accessory nerve. In the cord, growth cones are broad with filopodia until they near the external limiting membrane where they elongate. In the area of the turn growth cones are quite complex with numerous branches and filopodia, whereas within the nerve growth cones are narrow with few filopodia.

It was also possible to label the somata of spinal accessory neurons by placing crystals on the lateral region of the spinal cord as early as E11, and on the accessory nerve itself, thereafter. Many spinal accessory neurons on E11 possess an apical process directed toward the central canal. By E12 neurons are unipolar. By E13 many neurons have assumed a bipolar configuration and one or more dendrites with secondary branches have appeared. Our results demonstrate that axonal and dendritic processes can be revealed in striking detail from the earliest stages of motor neuron differentiation using diI. We show here that growth cone morphology changes with position along the accessory pathway. Use of diI in fixed tissue should allow study of process outgrowth in mammalian spinal cord in a way previously possible only for lower vertebrates.

382.2

RHOMBOMERES AND LONGITUDINAL BOUNDARIES IN THE CHICK EMBRYO BRAINSTEM SUBDIVIDE VESTIBULAR NEURONS ACCORDING TO AXONAL PATHWAY. J.C. Glover, Physiol.Inst., Univ.of Oslo, Norway

Vestibulospinal and vestibulo-ocular neuron groups can be distinguished on the basis of position and axonal pathway in the brainstem of the 11 day chicken embryo (Glover & Petursdottir, J.comp.Neurol.,270:25, 1988; Petursdottir & Glover, Neurosci.Abstr., 13:1504, 1987). To investigate how this spatial organization arises, retrograde tracers were injected into the vestibular axons as they were growing towards their targets. To label vestibulo-oculomotor neurons, the tracers were injected unilaterally into the medial longitudinal pathway at pontine levels. To label vestibulospinal neurons, they were injected unilaterally into the lateral longitudinal pathway at medullary levels.

At all stages, vestibulospinal and vestibulo-ocular neurons were spatially segregated, the former sandwiched in the longitudinal axis by the latter. Two-tracer studies, coupled with the visible morphological boundaries of the rhombomeres (r) on day 4, revealed that 1) vestibulospinal neurons projecting in the lateral pathway are restricted to r4, and 2) their caudal vestibulo-ocular neighbors neatly span the length of r5/6, and are subdivided into a contra-projecting column lying just medial to an ipsi-projecting column. Thus, axonal pathway is correlated with vestibular neuron position in an appealing geometric pattern that suggests that pathway choice may be determined by a simple array of segmental and mediolateral positional cues.

382.3

FORMATION OF THE MEDIAL LONGITUDINAL FASCICLE IN WILD-TYPE AND MUTANT ZEBRAFISH EMBRYOS. W. K. Metcalfe and K. Hatta*. Inst. of Neuroscience, U. of Oregon, Eugene, OR 97403

In 1941, J. Oppenheimer suggested that midbrain neurons may establish pathways that are necessary for hindbrain reticulospinal axons to project normally to the spinal cord. In zebrafish embryos, this pathway, the medial longitudinal fascicle (mlf) forms as a tight fascicle of axons descending on either side of the midline from reticulospinal neurons of the midbrain (the nucleus mlf) and hindbrain. In the cyclops mutant of zebrafish, *cyc-1((b16)*, midbrain axons do not form a normal mlf, and hindbrain axons are dispersed over the floor of the hindbrain rather than forming discrete fascicles as they descend. These observations are consistent with Oppenheimer's hypothesis.

To test this hypothesis in wild-type embryos, we examined the time course of axon outgrowth. We found that many hindbrain reticulospinal neurons began to grow axons directly along the appropriate pathway nearly simultaneously, regardless of their longitudinal position and before the arrival of the first descending fibers from the midbrain nucleus mlf. Further, we found that the hindbrain reticulospinal axons grew normally following laser ablation of the midbrain nucleus mlf.

We conclude that hindbrain reticulospinal axons are capable of growing along their normal pathways independently of influences from the midbrain nucleus mlf.

Supported by NIH grants NS17963, HD22486, and the Naitoh Foundation.

382.4

CLIMBING FIBERS CONTACT TARGET PURKINJE CELLS IN EMBRYONIC MOUSE CEREBELLUM

C.A. Mason and R. Blazeski*, Dept.of Pathology, College of Physicians and Surgeons of Columbia University, New York, N.Y. 10032.

Prior to synaptogenesis in complex brain regions, afferent axons must coordinate their ingrowth with the migration and settling of their target cells. We have witnessed three novel features of this process in embryonic mouse cerebellum by simultaneously labeling climbing fibers with DiI applied to the inferior olivary nuclei in fixed tissue, and Purkinje cells with antisera to calbindin (gift of S. Christakos). 1). At E14-15, olivary axons approach the cerebellum in a tract running under the pia, meeting the external granule layer as it spreads from the rhombic lip. By E15-16, olivary axons enter the cerebellum during Purkinje cell migration. The axon entry zone is surrounded by Purkinje cells and many axons enter this mass, while others grow toward migrating cells. By E18, fine, unbranched olivary axons project within the Purkinje cell zone, by now in a wide band beneath the external granule layer. 2) Growth cones display different forms during these stages: long and foliated in the tract leading to the cerebellum, complex with filopodia as they exit the tract, foreshortened, flat and lamellopodial as they grow toward targets, and minute and tapered within the Purkinje cell zone. 3) After relatively rapid entry and approach onto target cells, unbranched olivary axons make simple synaptic contacts with target Purkinje cells, and maintain this configuration from E18-P2.

These results constitute evidence that climbing fibers do not 'wait' to advance onto targets, as suggested by lower-resolution tracing methods, but pause after first interactions with targets and before arborization. In addition, consistent with our findings in the retinal axon pathway, growth cones change shape with locale and behavior.

382.5

EXPRESSION OF THE DISIALOGANGLIOSIDE GD3 IN DEVELOPING AXON TRACTS OF THE FETAL MOUSE BRAIN. **M.A. Edwards, G.A.Schwarting* and M. Yamamoto. Depts. of Devel. Neurobiol. and Biochem., E.K. Shriver Ctr., and Harvard Med. Sch., Waltham, MA 02145**

Previously, we reported that the monoclonal antibody 7C7, recognizes the ganglioside GD3 as its major antigenic species and immunocytochemically stains the early primary optic pathway of the mouse (E12-15) (Edwards and Yamamoto, Neurosci. Abstr. 11, 316, '85). In addition, dense staining was associated with proliferative neuroepithelial zones, as described by others using anti-GD3 antibodies in embryonic vertebrates. Further studies with 7C7 using various fixation protocols indicate that GD3 expression is concentrated transiently (2-4 days) in most or all fetal CNS axon systems. Comparisons were made with a new monoclonal antibody, PPATH, inferred to recognize acetylated GD3 based on antigen mobility with TLC and conversion to 7C7 reactivity with ammonia vapor treatment. PPATH stains only restricted ventricular zone sites and a subset of fetal CNS axon systems, including optic axons and trigeminal and vestibular fiber systems as far as the cerebellar rudiment. Ultrastructural observations reveal that both 7C7 and PPATH stain sites of axon-axon apposition as well as axon-radial glia contacts. (Funded by NIH Grants EY06080, HD21018, HD04147 and NS25580).

382.6

SELECTIVE EXPRESSION OF A NOVEL CADHERIN IN THE PATHWAYS OF DEVELOPING MOTOR- AND COMMISSURAL AXONS B.Ranscht and M.T.Dours (SPON: J.Dodd) La Jolla Cancer Research Foundation, NCI, La Jolla, CA 92037

Developing nerve fibers find the way to their targets with temporal and spatial precision even in the face of a changing environment. One possible explanation for this selectivity is that the pattern of neuronal connections is established by the interaction of neuronal cell surface components with molecules in the extracellular milieu. We have isolated and cloned a cell surface glycoprotein from chick embryo brains which is a novel member of the family of cadherin cell adhesion molecules. Our cDNAs exhibit 47 percent sequence identity with the extracellular domains of chick N-cadherin and less with E- and P-cadherin and L-CAM. In developing chicken embryos the expression of this new molecule has two striking features that suggest a role in establishing the pattern of nerve fiber growth. In the neural tube at the time of commissural axon growth, our antibodies recognize a small ventral area including the floor plate. This expression pattern correlates with the suggested role of the floor plate in controlling the pattern of commissural axon growth. In developing somites, staining is observed in a striking segmental pattern in the posterior region of the somitic sclerotome at the time of motor axon growth. This localization coincides with the establishment of the segmental pattern of motor nerve fibers, which is based on motor axons selecting only the anterior region of the somite segments to project into the periphery. It is an attractive hypothesis to speculate that cell adhesion molecules of the cadherin family could influence the pattern of neuronal connections in the developing nervous system.

382.7

SUBPLATE NEURONS PIONEER THE FIRST AXON PATHWAY OUT OF THE CEREBRAL CORTEX. S.K. McConnell, A. Ghosh and C.J. Shatz, Dept. of Neurobiology, Stanford Univ. School of Medicine, Stanford, CA 94305.

During the embryonic development of the mammalian cerebral cortex, the earliest-generated neurons form a transient structure known as the subplate (SP). SP neurons in the cat are born between E24 and E30; they reach a high degree of morphological maturity in fetal life, and disappear by cell death postnatally. To explore the possible roles of SP neurons in early cortical development, we placed tiny crystals of DiI into the cerebral cortex of cat fetuses at E26 and E30, times before the permanent neurons of the cortical plate have been generated. In E30 fetuses (but not E26), the DiI labeled long-distance axon pathways that headed directly toward, and sometimes entered, the internal capsule (the gateway from cortex to thalamus). There was a gradient of development of this pathway: temporal cortical injections labeled axons extending through the capsule and into the thalamus, whereas axons labeled from more dorsal injections did not yet reach these structures. Next, to identify the cells of origin of this pathway, we placed DiI in the internal capsule or thalamus of E30 fetuses. These experiments resulted in the retrograde labeling of temporal SP neurons and their axons, showing that SP cells form the early axon pathways. At later fetal ages (from E36 on), DiI-labeled axons could be traced even from the far dorsal areas of cortex (e.g., visual cortex) to the thalamus and tectum. Many of these axons must arise from SP neurons, since SP cells could be retrogradely labeled from these target structures well into fetal life. The present results suggest that one function of SP neurons is to pioneer the first axon pathways from the cortex to subcortical targets, at a time before the permanent neurons of the cerebral cortex have been generated. This raises the possibility that the pioneering axons of SP neurons may be required for the development of permanent subcortical projections from the deep-layer neurons of the cortex.

Supported by NIH grants EY02858 and EY06028.

382.8

ABLATION OF SUBPLATE NEURONS ALTERS THE DEVELOPMENT OF GENICULOCORTICAL AXONS. A. Ghosh, A. Antonini, S.K. McConnell and C. J. Shatz, Dept. of Neurobiology, Stanford Univ, Stanford, CA 94305.

During development of connections between thalamus and cortex, ingrowing thalamic axons wait in the subplate (SP) below the cortical plate (CP) for several weeks before growing into layer 4. The SP contains a special class of transient neurons, the SP neuron, which has led to the suggestion that interactions between SP neurons and waiting axons are essential for the normal development of thalamocortical projections. To investigate this suggestion, we (1) studied morphological changes in individual axons from the lateral geniculate nucleus (LGN) during development in fetal and neonatal cats by making DiI injections into the LGN and (2) ablated SP neurons by injecting kainic acid into the waiting zone. In normal development, as early as E30, LGN axons tipped with growth cones are present in the internal capsule. As the axons grow caudally towards visual cortex, they send out simple collaterals that are restricted to the SP and do not enter the CP. On reaching the visual cortex (E42), axons branch extensively within the SP. At about E55, axons begin to invade the CP and arborize, so that by P7 (birth=E65) a highly branched but fairly compact terminal arbor is restricted to layers 4 and 6.

Kainic acid injections into the SP zone at E42, when axons are waiting there, radically alters the trajectories of LGN axons. The lesion itself almost completely eliminates SP neurons but leaves the CP intact. LGN axons examined at E60 fail to form dense arborizations within the remaining zone of future white matter below the CP and instead run past the visual cortex in a tight fascicle of axons. By P5, LGN axons should normally have invaded the CP, but in lesioned cases they fail to do so and again continue past visual cortex, seemingly unaware of their correct destination. These observations indicate that subplate neuron-LGN axon interactions during the waiting period play a critical role in permitting thalamic afferents to select the appropriate cortical target area. Supported by NIH grant EY02858.

382.9

STIMULATION OF TECTAL CHEMOTROPIC INFLUENCE IN ANOPHTHALMIC MICE BY TRANSPLANT-DERIVED RETINAL AXONS M.H. Hankin & R.D. Lund, Dept. Neurobiol., Anat. & Cell Sci., Univ. Pittsburgh, Pittsburgh, PA 15261

Our previous work based on transplantation studies in rodents has indicated that retinal axons are guided to the tectum by several types of cues (Hankin and Lund, *J. Comp. Neurol.* (1987) 263:455). The outgrowth patterns in normal rat hosts exhibited by transplant-derived optic axons suggest that local outgrowth-promoting cues are expressed along the optic tract in a 200 um wide region just below the glia limitans (***subpial pathway***). The distance-dependent outgrowth towards the tectum by the earliest axons emerging from retinae placed in the midbrain parenchyma indicates that, in addition to these substrate pathway cues, optic axons may also be attracted to the tectum by diffusible factors emanating from the target region.

In the present study we have used the ocular retardation (or^J) mutant mouse as a transplant recipient to test whether optic innervation stimulates the tectal chemotropic influence (the genetic mutation in these mice leads to optic nerve aplasia during embryogenesis and by birth results in anophthalmia). Our results show that transplant-derived optic axons can innervate the or^J tectum via the subpial pathway. However, directed outgrowth towards the tectum through the or^J parenchyma takes place only in the presence of input to the tectum from a second retina placed along the subpial pathway.

It is suggested that the tectum in mammalian vertebrates produces a diffusible factor whose production (or release) is regulated by the initial population of ingrowing retinal axons. The possible function of this factor, in addition to that suggested by its chemotropic effect on transplant axons, may be to signal optic axons in the vicinity of the target to change from an elongation mode to one favoring the formation of terminal branches.

Supported by NIH grants NS26777 and RR05416 (MHH) and EY05308 (RDL).

382.10

AXON PATHFINDING IN THE EMBRYONIC MOUSE VISUAL SYSTEM. D.W.Sretavan. Lab. of Neurobiology, Rockefeller Univ., New York, NY 10021

At the mammalian optic chiasm, axons of retinal ganglion cells (RGC) project along either the ipsilateral or contralateral optic tract to reach CNS targets. In the mouse, ipsilaterally projecting RGCs are restricted to ventral temporal retina while those projecting contralaterally are found throughout the retina. This pattern of chiasmatic routing could result from specific axon growth at the chiasm or by initial randomly directed growth followed by elimination of incorrectly projecting RGCs. To differentiate between these possibilities, retinal projections were examined during the period of RGC neurogenesis between embryonic days 11 to 18 (E11-18; Drager, U., Proc. R. Soc. Lond. B224:57, 1985). By using either an anterograde tracer technique (small local retinal injections of fluorescent dye, DiI) or a retrograde labeling method (fluorescent latex microspheres injected into the optic tract), an adult-like pattern of axon routing at the chiasm can be demonstrated as early as E15. Thus, embryonic RGC axons make appropriate pathway choices at the optic chiasm.

The trajectories of individual axons were reconstructed in order to examine how these pathway choices are made. To do so, E14-E18 DiI labeled axons which have just grown past the chiasm into the contralateral or ipsilateral tract were photoconverted to form a DAB reaction product. Ipsilaterally and contralaterally projecting axons were found throughout all portions of the optic nerve and were intermixed at the entrance of the chiasm. Contralateral axons entering the chiasm close to the origin of the ipsilateral optic tract either grow well past this region without entering or once within this region turn away abruptly and head towards the origin of the contralateral tract. Similarly, ipsilateral axons up to 250μm away from the origin of the ipsilateral tract were observed to turn sharply at right angles and head directly into the ipsilateral tract crossing over axons growing towards the opposite side. These observations suggest that retinal axons are not merely passively channeled into the correct pathway but instead appear to be capable of active pathfinding at the optic chiasm.

382.11

SELECTIVE AXON GROWTH NOT AXON LOSS PRODUCES CONNECTIONAL DISTINCTIONS BETWEEN CALLOSAL AND SUBCORTICALLY PROJECTING LAYER 5 CORTICAL NEURONS. Susan E. Koester and Dennis D.M. O'Leary (SPON: G. Harding) Dept of Neurosurgery, Washington Univ Sch Med, St. Louis, MO 63110

In the cortex of adult rats, layer 5 neurons send axons through the corpus callosum to contralateral cortex or through the internal capsule to subcortical targets, but individual neurons reportedly do not have both connections. Here we confirm this separation and address whether it develops by extension of axon collaterals to both sets of targets with later elimination of one or the other (a phenomenon common in developing cortex) or by initially selective axon outgrowth. Retrograde tracers Fast Blue and Diamidino Yellow were injected in the subcortical path at the pyramidal decussation and in contralateral cortex, respectively, of adult and newborn rats. In 16 adults, no cortical neurons are double-labeled, indicating that none project to both sites. In 17 neonates, we found only one double-labeled cell, while in each brain hundreds of thousands of layer 5 neurons are single-labeled. This result rules out that this connectional distinction in adults is achieved by the elimination of long transient callosal or subcortical collaterals. To determine if shorter transient collaterals are extended subcortically by callosal neurons, we injected DiI into one cortical hemisphere of 7 aldehyde fixed E21 brains. In months, DiI retrogradely fills callosal neurons, including their axons and all branches. Two types of axons are seen in the white matter of the cortex opposite the injection: those which turn and extend through the callosum and those which branch, sending collaterals both to ipsilateral and contralateral cortex. None of the callosal neurons extend collaterals into or toward the internal capsule. We conclude that from early stages of axon extension, callosal and subcortically projecting cells are distinct classes of neurons and, apparently in response to cues present in cortex, initiate growth toward class-specific and non-overlapping sets of targets. (Support: NEI grant EY07025 & McKnight Foundation)

382.12

SPECIFIC OUTGROWTH SHAPES THE ORIENTED DENDRITIC ARBORS OF DEVELOPING NEURONS IN RAT SOMATOSENSORY CORTEX. A. Peinado and L.C. Katz. Laboratory of Neurobiology, The Rockefeller University, 1230 York Avenue, New York, NY 10021.

In several cortical and subcortical systems, including monkey striate cortex and the 3-eyed frog optic tectum, postsynaptic dendrites respond to borders between segregated afferent inputs. In the rat primary somatosensory cortex these biases are dramatic: spiny stellate neurons within layer 4 of the rat barrel cortex have their dendrites confined to the cortical territory of the thalamic afferents representing a single whisker. We examined the emergence of these biased dendritic arbors using brain slices from rats at postnatal days 4-11 (P4-P11), in which thalamocortical afferents were identified by anterograde transport of rhodamine injected into the ventrobasal thalamus at P2. Dendrites were visualized by intracellular injection of Lucifer Yellow.

Between P4 and P5, afferent labeling in layer 4 segregated from a continous band of label to increasingly obvious clusters. Even before clusters were completely segregated, the dendrites of postsynaptic cells were oriented away from the boundaries between afferent clusters. Dendrites growing towards a barrel border either terminated or turned abruptly upon encountering the boundary. At P4-5, most layer 4 dendrites still lacked their characteristic spines, yet they behaved like dendrites of adult animals. At later ages (P7-11) dendrites became longer, more highly branched, and spinous. With the increasing arbor size, initial biases became even more pronounced. We conclude that the dendritic bias observed in layer 4 cells results from early specific outgrowth, and not elimination of inappropriately situated dendrites. These results further suggest that the thalamic afferents themselves carry some signal that triggers dendritic growth. One difference between afferents in different barrels is the degree of correlation of electrical activity among incoming fibers. Postsynaptic dendritic growth may require a threshold level of temporally correlated inputs. Supported by NS08466 and the L.P. Markey Charitable Trust.

383.1

PAVLOVIAN CONDITIONING INDUCES REDUCTION OF BRANCHING VOLUME OF AN IDENTIFIED NEURON IN *HERMISSENDA*. D.L. Alkon, J. Dworkin*, H. Ikeno*, D.L. McPhie*, J.L. Olds, I.I. Lederhendler, L.D. Matzel, B.G. Schreurs*, A. Kuzirian*, and C. Collin*. NIH, NINDS, LMCN, Bethesda, MD 20892.

Storage of a learned association of discrete sensory stimuli has previously been shown in the mollusc *Hermissenda crassicornis* to be related to changes of protein phosphorylation, synthesis of specific proteins, and of membrane currents of a specific neuron, the medial Type B cell (causally implicated in the memory storage). Blind electrophysiologic measurements revealed that 4-5 days after training, conditioned Type B cells had significantly higher ($p < 0.02$ by ANOVA and post-hoc analyses) input resistance (inversely related to K^+ channel conductance) than cells from either Unpaired or Naive animals (which showed no between group differences). Images of Ni^{2+}-lysine-injected Type B cells' terminal branches were then analyzed with a computer-based system for measuring the limits of branching. Limiting boundary volume for the terminal arborization was obtained as a product of x, y, and z values. One way ANOVA and Scheffe contrasts demonstrated that both Unpaired and Naive Type B branch volumes (EV) were not different from each other but were significantly greater ($p < 0.01$) than volumes for the Paired animals. The degree of branching volume reduction was correlated (inversely) ($p < 0.014$) with the magnitudes of the blindly obtained input resistance values.

383.2

PHORBOL ESTER, AN ACTIVATOR OF PROTEIN KINASE C, INDUCES MORPHOLOGICAL CHANGES OF *HERMISSENDA* PHOTORECEPTORS. I. Lederhendler, R. Etcheberrigaray*, & D. L. Alkon. LMCN-NINDS, NIH, Bethesda, MD 20892.

In *Hermissenda* voltage-dependent K^+ currents within the Type B photoreceptor remain reduced for days following classical conditioning. Activation of protein Kinase C (PKC) induces changes in these same currents. PKC activation has also been implicated in learning-specific modifications of specific protein substrates in the photoreceptors. Since the Type B photoreceptor undergoes persistent conditioning-specific changes in structure (Alkon *et al.*, Soc. Neurosci. Abstr., 1989) we asked whether PKC activation alters cell shape. We isolated the eye, consisting of five photoreceptors, a single spherical lens, and a central area of screening pigment. We measured the area of individual photoreceptors from photographs obtained on an inverted microscope. In the presence of light, 220nM phorbol produced a significant increase (9.2 ± 3%) in the size of individual photoreceptors. Phorbol application in the absence of light, or application of 220nM inactive phorbol did not change the photoreceptors (ANOVA, $p<0.01$; $df = 2,17$). Because the photoreceptors were partially obscured by the screening pigment, any reduction in the size of the pigment could cause artifactual increases in the size of the photoreceptors, and therefore we measured the area enclosed by the screening pigment relative to the dimensions of the whole eye. The pigment increased equally in all the groups. However the overall dimensions of the eye increased only in the group which received phorbol in the presence of light (ANOVA, $p<0.005$; $df = 2,11$). The increases induced by phorbol-with-light in the photoreceptors' size are therefore independent of any increases in the pigment. These results suggest that phorbol activation of PKC induces morphological changes, which may be relevant to the learning process, in addition to biochemical and biophysical modifications of these cells.

383.3

ALAPROCLATE REDUCTION OF *HERMISSENDA* POTASSIUM CURRENTS MIMICS EFFECTS OF CLASSICAL CONDITIONING. C. Collin*, L.D. Matzel, M. Sakakibara,† and D.L. Alkon. LMCN-NINDS, NIH, Bethesda, MD 20892; †Dept Computer Sci, TUT, Toyohashi 440, Japan.

Alaproclate (ALA), a 5HT re-uptake inhibitor, also reduces calcium-dependent K^+ currents (I_c) in mammalian cultured neurons (Hedlund, Pharmacol. Toxicol, 61: 236, 1987). Behavioral experiments show enhancement of performance in a one-trial avoidance task in rats (Altman *et al*, Behav. Neur.Biol, 48: 49, 1987). Here we report that ALA also reduces the early voltage-dependent K^+ current (I_A) and the calcium-dependent K^+ current(I_c) in isolated type B photoreceptors of the marine snail *Hermissenda*, in two-electrode voltage-clamp experiments. 3 min after extracellular application of 250nM ALA, peak amplitudes of I_A and I_c were significantly reduced by 7 ± 2% and 12 ± 4% respectively (n = 4, $p<0.002$). Reductions of more than 50% in both currents were reached with 25 to 50 μM drug applications. This effect reversed spontaneously at concentrations lower than 10μM, but only partially at concentrations of 10 - 50μM. No recovery was observed for either current at concentrations larger than 50μM. There is no synaptic input to these axotomised cells, thus the effects of ALA could be mediated *via* intracellular messengers. To test this possibility, the effects of 10μM ALA were examined after intracellular injections of the calcium chelator EGTA, or bath application of 25μM TFP, a calmodulin-dependent protein kinase (Cam-K) inhibitor, or 200μM sphingosine, a protein kinase C (PKC) inhibitor. All three conditions prevented any ALA effect upon K^+ currents after 10 min. These results indicate that ALA reduction of K^+ currents is a calcium-dependent process, and is mediated by the modulation of Cam-K or PKC, or both, in a similar way to classical conditioning-induced changes in *Hermissenda*.

383.4

CHANGES IN A GTP-BINDING PROTEIN FOLLOWING ASSOCIATIVE CONDITIONING OF *HERMISSENDA*. T. J. Nelson and D. L. Alkon, Lab. of Molecular and Cellular Neurobiology, NINDS/NIH, Bethesda, MD 20892.

Classical conditioning of *Hermissenda* (*H*) increases the phosphorylation state of a 20 kDa protein (cp20) known to be a C-kinase substrate (Neary et al., 1981). To identify the proteins modified by conditioning, we trained *H* with paired light and rotation, dissected the eyes 24h after conditioning and analyzed the proteins by ion-exchange HPLC. Conditioning increased the peak area of cp20 by 3.3x (paired=1.23±.27, random=0.37±0.09, naive= 0.22±0.11 (mean±SD, p<.01)). We also purified cp20 from *H* CNS by ion-exchange HPLC followed by size-exclusion HPLC. A single peak from the 2nd column exhibited both GTPase and GTP binding activity, indicating cp20 is a G protein.

Iontophoretic injection of cp20 blocked the K^+currents I_{K+} (I_A) and $I_{Ca2+-K+}$ (I_C), which are also reduced after associative conditioning. I_A decreased from 47.5±7.3 to 34.8±5.7 nA(mean±SD,n-5,p<.05); I_C decreased from 30.5±3.2 to 21.1±3.3 (p<.005), whereas no change was observed after injection of KAc control solution, suggesting that cp20 may play a central role in mediating the reduction of these currents in vivo. Cp20 could play a role in memory storage analogous to that of *ras* in cell proliferation.

383.5

MECHANISMS OF EPENDYMIN PHOSPHORYLATION IN GOLDFISH BRAIN ECF. P.M. Nolan* and V.E. Shashoua. McLean Hospital, Harvard Medical School, Belmont, MA 02178.

Ependymin is a brain extracellular glycoprotein which consists of three related dimers. Much evidence has shown that this protein may be actively involved in the modification of neural circuits following memory consolidation. Recently, it has been suggested that its action may be mediated by a Ca^{2+}-dependent polymerization of ependymin, the stimulus being a reduction in extracellular Ca^{2+} concentration. Because of the importance linked with Ca^{2+}-dependent protein phosphorylation in events leading to memory formation, it was considered relevant to study ependymin phosphorylation. Ependymin or ECF fractions from goldfish brain were incubated with ^{32}P-ATP and were analyzed by SDS PAGE and autoradiography. In ECF, all three ependymin dimers appear to be phosphorylated whereas reduced monomers are not. Also in ECF, a band at approximately 130 kDa and a doublet at 18 kDa are phosphorylated. Experiments exploring the requirement for phosphorylation of ependymin were carried out to determine whether autophosphorylation or a possible extracellular protein kinase is required. No phosphorylation is seen with pure ependymin although pretreatment of ependymin with E. coli phosphatase results in monomer phosphorylation. In ECF, dimer phosphorylation is stimulated by 1mM EGTA and inhibited by higher concentrations (5-30 mM). The latter would suggest that polymerization of ependymin and its phosphorylation may be related events. We are currently exploring this possibility. This research was supported by NINCDS grant No: NS25748.

383.6

NEURAL NETWORK SIMULATION OF PERIPHERAL CUTANEOUS NERVE SECTION AND REGENERATION IN ADULT OWL MONKEYS. K.A.Grajski and M.M.Merzenich Coleman Labs., UCSF, San Francisco, CA 94143.

Thalamic and cortical consequences of peripheral cutaneous nerve section and regeneration are modelled by randomizing and progressively reestablishing peripheral-to-central targets in a simple multi-layer neural network operating under competitive conditions with a correlation-based (Hebbian) synaptic plasticity rule.

The network consists of three (15x15) layers: skin, subcortex and cortex. Projections between layers are topographically organized and refined by stimulus-driven activity. Intrinsic circuitry in the (sub) cortical layers includes local excitation and local inhibition, as well as mutually excitatory connections in the cortical layer. Model neurons linearly summate input and pass it through a sigmoidal nonlinearity to generate an output. Synaptic strength increases in direct proportion to pre- and post-synaptic activation; a passive decay acts to weaken unstimulated synapses. Competitive synaptic modification is enforced by increasing strengths at the expense of others.

The model reproduces the following experimental observations on the representation of the reinnervated skin: 1.) disrupted topographic organization in the thalamus, including large and discontinuous receptive fields; 2.) more complete cortical topography, characterized by small continuous receptive fields; 3.) small regions of incomplete cortical topography; and 4.) plasticity - newly co-activated skin sites across the denervation border are represented in both the thalamus and cortex. Research supported by NIH grants NS-10414,GM-07449, HRI, the Coleman Fund and the San Diego Supercomputer Center.

383.7

DYNAMIC ASPECTS OF RECEPTIVE FIELDS OF NEURONS CHRONICALLY RECORDED IN RAT VIBRISSA CORTEX. P.R.Kennedy & D.Banks* (Spon: M. Luskin), Bioeng. Center, Georgia Institute of Technology, Atlanta, Georgia 30332.

The ability to record cortical units continuously over months allows testing of the allegiance of receptive fields to these units following experimental manipulations. Such recording is now possible with a new electrode that records from neurites that grow from parent neurons onto its recording surface (P.R.Kennedy, J.Neurosci.Methods, 1989, in press). In the present experiments, the electrode was implanted in rat SM1 vibrissa cortex about 500 to 800 microns below the surface (layer 3b, 4; thalamocortical input layer) in order to record neural activity evoked by vibrissae deflections.

In 4 rats the electrode was placed just under the cortical surface (confirmed histologically in one rat). In other rats with deep placements, activity was recorded to jaw and neck, but not vibrissae. Manipulations were performed either by gluing adjacent vibrissae together or by trimming some vibrissae (thus allowing the untrimmed vibrissa(e) to be naturally and selectively stimulated). Recordings were performed under Ketamine sedation every 24 or 48 hours. In recent experiments, a touch-sensitive probe was used to deflect the vibrissae. Both the voltage output of this probe and the neural activity were archived simultaneously on an HP tape recorder for later analysis.

After determining the receptive field (RF) of the neural activty, two vibrissae not in the field were glued together. The rat separated these vibrissae overnight and the next day recordings revealed that these vibrissae evoked neural activity. The RF had widened in less than 24 hours. It contracted back to its original size within a week. These manipulations were repeated with similar results. In vibrissae trimming experiments, all vibrissae were cut off except C5. The RF contracted from 3 vibrissae (C4, C5 and D5) to 2 (C5 and D5). After C5 was cut off, the RF widened. When only vibrissa D5 was allowed to grow out, the RF moved ventrally to include C5, D5 and E5. After D5 was cut off, the RF widened again. Allowing E5 and E6 to grow out, produced a similar result. Deflecting the vibrissae with the touch-sensitive probe disclosed an average latency less than 10 ms between touch and response, and allowed analysis of responses as a percentage of the deflections.

These results suggest that receptive fields of chronically recorded neurons are dynamic when selective stimulation of vibrissae is employed.

383.8

IN VITRO ANTIGENIC AND ULTRASTRUCTURAL CHARACTERIZATION OF NEUROBLASTS DERIVED FROM A NEUROGENIC REGION OF THE ADULT CANARY BRAIN S.A.Goldman and W.Y.Clarke*, Dept. Neurology, Cornell Medical College., N.Y., N.Y. 10021.

The vocal control nucleus, HVc, of the songbird forebrain undergoes neurogenesis in adulthood, as ventricular zone precursor cells divide, migrate and differentiate into neurons (Goldman and Nottebohm, Proc. Natl. Acad. Sci. 80:2390-94, 1983). We now describe the in vitro antigenicity and ultrastructural maturation of adult-derived new neurons. The HVc ventricular zones of one year old canaries were placed into explant culture. One week later, the cellular outgrowths of selected explants were stained for a variety of neuron selective antigens. Morphologically-evident neurons were immunoreactive for MAP-2, neuron specific enolase, synaptic vesicle protein-2, and the tetanus toxin and A2B5 ligands. These cells failed to react with antisera against GFAP. Combined immunostaining and autoradiography of HVc cultures taken from brain exposed to ^{3}H-thymidine in vivo revealed a population of newly generated neurons positive for both MAP-2 and ^{3}H-thymidine. New neurons were also identified in plastic sections of adult explant outgrowths, and examined by electron microscopy of adjacent 80 nm. sections. These cells displayed typical neuronal ultrastructure, including dendrites with parallel arrays of microtubules, dendritic spines, multivesicular bodies, and immature dendro-dendritic synapses.

383.9

THE DEVELOPMENT OF LONG PROJECTIONS BY NEURONS BORN IN THE ADULT CANARY BRAIN. J. R. Kirn and F. Nottebohm, Rockefeller University, New York, N.Y. 10021.

Previous work from this laboratory indicates that neurons are born in the adult canary telencephalon. Many of these cells are incorporated into nucleus HVC (higher vocal center) where they become interneurons. Other new HVC neurons develop long projections to nucleus RA (robustus archistriatalis) and thus become part of the efferent pathway for song control. The present work employs [^{3}H]-thymidine and the retrograde tracer fluorogold to follow the birth, differentiation, and survival of HVC neurons born in adulthood. Thirty 18 month old male canaries were injected with [^{3}H] thymidine and killed 1,2,3,4,5 or 8 months later. Three days prior to sacrifice, flourogold was stereotaxically injected bilaterally into RA. Preliminary results suggest that roughly 28% of new HVC neurons project to RA by one month after [^{3}H]-thymidine treatment (n=3; range=15-36%). By 5 months, this fraction increases to 59% (n=2; range=46-71%). Thus, within the population of HVC neurons born in adulthood, there is a relative increase in the proportion of long-projection neurons with time.

383.10

SEASONAL AND SPECIES DIFFERENCES IN THE PRODUCTION OF LONG PROJECTION NEURONS IN ADULT BIRDS. A. Alvarez-Buylla and F. Nottebohm. The Rockefeller University, New York, NY 10021.

Combining retrograde tracers (fluorogold or rhodamine beads injected to RA) with [3H]-thymidine, we show that long projection neurons continue to be added to the Higher Vocal Center (HVC) of adult canaries and zebra finches. In both these species, about half of all HVC neurons are backfilled with fluorogold. However while in canaries 56% of the [3H]-thymidine labeled neurons projected to RA, in zebra finches only 23% of the labeled neurons were projection neurons. We compared May (n=3) and September (n=4) in canaries. The proportion of labeled projection neurons did not change between the two times of year. However the absolute number of all labeled neurons increased 6 fold in September when compared to May. (11.23 [sd=6.81] to 66.07 [sd=18.97] labeled neurons/mm2 and 6.65 [sd=3.59] to 33.8 [sd=11.30] labeled projetion neurons/mm2). New RA projection neurons may play a role in the motor learning of song that happens every year in adult canaries; however zebra finches do not learn new songs in adulthood.

383.11

HOUSING ADULT MALE RATS IN ENRICHED CONDITIONS INCREASES NEUROGENESIS IN THE DENTATE GYRUS. A.D. York[1]*, S.M. Breedlove[2], M.C. Diamond[3], and E.R. Greer[3]* (SPON: L. Proenza). Depts. Zoology[1], Psychology[2], and Physiology-Anatomy[3], University of California, Berkeley, CA 94720.

Postnatal neurogenesis has been shown in the granule cell layer of the dentate gyrus (DG) of the hippocampus. Adult rats housed in enriched conditions show changes in hippocampal neurohistology (Walsh, R.N., Intern. J. Neurosci., 12:33, 1981). The present study looked for changes in adult neurogenesis in DG due to different housing conditions. Twelve 60-day-old male Long-Evans rats were housed 30 days in enriched conditions: one common 70x70x45 cm cage with novel objects which changed daily. Eleven age-matched rats were housed in standard conditions: 3 rats per 11x19x8 cm cage with no objects. Rats were given a total dose of 5 uCi/g BW of ^{3}H-thymidine in 5 semi-weekly i.p. injections starting day 1 of differential housing. After standard autoradiography, 58 standardized regions of DG were examined in each animal. "Enriched" rats had significantly more heavily-labelled granule cells than did "standard" rats. More heavily-labelled neurons were seen in the anterodorsal quadrant and the endal limb of DG of "enriched" rats. The two groups had no differences in cell density or volume of whole DG, number of labelled glial cells, or number of labelled undifferentiated cells. Thus, neuroblasts appear to migrate to and mature in specific areas of the DG in response to enriched housing conditions.

383.12

CHRONIC HYPOXIA CAUSES MORPHOLOGICAL ALTERATIONS IN THE PHRENIC NUCLEUS OF YOUNG ADULT RATS. H.G. Goshgarian and X.-J. Yu*. Department of Anatomy/Cell Biology, Wayne State University Medical School, Detroit, MI 48201.

A recent study from our laboratory has shown that a spinal cord hemisection rostral to the phrenic nucleus in rats results in rapid morphological alterations of the normal ultrastructure of the phrenic nucleus. These alterations include: 1) increases in the number of double and multiple synapses contacting phrenic profiles, and 2) increases in both the length and number of phrenic dendrodendritic membrane appositions. The membrane appositions are due to the retraction of astroglial processes away from their normal position between adjacent dendrites. We have suggested that the morphological alterations may be related to the enhanced expression of a latent motor pathway which is responsible for restoring function to a paralyzed hemidiaphragm after spinal cord injury.

The present study was carried out to determine if prolonged hypoxia in non-injured rats causes alterations in the phrenic nucleus similar to those caused by spinal cord injury. Normal rats were subjected to chronic hypoxia in an atmosphere chamber for 48 hours. A Bioquant system was used to quantitatively analyze electron micrographs through the phrenic nucleus of chronically hypoxic and normal rats. The results in dicate that chronic hypoxia causes astroglial processes to retract away from their normal position in between adjacent phrenic dendrites, but it does not induce the synaptogenesis that is induced by spinal cord injury. Specifically, the mean length of the dendrodendritic appositions increased significantly from a normal value of 1.42 ± 0.09 um to 1.84 ± 0.08 um in hypoxic animals. Moreover, the mean number of dendrodendritic appositions in the normal phrenic nucleus (0.67 ± 0.08) increased significantly to 0.96 ± 0.10 in the phrenic nucleus of hypoxic rats. There was no significant difference between the mean number of double (0.71 ± 0.08) or multiple (0.80 ± 0.09) synapses in control animals as compared to the mean number of double (0.74 ± 0.09) or multiple (0.81 ± 0.1) synapses in the phrenic nucleus of hypoxic animals.

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384.1

ENDOGENOUS CCK PARTICIPATES IN NUTRIENT-INDUCED SUPPRESSION OF FOOD INTAKE BY A NON-ENDOCRINE MECHANISM. D. Yox, L. Brenner*, E. Simon* and R.C. Ritter. Dept of VCAPP, Washington State University, Pullman, WA 99164-6520.

To investigate the mechanism by which endogenous CCK participates in the suppression of food intake by intestinal nutrient infusion, we compared the effects of the CCK antagonist CR 1409 (Rotta Research) on suppression of sham ingestion and stimulation of amylase secretion by intestinal nutrient infusions. CCK stimulates pancreatic amylase secretion by an endocrine route of action, which we confirmed in these experiments by measuring plasma CCK concentrations. Suppression of food intake by intraintestinal maltose was abolished by CR 1409. However, maltose did not elevate intestinal amylase concentrations or increase plasma CCK. Intraintestinal oleate increased plasma CCK. Oleate also suppressed food intake and increased intestinal amylase concentration. Both of these effects were antagonized by CR 1409. Finally, casein increased plasma CCK and elevated intestinal amylase but did not suppress food intake. These results suggest that suppression of food intake by some intraintestinal nutrients is mediated by endogenous CCK. However, the CCK participating in suppression of food intake by intestinal nutrients is not reaching its target by an endocrine route. (Supported by NIH RO1 NS 20561).

384.2

EFFECTS OF ACUTE AND CHRONIC TREATMENT WITH POTENT CHOLECYSTOKININ (CCK) ANTAGONIST L-364,718 ON FEEDING AND PLASMA CCK IN RATS. RD Reidelberger and R-M Liehr*. Dept Physiol, Univ Kansas Med Sch, KC, KS 66103; VA Med Ctr, KC, MO 64128.

L-364,718 (L) stimulates feeding after acute (Reidelberger and O'Rourke, 1987) and chronic (Schneider et. al., 1988) treatment, thus supporting a role for CCK in control of food intake. We sought answers to the following questions: ***Exp. 1.*** Is the feeding response to L due to competitive antagonism of CCK action? ***Exp. 2.*** Is the feeding response to L due to its inhibitory effect on pancreatic secretion? ***Exp. 3.*** Is the return to baseline food intake after several days of L treatment due to elevated plasma CCK levels? ***Methods and Results***: ***Exp. 1.*** L (0 or 0.03 mg/kg) and CCK8 (1-128 nmol/kg, 25 min after L) were given i.p. to 1-h food deprived rats (288±4 g, *n=12/group*) during the dark phase. L inhibited CCK-8 action competitively (ED_{50} increased 16 fold with similar maximal inhibition). ***Exp. 2.*** Rats (413±3 g, *n=8-9/group*) received continuous duodenal infusion of bile-pancreatic juice or saline for 2 h; L (0 and 0.5 mg/kg i.v. on different days) was given 15 min after start of infusion. Infusion of pancreatic juice to mimic postprandial conditions did not alter the feeding response to L (37 vs 24% increase in controls). ***Exp. 3.*** Rats (130-175 g, *n=6-16/group*) fed ground chow ad libitum, received L (0, 0.5, or 1 mg/kg i.p.) every 8 h for 5 d; blood was obtained from fed and fasted rats 8-12 h after the last injection (3-7 h after lights on). Food intake was stimulated significantly only by 1 mg/kg of L, and only on days 1 (15%) and 2 (10%). L (1 mg/kg) significantly increased plasma CCK levels in fed animals (5.6±0.9 pM vs 3.8±0.5 pM), while neither dose altered fasting levels. ***Conclusions***: (1) Antagonism of CCK-8-induced suppression of feeding by L exhibited competitive-like kinetics. (2) Stimulation of feeding by L is apparently not due to its inhibitory effect on pancreatic enzyme secretion. (3) Chronic treatment with L transiently increased solid food intake, confirming results of Schneider et al. using liquid food. (4) Because CCK and L compete for the same binding sites, an increase in plasma CCK with time of L treatment may explain this transient effect.

384.3

THE SELECTIVE CCK RECEPTOR ANTAGONIST MK 329 BLOCKS THE ANORECTIC EFFECT OF D-FENFLURAMINE. S.J. Cooper, C.T. Dourish and D.J. Barber*. School of Psychology, University of Birmingham, Birmingham B15 2TT, U.K. and Merck Sharp & Dohme Research Labs., Harlow, Essex, CM20 2QR, U.K.

D-Fenfluramine reduces food intake in rats through a serotonergic mechanism that utilises 5-HT_1 receptors (Neill, J.C. and Cooper, S.J., Psychopharmacol., 97:213, 1989). We have investigated the further possibility that d-fenfluramine anorexia is dependent upon endogenous CCK activity. In the first set of experiments, d-fenfluramine (3.0 mg/kg, i.p.) significantly ($p<0.005$) reduced palatable food intake in nondeprived rats. This anorectic effect was blocked by the selective CCK receptor antagonist MK 329 (formerly L-364,718), injected in doses of 30 and 100 ug/kg (s.c.). Administered alone, MK 329 did not affect food intake. Furthermore, it did not block the anorectic effect of the selective dopamine D_2 receptor agonist quinpirole (0.3 mg/kg, s.c.) or that of the beta-carboline FG 7142 (10 mg/kg, i.p.). In further studies, d-fenfluramine (3.0 mg/kg, i.p.) significantly ($p<0.005$) reduced nocturnal free-feeding in rats over a 6 hr period. The anorectic effect was significantly attenuated by MK 329 (30 and 100 ug/kg). Hence, d-fenfluramine's anorectic effect appears to require endogenous CCK activity.

384.4

BLOCKADE OF CCK RECEPTORS REVERSES THE EFFECT ON GASTRIC EMPTYING BUT NOT FEEDING IN ZUCKER RATS. A.J.Strohmayer, R. von Heyn* D. Greenberg and L.Dornstein*, Dept Psychiatry, North Shore U Hosp - Cornell U Med Coll, Manhasset NY 11030, USA.

CCK slows gastric emptying (GE) and reduces food intake in rats. We have shown that the CCK receptor antagonist, L364,718 (>375 ug/kg), increased 30 min food intake in lean but not obese Zucker rats suggesting the obese did not release active CCK. Intragastric (IG) loads of trypsin inhibitor (TI) stimulate CCK release and inhibit GE. We measured GE in Zucker rats after IG TI or saline and with L364,718 pretreatment. TI (200 mg/kg) slowed GE in both genotypes. L364,718 (200 ug/kg, IP), completely reversed this effect in both genotypes. We conclude that TI inhibited GE by CCK release in both obese and lean rats. This mechanism may be distinct from the CCK inhibition of food intake, and obese Zucker rats may be deficient only in the food intake response.

Median % Remaining in Stomach

	Sal	TI	TI+L
Lean (n=3)	13	28	8
Obese (n=3)	25	50	19

384.5

IVT CHOLECYSTOKININ (CCK-8) REDUCES SINGLE MEAL SIZE IN THE BABOON OVER A 200-FOLD RANGE OF DOSAGES. P.K.Green, D.P.Figlewicz*,A.J.Sipols,D.Porte,Jr.,S.C.Woods. Dept. of Psychology, Univ. of Washington/Dept. of Med., Seattle Veterans Admin. Med. Research Ctr., Seattle, WA 98195.

CCK-8 reduces meal size when given intravenously in the baboon. We previously found that doses as low as 0.1 ug/kg, when given IVT, also decrease meal size, and that centrally-administered CCK is more effective in producing satiety than similar doses given IV. We have now extended the range of doses given IVT to as low as .01 ug/kg. Eight male baboons implanted with chronic intraventricular cannulae were adapted to a liquid diet (Ensure) on a fixed meal schedule. Food intake was measured in overnight fasted animals during a 30-min meal immediately following a 5-min IVT infusion of CCK-8 or CSF vehicle, each animal serving as its own control on an adjacent day. Doses ranged from .01 to 2.0 ug/kg. Not all animals received all eight dosages, but at least three animals received each of the lowest doses. Each dose produced a reduction of single meal size relative to the control condition. The lowest dose (.01 ug/kg) reduced meal size by 45±23% (n.s.); the next lowest dose tested (.025 ug/kg) reduced meal size 87±5% ($p<.10$). CCK's action at the lower doses was not reliably diminished relative to that observed after higher doses. These data provide further evidence of CCK's efficacy as a centrally-acting satiety factor in the baboon.

384.6

INHIBITORY EFFECTS OF CHOLECYSTOKININ DEVELOP THROUGH INTERACTION WITH DUODENAL SIGNALS. J.E. Cox, Dept. Psychology, Univ. Alabama at Birmingham, Birmingham, AL 35294.

Low doses of cholecystokinin octapeptide (CCK-8) produce marked impairment of runway performance if given after rats have consumed a partial meal of 30% sucrose (Cox, J.E., Behav. Brain Res., 21: 29, 1986). This effect is also produced by gastric sucrose infusion (Cox, J.E. & Smith, M.J., Neurosci. Abs. 13: 588, 1987). To further investigate the site of origin of feeding-contingent signals interacting with CCK-8, the current experiments assessed changes in runway performance produced by 1 ug/kg CCK-8 in conjunction with duodenal and hepatic portal infusions. Duodenal infusions of 30% sucrose were as effective as prefeeding and gastric infusions. After infusions, CCK-8 reduced running speed by 67% compared to tests with sucrose infusions and saline injections. Glucose appeared to be as effective as sucrose, whereas equiosmotic saline and mannitol were not. Intraportal glucose was relatively ineffective; some synergism with CCK-8 was produced by very large infusions, but the effect was relatively small. These results suggest that 1 ug/kg CCK-8 can reduce feeding motivation through synergism with a carbohydrate-sensitive signal generated within the small intestine. (Supported by NSF grant BNS-8606768.)

384.7

NEAR-COELIAC ARTERIAL CCK INJECTIONS SUPPRESS FOOD INTAKE AT HEPATIC PORTAL CCK LEVELS COMPARABLE TO THOSE OBSERVED POSTPRANDIALLY. N. Calingasan, S. Ritter, R. Ritter and L. Brenner*. Dept. of VCAPP, Washington State University, Pullman, WA 99164-6520.

Both exogenous and endogenous CCK appear to suppress food intake by acting on the vagal sensory innervation to organs within the hepatic portal drainage. To determine whether exogenous CCK can reduce feeding at physiological concentrations of hepatic portal CCK, we made automated measurements of liquid food intake before, during and after injection of CCK-8 through a chronic aortic catheter with its tip seated at the coeliac juncture. In a second group of rats, we made similar injections while collecting blood for CCK assay from the hepatic portal vein. In a third group we collected hepatic portal blood 10 min after a 10 ml spontaneously ingested liquid meal. Injection of 10, 30, 50 and 70 pmols of CCK-8 suppressed feeding in a dose dependent manner beginning within 1 min. While 10 pmol of CCK suppressed feeding in 50% of the rats, 30 pmol was the lowest dose to produce statistically significant suppression of preinjection intake (45.7 ± 10.6%). Near-coeliac injection of 30 pmol CCK produced portal plasma CCK concentrations averaging 3.1 ± 0.5 pM, compared to 0.6 ± 0.3 pM during saline injection. Ten min after a 10 ml liquid meal, portal CCK was 3.0 ± 0.5 pM, compared to 0.9 ± 0.4 pM in fasting controls. Thus, when infused near its site of action, exogenous CCK inhibits feeding at doses producing hepatic portal CCK concentrations similar to postprandial levels. The results are consistent with CCK's participation in the physiological process of satiation.

384.8

CAUDAL HINDBRAIN PARTICIPATION IN SUPPRESSION OF FEEDING BY CENTRAL AND PERIPHERAL BOMBESIN. E.E. Ladenheim and R.C. Ritter. Dept. of VCAPP, Washington State University, Pullman, WA 99164-6520

Our previous studies have shown that bombesin (BBS) infused into the fourth cerebral ventricle in rats suppresses food intake at doses 1/10 to 1/1000 of those required following lateral ventricular administration. These results suggest that centrally administered BBS acts in the caudal hindbrain to suppress feeding. In order to investigate the participation of caudal hindbrain structures in fourth ventricular BBS-induced suppression of feeding, we made lesions that destroyed the following: 1) the area postrema (AP) and 2) the AP and a majority of the adjacent medial nucleus of the solitary tract (mNST). In addition, we examined the role of the AP/NST in suppression of feeding following peripheral BBS administration. We have found that lesions which destroy the AP and extensively damage the mNST abolished suppression of food intake by fourth ventricular BBS and attenuated suppression by peripherally administered BBS. Lesions restricted to the AP attenuated fourth ventricular BBS-induced suppression of feeding but had no effect on suppression by peripheral BBS. These data suggest that the NST may be a common neural substrate for both central and peripheral BBS-induced suppression of feeding (Supported by NIH RO1 NS20561).

384.9

CLONIDINE MIMICS FEEDING PATTERN OF CARBOHYDRATE-PREFERRING RATS. G. Shor-Posner, G. Brennan, C. Ian *, R. Kalimi* and S.F. Leibowitz. The Rockefeller University, New York, New York 10021.

Recent work from this laboratory indicates that approximately 50% of albino Sprague-Dawley rats exhibit a pattern of carbohydrate preference expressed through ingestion of small, frequent meals. In light of evidence suggesting a role for a hypothalamic α 2 noradrenergic system in stimulating carbohydrate intake, this study examined the effects of the α 2 agonist, clonidine (CION: 3-25 ug/Kg, i.p.) on feeding patterns of self-selecting rats (N=12).

Computer-assisted analyses of feeding patterns after CION administration, at dark onset, revealed a dose-dependent increase in consumption of carbohydrate-rich meals. This response was apparent during the first 4 hr of the dark, when small, frequent and carbohydrate-rich meals were observed, and carbohydrate preference was increased from 45 to 64% ($p<0.01$), despite an overall decrease in total caloric intake. Little change was observed in the middle and late phases of the dark period. This meal pattern, after CION administration, appears similar to that exhibited by carbohydrate-preferring rats.

384.10

A RAPID ONSET SODIUM APPETITE INDUCED BY YOHIMBINE. A.K. Johnson, T.G. Beltz*, and G.L. Edwards. The Departments of Psychology and Pharmacology and The Cardiovascular Center, Univ. of Iowa, Iowa City, IA 52242

Male rats (n=9) were given several days of ad lib access to 1.8% NaCl (1.8%) and to tap water (TW). On separate days the fluid intake of animals was studied in 3 hr drinking tests following s.c. injection of isotonic saline (CONT) or 6 mg/kg of yohimbine (YOH). With both 1.8% and TW present YOH significantly increased the intake of both fluids (CONT=0.1±0.03 vs. 0.1±0.02; YOH=9.0±1.80 vs. 5.7±2.03 mls; TW to 1.8%, respectively). In a second series 1.8% was the only solution available to the rats during testing. YOH treatment induced a significant increase in intake of 1.8% at 1, 2, and 3 hrs (7.0±1.88, vs. 0.0; 17.0±4.97 vs. 0.0; 24.3±6.45 vs. 0.0; cumulative mls intake; YOH vs. CONT, respectively). YOH treated rats did not drink .001M quinine when it was offered as the only fluid in a 3 hr test. The YOH-induced ingestion of large quantities of hypertonic NaCl suggests that YOH treatment generates a sodium appetite in replete rats. This finding is consistent with other observations indicating that alpha-2 adrenoceptor antagonists can act to reduce central adrenergic inhibitory tone exerted by afferent input from sytemic volume/pressure receptors and that such an attenuation causes the activation of a constellation of responses consistent with the animals achieving an expansion of extracellular fluid volume.

384.11

EFFECTS OF SYSTEMIC CLONIDINE ON SIMULTANEOUS MEASUREMENTS OF FEEDING, LOCOMOTOR ACTIVITY AND RESPIRATORY GASES IN THE RAT. D.V. Coscina and J.W. Chambers*. Sect.of Biopsychology, Clarke Inst. of Psychiatry, Toronto, Canada, M5T 1R8.

The A2-adrenergic agent clonidine (CLON) has been shown to elicit feeding in sated rats when given peripherally. However, it has also been shown to depress locomotory activity as well as several metabolic indices. To help clarify the potential relationships which may exist among these variables, we recorded feeding, activity, oxygen consumption, and carbon dioxide output in adult male rats over a 3 hr period following subcutaneous CLON (50 ug/kg) or saline. Drug x time analyses of variance revealed no main effect of drug treatment. However, highly significant drug x time interactions were seen on all variables. The time course of each behavior remained essentially constant after CLON whereas it started relatively high and diminished after saline. These findings may indicate that CLON-induced feeding occurs to compensate for hypo-metabolic events induced by this drug treatment. Studies are underway to test this possibility further after direct CNS injections of this and related agonists.

384.12

OBESITY PRONE AND RESISTANT RATS DIFFER IN THEIR BRAIN ^{3}H PARAMINOCLONIDINE BINDING. B.E. Levin, Neurology Service, VA Med. Ctr., E.Orange, NJ 07019; Dept. of Neurosciences, N.J. Med. Sch., Newark, NJ 07103

Half the rats chronically fed a high energy diet develop diet-induced obesity (DIO); the rest are diet-resistant (DR). Before high energy diet exposure, rats prone to develop DIO have high plasma norepinephrine (NE) levels in response to i.v. glucose while DR-prone rats have little NE response. Here 28 chow-fed rats were tested for glucose-induced NE release. The 6 rats with the highest plasma NE responses (DIO-prone) had areas under the NE curve of 27803±4095 versus -2330±1127pg/ml/60min for the 6 lowest NE responders (DR-prone; P=0.0001). There were no differences between the groups in body weight or in plasma glucose or insulin responses to i.v. glucose. In these 12 rats, binding to brain α-adrenoreceptors was studied by receptor autoradiography using 1nM ^{3}H prazosin (PRZ; α_1-) and 1nM ^{3}H paraminoclonidine (PAC; α_2-). There were no differences in ^{3}H PRZ binding in any of 18 brain areas examined. However, the DR-prone rats had higher ^{3}H PAC binding (257-721%) compared to DIO-prone rats in 4 amygdalar nuclei, the lateral area, dorso- and ventromedial hypothalamic nuclei, the median eminence and medial dorsal thalamic nucleus. These pre-existing differences in brain ^{3}H PAC binding between rats which are prone to develop DIO or DR when subsequently fed a high energy diet may play an etiological role in the phenotypic expression of these weight gain traits.

385.1

GLUTAMATE IS CONTAINED IN AXON TERMINALS OF NEURONS IN THE ROSTRAL VENTROLATERAL MEDULLA THAT PROJECT TO THE SPINAL SYMPATHETIC INTERMEDIOLATERAL NUCLEUS. S.F. Morrison, J. Callaway*, T.A. Milner and D.J. Reis, Division of Neurobiology, Cornell Univ. Med. Coll., New York, NY 10021.

We have previously shown that (a) the glutamate receptor antagonist, kynurenic acid, blocks the excitation of sympathetic preganglionic neurons in the intermediolateral nucleus (IML) evoked by stimulation of the rostral ventrolateral medulla (RVL) and (b) within the IML, axon terminals with glutamate-like immunoreactivity (GLU-LI) primarily form asymmetric (excitatory) synapses with the dendrites of local neurons. To determine whether RVL neurons are a source of the glutamate-containing terminals in the IML, anterograde transport of *Phaseolus vulgaris*-leucoagglutinin (PHA-L) iontophoretically injected into the RVL was combined with glutamate immunocytochemistry in the same sections of thoracic spinal cord. By light microscopy, PHA-L immunoreactivity was found in fine varicose processes which had an overlapping distribution with perikarya and processes containing GLU-LI. Electron microscopic analysis revealed PHA-L immunoreactivity in axons and axon terminals within the IML. Terminals with PHA-L immunoreactivity ranged in diameter from 0.5-2.0 µm, contained numerous small clear and 0-3 large, dense-core vesicles, and formed primarily asymmetric synaptic contacts on small dendrites of IML neurons. Some of the PHA-L immunoreactive terminals also contained GLU-LI. We conclude that at least a portion of the input to the IML from the RVL uses glutamate as its transmitter. These findings support the hypothesis that the release of glutamate onto sympathetic preganglionic neurons plays a key role in the regulation of arterial pressure by reticulospinal, sympathoexcitatory pathways from the RVL. Supported by NIH HL18974.

385.2

SPINAL N-METHYL-D-ASPARTIC ACID (NMDA) RECEPTORS MEDIATE PRESSOR AND TACHYCARDIC RESPONSES EVOKED BY EXCITATION OF THE ROSTRAL VENTROLATERAL MEDULLA (RVM). M.K. Bazil* and F.J. Gordon (SPON: P.J. Conn). Dept. of Pharmacology, Emory Univ. Sch. of Med., Atlanta, GA 30322.

The purpose of these studies was to investigate a potential role for spinal NMDA receptors in the mediation of sympathoexcitatory responses evoked by L-glutamate (Glu) stimulation of the RVM. Rats were anesthetized with urethane, vagotomized, paralyzed and artificially ventilated. Microinjections of Glu (0.1-30 nmol/50 nl) into the RVM produced increases in mean arterial pressure (MAP) ranging from 9 ±2 to 60 ±8 mmHg and tachycardia of 4 ±1 to 55 ±10 bpm. Intrathecal (IT) administration of the NMDA receptor antagonist D-2-amino-7-phosphonoheptanoic acid (D-AP7; 100 nmol/10 µl) lowered MAP from 93 ±3 to 56 ±3 mmHg. After IT D-AP7, pressor responses evoked by microinjections of Glu into the RVM were reduced to 3 ±1 to 16 ±6 mmHg ($p < .01$). Tachycardic responses after IT D-AP7 averaged 3 ±1 to 30 ±6 bpm ($p < .01$). In other rats, IT NMDA (ED50=56 nmol/10 µl) increased MAP by 45 ±12 mmHg and heart rate by 57 ±15 bpm. IT kainic acid (ED50=3.7 nmol/10 µl) produced pressor responses of 39 ±6 mmHg and tachycardia of 42 ±7 bpm. Prior blockade of spinal NMDA receptors with IT D-AP7 abolished cardiovascular responses produced by IT NMDA without reducing those of kainic acid. These results indicate that: 1) cardiovascular responses evoked by Glu stimulation of RVM neurons requires synaptic activation of spinal NMDA receptors, and 2) excitatory amino acid neurotransmitters may participate in sympathoexcitation produced by activation of descending bulbospinal pathways. (Supported by NIH HL-36907 and American Heart Association)

385.3

EXCITATORY AMINO ACID (EAA) TRANSMISSION IN ROSTRAL VENTROLATERAL MEDULLA (RVL): EAA ANTAGONISTS AS POTENTIAL CENTRALLY ACTING ANTIHYPERTENSIVES? S.P. Arnerić, C.R. Kelley*, F.D. Tingley*, D.R. Luthin*, W.H. Cline, Department of Pharmacology, Southern Illinois University School of Medicine, Springfield, IL 62702

EAAs released from pathways arising from hypothalamic structures increase sympathetic drive from RVL-spinal neurons (Sun & Guyenet, Brain Res. 368:1, 1986). This study sought to determine: 1) Is release of the EAA glutamate (Glu) increased in RVL of spontaneously hypertensive rats (SHR)? 2) If so, are antagonists of N-methyl-D-aspartate (NMDA) receptors effective antihypertensives? Studies were performed in 4-6 wk (pre-hypertensive) and 12-16 wk (hypertensive) SHR and in age-matched normotensive Wistar-Kyoto (WKY) rats. Release of endogenous Glu was measured from micropunches of RVL and, for comparison, intermediolateral cell column (IML) using HPLC. No strain differences in spontaneous or K+ (35mM)-evoked release of Glu were seen at 4-6 wks ($p > 0.05$; N=4). In contrast, 12-16 wk SHR with established hypertension had marked 2- to 4-fold increases in spontaneous and K^+-evoked release of Glu in RVL ($p < 0.05$; N=4), but not IML ($p > 0.05$; N=6). Non-competitive NMDA antagonists MK-801 (1 mg/kg, s.c.) and dextromethorphan (DXT; 10 mg/kg, s.c.) significantly lowered arterial pressure (AP) by 20-35 mmHg in conscious 12-16 wk SHR ($p < 0.05$; N=3-12) but not WKY. In vitro and in vivo preparations suggest that MK-801 and DXT do not lower AP by interruption of pre- or postsynaptic peripheral sympathetic transmission. In particular, pressor responses to intravenous phenylephrine and norepinephrine (NE) are not attenuated and release of endogenous NE elicited by periarterial nerve stimulation from isolated-perfused mesenteric vasculature is not diminished by MK-801 or DXT (N=5). CONCLUSION: NMDA antagonists may lower AP by interrupting derangements in central sympathoexcitatory drive elicited by enhanced EAA neurotransmission (Supported by the American Heart Association., IL Affiliate).

385.4

INTERACTIONS OF NMDA AND ITS ANTAGONISTS DURING CAROTID CLAMPING. Y.WANG, M.C.KAO*, H.K.LEE*. DEPT. OF PHARMACOLOGY, NATIONAL DEFENSE MEDICAL CENTER, TAIPEI, TAIWAN, R.O.C.

The purpose of this experiment was to study the interactions of N-methyl-d-aspartate (NMDA) with baroreflex induced by temporary carotid clamping. Adult male Sprague -Dawley rats (250 - 300 g) were anesthetized with urethane (1.25 g/kg, i.p.). We found that clamping common carotids resulted in a reversible and reproducible hypertensive effect in the vagotomized animals. This reaction was blocked by intraventricular injection of NMDA antagonists, such as 2-amino- 7-phosphono-heptaneoate (AP-7) and phencyclidine. Furthermore, using pressure microejection and single unit recording, we found that clamping of common carotids resulted in excitation of the cardiovascular neurons of ventral lateral medulla (VLM). This evoked excitation, similar to that induced by NMDA, was blocked by locally applied AP-7. In conclusion, our experiment demonstrated that NMDA was involved in the hypertensive reaction during carotid clamping in the VLM.

385.5

DOES THE AREA POSTREMA MEDIATE THE PRESSOR EFFECTS OF ANGIOTENSIN II IN NORMOTENSIVE OR RENAL HYPERTENSIVE RATS? CJ Tseng, M Appalsamy*, D Robertson, R Mosqueda-Garcia. Dept. of Pharmacology, National Defense Medical Center, Taiwan and Vanderbilt University, Nashville, TN 37232.

We have previously reported that microinjection of angiotensin II (AII) into the area postrema (AP) of renal hypertensive rats (RHT) decreases BP and HR. In the present study we fully characterize the hypotensive response of AII and evaluate if the AP mediates the pressor response of AII in RHT rats. Sprague-Dawley RHT (2-kidney, 1-clip) and sham normotensive control rats (NT) were used 4 weeks after surgery. Under urethane anesthesia, BP was intra-arterially recorded and a microsyringe was stereotaxically positioned in the AP. Microinjection of AII (2 to 2,000 ng) or saralasin (96 ng) were done with recording of sympathetic nerve activity (SNA) from the left renal nerve.

Basal BP of RHT was significantly higher than in NT controls. A dose-dependent decrease in BP and HR was observed with AII in the RHT rats (peaked at 200 ng, -38±4 mmHg; -46±8 b/m for BP and HR, respectively), and with the two lowest doses of AII in the NT rats, whereas only the two highest doses of AII in the NT animals evoked a modest pressor effect (12±4 mmHg with 200 ng). In both groups of animals, all doses of AII decreased SNA. Saralasin increased BP and HR in the two groups of animals, and the AII antagonist was able to inhibit only the depressor effects of low doses of AII. The pressor effects remained unchanged in the normotensive group. These results suggest that a tonic depressor AII effect is present in the AP and indicate that inhibition of sympathetic activity mediates at least part of the hypotensive response of AII. The pressor effect of AII in the normotensive rats is probably not mediated in the AP. These results do not support a role of the AP for the pressor effects of AII.

385.6

ELECTROPHYSIOLOGICAL AND CARDIOVASCULAR EVIDENCE FOR ENDOTHELIN ACTIONS WITHIN THE AREA POSTREMA. A.V.Ferguson and P.Smith*. Dept.of Physiology, Queen's University, Kingston, CANADA K7L 3N6.

Vascular endothelial cells have recently been demonstrated to produce a 21 amino acid peptide named endothelin (ET), one of the most potent endogenous vasoconstrictors known to date. Recent studies suggest that this peptide has long lasting biphasic effects on cultured muscle cell membrane potential. In the rat binding sites for ET have now been localised in CNS regions such as the area postrema (AP). Therefore we have examined the potential cardiovascular and neuronal effects of ET within this circumventricular organ.

In urethane anaesthetised male Sprague Dawley rats microinjection of ET (0.2-5.0 pmol) into AP resulted in dose dependent biphasic changes in blood pressure (BP). Such effects consisted of initial rises followed by a longer lasting decrease in BP. The latter of these two affects appeared dominant at higher doses of ET (2.0 and 5.0 pmol). Optimal increases (15.5 ± 4.9mmHg), followed by maximal decreases (48.0 ± 8.5 mmHg), in BP were observed in response to 5.0 pmol ET. Such effects were specific to AP injection sites.

Extracellular single unit recordings revealed effects of intravenous ET on the spontaneous activity of AP neurons. The effect of this peptide on the activity of 15 AP neurons was examined in pentobarbital anaesthetised rats. A total of 7 of these neurons demonstrated increases in activity following systemic endothelin (1-20 pmol) followed by return to baseline. Higher doses of this peptide resulted in instability of AP such that neuronal recordings were seldom maintained throughout a test.

These data support a potential physiological role for ET within the rat AP. They suggest ET may activate AP neurons and thus influences cardiovascular function. These findings are in accordance with previous studies showing activation of AP neurons results in decreased BP.

Supported by The Heart and Stroke Foundation of Ontario.

385.7

SPINAL MICROINJECTIONS OF ANTAGONISTS TO SUBSTANCE P AND SOMATOSTATIN ATTENUATE THE EXERCISE PRESSOR REFLEX. L.B. Wilson*, P.T. Wall, K. Matsukawa*, L.W. McCallister and J.H. Mitchell* (SPON: P.M. Adams). Moss Heart Center, UT-Southwestern, Dallas, TX, 75235-9034.

We investigated at a spinal level the role of substance P (SP) and somatostatin (SOM) in the pressor response to static muscle contraction using anesthetized cats. The L_7 ventral root was electrically stimulated (3X motor threshold; 40 Hz; 0.1 msec) to elicit 2 types of triceps surae contractions: 1) a 1-min continuous, tetanic contraction, and 2) a 1-min period of intermittent, tetanic contractions. These were performed before and after the microinjection of the SP antagonist, (D-Pro^2-D-Phe^7-D-Trp^9)-substance P, or the SOM antagonist, cyclo(7-aminoheptanoyl-Phe-D-Trp-Lys-Thr[BZL]), into the L_7 dorsal horn region. Continuous and intermittent tetanic contractions increased mean arterial pressure (MAP) 51±5 and 51±13 mmHg, respectively, before microinjecting the SP antagonist, but only 33±4 and 28±5 mmHg after. Prior to the injection of the SOM antagonist, continuous and intermittent contractions increased MAP 38±4 and 40±8 mmHg, respectively, but only 21±6 and 28±6 mmHg following SOM antagonist. Microinjecting saline had no affect on the pressor response to either type of contraction. These data suggest that SOM and SP release into the dorsal horn of the spinal cord plays a role in the cardiovascular response to static muscle contraction.

385.8

PRAZOSIN INDUCED SYMPATHO-INHIBITION VIA PREJUNCTIONAL FACILITATION OF α_2-ADRENERGIC INHIBITION. M.C. Koss, J.A. Hey and T. Ito. Dept. Pharmacology, Univ. Okla. Hlth. Sci. Ctr., Oklahoma City, OK 73190.

We have previously reported, using CNS evoked electrodermal responses, that prazosin enhances inhibition mediated by alpha$_2$-adrenoceptor mechanisms with at least part of the effect being at the level of the spinal cord (Europ. J. Pharmacol. 158:225-231, 1988). The present study was undertaken to further evaluate this action of prazosin using spontaneous sudomotor activity. Tonic sympathetic-cholinergic electrodermal activity was measured in intact anesthetized and unanesthetized decerebrate and decerebrate-spinalized cats. Intravenous prazosin (3-300 µg/kg, i.v.) depressed spontaneous electrodermal activity in intact cats in a dose-dependent fashion. The alpha$_2$-adrenoceptor blocker, yohimbine (0.5 mg/kg i.v.), antagonized this prazosin-induced sympatho-inhibition and prazosin's effect was totally abolished by monoamine depletion. Prazosin also reduced tonic sudomotor activity in unanesthetized decerebrate cats, but had no effect in spinalized preparations. These results support the hypothesis that prazosin produces sympatho-inhibition via an alpha$_2$-adrenoceptor mediated mechanism, and that this effect appears to be prejunctional. It is proposed that prazosin acts at the level of the spinal cord to facilitate ongoing adrenergic inhibition arising from supra-spinal loci. (Supported by NSF and OCAST)

385.9

INVOLVEMENT OF GUANINE NUCLEOTIDE-BINDING PROTEIN IN GUANABENZ-INDUCED CARDIOVASCULAR SUPPRESSANT EFFECTS IN THE RAT. C.H. Chen* and S.H.H. Chan. Department of Psychiatry, Veterans General Hospital and Institute of Pharmacology, National Yang-Ming Medical College, Taipei 11221, Taiwan.

Previous work from our laboratory suggests that guanabenz, a potent antihypertensive agent, promotes its cardiovascular suppressant actions via the α_2-adrenoceptors, possibly located in the medullary nucleus reticularis gigantocellularis (NRGC). The present study further addresses the question of whether guanine nucleotide-binding protein(s) is involved in the circulatory depressive effects of the aminoguanidine compound, using male, adult Sprague-Dawley rats anesthetized with pentobarbital sodium. Pretreating animals by microinjecting pertussis toxin (125 or 250 ng) bilaterally into the NRGC significantly blunted the hypotensive and negative inotropic and chronotropic effects of guanabenz. Microinjection of Gi uncoupler (n-ethylmaleimide, 12.5 or 25 ng), Gs activator (cholera toxin, 125 or 250 ng), or adenylate cyclase activator (forskolin, 1.25 or 2.5 µg) into bilateral NRGC, however, did not appreciably affect the cardiovascular suppressant actions of guanabenz. These results suggest that a pertussis toxin-sensitive, cAMP-independent, guanine nucleotide-binding protein(s) located in the NRGC, possibly Go, may be involved in the expression of the circulatory depressive effects produced by the amino-guanidine compound.

385.10

LOCALIZATION OF SYMPATHETIC AND PARASYMPATHETIC PREGANGLIONIC NEURONS IN THE PIGEON SPINAL CORD AND BRAIN STEM AFTER INJECTIONS OF THE C-FRAGMENT OF TETANUS TOXIN INTO THE HEART. J.B. Cabot, N. Bogan* and J. Carroll*. Department of Neurobiology, State University of New York at Stony Brook, Stony Brook, NY 11794.

Previous studies in our laboratory have shown that the C-fragment of tetanus toxin (TTC) is transported retrogradely and transsynaptically by avian sympathetic preganglionic neurons (SPN's). Our aim in the present study was to take advantage of these transport properties in order to define anatomically the location(s) of putative "cardiac" sympathetic and parasympathetic preganglionic neurons. TTC was injected into the left ventricle (10 ul, 1-2 mg) of 5 pigeons. Twenty-four to 42 hrs later the animals were sacrificed and TTC-labeled neurons were visualized using a monoclonal antibody to TTC and standard immunohistochemical methods.

In all experiments, TTC-labeled parasympathetic preganglionic neurons were distributed bilaterally and restricted to: (a) the caudal portions of the nucleus ambiguus (nA; -0.70 to 0.30, obex = 0.0); and (b) a subset of cells in the ventrolateral subnucleus of the dorsal motor nucleus complex (VL; 0.70 to 0.80) (Katz and Karten, JCN, 242:358). The majority of TTC-labeled neurons were located in nA; most labeled cells were medium-sized, multipolar neurons. The limited number of TTC labeled cells within VL were similar morphologically to those labeled in nA.

Transneuronal TTC labeling of SPN's within spinal cord was much more difficult to detect. TTC labeling of SPN's was identified in only 3 of the 5 experiments. In these cases, TTC-labeled SPN's were: (a) distributed bilaterally; (b) located within the caudal portions of C14 (last brachial spinal segment), and throughout T1-4; and (c) restricted to the principal cell column (column of Terni). The majority of TTC-positive SPN's were located in T1-2. On average, fewer than 3 SPN's / 40 um transverse section (< 8% of the SPN's in T1-2) were TTC-positive in T1-2. (Supported by HL24103; JBC is an Established Investigator of the AHA.)

385.11

CENTRAL HISTAMINE DEPLETION ATTENUATES PRESSOR RESPONSE TO HYPERTONIC SALINE INFUSION. V. Schaumloffel* and S.L. Bealer (SPON: L. Share). Dept. Physiol. and Biophys., Univ. Tenn., Memphis, TN 38163.

The effect of central histamine (HA) depletion on the cardiovascular response to increased plasma osmolality (pOsm), induced by hypertonic saline infusion, was studied. Conscious male Sprague-Dawley rats were pretreated with alpha-fluoromethylhistidine (FMH, 500 µg icv), an irreversible inhibitor of the histamine synthetic enzyme, histidine decarboxylase, or vehicle (CONT). Two hours after FMH or vehicle, an iv infusion of 2.5 M (HT) or 0.17 M (ISO) NaCl was started (10 µl/100 g/min) and continued for 30 minutes. Mean arterial pressure (MAP), heart rate (HR), pOsm before and after infusion, and hypothalamic HA concentration were measured.

FMH pretreatment resulted in a 50% depletion in hypothalamic HA, but did not alter baseline MAP. ISO infusion had no effect on MAP or HR in either CONT or FMH rats. The pressor response to HT infusion was significantly attenuated in FMH rats (n=10) compared to CONT (n=10) (0.83 ± 0.17 vs. 1.37 ± 0.21 mm Hg blood pressure increase per mOsm increase in pOsm). Bradycardia was similar in CONT (-12 ± 8 beats/min) and FMH (-21 ± 9.5 beats/min) rats. These results suggest a role for brain histamine in osmotic regulation. (Supported by NIH grant HL 25877.)

385.12

SUBSTANCE P (SP) INNERVATION OF VAGAL CARDIOMOTOR NEURONS (VCN) IN THE NUCLEUS AMBIGUUS. M.M. Caverson[1] and T.-X. Zhang[2]. Departments of Anatomy[1] and Physiology[2], The University of Western Ontario, London, Canada N6A 5C1.

Central peptidergic pathways have been implicated in the control of the circulation. However, little is known about the peptidergic innervation of VCN. In the present study, two series of experiments were done in rats to investigate the SP innervation of VCN in nucleus ambiguus and the function of this innervation. In the first series, injections of wheat germ agglutinin (WGA) conjugated horseradish peroxidase (HRP) were made into either the right atrial myocardium or the pericardial sac. After a 1-3 day survival period, the animals were perfused transcardially and transverse brain stem sections were processed using HRP (TMB) histochemistry to reveal WGA retrogradely labeled perikarya and subsequently for SP-immunohistochemistry using the avidin-biotin (DAB) method. Within the nucleus ambiguus, a dense network of SP-immunoreactive fibers and presumptive nerve terminals were observed surrounding WGA labeled VCN. In the second series, microinjections of L-glutamate were made into the region of nucleus ambiguus in anesthetized C1 spinal cord transected rats to identify vagal cardioinhibitory sites. These sites were then tested with an injection of SP. SP elicited decreases in heart rate of 20-80 beats/min. These results provide evidence for the involvement of SP in the central control of VCN function. (Supported by MRC)

385.13

AFFERENT PATHWAYS MEDIATING SYSTEMIC SEROTONIN (5-HT)-INDUCED SYMPATHOINHIBITION. K.J. Varner, S.J. Lewis* and M.J. Brody, Dept. of Pharmacol. & Cardiovascular Center, University of Iowa, Iowa City, IA 52242.

Activation of cardiopulmonary (CP) vagal trunk afferents by intravenously (iv) injected 5-HT elicits the Bezhold-Jarisch reflex characterized by hypotension and sympathoinhibition. The purpose of this study was to determine the specific afferent pathways mediating 5-HT-induced sympathoinhibition. Experiments were performed in urethane-anesthetized rats prepared for recording arterial pressure and lumbar chain sympathetic nerve activity (LSNA). Administration of 5-HT (0.6-40 µg/kg) elicited dose-dependent decreases in LSNA (17-70%). Bilateral transection of the vagus nerve and superior laryngeal (SLN) nerve attenuated but did not abolish the decreases in LSNA. Subsequent bilateral transection of the glossopharyngeal (GL) nerves removed the remaining sympathoinhibitory responses at all doses. Section of GL nerves alone attenuated the 5-HT-induced falls in LSNA by approximately 30%. The GL contribution was abolished by removal of the superior cervical ganglion and SLN. These studies are the first demonstration that in addition to the vagus, afferents mediating 5-HT-induced sympathoinhibition also course in the GL nerve.

RETINA IV

386.1

HUMAN CONES CAN PROCESS ONE MILLION QUANTA PER MILLISECOND
C.W. Tyler, R.D. Hamer and L. Lei, (SPON: E.E. Sutter) Smith-Kettlewell Institute, San Francisco, CA 94115

How many quanta can be processed by the photochemical reactions of light transduction before saturation? With about 600 disks in each human peripheral cone, each disk surface appears to act as an independent transduction unit. Thus, linear response behavior should be expected at least up to levels of about 600 quanta absorbed per cone within the cone response time (i.e. simultaneously). Beyond this level, progressive response saturation would be expected if each disk surface were fully activated by absorption of a single quantum.

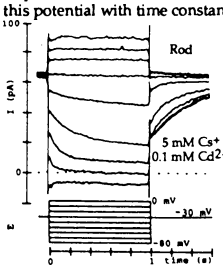
We measured human light response properties at high intensities, where brief flashes were at the psychophysical detection threshold - a 30' field of 660 nm at 35° eccentricity with an equiluminant white surround. Temporal integration thresholds for pulses of different durations analyzed response linearity as stimulus duration was reduced. Complete linearity with no evidence of saturation was obtained for durations down to 100 usec, where one million quanta were absorbed by each cone at threshold. This amounts to about 1000 quanta absorbed and processed linearly in each outer segment disc surface. These results strongly refute the hypothesis that each disk surface can be fully activated by a single quantum.
*Supported by NSF BNS 8696146 and NEI EY 6555 & 1P30 EY 6883. L.L. was supported by an Atkinson Fellowship.

386.2

The K^+ current that speeds photoreceptor responses to dim light. S. Barnes*, D.I. Beech* and B. Hille. Univ. of Washington Sch. of Med., Seattle WA 98195.

The voltage response of photoreceptors reaches a peak earlier than the photocurrent at all light intensities. Ionic mechanisms shaping the response to bright light are well characterized: the pronounced sag in the voltage waveform is produced by I_h, a Cs^+-sensitive cation current activated by hyperpolarization beyond -50 mV. But a different ion channel type must speed responses to dim light: even a 1 mV response peaks sooner than the corresponding photocurrent, and this is Cs^+-insensitive.

Using whole-cell patch-clamped, mechanically isolated rods and cones from salamander we have characterized a new potassium current, I_{Kx}, with the right properties to perform this role. I_{Kx} is almost maximally activated at a dark resting potential of -35 mV (activation curve midpoint -46 ± 7 mV, slope factor 5 ± 1 mV, s.d., n=23) and deactivates with hyperpolarization from this potential with time constants of 100-300 ms. It contributes about 40 pA of current at -35 mV in rods, accounting for the return (outward) flow of inward dark current. I_{Kx} is insensitive to 5 mM Cs^+ or 4-AP but is blocked by 5 mM Ba^{2+} or TEA (40% at 10 mM). The block by Ba^{2+} at -35 mV is due to an apparent 40 mV positive shift of the activation curve. The current is much smaller in cones (12 ± 4 pA). I_{Kx} runs-down in 30 minutes. We have not seen it modulated by GABA, glutamate, glycine, ACh, cadaverine or LHRH. Supported by NIH NS08174 and a McKnight Neuroscience Research Award.



386.3

VOLTAGE- AND TRANSMITTER GATED CURRENTS IN ISOLATED ROD BIPOLAR CELLS OF THE RAT RETINA.
Andreas Karschin* and Heinz Wässle (SPON: G.Pilar). Max-Planck-Institut für Hirnforschung, D-6000 Frankfurt, FRG.

We isolated bipolar cells from the adult rat retina using enzymatic and mechanical dissociation procedures. Based on their morphology and protein kinase C immunocytochemistry, isolated cells were identified as rod bipolar cells. Several voltage-gated conductances could be measured in the cells when studied with the whole-cell recording technique. In response to depolarizing voltage pulses, bipolar cells revealed a fast transient and a delayed sustained K^+ outward current, as well as a small Ca^{2+} inward current. In addition, an inwardly rectifying current, I_h, was observed upon hyperpolarization.

In almost all cells tested GABA- and glycine-activated Cl^- conductances could be antagonized by bicuculline/picrotoxin and strychnine, respectively. Local drug application revealed a higher GABA sensitivity at the bipolar axonal endings compared to their somatic/dendritic region. Similar experiments and also glycine-gated channels identified in excised somatic patches indicated a higher glycine sensitivity close to the bipolar cell body. Conductances induced by the putative photoreceptor transmitter glutamate and its agonists kainate, NMDA, and 2-amino-4-phosphonobutyric acid (APB) were observed in a proportion of the cells. In the majority of responding cells APB activated currents which reversed at 0 mV under symmetrical Cl^- conditions (Sol. electrode: CsCl; bath: NaCl). These responses were associated with the *opening* of ion channels. Only in a minority of cells (5%) did APB induce the *closure* of ion channels resulting in an outward current at negative clamp potentials.

386.4

TOPOGRAPHIC RELATIONSHIPS BETWEEN ROD-SIGNAL INTERNEURONS IN RABBIT RETINA. I.C. Gynther, H.M. Young* and D.I. Vaney*. Vision, Touch & Hearing Research Centre, Univ. of Queensland, St Lucia, Qld 4067, Australia

In mammalian retina, rod bipolar cells and AII amacrine cells are the second- and third-order neurons, respectively, in the rod-signal pathway. These two cell types have been identified in rabbit retina using selective neuronal markers and then characterized by Lucifer Yellow injection under direct visual control, enabling us to examine their dendritic morphologies, topographic distributions and structural relationships. The rod bipolar cells were stained in retinal wholemounts using protein kinase C immunohistochemistry (Negishi, K. et al., *Neurosci. Lett.*, 94: 247,1988). The rod bipolars reach maximum densities of 6,000 cells/mm^2 on the inferior and superior flanks of the horizontal streak, dropping to lower values over the peak visual streak. Their centre-to-periphery density ratio (3:1) is much less than that of the retinal ganglion cells (30:1). Rod bipolar axons give rise to multiple lobes within S5 of the inner plexiform layer, with the axonal-field diameter increasing from 11 µm in the visual streak to 20 µm in peripheral retina, and the axonal-field overlap ranging from 0.5 to 0.9.

In rabbit retina, the AII amacrine nuclei can be selectively labelled by injecting 5 µg Nuclear Yellow into the vitreous 2 days before enucleation and then incubating the eyecup in Ames medium for several hours; the stained retina is metachromatic under UV excitation, with the AII cells fluorescing yellow and other amacrines fluorescing blue. The AII amacrines reach a maximum density of 3,000 cells/mm^2 in the peak visual streak, and show a centre-to-periphery density ratio of 6:1. Although the somata of AII amacrines are distributed irregularly, their dendrites achieve uniform coverage of the retina; in the mid-periphery, the dendritic-field overlap of the sublamina *a* and *b* dendrites is 0.9 and 2.3, respectively. Photoconversion of Lucifer-filled AII cells with DAB followed by immunostaining of the rod bipolars using a contrasting chromagen, shows that the AII dendrites wrap around the axonal lobes of the bipolar cells.

386.5

POLYAXONAL AMACRINE CELLS OF THE RETINA. E. V. Famiglietti. Dept. of Anat. and Lions Sight Ctr., Univ. of Calgary, Calgary, AB, Can. T2N 4N1.

Two types of polyaxonal amacrine cell have been identified previously in rabbit retina (Famiglietti and Siegfried,'80, Famiglietti,'81, SNS Abs.). A third type (PA3) has been found resembling PA1 and PA2. All 3 give rise in the proximal dendritic tree to 1-6 "axons" of small and uniform caliber, branching sparsely at right angles, exhibiting boutons en passant, and extending for 2-3 mm in the same strata as the dendrites branch. PA1 amacrine cells are unique in the interstitial position (mid-IPL) of their cell bodies. Their wide dendritic trees are sparsely branched with zig-zag dendritic terminations, small dendritic spines near the cell body, and broad stratification at the a/b sublaminar border. Bodies of PA2 and PA3 cells are "displaced" to the ganglion cell layer. PA2 cells are smaller with branching at the stratum (S)4/5 border, and occasionally S1. PA3 cells resemble PA1 cells, but are more highly branched, multistratified (S1-S4), and have clusters of long spines. EM study of a PA2 cell reveals presynaptic dendrites, supporting an amacrine, rather than ganglion cell identity for PA amacrine cells.

(Supported by MRC & AHFMR of Canada)

386.6

SYNAPTIC CONNECTIONS OF AII AMACRINE CELLS IN THE RABBIT RETINA. E. Strettoi*, E. Raviola, and R.F. Dacheux*, Istituto di Neurofisiologia del CNR, Pisa, Italy and Dept. of Anatomy and Cell Biology, Harvard Medical School, Boston, MA 02115.

In the rabbit retina the scotopic information generated in rod photoreceptors is conveyed to the inner plexiform layer (IPL) through a single type of rod bipolar that, in turn, has its major output onto the narrow-field, bistratified (AII) amacrine cell. We have examined the synaptic connections of this amacrine cell type by the electron microscopical analysis of serial ultrathin sections. In the vitreal part of the IPL (on-sublamina) the AII dendrites receive ribbon synapses from rod bipolars; in this sublamina, the transfer of scotopic information to the on-channel is mediated by large gap junctions between AII dendrites and the axonal arborization of cone bipolars. In the scleral part of the IPL, scotopic information reaches the off-channel through chemical synapses established by the lobular appendages of AII amacrines on the axonal arborization of cone bipolars. Only exceptionally, either the main dendrite of the AII or its lobular appendages are presynaptic to ganglion cell dendrites in the scleral half of the IPL. Thus, the rod pathway in the rabbit retina consists of five neurons arranged in series: rods, rod bipolars, AII amacrines, cone bipolars and ganglion cells. Supported by grants EY01344 and EY03011.

386.7

GAP JUNCTIONS BETWEEN AII AMACRINE SOMAS IN CAT RETINA. N.Vardi & P.Sterling. Anat Dept, U of P, Phila PA 19104

The AII amacrine cell is a narrow-field interneuron on the pathway from rod bipolars to ganglion cells. Neighboring AII cells are potentially coupled in sublamina b via large gap junctions ($0.5 \pm 0.3\mu m^2$) between their dendrites and b_1 cone bipolar axons and also by occasional, smaller junctions directly between AII dendrites. We report that neighboring AII cells are also extensively coupled by gap junctions between their somas in the inner nuclear layer and between somas and AII processes in sublamina a. We studied electron micrographs of serial, radially cut, thin sections in a patch of retina 25 x 90 μm within the area centralis. The patch contained 43 amacrine cells of which 12 were AII. In every case (4) where AII somas abutted they were connected by a large, discoid gap junction ($3 \pm 2.5\mu m^2$). These were the only gap junctions observed between amacrine somas. Where neighboring AII somas were not in apposition, they were coupled via large ($1\mu m^2$) gap junctions between their appendages and the somas and also between their appendages in sublamina a. Apparently, the quantal signal carried by a rod bipolar cell is first amplified by multiple, chemical synapses onto the AII and then pooled spatially with other such signals into an AII syncytium created by these extensive electrical synapses.

386.8

LOCALIZATION OF GLUTAMATE DECARBOXYLASE (GAD) mRNA IN THE RAT RETINA BY IN SITU HYBRIDIZATION HISTOCHEMISTRY. M.F.Humphrey, K. Anderson*, C. Sternini and N. Brecha. Dept. of Psychology, University of Western Australia, Australia; Depts. of Medicine and Anatomy & Cell Biology and CURE, UCLA School of Medicine and VAMC-West Los Angeles, LA, CA.

The cellular expression of GAD mRNA was examined in the adult and developing rat retina using in situ hybridization histochemistry with a ^{35}S-labeled rat GAD-encoding RNA probe. Cryostat and paraffin sections were incubated with antisense or sense RNAs at 55°C, treated with RNase A, washed at high stringency (0.1x SSC @ 60°C) and then autoradiographed. In adult retina, GAD mRNA was expressed in numerous cells located in the inner INL and GCL, and in some cells within the IPL. There was negligible labeling of INL cells located adjacent to the OPL. A similar distribution and density of labeled cells were observed in retinas at postnatal day (PND) 5, 10 and 20. However, in PND 1 retinas, labeled cells were only located in the INL adjacent to the presumptive IPL border. No significant label was observed in sections incubated with sense RNA probes. This pattern of GAD mRNA expression closely resembles the distribution of GAD- and GABA-like immunoreactivity in the adult rat retina suggesting that the majority of cells containing GAD mRNA-encoding transcripts are amacrine and displaced amacrine cells.

We would like to thank A. Tobin for the rat GAD cDNA. Supported by NIH grants EY 04067, DK 40469, VAMC Research Funds and NH&MRC (Aust) grant 890174.

386.9

EFFECTS OF GLUTAMATE AGONISTS AND ANTAGONISTS ON ISOLATED GOLDFISH RETINAL GANGLION CELLS. Bruce Yazejian, B.N. Cohen* and G.L. Fain. Jules Stein Eye Institute, UCLA School of Medicine Los Angeles, CA 90024-1771.

We have used patch-clamp techniques to explore the effects of glutaminergic agonists and antagonists on ganglion cells isolated from the goldfish retina. Most cells responded to kainate (KA), quisqualate (QA) and AMPA 2-36 hrs after retinal dissociation, as well as to glutamate (Glu), aspartate, and NMDA when they were co-applied with 1 μM glycine (Gly). We saw responses to QA and AMPA at concentrations as low as 0.3 μM, and responses to Glu plus Gly at 1 μM. Responses to Glu, QA and AMPA reached saturation near 30 μM but even 30 mM KA failed to fully saturate the whole-cell response. Under our recording conditions (symmetric Na^+, 1mM Ca_o^{2+}, and no Mg^{2+}), most of the Glu response appeared to be mediated by NMDA-type channels; NMDA-activated single-channel currents had a slope conductance of 24 pS between -80 mV and 0 mV (10°C). Kynurenic acid competitively blocked responses to KA, QA and AMPA but was much less potent against QA than KA or AMPA. KA-activated currents were also competitively blocked by QA and AMPA. Low external pH antagonized the current activated by KA. Supported by NIH EY 01844 and NRSA 06092.

386.10

VOLTAGE-ACTIVATED SODIUM AND CALCIUM CURRENTS IN GOLDFISH RETINAL GANGLION CELLS. A. T. Ishida. Department of Animal Physiology, University of California, Davis CA 95616.

Although it has long been known that retinal ganglion cells produce sodium (Na)-dependent action potentials (e.g. Murakami & Snigematsu, 1970), it has recently been shown that they can also produce calcium (Ca)-dependent action potentials in situ (e.g. Murakami & Takahashi, 1988). We have recently described methods for identifying ganglion cells in vitro after dissociation from adult goldfish retinas (Ishida & Cohen, 1988), and have now identified two voltage-activated currents which should contribute to the action potentials described above. One current (i_{Na}) appears to be carried by Na ions, whereas the other (i_{Ca}) appears to be carried inwardly by Ca ions. These currents were recorded with the whole-cell patch-clamp method, from single ganglion cells within 3-36 hours after dissociating them from adult goldfish (Carassius auratus) retinas. Patch-electrodes were usually filled with (in mM) 140 CsCl, 3 NaCl, 10 EGTA, 0.5 $CaCl_2$, 2 $MgCl_2$, 3 HEPES; the bath contained 140 NaCl, 2.5 $CaCl_2$, 10 glucose, 10 HEPES; pH 7.5; routinely, holding potential was -95 mV. Large, transient i_{Na} was elicited in all ganglion cells by step depolarizations positive to -50mV. Inward i_{Na} decreased when external [Na] was decreased, and appeared to be completely blocked by 300 nM tetrodotoxin (TTX). In the presence of 300 nM TTX, i_{Ca} with a transient peak and sustained plateau was elicited by step depolarizations positive to -50 mV. Inward i_{Ca} increased when external [Ca] was increased, was augmented by BAY K8644 (100 nM), and was blocked by 3 mM Co or 20 μM Cd. In preliminary experiments, i_{Ca} appeared to be unaffected by the $GABA_B$ agonist baclofen (30 μM).

Supported by NIH grant EY08120.

386.11

KYNURENIC ACID REVERSES THE EFFECTS OF A DOPAMINE D_1 ANTAGONIST ON ON-CENTER GANGLION CELLS IN THE RABBIT RETINA. R.J.Jensen. Southern College of Optometry, Memphis, TN 38104.

Dopamine D_1 antagonists increase the spontaneous activity and reduce the light-evoked responses in On-center ganglion cells (Neuroscience 17:837, 1986). A major target of dopaminergic neurons in mammalian retinas appears to be rod AII amacrine cells. These cells form electrical synapses with depolarizing cone bipolar cells, which in turn make chemical synapses with On-center ganglion cells. Since the bipolar cells are thought to use an excitatory amino acid (EAA) transmitter, I examined the possibility that the effects of D_1 antagonists on On-center ganglion cells were due to an increased release of an EAA from the bipolar cells.

Ganglion cell action potentials were recorded extracellularly with microelectrodes from a rabbit retinal strip preparation. The general EAA antagonist kynurenic acid, bath-applied at 380-600 uM, reversed the effects of the D_1 antagonist (+)-SCH 23390 on both On-center brisk-sustained cells and On-center brisk-transient cells. The spontaneous activity and light-evoked responses in these cells were now similar to control records.

In conclusion, dopamine D_1 antagonists appear to disinhibit AII amacrine cells which results in an increased release of an EAA onto On-center ganglion cells.

386.12

FACILITATION IN ON-OFF DIRECTIONALLY SELECTIVE GANGLION CELLS OF THE RABBIT RETINA. Norberto M. Grzywacz and Franklin R. Amthor. MIT E25-201, Cambridge, MA 02139, and Dept. Psychol., Univ. Alabama at Birmingham.

Although Barlow and Levick (1965) reported that inhibition underlies directional selectivity in ON-OFF retinal ganglion cells, they also observed small facilitations for preferred direction motions. These facilitations appeared in two-slit apparent motions, which elicited cell responses larger than the sum of the responses to the isolated slits. Due to the reported smallness, this effect has been neglected. However, we found that sometimes this facilitation augments responses by threefold or more. We investigated the facilitation's spatiotemporal properties with two-slit apparent motions. Facilitation only occurs if the first slit precedes the second by at least 100 *msec* and remains on. This effect is sustained, occurring even after a 700 *msec* delay. Although the strongest facilitations happen for small slit separation, they often occur for separations up to 200 μm. Facilitation occurs for most motions, whose projections to the preferred-null axis point to the preferred direction. However, facilitation occurs, in some cases, even when the projections point to the null direction. Our data suggest that facilitation is directionally selective. Particularly, it is strongest for small slit separation, even if at a larger separation, the first slit would elicit more response alone. This shows that facilitation does not depend directly on the first slit's response and thus, it is not the cell's threshold masked by null-direction inhibition. Alternative mechanisms include a depolarizing shunting synapse (Torre and Poggio, 1978) or a first-transient-then-sustained excitatory synapse. *Supported by NSF (BNS-8809528), NIH (EY05070), and Sloan.*

386.13

CENTRAL PROJECTIONS OF RABBIT RETINAL GANGLION CELLS M.L. Pu* & F. R. Amthor[1]. *School of Optometry
Dept. of Psychology & Neurobiology Research Center[1]
Univ. of Alabama at Birmingham 35294

The ganglion cells that project to specific brain nuclei in rabbit have been identified by retrograde transport of fluorescent dyes, followed by intracellular recording and staining of labeled somas. Focal injections of Fast Blue and Rhodamine latex beads were made in CNS areas includng the dLGN, pretectum, and superior colliculus. Fluorescent ganglion cell somas in the retina were then intracellularly impaled in an isolated, living retina preparation and stained with Lucifer Yellow or Rhodamine-HRP to reveal the the projecting cells' dendritic morphologies. Because the dendritic morphologies of most major classes of retinal ganglion cells in rabbit retina have now been directly identified by intracellular recording and staining (Amthor et al. JCN '89), the physiology of many of these cells is known.

At least 6 ganglion cell classes project to the dLGN. Cell types labeled varied with injection location within the dLGN. In some cases all the injected cells were alpha-like (brisk-transient physiology), but in other cases beta-like (brisk-sustained physiology), sparse dendritic trees (sluggish physiologies), and bistratified cells (On-Off directionally selective) were labeled.

Focal injections in the nucleus of the optic tract (NOT) labeled unistratified cells in the visual streak corresponding to the On directionally selective (DS) ganglion cells. Other pretectal injections revealed On and On-Off DS ganglion cell classes in the visual streak.

Supported by EY05070

LINGAND-GATED ION CHANNELS I

387.1

CHANNEL PERMEANT CATIONS BLOCK NONCOMPETITIVE INHIBITOR BINDING TO THE NICOTINIC ACETYLCHOLINE RECEPTOR. J.M. Herz, T.P. Erlinger* and S.J. Kolb*. Institute for Neuroscience and Cell Research Institute, Univ. of Texas at Austin, Austin, TX. 78713.

Electrophysiological studies have shown that permeant cations bind to certain sites within the receptor channel as part of the permeation process. Since allosterically coupled noncompetitive inhibitor (NCI) ligands are postulated to bind within the channel, we have investigated whether NCI ligands and cations bind to a common site. Using ethidium as a probe of the NCI site, we have shown that bound ethidium can be completely displaced from Torpedo AChR by inorganic monovalent cations (rank order $Tl^+ > K^+ > Na^+ \sim Li^+ > Cs^+$). The cation K_ds vary markedly and range from 1.7 to 200 mM. Hill plots of the competitive dissociation of ethidium in the presence of carbamylcholine show slopes of 1.0 indicating the cations bind to a single class of independent sites, as previously shown for PCP and HTX. Forward titrations of ethidium in the presence of 5 and 20 mM Tl^+ and 100 µM carbamylcholine were carried out. Scatchard analysis revealed a reduction in the equilibrium affinity and no changes in $B_{(max)}$ indicating a competitive interaction. The divalent cations, Ca^{2+} and Mg^{2+} (K_d= 45 and 86 mM, respectively) also displaced ethidium. Inhibition of ethidium binding by Tl^+, K^+ and Mg^{2+} was reversed after removal of these ions by ultracentrifugation. The kinetics of ethidium dissociation induced by the addition of Tl^+, Na^+, Mg^{2+}, Ca^{2+} and PCP are similar indicating that the dissociation rate of ethidium is the rate limiting step. Our cation K_ds are in close agreement with the K_ds for channel saturation calculated from single channel conductances for these cations (Adams, et al. (1981) J. Gen Physiol. 78, 593-615). We suggest that the cation binding site(s) is the same site to which noncompetitive inhibitors bind and block channel function.

387.2

AN α-BUNGAROTOXIN BINDING PROTEIN FROM *APLYSIA* ISOLATED BY BROMOACETYLCHOLINE AFFINITY CHROMATOGRAPHY
J. T. McLaughlin and E. Hawrot Dept. of Pharmacology, Yale Univ. School of Medicine New Haven, CT. 06510

The body wall musculature of *Aplysia californica* contains an α-bungarotoxin (α-BTX) binding protein that is biochemically similar to a functional acetylcholine receptor (AChR) in *Aplysia* ganglia. Earlier studies (McLaughlin and Hawrot, *Mol. Pharm.* 35, in press) had established that both *Aplysia* α-BTX binding activities resided on multimeric proteins resistant to dissociation in SDS. We have now used affinity chromatography on bromoacetylcholine-coupled Affigel 15 to isolate the *Aplysia* α-BTX binding activity from muscle membrane extracts. Analysis of eluted samples by SDS gel electrophoresis reveals a major polypeptide at 260 kDa (which retains α-BTX binding activity) and minor polypeptides at 60 kDa and 45 kDa. The 260 kDa polypeptide was further purified by electro-elution from SDS gels. Amino acid composition analysis of the electroeluted sample was characteristic of an integral membrane protein, and was similar to that of the AChR from *Torpedo*. When the electroeluted protein is re-analyzed on SDS gels the minor polypeptides reappear despite inclusion of protease inhibitors throughout the isolation. This suggests that the smaller polypeptides eluted from the affinity column may be subunits of the partially dissociated 260 kDa protein. In combination with earlier crosslinking studies that identified an α-BTX binding subunit in both *Aplysia* tissues of 58-65 kDa, we conclude that the *Aplysia* α-BTX binding protein is a multisubunit AChR which appears to include at least two distinct polypeptides, including a 60 kDa ligand-binding subunit .

387.3

EXPRESSION OF RAT MUSCLE ACETYLCHOLINE RECEPTOR EPSILON SUBUNIT IN XENOPUS OOCYTES. P.Camacho, R. H. Goodman*, G. Mandel*, and P. Brehm. Dept. of Physiology., Tufts Univ. Med. Sch., Boston, MA 02111 and Div. Mol. Med., New England Med. Ctr., Boston, MA 02111.

The nicotinic acetylcholine receptor channel from muscle is a pentameric protein assembled from alpha, beta, gamma, and delta subunits. A fifth subunit, epsilon, has been cloned from calf and more recently, from rat (Criado et al., Nucleic Acids Res.,16,1988 and Camacho et al., Biophysics J.,55,1989). Using the Xenopus oocyte translation system, we have studied the single channel properties of acetylcholine receptor channels expressed from synthetic mRNA transcripts. We have obtained functional expression of AChR channels by injecting alpha, beta and delta from mouse, in conjunction with mRNA transcripts from rat epsilon subunit. Injection of mRNAs encoding alpha, beta, delta, epsilon subunits results in inward currents of several microamperes at the macroscopic level. Single channel recordings from outside-out patches revealed two amplitude classes of ACh-activated channels. Both had briefer open times and higher conductances than those obtained by alpha, beta, gamma and delta. Injection of alpha, beta and gamma subunit RNAs result in the expression of substantial macroscopic currents. However, in one experiment injection of alpha, beta and epsilon transcripts did not express functional channels, suggesting that receptors containing epsilon may be conditional on the presence of delta subunit.

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387.4

SYNTHESIS OF A CHANNEL PROTEIN AND CHARACTERIZATION OF ITS SINGLE CHANNEL PROPERTIES. M. Montal, M.S. Montal* and J. M. Tomich*, UCSD, La Jolla, CA 92093 and Children's Hospital, Los Angeles, CA 90027

A 23-mer peptide with the sequence of the M2 segment of the *Torpedo* acetylcholine receptor δ-subunit (EKMSTAISVLLAQAVFLLLTSQR) forms cation-selective channels in lipid bilayers (*PNAS* 85:8703-8707). Channel formation presumably involves self-assembly of conductive oligomers. Here, we synthesized a tethered parallel tetramer with M2δ peptides attached to a multifunctional carrier template (*Tetrahedron* 44:771-785): a 9-aminoacid backbone $K^*KK^*PGK^*EK^*G$ with K containing N^{α} -tboc, N^{ε}-fmoc(*) to generate 4 branch points. M2δ was then attached to template in a stepwise manner at the 4 base-deprotected K side chains. The complete 101 residue protein was cleaved in HF and purified by RP-HPLC. It migrates as a single band in SDS-PAGE (15%) with apparent M_r 11 000. The synthetic channel protein does indeed form channels in phosphatidyl choline bilayers. The single channel conductance γ, channel open (τ_o) and closed (τ_c) lifetimes and percent open time (Po) in symmetric 0.5M NaCl or KCl (10 mM Hepes, 0.5mM $CaCl_2$, pH 7.5) at 100 mV are:

NaCl-γ=18 pS; τ_{o1}=0.8 ms, τ_{o2}=25 ms; τ_{c1}=1.0 ms,τ_{c2}= 17 ms; Po=66%.

KCl- γ=24 pS; τ_{o1}=1.7ms, τ_{o2}=11 ms; τ_{c1}=0.9 ms,τ_{c2}= 4.5 ms; Po=47%.

Membrane conductance increased in discrete steps, integral multiples of these elementary conductances, indicating that the tethered tetramer is the conductive species. We conclude that a four-helix bundle protein is a plausible structure underlying the conductance events.

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387.5

SINGLE-CHANNEL CURRENTS ACTIVATED BY GABA, MUSCIMOL AND ISOGUVACINE IN OUTSIDE-OUT MEMBRANE PATCHES FROM CULTURED CHICK CEREBRAL NEURONS. D.K.Mistry* and J.J.Hablitz, Neurobiology Res Ctr, Univ. of Al. at Birmingham.

Single-channel currents activated by GABA (500nM-1μM) muscimol (200-500nM) and isoguvacine (500nM-2μM) were recorded from outside-out patches in isotonic Tris-Cl solutions. Under these conditions GABA-activated channels had multiple conductance states of 12, 19, 26 and 31 pS. Transitions between this and the other conductance levels were found. Muscimol and isoguvacine activated similar conductance levels. For all agonists, the main conductance level was around 26 pS. I-V relations for all agonists were linear and reversed around 0 mV. Analysis of the main-state openings gated by GABA revealed two time constants of 0.15 ± 0.04 and 2.07± 0.37 ms (n=5). Similar time constants of 0.48 ± 0.22 and 2.21 ± 0.43 ms (n=4) were obtained for isoguvacine. Open time distributions for muscimol were best described by the sum of three exponentials having time constants of 0.16 ± 0.08, 1.15 ± 0.43 and 6.53 ± 4.07 ms. GABA, muscimol and isoguvacine activate similar multiple conductance levels in chick cerebral neurons. Opentime kinetics for GABA and isoguvacine are similar while muscimol openings displayed an additional longer open state. Additionally, in some patches the occurrence of subconductance levels were more frequent with muscimol and isoguvacine. Supported by NS11535, NS18145 and NS22373.

387.6

TWO NOVEL $GABA_A$ RECEPTOR SUBUNITS EXIST IN DISTINCT NEURONAL SUBPOPULATIONS. B.D. Shivers, I. Killisch*, R. Sprengel*, H. Sontheimer*+, M. Köhler*, P.R. Schofield* and P.H. Seeburg*. Center for Molecular Biology, INF 282 and +Institute for Neurobiology, INF 364, University of Heidelberg, D-6900 Heidelberg, F.R.G.

Two cDNAs encoding novel $GABA_A$ receptor subunits were isolated from a rat brain library. These subunits, named γ_2 and δ, share approximately 35% sequence identity with α and β subunits and, like these, form functional GABA-gated chloride channels when expressed alone in cultured cells. The γ_2 subunit represents the rat homolog of the recently described human γ_2 subunit shown to be important for benzodiazepine (BZ) pharmacology (D.B. Pritchett et al., Nature, 338:582, 1989). Cellular localization of the mRNAs encoding the γ_2 and δ subunits in rat brain revealed that largely distinct neuronal subpopulations express the two subunits. The distribution of the δ subunit resembles that of high affinity $GABA_A$ receptors labeled by ^{3}H-muscimol while the localization of the γ_2 subunit resembles that of $GABA_A$/BZ receptors labeled with ^{3}H-flunitrazepam. These findings have implications for the subunit composition of two different $GABA_A$ receptor subtypes and for information processing in neural networks using GABA for signaling.

387.7

HUMAN $GABA_A$ RECEPTORS ASSEMBLED FROM DIFFERENT COMBINATIONS OF CLONED SUBUNITS HAVE DIFFERENT ELECTROPHYSIOLOGICAL PROPERTIES

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Cultured human embryonic kidney cells were transiently transfected with cDNA clones encoding α_1, β_1, and γ_2 subunits of the human $GABA_A$ receptor. Chloride currents activated by GABA were examined using patch clamp in the whole cell and outside-out configurations (symmetrical Cl-, 140 mM). In cells transfected with cDNAs encoding the α_1 and β_1 subunits whole cell currents (-60 mV) elicited by fast application of 10 μM GABA desensitized markedly to 16 ± 4% (mean ± SD, n=4) of the initial peak current after 10 sec. In contrast, cells transfected with cDNAs encoding the α_1, β_1, and γ_2 subunits (74 ± 18 % of peak after 10 sec., n=4, 10 μM GABA) or only α_1 and γ_2 (no detectable desensitization over 10 sec., n=5, 30 μM GABA) showed much less desensitization. Single channel GABA-activated currents in outside-out patches (-50 mV) from cells transfected with DNAs encoding α_1 and β_1 subunits had a mean conductance of 19.9 ± 1.2 pS (n=4, 10 μM GABA). The single channel conductance was higher in cells cotransfected with DNA encoding the γ_2 subunit: α_1, β_1, and γ_2 transfected together yielded a mean single channel conductance of 36.4 ± 2.5 pS (n=4, 10 μM GABA); cells transfected with α_1 and γ_2 showed a mean conductance of 34.5 ± 2.5 pS (n=3, 30 μM GABA). The reduced desensitization and higher single channel conductance upon cotransfection with DNA encoding the γ_2 subunit may result from the formation of a new $GABA_A$ receptor subtype or the expression of a mixture of subtypes.

387.8

PRIMARY CULTURES OF MOUSE SPINAL CORD EXPRESS THE NEONATAL ISOFORM OF THE INHIBITORY GLYCINE RECEPTOR. W. Hoch*, H. Betz and C.-M. Becker*. ZMBH, Universität Heidelberg, Im Neuenheimer Feld 282, D-6900 Heidelberg, FRG.

Glycine receptor expressed by primary cultures of spinal cord is predominantly of the recently identified neonatal isoform characterized by a low affinity for strychnine. Its ligand binding subunit differs from that of adult receptor in antigenic epitopes and molecular weight. Whereas in vivo the neonatal receptor isoform is completely replaced by the adult isoform within three weeks after birth, this exchange of subtypes is not seen in culture. However, the increased expression of the cytoplasmic glycine receptor-associated 93 kD protein occurring after birth is also observed in culture. Purification of glycine receptor from cultures yielded polypeptides of 49 kD and 93 kD suggesting that the membrane-spanning core of the neonatal receptor may be a homooligomer composed of 49 kD subunits. Pulse-labeling experiments revealed the 49 kD subunit to be a metabolically stable glycoprotein (half life ≈2 days).